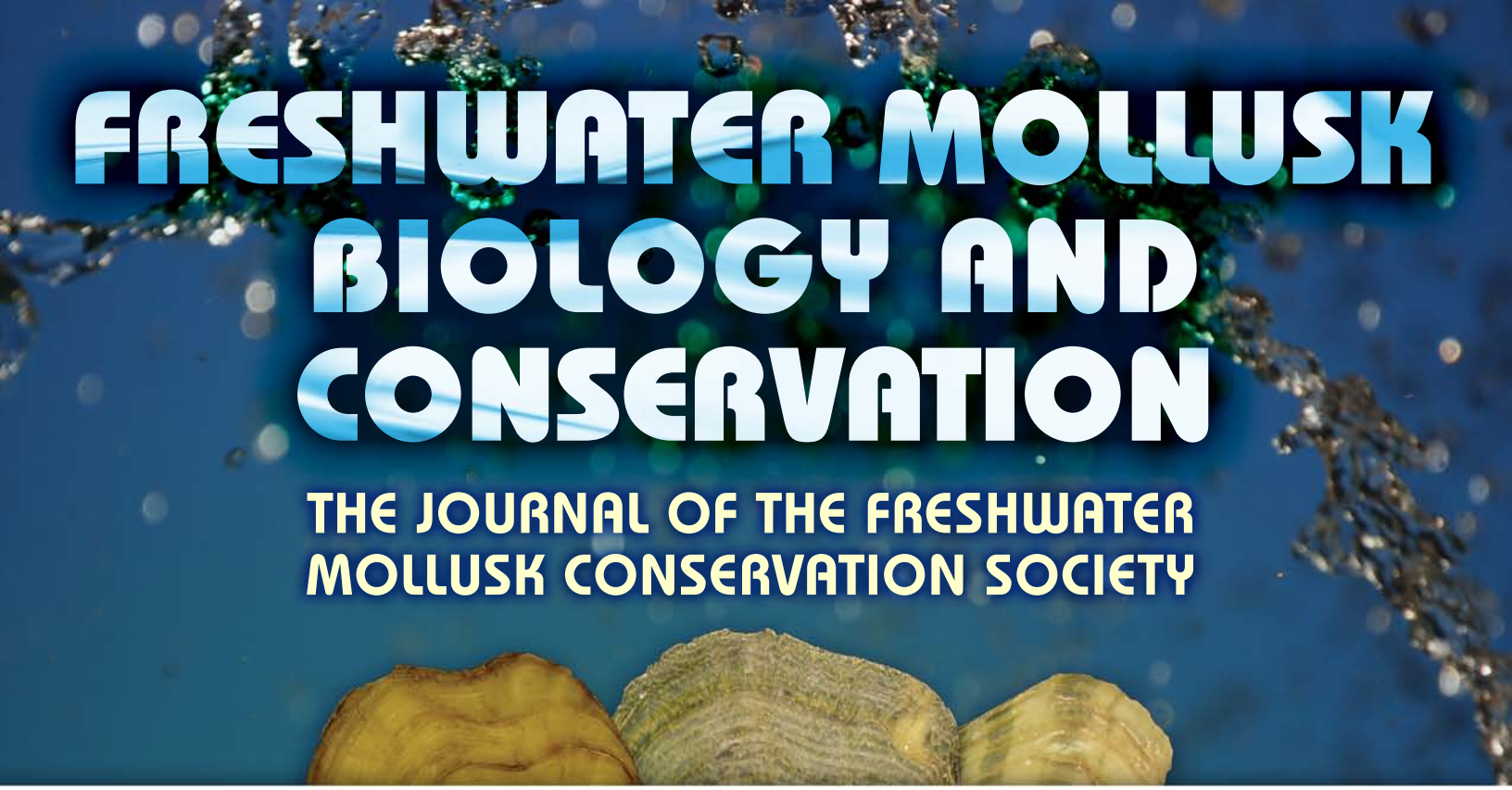

FRESHWATER MOLLUSK BIOLOGY AND CONSERVATION

THE JOURNAL OF THE FRESHWATER MOLLUSK CONSERVATION SOCIETY

VOLUME 19 NUMBER 2 SEPTEMBER 2016

Freshwater Mollusk Biology and Conservation

ISSN 2472-2944

Editorial Board

Freshwater Mollusk Biology and Conservation 19:1–18, 2016

REGULAR ARTICLE

QUANTITATIVE MONITORING OF FRESHWATER MUSSEL POPULATIONS FROM 1979–2004 IN THE CLINCH AND POWELL RIVERS OF TENNESSEE AND VIRGINIA, WITH MISCELLANEOUS NOTES ON THE FAUNA

Steven A. Ahlstedt[1], Mark T. Fagg[2], Robert S. Butler[3], Joseph F. Connell[4], and Jess W. Jones[5*]

[1] *P.O. Box 460, Norris, TN 37828 USA*
[2] *551 Ravenswood Drive, Morristown, TN 37814 USA*
[3] *U.S. Fish and Wildlife Service, 160 Zillicoa Street, Asheville, NC 28801 USA*
[4] *108 Orchard Circle, Oak Ridge, TN 37830 USA*
[5] *U.S. Fish and Wildlife Service, Department of Fish and Wildlife Conservation, 106a Cheatham Hall, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 USA*

ABSTRACT

The Clinch and Powell rivers, Tennessee (TN) and Virginia (VA), upstream of Norris Reservoir, TN, are known for high freshwater mussel species diversity and endemism. Collectively, these rivers harbored at least 56 species historically and 49 are extant, many of which now survive only in the Clinch or Powell rivers or a few other streams. Among an unparalleled 24 federally endangered mussel species known from these rivers, 20 species are considered extant. We sampled 0.25 m^{-2} quadrats at six Clinch River sites and four Powell River sites for a total of 4–6 sample years at each site. Overall trends were highly significant in the Clinch River, with mean mussel density at combined sites in each state increasing from 16.5 m^{-2} to 41.7 m^{-2} ($p < 0.0001$) at sites in TN but declining from 12.0 m^{-2} to 3.3 m^{-2} ($p < 0.001$) at sites in VA. Cumulative species richness was 39, ranging from 36 in TN to 27 in VA. Greater density in the Clinch River, TN, was due primarily to increases in *Epioblasma capsaeformis*, *Medionidus conradicus*, and *Ptychobranchus subtentus*, which were rare or undetected at most sites in 1979, but increased five- to ten-fold by 2004. Conversely, at Pendleton Island, VA, which was the best site for mussels in the river circa 1980, the decline in density was highly significant, from 24.6 m^{-2} in 1979 to 4.6 m^{-2} ($p < 0.001$) in 2004. In the Powell River, there was also a highly significant decline in mean mussel density at combined sites from 8.7 m^{-2} to 3.3 m^{-2} ($p < 0.001$), with a total of 33 species documented. Though species diversity remains relatively high, our results confirm that mussel populations have declined in large reaches of each river over the 25-year study period.

KEY WORDS - Clinch and Powell rivers; biodiversity hotspot; freshwater mussels; endangered species; mussel population declines.

INTRODUCTION

The Clinch River and its largest tributary Powell River are located in northeastern TN and southwestern VA and are part of the upper Tennessee River drainage (Figure 1). The Tennessee River drainage supports the highest freshwater mussel diversity of any comparably-sized river system in the world. More than 105 species are known from this drainage, with at least 36 species endemic to it or shared only with the Cumberland River drainage (Parmalee and Bogan 1998). Collectively, upland portions of these two drainages are known as the Tennessee-Cumberland Province (Haag 2010). Mussel diversity was highest in the mainstem Tennessee River and its large tributaries, but impoundments created during the 1920s through

*Corresponding author: Jess_Jones@fws.gov

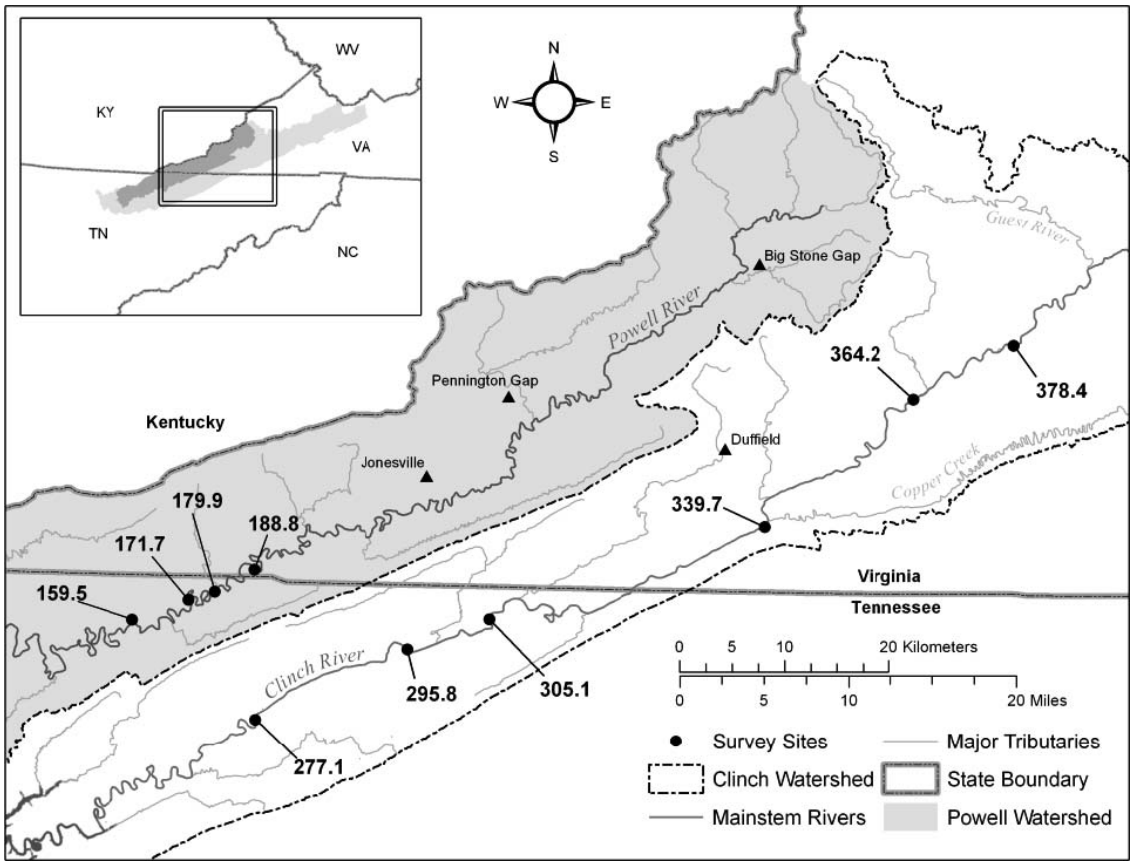

Figure 1. The Clinch and Powell river watersheds showing locations of sites (in RKM) sampled from 1979–2004.

1970s destroyed most large-river habitats (Haag 2009). The lower Clinch River was impounded by Watts Bar Dam on the Tennessee River, and Melton Hill and Norris dams on the Clinch River (river km [RKM] 37.0 and 128.5, respectively). Norris Dam impounds the river to about river km 249 as well as the lower 90 km of the Powell River, and the dam effectively isolated these two rivers and eradicated at least 10 additional species from the drainage (Ortmann 1918; Ahlstedt 1991a). Nevertheless, the free-flowing upper reaches of the Clinch and Powell rivers are among the most important remaining riverine habitats in the Tennessee River drainage, and they support a large percentage of the surviving mussel fauna of the region (Johnson et al. 2012; Jones et al. 2014).

Among the 56 species known historically from the Clinch and Powell river mainstems upstream of Norris Reservoir, 24 are now federally endangered under the Endangered Species Act (ESA), though 4 of these listed species are considered extinct or extirpated from these rivers (Table 1). Further, an additional seven of the extant species are included in a petition for possible federal listing. The Clinch River harbors the largest remaining population (namely, *Dromus dromas*, *Epioblasma brevidens*, *E. capsaeformis*, *Fusconaia cor*, *F. cuneolus*, *Hemistena lata*, *Ptychobranchus subtentus*, *Quadrula cylindrica strigillata*), or one of the few existing populations (e.g., *Cyprogenia stegaria*, *E. florentina aureola*, *Lemiox rimosus*, *Pegias fabula*, *Pleurobema plenum*, *Q. sparsa*, *Venustaconcha trabalis* [studies by Kuehnl (2009) and Lane et al. (2016) have shown that *Villosa trabalis* belongs in the genus *Venustaconcha*, and that *Villosa perpurpurea* is a synonym; see Discussion]), of 15 endangered mussels, in addition to large populations of several other imperiled species (Jones et al. 2014; Table 1). Fifteen of 19 endangered species are considered extant in the Powell River, which itself harbors 1 of only 2 extant populations of *D. dromas*, *Q. cylindrica strigillata*, *Q. intermedia*, and *Q. sparsa* (Johnson et al. 2012).

Various malacologists have reported on the mussels in the Clinch and Powell rivers over the last century. Arnold E. Ortmann (1918), Carnegie Museum, Pittsburgh, Pennsylvania, reported the only systematic pre-impoundment collection records in the study area, including several records from Adams (1915). In the 1960s and 1970s, David H. Stansbery, Ohio State University Museum of Biological Diversity, Columbus, Ohio, and his students made scores of collections in the study area, documenting declines in species richness since Ortmann's (1918) collections, many from areas now inundated by Norris Reservoir (Stansbery 1973). Greater survey effort and interest in conserving the mussel fauna accelerated in the mid-1970s following passage of the ESA in

Table 1. Scientific names, and federal and Freshwater Mollusk Conservation Society (FMCS; J.D. Williams, Florida Museum of Natural History, unpub. data) status of mussel species known from the Clinch and Powell river mainstems upstream of Norris Reservoir in TN and VA. √ = extant and sampled during our study, √x = extant but not sampled or recognized during our study, * = very rare, - = no federal status, CS = currently stable, E = endangered, EX = extirpated, FE = federally endangered, NR = not reported, P = petitioned for federal listing, RI = sampled during our study and subsequently considered extirpated but now extant from reintroduction, T = threatened, V = vulnerable, and X = extinct. Species list and study area status updated from Johnson et al. (2012) and Jones et al. (2014).

Scientific Name	Clinch	Powell	Federal	FMCS
(1) *Actinonaias ligamentina*	√	√	-	CS
(2) *Actinonaias pectorosa*	√	√	-	T
(3) *Alasmidonta marginata*	√*	√*	-	V
(4) *Alasmidonta viridis*	√x*	NR	-	V
(5) *Amblema plicata*	√	√	-	CS
(6) *Anodontoides ferrusacianus*	NR	EX	-	CS
(7) *Cumberlandia monodonta*	√	√x*	FE	E
(8) *Cyclonaias tuberculata*	√	√	-	V
(9) *Cyprogenia stegaria*	√	EX	FE	E
(10) *Dromus dromas*	√	√	FE	E
(11) *Elliptio crassidens*	√*	√*	-	V
(12) *Elliptio dilatata*	√	√	-	V
(13) *Epioblasma brevidens*	√	√	FE	E
(14) *Epioblasma capsaeformis*	√	√ RI	FE	E
(15) *Epioblasma florentina aureola*	√x	NR	FE	E
(16) *Epioblasma haysiana*	X	X	-	X
(17) *Epioblasma lenior*	X	X	-	X
(18) *Epioblasma torulosa gubernaculum*	X	X	FE	X
(19) *Epioblasma triquetra*	√	√*	FE	T
(20) *Fusconaia cor*	√	√*	FE	E
(21) *Fusconaia cuneolus*	√	√*	FE	E
(22) *Fusconaia subrotunda*	√	√	P	E
(23) *Hemistena lata*	√	√x	FE	E
(24) *Lampsilis abrupta*	√x	NR	FE	T
(25) *Lampsilis fasciola*	√	√	-	CS
(26) *Lampsilis ovata*	√	√	-	V
(27) *Lasmigona costata*	√	√	-	CS
(28) *Lasmigona holstonia*	√x*	EX	P	V
(29) *Lemiox rimosus*	√	√*	FE	E
(30) *Leptodea fragilis*	EX	EX	-	CS
(31) *Leptodea leptodon*	EX	NR	FE	E
(32) *Ligumia recta*	√*	√*	-	V
(33) *Medionidus conradicus*	√	√	P	T
(34) *Pegias fabula*	√x*	EX	FE	E
(35) *Plethobasus cyphyus*	√	√	FE	E
(36) *Pleurobema cordatum*	√*	NR	-	V
(37) *Pleurobema oviforme*	√	√*	P	T
(38) *Pleurobema plenum*	√	NR	FE	E
(39) *Pleurobema rubrum*	√	NR	P	E
(40) *Pleurobema sintoxia*	√x*	NR	-	V
(41) *Pleuronaia barnesiana*	√	√*	P	V
(42) *Pleuronaia dolabelloides*	√	√*	FE	E
(43) *Potamilus alatus*	√	√*	-	CS
(44) *Ptychobranchus fasciolaris*	√	√	-	V
(45) *Ptychobranchus subtentus*	√	√	FE	E
(46) *Quadrula cylindrica strigillata*	√	√	FE	E
(47) *Quadrula intermedia*	EX	√	FE	E
(48) *Quadrula pustulosa*	√	√	-	CS

Table 1, continued.

Scientific Name	Clinch	Powell	Federal	FMCS
(49) *Quadrula sparsa*	√x*	√	FE	E
(50) *Strophitus undulatus*	√x	√x*	-	CS
(51) *Toxolasma lividum*	√x*	EX	P	V
(52) *Truncilla truncata*	√*	√x*	-	CS
(53) *Venustaconcha trabalis*	√*	EX	FE	E
(54) *Villosa fabalis*	EX	NR	FE	E
(55) *Villosa iris*	√	√	-	CS
(56) *Villosa vanuxemensis*	√	√	-	V
Total Species Known	55	47		
Total Species Extant	48	37		
Total Species Extant Sampled 1979–2004	39	33		
Total Listed Species Known	24	19		
Total Listed Species Extant	20	15		
Total FMCS Imperiled Species Extant	39	28		

1973. These surveys include the Clinch River (Stansbery 1973; Bates and Dennis 1978; Stansbery et al. 1986; Dennis 1989; Ahlstedt 1991a; Church 1991; Jones et al. 2014), the Powell River (Ahlstedt and Brown 1979; Dennis 1981; Ahlstedt 1991b; Wolcott and Neves 1994; Johnson et al. 2012), or both rivers (Neves et al. 1980; Dennis 1985; Ahlstedt and Tuberville 1997).

The goal of our study was to quantify changes in the mussel fauna of the upper Clinch and Powell rivers over a 25-y period (1979–2004). Our primary objective was to quantitatively sample multiple fixed sites in both rivers and evaluate species richness, density and population trends during this period. Secondary objectives were to: 1) compare our results with previous and more recent collection data, 2) compile a comprehensive list of mussels known from the study area and their conservation status, and 3) generate a timeline of anthropogenic impacts that have potentially affected the status of the fauna during the past and into the future.

METHODS

Study Area

The watersheds of the Clinch River and its tributary Powell River form a large portion of the headwaters of the upper Tennessee River drainage in northeastern TN and southwestern VA (Figure 1). These drainages occur primarily in the Ridge and Valley Physiographic Province, with a small portion in the Appalachian Plateaus Physiographic Province in VA. The study area incorporates the free-flowing mainstems of these rivers upstream of Norris Reservoir, TN. The upper Clinch River watershed contains an area of 3,721 km^2, while the upper Powell River watershed contains 2,471 km^2. Land cover is primarily agriculture and mixed forest, with small towns scattered in the drainages and fossil fuel extraction in the Appalachian Plateaus headwaters. Industry is limited, but two coal-fired power plants are located on the upper Clinch River.

Site Selection and Sampling Methodology

We selected sampling sites during float surveys via canoe and small watercraft in 1979 (Ahlstedt 1991a, b; Table 2; Figure 1). Selected sites had aggregations of mussels in shoals—habitat patches having shallow water and swift flows over primarily gravel and cobble substrates—that also offered easy access. We identified sampling sites by RKM and landmarks on 7.5-minute topographic maps. We initially selected 11 sites on the Clinch River and 15 sites on the Powell River (Ahlstedt and Tuberville 1997). However, due to severe mussel declines at some sites, as well as time and funding constraints, sampling sites were reduced to six in the Clinch River (three each in TN and VA) and four in the Powell River (three in TN and one in VA) for data analyses.

Quantitative mussel sampling was conducted by randomly placing 0.25 m^2 quadrats on substrate in the shoal habitat of each site. Using mask and snorkel, surveyors searched for mussels by excavating substrate from quadrats to a depth of ~15 cm or until hardpan or bedrock was reached. Once live mussels were identified and recorded, we returned them to the substrate. Over the 25-y period, we conducted four (at Pendleton Island, VA) or five (at all other sites) sampling events at each Clinch River site, and six sampling events at each Powell River site (Table 3; Appendices I and II).

Data Analysis

We calculated mean density for each species and the assemblage at each site and year of sampling (Table 3; Appendices I and II). The 1979 quadrat data from all sites in the Clinch and Powell rivers were not available for analysis, only the mean values per site were available for that year,

Table 2. Location and quadrat sample sizes of the ten long-term fixed-station monitoring sites for mussels in the Clinch and Powell rivers, TN and VA, sampled from 1979–2004.

River/RKM	Site	Lat./Long.	No. Quadrats
Clinch River			
CRKM 277.1	Swan Island, Hancock Co., TN	36.2834N 83.1726W	40
CRKM 295.8	Brooks Island, Hancock Co., TN	36.3216N 83.0739W	26
CRKM 305.1	Kyles Ford, Hancock Co., TN	36.3403N 83.0233W	41
CRKM 339.7	Speers Ferry, Scott Co., VA	36.3858N 82.4455W	40
CRKM 364.2	Pendleton Island, Scott Co., VA	36.4542N 83.3526W	40
CRKM 378.4	Semones Island, Scott Co., VA	36.4833N 82.2905W	40
Powell River			
PRKM 159.5	Buchanan Ford, Claiborne Co., TN	36.3329N 83.2525W	40
PRKM 171.7	McDowell Shoal, Hancock Co., TN	36.3442N 83.2200W	40
PRKM 179.9	Bales Ford, Hancock Co., TN	36.3503N 83.2011W	20
PRKM 188.8	Fletcher Ford, Lee Co., VA	36.3614N 83.1741W	42

which were previously recorded by Ahlstedt and Tuberville (1997). Hence, all analyses were restricted to mean density values per site and year. We used a generalized linear model (GLM) to test for significance of trends in mean density of the mussel assemblage over time to make four comparisons: 1) sites in the Clinch River, VA; 2) sites in the Clinch River, TN; 3) Pendleton Island, VA; and 4) sites in the Powell River, TN and VA (Figure 2). We implemented the GLM using a Poisson distribution and log link function in the program R (R Development Core Team 2006) and test results were considered significant at α=0.05. Mean density values of the portion of our study from 1979–1994 were reported by Ahlstedt and Tuberville (1997). Mean mussel density at Pendleton Island in 1987 was obtained from Dennis (1989), who used similar sampling methods as our study.

Table 3. Mussels per meter squared, number of species, number of endangered species, mean values and associated 95% confidence intervals (CI) for sampling sites in the Clinch and Powell rivers, TN and VA, sampled from 1979–2004. Non-overlapping CI's among sites in each respective river are significantly different at the 0.05 alpha level. Numbers in parentheses under each site location are the total number of species collected at the site over the study.

	Sample Year							
	1979	1983	1988	1994	1999	2004	Mean	±95% CI
Clinch River Site (CRKM)								
Swan Island, TN (CRKM 277.1)								
Per meter squared	7.0	-	1.6	10.6	11.4	29.4	12.0	9.2
Species (23)	11	-	9	17	16	17	14.0	3.3
Endangered Species (8)	4	-	3	6	7	6	5.2	1.4
Brooks Island, TN (CRKM 295.8)								
Per meter squared	11.4	-	9.7	13.7	40.8	21.3	19.4	11.2
Species (29)	15	-	10	15	16	20	15.2	3.1
Endangered Species (10)	4	-	3	6	5	7	5.0	1.4
Kyles Ford, TN (CRKM 305.1)								
Per meter squared	31.0	-	14.1	37.6	95.9	74.3	50.6	29.3
Species (31)	27	-	19	20	23	23	22.4	2.7
Endangered Species (12)	11	-	7	8	10	11	9.4	1.6
Speers Ferry, VA (CRKM 339.7)								
Per meter squared	3.7	-	2.7	2.9	4.8	3.7	3.6	0.7
Species (22)	11	-	13	10	9	10	10.6	1.3
Endangered Species (8)	3	-	4	2	2	4	3.0	0.9
Pendleton Island, VA (CRKM 364.2)								
Per meter squared	24.6	-	-	11.2	12.4	4.6	13.2	7.3
Species (23)	21	-	-	13	13	10	14.3	4.1
Endangered Species (6)	6	-	-	3	2	1	3.0	1.9

Table 3, continued.

	Sample Year							
	1979	1983	1988	1994	1999	2004	Mean	±95% CI
Semones Island, VA (CRKM 378.4)								
Per meter squared	-	7.7	4.6	6.5	4.2	1.7	4.9	2.0
Species (17)	-	14	11	10	9	6	10.0	2.6
Endangered Species (5)	-	2	3	2	1	1	1.8	0.7
Powell River Site (PRKM)								
Buchanan Ford, TN (PRKM 159.5)								
Per meter squared	10.9	21.8	3.5	5.5	5.1	8.0	9.1	5.4
Species (24)	14	15	7	9	7	11	10.5	2.8
Endangered Species (8)	2	5	-	2	2	1	2.4	1.2
McDowell Shoal, TN (PRKM 171.7)								
Per meter squared	5.5	2.3	3.3	1.8	2.8	1.4	2.9	1.2
Species (22)	16	10	13	8	10	7	10.7	2.7
Endangered Species (8)	6	2	3	1	1	1	2.3	1.6
Bales Ford, TN (PRKM 179.9)								
Per meter squared	7.2	4.8	2.6	4.4	4.2	2.2	4.2	1.4
Species (19)	12	8	8	10	9	6	8.8	1.6
Endangered Species (7)	4	2	2	4	2	1	2.5	1.0
Fletcher Ford, VA (PRKM 188.8)								
Per meter squared	11.2	10.3	5.6	7.0	5.2	1.4	6.8	2.9
Species (23)	16	14	11	10	8	7	11.0	2.8
Endangered Species (8)	4	3	2	2	2	1	2.3	0.8

Conservation Status of the Fauna

We compiled a comprehensive list of mussels known from the upper Clinch and Powell river mainstems based on published literature and other records (Table 1). This list includes the population status of each species in the study area, federal status under the U.S. Fish and Wildlife Service (USFWS), and its global conservation status according to the Freshwater Mollusk Conservation Society (FMCS) (J.D. Williams, Florida Museum of Natural History, unpub, data). Finally, we generated a chronology of anthropogenic impacts from the literature and our personal observations over the last 40 y likely affecting the status of the mussel fauna (Table 4).

RESULTS

Clinch River

At the six study sites, we observed a total of 39 of 55 mussel species (71%) known from the Clinch River mainstem upstream of Norris Reservoir (Table 1; Appendix I). Species richness ranged from 36 in TN to 27 in VA, and among sites from 31 at Kyles Ford, TN, to 17 at Semones Island, VA. Over the sampling period, richness declined from 34 in 1979 to 29 in 2004 (Table 3). No other site yielded as many species during any intervening sampling year as did Kyles Ford, though richness dropped from 27 in 1979 to 23 species in both 1999 and 2004. Species richness increased from 11 to 17 species at Swan Island, TN, and 15 to 20 species at Brooks Island, TN, from 1979 to 2004. Sites in VA had lower species richness relative to those in TN, with the exception of Pendleton Island, VA, where 21 species were recorded in 1979. However, species richness declined to a low of 10 species at this site in 1999. At Speers Ferry, VA, species richness fluctuated from a high of 13 in 1988 to a low of 9 in 1999, and similarly at Semones Island from a high of 14 in 1988 to a low of 6 in 1999. Mussel diversity in Clinch River included 16 federally endangered species—14 in TN and 8 in VA. Endangered species ranged from 12 (Kyles Ford) to 8 (Swan Island) in TN and 8 (Speers Ferry) to 5 (Semones Island) in VA.

Mean mussel density among all sites combined on the TN side of the Clinch River increased significantly ($p < 0.001$) from 16.5 m^{-2} in 1979 to 41.7 m^{-2} in 2004 (Figure 2A). Density at the beginning and end of our study ranged from 7.0 to 29.4 m^{-2} at Swan Island, 11.4 to 21.3 m^{-2} at Brooks Island, and 31.0 to 74.3 m^{-2} at Kyles Ford, respectively, though the increasing trend was not uniform over all sampling periods (Table 3). Conversely, mean mussel density at sites on the VA side decreased significantly ($p < 0.001$) from 12.0 m^{-2} in 1979 to 3.3 m^{-2} in 2004 (Figure 2B). Over this period, density essentially remained unchanged at Speers Ferry (3.7 m^{-2}) but declined

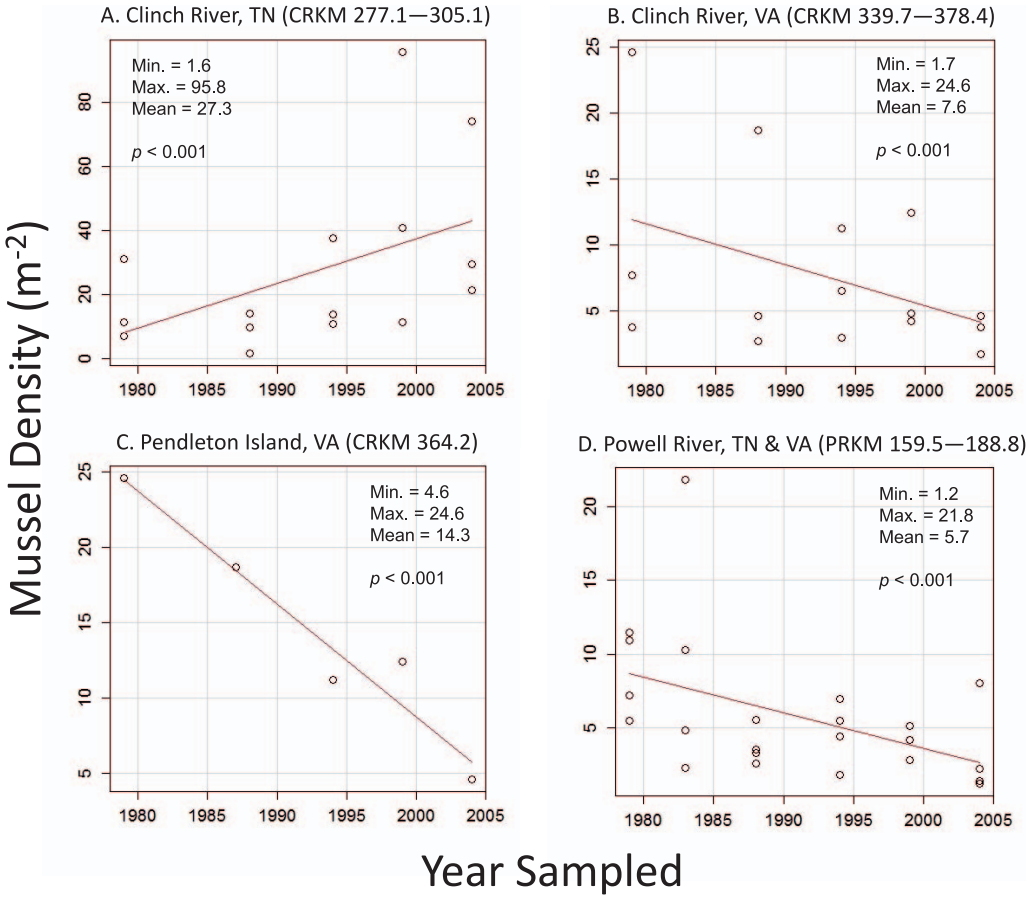

Figure 2. Time series plots and linear regression analyses of mean mussel density from 1979–2004 in reaches and sites in the Clinch and Powell rivers of TN and VA; data were collected using a random survey design. The mean density value of 18.7 mussels m^{-2} at Pendleton Island in 1987 was from data collected by Dennis (1989); data shown in panels B and C. Reported *p*-value indicates significance of the mussel density and year sampled trend.

significantly ($p < 0.001$) at Pendleton Island from 24.6 to 4.6 m^{-2} (Figure 2C) and also declined at Semones Island from 7.7 to 1.7 m^{-2} (Table 3).

Among species, *Actinonaias pectorosa* and *A. ligamentina* dominated overall abundance in the Clinch River at sites in both states (Appendix I). The next three most abundant species in VA were *Elliptio dilatata*, *Fusconaia subrotunda*, and *Medionidus conradicus*, while in TN they were *M. conradicus*, *Ptychobranchus subtentus*, and *Epioblasma capsaeformis*. *Ptychobranchus subtentus* was by far the most abundant endangered species reported, and was fourth in overall abundance. Peak densities of this species reached 20.3 m^{-2} in 1999 and 16.2 m^{-2} in 2004 at Kyles Ford. By 2004, the species had become more common at the two other TN sites (>3.0 m^{-2} per sample) but remained uncommon at VA sites (<0.3 m^{-2} per sample). Other relatively common listed species (~1 m^{-2} per sample) by the end of our study were *E. capsaeformis* at all three TN sites, *Dromus dromas* at Swan Island, and *E. triquetra* at Brooks Island.

A total of 55 species are known historically from the Clinch River and we consider 48 species to be extant, including 20 of 24 federally endangered species (Table 1). Overall, 39 of the extant species in the river are imperiled. The USFWS has been petitioned to list under the ESA seven imperiled species known from and considered extant in the river.

Powell River

At the four sites, we observed a total of 33 of 47 mussel species (70%) known from the mainstem Powell River upstream of Norris Reservoir (Table 1; Appendix II). Species richness was 26 in 1979 but declined to 14 by 2004; among sites, it ranged from 24 at Buchanan Ford, TN, to 19 at Bales Ford, TN (Table 3). Between 1979 and 2004, richness declined from 16 to 7 species at both McDowell Shoal, TN, and Fletcher Ford, VA, and from 12 to 6 species at Bales Ford. At Buchanan Ford the decline was 14 to 11 species. Powell River diversity included 12 endangered species, where each site had eight endangered species except Bales Ford (7 endangered species).

Mean mussel density among all sites combined declined significantly (8.8 to 3.2 m^{-2}; $p < 0.001$) over the study period (Figure 2D) and was most severe at Fletcher Ford (11.2 to 1.4

Table 4. Chronology of some significant perturbations that have occurred in the Clinch and Powell rivers.

Year(s)	Perturbation	Source
1870–1920	The Clinch and Powell river watersheds are initially logged, releasing massive quantities of sediment into the rivers.	Caudill (1963)
Mid to late 1800s	Logs floated downstream in the Clinch and Powell rivers to markets in Knoxville, TN, likely results in shoal habitat disruption and increased sedimentation.	Caudill (1963)
1881	Deep mining for coal begins in southwestern VA.	Hibbard and Clutter (1990)
Late 1800s to early 1900s	Railroads expanded along rivers to haul coal out of southwestern VA.	Eby (1923), Woodward (1936)
1884–1936	Mussels harvested in the Clinch and Powell rivers for natural pearls.	Boëpple and Coker (1912)
1909–1940s	Mussels harvested in the Clinch and Powell rivers for button industry.	Boëpple and Coker (1912)
1913	Discharges of industrial and mine wastes in the upper Clinch and Powell rivers, VA.	Adams (1915), Ortmann (1918)
Beginning in 1900	Extreme soil erosion from row-cropping and other agricultural practices throughout the Clinch and Powell river watersheds.	Caudill (1963), Sagona (1990), Sagona and Carroll (1991), TNC (1992)
1936–1963	Three impoundments (Norris Dam, the lower Clinch River, 1936; Watts Bar Dam, the upper Tennessee River, 1942; Melton Hill Dam, the lower Clinch River, 1963) constructed by Tennessee Valley Authority (TVA) for flood control and electric power production results in major loss or alteration of habitat throughout ~240 kilometers of the lower Clinch River and ~80 kilometers of the lower Powell River, isolating and fragmenting mussel and fish populations, and blocking movements of migratory host fishes.	Cahn (1936), Hickman (1937), Masnik (1974), Ahlstedt and Brown (1979), Ahlstedt (1991a)
1943	Surface mining for coal begins in southwestern VA.	Caudill (1963)
1950s to present	Black-water releases (coal fines) into the Clinch and Powell rivers from preparation plants located in southwestern VA.	Carriker (1981), TN/VA Joint Task Force (1985), TNC (1992)
1960s–1970s	Mussels harvested in the Clinch River for cultured pearl industry.	Tennessee Wildlife Resources Commission Proclamation 80-14 [1980]
1960s–1970s	Mussels harvested in the Clinch and Powell rivers and sold to biological supply companies for dissection in high school and college biology classes.	Tennessee Wildlife Resources Commission Proclamation 80-14 [1980]
1967	Massive fly-ash spill from the Clinch River Steam Plant at Carbo, VA, kills thousands of mussels and fishes over ~120 kilometers of river. Macroinvertebrates largely recover within a few months but mollusks do not.	Cairns et al. (1971), Crossman (1973), Raleigh et al. (1978), Stansbery et al. (1986)
1970s	The Powell River upstream of Pennington Gap, VA, was so adversely affected from coal mining operations that it was dredged to remove contaminants.	EPA (2002)
1970	Sulfuric acid spill from the Clinch River Steam Plant at Carbo, VA, kills thousands of mussels and fishes for ~24 kilometers of river. Fishes and macroinvertebrates largely recover within a few months but mollusks do not.	Cairns et al. (1971), Crossman (1973), Raleigh et al. (1978)
1972	On December 25th the Powell River was observed black from coal fines.	EPA (2002)
1977	A 100-y flood in the Clinch and Powell rivers strands many mussels along shorelines.	TVA (1978), S.A. Ahlstedt (pers. obs.)

Table 4, continued.

Year(s)	Perturbation	Source
1978	A low-head bridge was constructed after the 100-y flood on McDowell Shoal, the richest mussel shoal habitat in the Powell River, TN. This bridge was washed-out in 1979 and removed from the river, highly destabilizing shoal habitat.	S.A. Ahlstedt (pers. obs.)
1979	Black-water releases were observed draining into the Clinch River from a preparation plant located in the Lick Creek drainage near St. Paul, VA.	S.A. Ahlstedt (pers. obs.)
1978	Black-water releases from a settling pond into the Powell River from a preparation plant at Big Stone Gap, VA. Discharged water was thought to have affected ~200 river kilometers.	Carriker (1981), S.A. Ahlstedt (pers. obs.)
1982–1986	A Powell River mussel die-off is recorded, but its cause is unknown.	Ahlstedt and Jenkinson (1987)
1983–1988	Record low flows are caused by a prolonged drought in the Clinch and Powell rivers.	Ahlstedt and Tuberville (1997)
1983	A deep-mine blowout occurs on Bull Run Creek, a Clinch River tributary near Carfax, VA, creating a large slug of muddy water entering the Clinch River.	S.A. Ahlstedt (pers. obs.)
1986–1988	A Clinch River mussel die-off is observed, but its cause is unknown.	S.A. Ahlstedt (pers. obs.)
1992	Sediment toxicity to juvenile mussels is documented on the VA side of the Clinch and Powell rivers.	Olem (1980), McCann and Neves (1992)
1991–2004	Biological health of fish and macroinvertebrates are generally poor in tributary streams in the Clinch and Powell river drainages based on Index of Biotic Integrity sampling.	Angermeir and Smogar (1993), O'Bara et al. (1994), Ahlstedt and Tuberville (1997)
1996	An accidental black-water spill occurs in the North Fork Powell River, a major tributary to the upper Powell River, St. Charles, VA.	B. Evans (USFWS, pers. comm.)
1998	A truck accidentally dumps a rubber processing chemical into the Clinch River at Cedar Bluff, VA, resulting in a ~10 river kilometer kill of mussels, snails, fishes, and benthic macroinvertebrates.	Jones et al. (2001), Schmerfeld (2006)
2001–2004	Mussels are observed dead with meat inside their shells in the Clinch River (e.g., *Amblema plicata, Fusconaia subrotunda*).	S.A. Ahlstedt (pers. obs.)
2002–2003	Six black-water release events are documented in the Clinch and Powell river drainages.	B. Evans (USFWS, pers. comm.)

m^{-2}; Appendix II). Only at Buchanan Ford and Fletcher Ford did density ever exceed 10 m^{-2}, but decades ago in 1979 and 1983. By 2004, density ranged from 1.4 to 2.2 m^{-2} among sites, except at Buchanan Ford where it remained comparatively high at 8.0 m^{-2}. Declines were steep at other sites over 25 y, varying from 69% at McDowell Shoal to 88% at Fletcher Ford (Table 3; Appendix II).

Actinonaias pectorosa and *A. ligamentina* were also the co-dominant species in the Powell River, though their densities over the study averaged only 2.0 m^{-2} and 1.6 m^{-2} per sample, respectively (Appendix II). *Medionidus conradicus*, *Fusconaia subrotunda*, and *Elliptio dilatata* were next in abundance, but relatively uncommon, averaging <0.6 m^{-2} per sample. Endangered species density declined over the 25 y at all sites, and specimens were nearly always uncommon or rare (≤ 0.4 m^{-2} per sample). Among these, only *Dromus dromas* and *Plethobasus cyphyus* were found at each site, while *Epioblasma capsaeformis*, *F. cuneolus*, and *Quadrula cylindrica strigillata* were not found in quadrats after 1983.

A total of 47 species are known from the Powell River, and we consider 37 species to be extant, including 15 of 19 federally endangered species (Table 1). Overall, 28 of the extant species in the river are imperiled, 4 of which the USFWS has been petitioned to list under the ESA. Two other petitioned species are considered extirpated in the river.

Threats

We documented >30 anthropogenic trends, activities, or explicit events that have likely affected the mussel fauna in the

study area since the late 1800s (Table 4). They range from general changes in land-use (e.g., widespread logging, coal extraction, railroad construction) and direct exploitation (e.g., pearling, harvest) to catastrophic site-specific incidents (e.g., toxic spills ca. 1970 and 1998 in the Clinch River, VA). Most perturbations are based on the literature or personal communications with agency personnel, while others include personal observations by the authors.

DISCUSSION

Overview of the Mussel Faunas

A total of 48 of 55 species recorded from the mainstem Clinch River upstream of Norris Reservoir are considered extant, representing a faunal loss of 13% (Table 1). Our total species richness relative to that recorded by Jones et al. (2014) was 39 to 38, who quantitatively observed all 38 species in TN and 26 species in VA during 2004–2009. They sampled at our six sites plus three more sites in VA and seven more sites in TN. Based on this combined sampling over 30 y, we consider *Leptodea fragilis*, *Quadrula intermedia*, and *Villosa fabalis* to be extirpated from the Clinch River, while *Epioblasma haysiana*, *E. lenoir*, and *E. torulosa gubernaculum* are now extinct. All six species likely persisted in the river until the early 1970s to mid-1980s. Though we did not detect *Epioblasma florentina aureola* during our sampling, we consider it extant in the upper Clinch River mainstem in VA, despite the catastrophic pollution spill in 1998 that killed at least 182 individuals of this critically endangered species (Jones et al. 2001; Schmerfeld 2006; Table 4). We also did not observe *Toxolasma lividum*—an FMCS vulnerable petitioned species not reported alive for decades—but consider it extant based on shells collected in TN since the mid-1990s (Jones et al. 2014), and it being a small and easily overlooked species that primarily occurs in seldom-sampled stream margins. *Pleurobema sintoxia* was considered extirpated (Jones et al. 2014), until fresh-dead shells collected from muskrat middens in Hancock County, TN, in 2013 confirmed its continued presence (S.A. Ahlstedt, unpub. data). It is possible that this species has been confused with individuals of *P. cordatum* or *P. plenum* over the past few decades.

Other Clinch River records need clarification. *Epioblasma stewardsonii* was reported erroneously from the Clinch River upstream of Norris Reservoir by Stansbery (1973) (and repeated by Jones et al. 2014) based on an Ortmann (1918) record actually reported from a pre-impoundment site. Though Ortmann (1918) reported both forms of *Quadrula cylindrica*—the headwater subspecies *Q. c. strigillata* and the nominate subspecies *Q. c. cylindrica* (as *Q. cylindrica*)—from the currently unimpounded upper Clinch River, we do not recognize the occurrence of the nominate subspecies in our study area. Our viewpoint is supported by Stansbery (1973) and USFWS (2004). We accept the federally endangered *Leptodea leptodon* as part of the study area mussel fauna based on a museum specimen (U.S. National Museum 150158) with the stream of origin missing from the label (only "Scott County, Virginia" appears for a locality). In all likelihood the specimen is from Clinch River (Williams et al. 2008), which represents a new state record for VA. Probably collected in the early 1900s, the species is now extirpated from the study area. Jones et al. (2014) also recognized this specimen but their position was equivocal, stating that it may have been collected from either the Clinch River or North Fork Holston River. Lastly, recent mitochondrial DNA and soft anatomy data has shown that *Villosa perpurpurea* and *Villosa trabalis* in the Clinch River are the same species, which makes the former taxon a synonym of the latter taxon based on priority (Lane et al. 2016). Further, these data show that the species actually belongs in the genus *Venustaconcha* (Kuehnl 2009; Lane et al. 2016). These taxonomic name changes are reflected in our paper accordingly.

A total of 37 of 47 species recorded from the mainstem Powell River upstream of Norris Reservoir are considered extant, representing a 21% faunal loss (Table 1). Johnson et al. (2012) stated that the Powell River had "likely lost one-third of its species" over the last century. Our estimate of decline reflects an optimistic view that several species may continue to exist but at abundance levels difficult to detect, especially by quadrat sampling. For example, *Strophitus undulatus* was rediscovered in 2013 in Claiborne Co., TN, after a nearly 40-y absence from collections (T. Lane, Virginia Tech, unpub. data). Regardless, based on Johnson et al. (2012) and our study, we consider *Lasmigona holstonia*, *Leptodea fragilis*, *Pegias fabula*, *Toxolasma lividum*, and *Venustaconcha trabalis* to be extirpated from the Powell River, and *Epioblasma haysiana*, *E. lenoir*, and *E. torulosa gubernaculum* to be extinct (Table 1). Of note, while *L. holstonia* likely is extirpated from the mainstem, it still occurs in at least one headwater tributary, South Fork Powell, VA (R.S. Butler, unpub. data). With the exception of *L. fragilis* (observed only in 1979), most of these species had likely disappeared by the 1960s. Two additional species, *Epioblasma lewisii* and *Villosa fabalis*, occurred in the Powell River a century ago (Ortmann 1918), but were reported only from sites inundated by Norris Reservoir. Herein, we report *Cyprogenia stegaria* for the first time from the Powell River, based on collections made several decades ago at McDowell Shoal but overlooked in previous studies (S.A. Ahlstedt, unpub. data). No additional records of this species are known, indicating that it is likely extirpated from the river.

Another record warrants discussion. We also include *Anodontoides ferussacianus* in Table 1 based on a record in Ortmann (1918) of two specimens from an unspecified site on Powell River, Lee County, VA, likely collected well over a century ago. Ortmann (1918) considered the record to be unequivocal, indicating he must have personally studied the specimens. The Powell River record has subsequently been overlooked; the genus is not reported anywhere else in the Tennessee River drainage, and it represents another addition to the VA mussel fauna. Since *A. ferussacianus* is primarily a

smaller stream Midwestern species (Watters et al. 2009) with a range well over a thousand river kilometers from the study area, the form from the Powell River is unlikely the same species. The specimens may actually represent *A. denigrata* from the adjacent upper Cumberland River drainage (located across the drainage divide in Kentucky), or possibly an undescribed species of *Anodontoides*.

Among the 24 federally listed species historically known upstream of Norris Reservoir in the Clinch River, 19 of them were shared with the Powell River; 20 and 15 listed species, respectively, are considered extant (Table 1). Endangered species now comprise 40–50% of the Clinch and Powell River mussel faunas. Such high levels of endangered species richness are unparalleled among diverse freshwater faunas of North America. The similarity of endangered species richness over time suggests that declines in the two rivers documented over the past century have been roughly parallel, having affected their faunas to similar degrees, though overall species losses are higher in the Powell River (21% vs. 13%; Table 1). About three-quarters of the extant fauna in these rivers are now comprised of imperiled species.

Clinch River Mussel Declines in VA

The decline in mussel density among Clinch River, VA, sites was highly significant over the study period, and species richness also decreased (Table 3; Figure 2B; Appendix I). Precipitous declines by more than 90% were observed at Pendleton Island (highly significant; Figure 2C) and at Semones Island (untested). These two sites occur in the lower half of a 68-km reach considered a "dead-zone" due to a severe decline in mussel density over more than a 30-y period (Jones et al. 2014). Further, if the 2009 data observed at the three VA sites by Jones et al. (2014) are included in the GLM analysis (1979–2009), the declining trend in mussel density over time remains highly statistically significant (J.W. Jones, unpub. data).

The density decline at Pendleton Island observed during our study continues (Figure 2C); sampling in 2009 produced a density of only 0.7 $m^{-2,}$ dropping from 4.6 m^{-2} in 2004 (Jones et al. 2014). The decline of the mussel fauna at this site is notable for several reasons. In the 1970s the site harbored 46 species, making it arguably the most diverse site in the country at the time (Jones et al. 2014). In 1979, mussel density was second only to Kyles Ford. Species that were once common are now rare (e.g., *Actinonaias* spp., *Cyclonaias tuberculata*, *Fusconaia subrotunda*, *Lampsilis ovata*). Many of the remaining species are relatively long-lived, and several short-lived species are already extirpated, an indication of recruitment failure. We recorded the short-lived (~5 y; Haag 2012) *Leptodea fragilis* at Pendleton Island in 1979 but nowhere in the Clinch River since then, indicating that it is likely extirpated from the site and river.

Declines on the VA side of the Clinch River also were evident among endangered mussels. By 2004, endangered species were very rare, extirpated, or existed at levels difficult to detect using standard quantitative sampling techniques. Three of six listed species we observed at Pendleton Island in 1979—*Fusconaia cuneolus*, *Ptychobranchus subtentus*, and *Quadrula cylindrica strigillata*—were common (1.1–1.3 m^{-2}) at that time, but have not been observed in quantitative samples since 1999. Among listed mussels, only *Fusconaia cor* was sampled in 2004. The decline of *Fusconaia cuneolus* at this site is noteworthy, since it was the most common mussel (2.3 m^{-2}) among the endangered species at the site during the 1970s and fourth in relative abundance, comprising 11.6% of the entire mussel assemblage (Dennis 1989). The last record of *Quadrula intermedia* in the Clinch River was a fresh dead shell at Pendleton Island in 1983 (Ahlstedt 1991a). Further, *Epioblasma torulosa gubernaculum* was also last observed at this site in 1983 (Jones et al. 2014) and at Kyles Ford during 1973–1975 sampling (Dennis 1985). The Clinch River, VA, also was the final refugium for *E. haysiana*, last collected as shells in 1970 (R. Muir, U.S. Geological Survey [USGS] retired, pers. comm.) and in 1984 near Cleveland, VA (R.J. Neves, USGS retired, pers. comm.), and one of the last refugia for *E. lenoir*, last collected as a shell in the 1960s near St. Paul, VA (Haag 2009).

Speers Ferry had the best mussel fauna among VA sites that we studied. Though the mussel assemblage at this site occurs at a moderate density (>3.7 m^{-2} since 1999), recruitment is evident and density appears to be increasing, last recorded at 5.0 m^{-2} in 2009 (Jones et al. 2014). *Medionidus conradicus* was the most common species and the only mussel with a density >1.0 m^{-2} since 1999. Despite its abundance, this Tennessee-Cumberland province regional endemic species is considered threatened by FMCS and is petitioned for federal listing. The federally endangered *Epioblasma brevidens* and *Ptychobranchus subtentus* appear to be increasing in density in recent years, as have *Elliptio dilatata* and *Lampsilis fasciola*, though densities for all four species remain low (<0.6 m^{-2} since 1999). In contrast, the endangered *Venustaconcha trabalis* was last sampled there in 1988 and now is rare in the upper river mainstem.

Improvement of the Mussel Fauna in the Clinch River, TN

Mean mussel density increased significantly at TN sites in the Clinch River from 1979–2004 (Figure 2A). Several species account for most of the general increase, particularly *Actinonaias pectorosa*, *Medionidus conradicus*, and *Ptychobranchus subtentus*, but also *Lampsilis fasciola*, *P. fasciolaris*, and the FMCS endangered and petitioned for listing *Fusconaia subrotunda*. The latter species is now common (1.4 and 1.7 m^{-2}) at Brooks Island and Kyles Ford, respectively (Appendix I); Clinch River likely represents its largest population range-wide. Densities of some of the rarer endangered species have generally increased, such as *Cyprogenia stegaria*, *Dromus dromas*, *Epioblasma capsaeformis*, and *Epioblasma brevidens*. *Pleurobema rubrum*, an FMCS endangered species that also has

been petitioned for listing, was not detected during our study. Nevertheless, it remains a rare species in the Clinch River, which represents one of its largest population's rangewide. Several other species have maintained relatively stable abundance levels since 1979, namely *A. ligamentina*, *Cyclonaias tuberculata*, and *Lampsilis ovata*, or have occurred at low densities (<0.5 m^{-2}) and were sporadically observed during our study, such as *Lasmigona costata* and *Amblema plicata*.

The population of *Epioblasma capsaeformis* in the Clinch River has varied tremendously since the 1970s, highlighting how population trends differ within species over time. The species was common during 1973–1975 sampling, representing 34.0% of mussel abundance at Speers Ferry and 17.7% at Kyles Ford (Dennis 1985, 1989). It declined over the next decade and by 1987, Dennis (1987) warned that *E. capsaeformis* had become "all but extirpated from Speers Ferry and Kyles Ford." The species remained generally uncommon in the river through the early 1990s while disappearing from several other rivers (e.g., Powell River), prompting its listing as endangered in 1997. Our data show that its population then began to increase appreciably by 2004. By 2009, *E. capsaeformis* became the second most abundant mussel on the TN side of the Clinch River (Jones et al. 2014), even exceeding abundance levels observed in the mid-1970s. The decline of this and other mussel populations in the mid-1980s may have been initiated by combined effects of a prolonged drought and chronic pollution (Ahlstedt and Tuberville 1997). Environmental conditions may have remained sub-optimal until ca. 1999 when favorable conditions allowed the species to recover (>1.0 m^{-2}).

The TN section of the Clinch River is not without some species losses and declines in density. Notably, *Leptodea fragilis* and *Quadrula intermedia* likely were extirpated from this reach by the mid-1970s. Though not collected in our quantitative sampling since 1979, *Truncilla truncata* was collected from the TN reach during quantitative sampling in 2005 (Jones et al. 2014). The species has either declined drastically since being relatively common circa 1980, or it may survive in habitats infrequently sampled, such as in pools (Ahlstedt 1991a). Species richness, number of listed species, and density at the three TN sites reached their lowest levels in 1988, which was attributed to summer drought conditions from 1983 to 1988 (Ahlstedt and Tuberville 1997).

Decline of the Powell River Mussel Fauna in TN and VA

The downward trend in mussel diversity and abundance in the Powell River has been evident for decades. A century ago, Ortmann (1918) reported the headwater sites in VA to be depauperate, noting that even common species were often absent. Surveys in the 1970s yielded 36–37 species (Ahlstedt and Brown 1979; Dennis 1981; Ahlstedt 1991b), while another study during 1988–1989 recorded 28 species (Wolcott and Neves 1994). The 1970s surveys yielded no mussels at sites in the uppermost Powell River where Ortmann (1918) reported 13 species. Ortmann (1918) reported several imperiled species now considered extirpated from the mainstem (e.g., *Lasmigona holstonia*, *Pegias fabula*, *Toxolasma lividum*, *Venustaconcha trabalis*; Table 1). Further, if the 2009 data collected from the three TN sites and one VA site by Johnson et al. (2012) are included in the GLM analysis (1979–2009), the declining trend in mussel density over time remains highly statistically significant (J.W. Jones, unpub. data). Collectively, we found 26 species in 1979, but only 14 in 2004 (Appendix II). The trend continues, as is evident in the significant decline in density over our study period (Figure 2D). Mussel density at Buchanan Ford fared better than our other three sites where declines were steep (Table 3; Appendix II).

Species once common in the Powell River have become increasingly rare, including *Actinonaias* spp. and *Medionidus conradicus*. *Fusconaia subrotunda* was once one of the more common and widespread mussels in the river, but it was not collected after 1994 (Appendix II). No species other than these four occurred at densities of >1.0 m^{-2} during our sampling regime. Lack of recruitment of these common mussels was noted in the late 1980s (Wolcott and Neves 1994). The FMCS vulnerable *Pleuronaia barnesiana*, a regional endemic and petitioned species, was also one of the most common species in the 1970s (Dennis 1985), but we found no evidence of it after 1983. Both *F. subrotunda* and *P. barnesiana* persist in the river but are rare (Johnson et al. 2012). *Quadrula pustulosa* is a common, widespread species not detected during our survey; it persists as the rarest of four *Quadrula* species, and ironically the only one that is not endangered (Johnson et al. 2012; Table 1). Another common species, *Strophitus undulatus* had not been reported from the river since the 1970s (Ahlstedt and Brown 1979; Dennis 1981) until found in 2013 in TN. Other common and widespread species, including *Alasmidonta marginata* and *Leptodea fragilis*, were not observed after 1979, while *Truncilla truncata* went undetected during our study. Though all three species were considered likely extirpated from the Powell River by Johnson et al. (2012), we believe *A. marginata* may persist. It is substantially longer-lived than *L. fragilis* (Watters et al. 2009) indicating that its extirpation would take longer to detect. Similarly, *S. undulatus* is very sporadic and has been perpetually rare in study area collections. We observed *Pleurobema oviforme*—a once common but now FMCS threatened regional endemic and petitioned species—only in 1979 and 1988 at Fletcher Ford. The species may persist but essentially at undetectable levels (Johnson et al. 2012).

Federally endangered mussels in the Powell River were always sporadic in occurrence in our quadrat samples, with no single species ever exceeding 0.6 m^{-2}. *Dromus dromas*, *Epioblasma brevidens*, and *Plethobasus cyphyus* represented the most frequently encountered endangered species in our study. We did not observe *Hemistena lata*, *Cumberlandia monodonta*, and *Lemiox rimosus*, though Ahlstedt and Brown (1979) and Dennis (1981) reported these species from three of our sites prior to 1979; recent data suggests that they remain in the river. A relatively fresh dead specimen of the deeply-

buried, easily overlooked *H. lata* was collected at Bales Ford in 1999 (J.W. Jones, unpub. data). A fresh dead specimen of *C. monodonta* was found during 2008–2009 (Johnson et al. 2012). This species usually occurs under large slab boulders (Stansbery 1967), a habitat type not well represented during our sampling. Lastly, 15 live individuals of *L. rimosus* were observed at five sites during 2008–2009 (Johnson et al. 2012). We did not observe *E. capsaeformis* after 1983, and the species was last reported in the river during 1988–1989 sampling upstream of our VA site (Wolcott and Neves 1994). Considered extirpated, it is now being reintroduced to multiple sites in TN and VA (Carey 2013). *Quadrula cylindrica strigillata*, *Q. intermedia*, and *Q. sparsa* were observed sporadically during our study. The population of *Q. sparsa* in the Powell River represents the only recruiting population known, underscoring its conservation importance. The other five endangered species considered extant—*E. triquetra*, *Fusconaia cor*, *F. cuneolus*, *Pleuronaia dolabelloides*, and *Ptychobranchus subtentus*—are very rare in the Powell River (Johnson et al. 2012).

Similar to the upper Clinch River, VA, mussel declines in the Powell River appear to have been driven by anthropogenic perturbations (Table 4). Change in the mussel fauna at McDowell Shoal epitomizes this decline in diversity and abundance. In the mid-1970s, 38 species were reported there, clearly making it the most productive site known in the river (Ahlstedt and Brown 1979, Dennis 1981; Ahlstedt 1991b; S.A. Ahlstedt, unpub. data). A mussel die-off lasting about three years, was reported by Ahlstedt and Jenkinson (1987) while conducting our 1983 sampling regime at this site; it was postulated that a toxic spill could have been the cause (Ahlstedt and Tuberville 1997). Ahlstedt and Jenkinson (1987) noted significant declines of the dominant species at the site, *Actinonaias ligamentina*, and total mussels sampled in quadrats between 1979 and 1983. Our data indicate that *A. ligamentina* never again achieved earlier densities. Collectively, we recorded 22 species in quadrats since 1979, but only 15 species since 1994 and 7 species in 2004. Though 17 species were recorded by Johnson et al. (2012) during qualitative sampling during 2008–2009, they found only 5 species in quadrats. Currently, several Powell River sites have higher species richness than McDowell Shoal. Fletcher Ford also has experienced a severe mussel decline since the late 1970s. In 1978, a density of 24.2 m^{-2} was calculated for the site (Neves et al. 1980). We recorded steady declines since 1979, with density declining to 1.4 m^{-2} by 2004.

Historical and Persistent Threats

European settlement of the Southern Appalachian Mountains brought with it vast changes to the landscape and its river drainages through logging, coal mining, railroads, and other activities (Eby 1923; Woodward 1936; Caudill 1963; Hibbard and Clutter 1990; Table 4). Riverine impacts and threats to the mussel fauna in the study area were documented a century ago; Ortmann (1918) noted specific activities detrimental to mussels, such as a wood extraction facility in the upper Powell River drainage near Big Stone Gap, VA. The post-impoundment collections made by Stansbery (1973) in the Clinch River clearly reflected a decline in species distribution and richness over the previous half century. Both authors anticipated further declines in the fauna based on trends and their observations.

Numerous perturbations in the study area have resulted in catastrophic impacts to the mussel fauna (Table 4). Some die-offs were directly attributable to chemical releases and spills (e.g., Cairns et al. 1971; Crossman 1973; Jones et al. 2001; Schmerfeld 2006), whereas others were less discernable (e.g., Ahlstedt and Jenkinson 1987; Jones et al. 2014). The decline of mussels in the Clinch River "dead zone" reach in VA, which includes Semones and Pendleton islands, likely was due to various poorly understood anthropogenic impacts over time (Krstolic et al. 2013; Johnson et al. 2014; Jones et al. 2014; Zipper et al. 2014). This faunal loss falls under the category of Haag's (2012) enigmatic declines, where all species are affected equally, and subsequent abundance of species post-impact is typically a function of pre-impact population size. The decline of the mussel fauna at Pendleton Island—especially the extinction of *E. torulosa gubernaculum*—represents one of the greatest losses to mussel conservation over the past 35 y. A long history of anthropogenic impacts to habitat quality in the Powell River has taken a similar toll on its fauna (McCann and Neves 1992; Wolcott and Neves 1994).

Natural resource exploitation has a long history in the Southern Appalachians and extraction of fossil fuels has often been implicated directly in mussel declines in the study area and elsewhere (Wolcott and Neves 1994; Ahlstedt and Tuberville 1997; Haag and Warren 2004; Warren and Haag 2005). The production of coal in VA peaked in 1990 and has since been in decline (Virginia Energy Patterns and Trends 2014). Coal mining and secondarily natural gas extraction nevertheless may pose the most significant threat, and spills from active and inactive coal processing waste ponds are common (Hampson et al. 2000; Table 4).

Impacts of coal mining on river fauna were reviewed by Hull et al. (2006). Mine-related pollutants that may impact mussels (e.g., water column ammonia, arsenic and other metals in sediments) were identified in the Clinch and Powell river drainages (Price et al. 2011). Though contaminants have declined in recent decades, total dissolved solids continue to rise in mined watersheds (Zipper et al. 2016). Research indicates that mussel populations were inversely correlated with deposited coal fines (Kitchel et al. 1981). Juvenile mussels tested in Powell River sediments sampled downstream of a coal processing facility had significantly lower survival rates ($p = 0.01$) than did juveniles tested in sediments from upstream of the facility (McCann and Neves 1992). Periodic heavy metal toxicity may have played a role in the mussel decline observed at McDowell Shoal in the mid-1980s (Ahlstedt and Jenkinson 1987; Ahlstedt and Tuberville 1997). In general, losses in mussel diversity and particularly abundance are greater on the VA side of the Powell River

(Johnson et al. 2012), though this is not apparent from data at our single VA site. The prevalence of resource extraction activities in the headwaters—first timber, then fossil fuels—may largely explain this continuing trend, first observed by Ortmann (1918). This phenomenon is mirrored in our data from the VA side of the Clinch River, and its cause may be similarly complex.

Stochasticity becomes an increasing threat to small, fragmented, and declining populations (Lande et al. 2003); many such mussel populations in the Clinch and Powell rivers are vulnerable to extirpation due to the absence of source populations for recolonization (Allendorf and Luikart 2007). Extinction debt models predict that in populations isolated by habitat destruction, even good competitors and abundant species are susceptible to eventual extirpation (Tilman et al. 1994; Hanski and Ovaskainen 2002). After the initial extinction of numerous mussel species in the early to mid-20th Century caused primarily by impoundments and secondarily water pollution in these rivers, a second extinction "wave" in the 21st Century may affect a broader suite of species due to effects from small population size and fragmentation (Haag 2009; Haag and Williams 2013).

Conservation and Population Restoration Efforts

Malacologists and resource managers in the region have written strategies to guide population restoration and conservation in streams like the Clinch and Powell rivers (Cumberlandian Region Mollusk Restoration Committee 2010; USFWS 2014). Culture facilities of the Virginia Department of Game and Inland Fisheries (VDGIF) and Virginia Tech have implemented a recovery program for increasing mussel diversity and abundance in these rivers. Various reintroduction methodologies have been attempted; translocation of adult mussels from large populations is the most cost-effective method for reestablishing historical populations, though density of available source populations is a limiting factor for most species (Carey et al. 2015). Researchers have refined culture methods for juveniles, allowing greater sizes for release and improved survival rates (Hua et al. 2015).

Epioblasma capsaeformis is the focus of concerted population restoration efforts in the upper Clinch River, VA, and Powell River, TN; survival has been high in both localities and evidence of recruitment documented in the Clinch River (Carey et al. 2015). Other endangered species that are being reintroduced or augmented include *E. brevidens* (Powell River, TN) and *Lampsilis abrupta* (Clinch River, TN and VA), in addition to several state priority species in VA in the Clinch River (VDGIF, unpub. data). The fortuitous abundance of Clinch River mussels in TN (e.g., *E. brevidens*, *E. capsaeformis*, *Medionidus conradicus*, *Ptychobranchus subtentus*) is serving as seed stock for most of these efforts and reintroductions elsewhere in the Tennessee River drainage (Hubbs 2016). Re-establishment of endangered species in historical river reaches increases spatial distribution, improves overall conservation status, and represents the primary means by which recovery under the ESA can be achieved (USFWS 2004).

Fewer species have become extirpated in the Clinch and Powell Rivers compared to many other southeastern United States rivers, and most probably did so prior to 1994. There remains the potential to lose additional species in both watersheds through continued downward spiral of small populations of some species, but positive advancements in research, culture, population reintroduction, habitat restoration, and conservation are providing the knowledge necessary to prevent further declines and extirpations. These collective efforts offer tangible hope for the conservation of the extant fauna, and to create a malacological preserve for imperiled species in the Clinch and Powell rivers.

ACKNOWLEDGMENTS

We wish to thank all of the landowners who graciously provided access to our sampling sites over the years and all the individuals at Tennessee Valley Authority, Tennessee Wildlife Resources Agency, and U.S. Geological Survey who assisted in sampling efforts from 1979–2004. The following individuals assisted with field sampling in 1999 and 2004: J. Aliucci, D. Garst, N. Johnson, R. Mair, B. Ostby, and J. Rash (Virginia Tech); R. Biggins, S. Chance, N. Eckert, B. Evans, and S. Hanlon (U.S. Fish and Wildlife Service); M. Pinder, and B. Watson (Virginia Department of Game and Inland Fisheries); Dr. B. Beaty and M. Dougherty (The Nature Conservancy); and C. Isaac, T. Lowe, and Q. Tolliver (Appalachian Technical Services, Inc.). B. Evans, and S. Hanlon (U.S. Fish and Wildlife Service) provided information on coal mining and observations of threats. We especially thank S. Hanlon for preparing Figure 1, and J. Garner (Alabama Department of Conservation and Natural Resources), Dr. W. Haag (U.S. Forest Service), and Dr. R. Neves (U.S. Geological Survey, retired) for reviewing and providing valuable comments on earlier drafts of the manuscript, and to Dr. T. Watters and two anonymous reviewers for their efforts during the editorial process. The views expressed in this article are the authors' and do not necessarily represent those of the U.S. Fish and Wildlife Service.

LITERATURE CITED

Adams CC. 1915. The variations and ecological distribution of the snails of the genus *Io*. National Academy of Sciences. Volume XII, 2nd Memoir.

Ahlstedt SA. 1991a. Twentieth century changes in the freshwater mussel fauna of the Clinch River (Tennessee and Virginia). Walkerana 5:73–122.

Ahlstedt SA. 1991b. Cumberlandian mollusk conservation program: mussel surveys in six Tennessee Valley streams. Walkerana 5:123–160.

Ahlstedt SA, Brown SR. 1979. The naiad fauna of the Powell River in Virginia and Tennessee. Bulletin of the American Malacological Union 1979:40–43.

Ahlstedt SA, Jenkinson JJ. 1987. A mussel die-off in the Powell River, Virginia and Tennessee, in 1983. Pages 21–28 in Neves RJ, editor. Proceedings of the workshop on die-offs of freshwater mussels in the

United States. Sponsored by U.S. Fish and Wildlife Service and Upper Mississippi River Conservation Committee. Blacksburg: Virginia Polytechnic Institute and State University.

Ahlstedt SA, Tuberville JD. 1997. Quantitative reassessment of the freshwater mussel fauna in the Clinch and Powell Rivers, Tennessee and Virginia. Pages 107–128 in Cummings KS, Buchanan AC, Mayer CA, Naimo TJ. Conservation and management of freshwater mussels II: initiatives for the future. Proceedings of a UMRCC Symposium, 16–18 October 1995, St. Louis, Missouri. Rock Island, Illinois: Upper Mississippi River Conservation Committee.

Allendorf FW, Luikart G. 2007. Conserving Global Biodiversity: Conservation and the Genetics of Populations. Oxford, United Kingdom: Blackwell Publishing.

Angermeier PL, Smoger RA. 1993. Assessment of biological integrity as a tool in the recovery of rare aquatic species. Virginia Department of Game and Inland Fisheries, Richmond.

Bates JM, Dennis SD. 1978. The mussel fauna of the Clinch River, Tennessee and Virginia. Sterkiana 69–70:3–23.

Boëpple JF, Coker RE. 1912. Mussel resources of the Holston and Clinch River, Tennessee and Virginia. U.S. Bureau of Fisheries Document No. 765:3–13.

Cahn AR. 1936. The molluscan fauna of the Clinch River below Norris Dam upon completion of that structure. Pages 1–27 in Three reports dealing with the clam-shell industry of the Tennessee River Valley. Norris, Tennessee: Tennessee Valley Authority.

Cairns J. Jr., Crossman JS, Dickman KL, Herrick EE. 1971. Chemical plants leave unexpected legacy in two Virginia rivers. Science 198:1015–1020.

Carey CS. 2013. An evaluation of population restoration and monitoring techniques for freshwater mussels in the upper Clinch River, Virginia, and refinement of culture methods for laboratory-propagated juveniles. Master's Thesis. Blacksburg: Virginia Polytechnic Institute and State University.

Carey CS, Jones JW, Butler RS, Hallerman E. 2015. Restoring the endangered Oyster Mussel (*Epioblasma capsaeformis*) to the upper Clinch River, Virginia: an evaluation of population restoration techniques. Restoration Ecology 23:447–454.

Carriker NE. 1981. Water quality in the Tennessee Valley–1980. Tennessee Valley Authority Report TVA/ONR/WR-81/2.

Caudill HM. 1963. Night Comes to the Cumberlands. Boston, Massachusetts: Little, Brown and Company.

Church GW. 1991. Survey of the family Unionidae in the upper Clinch River and Little River, Virginia. Charlottesville: Virginia Chapter of The Nature Conservancy.

Crossman JS. 1973. The biological recovery of the Clinch River following pollutional stress. Ph.D. Dissertation. Blacksburg: Virginia Polytechnic Institute and State University.

Cumberlandian Region Mollusk Restoration Committee. 2010. Plan for the population restoration and conservation of imperiled freshwater mollusks of the Cumberlandian Region. Asheville, North Carolina: U.S. Department of Interior, Fish and Wildlife Service.

Dennis SD. 1981. Mussel fauna of the Powell River, Tennessee and Virginia. Sterkiana 71:1–7.

Dennis SD. 1985. Distributional analysis of the freshwater mussel fauna of the Tennessee River system, with special reference to possible limiting effects of siltation. Nashville: Report No. 85-2, Tennessee Wildlife Resources Agency.

Dennis SD. 1987. An unexpected decline in populations of the freshwater mussel, *Dysnomia* (*Epioblasma*) *capsaeformis*, in the Clinch River of Virginia and Tennessee. Virginia Journal of Science 38:281–286.

Dennis SD. 1989. Status of the freshwater mussel fauna [at] Pendleton Island Mussel Preserve, Clinch River, Virginia. Sterkiana 72:19–27.

Eby BJ. 1923. The geology and mineral resources of Wise County and the coal bearing portion of Scott County, Virginia. Bulletin of the Virginia Geological Survey 24:1–617.

Haag WR. 2009. Past and future patterns of freshwater mussel extinctions in North America during the Holocene. Pages 107–128 in Turvey ST, editor. Holocene extinctions. Oxford, United Kingdom: Oxford University Press.

Haag WR. 2010. A hierarchical classification of freshwater mussel diversity in North America. Journal of Biogeography 37:12–26.

Haag WR. 2012. North American freshwater mussels: ecology, natural history, and conservation. Cambridge, United Kingdom: Cambridge University Press.

Haag WR, ML Warren Jr. 2004. Species richness and total population size of freshwater mussels in Horse Lick Creek, Kentucky in 2003. Oxford, Mississippi: U.S. Department of Agriculture, Forest Hydrology Laboratory.

Haag WR, Williams JD. 2014. Biodiversity on the brink: an assessment of conservation strategies for North American freshwater mussels. Hydrobiologia 735:45–60

Hampson PS., Treece MW Jr, Johnson GC, Ahlstedt SA, Connell JF. 2000. Water quality in the upper Tennessee River basin, Tennessee, North Carolina, Virginia, and Georgia 1994–98. U.S. Geological Survey Circular 1205.

Hanski I, Ovaskainen O. 2002. Extinction debt at extinction threshold. Conservation Biology 16:666–673.

Hibbard WR Jr, Clutter TJ. 1990. Virginia coal, an unabridged history. Blacksburg: Virginia Center for Coal and Energy Research, Virginia Polytechnic Institute and State University.

Hickman ME. 1937. A contribution to mollusca of east Tennessee. M.S. Thesis. Knoxville: University of Tennessee.

Hua D, Jiao Y, Neves RJ, Jones JW. 2015. Using PIT tags to assess individual heterogeneity in a mark recapture study of laboratory-reared juveniles of the endangered Cumberlandian combshell (*Epioblasma brevidens*). Ecology and Evolution 5:1076–1087.

Hubbs, D. 2016. 2015 Annual Mussel Recovery Activity report for Project 7775. Tennessee Wildlife Resources Agency, Camden.

Hull MS, Cherry DS, Neves RJ. 2006. Use of bivalve metrics to quantify influences of coal-related activities in the Clinch River watershed, Virginia. Hydrobiologia 556:341–355.

Johnson GC, Krstolic JL, Otsby BJK. 2014. Influences of water and sediment quality and hydrological processes on mussels in the Clinch River. Journal of the American Water Resources Association 50:878–897.

Johnson MS, Henley WF, Neves RJ, Jones JW, Butler RS, Hanlon SD. 2012. Freshwater mussels of the Powell River, Virginia and Tennessee: abundance and distribution in a biodiversity hotspot. Walkerana: Journal of the Freshwater Mollusk Conservation Society 15:83–98.

Jones J, Ahlstedt S, Ostby B, Beaty B, Pinder M, Eckert N, Butler R, Hubbs D, Walker C, Hanlon S, Schmerfeld J, Neves R. 2014. Clinch River freshwater mussels upstream of Norris Reservoir, Tennessee and Virginia: a quantitative assessment from 2004–2009. Journal of the American Water Resources Association 50:820–836.

Jones JW, Neves RJ, Patterson MA, Good CR, DiVittoria A. 2001. A status survey of freshwater mussel populations in the upper Clinch River, Tazewell County, Virginia. Banisteria 17:20–30.

Kitchel HE, Widlak JC, Neves RJ. 1981. The impact of coal-mining waste on endangered mussel populations in the Powell River, Lee County, Virginia. Richmond: Virginia State Water Control Board.

Krstolic JL, Johnson GC, Ostby BJK. 2013. Water quality, sediment characteristics, aquatic habitat, geomorphology, and mussel population status of the Clinch River, Virginia and Tennessee, 2009–2011. U.S. Geological Survey Data Series 802.

Kuehnl, K.F. 2009. Exploring levels of genetic variation in the freshwater mussel genus *Villosa* (Bivalvia Unionidae) at different spatial and systematic scales: implications for biogeography, taxonomy, and conservation. Ph.D. Dissertation. Columbus: Ohio State University.

Lande R, Engen S, Sæther B-E. 2003. Stochastic population dynamics in ecology and conservation. Oxford, United Kingdom: Oxford University Press.

Lane TW, Hallerman EM, Jones JW. 2016. Phylogenetic and taxonomic evaluation of extant populations of the endangered Cumberland bean (*Villosa trabalis*) and purple bean (*Villosa perpurpurea*). Conservation Genetics 17:1109-1124.

Masnik MT. 1974. Composition, longitudinal distribution, and zoogeography of the fish fauna of the upper Clinch system in Tennessee and Virginia. Ph.D. dissertation. Blacksburg: Virginia Polytechnic Institute and State University.

McCann MT, Neves RJ. 1992. Toxicity of coal-related contaminants to early life stages of freshwater mussels in the Powell River, Virginia. Asheville, North Carolina: U.S. Department of the Interior, Fish and Wildlife Service.

Neves RJ, Pardue GB, Benfield EF, Dennis SD. 1980. An evaluation of endangered mollusks in Virginia. Richmond: Virginia Commission of Game and Inland Fisheries.

O'Bara CJ, Eggleton MA, McAdoo LM, O'Brien SK. 1994. Clinch River biotic assessment part 1: macrobenthic and fish communities. Nashville: Tennessee Wildlife Resources Agency.

Olem H. 1980. Improving water quality of drainage from abandoned deep mines, St. Charles, Virginia. Chattanooga, Tennessee: Tennessee Valley Authority. 34 pp.

Ortmann AE. 1918. The nayades (freshwater mussels) of the upper Tennessee drainage with notes on synonymy and distribution. Proceedings of the American Philosophical Society 77:521–626.

Parmalee PW, Bogan AE. 1998. The freshwater mussels of Tennessee. Knoxville: University of Tennessee Press.

Price JE, Zipper CE, Jones JW, Franck C. 2011. Water and sediment quality in the Clinch and Powell Rivers of Virginia and Tennessee, 1964–2010. Blacksburg: Virginia Polytechnic Institute and State University.

R Development Core Team. 2006. A list of the Comprehensive R Archive Network (CRAN) sites. Available: www.cran.r-project.org (February 2015).

Raleigh RF, Bennett DH, Mohn LO, Maughan OE. 1978. Changes in fish stocks after major fish kills in the Clinch River near St. Paul, Virginia. American Midland Naturalist 99:1–9.

Sagona FJ. 1990. Assessing nonpoint pollution sources in the Tennessee Valley region of southwest Virginia. Richmond: Virginia Water Resources Conference, 22–24 April 1990.

Sagona FJ, Carroll TL. 1991. Aerial inventory of land uses and nonpoint pollution sources in the Powell and upper Clinch River watersheds. Chattanooga, Tennessee: Tennessee Valley Authority Report TVA/WR/WQ-91/14.

Schmerfeld J. 2006. Reversing a textbook tragedy. Endangered Species Bulletin 31:12–13.

Stansbery DH. 1967. Observations on the habitat distribution of the naiad *Cumberlandia monodonta* (Say, 1829). American Malacological Union Annual Reports for 1966:29–30.

Stansbery DH. 1973. A preliminary report on the naiad fauna of the Clinch River in the southern Appalachian Mountains of Virginia and Tennessee (Mollusca: Bivalvia: Unionidae). American Malacological Union Annual Reports for 1972:20–22.

Stansbery DH., Stein CB, Watters GT. 1986. The distribution and relative abundance of unionid mollusks in the Clinch River in the vicinity of Appalachian Power Company's Clinch River Plant at Carbo, Virginia (Clinch River Miles 264–270). Columbus: Ohio State University Research Foundation.

Tennessee Valley Authority. 1978. Flood of spring 1977 in the Tennessee River basin. Knoxville, Tennessee: Division of Water Management Flood Report WM-27-5-1.

Tennessee/Virginia Joint Task Force. 1985. Conserving the unique values of the Clinch and Powell Rivers. Nashville and Richmond: Report to the Governors of Tennessee and Virginia.

Tennessee Wildlife Resources Commission. 1980. Proclamation No. 80-14. Stating that the Clinch River from the Tennessee/Virginia state line (River mile 202.1) downstream to Norris Dam (River mile 79.8) be designated as a mussel sanctuary. Nashville.

The Nature Conservancy. 1992. Clinch Valley Bioreserve strategic plan. Abingdon: Virginia Chapter.

Tilman D, May RM, Lehman CL, Nowak MA. 1994. Habitat destruction and the extinction debt. Nature 371:65–66.

U.S. Environmental Protection Agency. 2002. Clinch and Powell Valley watershed ecological risk assessment. Washington DC: National Center for Environmental Assessment EPA/600/R-011050.

U.S. Fish and Wildlife Service. 2004. Recovery plan for Cumberland Elktoe (*Alasmidonta atropurpurea*), Oyster Mussel (*Epioblasma capsaeformis*), Cumberlandian Combshell (*Epioblasma brevidens*), Purple Bean (*Villosa perpurpurea*), and Rough Rabbitsfoot (*Quadrula cylindrica strigillata*). Atlanta, Georgia: U.S. Department of Interior.

U.S. Fish and Wildlife Service. 2014. Imperiled aquatic species conservation strategy for the upper Tennessee River basin. Gloucester, Virginia: U.S. Department of Interior.

Virginia Energy Patterns and Trends. 2014. Blacksburg: Electronic database maintained at Virginia Polytechnic Institute and State University. Available: http://www.energy.vt.edu/vept/coal/ (February 2015)

Warren ML Jr, Haag WR. 2005. Spatio-temporal patterns of decline of freshwater mussels in the Little South Fork Cumberland River, USA. Biodiversity and Conservation 14:1383–1400.

Watters GT, Hoggarth MA, Stansbery DH. 2008. The Freshwater Mussels of Ohio. Columbus: The Ohio State University Press.

Williams JD, Bogan AE, Garner JT. 2008. Freshwater mussels of Alabama and the Mobile Basin in Georgia, Mississippi, and Tennessee. Tuscaloosa, Alabama: The University of Alabama Press.

Wolcott LT, Neves RJ. 1994. Survey of the freshwater mussels of the Powell River, Virginia. Banisteria 3:3–14.

Woodward HP. 1936. Natural Bridge and Natural Tunnel, Virginia. Journal of Geology 44:604–616.

Zipper CE, Beaty B, Johnson GC, Jones JW, Krstolic J, Ostby BJK, Wolfe W. 2014. Freshwater mussel population status and habitat quality in the Clinch River, Virginia-Tennessee, USA: a featured collection. Journal of the American Water Resources Association 50:807–819.

Zipper CE, Donovan PF, Jones JW, Li J., Price JE, Stewart RE. 2016. Spatial and temporal correspondences among watershed mining, water quality, and freshwater mussel status in an Eastern USA River. Science in the Total Environment 541:603–615.

Appendix I. Summary of mussel density in the Clinch River at six sites sampled in TN and VA during quantitative surveys conducted from 1979–2004.
NA = data not available or collected.

	Swan Island, TN (CRKM 277.1)					Brooks Island, TN (CRKM 295.8)					Kyles Ford, TN (CRKM 305.1)					Speers Ferry, VA (CRKM 339.7)					Pendleton Island, VA (CRKM 364.2)					Semones Island, VA (CRKM 378.4)				
Scientific Name	1979	1988	1994	1999	2004	1979	1988	1994	1999	2004	1979	1988	1994	1999	2004	1979	1988	1994	1999	2004	1979	1988	1994	1999	2004	1983	1988	1994	1999	2004
(1) *Actinonaias ligamentina*	5.10	0.60	4.00	1.90	6.50	6.62	5.85	7.54	3.54	5.10	7.41	5.66	7.41	8.68	5.00	0.80	0.50	0.50	0.90	0.70	3.40	NA	2.60	2.50	0.50	1.90	1.00	1.60	0.70	0.10
(2) *Actinonaias pectorosa*	0.60	-	2.70	1.40	8.30	0.31	0.46	0.92	15.08	0.30	6.05	2.44	9.46	33.76	21.50	1.10	0.70	1.20	0.90	0.10	3.60	NA	3.40	4.30	1.80	2.50	1.80	1.90	1.60	0.80
(3) *Alasmidonta marginata*	-	-	-	-	0.10	-	-	-	-	-	-	0.10	0.10	-	-	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(4) *Amblema plicata*	-	0.10	0.10	-	-	0.15	-	-	0.46	-	0.39	0.29	-	-	-	-	0.10	-	-	-	0.80	NA	0.10	0.30	0.40	0.20	0.10	0.20	-	-
(5) *Cumberlandia monodonta**	-	-	-	-	-	-	-	-	-	-	0.78	0.10	0.10	0.88	0.10	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(6) *Cyclonaias tuberculata*	-	0.10	0.10	0.60	0.10	1.08	1.08	0.62	-	0.90	0.39	0.29	0.39	0.20	0.30	0.20	0.10	-	0.40	-	1.10	NA	0.50	0.70	0.60	0.20	0.30	0.50	0.20	-
(7) *Cyprogenia stegaria**	-	-	-	0.10	0.10	-	-	0.15	-	0.30	0.10	0.10	0.10	0.39	0.20	0.10	0.10	-	-	-	-	NA	-	-	-	-	-	-	-	-
(8) *Dromus dromas**	0.10	0.10	0.10	0.80	1.50	-	-	-	0.46	-	-	-	-	0.39	0.20	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(9) *Elliptio crassidens*	-	-	-	-	-	-	-	0.15	0.31	-	-	-	-	-	0.50	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(10) *Elliptio dilatata*	-	-	-	-	-	-	-	-	0.31	0.20	2.15	0.29	0.49	0.39	1.40	0.10	0.10	-	0.20	0.30	6.30	NA	1.40	0.80	0.20	0.10	0.10	0.10	0.10	-
(11) *Epioblasma brevidens**	-	0.20	0.30	0.60	0.60	-	0.31	0.31	-	1.10	0.10	-	0.29	0.88	0.30	-	0.10	-	0.60	0.30	-	NA	-	-	-	-	-	-	-	-
(12) *Epioblasma capsaeformis**	-	-	0.10	1.00	0.90	-	-	0.62	4.46	2.60	0.39	0.10	0.68	2.54	2.10	0.20	-	-	-	0.20	0.80	NA	-	-	-	0.10	-	-	-	-
(13) *Epioblasma triquetra**	0.10	-	0.10	0.10	-	0.15	-	0.31	0.31	0.90	0.10	-	-	0.10	0.10	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(14) *Fusconaia cor**	-	-	-	-	-	-	-	-	-	-	0.20	-	-	-	-	-	-	-	-	0.10	0.30	NA	0.40	-	0.20	0.30	-	0.10	-	-
(15) *Fusconaia cuneolus**	-	-	-	-	-	-	-	-	-	0.20	0.29	0.39	0.20	-	0.20	-	0.10	-	-	-	1.10	NA	0.30	0.70	-	-	0.20	0.10	0.20	-
(16) *Fusconaia subrotunda*	0.30	-	-	0.10	0.20	0.46	0.62	0.77	0.62	1.40	0.78	0.29	1.17	1.56	1.70	0.10	0.10	-	-	-	1.70	NA	2.00	1.90	0.10	1.00	0.70	1.70	0.20	0.20
(17) *Hemistena lata**	0.10	-	0.30	0.10	0.60	0.15	-	0.15	0.15	-	0.10	0.20	0.20	0.20	0.10	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(18) *Lampsilis fasciola*	-	-	0.20	0.30	0.50	-	0.15	0.15	0.31	0.60	0.20	-	0.10	0.49	0.70	-	-	0.10	0.20	0.30	0.20	NA	0.10	0.30	-	0.30	-	-	-	0.20
(19) *Lampsilis ovata*	0.20	0.10	-	0.10	0.10	0.92	0.15	-	0.15	0.90	0.10	-	0.10	0.10	0.10	0.30	0.10	-	-	-	0.50	NA	-	-	0.10	0.10	-	-	0.10	-
(20) *Lasmigona costata*	0.20	0.20	0.20	-	0.20	-	0.46	-	0.15	0.20	1.27	0.29	-	0.10	0.10	0.20	0.20	0.10	-	-	1.30	NA	0.10	0.20	-	0.10	0.10	0.20	0.10	-
(21) *Lemiox rimosus**	-	-	-	-	-	-	-	-	-	-	0.10	0.10	0.10	0.20	0.30	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(22) *Leptodea fragilis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	NA	-	-	-	-	-	-	-	-
(23) *Ligumia recta*	-	0.10	-	0.10	-	0.31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	NA	-	-	-	-	-	-	-	-
(24) *Medionidus conradicus*	0.10	-	1.00	2.00	2.20	-	-	0.46	5.69	0.50	2.44	2.24	10.34	22.15	20.10	-	0.40	0.50	1.30	1.30	0.20	NA	-	0.10	-	-	-	-	-	-
(25) *Plethobasus cyphyus**	-	0.10	-	-	-	0.15	0.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(26) *Pleurobema cordatum*	-	-	-	-	-	0.15	-	-	0.15	-	-	-	-	-	-	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(27) *Pleurobema oviforme*	-	-	-	-	-	-	-	-	-	0.20	0.29	-	-	-	-	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(28) *Pleurobema plenum**	-	-	-	-	-	-	-	-	-	0.90	-	-	-	-	-	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(29) *Pleurobema rubrum*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA	0.10	-	-	-	-	-	-	-
(30) *Pleuronaia barnesiana*	0.10	-	0.10	-	0.10	-	-	-	-	0.20	2.15	-	-	-	-	-	-	0.10	-	-	0.10	NA	-	-	-	0.10	-	-	-	-
(31) *Pleuronaia dolabelloides**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA	-	-	-	-	0.10	-	-	-
(32) *Potamilus alatus*	-	-	0.10	-	-	0.31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	NA	-	-	-	0.10	-	-	-	-
(33) *Ptychobranchus fasciolaris*	-	-	0.30	0.60	1.30	0.15	-	0.15	-	1.20	0.29	0.20	0.29	1.56	2.10	0.10	-	-	0.10	0.10	0.40	NA	0.10	0.30	0.30	0.70	0.10	0.10	1.00	0.30
(34) *Ptychobranchus subtentus**	0.10	-	0.80	1.60	6.10	0.15	0.46	1.08	8.62	3.40	4.20	0.59	5.56	20.29	16.20	0.50	-	0.10	0.20	0.30	1.10	NA	0.10	0.10	-	-	0.10	-	-	0.10
(35) *Quadrula cylindrica strigillata**	-	-	-	-	-	-	-	-	-	-	0.10	-	-	0.20	0.20	-	-	0.10	-	-	1.30	NA	-	-	-	-	-	-	-	-
(36) *Quadrula pustulosa*	-	-	-	-	-	0.31	-	0.31	-	-	0.10	0.29	0.10	0.10	-	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(37) *Strophitus undulatus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(38) *Truncilla truncata*	-	-	-	-	-	-	-	-	-	-	0.10	-	-	-	-	-	-	-	-	-	-	NA	-	-	-	-	-	-	-	-
(39) *Venustaconcha trabalis**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.10	-	-	-	0.10	NA	-	-	-	-	-	-	-	-
(40) *Villosa iris*	-	-	-	-	-	-	-	-	-	0.20	0.39	0.10	0.39	0.59	0.80	-	-	0.10	-	-	-	NA	-	0.20	0.40	-	-	-	-	-
(41) *Villosa vanuxemensis*	-	-	0.10	-	-	-	-	-	-	-	-	-	-	0.10	-	-	-	0.10	-	-	-	NA	-	-	-	-	-	-	-	-
Total per meter squared	7.00	1.60	10.60	11.40	29.40	11.37	9.69	13.69	40.77	21.30	30.96	14.06	37.57	95.85	74.30	3.70	2.70	2.90	4.80	3.70	24.60	NA	11.20	12.40	4.60	7.70	4.60	6.50	4.20	1.70

*Federally endangered species and dash mark "-" = 0.

Appendix II. Summary of mussel density in the Powell River at four sites sampled in TN and VA during quantitative surveys conducted from 1979–2004.

	Buchanan Ford, TN (PRKM 159.5)						McDowell Shoal, TN (PRKM 171.7)						Bales Ford, TN (PRKM 179.9)						Fletcher Ford, VA (PRKM 188.8)					
Scientific Name	1979	1983	1988	1994	1999	2004	1979	1983	1988	1994	1999	2004	1979	1983	1988	1994	1999	2004	1979	1983	1988	1994	1999	2004
(1) *Actinonaias ligamentina*	5.50	6.30	1.20	1.80	0.90	2.10	2.40	0.80	0.50	0.60	0.50	0.40	1.80	3.00	0.20	1.20	1.40	0.80	1.90	1.24	0.76	2.10	1.24	0.19
(2) *Actinonaias pectorosa*	1.60	10.10	1.00	2.70	2.90	3.40	1.00	0.80	0.10	0.20	0.90	0.30	1.60	0.40	0.60	0.80	1.40	0.20	4.86	4.38	2.67	2.76	2.38	0.57
(3) *Alasmidonta marginata*	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(4) *Amblema plicata*	0.20	-	-	0.10	-	0.20	0.30	0.20	0.60	0.30	0.10	0.20	-	-	-	-	0.20	-	0.10	-	0.10	0.19	-	-
(5) *Cyclonaias tuberculata*	0.10	-	0.10	0.10	-	0.10	-	-	-	0.10	0.40	-	0.20	-	-	-	-	-	0.10	-	0.10	0.19	0.19	0.19
(6) *Dromus dromas**	-	0.10	-	-	0.10	0.10	0.10	0.10	-	-	-	0.10	0.20	-	0.20	0.20	0.20	-	-	-	-	-	0.10	-
(7) *Elliptio crassidens*	0.20	-	-	-	-	-	0.20	0.10	0.10	-	-	-	-	-	-	-	-	-	-	0.10	-	-	-	-
(8) *Elliptio dilatata*	-	-	-	-	-	0.20	0.10	0.10	0.50	0.20	-	0.10	0.20	-	0.20	0.20	0.20	0.40	0.48	1.33	0.38	0.38	0.48	0.10
(9) *Epioblasma brevidens**	-	-	-	-	-	-	0.10	-	0.10	-	-	-	-	0.20	0.20	-	-	-	0.38	-	-	0.10	-	0.10
(10) *Epioblasma capsaeformis**	0.30	-	-	-	-	-	-	-	-	-	-	-	0.40	-	-	-	-	-	0.29	0.19	-	-	-	-
(11) *Epioblasma triquetra**	-	0.30	-	-	-	-	-	-	0.10	-	-	-	-	-	-	-	-	-	0.10	0.10	0.10	-	0.10	-
(12) *Fusconaia cor**	-	-	-	0.10	-	-	0.10	-	-	-	-	-	0.20	-	-	-	-	-	-	-	-	-	-	-
(13) *Fusconaia cuneolus**	-	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(14) *Fusconaia subrotunda*	1.00	1.40	0.30	-	-	-	0.10	0.10	0.30	0.10	-	-	-	0.20	0.60	-	-	-	0.38	0.95	0.38	0.86	-	-
(15) *Lampsilis fasciola*	-	0.50	-	0.10	-	0.40	0.20	-	-	-	0.10	-	-	0.20	0.20	0.20	-	0.20	0.38	0.29	-	-	0.10	-
(16) *Lampsilis ovata*	0.20	0.20	0.10	-	0.10	0.10	0.20	-	0.10	-	0.10	0.10	0.20	-	-	0.20	-	-	-	0.10	-	-	-	-
(17) *Lasmigona costata*	0.20	0.10	-	-	0.10	0.10	-	-	-	-	0.10	-	-	-	-	-	0.20	-	-	0.19	-	-	-	-
(18) *Leptodea fragilis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.19	-	-	-	-	-
(19) *Ligumia recta*	-	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(20) *Medionidus conradicus*	1.00	2.10	0.60	0.30	0.90	1.00	0.20	-	0.30	0.20	0.30	-	0.80	0.40	0.40	0.80	0.20	0.40	1.43	1.14	0.76	0.19	0.57	0.10
(21) *Plethobasus cyphyus**	0.10	0.10	-	-	-	-	0.10	-	-	-	-	-	-	0.20	-	0.40	-	0.20	-	-	-	0.10	-	-
(22) *Pleurobema oviforme*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.19	-	0.10	-	-	-
(23) *Pleuronaia barnesiana*	-	0.10	-	-	-	-	-	0.10	-	-	-	-	0.80	-	-	-	-	-	0.19	0.10	-	-	-	-
(24) *Pleuronaia dolabelloides**	-	-	-	-	-	-	-	-	-	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(25) *Potamilus alatus*	0.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(26) *Ptychobranchus fasciolaris*	0.10	0.20	0.20	0.20	-	0.30	0.10	0.10	0.10	-	0.20	-	0.20	0.20	-	-	0.20	-	0.10	0.10	0.10	0.10	-	0.10
(27) *Ptychobranchus subtentus**	-	-	-	0.10	-	-	-	-	-	-	-	-	0.60	-	-	0.20	-	-	-	0.10	-	-	-	-
(28) *Quadrula cylindrica strigillata**	-	-	-	-	-	-	0.10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(29) *Quadrula intermedia**	-	0.10	-	-	0.10	-	0.20	0.10	0.40	-	0.10	-	-	-	-	-	-	-	0.10	-	-	-	-	-
(30) *Quadrula sparsa**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.20	0.20	-	-	-	0.10	-	-	-
(31) *Villosa iris*	-	-	-	-	-	-	-	-	0.10	-	-	0.20	-	-	-	-	-	-	-	-	-	-	-	-
(32) *Villosa vanuxemensis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total per meter squared	10.90	21.80	3.50	5.50	5.10	8.00	5.50	2.50	3.30	1.80	2.80	1.40	7.20	4.80	2.60	4.40	4.20	2.20	11.17	10.31	5.55	6.97	5.16	1.35

*Federally endangered species and dash mark "-" = 0.

Freshwater Mollusk Biology and Conservation 19:19–26, 2016

REGULAR ARTICLE

GROWTH AND LONGEVITY ESTIMATES FOR MUSSEL POPULATIONS IN THREE OUACHITA MOUNTAIN RIVERS

Brandon J. Sansom[1,2,*], Carla L. Atkinson[1,3], and Caryn C. Vaughn[1]

[1]*Oklahoma Biological Survey, Department of Biology and Ecology and Evolutionary Biology Graduate Program, University of Oklahoma, 111 E. Chesapeake St., Norman, OK 73019, USA*

ABSTRACT

Freshwater mussels are a unique guild of benthic invertebrates that are of ecological and conservation importance. Age and growth determination are essential to better understand the ecological role of mussels, and to effectively manage mussel populations. In this study, we applied dendrochronology techniques and Ford-Walford analyses to determine growth parameters of mussel species collected in three Ouachita Mountain Rivers (Kiamichi, Mountain Fork, and Little Rivers). We collected six species of mussels, *Actinonaias ligamentina*, *Amblema plicata*, *Fusconaia flava*, *Ptychobranchus occidentalis*, *Quadrula pustulosa* and *Quadrula verrucosa*, created thin sections, and analyzed the internal annuli to determine growth and longevity estimates. Annual growth was validated in 12 of the 17 populations we sampled, and the series intercorrelation for the validated populations ranged from 0.108 to 0.477. The predicted average maximum validated age was 43 years, ranging from 15 to 79 years, while the growth constant (*K*) ranged from 0.038 to 0.137. Growth and longevity were inversely related. Growth patterns were more synchronous at local sites compared to river and regional scales, suggesting that local environmental conditions likely influence growth rates. This study provides the first reported growth parameters for mussels in Ouachita Mountain rivers of southeastern Oklahoma and will be useful in understanding the life history traits of these mussel populations.

KEY WORDS - Unionoida, Life History, Age, Growth Rate, Ouachita Mountain Rivers

INTRODUCTION

Freshwater mussels (Unionoida) are a unique guild of benthic invertebrates that are ecologically important, but are of conservation concern. Ecologically, mussels contribute to the overall structure and function of stream ecosystems. As filter feeders, mussels facilitate the transformation of nutrients that benefit primary (Allen et al. 2012; Atkinson et al. 2013) and secondary production (Howard and Cuffey 2006; Allen et al. 2012; Spooner et al. 2012), and help tighten downstream nutrient spirals, which increases the overall efficiency of streams per unit area (Atkinson et al. 2013). Mussel shells also provide habitat by increasing surface area for algae and macroinvertebrate colonization (Vaughn and Hakenkamp 2001).

From a conservation standpoint, mussels are a very diverse group of species. Nearly 300 species occur in North America (Graf and Cummings 2007; Bogan 2008) but almost 70% of the species have gone extinct or are currently listed as endangered, threatened, or of special concern (Williams et al. 1993; Neves 1999). Historically, the lack of age, growth, and longevity information hindered conservation efforts (Neves et al. 1997). Recent advances in methods to determine age and growth have improved the understanding of mussel life history (Anthony et al. 2001; Rypel et al. 2008; Haag 2009; Haag 2012), but increased efforts are still needed to understand differences among species, individual populations, or geographic regions of interest.

Mussels deposit growth rings, analogous to annual growth rings in trees or fish scales and otoliths, from which age and growth data can be interpreted. Validating the rate at which mussels produce rings is critical in order to obtain accurate age and growth estimates (Beamish and McFarlane 1983). Traditional mark-recapture methods have been

[2]Current Address: Civil, Structural, and Environmental Engineering, 207 Jarvis Hall, Buffalo, NY 14260

[3]Current Address: Department of Biological Sciences, University of Alabama, 300 Hackberry Lane, Tuscaloosa, AL 35487

*Corresponding Author: bsansom@buffalo.edu

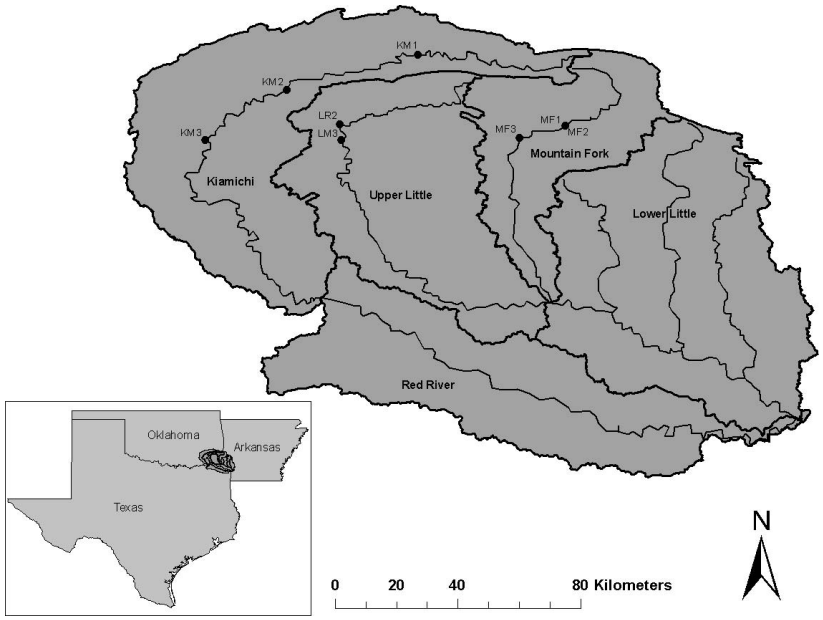

Figure 1. Regional map and locations of the eight sites where mussels were collected throughout the Kiamichi, Mountain Fork, and Little Rivers (K = Kiamichi River, L = Little River, and M = Mountain Fork River; numbers represent the site number for that specific river; M1 and M2 were too close to differentiate at this scale).

effective in validating annual ring deposition (Haag and Commens-Carson 2008), but can present bias in age and growth estimates due to handling and limited data ranges (Haag 2009). More recent approaches utilize common dendrochronological cross-dating techniques to validate ring production (Rypel et al. 2008; Haag and Rypel 2011; Sansom et al. 2013). This method, which typically uses shell thin sections to interpret growth rings, is less time intensive than mark-recapture, can result in larger sample sizes, and can also identify false or missing rings (Haag and Commens-Carson 2008). Recent advances in using this technique have improved our understanding of mussel life history (e.g. Rypel et al. 2008; Haag and Rypel 2011; Sansom et al. 2013). However, since individual populations often exhibit highly plastic growth patterns, growth often cannot be generalized within a species (Haag and Rypel 2011). Therefore, additional life history information regarding growth rates and longevity is needed from individual populations to provide meaningful management and conservation efforts at the population level (DeVries and Frie 1996; Campana and Thorrold 2001; Haag and Rypel 2011).

The aim of our study was to quantify the growth rates and variability of these rates within and across unionid freshwater mussel species in three watersheds in an understudied geographic region, the Ouachita Mountains. We applied dendrochronology techniques and Ford-Walford analyses to age and estimate growth rates of mussels, analyzed differences in growth rates within and across species, and compared our estimates to data from other regions.

METHODS

Study Sites and Shell Collection

Mussels were collected from three rivers (Kiamichi, Little, and Mountain Fork; Figure 1) during the summer of 2010 as part of a larger study (Atkinson et al. 2013; Atkinson et al. 2014). The rivers are tributaries of the Red River and share regional species pools. Headwaters and mid-reaches flow through the Ouachita Mountains ecoregion, with lower reaches flowing through the Gulf Coastal Plain ecoregion. The Ouachita Mountains ecoregion, which covers 46,500 km^2 in central Arkansas and southeastern Oklahoma (U.S.), is characterized by a sub-humid subtropical climate, mixed forests/woodlands, rugged mountains, broad valleys, and several large gravel-bed rivers (OEAT 2003). This region is a center of speciation for both terrestrial and aquatic organisms, with a large number of endemic species (Mayden 1985). Mussel diversity is noteworthy with >60 species, including 4 federally threatened or endangered species (Vaughn and Taylor 1999). Furthermore, these rivers support healthy and diverse mussel communities primarily due to relatively low anthropogenic impacts compared to other areas in the U.S. (Vaughn and Taylor 1999).

Mussels were quantitatively sampled from 8 sites across the three rivers. All sites were within the Ouachita Mountain ecoregion and were located upstream of any impoundments. We excavated 10, 0.25-m^2 quadrats randomly placed within each study site. Quadrats were excavated to a depth of 15 cm and all mussels were removed and identified to species. Five to ten individuals of the two or three most common species were

Table 1. Regression coefficients for linear regressions between shell height and shell length for each species (AL = *Actinonaias ligamentina*, AP = *Amblema plicata*, FF = *Fusconaia flava*, PO = *Ptychobranchus occidentalis*, QP = *Quadrula pustulosa*, QV = *Quadrula verrucosa*) in three rivers (K = Kiamichi, L = Little, M = Mountain Fork). Mean shell length % difference indicates the difference between the measured shell length and the predicted shell length using the regression coefficients.

Species and River	n	Intercept	Slope	R^2	Mean Shell Length % Difference
AL K	10	−14.750	1.913	0.880	3.10%
AP K	11	−8.281	1.473	0.846	3.29%
AP L	6	−19.256	1.693	0.891	5.10%
AP M	8	−12.449	1.666	0.922	2.28%
FF L	4	−7.009	1.459	0.971	2.39%
FF M	3	−114.763	4.025	0.999	0.05%
PO M	12	−4.817	2.307	0.942	1.94%
QP L	4	−14.226	1.480	0.922	1.27%
QP M	4	4.754	1.047	0.934	0.92%
QV L	7	−1.914	1.832	0.779	3.23%
QV M	2	15.703	1.844	NA	NA

collected from each site for tissue stoichiometric analyses (see Atkinson et al. 2013) and the shells of each individual were cleaned, marked, and cataloged for the purpose of this study.

Shell Preparation

Thin sections were created following standard methods for bivalves (Clark 1980; Neves and Moyer 1988). Each thin section was viewed and interpreted using a dissecting microscope by two individuals. True annuli were differentiated from non-annual rings following criteria in Haag and Commens-Carson (2008). Once the true annuli were agreed upon, we measured the annual growth increments using a linear encoder and digital readout in MeasureJ2X (Project J2X, VoorTech Consulting). Measurements, taken along the dorso-ventral growth increment between the prismatic and nacreous shell layers, began at the most recent complete growth year and proceeded towards the umbone. Due to extensive erosion on and around the umbone on most of the specimens, the early growth years were not measurable. The linear portion of the shell that was eroded was measured and used to determine the shell height and length for the first observable growth ring.

Quality Control

Growth pattern analysis and quality control measures followed dendrochronological methods described in Rypel et al. (2008) and Sansom et al. (2013). In short, the program COFECHA was used to remove age-related growth variation and generate a standardized index for each individual. Averaging the standardized index for each population created a master chronology. From that, each standardized index was compared to the master chronology to detect dating errors (i.e. false or missing rings). All potential errors flagged in COFECHA were re-examined, and if measurement errors occurred, the appropriate growth increments were re-measured and COFECHA was re-run.

Growth Parameters

After the quality control measures, we characterized growth among populations using the von Bertalanffy growth equation

$$L_t = L_\infty\left(1 - e^{-K(t-t_o)}\right) \quad (1)$$

where L_t is the length (mm) at a given time (t - age in years), L_∞ is the predicted mean maximum length (mm) for the population, K is the Brody's growth constant that depicts the rate at which the organisms approaches L_∞ (mm/year), and t_o is the theoretical time in which the L=0 (Ricker 1975). The growth increments measured between the internal annuli represent a change in shell height, rather than length. Since a length value is needed, we used linear regressions, grouped by species and river, between the shell height and length of our specimens to predict shell length. On average, these predictions resulted in <3% difference compared to actual length measurements (Table 1), and thus we used the regression parameters to predict the length at time t, based on the height at time t.

Furthermore, because we could not accurately assess age due to excessive erosion that masked the early years in many of our specimens, we used Ford-Walford plots to estimate the parameters L_∞ and K of equation one (see Anthony et al. 2001; Hornbach et al. 2014). Ford-Walford plots were created by regressing L_{t+1} on L_t, and using the slope and intercept to calculate L_∞ and K as:

$$L_\infty = \left(\frac{a}{1-\beta}\right) \quad (2)$$

$$K = -\ln\beta \quad (3)$$

where a is the y-intercept and β is the slope of the linear regression from the Ford-Walford plot. After determining the growth parameters for each population, we estimated age at length for the first identifiable growth ring for each individual as,

$$t = \ln\left[\frac{L_\infty - L_t}{L_\infty}\right]/(-K) \quad (4)$$

Following quality control for each population, we rounded the age estimate from equation four to the nearest whole number, and subsequently added the number of identifiable rings to determine the age of each individual.

Finally, we compared growth parameters between individual populations within each river, as well as comparisons between species across the three rivers. We examined bivariate relationships between growth rate (K), longevity (A_{max}), and

Table 2. Population growth parameters for the six species (AL = *Actinonaias ligamentina*, AP = *Amblema plicata*, FF = *Fusconaia flava*, PO = *Ptychobranchus occidentalis*, QP = *Quadrula pustulosa*, QV = *Quadrula verrucosa*) at eight sites in three rivers (K = Kiamichi, L = Little, M = Mountain Fork).

Site and Species	n	Intercept	Slope	R^2	K	L_∞	Max Age	Series Intercorrelation*	Cubic Spline*	Growth at Increment Skew
K1 AP	4	5.092	0.954	0.992	0.048	109.608	79	0.176	22	−0.523
K2 AL	5	15.633	0.893	0.986	0.113	146.689	30	0.411	38	−0.728
K3 AL	5	11.308	0.912	0.996	0.092	128.892	52	0.335	44	−0.925
K2 AP	5	8.978	0.902	0.981	0.103	91.335	38	0.139	24	−0.642
L2 AP	3	10.982	0.872	0.987	0.137	85.855	34	0.302	8	−0.692
L3 AP	3	6.308	0.934	0.992	0.068	95.641	53	0.256	2	−0.767
M1 AP	4	7.145	0.928	0.996	0.075	98.970	63	0.230	22	−0.863
M3 AP	4	6.513	0.922	0.994	0.081	83.947	46	-	-	−0.953
L2 FF	3	5.664	0.939	0.995	0.063	92.112	29	-	-	−0.378
M1 FF	3	3.366	0.962	0.995	0.038	89.162	64	-	-	−0.088
M1 PO	3	9.772	0.899	0.983	0.106	96.754	44	0.349	2	−1.093
M2 PO	4	7.156	0.941	0.990	0.061	121.679	32	0.108	22	−0.459
M3 PO	5	6.455	0.940	0.994	0.062	107.124	36	0.164	36	−0.397
L3 QP	4	5.041	0.951	0.984	0.051	102.251	32	-	-	0.178
M3 QP	4	7.376	0.888	0.971	0.119	65.747	25	-	-	−0.830
L2 QV	3	12.488	0.900	0.979	0.105	125.444	15	0.477	2	−0.816
L3 QV	4	10.989	0.902	0.994	0.104	111.669	34	0.330	40	−1.427

*Series intercorrelation and cubic spline are only listed for those populations that were statistically significant and validated.

maximum length (L_∞) using linear regression. All variables were $\log_{10}$ transformed. Additionally, because body size can strongly influence growth parameters (Calder 1984; Bonsall 2005), we examined growth patterns and longevity to length-standardized values of K and A_{max} by regressing both $\log_{10}$ transformed variables onto $\log_{10}$ transformed L_∞ and used the residuals in a separate regression (White and Seymour 2004; Haag and Rypel 2011). All regressions were done in JMP (v12.0.1, SAS Institute Inc.).

RESULTS

Shell Preparation

We collected mussel shells from eight different sites in the three rivers. Three sites were located on the Kiamichi and Mountain Fork Rivers, each, while two sites were on the Little River (Figure 1). We analyzed growth parameters for 69 shells from six different mussel species including, *Actinonaias ligamentina*, *Amblema plicata*, *Fusconaia flava*, *Ptychobranchus occidentalis*, *Quadrula pustulosa* and *Quadrula verrucosa* (Table 2).

Shell erosion prevented a complete analysis of internal growth rings on all specimens. On average, shell erosion accounted for approximately 46% of the total shell. This pattern was consistent between all species and sites. Therefore, we assumed that the juvenile and early adult years of growth were missed in our analysis, and the growth parameters presented here only characterize growth of adult mussels.

Quality Control

Quality control resulted in the identification of potential errors among eight individuals. In seven of the individuals, COFECHA suggested the highest series intercorrelation was obtained by shifting the chronology one year backwards (i.e. the most recent annual growth ring was likely overlooked in the initial measurement). After reanalyzing each shell, we confirmed that the last growth ring was overlooked, and repeated the quality control for those populations. For the eighth individual, the shell margin was cracked and we initially estimated that eight growth years were missing by comparing ring counts on the umbo to the rings we measured. COFECHA suggested that the highest series intercorrelation was obtained by shifting the chronology four years ahead. Because we could not confirm this based on the shell cross-section, this individual was removed from the analysis.

After quality control, our cross-dating methods supported the assumption of annual ring formation in 12 of the 17 populations in our study (Table 2). Of these 12 populations, the series intercorrelations were significant and ranged from 0.108 to 0.477, indicating that growth was synchronous among individuals within their respective population (Grissino-Mayer 2001; Black et al. 2005; Rypel et al. 2008). In the five populations that were not validated, all series intercorrelations were negative and indicate growth among these populations is not synchronous. No populations of *Fusconaia flava* or *Quadrula pustulosa* were validated, while only one population of *Amblema plicata* was not validated (Table 2). Despite not being able to validate annual ring production via cross-dating within five of our populations, we continued to

Table 3. Population growth parameters summarized for the six species (AL = *Actinonaias ligamentina*, AP = *Amblema plicata*, FF = *Fusconaia flava*, PO = *Ptychobranchus occidentalis*, QP = *Quadrula pustulosa*, QV = *Quadrula verrucosa*) in each river (K = Kiamichi, L = Little, M = Mountain Fork).

River and Species	n	Intercept	Slope	R^2	K	L_∞	Max Age	Series Intercorrelation*	Cubic Spline*	Growth at Increment Skew
K AL	10	12.784	0.907	0.989	0.098	137.083	52	0.295	6	−0.774
K AP	11	7.397	0.926	0.988	0.077	99.766	79	0.080	8	−0.629
L AP	6	7.945	0.913	0.989	0.091	91.664	53	0.212	40	−0.706
M AP	8	6.172	0.936	0.995	0.066	96.462	63	0.282	70	−0.470
L FF	4	6.467	0.927	0.992	0.076	88.430	29	-	-	−0.494
M FF	3	3.366	0.962	0.995	0.038	89.162	64	-	-	−0.088
L QP	4	5.041	0.951	0.984	0.051	102.251	32	-	-	0.178
M PO	12	7.257	0.932	0.990	0.070	107.166	44	-	-	−0.586
M QP	4	7.376	0.888	0.971	0.119	65.747	25	-	-	−0.830
L QV	7	12.417	0.889	0.990	0.117	112.093	34	0.176	40	−1.231

*Series intercorrelation and cubic spline are only listed for those populations that were statistically significant and validated.

conduct the Ford-Walford plots to estimate growth parameters. Validation of annual ring formation among other species at the same sites and in the same rivers suggests that climate conditions are conducive for the deposition of yearly growth rings.

Growth Parameters

Overall, growth and longevity varied greatly across both species and rivers (Table 2 and 3, respectively). For example, the population of *Amblema plicata* from Site 2 in the Little River had the highest growth constant ($K = 0.137$) with a moderate maximum predicted age (34 years), while another population of *Amblema plicata* from Site 1 in the Kiamichi River had the highest predicted age (79 years), with a low growth constant ($K = 0.048$). The lowest growth constant occurred in the *Fusconaia flava* population at Site 1 in the Mountain Fork River, but this river also had some of the higher growth rates at Site 3 and well as site 1 for *Quadrula pustulosa* and *Ptychobranchus occidentalis*, respectively.

Furthermore, growth was inversely related to longevity, and K explained ~24% of the variation in longevity (Figure 2a). This pattern remained true when the effect of size was removed (Figure 2b). There was no significant relationship between L_∞ and K.

Finally, patterns of growth showed higher synchrony among local populations within a river rather than a species wide growth trend for an entire river. For all populations that were validated, the local populations had a higher series intercorrelation than when the species of each of the populations were combined for an entire river (Tables 2 and 3).

DISCUSSION

In this study, we provide growth parameters for six mussel species across three rivers in southeastern Oklahoma. The maximum predicted age that was validated in our sample was 79 years old, while the average maximum, validated age across all six species was 43 years, and thus indicates a relatively long-lived life for these six mussel species. Growth rates were highly variable, ranging from 0.038 to 0.137, which indicates the range of life history traits among different species. The growth parameters presented in this study are the first to be reported for any mussel species in southeastern Oklahoma. Furthermore, we are the first to provide growth estimates for two species, *Ptychobranchus occidentalis* and *Fusconaia flava* (however, no populations of *F. flava* were validated having true growth annuli).

Examining the growth parameters at a species level, the growth constants (K) and maximum predicted length (L_∞) were within the range of previously reported studies on similar species. Only one similar growth study has been done in the Ouachita Mountain ecoregion (Christian et al. 2000). The only species analyzed by both Christian et al. (2000) and our study, *Amblema plicata*, had similar K and L_∞ estimates (Christian et al. (2000): $K = 0.13$, $L_\infty = 87.02$; our study: K ranged from 0.048 to 0.137, L_∞ ranged from 83.947 to 109.608). From a broader regional context, the growth parameters in our study were typically towards the lower range compared to previously reported studies (Haag and Rypel 2011; Hornbach et al. 2014). Additionally, the inverse relationship between maximum predicted age and growth rate (Figure 2a) is consistent with previously reported bivalve studies (Bauer 1992; Haag and Rypel 2011; Hochwald 2011).

Although we are confident in our methods to achieve both K and L_∞, the distribution of our data may have contributed to a reduction in both of these values. Because shell erosion was observed for the majority of the shells we collected and processed, our growth parameters do not include estimates for the juvenile years of growth, where we would expect higher growth rates. Furthermore, Haag (2009) found that K decreased as the range of shell size decreased and left-skewed datasets greatly underestimated K. In our dataset, we had a slight left-skew of the distribution of shell length at growth ring increments (see Tables 2 and 3 for skew breakdown

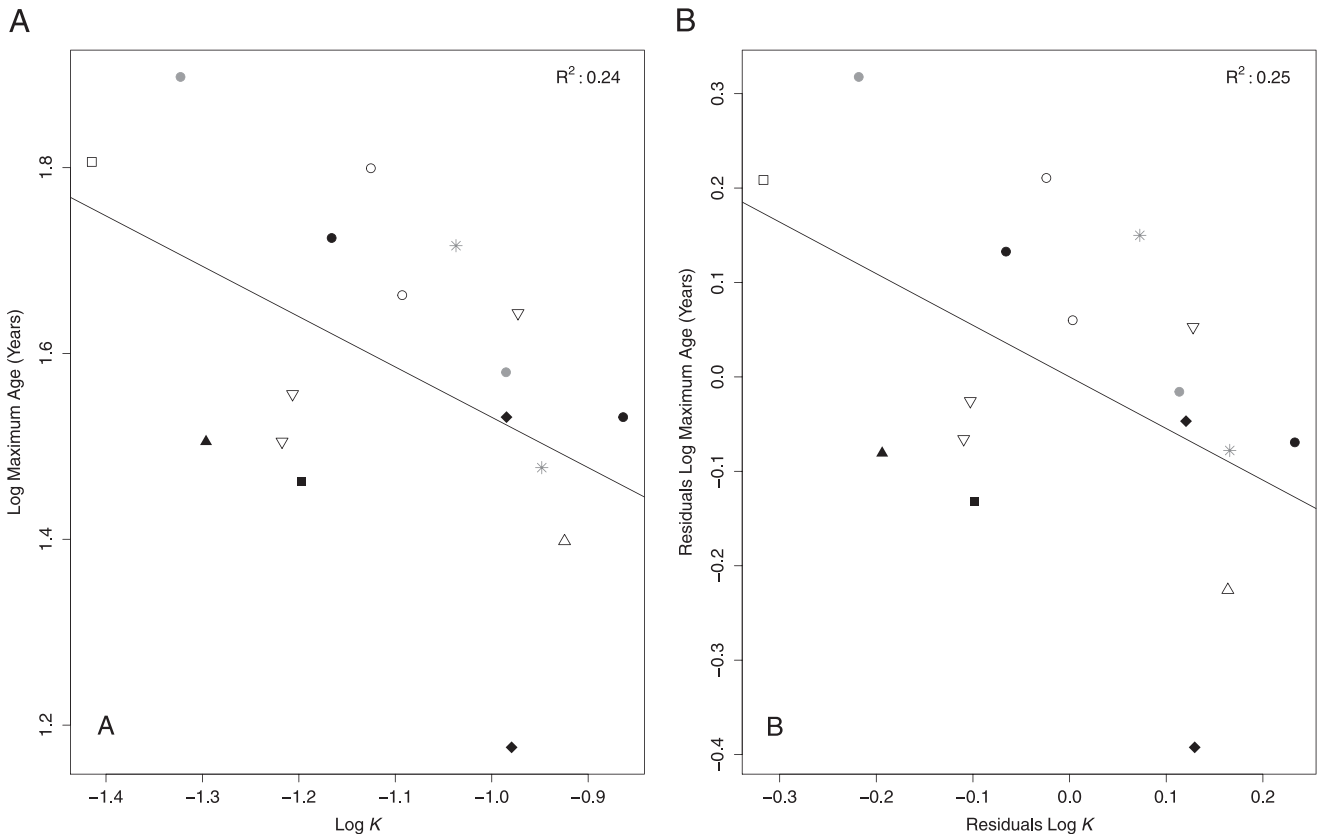

Figure 2. Mussel growth (K) and maximum predicted age (A_{max}) were inversely related (A). The growth rate (K) and maximum predicted age (A_{max}) were standardized by using the residuals of linear regressions between K and A_{max} against L_∞ to remove the effect of maximum predicted length. Regressing these residuals supported the negative relationship between maximum predicted age and growth rate (B). Rivers are differentiated by gray scale (Kiamichi River: gray symbols, Little River: black symbols, Mountain Fork River: open symbols); while mussel species are differentiated by symbols (*Actinonaias ligamentina*: ✳, *Amblema plicata*: •, *Fusconaia flava*: ■, *Ptychobranchus occidentalis*: ▼, *Quadrula pustulosa*: ▲, *Quadrula verrucosa*: ◆). Regression R^2 coefficients on both figures are for all species across all rivers.

among populations). Therefore, the combined effect of left-skew and lack of measuring juvenile growth could compensate for lower range of growth rates found in this study.

The validated age estimates (15-79 years) reported in this study are comparable to those found in Haag and Rypel (2011). It is important to note that age estimates using the von Bertalanffy growth equation have often been criticized for overestimating longevity (Haag 2009). In our study, we only used age estimates from the von Bertalanffy growth equation to predict the age at which the first observable ring was deposited. From there, we counted subsequent growth rings to obtain age estimations. This method reduced the potential for overestimating longevity throughout our dataset and removed bias in assigning an age to the first recognizable growth ring. Although on average we were only able to observe and measure growth for the latter half of the shell, the maximum predicted age of any specimen for the portion of the shell that was eroded was 11 years, and thus, our margin for error was greatly reduced.

Our approach to determine growth rates and longevity integrated dendrochronology dating techniques (Grissino-Mayer 2001; Black et al. 2005; Rypel et al. 2008) along with Ford-Walford regression plots (Anthony et al. 2001; Haag and Rypel 2011; Hornbach et al. 2014). Cross-dating allowed us to perform quality control measures on our data and identified populations with highly synchronous growth, which is indicative of regular ring formation (Grissino-Mayer 2001; Black et al. 2005; Rypel et al. 2008). While many of our specimens had large portions of eroded shells, our use of Ford-Walford plots allowed us to estimate growth rates and maximum predicted shell length for each population without having a full record of internal annuli. Furthermore, using equation four provided an unbiased age estimate to account for the portion of the shell that was eroded. Adding the subsequent, internal annuli to this age estimate provided the most accurate age estimates given large amount of erosion. When the effect of size was removed, the relationship between K and maximum age remained the same (Figure 2), suggesting that our methods to estimate growth parameters remained robust despite the shell erosion.

Overall, observed growth parameters among individuals between populations and across rivers were highly variable.

This was expected as each species likely has different life history traits (Coker et al. 1921; Stansbery 1967), and environmental conditions differ between watersheds and even local sites within a river. For example, discharge has been shown to negatively influence growth rates of freshwater mussels (Haag and Rypel 2011), and is known to strongly influence the quantity and quality of food resources (Atkinson et al. 2009), which can also impact growth rates. In our study, the higher series intercorrelations observed within local populations compared to a river scale suggest that local environmental conditions likely govern growth rates.

From a broader context, growth parameters are usually similar among species within specific tribes. Previous studies have shown that species belonging to the tribes Amblemini, Pleurobemini, and Quadrulini are typically categorized as long-lived and slow-growing (Haag and Rypel 2011). Species in the tribe Lampsilini are comparatively short-lived and fast-growing (Stansbery 1967), but can also overlap the long-lived, slow-growing tribes of Amblemini, Pluerobemini, and Quadrulini (Haag and Rypel 2011). Our results for growth and longevity at the tribe level are consistent with these documented patterns and are within the range of measurements made by Haag and Rypel (2011).

CONCLUSIONS

This study provides the first attempt to categorize growth parameters for mussel species in Ouachita Mountain rivers of southeastern Oklahoma. Growth and longevity information will be useful to understanding the life history traits of populations in southeastern Oklahoma. Using the parameters reported in this study, additional studies are in progress to assess how the growth and longevity of these mussel species are linked to environmental variables. Such studies will allow us to determine the impacts of climate change and the onset of an extended drought to the growth of these mussels, and allow us to provide better management options.

ACKNOWLEDGEMENTS

We would like to thank Robert Turner for the use of his laboratory and equipment to prepare the thin sections and Dr. Aondover Tarhule for the use of his laboratory and microscope for measuring growth rings. Thanks to Brent Tweedy and Janell Hartwell for taking pictures of shell cross-sections. Andrew Rypel and an anonymous reviewer provided helpful reviews and edits to the manuscript.

REFERENCES

Allen, D. C., Vaughn, C. C., Kelly, J. F., Cooper, J. T. and M. H. Engel. 2012. Bottom-up biodiversity effects increase resource subsidy flux between ecosystems. Ecology 93:2165-2174.

Anthony, J. L., Kessler, D. H., Downing, W. L. and J. A. Downing. 2001. Length-specific growth rates in freshwater mussels (Bivalvia: Unionidae): extreme longevity or generalized growth cessation? Freshwater Biology 46:1349-1359.

Atkinson, C. L, Golladay, S. W., Opsahl, S. P. and A. P. Covich. 2009. Stream discharge and floodplain connections affect seston quality and stable isotopic signatures in a coastal plain stream. Journal of the North American Benthological Society 28:360-370.

Atkinson, C. L., Vaughn, C. C., Forshay, K. J. and J. T. Cooper. 2013. Aggregated filter-feeding consumers alter nutrient limitation: consequences for ecosystem and community dynamics. Ecology 94:1359-1369.

Atkinson, C. L., Kelly, J. F. and C. C. Vaughn. 2014. Tracing consumer-derived nitrogen in riverine food webs. Ecosystems 17:485-496.

Bauer, G., 1992. Variation in the life span and size of the freshwater pearl mussel. Journal of Animal Ecology 61:425-436.

Beamish, R. J. and G. A. McFarlane. 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society 112:735-743.

Black, B. A., Boehlert, G. W. and M. M. Yoklavich. 2005. Using tree-ring crossdating techniques to validate annual growth increments in long-lived fishes. Canadian Journal of Fisheries and Aquatic Sciences 62:2277-2284.

Bogan, A. E. 2008. Global diversity of freshwater mussels (Mollusca, Bivalvia) in freshwater. Hydrobiologia 595:139-147.

Bonsall, M. B. 2005. Longevity and ageing: appraising the evolutionary consequences of growing old. Philosophical Transactions of the Royal Society of London B 361:119-135.

Calder, W. A. 1984. Size, Function and Life History. Harvard University Press, Boston, Massachusetts.

Campana, S. E. and S. R. Thorrold. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Canadian Journal of Fisheries and Aquatic Sciences 58:30-38.

Christian, A. D., Davidson, C. L., Posey II, W. R., Rust, P. J., Farris, J. L., Harris, J. L. and G. L. Harp. 2000. Growth curves of four species of commercially valuable freshwater mussels (Bivalvia: Unionidae) in Arkansas. Journal of the Arkansas Academy of Science 54:41-50.

Clark II, G. R. 1980. The study of shell structure and growth lines using thin sections. Pages 603-606 *in* D. C. Rhoads and R. A. Lutz, editors. Skeletal growth in aquatic organisms. Plenum, New York.

Coker, R. E., Shira, A. F., Clark, H. W. and A. D. Howard. 1921. Natural history and propagation of fresh-water mussels. Bulletin of the Bureau of Fisheries 37:79-181.

DeVries, D. R. and R. V. Frie. 1996. Determination of age and growth. Pages 483-512, *in* B. R. Murphy and D. W. Willis, editors. Fisheries Techniques, 2nd Edition. American Fisheries Society, Bethesda, Maryland.

Graf, D. L. and K. S. Cummings. 2007. Review of the systematics and global diversity of freshwater mussel species (Bivalvia : Unionoida). Journal of Molluscan Studies 73:291-314.

Grissino-Mayer, H. D. 2001. Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. Tree-Ring Research 57:205-221.

Haag, W. R. and A. M. Commens-Carson. 2008. Testing the assumption of annual shell ring deposition in freshwater mussels. Canadian Journal of Fisheries and Aquatic Sciences 65:493-508.

Haag, W. R. 2009. Extreme longevity in freshwater mussels revisited: sources of bias in age estimates derived from mark-recapture experiments. Freshwater Biology 54:1474-1486.

Haag, W. R. and A. L. Rypel. 2011. Growth and longevity in freshwater mussels: evolutionary and conservation implications. Biological Reviews 86:225-247.

Haag, W. R. 2012. North American freshwater mussels: natural history, ecology, and conservation. Cambridge University Press, New York.

Hochwald, S. 2001. Plasticity of life-history traits in *Unio crassus*. Pages 127-142 *in* K. Bauer and K. Wachtler, editors. Ecological Studies, Vol. 145, Springer-Verlag, Berlin.

Hornbach, D. J., Hove, M. C., Liu, H. T., Schenck, F. R., Rubin, D. and B. J.

Sansom. 2014. The influence of two differently sized dams on mussel assemblages and growth. Hydrobiologia 724:279-291.

Howard, J. K. and K. M. Cuffey. 2006. The functional role of native freshwater mussels in the fluvial benthic environment. Freshwater Biology 51:460-474.

Mayden, R. L. 1985. Biogeography of Ouachita Highland fishes. Southwestern Naturalist 30:195-211.

Neves, R. J. and S. N. Moyer. 1988. Evaluation of techniques for age-determination of fresh-water mussels (Unionidae). American Malacological Bulletin 6:179-188.

Neves, R. J. 1999. Conservation and commerce: management of freshwater mussel (Bivalvia : Unionoidea) resources in the United States. Malacologia 41:461-474.

Neves, R. J., Bogan, A. E., Williams, J. D., Ahlstedt, S. A. and P. W. Hartfield. 1997. Status of aquatic mollusks in the outheastern United States: a downward spiral of diversity. Pages 43-86 *in* G. W. Benz and D. E. Collins, editors. Aquatic fauna in peril: the southeastern perspective. Southeast Quatic Research Institute, Lenz Design and Communications, Decatur, GA.

OEAT (Ouachita Mountains Ecoregional Assessment). 2003. Nature Conservancy, p. 231, Little Rock, AR.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Fisheries Research Board of Canada 191:382.

Rypel, A. L., Haag, W. R. and R. H. Findlay. 2008. Validation of annual growth rings in freshwater mussel shells using cross dating. Canadian Journal of Fisheries and Aquatic Sciences 65:2224-2232.

Sansom, B. J., Hornbach, D. J., Hove, M. C. and J. S. Kilgore. 2013. Effects of flow restoration on mussel growth in a wild and scenic North American river. Aquatic Biosystems 9:6.

Spooner, D. E., Vaughn, C. C. and H. S. Galbraith. 2012. Species traits and environmental conditions govern the relationship between biodiversity effects across trophic levels. Oecologia 168:533-548.

Stansbery, D. H. 1967. Growth and longevity of naiades from Fishery Bay in Western Lake Erie. American Malacological Union, Inc. Annual Reports 34:10-11.

Vaughn, C. C. and C. M. Taylor. 1999. Impoundments and the decline of freshwater mussels: A case study of an extinction gradient. Conservation Biology 13:912-920.

Vaughn, C. C. and C. C. Hakenkamp. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology 46:1431-1446.

White, C. R. and R. S. Seymour. 2004. Does basal metabolic rate contain a useful signal? Mammalian BMR allometry and correlations with a selection of physiological, ecological, and life-history variables. Physiological and Biochemical Zoology 77:929-941.

Williams, J. D., Warren, M. L., Cummings, K. S., Harris, J. L. and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18:6-22.

Freshwater Mollusk Biology and Conservation 19:27–50, 2016

REGULAR ARTICLE

MICROHABITAT SUITABILITY AND NICHE BREADTH OF COMMON AND IMPERILED ATLANTIC SLOPE FRESHWATER MUSSELS

Tamara J. Pandolfo[1*], Thomas J. Kwak[2], and W. Gregory Cope[3]

[1] *North Carolina Cooperative Fish and Wildlife Research Unit, Department of Applied Ecology, North Carolina State University, Campus Box 7617, Raleigh, NC 27695 USA*
[2] *U.S. Geological Survey, North Carolina Cooperative Fish and Wildlife Research Unit, Department of Applied Ecology, North Carolina State University, Campus Box 7617, Raleigh, NC USA*
[3] *Department of Applied Ecology, North Carolina State University, Campus Box 7617, Raleigh, NC USA*

ABSTRACT

Knowledge of the habitat suitability of freshwater mussels (family Unionidae) is necessary for effective decision making in conservation and management. We empirically measured microhabitat use for 10 unionid mussel species, including the U.S. federally endangered *Alasmidonta heterodon*, at 20 sites in the Tar River basin, North Carolina, USA. We also quantified habitat availability at each site, and calculated habitat suitability for each mussel species. The majority of available habitat across all sites consisted of shallow, slow-moving water with penetrable silt or sand substrate. Among species, mean water depth of occupied habitats ranged 0.23 – 0.54 m, mean bottom velocity ranged 0.001 – 0.055 m/s, average mean-column velocity ranged 0 – 0.055 m/s, and mean substrate penetrability ranged 0.11 – 11.67 on an index scale. The most commonly measured dominant substrate materials were silt, sand, very coarse sand, pea gravel, and coarse gravel. The most common cover types were coarse woody debris and fine woody debris. These findings revealed a relationship between the niche breadth and conservation status of four species. Federally endangered *A. heterodon* consistently showed a narrower suite of suitable microhabitats than the common mussel *Elliptio complanata*. The range of suitable habitat characteristics for *Fusconaia masoni* and *Villosa constricta*, listed as North Carolina (USA) state endangered and special concern, respectively, was typically narrower than those of *E. complanata* and wider than those of *A. heterodon*. These habitat suitability criteria and relationships will be useful to guide identification of suitable sites for habitat protection, mussel relocation, or site restoration.

KEY WORDS - Unionid, habitat use, habitat availability, suitability, conservation, microhabitat

INTRODUCTION

Freshwater ecosystems are losing biodiversity at a higher rate than terrestrial or marine systems (Ricciardi and Rasmussen 1999; Dudgeon et al. 2006). Among North American freshwater species, 39% of fishes, 48% of crayfishes, and 74% of gastropods are considered to be extinct or imperiled (Taylor et al. 2007; Jelks et al. 2008; Johnson et al. 2013). Among the most imperiled aquatic taxonomic groups in North America are freshwater mussels (order Unionida); of the 297 species of freshwater mussel in North America, 72% are at risk, including the 37 species that are already presumed extinct (Williams et al. 1993; Lydeard et al. 2004, Master et al. 2000).

These widespread declines in freshwater fauna have been broadly attributed to habitat degradation, contaminants, stream fragmentation, flow alteration, and the presence of nonindigenous species (Neves et al. 1997; Richter et al. 1997; Strayer et al. 2004; Dudgeon et al. 2006; Cope et al. 2008; Jelks et al. 2008). Among these and many other possible causes, habitat degradation or destruction is ranked the most detrimental threat to about 50% of the imperiled species in the United States (Richter et al. 1997). For freshwater mussels in the eastern United States, some of the greatest contributors to mussel decline

*Corresponding Author: tjpandol@ncsu.edu

are habitat degradation due to increased sediment load from agricultural land use, mining impacts, and urbanization (Richter et al. 1997; Diamond et al. 2002; Gillies et al. 2003). The role of habitat preservation in the conservation of animals is clear, and a lack of information regarding the habitat requirements of freshwater species impedes conservation (Abell 2002). Freshwater mussels may represent an extreme case for the importance of elucidating habitat requirements because many imperiled mussels may require human intervention to persist. It is critical to identify optimal and suitable habitat characteristics to assist in habitat protection, management, and restoration, as well as mussel relocation site selection.

The majority of published habitat studies conducted with freshwater mussels have developed habitat models to predict mussel distribution and abundance (e.g., Brim Box et al. 2002; McRae et al. 2004; Gangloff and Feminella 2007; Allen and Vaughn 2010). These modeling efforts have met with mixed success (Layzer and Madison 1995; Johnson and Brown 2000), but there is currently general agreement that microhabitat characteristics alone are not effective predictors of mussel distribution (e.g., Strayer and Ralley 1993; Haag and Warren 1998; Brim Box et al. 2002). Freshwater mussel habitat preferences also have been examined in controlled laboratory studies (Michaelson and Neves 1995; Downing et al. 2000). However, research on habitat suitability indices for freshwater mussels is lacking (but see Layzer and Madison 1995).

Habitat suitability indices have been widely developed for fishes and other aquatic organisms (e.g., Hamilton and Nelson 1984; Raleigh et al. 1986; Simon and Cooper 2014). A primary application of habitat suitability indices is to conduct instream flow modeling (Bovee 1986; Annear et al. 2004). Such models apply site-specific stream flow and habitat suitability data for a species to project how the availability of suitable habitat may change with fluctuations in stream flow, which is especially applicable to regulated river systems. Habitat suitability indices provide the biological input for instream flow models, and describe the relative importance, or suitability, of different microhabitats based on measures of habitat use in proportion to availability of that habitat. The application of habitat suitability indices for aquatic species extends beyond flow modeling. They are also relevant for use in varied applications, such as targeted field surveys (Midway et al. 2010), animal relocations or reintroductions (Fisk et al. 2014), site restoration (Quinn and Kwak 2000; Hewitt et al. 2009; Fisk et al. 2015; Yao et al. 2015), conservation planning (Spooner et al. 2011), or more complex species distribution or niche modeling efforts (Elith and Leathwick 2009).

In this study, we investigated the habitat suitability of common and imperiled mussel species in a lotic ecosystem of the eastern United States. We measured microhabitat use and habitat availability to determine habitat suitability for a suite of microhabitat parameters for 10 species of freshwater mussels. These suitability results can be used to infer relative selectivity of freshwater mussels for a variety of microhabitats and target suitable ranges of habitat parameters for conservation and management (Johnson 1980).

METHODS

Field Surveys

We selected twenty sites within the upper Tar River basin, North Carolina, USA, from three subbasins with similar drainage areas: the Upper Tar, Swift Creek, and Fishing Creek subbasins (Figure 1). Sites were selected to reflect a range of environmental conditions (e.g., land use, stream size, etc.) and for accessibility via bridge crossings. We targeted sites with known occurrences of rare species, particularly *Alasmidonta heterodon*, based on documented occurrences and the past mussel survey data and experience of the North Carolina Wildlife Resources Commission (NCWRC) personnel. We conducted freshwater mussel snorkel surveys in the summer of 2010. Mussel surveys began at the start location of prior surveys by the NCWRC and where habitat appeared amenable for mussels (e.g., away from bridge pools). Mussel surveys continued for 6 person-hours, and the length of the survey reach depended on the number of survey personnel and size of the stream, but ranged from about 100 m to 500 m. We conducted surveys of mussel microhabitat use concurrent with freshwater mussel surveys. We flagged precise mussel locations, and we measured microhabitat characteristics at these precise locations. For the most common species, *Elliptio complanata*, up to 20 individuals were flagged per site and their data recorded. For all other species, microhabitat characteristics were measured for all mussels detected during a survey.

We recorded measurements of six microhabitat parameters for each mussel location for base-flow conditions, including water depth (m), bottom water velocity (m/s), mean-column water velocity (m/s), substrate penetrability (index), dominant substrate type, and closest cover type. Depth and velocity measurements were included as an indication of conditions at base flow, and these measurements are always included in standard habitat suitability criteria in support of the IFIM methodology (Bovee 1986). Substrate penetrability was included as a quantitative measure of the compaction of the substrate. It is indicative of the degree of embeddedness or sedimentation at a site, and is an important consideration for burrowing organisms. Dominant substrate type is a categorical indicator of substrate composition. Closest cover type was included as an indicator of potential flow refugia for mussels. In addition, some species are anecdotally associated with certain cover types (e.g., *Alasmidonta heterodon* is associated with root structures; personal communication T. R. Black, N.C. Wildlife Resources Commission), and we wanted to investigate such associations. All of these parameters are useful in describing species' basic habitat requirements or niche, with some focus on factors attributing to mussel decline (i.e., substrate penetrability as a measure of sedimentation).

We measured depth and water velocity using a top-set wading rod and a Marsh-McBirney Model 2000 digital flow meter, with bottom velocity measured at the stream bed and mean velocity measured at 60% of depth (Bain and Stevenson

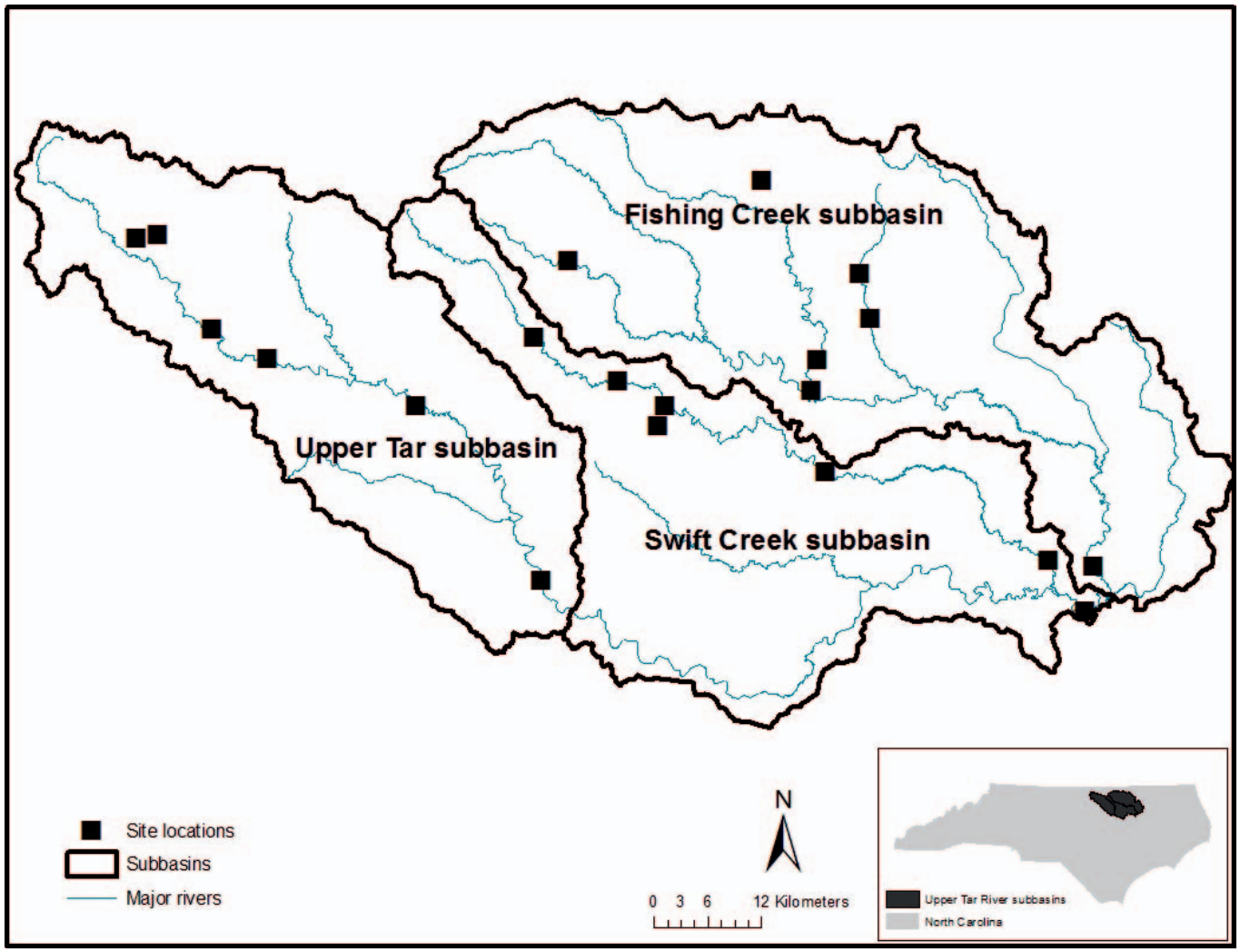

Figure 1. Locations of 20 mussel and habitat survey sites in the Tar River basin, North Carolina, USA.

1999). Dominant substrate type (Table 1) was assessed visually based on a modified Wentworth particle size scale (Bovee and Milhous 1978). We measured substrate penetrability using the index scale of a Lang Penetrometer (Johnson and Brown 2000). Force-pound conversions for the index measurements were provided by the manufacturer for comparison (Table 1). The closest cover type was the nearest material, upstream or downstream, that could slow water velocity or provide shelter for a mussel (Table 1). Where appropriate, the Wentworth particle size scale was used to determine the type of cover (e.g., boulders). Woody debris was considered fine with a diameter of <10 cm, and coarse with a diameter >10 cm. Vegetation was considered cover if the plant was rooted and stable. Trash was considered cover if it was large enough to be stable during moderate flows, e.g., tires, furniture. We measured mussel survey reach lengths using a digital rangefinder, and we recorded GPS coordinates to ensure habitat availability surveys would be conducted at locations corresponding to mussel micrhabitat use surveys.

We assessed microhabitat availability by conducting instream habitat surveys at each site under base-flow conditions. At each site, we determined a mean stream width and then, starting with the placement of the first cross-sectional transect within the mussel survey reach based on a location determined by a random number generator, 10 transects were spaced every two mean-stream-widths apart to determine the end of the survey reach (Simonson et al. 1994). At a minimum of 10 equally-spaced points within each transect, we measured six microhabitat parameters to characterize microhabitat availability. These were the same parameters measured in the mussel microhabitat use assessment.

Habitat Suitability Analysis

Mussel species with at least five individuals sampled at one site were considered for further analysis. Nine species met this criterion, including *E. complanata*, *E. icterina, E. congaraea*, *E. roanokensis*, *E. fisheriana*, *Alasmidonta heterodon*, *Villosa*

Table 1. Classification and abbreviations of substrate, cover type, and substrate penetrability for habitat use and availability analyses.

Covariate	Value	Abbreviation/ Index
Substrate	mm	
Silt-clay	<0.062	Silt
Sand	0.062-1	Sand
Very coarse sand	1-2	VCS
Pea gravel	2-4	PG
Fine gravel	4-8	FG
Medium gravel	8-16	MG
Coarse gravel	16-32	CG
Very coarse gravel	32-64	VCG
Small cobble	64-130	SC
Large cobble	130-250	LC
Small boulder	250-500	SB
Medium boulder	500-1,000	MB
Large boulder	1,000-2,000	LB
Very large boulder	2,000-4,000	VLB
Mammoth boulder/bedrock	>4,000	Bedrk
Cover Type		
Coarse woody debris		CWD
Fine woody debris		FWD
Vegetation		Veg
Roots		Roots
Undercut bank		Bank
Small boulder		SB
Medium boulder		MB
Very large boulder		VLB
Mammoth boulder/bedrock		Bedrk
Tire, trash, misc.		Other
Substrate Penetrability	Force pounds	
(highest penetrability,	3.57	1
lowest compaction)	4.64	2
	5.72	3
	6.79	4
	7.86	5
	8.94	6
	10.01	7
	11.09	8
	12.16	9
	13.24	10
	14.31	11
	15.39	12
	16.46	13
	17.54	14
	18.61	15
	19.68	16
	20.76	17
	21.83	18
(lowest penetrability,	22.91	19
most compaction)	23.98	20

constricta, *Fusconaia masoni*, and an undescribed *Lampsilis* species. Data were limited for an endemic federally endangered species, *E. steinstansana*. Though this species did not meet the analysis criteria, because the species is so rare and information on the species is so scarce, we have included an anecdotal analysis of the habitat suitability for the three individuals sampled.

We calculated and graphed microhabitat suitability values as distributions for each of the 10 investigated species using the microhabitat use and availability data. For each habitat parameter, we calculated suitability by dividing microhabitat use at a site by availability at that site over a range of values for each parameter (Bovee 1986). Availability data for only the individual sites where each mussel species was found were used in suitability calculations. Each habitat parameter's entire range of values was normalized to a maximum of 1.0 to provide a scale where 1.0 indicates the most optimal, or suitable habitat, and 0 indicates the least suitable. When a mussel species was encountered at more than one site, data from multiple sites were combined by weighting suitability for each site by the number of individuals at that site, and then summing the weighted suitability values and again normalizing to a maximum of 1.0. In cases where proportional use for a particular interval or category of a parameter was greater than its availability, we set suitability to 1.0 because the suitability scale is proportional and reaches its maximum at 1.0 (i.e., optimal range of the parameter).

We further analyzed data for *A. heterodon*, *F. masoni*, *V. constricta*, and *E. complanata*, because sufficient sample sizes were attained and these species represent a range of conservation statuses (i.e., endangered to common). We graphed the habitat suitability of the six parameters for these four species together to compare the range of suitability according to species and conservation status. Data for these species were analyzed using a bootstrap, two-sample Kolmogorov-Smirnov test (R statistical software; Sekhon 2011) to test for significant differences between habitat use and habitat availability distributions (i.e., non-random use of habitat by a mussel species) and pairwise comparisons of cumulative habitat suitability between species for each parameter, except closest cover type. Closest cover type was a categorical variable, and thus, a likelihood ratio chi-square test was used to test for differences between use and availability and habitat suitability between species (JMP statistical software, SAS, Cary, North Carolina).

RESULTS

Microhabitat Use and Availability

The most ubiquitous species, *E. complanata,* was represented by 357 individuals from 20 sites (Table 2), whereas the rarest species, *E. steinstansana*, was represented by three individuals from two sites. Among species, mean depth of occupied habitats ranged 0.23 – 0.54 m, mean bottom velocity

Table 2. Count (*N*), mean, range, and standard deviation (SD) of habitat availability for 20 sites and microhabitat use measurements for 10 species of freshwater mussels of depth (m), bottom and mean-column velocity (m/s), substrate penetrability, dominant substrate, and cover type.

	N		Depth (m)		Bottom Velocity (m/s)		Mean Velocity (m/s)		Substrate Penetrability (Index)		Dominant Substrate (Type)		Closest Cover (Type)	
Species	indiv	sites	Mean (Range)	SD	Mean (Range)	SD	Mean (Range)	SD	Mean (Range)	SD	Mode	Types	Mode	Types
Alasmidonta heterodon	19	2	0.26 (0.08 - 0.55)	0.12	0.003 (0.00 - 0.02)	0.01	0.004 (0.00 - 0.02)	0.01	0.11 (0 - 1.5)	0.36	VCS, PG	6	CWD	4
Elliptio complanata	357	20	0.38 (0.02 - 1.36)	0.24	0.019 (0.00 - 0.31)	0.05	0.047 (0.00 - 0.64)	0.08	3.42 (0 - 20)	4.93	Silt	14	CWD	9
Elliptio congaraea	22	3	0.53 (0.24 - 0.80)	0.17	0.055 (0.00 - 0.43)	0.10	0.074 (0.00 - 0.56)	0.13	6.98 (0 - 18.5)	5.94	Sand	9	CWD	5
Elliptio fisheriana	11	2	0.30 (0.10 - 0.53)	0.15	0.017 (0.00 - 0.04)	0.01	0.033 (0.00 - 0.06)	0.02	8.36 (0 - 20)	7.50	Sand	6	CWD	5
Elliptio icterina	62	7	0.43 (0.06 - 1.02)	0.24	0.016 (0.00 - 0.31)	0.05	0.051 (0.00 - 0.52)	0.08	5.60 (0 - 20)	5.84	Sand	11	CWD	7
Elliptio roanokensis	13	2	0.52 (0.14 - 1.19)	0.33	0.050 (0.00 - 0.18)	0.06	0.151 (0.00 - 0.32)	0.10	3.42 (0 - 16)	4.65	Sand, CG	5	CWD	3
Elliptio steinstansana	3	2	0.47 (0.13 - 0.65)	0.29	0.013 (0.00 - 0.04)	0.02	0.023 (0.00 - 0.07)	0.04	11.67 (7 - 14.5)	4.07	Sand	2	CWD	2
Fusconaia masoni	14	2	0.54 (0.41 - 0.69)	0.08	0.036 (0.00 - 0.14)	0.06	0.080 (0.00 - 0.30)	0.11	9.29 (0 - 14.5)	4.08	Sand	5	CWD	4
Lampsilis sp.	5	1	0.23 (0.07 - 0.40)	0.13	0.000 (0.00 - 0.00)	<0.01	0.004 (0.00 - 0.01)	0.01	NA	NA	NA	5	CWD	3
Villosa constricta	24	2	0.48 (0.05 - 0.86)	0.25	0.001 (0.00 - 0.02)	<0.01	0.010 (0.00 - 0.05)	0.02	6.98 (0 - 13)	4.92	Sand	8	FWD	4
Habitat availability	2,398	20	0.35 (0.00 - 2.70)	0.34	0.013 (−0.05 - 0.65)	0.07	0.037 (−0.05 - 0.89)	0.10	4.19 (0 - 20)	6.26	Silt	15	CWD	10

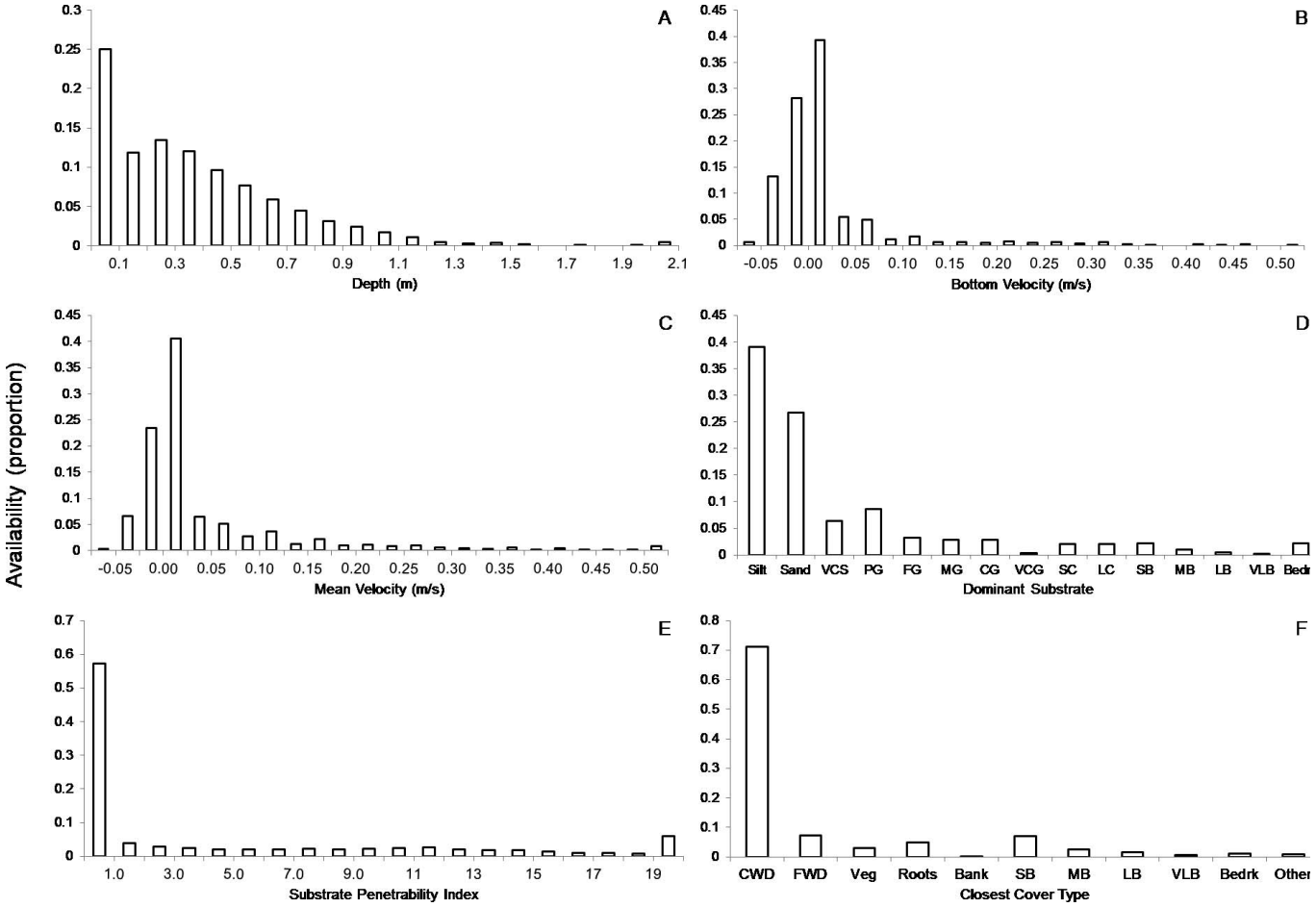

Figure 2. Availability of six microhabitat parameters from 20 sites in the Tar River basin, North Carolina, USA.

ranged 0.001 – 0.055 m/s, average mean-column velocity ranged 0 – 0.055 m/s, and mean substrate penetrability ranged 0.11 – 11.67 on an index scale. The most commonly measured dominant substrate materials were silt, sand, very coarse sand, pea gravel, and coarse gravel. The most common cover types were coarse woody debris and fine woody debris.

We surveyed habitat availability at a mean of 120 (range 80 – 161) points within each of 20 sites (Table 2, Figure 2). The majority of available habitat across all sites consisted of shallow, slow-moving water with penetrable silt or sand substrate. The most abundant cover type was coarse woody debris.

Habitat Suitability Distributions

Habitat suitability distributions for depth, bottom velocity, mean velocity, substrate penetrability, dominant substrate, and closest cover type varied among species (Figures A1 – A10), reflecting differences among habitat niches occupied, but influenced by the range in sample sizes (i.e., suitability distributions of species with the greatest numbers of habitat use measurements, *E. complanata* and *E. icterina*, more closely resembled a continuous distribution).

Differences among species microhabitat suitability were evident (Table 3). For example, *A. heterodon* tended to occupy shallow, slow-flowing sites with penetrable silt, coarse sand, and gravel. Tree roots and vegetation provided suitable cover, in addition to woody debris. *V. constricta* also utilized shallow slow-flowing locations, but moderately penetrable gravels and cobble were the most suitable substrates. Boulders and woody debris provided the most suitable cover. Suitable habitat for *F. masoni* was similar to that of *V. constricta*, but slightly deeper and faster flowing water was more suitable. The undescribed *Lampsilis* species was most suited to habitats like those preferentially occupied by *V. constricta*.

The most common species, *E. complanata*, was at least marginally suited to almost all available habitat. The most suitable habitats for this species were shallow, slow-flowing sites with penetrable substrates. *E. icterina* had similar suitability, but moderately penetrable coarse sand was its most suitable substrate. *E. congaraea* occurred in slightly deeper water with slow velocity, though it tolerated even the swiftest flows (> 0.50 m/s). Many substrates were suitable for *E. congaraea*, but silt was not. *E. fisheriana* was suited to

Table 3. Most suitable conditions (habitat suitability ≥ 0.5) of depth, bottom and mean-column velocity, substrate penetrability, dominant substrate, and cover type for 10 species of freshwater mussels. Habitat conditions with optimal suitability (habitat suitability = 1.0) are shown in bold.

Species	Depth (m)	Bottom Velocity (m/s)	Mean Velocity (m/s)	Substrate Penetrability (Index)	Dominant Substrate (Type)	Closest Cover (Type)
Alasmidonta heterodon	**0.30–0.39** 0.10–0.29	**0–0.024**	**0–0.024**	**0–0.9** 1.0–1.9	**VCS** Silt, PG, FG, MG	**FWD** Veg
Elliptio complanata	**0.30–0.39** 0.10–0.69	**0–0.024**	**0–0.024** 0.025–0.074, 0.100–0.124	**0–0.9** 1.0–3.9, 5.0–6.9, 8.0–10.9	**VCG** Silt, Sand, VCS, PG, FG, MG, CG, SC, LC	**FWD** Bank
Elliptio congaraea	**0.60–0.69** 0.40–0.49	**0–0.024**	**0–0.024** 0.200–0.224	**3.0–3.9, 8.0–8.9** 5.0–5.9, 9.0–9.9, 13.0–13.9	**VCS**, **VCG** Sand, CG, LC	**SB** FWD, MB
Elliptio fisheriana	**0.50–0.59** 0.30–0.39	**0.025–0.049**	**0.025–0.049** 0.050–0.074	**9.0–9.9** 12.0–12.9	**SC** Sand, CG, VCG, MB, Bedrk	**FWD** MB, Bedrk
Elliptio icterina	**0.50–0.59** 0.10–0.29, 0.60–0.69	**0–0.024**	**0–0.024** 0.050–0.074, 0.100–0.124	**8.0–8.9** 10.0–11.9, 13.0–13.9	**VCS** VCG	**FWD**
Elliptio roanokensis	**0.20–0.29**, 0.70–0.89	**0.050–0.074** 0–0.124, 0.175–0.199	**0.225–0.249** 0.100–0.124, 0.300–0.324	**6.0–7.9** 1.0–2.9, 16.0–16.9	**CG**	**FWD** SB, Other
Elliptio steinstansana	**0.60–0.69**	**0.025–0.049**	**0.050–0.074**	**7.0–7.9** 13.0–14.9	**VCS**	**SB** CWD
Fusconaia masoni	**0.40–0.49** 0.50–0.69	**0–0.024**	**0–0.024** 0.100–0.124, 0.175–0.224, 0.250–0.274, 0.300–0.324	**7.0–7.9** 6.0–8.9, 14.0–14.9	**PG** FG, LC	**SB** FWD, MB
Lampsilis sp.	**0.40–0.49**	**0–0.024**	**0–0.024**	NA	**SC** VCG, LC	**FWD** SB
Villosa constricta	**0.60–0.69** 0.10–0.19, 0.50–0.59	**0–0.024**	**0–0.024**	**7.0–7.9** 4.0–4.9, 6.0–6.9, 10.0–11.9, 13.0–13.9	**VCG** FG, MG, SC	**SB** FWD

Table 4. Results from two-sample Kolmogorov-Smirnov tests (*D*-statistic and *p*-value) and likelihood ratio chi square test (χ^2-statistic and *p*-value) of the difference between microhabitat use and microhabitat availability distributions. Statistically significant results ($p < 0.05$, in bold font) indicate non-random use of habitat.

	Depth (m)		Bottom Velocity (m/s)		Mean Velocity (m/s)		Substrate Penetrability		Dominant Substrate		Closest Cover	
Species	*D*	*p*	*D*	*p*	*D*	*p*	*D*	*p*	*D*	*p*	$\chi 2$	*p*
Alasmidonta heterodon	0.579	**0.001**	0.833	**<0.001**	0.667	**0.018**	0.850	**<0.001**	0.364	0.328	107.6	**<0.001**
Elliptio complanata	0.478	**<0.001**	0.541	**<0.001**	0.480	**<0.001**	0.498	**<0.001**	0.230	**<0.001**	1,182.0	**<0.001**
Fusconaia masoni	0.750	**<0.001**	0.571	**0.010**	0.353	0.124	0.703	**<0.001**	0.708	**<0.001**	328.2	**<0.001**
Villosa constricta	0.524	**0.004**	0.800	**0.002**	0.444	0.256	0.588	**<0.001**	0.300	0.229	161.8	**<0.001**

shallow, slow-flowing habitats with moderately penetrable substrates. *E. roanokensis* was suited to coarse gravel habitats with deeper and swifter water than the other species. The federally endangered *E. steinstansana* was anecdotally associated with moderately penetrable coarse sand and slow velocity with woody debris and boulders as cover.

Non-random Habitat Selectivity

We tested habitat use of *E. complanata*, *A. heterodon*, *F. masoni*, and *V. constricta* against habitat availability to detect randomness in habitat selectivity among species (Table 4). Depth, bottom velocity, substrate penetrability, and closest cover type were non-randomly selected among all four species. *E. complanata* exhibited non-random habitat use for all six measured microhabitat parameters. Mean-column velocity use was also non-random for *A. heterodon*, and *F. masoni* exhibited non-random use of dominant substrate.

Habitat Suitability Among Conservation Statuses

Habitat suitability for four species with different conservation statuses, *E. complanata*, *A. heterodon*, *F. masoni*, and *V. constricta*, was plotted together for relative species comparisons (Figure 3). Most suitable depths for *A. heterodon*, *F. masoni*, and *E. complanata* ranged 0.3 – 0.5 m, whereas slightly deeper waters of 0.6 – 0.7 m were most suitable for *V. constricta*. All four species were suited to velocities up to 0.025 m/s, which were also the most widely available. A range of substrates could be considered at least moderately suitable for all species, but the species differed in substrate penetrability suitability. *A. heterodon* was suited to the most penetrable substrates, although those were the only substrates available at the sites where it occurred. *E. complanata* was also most suited to highly penetrable substrates, whereas *V. constricta* and *F. masoni* found mid- to high-range compaction most suitable. Woody debris was suitable cover for all four species. *V. constricta* and *F. masoni* also utilized boulders, and *A. heterodon* was associated with vegetation, roots, and undercut banks. Federally endangered *A. heterodon* consistently showed a narrower suite of suitable microhabitats than the common mussel *E. complanata*. The range of suitable habitat characteristics for *F. masoni* and *V. constricta*, listed as North Carolina (USA) state endangered and special concern, respectively, was typically narrower than those of *E. complanata* and wider than those of *A. heterodon*.

Statistical analysis confirmed that differences in habitat suitability among mussels of different conservation statuses were significant (Table 5). Among 36 between-species comparisons of 6 habitat suitability variables, 22 (61%) detected significantly different distributions. Habitat suitability of *E. complanata* differed significantly from that of federally endangered *A. heterodon* and NC state endangered *F. masoni* for all six parameters measured. Habitat suitability of *E. complanata* significantly differed from that of NC state special concern *V. constricta* for four of six parameters: bottom and mean velocity, substrate penetrability, and closest cover type. There were no significant differences detected among any of the uncommon species (*A. heterodon*, *F. masoni*, and *V. constricta*) for depth, bottom velocity, or dominant substrate. *A. heterodon* and *F. masoni* exhibited significantly different habitat suitability distributions for mean velocity. All species differed significantly from one another in suitability of closest cover type and substrate penetrability, with the exception of *F. masoni* and *V. constricta* for substrate penetrability.

DISCUSSION

Relationship Between Freshwater Mussels and Microhabitat

Our results indicated that freshwater mussels generally occupied microhabitat non-randomly and that mussel conservation status may correspond to niche breadth. Although freshwater mussels are broadly described as habitat generalists (Tevesz and McCall 1979), results of this study demonstrated that some characteristics are more suitable than others when habitat use is adjusted for availability. Habitat requirements are thought to be one of the primary controls on animal distribution and abundance (Haag and Warren 1998). However, defining this relationship for freshwater mussels has been complicated. The value of traditional microhabitat parameters, such as depth and substrate type, is greatly surpassed by complex hydraulic variables, which influence

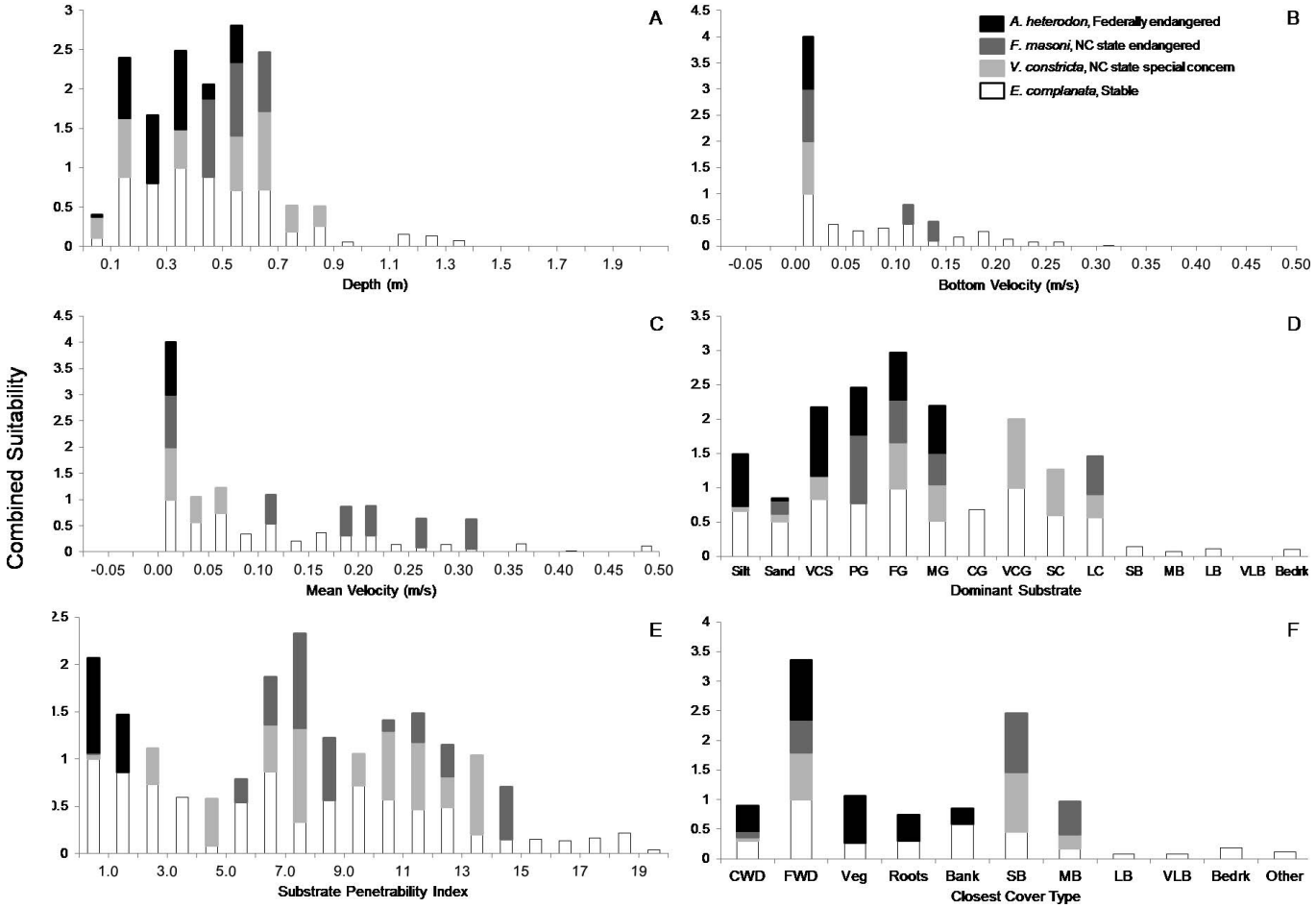

Figure 3. Habitat suitability distributions for four freshwater mussel species with different conservation status: federally endangered *Alasmidonta heterodon*, North Carolina (USA) state endangered *Fusconaia masoni*, North Carolina state special concern *Villosa constricta*, and stable *Elliptio complanata*. Combined suitability is for relative comparison only.

substrate stability, in the ability to predict the distribution and abundance of freshwater mussels (Layzer and Madison 1995; Zigler et al. 2008; Allen and Vaughn 2010). Despite the general lack of broad predictive value, multiple investigators have found correlative relationships between some microhabitat parameters and freshwater mussel occurrence and abundance (Salmon and Green 1983; Strayer and Ralley 1993; Johnson and Brown 2000). These mixed conclusions suggest that microhabitat may not directly control mussel occurrence *per se*, but it is a factor influencing the distribution of freshwater mussels (Strayer and Ralley 1993; Layzer and Madison 1995; Haag and Warren 1998; Downing et al. 2000; Strayer 2008). Habitat is almost certainly a limiting factor in mussel distributions, but the relationship is complex and involves dynamics at multiple interacting spatial and temporal scales (e.g., McRae et al. 2004, Pandolfo 2014).

Further complicating these relationships is the fact that some parameters are indicative of conditions at multiple scales. In this study, all parameters were measured at a microhabitat scale, and habitat use measurements in particular were taken at precise mussel locations. However, these data can also provide information on habitat conditions at the macrohabitat scale, or even at the reach scale. For instance, measures of substrate penetrability can reflect bank erosion in a reach or overall land use in a watershed.

Substrate composition and flow are among the most often measured habitat characteristics in mussel habitat studies, and they are also the parameters most often found to correlate with freshwater mussel occurrence (Salmon and Green 1983; Holland-Bartels 1990; Strayer and Ralley 1993; Johnson and Brown 2000), though there is not always a strong relationship (Neves and Widlak 1987; Strayer et al. 1994; Layzer and Madison 1995; Haag and Warren 1998). The microhabitat parameters that we examined in this study were aligned with these two characteristics: water depth, velocity, dominant substrate, substrate penetrability, and cover type. Depth, velocity, and substrate penetrability were selected non-randomly by all four species tested (*E. complanata*, *A. heterodon*, *V. constricta*, and *F. masoni*). This further supports the notion that freshwater mussels are responding to habitat gradients and findings of previous studies that demonstrate the importance of flow and substrate stability for freshwater

Table 5. Results from two-sample Kolmogorov-Smirnov tests and likelihood ratio chi-square tests (*p*-value) of the difference between cumulative habitat suitability distributions for four mussel species with different conservation statuses. Statistically significant comparisons ($p < 0.05$, in bold font) indicate non-random differences in habitat suitability between species.

	Parameter					
	Depth			Dominant substrate		
Species	*E. com*	*F. mas*	*V. con*	*E. com*	*F. mas*	*V. con*
Alasmidonta heterodon	**0.0426**	0.5420	0.6981	**0.0072**	0.3325	0.4013
Elliptio complanata		**0.0043**	0.2015		**0.0057**	0.1570
Fusconaia masoni			0.3252			0.6636
Villosa constricta						
	Bottom Velocity			Substrate Penetrability		
	E. com	*F. mas*	*V. con*	*E. com*	*F. mas*	*V. con*
Alasmidonta heterodon	**0.0011**	0.4913	1.0000	**<0.0001**	**0.0325**	**0.0114**
Elliptio complanata		**0.0155**	**0.0009**		**0.0005**	**0.0084**
Fusconaia masoni			0.4968			0.5567
Villosa constricta						
	Mean Velocity			Closest Cover		
	E. com	*F. mas*	*V. con*	*E. com*	*F. mas*	*V. con*
Alasmidonta heterodon	**<0.0001**	**0.0662**	0.4918	**<0.0001**	**<0.0001**	**<0.0001**
Elliptio complanata		**0.0060**	**0.0002**		**<0.0001**	**<0.0001**
Fusconaia masoni			0.1115			**0.0003**
Villosa constricta						

mussel habitat (e.g., Layzer and Madison 1995, Allen and Vaughn 2010).

In those studies that observed a correlation among mussels and microhabitat, mussel abundance, recruitment, and density were most often positively associated with slow to moderate flows and moderately coarse substrates with few fines (e.g., Salmon and Green 1983; Holland-Bartels 1990; McRae et al. 2004; Geist and Auerswald 2007). Measures of substrate compaction with a penetrometer have been applied in a limited number of studies (Johnson and Brown 2000; Geist and Auerswald 2007), and those studies have shown that this microhabitat measure is relevant to mussel ecology. Sediment compaction was positively related to mussel abundance, but negatively affected recruitment (Johnson and Brown 2000; Geist and Auerswald 2007).

The common mussel, *E. complanata*, exhibited non-random selectivity of all habitat parameters tested. However, suitability values for dominant substrate indicated a broad substrate suitability ranging in size from silt to large cobble. Other studies of *E. complanata* have found a similar broad tolerance of substrate types. In the coastal plain of the Apalachicola, Chattahoochee, and Flint River basins in Alabama, Georgia, and Florida, USA, the presence of *E. complanata* and *E. icterina* was not correlated with substrate composition (Brim Box et al. 2002). A study of *E. complanata* in Virginia, USA, found no habitat characteristics that explained the mussels' clumped distribution (Balfour and Smock 1995). In a laboratory study, *E. complanata* most commonly occurred in muddy substrates, which differed from the sand and gravel that were most commonly occupied in their lake environment (Downing et al. 2000). In the Hudson River, New York, USA, low percentages of fine sand were significantly correlated with the abundance of unionids, including *E. complanata* (Strayer et al. 1994), and in the Neversink River, New York, USA, high percentages of medium sand were correlated with the occurrence of *E. complanata* and other species (Strayer and Ralley 1993). These cumulative results concur to describe the wide niche breadth of *E. complanata* that is reflected in its ubiquitous distribution throughout eastern North America (Johnson 1970).

We found that the federally endangered *A. heterodon* was most suited to slow flowing, shallow locations with fine to medium-fine substrate. These results generally agree with habitat suitability criteria from the Delaware River suggesting moderately deep, slow-flowing water, and laboratory studies that confirm a preference for slow to moderate velocity (Michaelson and Neves 1995; Parasiewicz et al. 2012). Field and laboratory studies also suggest fine sand substrates are most suitable for *A. heterodon* (Strayer and Ralley 1993;

Michaelson and Neves 1995). Empirically, the other federally endangered species in the Tar River basin, *E steinstansana*, often occurs in fast-flowing, well-oxygenated water and relatively silt-free substrate composed of gravel or coarse sand (USFWS 1992). The very limited data on *E. steinstansana* from this study suggest a slow velocity with moderately compacted sand or coarse sand substrate.

The importance of microhabitat influence on mussel distribution may depend on the species (Huehner 1987; Brim Box et al. 2002). Minor microhabitat differentiation among species has been shown in some species (Salmon and Green 1983; Holland-Bartels 1990). In the Mississippi River, USA, mussels occurred in a broad range of sediment types that indicated a general lack of species differences; the endangered *L. higginsii* was present in habitats similar to those as the most common species, *A. plicata* (Holland-Bartels 1990). Subtle differences in habitat dynamics among mussel species have been found, however, and they could be broadly grouped into those with affinities for fine to medium-fine sands and those with coarser sand affinities (Holland-Bartels 1990). These slight microhabitat differences among species may explain niche partitioning that allows the coexistence of numerous mussel species within a single bed (Salmon and Green 1983). However, habitat is certainly not the only factor that determines mussel distribution; species traits, distribution of host fishes, and availability of resources are all important factors as well (Haag and Warren 1998, Strayer 2008, Schwalb et al. 2013).

Species Differences in Habitat Suitability Distributions

We found evidence of both subtle and distinct species differences in habitat suitability distributions among the 10 species examined. There was evidence of some species occupying habitat non-randomly for specific parameters, whereas other species occupied habitat randomly for the same parameter. For instance, *A. heterodon* and *E. complanata* appeared to select mean velocity non-randomly whereas this was not true for *F. masoni* and *V. constricta*. There was also evidence of differences among species related to their conservations status. Significant differences between habitat suitability distributions for the common species, *E complanata*, and the rarer species, *A. heterodon*, *F. masoni*, and *V. constricta*, suggest that, for these species, conservation status serves as a proxy for niche breadth and degree of habitat specialization. Conservation status was positively related to the range of suitable habitats for a species, which suggests, as would be expected, that the rarest mussels have narrower microhabitat niches than ubiquitous species. Results also show that the most ubiquitous species, *E. complanata*, was the only one that demonstrated non-random habitat use for all habitat parameters. It is relevant, however, that the sample size for this species was much larger than that of the other species, and statistical significance may have been more likely due to greater statistical power.

Utility of Habitat Suitability Distributions

Habitat suitability index models are a useful method for identifying environmental factors that may limit species occurrence, but these relationships are not necessarily causal and should be considered primarily as a premise for further investigation and management planning (Morrison et al. 1998). Absolute statements regarding the suitability of habitats are not recommended, but relative comparisons of suitability distributions can be informative (Johnson 1980). Any habitat suitability study is constrained by the researcher's options and choice of available habitat, and suitable conditions that were not measured or present in the defined study area may exist. However, given a region with similar habitat characteristics (e.g., coastal plain systems), results of this study represent a valid relative comparison of the suitability of a variety of habitat components (Johnson 1980).

Another consideration in the applicability of habitat suitability studies is that the use of habitat by an animal does not necessarily imply active selection, rather than an unmotivated presence (Johnson 1980; Beyer et al. 2010). In addition, substrate use by freshwater mussels is probably more complex than can be measured via simple microhabitat use (Layzer and Madison 1995). Mussels may require combinations of fine substrate materials for burrowing, and also coarser substrates to function as cover and velocity breaks (Layzer and Madison 1995). It is also possible that the apparently random habitat use measured by some parameters (mean velocity, substrate, and cover) for three species in this study was influenced by low sample size. In some cases, the lack of correlation between substrate and freshwater mussel distribution or abundance may be due to an inadequate sampling effort (Brim Box and Mossa 1999). In this study, *E. complanata* had the largest sample size, and non-random habitat use was detected for all six measured habitat variables. The species with fewer microhabitat use measurements exhibited both random and non-random use of habitat according to the particular parameter. This may be due to the lack of statistical power or adequate representation of suitability distributions in these samples, or it may be due to an actual ecological difference among species.

The complications arising from the limited number of rare mussels encountered during habitat use surveys is a common problem when working with rare species (Brim Box et al. 2002). Our results indicate that rarer mussel species may have a narrower and significantly different habitat suitability distribution than the most common species. However, this association is not unequivocal because of the confounded issue of limited sample size inherent in the study of rare species. The ability to detect and measure microhabitat use for representative numbers of rare species was limited, even with the intensive sampling effort in this study. The typical level of effort applied in timed search mussel assemblage surveys in streams is 1.5 person-hours per site (Metcalfe-Smith et al. 2000), yet we expended 6.0 person-hours of effort at each sampling site in this study, suggesting that the low sample sizes for some species reflect

actual low site densities, rather than low detection probability. This was particularly the case with the federally endangered *E. steinstansana*, of which only three individuals were found. This highlights the difficulty of studying the rarest species; it is often very difficult to collect information on the species in greatest need of conservation and in which we are most concerned. Future research that aims to characterize the microhabitat of rare species would be enhanced by using sampling designs and methods, such as adaptive sampling, that will allow these species to be sampled more frequently (Brim Box et al. 2002, Strayer and Smith 2003).

Habitat Suitability Distributions in Mussel Conservation

Quantitative methods of habitat assessment, such as habitat suitability indices, are more valuable and ecologically relevant than anecdotal descriptions of habitat (Bovee 1986). The habitat suitability method adopted in this study empirically measured habitat use and availability independently for each site, thus allowing the relative selectivity for habitats to be quantified (Bovee 1986). Microhabitat characteristics that are associated with mussel occurrence can be simply and quickly assessed in the field, making habitat suitability a useful tool in practical applications, if not in predictive modeling exercises. This knowledge can be useful in targeting field surveys for rare species (Midway et al. 2010), identification of relocation sites for imperiled species (Fisk et al. 2014), or for the planning of conservation measures, including site restoration (Quinn and Kwak 2000; Fisk et al. 2015). Microhabitat is one in a scale and suite of variables to be considered, it may not limit or predict distribution on its own, but neither is it inconsequential. It appears that no one scale and approach of habitat assessment may adequately describe the ecological relationships between freshwater mussel populations and their dynamic environment.

Habitat degradation is among the most prominent threats facing freshwater mussels, and the habitat requirements of mussels must be understood to develop the best spatial scale and specific conservation practices to protect them from future decline. The assessment of microhabitat can be useful in quantifying suitable and optimal habitat to guide conservation strategies and management plans for endangered mussel species (Johnson and Brown 2000). Microhabitat preferences are already being used to relocate the endangered *Margaritifera hembeli* to suitable sites when their beds are threatened by channel alterations (Johnson and Brown 2000). Habitat suitability criteria such as those we developed for 10 species in this study can similarly be used to target habitat protection, mussel relocations, reintroductions, or site restoration within acceptable macrohabitats.

ACKNOWLEDGEMENTS

This research was funded by the U.S. Geological Survey National Climate Change and Wildlife Science Center through Research Work Order 171. Daniel Weaver, Elissa Buttermore, Jennifer Archambault, Angela White, Bobby Cope, and Michael Fisk provided assistance with habitat measurements, and Rob Nichols, Ryan Heise, Tyler Black, and Brena Jones of the North Carolina Wildlife Resources Commission provided assistance with mussel surveys. Barbara Doll offered helpful suggestions in stream habitat parameter measurement methods, and David Strayer provided a constructive manuscript review. The North Carolina Cooperative Fish and Wildlife Research Unit is jointly supported by North Carolina State University, North Carolina Wildlife Resources Commission, U.S. Geological Survey, U.S. Fish and Wildlife Service, and Wildlife Management Institute. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

REFERENCES

Allen, D. C., and C. C. Vaughn. 2010. Complex hydraulic and substrate variables limit freshwater mussel species richness and abundance. Journal of the North American Benthological Society 29: 383-394.

Annear, T., Chisholm, I., Beecher, H., Locke, A., Aarrestad, P., Coomer, C., Estes, C., Hunt, J., Jacobson, R., Jobsis, G., Kauffman, J., Marshall, J., Mayes, K., Smith, G., Wentworth, R., and C. Stalnaker. 2004. Instream flows for riverine resource stewardship, revised edition. Instream Flow Council: Cheyenne, Wyoming.

Bain, M. B., and N. J. Stevenson, editors. 1999. Aquatic habitat assessment: common methods. American Fisheries Society, Bethesda, Maryland.

Bovee, K. D. 1986. Development and evaluation of habitat suitability criteria for use in the instream flow incremental methodology. U.S. Fish and Wildlife Service, Washington, D. C.

Bovee, K.D., and R. Milhous. 1978. Hydraulic simulation in instream flow studies: Theory and techniques. U.S. Fish and Wildlife Service, Washington, D. C.

Brim Box, J., Dorazio, R. M., and W. D. Liddell. 2002. Relationships between streambed substrate characteristics and freshwater mussels (Bivalvia: Unionidae) in Coastal Plain streams. Journal of the North American Benthological Society 21: 253-260.

Brim Box, J., and J. Mossa. 1999. Sediment, land use, and freshwater mussels: prospects and problems. Journal of the North American Benthological Society 18: 99-117.

Diamond, J. M., Bressler, D. W., and V. B. Serveiss. 2002. Assessing relationships between human land uses and the decline of native mussels, fish, and macroinvertebrates in the Clinch and Powell River watershed, USA. Environmental Toxicology and Chemistry 21:1147-1155.

Downing, J. A., van Leeuwen, H., and L. A. DiPaulo. 2000. Substratum patch selection in the lacustrine mussels *Elliptio complanata* and *Pyganodon grandis grandis*. Freshwater Biology 44: 641-648.

Dudgeon, D., Arthington, A.H., Gessner, M. O., Kawabata, Z., Knowler, D. J., Leveque, C., Naiman. R. J., Preiur-Richard, A., Soto, D., Stiassny, M. L. J., and C. A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81: 163-182.

Elith, J., and J. R. Leathwick. 2009. Species distribution models: ecological explanation and prediction across space and time. Annual Review of Ecology, Evolution, and Systematics 40: 677-697.

Fisk, J. M. II, Kwak, T. J., and R. J. Heise. 2014. Modelling riverine habitat for robust redhorse: assessment for reintroduction of an imperilled species. Fisheries Management and Ecology 21: 57-67.

Fisk, J. M. II, Kwak, T. J., and R. J. Heise. 2015. Effects of regulated river flows on habitat suitability for the robust redhorse. Transactions of the American Fisheries Society 144: 792-806.

Gangloff, M. M., and J. W. Feminella. 2007. Stream channel geomorphology influences mussel abundance in southern Appalachian streams, U.S.A. Freshwater Biology 52: 64-74.

Geist, J., and K. Auerswald. 2007. Physicochemical stream bed characteristics and recruitment of the freshwater pearl mussel (*Margaritifera margaritifera*). Freshwater Biology 52: 2299-2316.

Gillies, R. R., Brim Box, J., Symanzik, J., and E. J. Rodemaker. 2003. Effects of urbanization on the aquatic fauna of the Line Creek watershed, Atlanta-a satellite perspective. Remote Sensing of Environment 86:411-422.

Haag, W. R., and M. L. Warren Jr. 1998. Role of ecological factors and reproductive strategies in structuring freshwater mussel communities. Canadian Journal of Fisheries and Aquatic Sciences 55: 297-306.

Hamilton, K., and P. C. Nelson. 1984. Habitat suitability index models and instream flow suitability index curves: white bass. U.S. Fish and Wildlife Service, Washington, D. C.

Hewitt, A. H., Kwak, T. J., Cope, W. G., and K. H. Pollock. 2009. Population density and instream habitat suitability of the endangered Cape Fear shiner. Transactions of the American Fisheries Society 138: 1439-1457.

Holland-Bartels, L. E. 1990. Physical factors and their influence on the mussel fauna of a main channel border habitat of the upper Mississippi River. Journal of the North American Benthological Society 9: 327-335.

Huehner, M. K. 1987. Field and laboratory determination of substrate preferences of unionid mussels. Ohio Journal of Science 87: 29-32.

Jelks, H. L., Walsh, S. J., Burkhead, N. M., Contreras-Balderas, S., Diaz-Pardo, E., Hendrickson, D. A., Lyons, J., Mandrak, N. E., McCormick, F., Nelson, J. S., Platania, S. P., Porter, B. A., Renaud, C. B., Schmitter-Soto, J. J., Taylor, E. B., and M. L. Warren Jr. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33: 372-407.

Johnson, D. H. 1980. The comparison of usage and availability measurements for evaluating resource preference. Ecology 61: 65-71.

Johnson, P. D., Bogan, A. E., Brown, K. M., Burkhead, N. M., Cordeiro, J. R., Garner, J. T., Hartfield, P. D., Lepitzki, D. A. W., Mackie, G. L., Pip, E., Tarpley, T. A., Tiemann, J. S., Whelan, N. V., and E. E. Strong. 2013. Conservation status of freshwater gastropods of Canada and the United States. Fisheries 38: 247-282.

Johnson, P. D., and K. M. Brown. 2000. The importance of microhabitat factors and habitat stability to the threatened Louisiana pearl shell, *Margaritifera hembeli* (Conrad). Canadian Journal of Zoology 78: 271-277.

Johnson, R. I. 1970. The systematics and zoogeography of the Unionidae (Mollusca: Bivalvia) of the southern Atlantic Slope region. Bulletin of the Museum of Comparative Zoology, Harvard University 140: 263-449.

Layzer, J. B., and L. M. Madison. 1995. Microhabitat use by freshwater mussels and recommendations for determining their instream flow needs. Regulated Rivers: Research and Management 10: 329-345.

Lydeard, C., Cowie, R. H., Ponder, W. F., Bogan, A. E., Bouchet, P., Clark, S. A., Cummings, K. S., Frest, T. J., Gargominy, O., Herbert, D. G., Hershler, R., Perez, K. E., Roth, B., Seddon, M., Strong, E. E., and F. G. Thompson. 2004. The global decline of nonmarine mollusks. BioScience 54: 321-330.

Master, L. L., Stein, B. A., Kutner, L. S., and G. A. Hammerson. 2000. Vanishing assets: Conservation status of U.S. species. Pages 93-118 *in* B. A. Stein, L. S. Kutner, and J. S. Adamas, editors. Precious heritage: the status of biodiversity in the United States. Oxford University Press, Oxford.

McRae, S. E., Allan, J. D., and J. B. Burch. 2004. Reach- and catchment-scale determinants of the distribution of freshwater mussels (Bivalvia: Unionidae) in south-eastern Michigan, USA. Freshwater Biology 49: 127-142.

Metcalfe-Smith, J. L, Di Maio, J., Stanton, S. K., and G. L. Mackie. 2000. Effect of sampling effort on the efficiency of the timed search method for sampling freshwater mussel communities. Journal of the North American Benthological Society 19: 725-732.

Michaelson, D. L., and R. J. Neves. 1995. Life history and habitat of the endangered dwarf wedgemussel *Alasmidonta heterodon* (Bivalvia: Unionidae). Journal of the North American Benthological Society 14: 324-340.

Midway, S. R., Kwak, T. J., and D. D. Aday. 2010. Habitat suitability of the Carolina madtom, an imperiled, endemic stream fish. Transactions of the American Fisheries Society 139: 325-338.

Morrison, M. L., Marcot, B. G., and R. W. Mannan. 1998. Wildlife-habitat relationships: concepts and applications 2nd edition. University of Wisconsin Press, Madison.

Neves, R. J., Bogan, A. E., Williams, J. D., Ahlstedt, S. A., and P. W. Hartfield. 1997. Status of aquatic mollusks in the southeastern United States: a downward spiral of diversity. Pages 43-85 *in* G. W. Benz and D. E. Collins, editors. Aquatic fauna in peril: the southeastern perspective. Southeastern Aquatic Research Institute, Decatur, Georgia.

Neves, R. J., and J. C. Widlak. 1987. Habitat ecology of juvenile freshwater mussels (Bivalvia: Unionidae) in a headwater stream in Virginia. American Malacological Bulletin 5: 1-7.

Pandolfo, T. J. 2014. Biotic and abiotic influences on common and imperiled freshwater mussels at multiple spatial and temporal scales with inferences to global change. Doctoral dissertation. North Carolina State University, Raleigh.

Paraciewicz, P., Castelli, E., Rogers, J. N., and E. Plunkett. 2012. Multiplex modeling of physical habitat for endangered freshwater mussels. Ecological Modelling 228: 66-75.

Quinn, J. W., and T. J. Kwak. 2000. Use of rehabilitated habitat by brown trout and rainbow trout in an Ozark tailwater river. North American Journal of Fisheries Management 20: 737-751.

Raleigh, R. F., Zuckerman, L. D., and P. C. Nelson. 1986. Habitat suitability index models and instream flow suitability curves: brown trout. U.S. Fish and Wildlife Service, Washington, D. C.

Ricciardi, A., and J. B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. Conservation Biology 13: 1220-1222.

Richter, B. D., Braun, D. P., Mendelson, M. A., and L. L. Master. 1997. Threats to imperiled freshwater fauna. Conservation Biology 11: 1081-1093.

Salmon, A., and R. H. Green. 1983. Environmental determinants of unionid clam distribution in the Middle Thames River, Ontario. Canadian Journal of Zoology 61: 832-838.

Schwalb, A. N., Morris, T. J., Mandrak N. E., and K. Cottenie. 2013. Distribution of unionid freshwater mussels depends on the distribution of host fishes on a regional scale. Diversity and Distributions 19:446-454.

Sekhon, J. S. 2011. Multivariate and propensity score matching software with automated balance optimization: The Matching Package for R. Journal of Statistical Software 42: 1-52.

Simon, T. P., and N. J. Cooper. 2014. Habitat suitability index relationships for the northern clearwater crayfish, *Orconectes Propinquus* (Decapoda: Cambaridae). Fisheries and Aquaculture Journal 5: 098. doi: 10.4172/2150-3508.100098

Simonson, T. D., Lyons, J., and P. D. Kanehl. 1994. Quantifying fish habitat in streams: transect spacing, sample size, and a proposed framework. North American Journal of Fisheries Management 14: 607-615.

Spooner, D. E., Xenopoulos, M. A., Schneider, C., and D. A. Woolnough. 2011. Coextirpation of host-affiliate relationships in rivers: the role of climate change, water withdrawal, and host-specificity. Global Change Biology 17: 1720-1732.

Strayer, D. L. 2008. Freshwater mussel ecology: a multifactor approach to distribution and abundance. University of California Press, Berkeley and Los Angeles.

Strayer, D. L., Downing, J. A., Haag, W. R., King, T. L., Layzer, J. B., Newton, T. J., and S. J. Nichols. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. BioScience 54: 429-439.

Strayer, D. L., Hunter, D. C., Smith, L. C., and C. K. Borg. 1994. Distribution,

abundance, and roles of freshwater clams (Bivalvia, Unionidae) in the freshwater tidal Hudson River. Freshwater Biology 31: 239-248.

Strayer, D. L., and J. Ralley. 1993. Microhabitat use by an assemblage of stream-dwelling unionaceans (Bivalvia), including two rare species of *Alasmidonta*. Journal of the North American Benthological Society 12: 247-258.

Strayer, D. L., and D. R. Smith. 2003. A Guide to Sampling Freshwater Mussel Populations. Monograph 8. American Fisheries Society, Bethesda, Maryland.

Taylor, C. A., Schuster, G. A., Cooper, J. E., DiStefano, R. J., Eversole, A. G., Hamr, P., Hobbs III, H. H., Robison, H. W., Skelton, C. E., and R. F. Thoma. 2007. A reassessment of the conservation status of crayfishes of the United States and Canada after 10+ years of increased awareness. Fisheries 32: 372-389.

Tevesz, M. J. S, and P. L. McCall. 1979. Evolution of substratum preference in bivalves (Mollusca). Journal of Paleontology 53: 112-120.

USFWS (U.S. Fish and Wildlife Service). 1992. Revised Tar River Spinymussel Recovery Plan. U.S. Fish and Wildlife Service, Atlanta, Georgia.

Williams, J. D., Warren, M. L., Cummings, K. S., Harris, J. L., and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18(9): 6-22.

Yao, W., Rutschmann, P., and Sudeep. 2015. Three high flow experiment releases from Glen Canyon Dam on rainbow trout and flannelmouth sucker habitat in Colorado River. Ecological Engineering 75: 278-290.

Zigler, S. J., Newton, T. J., Steuer, J. J., Bartsch, M. R., and J. S. Sauer. 2008. Importance of physical and hydraulic characteristics to unionid mussels: a retrospective analysis in a reach of a large river. Hydrobiologia 598: 343-360.

Appendices

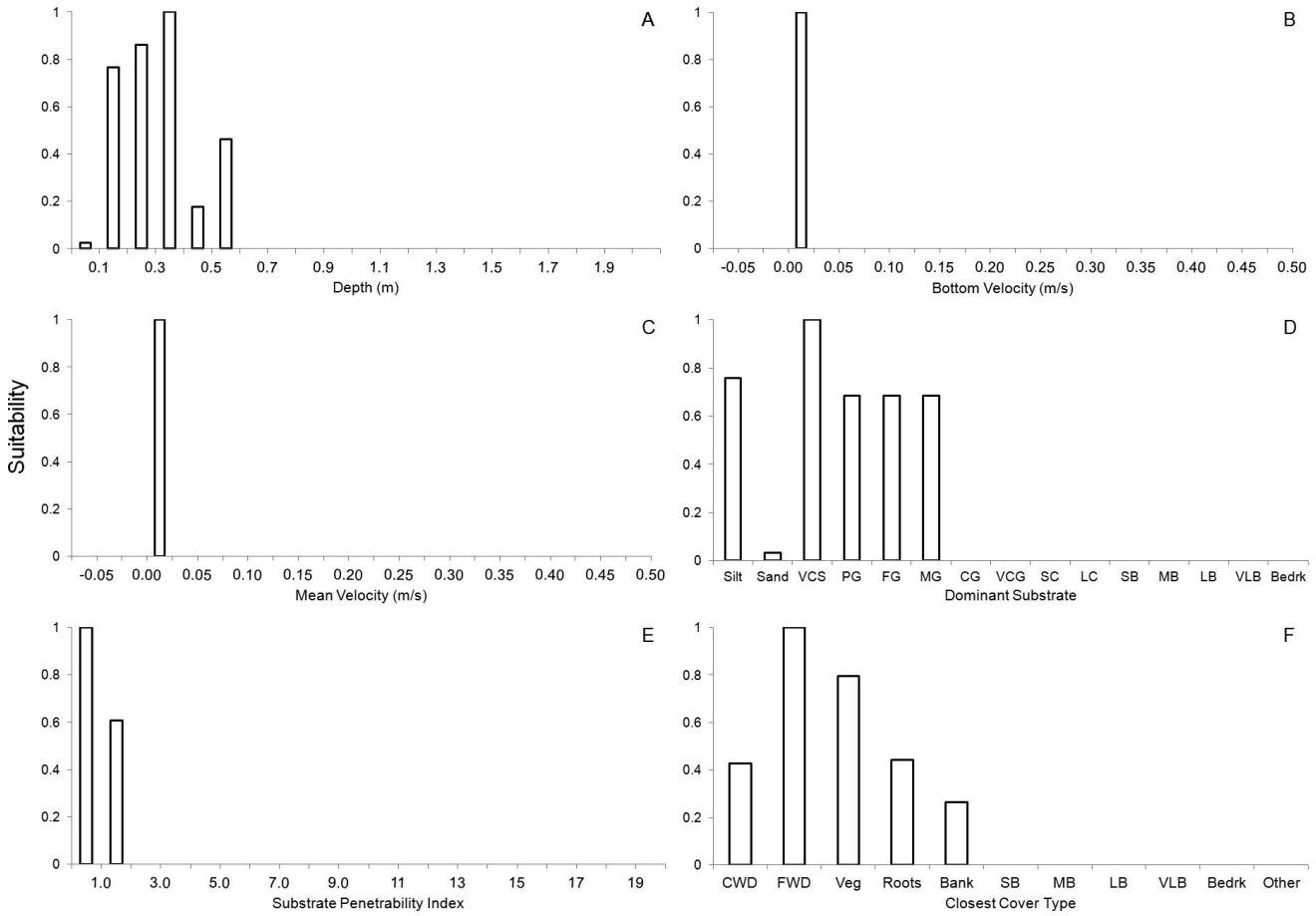

Figure A1. Microhabitat suitability distributions for *Alasmidonta heterodon*. Suitability for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

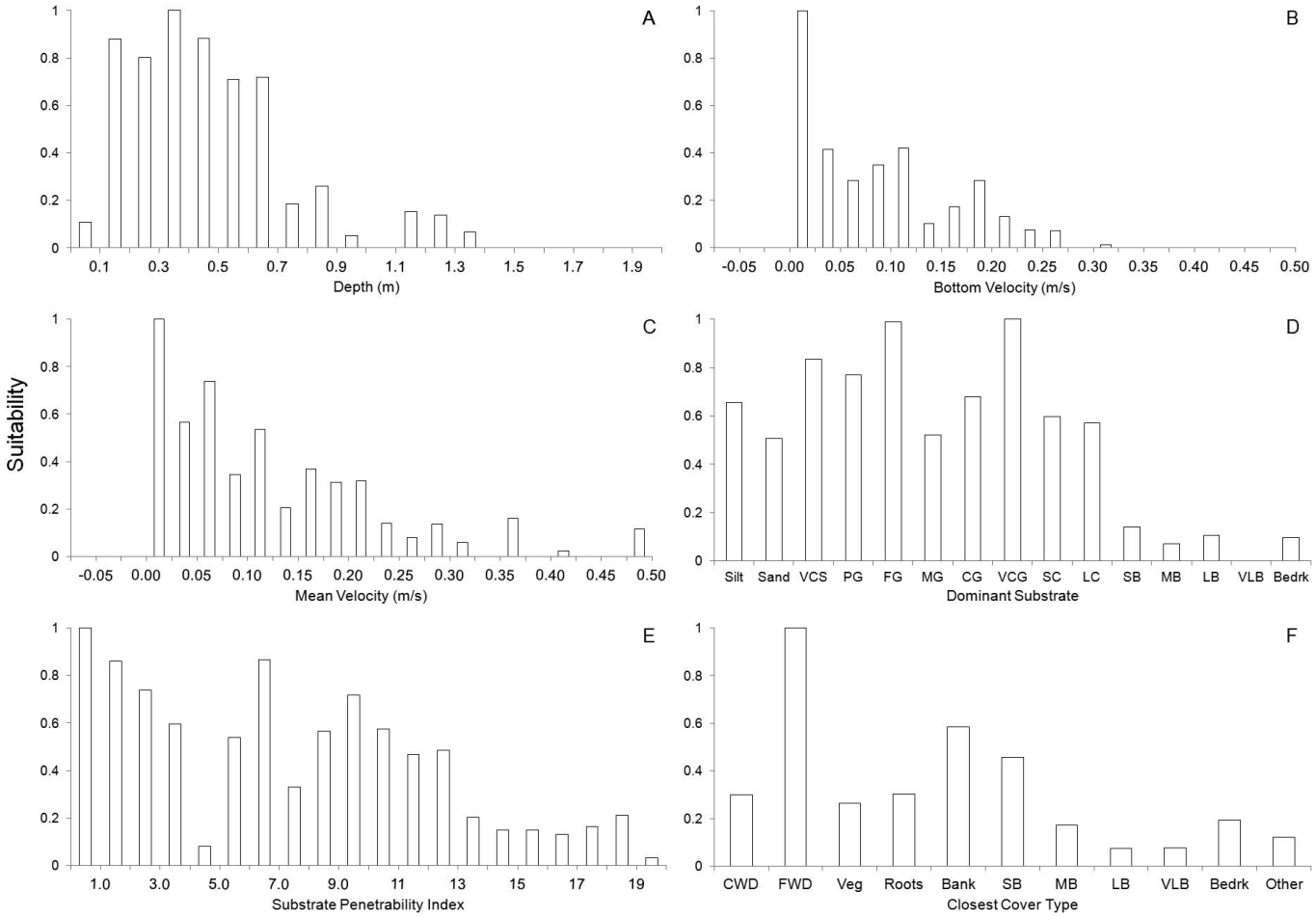

Figure A2. Microhabitat suitability distributions for *Elliptio complanata*. Suitability for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

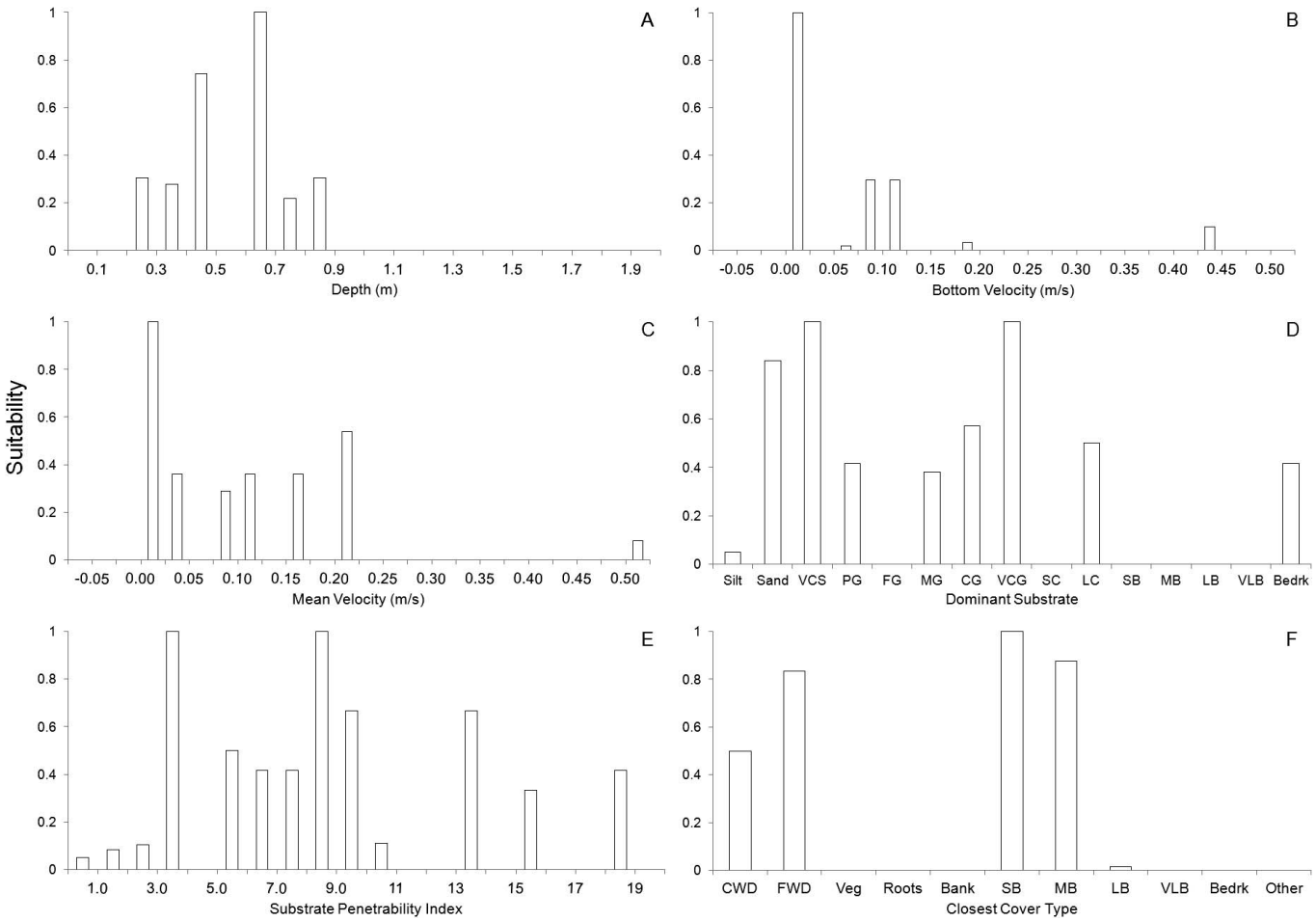

Figure A3. Microhabitat suitability distributions for *Elliptio congaraea*. Suitability for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

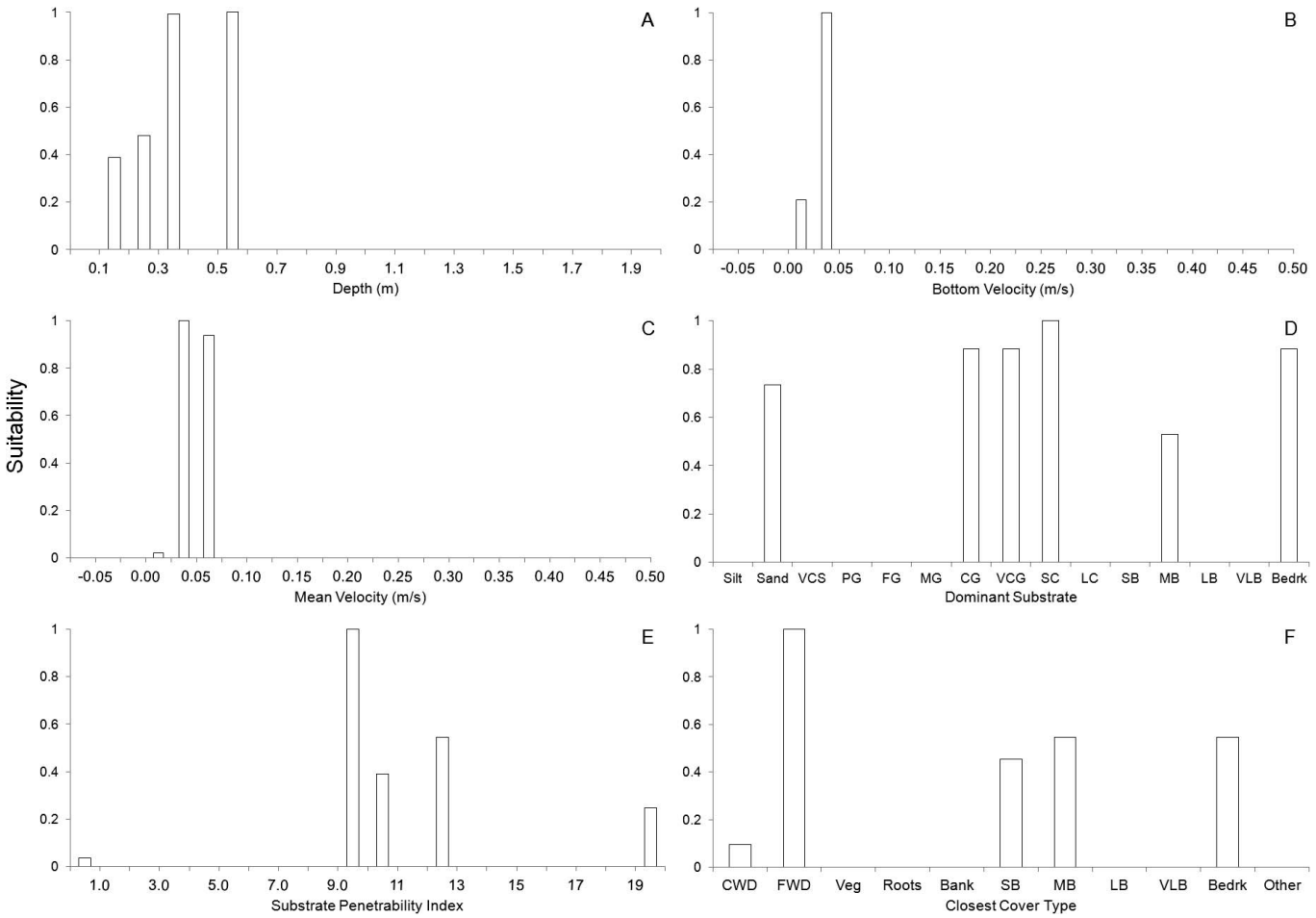

Figure A4. Microhabitat suitability distributions for *Elliptio fisheriana*. Suitability for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

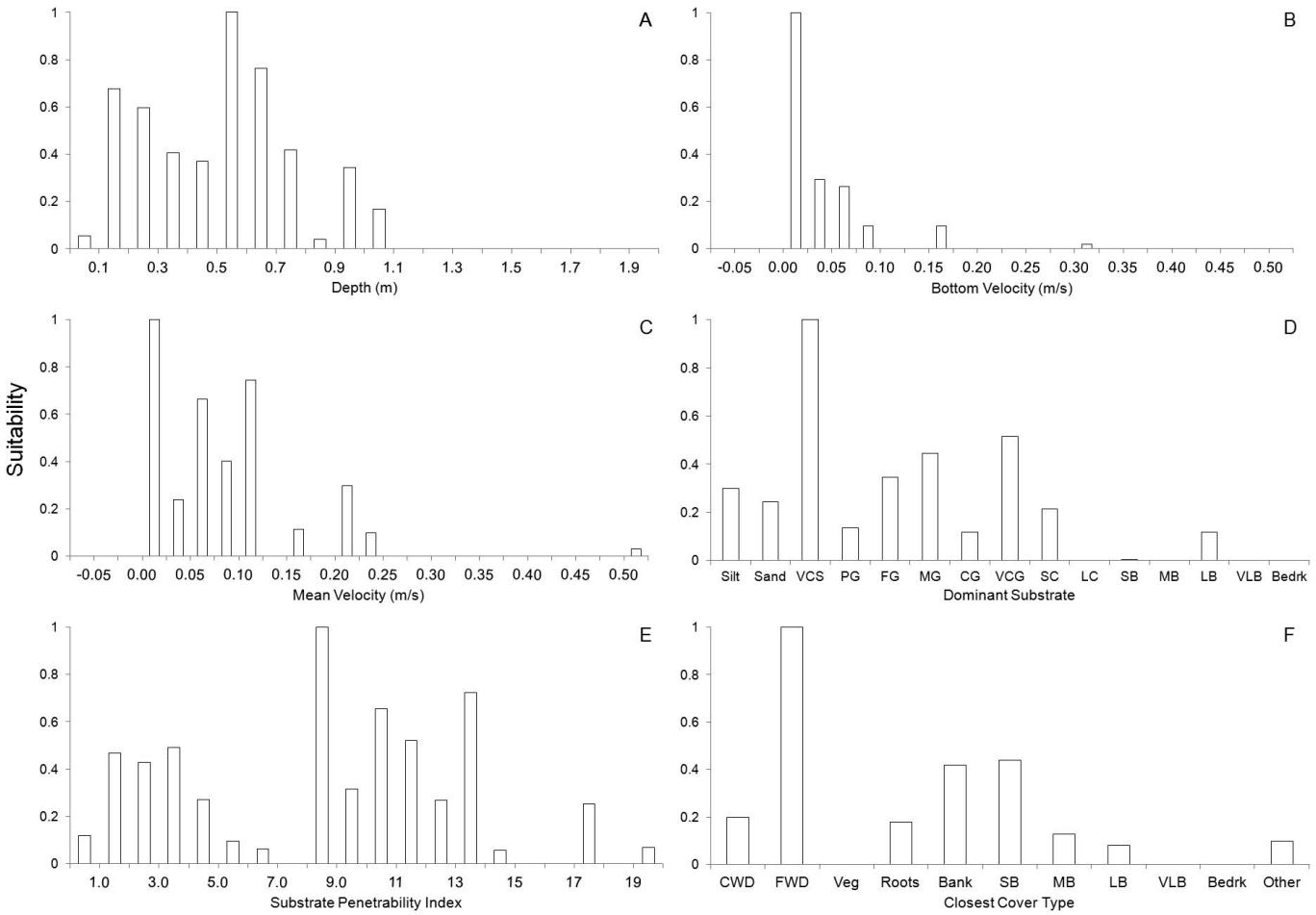

Figure A5. Microhabitat suitability distributions for *Elliptio icterina*. Suitability for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

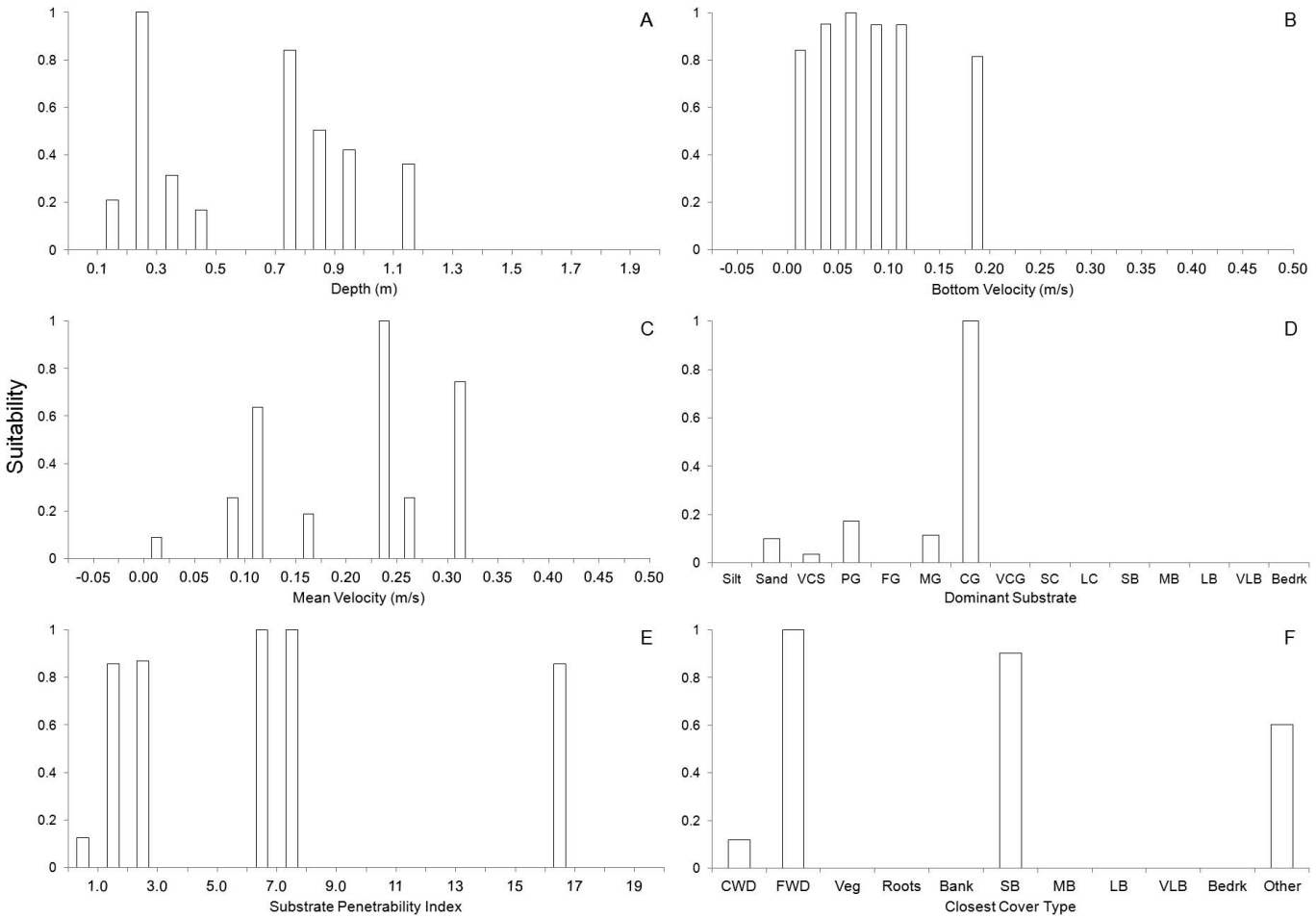

Figure A6. Microhabitat suitability distributions for *Elliptio roanokensis*. Suitability for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

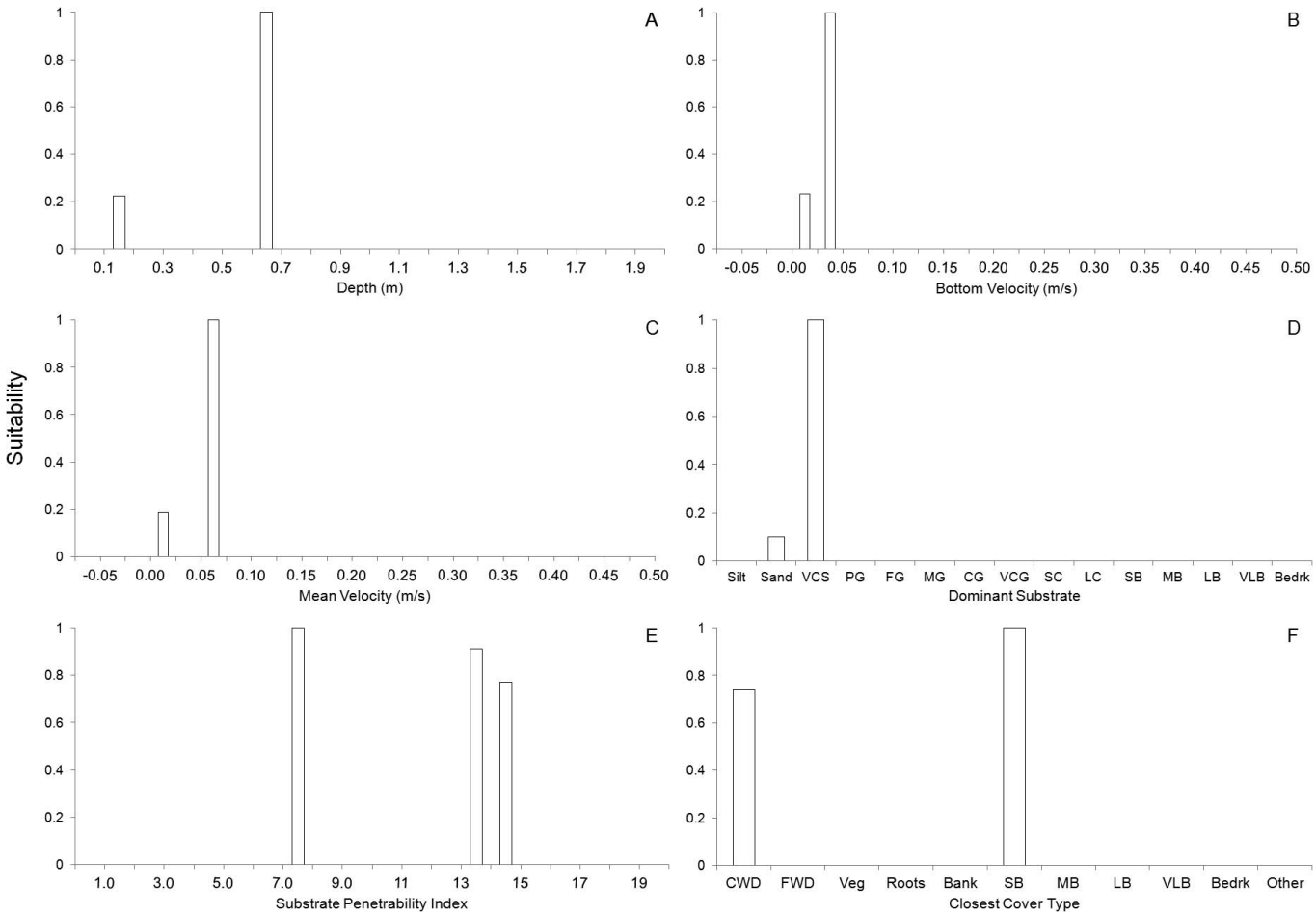

Figure A7. Microhabitat suitability distributions for *Elliptio steinstansana*. Suitability for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

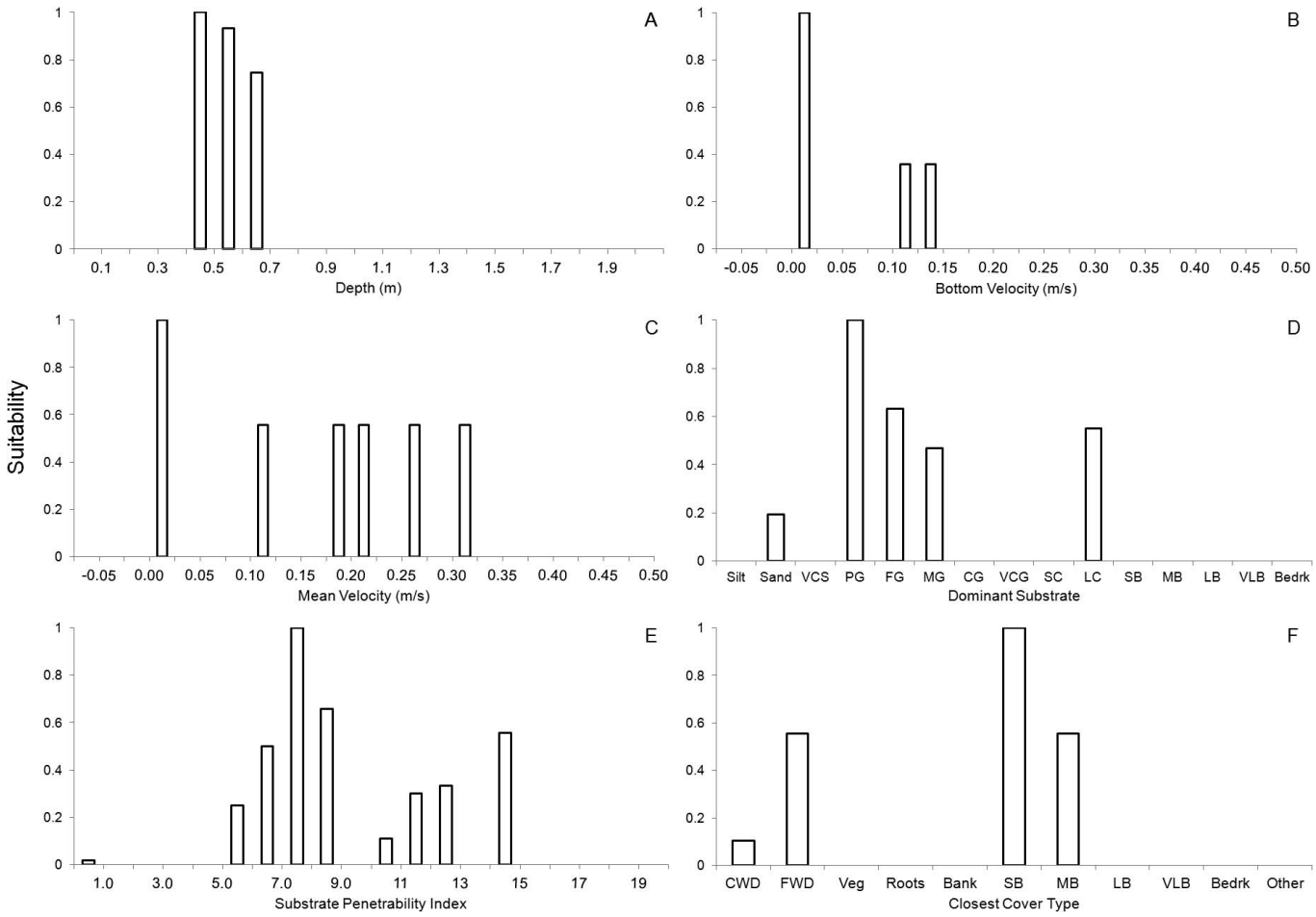

Figure A8. Microhabitat suitability distributions for *Fusconaia masoni*. Suitability for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

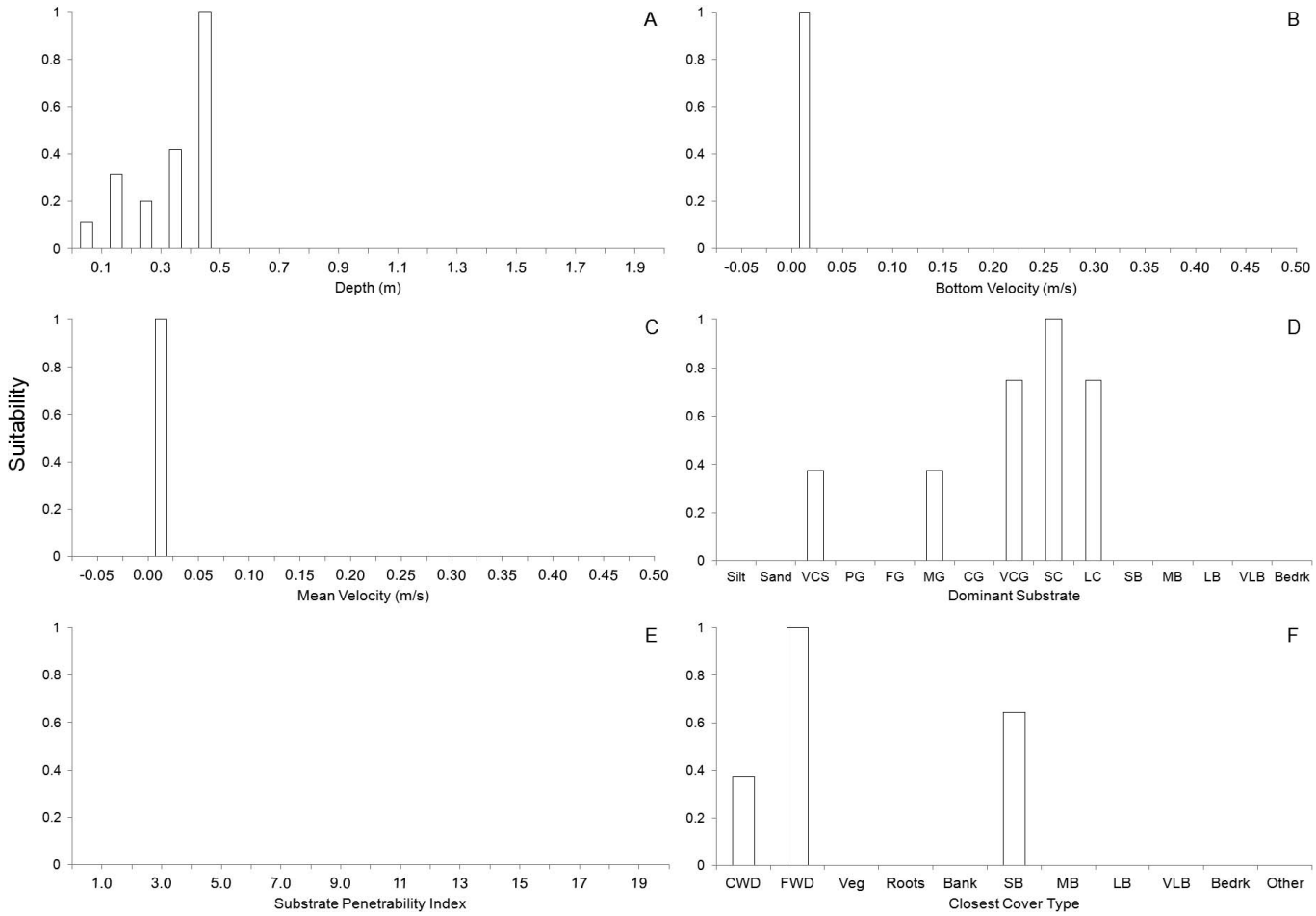

Figure A9. Microhabitat suitability distributions for an undescribed *Lampsilis*. Suitability for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

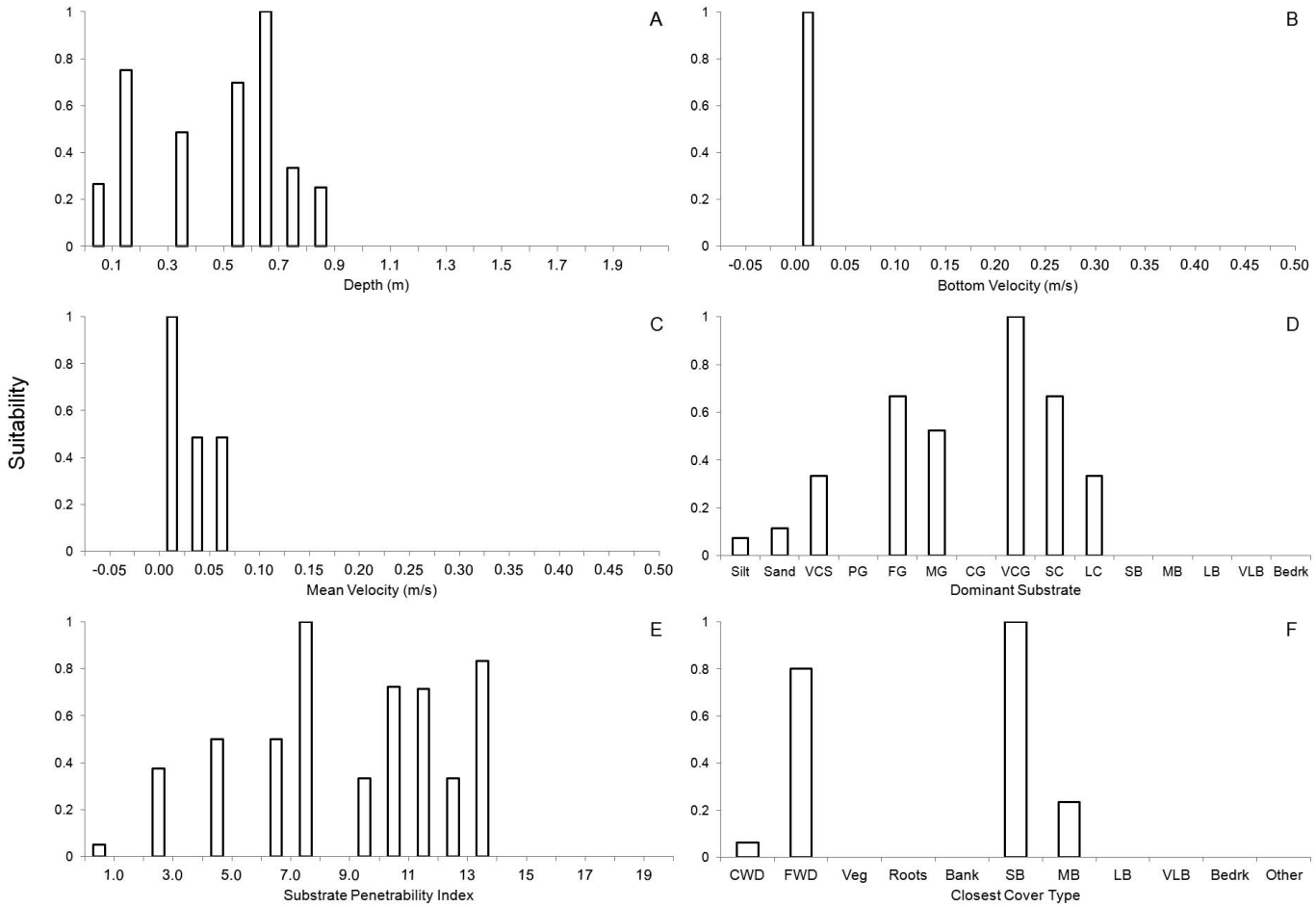

Figure A10. Microhabitat suitability distributions for *Villosa constricta*. Suitability for depth (A), bottom velocity (B), mean velocity (C), dominant substrate (D), substrate penetrability index (E), and cover type (F) are graphed from 0 (least suitable) to 1 (most suitable).

Freshwater Mollusk Biology and Conservation 19:51–55, 2016

REGULAR ARTICLE

USE OF SIDE-SCAN SONAR TO LOCATE *TULOTOMA MAGNIFICA* (CONRAD, 1834) (GASTROPODA: VIVIPARIDAE) IN THE ALABAMA RIVER

Jeffrey T. Garner[1*], Michael L. Buntin[2], Todd B. Fobian[2], Jesse T. Holifield[2], Thomas A. Tarpley[2], and Paul D. Johnson[2]

[1] *Alabama Division of Wildlife and Freshwater Fisheries, 350 Co. Road 275, Florence, AL 35633 USA*
[2] *Alabama Division of Wildlife and Freshwater Fisheries, 2200 Highway 175, Marion, AL 36756 USA*

ABSTRACT

***Tulotoma magnifica* is a federally threatened freshwater gastropod endemic to the Mobile Basin in Alabama. It was considered extirpated from the Alabama River until its rediscovery there in 2006. *Tulotoma* occurs primarily in colonies in large interstitial spaces beneath boulders and in bedrock crevices. We used side-scan sonar to identify boulder habitat in the Alabama River and to focus dive surveys at those sites. Eighty-five sites with potential *Tulotoma* habitat were identified with sonar and assessed by a diver. Colonies were found at five locations, three of which were previously unknown. Side-scan sonar greatly increased efficiency during this survey and was a useful tool.**

KEY WORDS - *Tulotoma*, snail, Mobile River Basin

INTRODUCTION

Tulotoma magnifica (Tulotoma) (Conrad, 1834) comprises a monotypic genus within the Viviparidae and is endemic to the Mobile Basin of Alabama. It was considered extinct until its rediscovery in 1988 (Hershler et al. 1990). *Tulotoma* was listed as endangered under the U.S. Endangered Species Act in 1991 but was reclassified as threatened in 2011 based on improvements in a large Coosa River (Jordan Dam tailwater) population and discovery of several smaller, previously unknown populations (USFWS 2011). The snail is relatively large, up to 50 mm in height, and has a distinctive, moderately heavy shell usually adorned with variable, spirally arranged nodules. Similar to other viviparids, *Tulotoma* is ovoviviparous, retaining eggs in a chamber of the mantle cavity until they hatch (Johnson 2004; Johnson et al. 2013). *Tulotoma* is generally found in colonies under large rocks or in bedrock crevices in flowing water of large streams. Suitable habitat usually has a bottom roughness value greater than 2 (on a scale of 0-5), boulder density greater than 2/m^2, rocks of dissimilar sizes, and current velocity sufficient to prevent silt accumulation (Christman et al. 1997). In tributaries of the Coosa River, *Tulotoma* consistently used larger and taller rocks, but water depth, current speed, and abundance of co-occurring gastropod species were not significantly related to *Tulotoma* occurrence (DeVries et al. 2003).

The type locality for *T. magnifica* is the Alabama River at Claiborne, Monroe Co., Alabama, but *Tulotoma* is reported historically from only one other Alabama River site (Hershler et al. 1990). It formerly was considered restricted to the main channels of the Coosa and Alabama rivers and it was thought that impoundment and channelization of these rivers in the 20th century drove the species to extinction (Stein 1976). However, populations were discovered subsequently in the lower, unimpounded reaches of some larger Coosa River tributaries, as well as in a short free-flowing reach of the Coosa River downstream of Jordan Dam (Hershler et al. 1990). A small *Tulotoma* population was discovered in 2006 in the Alabama River downstream of Claiborne Dam, near the type locality, and a larger population was discovered in 2008 near Selma (J. T. Garner, unpublished data). Another small population was discovered in 2008 downstream of Millers Ferry Dam (J. Powell, US Fish and Wildlife Service, personal communication).

*Corresponding Author: bleufer@aol.com

The Alabama and Coosa rivers flow through different physiographic provinces and provide different stream habitats. The middle and lower reaches of the Coosa River where *Tulotoma* was widespread historically flow through the Valley and Ridge and Piedmont physiographic provinces to the Fall Line. Streams in these regions have relatively high gradients with frequent bedrock and boulder substrates. Just downstream of the Fall Line, the Coosa and Tallapoosa rivers join to form the Alabama River, which flows across the East Gulf Coastal Plain physiographic province. Streams in the Coastal Plain have lower gradients and substrates of unconsolidated and finer sediments with only localized outcroppings of bedrock and associated cobble and boulders.

We conducted a comprehensive survey of the Alabama River in 2010 to better understand the current distribution of *Tulotoma*. We used side-scan sonar to locate suitable boulder habitat and sampled these habitats by diving. Our study appears to be the first to use this technique for detecting a specific freshwater snail species.

METHODS

The study section included 388 km of the Alabama River, extending from Alabama River km (ARK) 38 upstream to the river's origin at the confluence of the Coosa and Tallapoosa rivers (ARK 491, Figure 1). Within this section, we conducted surveys only in riverine reaches downstream of Robert F. Henry, Millers Ferry, and Claiborne dams, as well as the area downstream of the confluence of the Coosa and Tallapoosa rivers. Areas of slack water and extensive sediment deposition immediately upstream of dams were assumed to provide unsuitable habitat for *Tulotoma* and were not surveyed. These areas generally have interstitial spaces underneath boulders filled with sediment, leaving no space for *Tulotoma*. The lower 38 km section of the Alabama River is considered outside of the historical range of *Tulotoma* and previous surveys indicated this section has little boulder habitat (J. T. Garner, unpublished data).

This study was carried out from 5 August through 3 November 2010. We systematically surveyed the study section with a Humminbird 1197c side-scan sonar unit (Johnson Outdoors Marine Electronics, Inc., Eufaula, AL) mounted on a flat-bottomed jon boat. The sonar unit was set to scan 60 m on each side of the boat resulting in a total coverage of 120 m perpendicular to the path of the boat. We made a single pass through each reach by steering a downstream course about 45 m from and parallel to the river bank at an average speed of 8.8 km/h (range: 7-10 km/h). The river is wider than 120 m throughout its length, and time did not allow multiple passes necessary to cover the entire river bottom. However, based on our prior experience on the river (20 years, including 191 h of diving bottom time, J. T. Garner), exposed boulders occur primarily on the outer bank of river bends or other areas where bank scour occurs. We focused our surveys on these areas and did not survey the inner bank or other depositional areas. In straight river reaches, we chose a course based on bank features (e.g., rocks, bluff banks) that suggested the presence of suitable habitat, again based on previous experience. Areas of potential habitat located by sonar were marked with flagging tape on the adjacent bank or an anchored buoy. The sonar boat relayed to a separate dive boat specific site information such as water depth, distance from the bank, habitat area, and irregular bottom features.

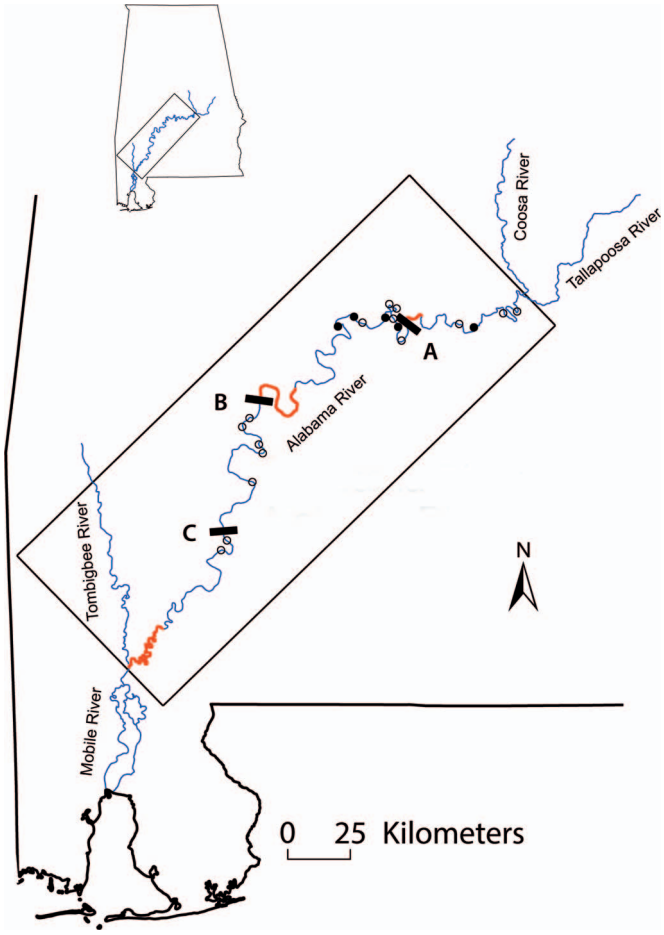

Figure 1. Map of the Alabama River showing location of potentially suitable habitat for *Tulotoma* identified by side-scan sonar. Closed circles indicate sites where *Tulotoma* was found by divers, and open circles indicate sites with apparently suitable habitat but where *Tulotoma* was not found. Red reaches are those that were not surveyed, and dams are represented as solid black lines (A – Robert F. Henry Dam, B – Millers Ferry Dam, C – Claiborne Dam). Inset map shows the location of the Alabama River in Alabama. Note that a portion of the Tombigbee River lies within the study area box, but it was not surveyed.

All sites having boulder substrate identified by sonar (Figure 2) were examined by a diver working from an anchored boat equipped with surface-supplied air. All dives were performed by J.T. Garner, and searches were visual or by touch, depending on visibility, with emphasis on areas underneath boulders. Searches were carried out within the radius of the 30 m air line connecting the diver to the boat. Dive duration was not standardized and depended on the amount of suitable habitat present and physical characteristics

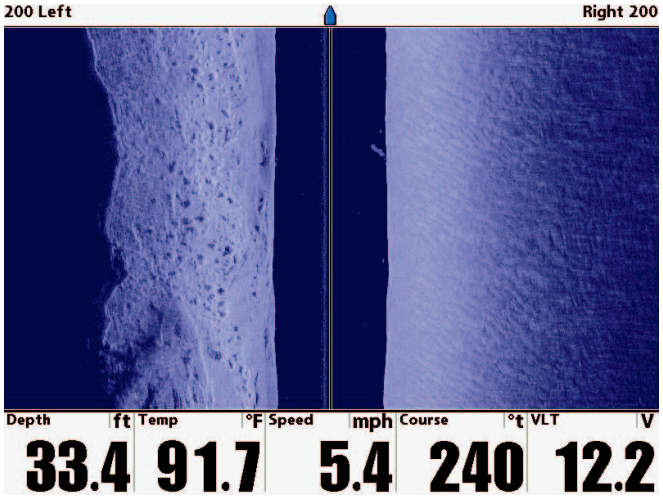

Figure 2. Side-scan sonar screen view of Alabama River substrate. The left side of the image shows boulder habitat along the channel slope potentially suitable for *Tulotoma*. The dark vertical column in the center of the image represents the water column. The right side of the image shows unstable sand substrate where *Tulotoma* are unlikely to be present.

of the site. For example, at some sites boulders were embedded in the sediment and provided no interstitial spaces for *Tulotoma*, which shortened dive times.

Dives were terminated when the diver had searched all suitable habitat within the 30 m radius, when it became evident that habitat was unsuitable, or when *Tulotoma* was encountered. If *Tulotoma* was encountered we made a brief assessment of the relative size and age structure of the population but terminated the dive shortly thereafter to limit habitat disturbance and potential mortality. The diver collected all snails encountered during a dive and placed them in a mesh bag, with the exception of *Elimia* spp. and *Pleurocera prasinata*, which were too numerous to collect at some sites but were easily distinguished from *Tulotoma* even with little or no visibility. Snails were brought to the surface for identification and all *Tulotoma* were measured, photographed, and released. Vouchers of all unprotected taxa were retained.

To determine if efficiency was increased by using sonar, we recorded effort expended by the sonar study (as person-days) and compared this with the estimated effort necessary to carry out the survey without sonar. For this estimation, sites potentially having suitable boulder substrate were identified from Alabama River aerial photographs based on our previous experience on the river. Likely sites included river bends and straight reaches of at least two kilometers. Without the benefit of sonar, we assumed that an average of four dives/site (using a two-person crew) would be necessary to detect *Tulotoma*, and five dives/day could be completed (the average number completed during the sonar study). As such, the total number of person-days required for surveying the study section without sonar was estimated as (number of likely sites x 4 x 2)/5. Total effort for the sonar method included effort of the sonar team and the dive team.

Table 1. Alabama River localities with apparent suitable *Tulotoma* habitat.

River km	River reach	Latitude	Longitude
Sites where *Tulotoma* was found			
433.2	Robert F. Henry Dam pool	32.34498°	−86.49345°
372.6	Millers Ferry Dam pool	32.34170°	−86.81513°
348.7	Millers Ferry Dam pool	32.38513°	−86.86703°
330.1	Millers Ferry Dam pool	32.40365°	−87.02458°
318.6	Millers Ferry Dam pool	32.36907°	−87.04978°
Sites where no *Tulotoma* was found			
102.5	Claiborne Dam tailwaters	31.54770°	−87.57605°
113.5	Claiborne Dam tailwaters	31.59195°	−87.54173°
145.2	Claiborne Dam pool	31.79082°	−87.42068°
170.3	Claiborne Dam pool	31.93500°	−87.47857°
181.2	Claiborne Dam pool	31.90915°	−87.38153°
186.7	Claiborne Dam pool	31.94810°	−87.39798°
199.1	Claiborne Dam pool	32.00785°	−87.47445°
205.2	Claiborne Dam pool	32.03095°	−87.43080°
334.6	Millers Ferry Dam pool	32.38178°	−86.99313°
360.5	Millers Ferry Dam pool	32.42667°	−86.82567°
361.6	Millers Ferry Dam pool	32.42093°	−86.83343°
362.6	Millers Ferry Dam pool	32.42047°	−86.83782°
364.5	Millers Ferry Dam pool	32.40682°	−86.84618°
365.3	Millers Ferry Dam pool	32.39433°	−86.83777°
367.7	Millers Ferry Dam pool	32.37555°	−86.82183°
376.6	Millers Ferry Dam pool	32.30872°	−86.81325°
427.4	Robert F. Henry Dam pool	32.35688°	−86.54065°
458.7	Robert F. Henry Dam pool	32.39465°	−86.35020°
474.6	Robert F. Henry Dam pool	32.41657°	−86.31742°

RESULTS

Eighty-five Alabama River sites with boulder habitat were identified using side-scan sonar and assessed for the presence of *Tulotoma* by the diver. Overall, dives averaged 30 min in duration, with a range of 11–93 min, depending primarily on habitat suitability and whether and how quickly *Tulotoma* was encountered. Dives at sites that provided little *Tulotoma* habitat (N = 58) averaged 27 min in duration, dives in apparent good habitat, but during which no *Tulotoma* was encountered (N = 19) averaged 41 min in duration. Because dives were halted soon after *Tulotoma* was found, dives at those sites (N = 5) were also of relatively short duration, averaging 29 min.

Tulotoma was found at five sites (Table 1) irregularly distributed in the upstream half of the study reach (Figure 1). These sites included the river reach adjacent to Selma (ARK 330.1), where *Tulotoma* was found previously. The Selma site appeared to support the largest population of all five sites. *Tulotoma* was locally abundant (some boulders harboring over 100 individuals). One of the other sites, ARK 372.6, was near another previously known occurrence (ARK 372.9, found September 2008). Previously unknown populations were discovered at ARK 433.2 (near the mouth of Pintlala Creek, Robert F. Henry Dam pool), ARK 348.7 (Cunningham Bluff, Millers Ferry Dam pool), and ARK 318.6 (Millers Ferry Dam

pool, upstream of the mouth of Cahaba River). The number of *Tulotoma* encountered varied at the other sites, but only a single individual was encountered at ARK 348.7. All sites except ARK 348.7 harbored a wide range of size classes from juveniles to adults.

Our sonar survey required a total of 64 person-days to perform, including both the sonar and dive teams. Both teams required two workers each, the dive team with a diver and tender and the sonar team with a boat driver and sonar operator. A total of 14 d (28 person-days) was required to complete the sonar survey (average = 25 km of river surveyed/day), and 18 d (36 person-days) were needed by the dive team to examine all potential sites identified by the sonar team (average = five dives/day). We estimated that the total effort required to survey the same river section without sonar was 280 person-days. This estimate included 175 sites identified from aerial photographs as potentially providing suitable habitat.

DISCUSSION

Tulotoma has long been known to occur almost exclusively under large rocks and in bedrock crevices. Side-scan sonar proved a valuable tool for locating boulder habitat and greatly improved sampling efficiency. We were able to complete our survey in about 20% of the time we estimated would be required to survey the same river section without the use of sonar. Previous scientific uses of side-scan sonar in freshwater lakes and rivers include habitat assessments, sediment studies, and surveys of fish, unionids, and Zebra Mussels (Duncan and Kubecka 1996; Haltuch and Berkman 2000; Woodruff et al. 2001; Kaeser and Litts 2008, 2010; Gonzalez-Socoloske et al. 2009; Powell et al. 2015). We found no previous studies that used sonar for a survey of a freshwater gastropod.

Our observations provided additional detail about the habitat preference of *Tulotoma*. *Tulotoma* occurred exclusively under boulders composed of dense, hard rock and never under brittle siltstone; siltstone boulders were common at some sites and often fell apart when overturned. The amount of interstitial space and sediment underneath the boulders also appeared to be important factors for the occurrence of *Tulotoma*. Boulders that were embedded or had interstitial spaces choked with sediment (generally sand) held no *Tulotoma*. Boulders lying on bedrock or over other boulders often had larger interstitial spaces than those lying on gravel or sand, but these large spaces were sometimes kept free of silt by currents. No *Tulotoma* were found in these habitats, suggesting that at least some silt is necessary for colonization by the species.

Tulotoma was not encountered at 80 of the sites assessed. However, habitat at some of these sites appeared suitable, and most were in upper reaches of Millers Ferry Dam pool, which harbors four of the seven known *Tulotoma* populations in the Alabama River. It is possible that small populations of *Tulotoma* exist at some of these sites, but their detection would require more dive time than could be expended at any one site in this study.

Tulotoma was not encountered during this survey at two sites where it was found previously. In 2006, a small colony of *Tulotoma* was found in Claiborne Dam tailwaters at ARK 113.5 (J. T. Garner, unpublished data). At that time, only seven individuals were observed, ranging in size from 4 to 22 mm in shell height, during a total of 7 h, 35 min bottom time over 3 d. In 2008, six individuals were encountered in the upper reaches of Claiborne Dam Pool (J. R. Powell, personal communication). Again, detecting these small populations would require considerable dive time, which was not feasible in our study because of the large number of sites we surveyed.

We discovered three previously unknown populations of *Tulotoma* in the Alabama River and confirmed the persistence of two previously known populations. The population we found at ARK 372.6 probably is contiguous with a population previously found at ARK 372.9. At least one of these populations (ARK 330.1) appears to be large, but we were unable to conduct thorough population assessments at any site. However, evidence of recent recruitment was evident at all but one site. These findings support the recent downlisting of this species from endangered to threatened (USFWS 2011). More focused surveys of known populations or other potentially suitable sites are needed to assess population size and extent. Side-scan sonar was a valuable tool in our survey and can increase the efficiency of future efforts.

ACKNOWLEDGEMENTS

This survey was conducted using American Recovery and Restoration Act funds from the U.S. Army Corps of Engineers. We thank Wade Taylor and Bill Stark (ADCNR) for field assistance, Paul Hartfield (USFWS) for advice, Jeff Powell (USFWS) for help in securing funds, Adam Kaeser for training with the side-scan sonar, and Charlotte LaGrone and Stacy Norris (ADCNR) for administrative guidance. The editor, Wendell Haag, and two anonymous reviewers greatly improved this manuscript.

LITERATURE CITED

Christman, S. P., E. L. Mihalcik, and F. G. Thompson. 1997. *Tulotoma magnifica* (Conrad, 1834) (Gastropoda: Viviparidae) population status and biology in the Coosa River, Alabama. Malacological Review 29:17-63.

De Vries, D. R., D. L. Armstrong, Jr., M. Topolski, W. E. Pine, III, J. A. Johnson, R. A. Dunham, L. Robison, J. DiBona, K. Norgren, P. Hartfield, and S. Cook. 2003. Distribution, habitat use, and genetics of *Tulotoma magnifica*. Southeastern Naturalist 2:35-58.

Duncan, A., and J. Kubecka. 1996. Patchiness of longitudinal fish distributions in a river as revealed by a continuous hydroacoustic survey. ICES Journal of Marine Science 53:161-165.

Gonzalez-Socoloske, D., L. D. Olivera-Gomez, and R. E. Ford. 2009. Detection of free-ranging West Indian manatees *Trichechus manatus* using side-scan sonar. Endangered Species Research 8:249-257.

Haltuch, M. A., and P. A. Berkman. 2000. Geographic information systems (GIS) analysis of ecosystem invasion: Exotic mussels in Lake Erie. Limnology and Oceanography 45:1778-1787.

Hershler, R., J. M. Pierson, and R. S. Krotzer. 1990. Rediscovery of *Tulotoma magnifica* (Conrad) (Gastropoda: Viviparidae). Proceedings of the Biological Society of Washington 103:815-824.

Johnson, P. D. 2004. *Tulotoma magnifica*. Page 120 *in*: R. E. Mirarchi, J. T. Garner, M. F. Mettee, and P. E. O'Neil (eds.). Alabama Wildlife. Volume 2: Imperiled aquatic mollusks and fishes. The University of Alabama Press, Tuscaloosa, Alabama. 255 pages.

Johnson, P. D., A. E. Bogan, K. M. Brown, N. M. Burkhead, J. R. Cordiero, J. T. Garner, P. D. Hartfield, D. A. W. Lepitzki, G. L. Mackie, E. Pip, T. A. Tarpley, J. S. Tiemann, N. V. Whelan, and E. E. Strong. 2013. Conservation status of freshwater gastropods of Canada and the United States. Fisheries 38:247-282.

Kaeser, A. J., and T. L. Litts. 2008. An assessment of deadhead logs and large woody debris using side scan sonar and field surveys in streams of southwest Georgia. Fisheries 33:589-597.

Kaeser, A. J., and T. L. Litts. 2010. A novel technique for mapping habitat in navigable streams using low-cost side scan sonar. Fisheries 35:163-174.

Powell, J., S. K. Brewer, J. M. Long, and T. Campbell. 2015. Evaluating the use of side-scan sonar for detecting freshwater mussel beds in turbid river environments. Hydrobiologia 743:127-137.

Stein, C. B. 1976. Gastropods. Pages 1-41 *in*: Boschung (ed.). Endangered and threatened plants and animals of Alabama. Bulletin Alabama Museum of Natural History, 2.

USFWS (U.S. Fish and Wildlife Service). 2011. Endangered and threatened wildlife and plants; reclassification of the Tulotoma Snail from endangered to threatened. Federal Register, 76:106(2 June 2011):31866-31874.

Woodruff, J. D., W. R. Geyer, C. K. Sommerfield, and N. W. Driscoll. 2001. Seasonal variation of sediment deposition in the Hudson River estuary. Marine Geology 179:105-119.

Freshwater Mollusk Biology and Conservation 19:56–68, 2016

REGULAR ARTICLE

GENETIC STRUCTURE OF FAUCET SNAIL, *BITHYNIA TENTACULATA* POPULATIONS IN NORTH AMERICA, BASED ON MICROSATELLITE MARKERS

Kathryn E. Perez[1*], Rebecca L. Werren[2], Christopher A. Lynum[3], Levi A. Hartman[2], Gabor Majoros[4], and Rebecca A. Cole[5]

[1] *Department of Biology, University of Texas Rio Grande Valley, 1201 W. University Dr., Edinburg, TX 78539 USA*
[2] *Department of Biology, University of Wisconsin-La Crosse, 1725 State St., La Crosse, WI 54601 USA*
[3] *Department of Biology, University of Massachusetts, Boston, 100 Morrissey Blvd, Boston, MA 02125 USA*
[4] *Department of Parasitology and Zoology, Faculty of Veterinary Science, Szent István University Budapest, P.O.B. 2. H-1400, Hungary*
[5] *United States Geological Survey, National Wildlife Health Center, 6006 Schroeder Rd, Madison, WI 53711 USA*

ABSTRACT

***Bithynia tentaculata* is believed to have been extirpated from North America during the last glacial maximum. It was reintroduced into North America via the Great Lakes basin in the 1800's and has recently been expanding its geographic range. This snail serves as intermediate host for three trematodes that cause extensive recurring morbidity and mortality events in migratory water birds along the Mississippi River. Using twelve microsatellite loci for ~200 individual snails from 11 populations in North America and Europe, we examined one of the three major geographic regions from which founding populations into the Great Lakes typically originate. Our data supports a single recolonization of North America into the Great Lakes Basin followed by subsequent introduction events from the Great Lakes to other large watersheds in North America. However, additional watersheds in Europe require sampling to confirm this result. No populations with genetic signatures indicative of North American glacial relics were found. The initial invasion of North America was likely not from the Ponto-Caspian basin, the usual source of freshwater invasive species to the Laurentian Great Lakes.**

KEYWORDS - faucet snail, phylogeography, invasive species, Mississippi River

INTRODUCTION

The Laurentian Great Lakes of North America have been a hotspot for invasion by exotic species. Many ecologically damaging aquatic invasive species have been introduced into the United States (U.S.) via this route (Mills et al. 1993). Molecular data have been used to determine the source of invasion of various aquatic invaders. For example, using the mitochondrial cytochrome oxidase I gene, Gelembiuk et al. (2006) concluded that the source of invasion of zebra (*Dreissena polymorpha*) and quagga mussels (*D. bugensis*) into the Great Lakes was the Ponto-Caspian Sea basin (the Black, Caspian, and Azov Seas and their surrounding watersheds). This is congruent with other studies that have shown that the Ponto-Caspian Sea basin has been an important source of many aquatic invaders into the Great Lakes (Lee & Bell 1999, Ricciardi & MacIsaac 2000). Up to 70% of recent invaders in the Great Lakes (1985-2000) trace their source

*Corresponding Author: perezke@gmail.com

population to this region (Ricciardi & MacIsaac 2000, Brown & Stepien 2009, Keller et al. 2010). However, this is not the only possible source of aquatic invasive species. Shipping routes from European waters to the Great Lakes commonly originate in three locations: (1) the Black/Mediterranean Seas, (2) the North Sea, (3) or the Baltic Sea (Ricciardi & MacIsaac 2000, Grigorovich et al. 2003, Brown & Stepien 2009). These usual source regions for invaders into the Great Lakes provide an excellent starting point for comparing the genetic structure of invasive and potential source populations.

Bithynia tentaculata (*L*., 1758) had a Holarctic distribution prior to the last glacial maximum with shells found in Pleistocene fossil deposits from Lake Michigan, Illinois, U.S.A (Baker 1928). It is believed to have been extirpated from North America by glaciation events with subsequent recolonization through human-mediated introduction. Following the last glacial maximum, the first North American record of *B. tentaculata* was in Lake Michigan (presumably by passage through the Great Lakes Waterway, via the Hudson River) in 1871 (Baker 1928, Mills et al. 1993). It was speculated at that time that the snail was carried into Lake Michigan through ballast of timber ships arriving from Europe (Baker 1928). The species then spread throughout the Great Lakes region and into other U.S. waterways. It is now widespread in the Great Lakes, Northern Atlantic Coast drainages, isolated lakes in Montana, and most recently, in the Upper Mississippi River and Wolf River drainages, WI (Sauer et al. 2007)

Since the snail's introduction into the Mississippi River, first recorded in 2002 (National Wildlife Health Center, Madison, WI, unpublished data), parasites carried by *B. tentaculata* have caused recurring morbidity and mortality events in water bird populations during spring and fall migrations. The intestinal trematodes *Cyathocotyle bushiensis*, *Sphaeridiotrema globulus*, *S. pseudoglobulus* and *Leyogonimus polyoon* (Sauer et al. 2007, Mitchell & Cole 2008) cause intestinal hemorrhage and extensive mucosal damage. One snail can be infected with hundreds of infectious larval trematodes (Cole, unpublished data) and thus, by eating a small number of snails, a bird can receive a lethal infection in a short period of time (Sauer et al. 2007). From 2002-14 over 135,000 water birds consisting of 17 species have died in mortality events in Wisconsin, Minnesota and Illinois. These mortalities have been attributed to the four trematodes transmitted by *B. tentaculata*. The majority of these events have occurred in navigation pools (long stretches of river between dams) 7-11 of the Mississippi River and were predominately lesser scaup (*Aythya affinis*) and American coot (*Fulica americana*) (National Wildlife Health Center, Madison, WI, unpublished data).

Negative interactions between invasive species and native species are a leading cause of animal extinctions (Claver & Garcia-Berthou 2005) and freshwater gastropods are a highly threatened freshwater fauna with 74% of species categorized as imperiled or extinct (Johnson et al. 2013). In eutrophic lakes in upstate New York, *B. tentaculata* contributed to the decline of populations of the pleurocerids *Elimia livescens*, *E. virginica*, and *Pleurocera acuta* (Harman 1968, Harman 1968, Harman & Forney 1970, Jokinen 1992). The proposal that the ability of *B. tentaculata* to both graze and filter-feed contributed to their competitive ability was supported by a finding that *B. tentaculata* adds biomass approximately 10 times faster than pleurocerids (Harman & Forney 1970) due to the higher efficiency of carbon and nitrogen assimilation associated with filter-feeding (Tashiro & Colman 1982). While these pleurocerids are relatively common – this is indicative that this introduced species is a potential competitor with other native pleurocerids. Furthermore, an initial study indicated native snails could suffer negative consequences from *B. tentaculata* invasion, largely due to increased exposure to trematode parasite larvae transmitted at high densities of *B. tentaculata* (Sandland et al. 2013). A further study in an experimental setting found several native snail species and *B. tentaculata* were equally infected with the larval stage (metacercariae) of an echinostome parasite suggesting a potentially positive effect of the invasive snail on natives may occur by diluting the parasite load of the entire snail community (Gladosky & Sandland 2014); however, this does not consider the ability of *B. tentaculata* to form very high population densities, which would serve to enhance overall parasite abundance and pose a threat to native snails.

Understanding the history of invasion and parent populations of *B. tentaculata* may lead to precautionary steps to be implemented to limit the spread of this species. Use of microsatellite data can be helpful in understanding the routes of introduction and pinpointing parent populations (Stepien et al. 2005, Brown & Stepien 2009). In this study, we used microsatellite data to determine the colonization route of the invasive populations of *B. tentaculata* into and throughout North America. We distinguish between four alternative hypotheses of potential colonization routes: (1) a single population of *B. tentaculata* was introduced into the Great Lakes from a single source population and has since dispersed; (2) *Bithynia tentaculata* were introduced multiple times into the Great Lakes from multiple sources and have since dispersed; (3) there were multiple introductions of *B. tentaculata* from Europe into geographically distinct locations within North America; (4) while some invasive populations may have been introduced from Europe, some populations may be glacial relics that persisted in North America.

METHODS

Bithynia tentaculata samples (Figure 1) were stored at –20°C in 70-100% ethanol after collection and were deposited in the Field Museum of Natural History (F numbers 344681-344697). This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. As an invertebrate, this species is exempt from the approval process of the Institutional Animal Care and Use Committees at UWL

Figure 1. Collection sites of *Bithynia tentaculata* are indicated with filled black squares. Stars represent potential origination shipping routes for colonization from Europe. Locality information presented in Table 1.

and University of Texas Rio Grande Valley. Total cellular DNA was extracted from a snip of foot tissue using the CTAB (Cetyltrimethylammonium bromide) method (Saghai-Maroof et al. 1984). Primers that target twelve loci were used to determine allele frequencies as described by Henningsen et al. (2010). DNA was amplified via PCR with a CAG tagged primer along with the associated primers, with and without a tag. PCR was performed in a 20uL reaction volume with the amplification mixture at concentrations as follows: *Taq* DNA Polymerase Thermopol Buff-2 0.05 U/μL, 0.15 mM dNTP, 1.5 mM $MgCl_2$, 25 μg/mL BSA, and 1X *Taq* Thermopol buff-2 buffer. Tagged primers were included at a concentration of 0.025 μM, untagged primers at a concentration of 0.25 μM, and 2 μL of DNA template was added to each reaction. Cycling conditions consisted of 4 min at 94°C; followed by 32 cycles of: 94°C for 30 s, 55°C for 30 s, 65°C for one min followed by a final extension at 65°C for 3 min. Samples were then diluted with water 1:10 and genotyped at the University of Wisconsin Biotechnology center, on an ABI 3730xl Genetic Analyzer. Output files were analyzed using the auto run setting in GeneMarker® (Holland et al. 2008) with a GS500 size standard and ABI template, to determine the size of alleles present at each locus.

A Bayesian analysis in STRUCTURE v 2.2 (Pritchard et al. 2000, version 2. from http://pritch.bsd.uchicago.edu/sofware/structure2_1.html.) was used to infer the number of populations (K) using Markov Chain Monte Carlo (MCMC), with five independent runs of 100,000 steps following a 100,000 step burn-in for each K from K=1 to K=12 (these represent the maximum and minimum possible values for K based on the number of populations sampled plus one). A test run of 200,000 steps and a 200,000 burn in was conducted to see if this level of iterations were required. The model assumed correlated allele frequencies among populations, sampling locations were informative about ancestry (LOCPRIOR), and followed an admixture model with a single value of lambda (λ=1.0) inferred for all populations. K was estimated based on the log likelihood score and posterior probability of K, Ln P(D) also known as *L(K)* (Falush et al. 2007) as well as the rate of change in the log likelihood score (ΔK) (Evanno et al. 2005). The log likelihood score was calculated following Evanno et al. (2005): $\Delta K = m|L(K+1) - 2L(K) + L(K-1)|\ /s[L(K)]$ for each K. Three values were used to estimate K, *L(K)*, ΔK, and α. The best estimate of K is identified as the maximal value of *L(K)*, yet as the true K is reached, *L(K)* at larger K values will plateau or even increase slightly (Evanno et al. 2005). The rate of change in the log likelihood score, ΔK, will be the highest at the true K. Finally, the lowest value for α indicates that most individuals are essentially from one population or another. The posterior probability of K, *L(K)*, Ln(K), and α were output directly from the program, and ΔK was calculated using the equation above for K=1 to K=12. Once K was estimated, 5 runs of STUCTURE was used to calculate F_{ST}, H_E, and H_O for each of the K populations. Average expected and observed heterozygosity (H_O, N_A) for each of the three populations clusters determined by STUCTURE were calculated in Microsoft Excel. Finally, the North American populations

Table 1. Eleven populations examined for 12 microsatellite loci with statistics summarizing genetic variation within populations. Data presented are latitude, longitude, number of individuals (n), number of microsatellite alleles (N_A), observed heterozygosity (H_O) and expected heterozygosity (H_E) averaged across loci. Dunaremete, Baracska, and Botanical Garden are all collection sites in the Danube River drainage. The order of populations matches the population number in Figure 2.

	Population	Latitude	Longitude	N	N_A	H_O	H_E
1	River Zala, Hungary	46.871633	16.787572	17	22	0.344	0.511
2	Lake Balaton, Hungary	46.763097	17.266496	9	8	0.381	0.403
3	Dunaremete, Hungary	47.884563	17.436472	6	18	0.339	0.515
4	Baracska, Hungary	47.287274	18.757078	1	1	—	—
5	Botanical Garden, Budapest, Hungary	47.485031	19.085412	21	43	0.589	0.582
6	Lake Winnibigoshish, Minnesota, U.S.	47.431292	−94.196227	10	17	0.466	0.620
7	Georgetown Lake, Montana, U.S	46.181239	−113.286868	3	7	0.750	0.750
8	Rattlesnake Reservoir, Montana, U.S.	45.90345	−108.426982	18	17	0.463	0.629
9	Upper Mississippi River (Pool 7), Wisconsin, U.S.	43.8669095	−91.3070842	65	39	0.409	0.557
10	Lake Winnebago, Wisconsin, U.S.	43.806288	−88.402219	6	12	0.521	0.533
11	Ottawa River, Canada	45.793924	−76.99684	7	9	0.389	0.589

were run separately to determine if the greater European diversity masked internal structure in North American populations.

Genepop v 4.0.10 (Raymond & Rousset 1995), was used to estimate number of alleles (N_A) for each population. GenAlEx 6.501 (Peakall & P.E. 2006, Peakall & Smouse 2012) was used to perform an analysis of molecular variance to calculate F_{ST} and F'_{ST} of the three population clusters (regions) determined by STRUCTURE. We used these combinational, regional groups rather than individual populations to increase the sample size of each group. This conforms with the findings of Hale et al. (2012) that 25-30 individuals are needed per "group" for accuracy of microsatellite data. When grouping by the regions Danube (n=27), Lake Balaton (n=26), and North America (n=109) we have sufficient sampling for comparison among regions, although not comprehensive sampling for any region. GenAlEx v. 6.501 was also used to perform a genetic distance based Principal Coordinates Analysis (PCoA) on alleles from all populations.

As a final examination of patterns of population genetic structure we used Bottleneck 1.2.02 (Cornuet & Luikart 1996) to examine each of the 3 regions for signs of a recent genetic bottleneck using a Wilcoxon sign-rank test, to accommodate our limited loci and sampling under the two-phase-model of evolution as recommended for microsatellite loci (Luikart & Cornuet 1997). In a genetic bottleneck, reduced population size results in loss of alleles and a decline in heterozygosity, recent bottlenecks should appear to have higher than expected genetic diversity compared to expectations from Hardy-Weinberg equilibrium.

RESULTS

Eleven populations of *Bithynia tentaculata* were sampled for 12 microsatellite loci from the native European range and from North America (Table 1). Structure runs with 200,000 burn-in and iterations were not different from 100,000 burn-in and 100,000 iterations all further analyses were carried out using the latter settings.

All three *ad hoc* estimates for K from our analysis using STRUCTURE, considered together, suggest a best estimate of K=3 (data not figured). All eleven populations grouped into one of these 3 clusters and provided the rationale for combining populations in further analyses. The three clusters include 1) the Danube population (all populations in the Danube River Basin, Hungary), 2) Lake Balaton population (includes Lake Balaton and River Zala—which flows into Lake Balaton, Hungary), and 3) North America. The F_{ST} value for the combined Danube populations is much lower than that for North America or Lake Balaton (Table 2). The North American populations analyzed without the European data resulted in K=1.

In Figure 2 which illustrates all eleven populations as well as the three combined populations, Lake Balaton stands out as being the least intermixed (also Table 2 with the highest among population F_{ST} value, 0.3379). The Danube has some contribution from Lake Balaton and from the population that is the source of the North American populations. The North American populations in Canada and Montana are the most heterogeneous, although lacking unique alleles. Examination for genetic signatures of a recent population bottleneck found no signature of this event in the Danube (Wilcoxon sign-rank test for two-phase-model, $P = 0.57$), and Lake Balaton regions ($P = 0.688$), however the North American region shows the signature of a recent bottleneck ($P = 0.012$).

Table 2. Results of AMOVA on populations grouped by regions. Genetic differentiation among populations, F_{ST} (+SD) and expected heterozygosity, H_E (+SD) for K=3.

Cluster	n	F_{ST}	H_E
Danube	28	0.1614+0.0010	0.6261+0.0001
North America	109	0.3133+0.0015	0.5238+0.0003
Lake Balaton	26	0.3379+0.0025	0.5613+0.0001

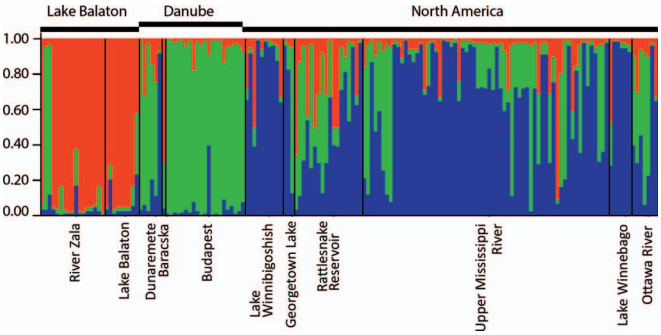

Figure 2. STRUCTURE output of Q (or proportion of each individual attributed to each cluster). Clusters indicated by color, population by number: (Lake Balaton Cluster: 1-River Zala HUN, 2-Lake Balaton HUN; Danube River Cluster: 3-Dunaremete HUN, 4-Baracska HUN, 5-Botanic Garden HUN; North American Cluster: 6-Lake Winnibigoshish MN, 7-Georgetown Lake MT, 8-Rattlesnake Reservoir MT, 9-Upper Mississippi River, 10-Lake Winnebago WI, 11-Ottawa River, Canada).

The number of alleles at 12 microsatellite loci ranged from 1-39. In most populations, observed heterozygosity was lower than expected under Hardy-Weinberg equilibrium (Table 1). Allele-frequency divergence among these three populations is shown in Table 3. The two European populations are more similar to each other (0.1160) than to the North American population, which is roughly equally different from the two European populations (0.1484, 0.1547). The Danube River population had the largest number of private alleles (21), followed by North America (11) and the fewest in Lake Balaton (8).

Most loci not only had different frequencies, but usually unique alleles in each population. In general, allelic diversity is highest in the Danube populations, followed by North America, then Lake Balaton (Table 1). For example, a single well-sampled locus, Bt03, displays very different allele frequencies across all populations, each population also has unique alleles at this locus (not figured). This pattern is repeated in most other loci. However, some population structure was observed. Allele frequencies are more similar in populations such as the Montana Lakes and Ottawa River (i.e. those outside the areas adjacent to Lake Michigan) and have fewer alleles, none unique to those populations (not figured).

A principal coordinates analysis (PCoA) of the microsatellite allele data across all populations (excluding Baracska, a Hungarian population represented by a single individual) resulted in three significant PCoA axes, axis one explains 17% of the variation present, axis two 13.19 %, and axis three 9.28 %. A scatterplot showing all individuals grouped by population on axis one and two is shown in Figure 3. On both axis one and two (Figure 3) the Upper Mississippi River population and Lake Winnibigoshish populations have the widest variation in allelic diversity. The other populations are restricted to the lower left quadrant of the graph, encompassed within the diversity of those two populations. Axis three (not figured) distinguishes the European populations from Lake Winnebago, WI and the Montana populations.

Table 3. Allele frequency divergence among regions (net nucleotide distance) calculated using STRUCTURE.

	Danube	North America
Danube	—	—
North America	0.1483	—
Lake Balaton	0.1160	0.1547

DISCUSSION

The goal of this study was to distinguish among four alternative hypotheses for introduction of *B. tentaculata* into North America. Hypothetical scenarios were proposed considering what is known of the possible invasion route and history of the species. The expected genetic consequences of each scenario are proposed based on the patterns observed in a review of genetic consequences of invasion in 80 species of animals, plants, and fungi by Dlugosch and Parker (2008) and glacial refugia by Maggs et al. (2009).

Hypothesis One incorporated a single introduction scenario: *B. tentaculata* was introduced once into the Great Lakes and dispersed into other North American watersheds. If this hypothesis were supported we would expect a low F_{ST} in North American populations compared to European populations, similar allele frequencies to the source, and very few private alleles in North America compared to European source. Hypothesis Two was developed around a multiple introduction scenario into the Great Lakes; after which *B. tentaculata* dispersed. If this hypothesis were correct, we would expect a higher F_{ST}, and greater heterogeneity in North America and many alleles from all across the founding European populations (albeit at lesser frequencies) and many private alleles if comparing North American population against a single source population. Hypothesis Three described a scenario with introductions from more than one European source population into the different North American watersheds in which *B. tentaculata* has now been found. If this hypothesis were correct, we would expect a high F_{ST}, very different allele frequencies both within North American populations and between North American and European populations, as well as private alleles unique to different North American populations. Hypothesis Four incorporated a European source of introduction for some of the North American populations, while other North American populations were assumed glacial relics. If this hypothesis were correct, we would expect a signal similar to Hypothesis Three but with private alleles in the glacial relic populations that are different from other North American and European populations.

Populations in Hungary compared to populations in North America

The *B. tentaculata* samples collected in Hungary are all part of the Ponto-Caspian Basin which contributes to the Black sea colonization route, and has contributed >70% of Great

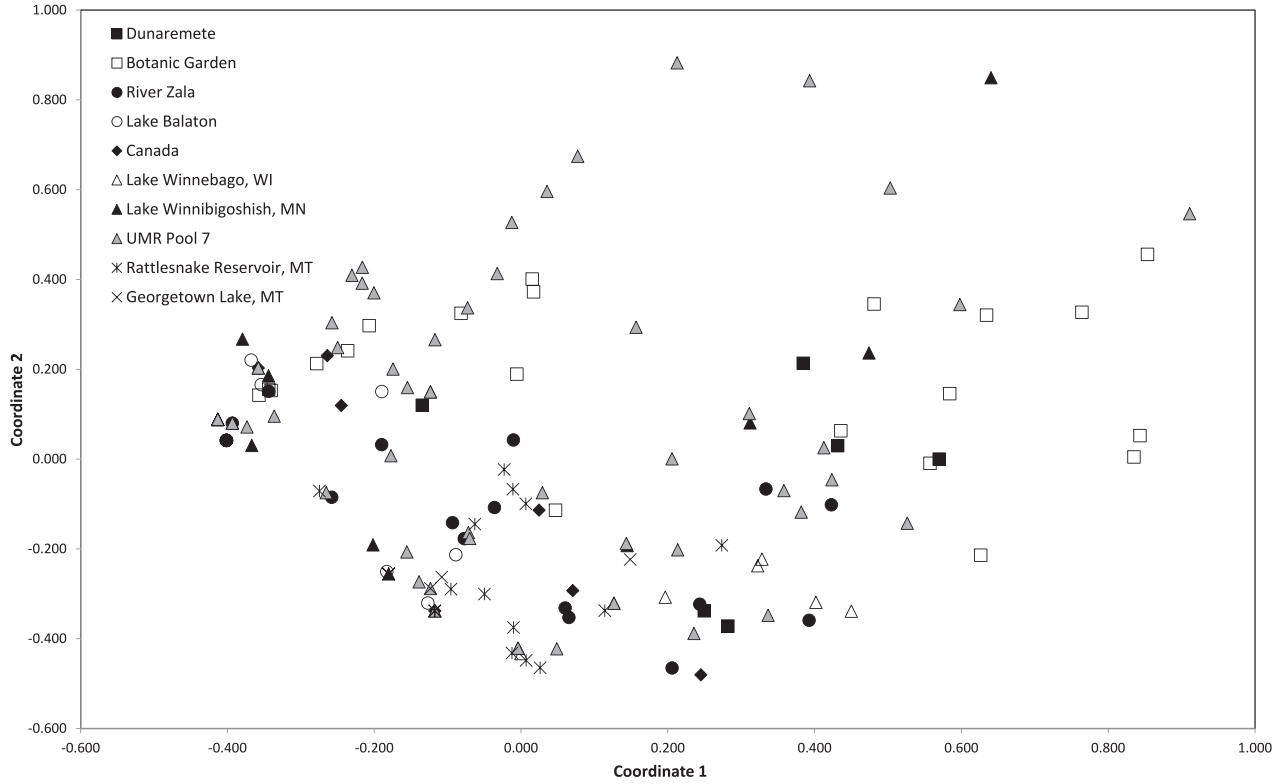

Figure 3. Genetic comparisons between populations using a Principal coordinates analysis of the genetic distance matrix from the microsatellite allele data. Similar shapes represents populations from the same region or drainage basin. Axis 1 represents 17% and axis 2 represents 13.19% of the variation present.

Lakes invaders (Ricciardi 2001, Gelembiuk et al. 2006). The concordance of the *ad hoc* estimates given in STRUCTURE (Figure 2) grouped all 11 populations into one of three clusters (K). However, Evanno et al. (2005) warn that these estimates must also coincide with scenarios that are biologically significant. Considering the *a priori* knowledge of sampling sites, K=3 corresponds with what would be predicted based on sampling localities from North America and two different Hungarian watersheds, the Danube River (Dunaremete, Baracska, and the Botanic Garden in Budapest sites) and Lake Balaton (Lake Balaton and River Zala sites) watersheds.

Examination of allele frequencies and variability (Figure 2, Figure 3, Table 3) from the Danube River and Lake Balaton supports the idea that the populations could be genetically distinct with the North American samples grouped as a third distinct population. There appears to be some shared alleles with alleles found in the Hungarian populations also present in the North American populations (Figure 2). This may indicate some contribution to ancestry of or a shared common ancestry with the North American populations, however additional European populations must be sampled for a robust comparison to be made. Some preliminary estimates can be made from the allele-frequency divergences of each population (Table 3). Even though the two Hungarian populations are distinct (Figure 3), they are more similar to each other than either is to the North American population (Table 2). This may indicate that the Hungarian populations are not the source of the North American populations. This inference is also supported by the number of private alleles, as there are more private alleles in samples from Hungary than in samples from North America, supporting the idea that Hungary is not the source of the North American populations. However, this is not definitive; the source could be from further downstream on the Danube, which would explain some of the same alleles present seen in the STRUCTURE figure (Figure 2) and as overlap in the PCoA (Figure 3).

These data do not support the Black sea colonization route as the source of the North American invasion, however this route cannot be definitely ruled out considering its large range and our limited sampling. Samples from further downstream on the Danube River, or within the Black Sea, will be necessary before this entire watershed can be definitively excluded as a source for the North American invasion. Sampling of the other two likely colonization routes through the North Sea and the Baltic Sea is also needed to determine if those routes may be the source population of the North American invasion.

Populations within North America

The North American populations were all combined into a single population by the *ad hoc* estimates in STRUCTURE which points toward a recent shared ancestry of all the populations within North America. Further when analyzed

separately to determine if the European diversity masked variation within North American, STRUCTURE found the most support for a single North American population. This evidence supports Hypotheses one or two that *B. tentaculata* was introduced into the Great Lakes and dispersed from there. This is supported by few private alleles in North America (compared to the European populations) and that the North American F_{ST} is relatively low compared to the native European populations. Another source of data offers some support for this scenario. In a relatively recent M.S. thesis Whalen (2011) used 11 microsatellite loci of which 4 overlap with this study and several of the same populations but also including 2 population not included in this study which are from Eastern Wisconsin near the Great Lakes. They found the populations near the Great Lakes (e.g. Lake Winnebago, WI) were probably "parent" populations to the Lake Onalaska and Lake Winnibigoshish populations included in both studies.

The STRUCTURE analysis does not appear to support Hypothesis Three, that there are multiple European source populations for the North American populations, unless Europe has very homogeneous populations, which is unlikely with the amount of divergence we observed in just the two Hungarian watersheds. Hypothesis Four also appears unlikely given our data set, though it is hard to distinguish private alleles from potential glacial relics without more extensive sampling of specimens from Europe for comparison. It is difficult at this point to confirm whether Hypothesis One or Two is more likely, as there were not enough data from European specimens to compare source populations. However, by comparing allele frequencies among populations, we can get a hint of which hypothesis (One or Two) is more likely.

With the exception of two loci, Bt22 and Bt40, the allele frequencies across the three populations differ, once again supporting the hypothesis of a different source population for invasion than those sampled in Hungary (data not figured). There were alleles present in snails from North America that were absent in snails collected in Hungary. While these may be attributed to new alleles arising in the population, such a scenario is unlikely across so many loci. There were also alleles present in the Hungarian populations that are not in snails from North America. Founder effects could account for this; however, if this were the case, the other alleles present would most likely be at similar frequencies, which they were not. It is possible that some snails from Hungary were mixed with other European source populations that then gave rise to the North American populations. If this were the case, there would likely be more heterogeneity in the North American populations (Figure 2). Most of the North American loci are dominated by one allele indicating a significant recent genetic bottleneck occurred, this is also supported by the Bottleneck analysis finding the signature of a recent bottleneck only in the North American population. This signature could be due to a relatively small initial invasive population from a single source population and subsequent bottlenecks with colonization of additional watersheds. This coincides with other invasions into the Great Lakes that have been shown to be from a single source population (Ricciardi & MacIsaac 2000, Brown & Stepien 2009). At this point it is still speculative, but Hypothesis One is more likely, and the higher F_{ST} (Table 3) may be an artifact of founder effects which can elevate F_{ST} levels (Weir & Cockerham 1984).

Our data suggest *B. tentaculata* has dispersed across the U.S. from a single initial colonization, not from multiple invasions from different sources. It also appears that all sampled North American populations are recent recolonizations, not glacial relic populations. Given the few populations sampled in the European range of *B. tentaculata*, the European source of the introduction into North America is still unknown, and will require further study of the European range. However, it does appear, based on the data available, that the most common route for invasion into the Great Lakes, from the Ponto-Caspian Sea Basin through the Black sea, is not the likely source of introduced *Bithynia tentaculata* in North America.

ACKNOWLEDGEMENTS

The authors would like to thank individuals that contributed their insight, time, and specimens to the project: Russell Minton, Anindo Choudbury, Jenny Sauer, Roger Haro, Mark Sandheinrich, Ben Walker, Greg Sandland, Rachelle Amundson, Adam Prise, Kirk Gallant, and Josh Laurila. This project was funded by the UWL River Studies Program, United States Geological Survey, Upper Mississippi Environmental Research Center, National Great Rivers Research and Education Center, University of Wisconsin Institute for Race and Ethnicity, the UWL faculty research grant program, and the UWL College of Science and Health Dean's Fellowship program. Several anonymous reviewers contributed greatly to the improvement of the manuscript, we appreciate their critical insights. Use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

LITERATURE CITED

Baker, F.C. 1928. The american *Bithynia* not wholly an introduced species. *Illinois State Academy of Science* 46: 56-62, 51 pl.

Brown, J.E. & Stepien, C.A. 2009. Invasion genetics of the Eurasian round goby in North America: tracing sources and spread patterns. *Molecular Ecology* 18: 64-79.

Claver, M. & Garcia-Berthou, E. 2005. Invasive species are a leading cause of animal extinctions. *Trends in Ecology and Evolution* 20: 110.

Cornuet, J.M. & Luikart, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144: 2001-2014.

Dlugosch, K.M. & Parker, I.M. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* 17: 431-449.

Evanno, G., Regnaut, S. & Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611-2620.

Gelembiuk, G.W., May, G.E. & Lee, C.E. 2006. Phylogeography and

systematics of zebra mussels and related species. *Molecular Ecology* 15: 1033-1050.

Gladosky, C. & Sandland, G. 2014. Assessing host competency between native and invasive snail species exposed to the native parasite *Echinostoma revolutum*. *Aquatic Invasions* 9: 87-93.

Grigorovich, I., Colautti, R., Mills, E., Holeck, K. & MacIsaac, H. 2003. Ballast mediated animal introductions in the Laurentian Great Lakes: retrospective and prospective analyses. *Canadian Journal of Fisheries and Aquatic Sciences* 60: 740-756.

Hale, M.L., Burg, T.M. & Steeves, T.E. 2012. Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. *PLoS ONE* 7: doi:10.1371/journal.pone.0045170.

Harman, W.N. 1968. Interspecific competition between *Bithynia* and pleurocerids. *Nautilus* 82: 72-73.

Harman, W.N. 1968. Replacement of pleurocerids by *Bithynia* in polluted waters of central New York. *Nautilus* 81: 77-83.

Harman, W.N. & Forney, J.L. 1970. Fifty years of change in the molluscan fauna of Oneida Lake, New York. *Limnology and Oceanography* 15: 454-460.

Henningsen, J.P., Lance, S.L., Jones, K.L., Hagen, C., Laurila, J., Cole, R.A. & Perez, K.E. 2010. Development and characterization of 17 polymorphic microsatellite loci in the faucet snail, *Bithynia tentaculata* (Gastropoda: Caenogastropoda: Bithyniidae). *Conservation Genetics Resources* 2: 247-250.

Holland, B., Clarke, A. & Meudt, H. 2008. Optimizing automated AFLP scoring parameters to improve phylogenetic resolution. *Systematic Biology* 57: 347-366.

Johnson, P.D., Bogan, A.E., Brown, K.M., Burkhead, N.M., Cordeiro, J.R., Garner, J.T., Hartfield, P.D., Lepitzki, D.A.W., Mackie, G.L., Pip, E., Tarpley, T.A., Tiemann, J.S., Whelan, N.V. & Strong, E.E. 2013. Conservation Status of Freshwater Gastropods of Canada and the United States. *Fisheries* 38: 247-282.

Jokinen, E. 1992. *The Freshwater Snails (Mollusca: Gastropoda) of New York State*. The University of the State of New York, The State Education Department, The New York State Museum, Albany, New York.

Keller, R.P., Drake, J.M., Drew, M.B. & Lodge, D.M. 2010. Linking environmental conditions and ship movements to estimate invasive species transport across the global shipping network. *Diversity & Distributions* 17: 93-102.

Lee, C. & Bell, M. 1999. Causes and consequences of recent freshwater invasions by saltwater animals. *Trends in Ecology and Evolution* 14: 284-288.

Luikart, G. & Cornuet, J.M. 1997. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conservation Biology* 12: 228-237.

Maggs, C.A., Castilho, R., Foltz, D., Hensler, C., Jolly, T., Olsen, J., Perez, K.E., Stam, W., Vainola, R., Viard, F. & Wares, J. 2009. Evaluating signatures of glacial refugia for North Atlantic marine organisms. *Ecology* 89: s108-122.

Mills, E.L., Leach, J.H., Carlton, J.T. & Secor, C.L. 1993. Exotic species in the Great Lakes - a history of biotic crises and anthropogenic introductions. *Journal of Great Lakes Research* 19: 1-54.

Mitchell, A.J. & Cole, R.A. 2008. Survival of the faucet snail after chemical disinfection, pH extremes, and heated water bath treatments. *North American Journal of Fisheries Management* 28: 1597-1600.

Peakall, R. & P.E., S. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288-295.

Peakall, R. & Smouse, P. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537-2539.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Raymond, M. & Rousset, F. 1995. GenePop (version 3.4): population genetics software for exact test and ecumenicism. *Journal of Heredity* 86: 248-249.

Ricciardi, A. 2001. Facilitative interactions among aquatic invaders: is an 'invasional meltdown' occurring in the Great Lakes? *Canadian Journal of Fisheries and Aquatic Sciences* 58: 13p.

Ricciardi, A. & MacIsaac, H. 2000. Recent mass invasion of the North American Great Lakes by Ponto-Caspian species. *Trends in Ecology and Evolution* 15: 62-65.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. & Allard, R.W. 1984. Ribosomal DNA spacer length variation in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proceedings of the National Academy of Sciences of the United States of America* 81: 8018-8021.

Sandland, G.J., Houk, S., Walker, B., Haro, R.J. & Gillis, R. 2013. Differential patterns of infection and life-history expression in native and invasive hosts exposed to a trematode parasite. *Hydrobiologia* 701: 89-98.

Sauer, J.S., Cole, R.A., Nissen, J.M., Upper Midwest Environmental Sciences Center (Geological Survey) & Geological Survey (U.S.) (2007). Finding the exotic faucet snail (*Bithynia tentaculata*): investigation of waterbird die-offs on the Upper Mississippi River National Wildlife and Fish Refuge. Reston, Va., U.S. Geological Survey**:** 3 p.

Stepien, C.A., Brown, J.E., Neilson, M.E. & Tumeo, M.A. 2005. Genetic diversity of invasive species in the Great Lakes versus their Eurasian source populations: Insights for risk analysis. *Risk Analysis* 25: 1043-1060.

Tashiro, J.S. & Colman, S.D. 1982. Filter-feeding in the freshwater prosobranch snail *Bithynia tentaculata*: bioenergetic partitioning of ingested carbon and nitrogen. *American Midland Naturalist* 107: 114-132.

Weir, B.S. & Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38: 1358-1370.

Whalen, S.J. (2011). Analysis of the genetic structure of *Bithynia tentaculata* snail populations in Wisconsin and Minnesota. Master's thesis, University of Minnesota, Mankato.

Appendix 1. List of all specimens genotyped. Localities, latitude, and longitude are listed as well as specimen identification number and Field Museum accession number (F-number).

F Number	DNA Numbers	Locality	latitude	longitude
344681.1	1669	Ottawa River, Canada	45.7939	−76.9968
344681.2	1671	Ottawa River, Canada	45.7939	−76.9968
344681.3	1672	Ottawa River, Canada	45.7939	−76.9968
344681.4	1673	Ottawa River, Canada	45.7939	−76.9968
344681.5	1675	Ottawa River, Canada	45.7939	−76.9968
344681.6	1874	Ottawa River, Canada	45.7939	−76.9968
344681.7	1875	Ottawa River, Canada	45.7939	−76.9968
344681.8	1877	Ottawa River, Canada	45.7939	−76.9968
344681.9	1893	Ottawa River, Canada	45.7939	−76.9968
344681.10	1894	Ottawa River, Canada	45.7939	−76.9968
344682.1	1868	Baracska, Hungary	47.2873	18.7571
344683.1	1820	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.2	1896	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.3	1897	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.4	1899	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.5	1900	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.6	1902	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.7	1905	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.8	1909	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.9	1915	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.10	1923	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.11	1927	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.12	1928	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.13	1930	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.14	1931	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.15	1934	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.16	1937	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.17	1941	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.18	1944	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.19	1945	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.20	1947	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.21	1949	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.22	1950	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344683.23	1951	Botanic Garden near Eotvos Lorant Univ., Budapest, Hungary	47.4850	19.0854
344684.1	1854	Dunaremete, Hungary	47.8846	17.4365
344684.2	1855	Dunaremete, Hungary	47.8846	17.4365
344684.3	1856	Dunaremete, Hungary	47.8846	17.4365
344684.4	1857	Dunaremete, Hungary	47.8846	17.4365
344684.5	1858	Dunaremete, Hungary	47.8846	17.4365
344684.6	1859	Dunaremete, Hungary	47.8846	17.4365
344685.1	1788	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.2	1792	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.3	1793	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.4	1794	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.5	1795	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.6	1797	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.7	1801	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.8	1802	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.9	1803	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.10	1807	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665

Appendix 1, continued.

F Number	DNA Numbers	Locality	latitude	longitude
344685.11	1809	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.12	1810	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.13	1811	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.14	1812	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.15	1813	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.16	1814	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.17	1816	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.18	1817	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.19	1818	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.20	1826	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.21	1827	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344685.22	1828	Lake Balaton, near Keszthely, Hungary	46.7631	17.2665
344686.1	1865	Lipot, Hungary	47.8661	17.4860
344687.1	1849	Northern Part of Budapest, Hungary	47.5128	19.0427
344687.2	1850	Northern Part of Budapest, Hungary	47.5128	19.0427
344687.3	1851	Northern Part of Budapest, Hungary	47.5128	19.0427
344687.4	1852	Northern Part of Budapest, Hungary	47.5128	19.0427
344687.5	1853	Northern Part of Budapest, Hungary	47.5128	19.0427
344688.1	1765	River Zala, Hungary	46.8716	16.7876
344688.2	1766	River Zala, Hungary	46.8716	16.7876
344688.3	1767	River Zala, Hungary	46.8716	16.7876
344688.4	1768	River Zala, Hungary	46.8716	16.7876
344688.5	1769	River Zala, Hungary	46.8716	16.7876
344688.6	1770	River Zala, Hungary	46.8716	16.7876
344688.7	1771	River Zala, Hungary	46.8716	16.7876
344688.8	1772	River Zala, Hungary	46.8716	16.7876
344688.9	1773	River Zala, Hungary	46.8716	16.7876
344688.10	1774	River Zala, Hungary	46.8716	16.7876
344688.11	1775	River Zala, Hungary	46.8716	16.7876
344688.12	1776	River Zala, Hungary	46.8716	16.7876
344688.13	1777	River Zala, Hungary	46.8716	16.7876
344688.14	1778	River Zala, Hungary	46.8716	16.7876
344688.15	1779	River Zala, Hungary	46.8716	16.7876
344688.16	1780	River Zala, Hungary	46.8716	16.7876
344688.17	1781	River Zala, Hungary	46.8716	16.7876
344688.18	1782	River Zala, Hungary	46.8716	16.7876
344688.19	1783	River Zala, Hungary	46.8716	16.7876
344688.20	1784	River Zala, Hungary	46.8716	16.7876
344688.21	1785	River Zala, Hungary	46.8716	16.7876
344689.1	1703	Lake Winnebagoshish, MN	47.4313	−94.1962
344689.2	1704	Lake Winnebagoshish, MN	47.4313	−94.1962
344689.3	1705	Lake Winnebagoshish, MN	47.4313	−94.1962
344689.4	1706	Lake Winnebagoshish, MN	47.4313	−94.1962
344689.5	1707	Lake Winnebagoshish, MN	47.4313	−94.1962
344689.6	1708	Lake Winnebagoshish, MN	47.4313	−94.1962
344689.7	1709	Lake Winnebagoshish, MN	47.4313	−94.1962
344689.8	1710	Lake Winnebagoshish, MN	47.4313	−94.1962
344689.9	1713	Lake Winnebagoshish, MN	47.4313	−94.1962
344689.10	1714	Lake Winnebagoshish, MN	47.4313	−94.1962
344689.11	1715	Lake Winnebagoshish, MN	47.4313	−94.1962
344689.12	1717	Lake Winnebagoshish, MN	47.4313	−94.1962

Appendix 1, continued.

F Number	DNA Numbers	Locality	latitude	longitude
344689.13	1720	Lake Winnebagoshish, MN	47.4313	−94.1962
344689.14	1722	Lake Winnebagoshish, MN	47.4313	−94.1962
344689.15	1724	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.1	1878	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.2	1879	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.3	1880	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.4	1881	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.5	1882	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.6	1883	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.7	1884	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.8	1885	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.9	1886	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.10	1887	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.11	1888	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.12	1889	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.13	1890	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.14	1891	Lake Winnebagoshish, MN	47.4313	−94.1962
344697.15	1892	Lake Winnebagoshish, MN	47.4313	−94.1962
344690.1	1655	Georgetown Lake, Montana	46.1812	−113.2869
344690.2	1656	Georgetown Lake, Montana	46.1812	−113.2869
344690.3	1657	Georgetown Lake, Montana	46.1812	−113.2869
344690.4	1658	Georgetown Lake, Montana	46.1812	−113.2869
344691.1	1659	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.2	1660	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.3	1661	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.4	1663	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.5	1666	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.6	1727	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.7	1728	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.8	1729	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.9	1730	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.10	1732	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.11	1734	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.12	1735	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.13	1742	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.14	1746	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.15	1755	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.16	1756	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.17	1757	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.18	1760	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.19	1761	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.20	1763	Rattlesnake Reservior, MT	45.9035	−108.4270
344691.21	1764	Rattlesnake Reservior, MT	45.9035	−108.4270
344692.1	1456	Pool 7, Cormorant Is. La Crosse, WI	43.9028	−91.2985
344692.2	1457	Pool 7, Cormorant Is. La Crosse, WI	43.9028	−91.2985
344692.3	1458	Pool 7, Cormorant Is. La Crosse, WI	43.9028	−91.2985
344692.4	1460	Pool 7, Cormorant Is. La Crosse, WI	43.9028	−91.2985
344692.5	1462	Pool 7, Cormorant Is. La Crosse, WI	43.9028	−91.2985
344692.6	1463	Pool 7, Cormorant Is. La Crosse, WI	43.9028	−91.2985
344692.7	1464	Pool 7, Cormorant Is. La Crosse, WI	43.9028	−91.2985
344692.8	1465	Pool 7, Cormorant Is. La Crosse, WI	43.9028	−91.2985

Appendix 1, continued.

F Number	DNA Numbers	Locality	latitude	longitude
344693.1	1466	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344693.2	1467	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344693.3	1469	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344693.4	1470	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344693.5	1471	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344693.6	1472	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344693.7	1473	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344693.8	1474	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344693.9	1475	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344692.9	1478	Pool 7, Cormorant Is. La Crosse, WI	43.9028	−91.2985
344692.10	1479	Pool 7, Cormorant Is. La Crosse, WI	43.9028	−91.2985
344692.11	1480	Pool 7, Cormorant Is. La Crosse, WI	43.9028	−91.2985
344693.10	1484	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344693.11	1485	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344693.12	1488	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344693.13	1489	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344693.14	1491	Pool 7, Broken Gun Is. E. La Crosse, WI	43.9133	−91.2889
344694.1	1493	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.2	1494	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.3	1496	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.4	1497	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.5	1498	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.6	1500	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.7	1501	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.8	1520	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.9	1521	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.10	1523	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.11	1524	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.12	1525	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.13	1526	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.14	1527	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.15	1528	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344694.16	1529	Pool 7, Arrow Head Is. E., La Crosse, WI	43.8996	−91.2863
344695.1	1624	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.2	1625	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.3	1628	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.4	1629	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.5	1631	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.6	1632	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.7	1634	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.8	1636	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.9	1637	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.10	1638	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.11	1639	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.12	1641	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.13	1644	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.14	1645	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.15	1646	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.16	1647	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.17	1650	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.18	1653	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863

Appendix 1, continued.

F Number	DNA Numbers	Locality	latitude	longitude
344695.19	1679	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.20	1680	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.21	1681	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.22	1682	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.23	1683	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.24	1684	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.25	1685	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.26	1687	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.27	1689	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.28	1691	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.29	1693	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.30	1694	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.31	1695	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.32	1696	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.33	1697	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.34	1698	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.35	1699	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.36	1701	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344695.37	1702	Pool 7 of Mississippi River, La Crosse, WI	43.8996	−91.2863
344696.1	2056	Lake Winnebago, WI	43.9670	−88.5228
344696.2	2058	Lake Winnebago, WI	43.9670	−88.5228
344696.3	2059	Lake Winnebago, WI	43.9670	−88.5228
344696.4	2063	Lake Winnebago, WI	43.9670	−88.5228
344696.5	2065	Lake Winnebago, WI	43.9670	−88.5228
344696.6	2066	Lake Winnebago, WI	43.9670	−88.5228
344696.7	2068	Lake Winnebago, WI	43.9670	−88.5228
344696.8	2069	Lake Winnebago, WI	43.9670	−88.5228
344696.9	2071	Lake Winnebago, WI	43.9670	−88.5228
344696.10	2072	Lake Winnebago, WI	43.9670	−88.5228
344696.11	2073	Lake Winnebago, WI	43.9670	−88.5228
344696.12	2074	Lake Winnebago, WI	43.9670	−88.5228
344696.13	2076	Lake Winnebago, WI	43.9670	−88.5228

Freshwater Mollusk Biology and Conservation 19:69–79, 2016

REGULAR ARTICLE

LIFE STAGE SENSITIVITY OF A FRESHWATER SNAIL TO HERBICIDES USED IN INVASIVE AQUATIC WEED CONTROL

Jennifer M. Archambault[1*] and W. Gregory Cope[1]

[1] *Department of Applied Ecology, North Carolina State University, Campus Box 7617, Raleigh, NC 27695 USA*

ABSTRACT

Invasive aquatic plants like hydrilla (*Hydrilla verticillata*) threaten native species in many ways, ultimately degrading overall habitat quality and quantity. Aquatic herbicides are often chosen as a control and management strategy, but few peer-reviewed studies address their effects on non-target organisms, especially native freshwater mussels and snails. The aim of this study was to assess the life stage sensitivity of a rare snail, *Somatogyrus virginicus* (Lithoglyphidae), to two aquatic herbicides (dipotassium salt of endothall and fluridone). We collected adult snails, cultured their eggs on a vinyl card substrate, exposed adults and eggs in 96-h static-renewal experiments, and monitored eggs through hatching. Because fluridone is typically applied for $\geq$ 60 d, an additional treatment was exposed in static-renewal through hatching (30 d total) to improve environmental relevance. Eggs present on the shells of adult snails were also monitored. Endpoints were adult survival and egg hatching success. Fluridone did not affect adult snail survival at concentrations up to 1500 µg/L, and in the test with eggs on vinyl cards, fluridone did not significantly delay ($p = 0.12$) or influence overall hatching success ($p = 0.22$), including in the 30-d exposure (Dunnett's $p = 0.09$). However, fluridone significantly delayed hatching of eggs on adult shells ($p < 0.01$) and reduced their overall hatching success ($p < 0.01$). The 96-h median effect concentration (EC50) for fluridone on hatching success of eggs on adults was 1334 µg/L (95% CI, 1215 – 1466 µg/L). For endothall, the adult 96-h median lethal concentration (LC50) was 223 mg/L (157 – 318 mg/L). Endothall negatively affected hatching success in both egg tests by delaying hatching ($p < 0.01$ in both tests) and by reducing overall hatching success ($p = 0.04$ for eggs on cards, and $p < 0.01$ for eggs on adults). The endothall 96-h EC50s for egg hatching success were 54.1 mg/L (95% CI, 35.6 – 82.2 mg/L; eggs on adults) and 83.4 mg/L (95% CI, 60.4 – 115.2 mg/L; eggs on cards). Neither herbicide had toxic effects to either life stage at concentrations typically prescribed for control of hydrilla (5 – 15 µg/L fluridone and 1 – 5 mg/L endothall). However, applying the minimum amount of herbicide needed for effective weed control is recommended for ensuring safety of non-target organisms.

KEYWORDS - Gastropoda, prosobranch snail, hatching success, Panhandle Pebblesnail, dipotassium salt of endothall (Aquathol-K®), fluridone (Sonar-Genesis®)

INTRODUCTION

Understanding the effects of toxicants on rare and imperiled species in environments laden with contaminants is as critical to achieving conservation goals as is understanding life history and habitat requirements. Toxicological and other studies on freshwater mollusks (mainly freshwater mussels) have increased over the past ~20 years (Cope et al. 2008; FMCS 2016), but they still number far fewer than studies of other taxa (e.g., fishes, insects, and other invertebrates). Gastropods – especially gill-breathing species in the clades Caenogastropoda and Neritimorpha (formerly known from the subclass Prosobranchia) – are represented by just a few recent studies (Besser et al. 2009, 2016; Archambault et al. 2015; Poznanska et al. 2015; Gibson et al. 2016) despite their high imperilment rates and importance to the functional ecology of freshwater systems (Johnson et al. 2013).

Invasive plants and animals are another credible and widely documented threat to freshwater mollusks, and resource managers must often balance their control with

*Corresponding Author: jmarcham@ncsu.edu

conserving native species. For example, researchers have long worked to identify chemicals to combat invasive mollusks (e.g., Zebra Mussels (*Dreissena polymorpha*)) without harming non-target species, including native mussels (e.g., Waller et al. 1993; Cope et al. 1997; Meehan et al. 2014). The effects of herbicides used to combat invasive aquatic plants, such as hydrilla (*Hydrilla verticillata,* Hydrocharitaceae), on non-target organisms has also been investigated (Hamelink et al. 1986; Keller 1993; Paul et al. 1994; Yi et al. 2011), including the most recent study on freshwater mussels and snails (Archambault et al. 2015). Hydrilla is an aquatic invasive weed non-native to the United States (US), and is included on the Federal Noxious Weed List (USDA APHIS 2012). It can form vast monocultures, shade out native vegetation (FWC 2013), alter water quality parameters including dissolved oxygen (Pesacreta 1988), and can serve as a vector for a neurotoxic cyanobacteria that affects waterfowl and their predators (Wiley et al. 2008; Williams et al. 2009). Hydrilla has been frequently dispersed anthropogenically via boat motors, trailers, and angling gear, and eradication or long-term maintenance control is difficult (Langeland 1996).

The most common hydrilla control methods include application of aquatic herbicides, introduction of non-native (to the US) Grass Carp (*Ctenopharyngodon idella*), and mechanical removal (Langeland 1996). Fluridone (market name Sonar®; CAS number 59756-60-4), typically prescribed for one to four months, and the dipotassium salt of endothall (market name Aquathol®; CAS number 2164-07-0), typically prescribed two to three times during the growing season, each for a period of days, are among the most commonly used aquatic herbicides for control of hydrilla (Archambault et al. 2015). The impetus for this study was the persistence of hydrilla in the Eno River, located in the Piedmont region (Durham and Orange Counties), North Carolina, USA – a river with high biodiversity, high rates of endemism, and the presence of threatened and endangered species (Smith et al. 2002; NCWRC 2015; LeGrand et al. 2013; NatureServe 2013) – where the targeted use of herbicides has been recommended as the most appropriate hydrilla control method. However, more information on the potential effects to non-target organisms was needed, especially for the Panhandle Pebblesnail (*Somatogyrus virginicus*), whose habitat has been invaded by hydrilla and where herbicide applications would occur.

Somatogyrus virginicus (Lithoglyphidae) is a rare, non-pulmonate snail in the clade Caenogastropoda; species in this genus have an annual reproductive ecology in which most adults die soon after breeding (Johnson et al. 2013). *Somatogyrus virginicus* has a limited and patchy distribution in Atlantic Slope streams of Virginia, North Carolina, and South Carolina (USA; NatureServe 2013), and the Eno River has the only confirmed population in North Carolina (LeGrand et al. 2013), where it has been identified as a species of greatest conservation need in the North Carolina Wildlife Action Plan (NCWRC 2015). The Eno River, which is also culturally important as a recreational destination and municipal drinking water source, supports a variety of other rare species, including the Carolina Madtom (*Noturus furiosus*, state-listed threatened), and one state-threatened (*Lampsilis radiata*) and three state-endangered (*Fusconaia masoni, Lampsilis cariosa, Lasmigona subviridis*) freshwater mussels (LeGrand et al. 2013).

Like other lithoglyphids, *S. virginicus* lays its eggs in spring, with timing of reproduction and development of eggs influenced by stream temperature (P. Johnson, Alabama Aquatic Biodiversity Center, personal communication). Those in the Eno River begin laying eggs in mid- to late-April when the water temperature approaches $\sim$ 17°C and continues through mid-May, often depositing them on a clean surface of silt-free rocks with riffleweed (*Podostemum ceratophyllum*), an aquatic plant that provides habitat for the snails. Eggs are most abundant (e.g., hundreds per rock) within stream riffle habitat and are deposited individually in a clear, hard casing. The duration of development is dependent upon temperature, typically requiring 2 – 4 weeks before hatching (P. Johnson, personal communication, and author personal observations).

Prior to the recent study by Archambault et al. (2015) on the effects fluridone and the dipotassium endothall on freshwater mussels and juvenile *S. virginicus*, peer-reviewed toxicity data were limited to only a few studies of other freshwater invertebrates and fishes (Crosby and Tucker 1966; Hamelink et al. 1986; Paul et al. 1994; Yi et al. 2011). The toxicity thresholds for freshwater mollusks ranked among the lowest (i.e., most sensitive) compared to fishes and other invertebrates, but concentrations associated with acute toxicity were still greater than the concentrations typically prescribed for controlling invasive aquatic weeds ($\sim$10 to 100 times greater; Archambault et al. 2015). The potential risks of such aquatic herbicides to freshwater mollusks should be assessed and balanced appropriately against the significant biological threat posed by invasive aquatic weeds like hydrilla.

Fluridone (market formulation liquid Sonar-Genesis®) and the dipotassium salt of endothall (hereafter, simply 'endothall'; market formulation Aquathol-K®) were considered for management of hydrilla in the Eno River. The complex management situation of snail habitat juxtaposed with dense stands of hydrilla and, therefore, snail reproduction and egg development with timing and location of herbicide applications required a thorough assessment of potential hazards of these herbicides to the life stages of *S. virginicus*. An earlier study reported the acute median lethal concentrations (LC50s) of fluridone to *S. virginicus* juveniles (409 – 500 µg/L, 96 h test to 48 h post-exposure; Archambault et al. 2015), but effects on snail eggs and adults, effects from longer duration exposures, and effects from other chemicals (e.g., endothall) have not been studied. The aims of this study were to determine the effects of two herbicides used for control and management of hydrilla and other aquatic weeds on *S. virginicus* eggs and adults so that the species' sensitivity can be holistically understood with information from multiple life stages; to expand the toxicological data base for gill-breathing snails in Caenogastropoda and related clades; and to assess the

results in the context of typically prescribed invasive plant control methods in high-biodiversity ecosystems.

METHODS

Test Organisms

Adult *S. virginicus* were collected from the Eno River when their eggs were abundant on river rocks to ensure they were reproductively active (120 snails on 7 May 2014 and 255 snails on 13 April 2015). Upon collection, snails were placed in sanitized Naglene® bottles filled with river water, placed in a cooler to maintain the ambient water temperature, and immediately transported (~45 min travel duration) to our laboratory at North Carolina State University (Raleigh, USA). Average shell height, as measured from the apex to the base of the aperture, perpendicular to the spiral axis was 4.45 mm (± 0.43, SD) in 2014 and 4.32 mm (± 0.42 mm) in 2015. Snails were acclimated from river water to the test water by placing them in a 50:50 solution of river/reconstituted water for 2 h, then further diluting the river water to a 25:75 ratio with reconstituted water, and held for an additional 2 h before being placed in 100% reconstituted water (ASTM 2007; 2013). ASTM reconstituted soft water (ASTM 2007) was selected because it most closely approximated the water quality parameters in the native range of *S. virginicus*.

Egg culture.—Ten (in 2014) or 12 (in 2015) snails were placed in each of 12 (in 2014) or 18 (in 2015) beakers containing 300 mL of water and a 5x8-cm card cut from a section of vinyl siding, which was suggested as an appropriate substrate for egg deposition (P. Johnson, Alabama Aquatic Biodiversity Center, personal communication). All vinyl cards were oriented with the rough surface facing downward and the smooth side facing upward. The first study was smaller to minimize collection of animals, given the uncertainty of potential success with culturing eggs of gill-breathing snails in a laboratory setting for the first time. Typically, current culture methods focus on augmenting wild populations, and are accomplished with large numbers of adult snails grown in outdoor pools sourced with food-rich pond water (P. Johnson, Alabama Aquatic Biodiversity Center, personal communication), whereas we needed to produce eggs on discrete units for individual exposure in independent experimental replicates. Water was renewed (100% volume) twice per week during the egg culture phase, and each chamber received a one-time dose of $<$ 1 mL of Instant Algae® Nannochloropsis (Nanno 3600; Reed Mariculture, Campbell, California, USA) concentrate to aid establishment of a biofilm on which the adult snails might feed. Eggs were counted twice per week until it was determined there was a sufficient quantity for testing, which took 7 – 10 d. The initial egg count on vinyl cards at the beginning of the experiments averaged 47 per card in 2014 (range 10 – 91; age $\leq$ 10 d) and 15 per card in 2015 (range 5 – 29; age $\leq$ 5 d).

Experimental Conditions

We selected herbicide treatment concentrations based on recommended application rates for treatment of hydrilla, herbicide label maximum application rates, and acute toxicity data reported for other taxa in the peer-reviewed literature (Crosby and Tucker 1966; Sanders 1969; Hamelink et al. 1986; Paul et al. 1994; SePRO 2010, 2011; Yi et al. 2011; UPI 2011, 2012), including the only known toxicities of fluridone and endothall to other freshwater mollusks (Keller 1993; Archambault et al. 2015). Sonar – Genesis® (fluridone), labeled as 0.5 lb/gal (59,913 mg/L) was provided by the SePRO Corporation Research and Technology Campus (Whitakers, North Carolina, USA) and was stored refrigerated until use in toxicity tests. Before use, the fluridone was diluted to a working stock of 1500 µg/L (parts per billion) active ingredient, as formulated. Acute test concentrations of fluridone ranged from 5 to 1000 µg/L in the exposure of eggs on vinyl cards, with an additional chronic (30-d) test treatment at 5 µg/L. Test concentrations for adult snails ranged from 5 to 1500 µg/L. Endothall (Aquathol-K®; United Phosphorus, Inc., King of Prussia, Pennsylvania, USA), labeled as 4.23 lb/gal (~506,866 mg/L), was obtained from personnel in the Aquatic Plant Management Program in the Department of Crop Science, North Carolina State University, and subsequently diluted to a working stock of 1000 mg/L (parts per million) active ingredient, as formulated. Test concentrations of endothall ranged from 5 to 100 mg/L in the exposure of eggs on vinyl cards, and from 1 to 1000 mg/L in the adult snail test. Composite water samples (10 mL from each of 3 replicates, 30 mL total volume) were collected for herbicide concentration verification prior to placing organisms into the chambers, and again at 48-h; samples were stored at 4°C until they were shipped to the SePRO Corporation analytical laboratory (fluridone quantified via HPLC) or the US Army Engineer Research and Development Center's Environmental Laboratory (endothall quantified via immunoassay; Gainesville, Florida, USA).

As in the culture phase, all experiments were static-renewal tests conducted in reconstituted soft water (ASTM 2007), with 90 – 100% water renewal at 48 h during the tests, and 3x/wk during the observation period following the tests. No formalized guidelines (e.g., ASTM) exist for conducting acute or chronic toxicity tests with freshwater snails, so quality assurance and control were ensured by conducting all tests according to guidelines for other freshwater mollusks (ASTM 2013), as per protocol in other recently published studies (Besser et al. 2009, 2016; Archambault et al. 2015). Tests were conducted in light- and temperature-controlled environmental chambers (Precision Model 818, Thermo Fisher Scientific, Marietta, Ohio, USA), held at 20°C and a light:dark cycle of 16:8 h (3678 lux). During the post-exposure observation period, temperature conditions were adjusted to approximate the natural river conditions, encouraging timely development of eggs. The final temperature was 23.5°C in 2014 and 23°C in 2015. In exposures with adults, six (in 2014) or seven (in

2015) snails were placed in each of three replicates per treatment, including in controls (0 μg/L). Because the adults were carrying embryos on their shells, we used the opportunity to observe them throughout the experiment, and afterward, transferred the adults to untreated reconstituted water to observe the embryos through hatching. Adult snail shells had an average of 9 total embryos per replicate in 2014 (range 1 – 20) and 12 embryos per replicate in 2015 (range 4 – 21). Eggs on adult shells ranged from freshly laid to final developmental stages because many were present when the adult snails were collected from the river and they continued to deposit eggs on shells or beaker surfaces while in the laboratory. In exposures of eggs on vinyl cards, each card was distributed to one of three independent replicates per treatment. Mean water quality conditions among experiments were 30.0 mg $CaCO_3$/L alkalinity, 42.0 mg $CaCO_3$/L hardness, 261 μS/cm conductivity, 7.78 pH, and 8.49 mg/L dissolved oxygen ($n = 4$ for alkalinity and hardness, $n = 15$ for all other variables). After the experiments, each chamber was dosed with < 1 mL of Nanno 3600 concentrate to aid establishment of a biofilm on which the snails and hatchlings might feed.

Data Collection and Statistical Analysis

At the end of each 96-h exposure, survival of adult snails was assessed by viewing them under a stereomicroscope and observing for righting behavior or movement within five minutes, an endpoint used in other studies and similar to assessment guidelines established for other freshwater mollusks (Besser et al. 2009, 2016; ASTM 2013; Archambault et al. 2015). Eggs were assessed after 96 h and three times weekly for viability until hatching senesced in each test (18 – 26 d post-exposure) by observing for vibrant yellow yolks, their characteristic constant rotation, and embryo development. Yolks/embryos that separated, stopped moving, lost color (turned white), stopped developing, or aborted were documented as non-viable.

The effects of herbicide concentration on survival of adult snails and on hatching success of snail eggs were analyzed by using survival data to generate median lethal/effective concentrations (LC50, EC50) and 95% confidence intervals (CI) via the Trimmed Spearman-Karber method (Comprehensive Environmental Toxicity Information Software (CETIS)™, v1.8.0.12, Tidepool Scientific, LLC, McKinleyville, California, USA). The LC50 or EC50 was defined as the concentration that caused mortality (LC50) or observed effect (i.e., lack of hatching; EC50) in 50% of the individuals in the exposed sample, and the LC05/EC05 was defined as the concentration that caused mortality/effect in 5% of the sample. LC and EC values were considered significantly different when their 95% CIs did not overlap (i.e., $\alpha = 0.05$).

The effect of herbicide concentration on hatching success was further analyzed using a repeated measures analysis of variance (PROC MIXED; SAS version 9.4; SAS Institute, Inc., Cary, North Carolina, USA). Significant effects ($\alpha = 0.05$) of herbicide concentration were further analyzed using a Dunnett's post-hoc test to elucidate toxic effects compared to controls.

RESULTS

Herbicide Concentration Analysis

Exposure accuracy (i.e., measured herbicide concentration compared to target concentration) was calculated as: exposure accuracy $= (P_m)/(P_t) \bullet 100$, where P_m is the measured herbicide concentration and P_t is the target concentration. The mean exposure accuracies are an average among all treatments sampled (all concentrations for fluridone and 0 – 100 mg/L for endothall because samples from the highest concentrations exceeded the dilution curve for analysis). They include sample results from both the test start (time zero) and 48-h time points (prior to solution renewal). The mean exposure accuracy of fluridone in experiments was 114.3% (range 99 – 154%) of target treatment concentrations. The mean exposure accuracy in endothall experiments was 93.6% (range 80 – 108%) of target treatment concentrations. All results were, therefore, expressed based on target concentrations. Post-hatch mortality was minimal in all egg tests and was similar among treatments within a given test; therefore, any post-hatch mortality was considered an effect of holding conditions rather than a treatment effect. Accordingly, the following statistical analyses of treatment effects were based on the ratio of total eggs hatched/initial egg count.

Fluridone

Hatching success.—In the exposure with eggs on vinyl cards, hatchlings began appearing 19 d after exposure, allowing four observation time points to be used in the analysis. Fluridone did not significantly affect hatching success ($p = 0.22$) (Table 1, Figure 1A). While no treatments were significantly different from controls at the $\alpha = 0.05$ level, a comparison of the treatment continuously exposed at 5 μg/L yielded a p-value of 0.09, trending lower than others (all other comparisons had p-values ranging from 0.13 to 0.57) (Table 1, Figure 1A).

In the exposure of eggs laid on adults, hatchlings were present 5 d after exposure and eight time points were used in the analysis. Fluridone significantly decreased hatching success ($F_{5,12} = 15.55$, $p < 0.01$), and its effect was dependent on time ($F_{35,84} = 14.07$, $p < 0.01$) (Table 1, Figure 1B). Hatching was delayed in the 500, 1000, and 1500 μg/L treatments (e.g., significantly lower on day 19 compared to controls). Further, overall hatching success was lower in the 500 and 1500 μg/L treatments compared to controls (Dunnett's $p = 0.05$ and $p < 0.01$, respectively) (Figure 1B).

Median lethal concentrations.—A fluridone 96-h LC50 for adult snails could not be calculated due to lack of mortality; most snails survived in all treatments, including the highest

Table 1. Results of repeated measures analysis of variance of the effects of fluridone on *Somatogyrus virginicus* hatching success.

Effect	Numerator degrees of freedom	Denominator degrees of freedom	F value	p-value
Eggs on cards				
day	3	36	254.59	<0.0001
fluridone	5	12	1.65	0.2198
fluridone*day	15	36	1.61	0.1206
Eggs on adults				
day	7	84	408.23	<0.0001
fluridone	5	12	15.55	<0.0001
fluridone*day	35	84	14.07	<0.0001

Table 2. Results of repeated measures analysis of variance of the effects of endothall on *Somatogyrus virginicus* hatching success.

Effect	Numerator degrees of freedom	Denominator degrees of freedom	F value	p-value
Eggs on cards				
day	3	24	75.27	<0.0001
endothall	3	8	4.29	0.0441
endothall*day	9	24	3.91	0.0036
Eggs on adults				
day	7	112	42.54	<0.0001
endothall	7	16	6.14	0.0013
endothall*day	49	112	3.87	<0.0001

treatment of 1500 µg/L. Likewise, a 96-h EC50 for egg hatching success could not be determined in the exposure of eggs on cards because of high hatching rates in all treatments. The 96-h EC50 for hatching success of eggs on adults was 1334 µg/L (95% CI, 1215 – 1466 µg/L). The only fluridone EC05 derived was for the same test, and was 288 µg/L (95% CI, 0 – 593 µg/L).

Endothall

Hatching success.—Somatogyrus eggs on vinyl cards began hatching 14 d after the end of the exposure, allowing four observation time points to be used in statistical analysis. Endothall had a significant fixed effect on overall hatching success ($F_{3,8} = 4.29$, $p = 0.04$), and the Dunnett's post-hoc analysis showed that hatching success was significantly lower in the 100 mg/L treatment compared to control ($p = 0.02$), but not in other treatments (p-values ≥ 0.11) (Table 2, Figure 2A). In addition to the main effect, the significant treatment-time interaction ($F_{9,24} = 3.91$, $p < 0.01$) provided evidence of a delay in hatching (i.e., eggs took longer to hatch at high concentrations). For example, hatching in the 100 mg/L treatment on day 17 was significantly less than control (Figure 2A).

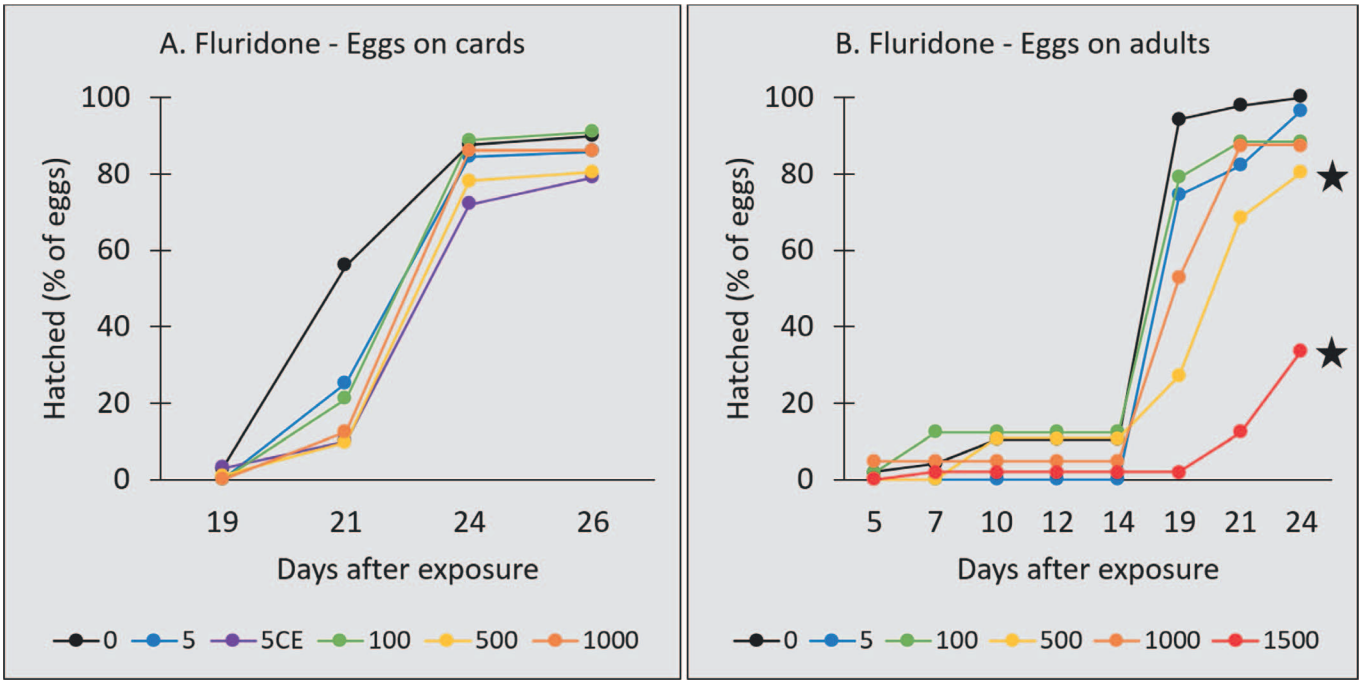

Figure 1. Mean percent of *Somatogyrus virginicus* eggs on vinyl cards (A) and on adult snail shells (B) counted initially in each fluridone treatment that hatched by each observation time point. Warmer colors represent higher concentrations (in µg/L), as legend indicates. Notes: 5CE in panel A denotes the continuously-exposed static-renewal treatment that received fluridone throughout observation period. Black stars indicate significantly lower overall hatching success at final time point, compared to control (Dunnett's $p \leq 0.05$). Standard errors for each data point are listed in the Appendix.

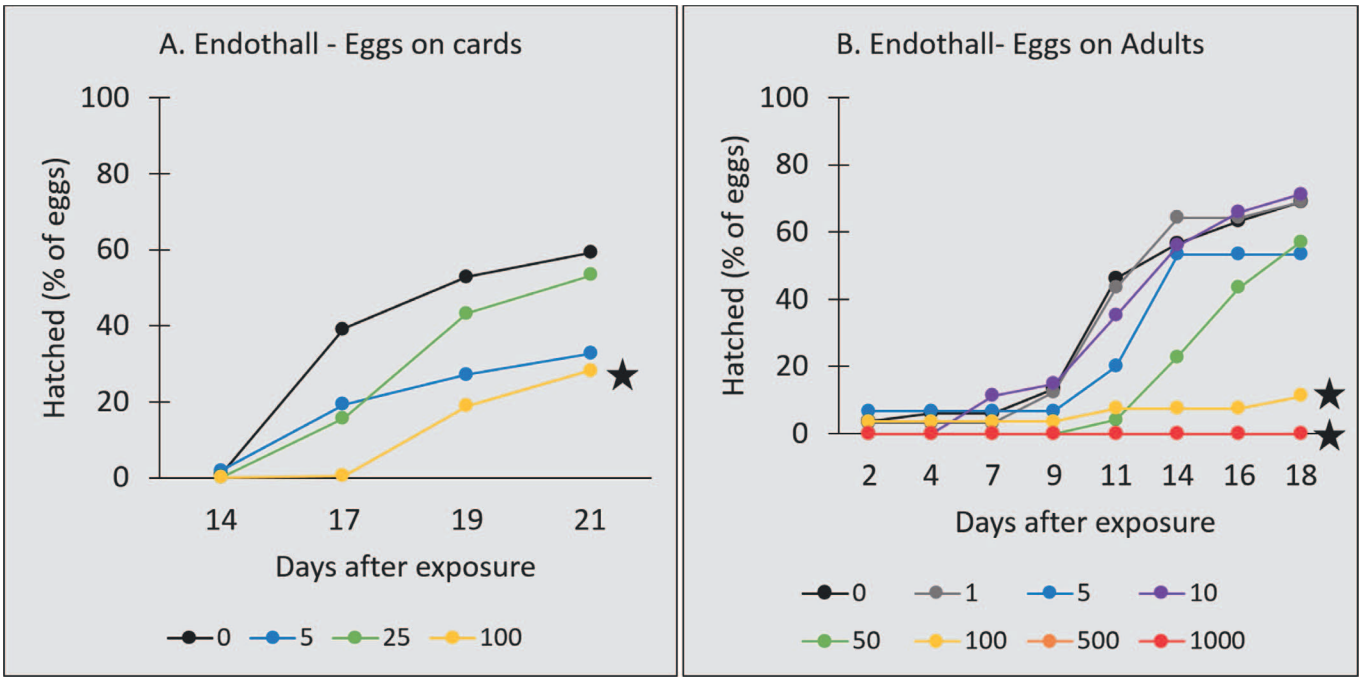

Figure 2. Mean percent of *Somatogyrus virginicus* eggs on vinyl cards (A) and on adult snail shells (B) counted initially in each endothall treatment that hatched by each observation time point. Warmer colors represent higher concentrations (in mg/L), as legend indicates. Black stars indicate significantly lower overall hatching success at final time point, compared to control (Dunnett's $p \leq 0.05$). Responses in the 500 and 1000 mg/L were the same and overlap. Standard errors for each data point are listed in the Appendix.

The first hatchlings from eggs on adults appeared 2 d post-exposure, and eight observation time points were used in analysis. In addition to the significant fixed effect of endothall on overall hatching success ($F_{7,16} = 6.14$, $p < 0.01$), the treatment-time interaction ($F_{49,112} = 3.87$, $p < 0.01$) again provided evidence of a hatching delay at higher concentrations (Table 2, Figure 2B). A Dunnett's post-hoc test of the main effect of treatment showed significantly poorer hatching success in the 100, 500, and 1000 mg/L concentrations ($p = 0.03$, 0.01, and 0.01, respectively), but not in lower concentrations (p-values ≥ 0.24) (Figure 2B).

Median lethal concentrations.—Both the 48-h and 96-h LC50s for adult snails exposed to endothall were 223 mg/L (95% CI, 157 – 318 mg/L). Responses were the same at both time points, because mortality occurred within the first 48 h. LC05s could not be determined for either time point due to lack of partial mortality responses – all snails survived in all treatments from 0 – 100 mg/L endothall, and no snails survived in the 500 and 1000 mg/L treatments. Most surviving snails remained alive and active during the observation of eggs on their shells following the exposure with no differences among treatments, indicating there was no latent effect of the acute duration of endothall exposure on adults. The 96-h EC50s for egg hatching success were 54.1 mg/L (95% CI, 35.6 – 82.2 mg/L; eggs on adults) and 83.4 mg/L (95% CI, 60.4 – 115.2 mg/L; eggs on cards) in the two separate tests. The EC50 results from the two tests were not significantly different, based on comparison of the overlapping 95% confidence intervals.

DISCUSSION

Comparative Toxicity

Based on LC50s and EC50s determined in our study, the *S. virginicus* egg and adult life stages appear less acutely sensitive to fluridone than its previously-tested juvenile life stage (Archambault et al. 2015). Compared to the known acute toxicity of fluridone to other aquatic organisms, the egg and adult life stages of *S. virginicus* are more sensitive to fluridone than most other species (Hamelink et al. 1986; Paul et al. 1994; Yi et al. 2011; Archambault et al. 2015). The greater sensitivity of the snails' egg and adult life stages to fluridone than other species is in agreement with that of other freshwater mollusks (including the *S. virginicus* juvenile life stage), all of which were found to be more sensitive than nearly every other organism for which fluridone toxicity values have been published (LC50 range 1300 – 32,000 µg/L, except *Arrenurus* spp. (10 – 891 µg/L); Archambault et al. 2015).

This study produced the first *Somatogyrus* LC50 and EC50s for endothall. Based on these values, the egg life stage is more sensitive than that of adults, whose LC50 value was 2.7 – 4.1 times greater than the egg EC50s. The sensitivity of juvenile *S. virginicus* to endothall has not yet been determined. If the juvenile life stage is more sensitive, as the fluridone data

indicates (Archambault et al. 2015), determining juvenile sensitivity to endothall may be prudent, especially because they would be present during summer applications of herbicides for aquatic weed control. The adult snail LC50 for endothall is approximately 6 – 7 times greater, and the egg EC50s are approximately 1.6 – 2.7 times greater, than the LC50s reported for the freshwater mussel *Lampsilis siliquoidea* (31 – 34 mg/L), the only freshwater mollusk for which endothall dipotassium salt toxicity data are published (Archambault et al. 2015). Keller (1993) evaluated the toxicity of Hydrothol 191 (CAS number 66330-88-9), a mono-amine salt of endothall to in-vitro propagated *Anodonta* (now *Utterbackia*) *imbecillis* and reported an LC50 of 4.85 mg/L. Another experiment in our laboratory with the dipotassium salt of endothall and in-vitro propagated *Lampsilis cardium* resulted in a 96-h LC50 of 137 mg/L (105 – 178 mg/L) (J. Archambault, unpublished data). Together, these findings indicate that mollusks may exhibit a wide range of tolerance to endothall formulations, even within a genus or among life stages. Compared to the known acute toxicity values of endothall to other non-molluscan aquatic organisms (16 – 130 mg/L (Crosby and Tucker 1966; Sanders 1969; Paul et al. 1994)), the adult life stage of *S. virginicus* is more tolerant of endothall than other species, having the highest acute LC50 value, and *S. virginicus* egg EC50s are in the middle of that range. That contrasts with some of their freshwater mussel counterparts, whose LC50s occur at the sensitive end of the known toxicity range (Archambault et al. 2015).

Relative Risk

Fluridone is typically applied at a rate of 5 to 15 μg/L for hydrilla control, with a maximum allowable application rate of 150 μg/L (SePRO 2010), and its application is most effective once plants are emerging from winter senescence and actively growing (e.g., May for hydrilla in the Eno River) to ensure maximum exposure to the product. Because of the similar spring timing of reproduction in *S. virginicus* and the growth of hydrilla, herbicide application during snail egg development and hatching overlap, and would likely be similar in other locations in the southeastern US where lithoglyphids co-occur with invasive plants. The negative effects of fluridone on *S. virginicus* egg hatching were due to delayed hatching and lower hatching rates in the highest concentrations tested (i.e., $\geq$ 500μg/L, Figure 1), indicating fluridone poses a minimal risk of harm compared to the potentially substantial risk of habitat degradation posed by hydrilla or other invasive aquatic weeds. Negative effects were not observed in the environmentally relevant range of concentrations in either egg test, providing consistent results from both 96-h exposures (Figure 1). Despite the lack of a statistically significant effect on hatching success in the 30-d exposure of 5 μg/L fluridone, the results were lower than the 96-h treatments of all other concentrations in the same test, and may be biologically relevant (Figure 1A). The 30-d exposure was about half to one-third as long as the typical treatment duration for fluridone in flowing waters. Laboratory conditions are vastly different from the natural swift river environment of *S. virginicus*, likely rendering longer duration studies in the laboratory impractical. Fluridone's primary degradation pathway is photolysis, and according to the Sonar Genesis product label, it may be less effective if in contact with highly organic sediments (SePRO 2010); however, water concentrations are typically monitored to maintain the target treatment concentrations during an herbicide application. Other factors that may reduce exposure of non-target organisms like *S. virginicus* include uneven mixing within complex habitats of a river course and proximity of the treatment area to species of concern (if not overlapping, as in our study area).

The acute exposures of endothall to *S. virginicus* eggs had a significant negative effect on hatching success at higher concentrations ($\geq$ 100 mg/L) in both tests (Figure 2), but not at concentrations typically prescribed for invasive aquatic plant control (1 – 5 mg/L). However, rates up to 150 mg/L are authorized for use on the product label (UPI 2011), and such concentrations in high biodiversity ecosystems should be avoided based on our findings, especially because the acute test durations are environmentally relevant for prescribed endothall applications, and endothall application would likely overlap with gastropod egg development when the target plants are actively growing.

Adult snails were unaffected by 96-h exposure to both herbicides in the label recommended application ranges. Further, any latent mortality following the tests and documented during the observation of eggs on their shells was sporadic, minimal, and equivalent in all treatments, including controls. Moreover, the risk of exposure to adult *S. virginicus* is minimized because most adults of this snail species will have already reproduced and are likely to die naturally before, or in the early phase of, any field application of herbicides. The egg and hatchling/juvenile life stages would be the most exposed and potentially vulnerable to any negative effects of herbicides.

At environmentally relevant concentrations (those typically applied to control hydrilla and other aquatic weeds), fluridone and endothall pose a minimal risk to all life stages of *S. virginicus*, compared to the potential risk that hydrilla infestation poses by degrading physical habitat and water quality. In riverine situations, such as in the Eno River of North Carolina, stands of hydrilla often grow directly within, and adjacent to, optimal snail habitat. In the swift-flowing riffles with clean rocks and riffleweed that provide snail habitat, hydrilla may shade out the native preferred vegetation and reduce water velocity, facilitating increased siltation. During our field collections of adult snails– even within an occupied riffle – snails were often more abundant in the swiftest flowing portion of the stream reach, despite available riffleweed habitat throughout the riffle. Snails were often more difficult to find in abundance or seemingly absent in slower portions of the riffle, where the slightest layer of sediment was apparent on rocks. The genus *Somatogyrus* has a strong foot

compared to many other freshwater snails (P. Johnson, Alabama Aquatic Biodiversity Center, personal communication), a possible adaptation for living in clean, swift water habitats where they are most often encountered. Because these and many other snails in the gill-breathing clades, Caenogastropoda and Neritimorpha, are simultaneously imperiled and geographically restricted, conservation of high quality habitat is imperative. We recommend that resource managers apply our findings in protecting freshwater habitats infested by aquatic weeds, while also recognizing their limitations. For example, selecting aquatic herbicide treatment prescriptions that use the minimum necessary concentrations to achieve effective control of invasive aquatic plants would be prudent because detrimental effects on egg hatching success were observed within the application range allowed on existing endothall labels, and because higher fluridone concentrations were not tested over relevant treatment durations (e.g., 30 – 90 d).

ACKNOWLEDGEMENTS

Funding for this project was provided, in part, by the U.S. Fish and Wildlife Service Aquatic Invasive Species Program through a sub-award (no. FWS-800-037-2014-NCSU) with the Gulf States Marine Fisheries Commission. This project was also supported by the respective institution of the authors, and in-kind contributions of materials and services by those acknowledged below. The members of the Eno River Hydrilla Management Task Force and their respective agencies/municipalities provided impetus and context for this research. Test organisms were collected from the Eno River with permission from the North Carolina Division of Parks and Recreation. Dr. Rob Richardson (Department of Crop Science, North Carolina State University) supplied Aquathol – K. SePRO Corporation supplied Sonar – Genesis and performed analytical chemistry for verification of fluridone concentrations. Dr. Mike Netherland (US Army ERDC, Gainesville, FL) provided analytical chemistry for verification of endothall concentrations. Sean Buczek, Ani Popp, Bobby Cope, Mary Siliman, and Joseph McIver provided laboratory assistance. Shannon Auell (Department of Crop Science, North Carolina State University) assisted in field collection of snails. Janet Kim (Department of Statistics, North Carolina State University) assisted with statistical analyses.

REFERENCES

Archambault J. M., C. M. Bergeron, W. G. Cope, R. J. Richardson, M. A. Heilman, J. E. Corey III, M. D. Netherland, and R. J. Heise. 2015. Sensitivity of freshwater molluscs to hydrilla-targeting herbicides: providing context for invasive aquatic weed control in diverse ecosystems. Journal of Freshwater Ecology 30:335-348.

[ASTM] American Society for Testing and Materials. 2007. Standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. E729-96. West Conshohocken (PA): ASTM International.

[ASTM] American Society for Testing and Materials. 2013. Standard guide for conducting laboratory toxicity tests with freshwater mussels. E2455-06. West Conshohocken (PA): ASTM International.

Besser J. M., D. L. Hardesty, I. E. Greer, and C. G. Ingersoll. 2009. Sensitivity of freshwater snails to aquatic contaminants: survival and growth of endangered snail species and surrogates in 28-day exposures to copper, ammonia and pentachlorophenol. Administrative report CERC-8335-FY07-20-10, submitted to US Environmental Protection Agency, Office of Research and Development, Duluth (MN).

Besser J. M., R. A. Dorman, D. L. Hardesty, and C. G. Ingersoll. 2016. Survival and growth of freshwater pulmonate and nonpulmonate snails in 28-day exposures to copper, ammonia, and pentachlorophenol. Archives of Environmental Contamination and Toxicology 70:321-331.

Cope W. G., M. R. Bartsch, and L. L. Marking. 1997. Efficacy of candidate chemicals for preventing attachment of zebra mussels (*Dreissena polymorpha*). Environmental Toxicology & Chemistry 16:1930-1934.

Cope W. G., R. B. Bringolf, D. B. Buchwalter, T. J. Newton, C.G. Ingersoll, N. Wang, T. Augspurger, F. J. Dwyer, M. C. Barnhart, R. J. Neves, and E. Hammer. 2008. Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants. Journal of the North American Benthological Society 27:451-462.

Crosby D. G., and R. K. Tucker. 1966. Toxicity of aquatic herbicides to *Daphnia magna*. Science 154:289-291.

[FMCS] Freshwater Mollusk Conservation Society. 2016. A national strategy for the conservation of freshwater mollusks. Freshwater Mollusk Biology and Conservation 19(1): in press.

[FWC] Florida Fish and Wildlife Conservation Commission. 2013. Weed alerts, hydrilla [Internet]. Tallahassee (FL): FWC; [cited 2013 Dec 12]. Available from: http://myfwc.comwildlifehabitats/invasive-plants/weed-alerts/hydrilla/.

Gibson K. J., J. M. Miller, P. D. Johnson, and P. M. Stewart. 2016. Toxicity of sodium dodecyl sulfate to federally threatened and petitioned freshwater mollusk species. Freshwater Mollusk Biology and Conservation 19: 29–35.

Hamelink J. L., D. R. Buckler, F. L. Mayer, D. U. Palawski, and H. O. Sanders. 1986. Toxicity of fluridone to aquatic invertebrates and fish. Environmental Toxicology & Chemistry 5:87-94.

Johnson PD, Bogan AE, Brown KM, Burkhead NM, Cordeiro JR, Garner JT, Lepitzki DA, Mackie GL, Pip E, Tarpley TA, et al. 2013. Conservation status of freshwater gastropods of Canada and the United States. Fisheries 38(6):247-282.

Keller AE. 1993. Acute toxicity of several pesticides, organic compounds, and a wastewater effluent to the freshwater mussel, *Anodonta imbecilis, Ceriodaphnia dubia*, and *Pimephales promelas*. Bulletin of Environmental Contamination Toxicology 51:696-702.

Langeland, K. A. 1996. Hydrilla verticillata (L.F.) Royle (Hydrocharitaceae), "The perfect aquatic weed". Castanea 61:293-304.

LeGrand H. E., Jr., Finnegan JT, Hall SP, Leslie AJ, Ratcliffe JA. 2013. Natural heritage program list of the rare animal species of North Carolina 2012. NC Natural Heritage Program. Raleigh (NC).

Meehan, S. A. Shannon, B. Gruber, S. M. Rackl, and F. E. Lucy. 2014. Ecotoxicological impact of Zequanox®, a novel biocide, on selected non-target Irish aquatic species. Ecotoxicology and Environmental Safety 107:148-153.

NatureServe. 2013. NatureServe Explorer: an online encyclopedia of life [Internet]. Arlington (VA): Version 7.1. NatureServe; [cited 2013 Dec 12]. Available from: http://www.natureserve.org/explorer.

[NCWRC] NC Wildlife Resources Commission. 2015. North Carolina wildlife action plan. Raleigh (NC).

Pesacreta G. J. 1988. Water chemistry from North Carolina piedmont impoundments with hydrilla (Hydrilla verticillata (L.f.) Royle) [dissertation]. Raleigh (NC): North Carolina State University.

Paul E. A., H. A. Simonin, J. Symula, and R. W. Bauer. 1994. The toxicity of diquat, endothall, and fluridone to the early life stages of fish. Journal of Freshwater Ecology 9:229-239.

Poznanska M. T. Kakareko, T. Gulanicz, L. Jermacz, and J. Kobak. 2015. Life on the edge: survival and behavioural responses of freshwater gill-breathing snails to declining water level and substratum drying. Freshwater Biology 60:2379-2391.

Sanders H. O. 1969. Toxicity of pesticides to the crustacean *Gammarus lacustris*. Technical Paper of the Bureau of Sport Fisheries and Wildlife, No. 25. Washington, DC.

SePRO Corporation. 2010. Sonar Genesis Aquatic Herbicide label [Internet]. Carmel (IN): SePRO Corporation; [cited 2013 May 16]. Available from: http://sepro.com/default.php?page=labels.

SePRO Corporation. 2011. Sonar Genesis Aquatic Herbicide Material Data Safety Sheet [Internet]. Carmel (IN): SePRO Corporation; [cited 2013 May 16]. Available from: http://sepro.com/default.php?page=labels.

Smith R. K., P. L. Freeman, J. V. Higgins, K. S. Wheaton, T. W. FitzHugh, K. J. Ernstrom, and A. A. Das. 2002. Priority areas for freshwater conservation action: a biodiversity assessment of the southeastern United States. The Nature Conservancy; Arlington (VA).

[USDA APHIS] US Department of Agriculture Animal and Plant Health Inspection Service. 2012. Federal noxious weed list [Internet]. Washington (DC): USDA APHIS; [cited 2013 Dec 11]. Available from: http://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/index.shtml.

[UPI] United Phosphorus, Inc. 2011. Aquathol K Aquatic Herbicide label [Internet]. King of Prussia (PA): United Phosphorus, Inc.; [cited 2013 Jan 11]. Available from: http://www.cdms.net/LabelsMsds/LMDefault.aspx?manuf=111&t=

[UPI] United Phosphorus, Inc. 2012. Aquathol K Aquatic Herbicide Material Data Safety Sheet [Internet]. King of Prussia (PA): United Phosphorus, Inc.; [cited 2013 Jan 11]. Available from: http://www.cdms.net/LabelsMsds/LMDefault.aspx?manuf=111&t=

Waller D. L., J. J. Rach, W. G. Cope, L. L. Marking, S. W. Fisher, and H. Dabrowska. 1993. Toxicity of candidate molluscicides to zebra mussels (*Dreissena polymorpha*) and selected nontarget organisms. Journal of Great Lakes Restoration 19:695-702.

Wiley F. E., M. J. Twiner, T. A. Leighfield, S. B. Wilde, F. M. Van Dolah, J. R. Fischer, and W. W. Bowerman. 2008. An extract of *Hydrilla verticillata* and associated epiphytes induces avian vacuolar myelinopathy in laboratory mallards. Environmental Toxicology 24:362-368.

Williams J. B. Jr, C. Puchulutegui, J. H. Landsberg, and S. K. Williams. 2009. The cyanobacterium (Order Stigonomatales) suspected of causing avian vacuolar myelinopathy is confirmed in Florida fresh waters. Journal of Freshwater Ecology 24:309-314.

Yi S. A., B. M. Francis, M. J. Wesley, and D. J. Soucek. 2011. Toxicological effects of the aquatic herbicide, fluridone, on male water mites (Hydrachnidiae: Arrenurus: Megaluracarus). Ecotoxicology 20:81-87.

Appendix

Mean percent of snail eggs hatched and associated standard error for each treatment and time point shown in figures.

Table A1. Fluridone eggs on cards; corresponds with Figure 1A.

Treatment (µg/L)	Days after exposure	Mean (% hatched)	Standard Error
0	19	2.90	2.90
5		0.00	0.00
5CE		3.03	3.03
100		0.00	0.00
500		1.15	1.15
1000		0	0
0	21	55.99	7.71
5		25.27	7.06
5CE		9.98	5.32
100		21.07	3.48
500		9.75	5.10
1000		12.50	7.22
0	24	87.58	8.46
5		84.43	13.69
5CE		72.01	15.76
100		88.89	5.56
500		78.25	3.23
1000		86.11	7.35
0	26	89.96	6.22
5		85.71	14.29
5CE		78.97	13.23
100		91.00	14.29
500		80.55	13.23
1000		86.11	5.56

Table A2. Fluridone eggs on adult shells; corresponds with Figure 1B.

Treatment (µg/L)	Days after exposure	Mean (% hatched)	Standard Error
0	5	2.08	2.08
5		0	0
100		1.96	1.96
500		0	0
1000		4.76	4.76
1500		0	0
0	7	4.17	4.17
5		0	0
100		12.25	7.22
500		0	0
1000		4.76	4.76
1500		2.08	2.08
0	10	10.42	5.51
5		0	0
100		12.25	7.22
500		10.82	5.53
1000		4.76	4.76
1500		2.08	2.08
0	12	10.42	5.51
5		0	0
100		12.25	7.22
500		10.82	5.53
1000		4.76	4.76
1500		2.08	2.08
0	14	10.42	5.51
5		0	0
100		12.25	7.22
500		10.82	5.53
1000		4.76	4.76
1500		2.08	2.08
0	19	94.21	3.22
5		74.52	2.49
100		79.12	2.17
500		27.19	7.42
1000		52.98	9.91
1500		2.08	2.08
0	21	97.92	2.08
5		82.06	6.63
100		88.33	7.26
500		68.54	7.18
1000		87.50	7.22
1500		12.42	4.40
0	24	100	0
5		96.67	3.33
100		88.33	7.26
500		80.29	3.65
1000		87.50	7.22
1500		33.71	9.88

Table A3. Endothall eggs on cards; corresponds with Figure 2A.

Treatment (mg/L)	Days after exposure	Mean (% hatched)	Standard Error
0	14	0.93	0.93
5		1.85	1.85
25		0	0
100		0	0
0	17	39.29	7.43
5		19.30	9.68
25		15.72	5.65
100		0.44	0.44
0	19	52.96	8.77
5		27.23	10.99
25		43.19	4.02
100		18.79	1.21
0	21	59.14	8.75
5		32.73	14.07
25		53.13	4.00
100		28.26	1.42

Table A4. Endothall eggs on adults; corresponds with Figure 2B.

Treatment (mg/L)	Days after exposure	Mean (% hatched)	Standard Error
0	2	3.70	3.70
1		3.17	3.17
5		6.67	6.67
10		0.00	0
50		0.00	0
100		3.70	3.70
500		0	0
1000		0	0
0	4	5.93	3.23
1		3.17	3.17
5		6.67	6.67
10		0.00	0
50		0.00	0
100		3.70	3.70
500		0	0
1000		0	0
0	7	5.93	3.23
1		3.17	3.17
5		6.67	6.67
10		11.11	11.11
50		0.00	0
100		3.70	3.70
500		0	0
1000		0	0
0	9	13.33	10.18
1		12.48	3.80
5		6.67	6.67
10		14.81	9.80
50		0.00	0
100		3.70	3.70
500		0	0
1000		0	0
0	11	46.30	8.17
1		43.46	5.14
5		20.00	20.00
10		35.19	8.07
50		4.17	4.17
100		7.41	7.41
500		0	0
1000		0	0
0	14	56.67	13.47
1		64.25	3.74
5		53.33	29.06
10		56.02	3.62
50		22.97	6.01
100		7.41	7.41
500		0	0
1000		0	0
0	16	63.33	6.94
1		64.25	3.74
5		53.33	29.06
10		65.74	5.63
50		43.38	12.28
100		7.41	7.41
500		0	0
1000		0	0
0	18	68.89	5.88
1		68.89	5.88
5		53.33	29.06
10		71.30	8.23
50		57.05	22.33
100		11.11	11.11
500		0	0
1000		0	0

Freshwater Mollusk Biology and Conservation 19:80–87, 2016

REGULAR ARTICLE

ASSESSMENT OF A SHORT-DISTANCE FRESHWATER MUSSEL RELOCATION AS VIABLE TOOL DURING BRIDGE CONSTRUCTION PROJECTS

Jeremy S. Tiemann[1]*, Michael J. Dreslik[1], Sarah J. Baker[1], and Christopher A. Phillips[1]

[1] *Illinois Natural History Survey, Prairie Research Institute, University of Illinois, 1816 South Oak Street, Champaign, IL 61820 USA*

ABSTRACT

Freshwater mussels have undergone dramatic population declines due largely to habitat alteration. A commonly employed measure to minimize the effects of anthropogenic habitat disturbance on mussels is short-distance relocations of individuals. However, quantified survival data are lacking to gauge the success of relocations. To evaluate the suitability of short-distance relocations as a conservation tool for freshwater mussels, we experimentally relocated two common species, Mucket (*Actinonaias ligamentina*) and Plain Pocketbook (*Lampsilis cardium*), in an active construction zone. We marked 100 mussels with passive integrated transponders, released them ~200 m upstream of the construction site, and monitored them monthly throughout the spring and summer 2013-2015. We used Cormack-Jolly-Seber models to estimate apparent survival rates and found survival was lowest the first two months after relocation but increased and stabilized thereafter. Our models predict 93% of the relocated *A. ligamentina* and 71% of the *L. cardium* remained alive three years post-relocation. We conclude short-distance relocations are a viable minimization tool for protecting freshwater mussels at bridge construction sites, but further study is needed examine the factors driving the initial mortality.

KEYWORDS - relocation, translocation, bridge construction, habitat alteration, PIT tags

INTRODUCTION

The precipitous decline of freshwater mussels in North America has been well documented and is attributed to anthropogenic habitat alterations (Williams et al. 1993; Lydeard et al. 2004; Strayer et al. 2004). Despite efforts to conserve and protect remaining mussel populations, anthropogenic habitat alterations often continue to affect biologically significant areas. One example is the instream work, such as the creation of temporary dams or crane pads, required during construction of new bridges or repairing existing ones. Instream work can cause direct mortality of freshwater mussels in the construction zone, or indirect mortality through increased siltation or altered water levels (Oblad 1980; Trdan and Hoeh 1993).

According to the U.S. Department of Transportation, a quarter of the approximate 607,380 bridges in the United States are structurally deficient or functionally obsolete (Islam et al. 2014; Lo 2014). Therefore, one would expect an increased need for instream work for repairs or replacement, and thus an increased need for biological mitigation and disturbance minimization techniques to help conserve freshwater mussels (Miller and Payne 2006). Frequently, short-distance relocation of mussels out of the construction zone is the preferred minimization method as it is both time and cost-effective (Oblad 1980; Trdan and Hoeh 1993; Dunn and Sietman 1997). However, relocation effectiveness (e.g., recovery and survival) is not well documented (Cope and Waller 1995; Cope et al. 2003). Follow-up monitoring is often short-term, published in obscure gray literature, and fails to identify mortality or detectability rates (Cope and Waller 1995; Cope et al. 2003). Additionally, little is known regarding what environmental or species-specific factors affect relocation success. Therefore, despite its widespread use, little support exists for short-distance relocation as an effective minimization tool for protecting freshwater mussels at bridge construction sites.

*Corresponding Author: jtiemann@illinois.edu

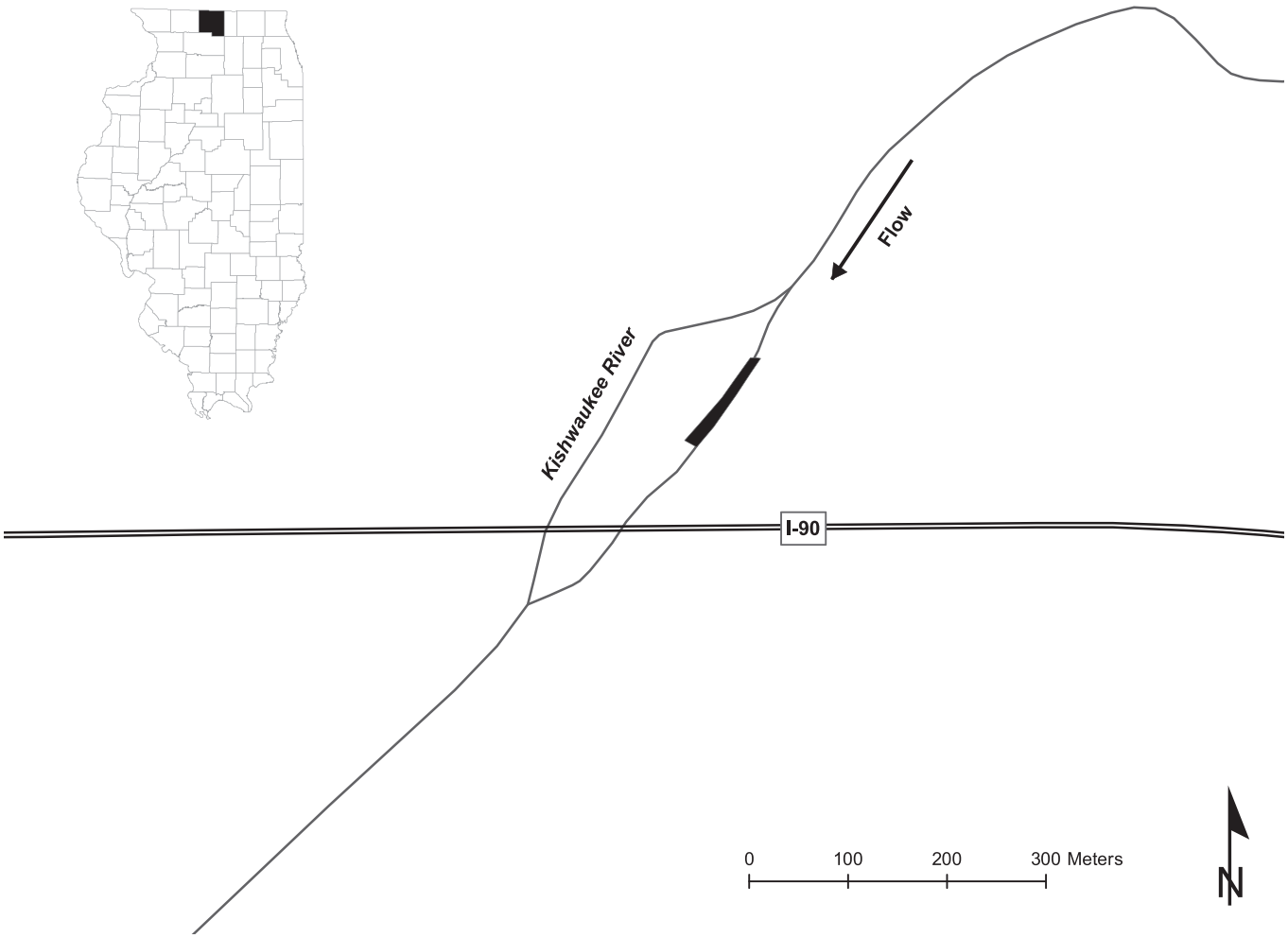

Figure 1. Kishwaukee River (Rock River drainage) at the Interstate 90 bridge, southeastern edge of Rockford, Winnebago and Boone counties, Illinois (42.24721°N, 88.94394°W). The blackened polygon indicates the relocation area.

To assess the efficacy of short-distance relocations of freshwater mussels, we experimentally relocated 100 individuals during a bridge reconstruction project on the Jane Addams Memorial Tollway (I-90) over the Kishwaukee River in northern Illinois. We estimated apparent survival rates for two mussel species over three years while examining several factors potentially influencing survival, including individual size, species, time and environmental measurements. Tracking apparent survival rates over a prolonged period allows us to better determine if short-distance relocations are a predictable and viable conservation tool for minimizing the effects of bridge construction on freshwater mussels.

METHODS

Study Area

The study site was located in the Kishwaukee River (Rock River drainage) at the Interstate 90 bridge, southeastern edge of Rockford, Winnebago and Boone counties, Illinois (Figure 1). The study area was bordered by land owned by the Winnebago County Forest Preserve District and the Boone County Conservation District. At base flow, the stream was approximately 53 m wide, 1 m deep, and had a flow rate of <0.15 m/sec. The streambed was sandy gravel; no aquatic vegetation or undercut banks were evident, but isolated, small patches of wood debris were present. This reach of the Kishwaukee River is biologically significant and rated as a Unique Aquatic Resource because of high freshwater mussel and fish diversity, including rare taxa (Bertrand et al. 1996; Shasteen et al. 2013). The Kishwaukee River basin is characterized by open oak woodland and prairie country on low undulating land, but the landscape is primarily agriculture with croplands accounting for nearly two-thirds of the surface area (Page et al. 1992; Shasteen et al. 2013). The flow of the Kishwaukee River is unimpeded except for a $\sim$3.5 m dam in Belvidere, approximately 10 km upstream of our study area (Page et al. 1992).

Table 1. List of the 22 additive models assembled to assess the survival of 100 relocated mussels in response to a short-distance relocation experiment in the Kishwaukee River at the I-90 bridge, Winnebago/Boone counties, Illinois. All models included intercepts (Int) for apparent survival rates and individual detection probabilities. Variable include species, time, shell length, maximum flow rate in the previous month, water depth at census, flow rate at census, and air temperature at census.

Covariates	Apparent Survival	Individual Detection
0 - Null	Int	Int
1	Int	Depth, Int
1	Int	Flow, Int
1	Int	Length, Int
1	Int	Species, Int
1	Int	Temp, Int
1	Int	time, Int
1	Length, Int	Int
1	Max Flow, Int	Int
1	Species, Int	Int
1	time, Int	Int
2	Length, Int	Length, Int
2	Species, Int	Species, Int
2	time, Int	time, Int
4	Species, time, Int	Species, time, Int
5	Species, time, Int	Species, time, Temp, Int
6	Species, time, Int	Species, Time, Depth, Temp, Int
6	time, Max Flow, Int	time, Depth, Flow, Temp, Int
7	Species, time, Int	Species, time, Depth, Flow, Temp, Int
8	Species, time, Max Flow, Int	Species, time, Depth, Flow, Temp, Int
10	Species, time, Length, Max Flow, Int	Species, time, Length, Depth, Flow, Temp, Int
14 - Global	Species, time, Length, Depth, Flow, Max Flow, Temp, Int	Species, time, Length, Depth, Flow, Max Flow, Temp, Int

Survey Techniques

We conducted a qualitative, haphazard survey of the freshwater mussel fauna in the Kishwaukee River at the I-90 bridge (Figure 1) in May 2013 before bridge reconstruction. The fauna comprised 15 species, including the state-threated Black Sandshell (*Ligumia recta*), but was dominated by the Mucket (*Actinonaias ligamentina*) and Plain Pocketbook (*Lampsilis cardium*). These two common species were used to assess apparent survival rates in response to a short-distance relocation. During the same 2013 survey, we collected 58 adult Muckets (85-137 mm, mean size = 115 mm) and 42 adult Plain Pocketbooks (17 females – 81-124 mm, mean size = 104 mm; and 25 males – 60-127 mm, mean size = 103 mm) in the vicinity of the I-90 bridge. Passive integrated transponder (PIT) tags are an effective tool for monitoring relocated mussels (Kurth et al. 2007), therefore, we externally outfitted individuals with 12.5 mm, 134.2 kHz PIT tags (BioMark, Inc., Boise, ID) using Devcon marine grade epoxy (Danvers, MA). Tagged mussels were held in damp towels overnight while the epoxy cured and then relocated the next morning, resulting in a handling time of approximately 16h. Tagged mussels were relocated to a 100 m area approximately 200 m upstream of the construction site in the eastern channel (Figure 1). We chose the eastern channel for relocation because habitat was comparable to the source site (e.g., sandy gravel run with moderate current), and we wanted to eliminate any siltation effects resulting from the bridge construction. Animals were deposited on the streambed surface and not buried. Marked individuals were monitored monthly with an aquatic PIT tag reading system (BioMark FS2001F-ISO or BioMark HPR Plus with portable BP antennas) from July-October 2013, May-October 2014 and April-September (sans June) 2015; weather and water conditions (e.g., ice or high flows) prohibited sampling at other times. We scanned the relocation area plus a 75 m buffer downstream for marked mussels during each monitoring event.

Statistical Analysis

We conducted survival analyses in R (R Core Team 2015) using the RMark package (Laake 2013) with Cormack-Jolly-Seber models. We modeled the effects of species, time, shell length (mm), maximum flow rate in the previous month (m/sec), water depth at census (m), flow rate at census (m/sec), and air temperature at census (°C) as covariates affecting individual detection probabilities and apparent survival rates (Table 1). Water-related covariates were taken from the nearby Kishwaukee River, Belvidere, IL gauging station (USGS 05438500) located approximately 9 km upstream and air temperature was taken on site. We fit 22 survival models, which included an intercept only model (null), global model (all covariates), all single effects models, and a series of step-

Table 2. AIC results for 22 Cormack-Jolly-Seber survival models including the global and null models for a short-distance relocation experiment for 100 mussels in the Kishwaukee River at the I-90 bridge, Winnebago/Boone counties, Illinois. Where Ψ = apparent survival, p = individual detection probability, K = number of parameters, S = Species, t = time, L= initial mussel length, D = depth, F = flow, MF = max flow, and T = temp.

Rank	Model	K	Deviance	AIC_C	ΔAIC_C	w_i
1	$\Psi_{(S+t)}$, $p_{(S+t)}$	32	1001.36	1765.52	0.00	1.00
2	Global	16	1751.01	1783.78	18.26	0.00
3	$\Psi_{(S+t+MF)}$, $p_{(S+t+D+F+T)}$	10	1767.68	1787.99	22.47	0.00
4	$\Psi_{(t)}$, $p_{(t)}$	30	915.26	1790.51	24.99	0.00
5	$\Psi_{(S+t+L+MF)}$, $p_{(S+t+L+D+F+T)}$	12	1766.74	1791.17	25.66	0.00
19	Null	2	1035.35	1851.93	86.41	0.00

wise models where we eliminated from the global model until we reached the species and time effects only (Table 1). To determine the best-fit model, we used an AIC approach (Burnham and Anderson 1998), whereby our 95% confidence set of candidate models included those with Akaike weights summing to 0.95. Finally, all graphics were produced using ggplot2 in R (Wickham 2009).

RESULTS

Of the 22 models analyzed, the top model included both species and time effects on apparent survival rates and individual detection probabilities (Table 2). The species and time model carried high support despite consisting of 32 parameter estimates (Table 2). None of the other 21 models had any significant support suggesting individual length and environmental covariates (depth, flow rate, maximum flow rate and temperature) had no discernable effects on apparent survival rates or individual detection probabilities (Table 2).

Individual detection probabilities varied by species and over time with probabilities lower for *A. ligamentina* versus *L. cardium*, but confidence intervals broadly overlapped (Table 3; Figure 2). Probabilities varied between 0.392 – 0.587 for *A. ligamentina* and 0.479 – 0.669 for *L. cardium* (Table 3; Figure 2). Although individual detection probabilities fluctuated, they appeared fairly stable (Table 3; Figure 2). We observed the lowest detection probabilities for the May 2014 sample and the highest for the May 2015 survey (Table 3; Figure 2).

Apparent survival rates differed for each species but showed little monthly variation (Table 3; Figure 3). Overall, the first two months post-relocation had the lowest apparent survival rates for both species (Table 3; Figure 3). The apparent survival rates rapidly increased thereafter, except for a small decrease that occurred around the time the earthen causeway at the bridge was removed post-construction (Table 3; Figure 3). For *A. ligamentina*, apparent survival rates were lowest between the first two survey transitions ($\sim$0.966) then rose to $\sim$1.000 survival throughout the remainder of the study (Table 3; Figure 3). Apparent survival rates for *L. cardium* were lowest between the first two survey transitions ($\sim$0.848) but then rapidly rose to $\sim$0.995 (Table 3; Figure 3). From our initial relocation of 58 *A. ligamentina* and 42 *L. cardium*, our models predict we have 54 (95% C.I. 45,56) and 30 (95% C.I. 14,35) surviving individuals of each species, respectively, and equates to 93.1% (77.6% – 95.6%) of the relocated *A. ligamentina* and 71.4% (33.3% – 83.3%) of the *L. cardium* surviving to the last survey.

DISCUSSION

Our data suggested short-distance relocation is a viable tool for mussel conservation but will not eliminate all mortality. In our study, *A. ligamentina* and *L. cardium* had comparable detection rates and our models predicted 93% of the relocated *A. ligamentina* and 71% of the *L. cardium* were alive three years post-relocation. Previous studies have shown recovery (=detectability) and survival rates are highly variable among relocations and are dependent upon biotic and abiotic factors, including environmental conditions and handling stress (Dunn et al. 2000; Bolden and Brown 2002; Villella et al. 2004). In a review of 33 papers on mussel relocation, Cope and Waller (1995) reported a mean mortality of relocated mussels at 49% based on an average recovery rate of 43%. Recovery and survival rates have been reported as low as $<$10% (Sheehan et al. 1989; Cope and Waller 1995, and references therein) and as high as $>$90% (Dunn and Sietman 1997; Peck et al. 2014). In our study, the greatest mortality occurred the first two months post-relocation.

Survivorship

Four stress related factors can explain the early decrease in apparent survival rates for the relocated individuals, but unfortunately, they are not mutually exclusive. First, some mussels might already have been in a stressed state given localized construction activities, and/or simply were in poorer body condition before relocation. Second, our prolonged handling time might have exacerbated or initiated a stressed condition of the mussels. Third, animals became stressed when placed in unfamiliar habitat in the release area. Finally, our placement did not include burying mussels; thus, they might have incurred additional stress seeking proper refuge. All four stress related factors could have individually, or more likely synergistically, manifested in the initial decrease in apparent survival rates. Of the four factors, we feel the first two coupled together – poor body condition and prolonged handling time –

Table 3. Transformed parameter estimates (real), standard errors, and 95 % confidence intervals for the species and time Cormack-Jolly-Seber survival model.

Individual Detection Probability

	Actinonias ligamentina				*Lampsilis cardium*			
Sample	Est.	S_{err}	Lower CI	Upper CI	Est.	S_{err}	Lower CI	Upper CI
Jul 2013	0.518	0.039	0.442	0.593	0.605	0.040	0.523	0.681
Aug 2013	0.445	0.038	0.372	0.521	0.533	0.042	0.451	0.614
Sep 2013	0.559	0.036	0.488	0.627	0.643	0.037	0.567	0.713
Oct 2013	0.576	0.041	0.495	0.653	0.659	0.041	0.575	0.734
May 2014	0.392	0.044	0.310	0.481	0.479	0.049	0.384	0.575
Jun 2014	0.531	0.028	0.475	0.586	0.617	0.033	0.552	0.679
Jul 2014	0.562	0.026	0.511	0.612	0.647	0.030	0.586	0.703
Aug 2014	0.562	0.027	0.509	0.614	0.647	0.031	0.584	0.704
Sep 2014	0.526	0.033	0.461	0.591	0.613	0.037	0.539	0.681
Oct 2014	0.579	0.032	0.515	0.640	0.662	0.034	0.591	0.725
Apr 2015	0.516	0.032	0.452	0.578	0.603	0.037	0.529	0.672
May 2015	0.587	0.030	0.528	0.644	0.669	0.033	0.601	0.731
Jul 2015	0.497	0.036	0.427	0.567	0.584	0.041	0.502	0.661
Aug 2015	0.574	0.037	0.500	0.644	0.657	0.040	0.575	0.731
Sep 2015	0.575	0.032	0.511	0.637	0.658	0.036	0.584	0.725

Apparent Survival Rates

	Actinonias ligamentina				*Lampsilis cardium*			
Transition	Est.	S_{err}	Lower CI	Upper CI	Est.	S_{err}	Lower CI	Upper CI
Jun 2013 - Jul 2013	0.965	0.016	0.915	0.986	0.847	0.043	0.743	0.913
Jul 2013 - Aug 2013	0.966	0.016	0.916	0.986	0.848	0.044	0.742	0.915
Aug 2013 - Sep 2013	0.998	0.001	0.991	1.000	0.992	0.006	0.964	0.998
Sep 2013 - Oct 2013	0.999	0.001	0.993	1.000	0.993	0.005	0.970	0.998
Oct 2013 - May 2014	0.999	0.001	0.994	1.000	0.993	0.005	0.974	0.998
May 2014 - Jun 2014	0.996	0.002	0.988	0.999	0.981	0.009	0.952	0.993
Jun 2014 - Jul 2014	0.999	0.001	0.996	1.000	0.993	0.004	0.981	0.998
Jul 2014 - Aug 2014	0.997	0.002	0.991	0.999	0.987	0.008	0.959	0.996
Aug 2014 - Sep 2014	0.999	0.001	0.996	1.000	0.995	0.003	0.984	0.999
Sep 2014 - Oct 2014	0.999	0.001	0.996	1.000	0.995	0.003	0.984	0.999
Oct 2014 - Apr 2015	0.999	0.001	0.997	1.000	0.996	0.003	0.986	0.999
Apr 2015 - May 2015	0.998	0.002	0.986	1.000	0.992	0.009	0.933	0.999
May 2015 - Jul 2015	0.999	0.001	0.994	1.000	0.996	0.004	0.971	1.000
Jul 2015 - Aug 2015	0.998	0.003	0.966	1.000	0.989	0.016	0.847	0.999
Aug 2015 - Sep 2015	1.000	0.001	0.994	1.000	0.998	0.003	0.972	1.000

likely caused stress-induced mortality. We did not collect hemolymph to measure physiological responses so we can only speculate the cause.

We feel some of the individuals might have been in a stressed state and were in poor body condition before our relocation, which occurred only a few months after the drought of 2011–2012 subsided. During the drought, the Kishwaukee River did not dry completely, but several hundred dead and dying mussels were found on exposed areas in our source area while others appeared lethargic (e.g., slow to respond to shadows and touch) in water temperatures exceeding 35°C (J.S. Tiemann, personal observation). Drought, with its extended periods of high water temperatures and reduced stream velocity, could have adversely affected physiological responses and might have decreased the amount of energy available for key biological processes, such as survival (Gasner et al. 2015; Vaughn et al. 2015).

A likely second coupling factor was our handling time. Reducing handling time can become tricky and potentially problematic if animals need to be marked to allow monitoring. Dunn et al. (2000) recommended reducing handling times and avoiding extreme temperature conditions while keeping the

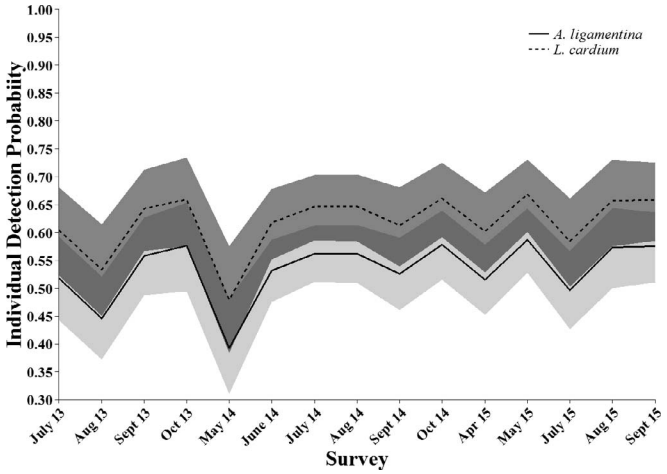

Figure 2. Individual detection probabilities by species to survey, with 95% confidence intervals shaded, a short-distance relocation experiment for 100 mussels in the Kishwaukee River at the I-90 bridge, Winnebago/Boone counties, Illinois.

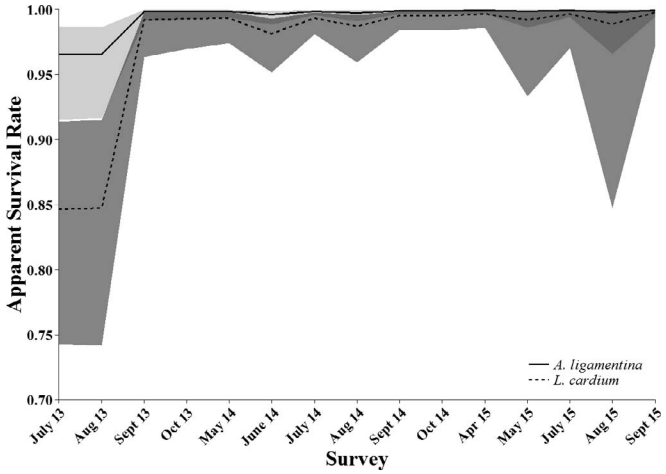

Figure 3. Apparent survival rates by species to survey, with 95% confidence intervals shaded, a short-distance relocation experiment for 100 mussels in the Kishwaukee River at the I-90 bridge, Winnebago/Boone counties, Illinois.

animals moist when conducting relocations. However, the use of PIT tags requires more handling time than other methods, such as plastic tags or glitter glue, because most epoxies need ~12 h to cure completely. Future projects affixing PIT tag with epoxy or cement should consider faster-drying brands (e.g., Fuji Glass Ionomer Luting Cement recommended by Hua et al. 2015) to reduce holding time. We do not feel the mass of the epoxied PIT tag caused stress given the sizes of mussels (mean size was 115 mm for *A. ligamentina* and 103 mm for *L. cardium*) and the minimal amount of epoxy used for the 12.5 mm tags. Although a potentially large upfront cost (e.g., purchasing readers and tags, plus manpower to affix tags), monitoring can be less costly (e.g., less manpower to monitor) and can be done when conditions are less favorable (e.g., slightly turbid or cold waters) compared to hand-picking for animals marked in some other manner (e.g., plastic tags or glitter glue). We believe that PIT tags have several advantages over other methods (e.g. plastic tags) that justify the longer handling times, mainly the two-fold recovery rate over visual tags (Kurth et al. 2007).

We do not believe unfamiliar habitat in the release area caused an initial reduction in apparent survival rate. The lower Kishwaukee River, including both the construction zone and the relocation area upstream of the bridge, is predominantly sandy gravel runs with moderate flow and mussel densities <1 individual/m^2 (J.S. Tiemann, unpublished data). Per the recommendations of previous studies (e.g., Dunn and Sietman 1997; Dunn et al. 2000), the relocation area consisted of suitable habitat and was large enough to harbor both the resident fauna and individuals being relocated. Habitat stability and diversity in the relocation area is a critical factor because the type of preferred habitat varies by species being relocated (Sheehan et al. 1989; Dunn 1993; Dunn and Sietman 1997). Selection of suitable relocation sites should be species specific if quantitative information on the habitat requirements of individual species is known (Cope and Waller 1995; Hamilton et al. 1997). The benefit of short-distance, intra-stream relocations can often help eliminate issues with habitat similarity and suitable host fishes (Havlik 1997).

We do not feel our placement method of relocated mussels caused a reduction in apparent survival rates. Our placement method was not extraneous and was similar to standard practices in Illinois (K.S. Cummings, Illinois Natural History Survey, personnel communication). However, several previous projects involving either PIT tags (e.g., Newton et al. 2015) or relocation (e.g., Dunn et al. 2000) hand planted mussels. Therefore, future projects could assess the differences in placement methods (e.g., burying mussel vs. depositing them on the streambed surface).

The lower apparent survival rate of *L. cardium* should be approached with caution. Most (six of nine) dead individuals were discovered after the earthen causeway was removed, which was three years post-relocation; all of these individuals were recorded alive at least one to two months post-relocation. We are reluctant to speculate the cause of this observation. One possibility is once the causeway was breached, a sudden pulse in water and subsequent drop in water levels caused mussels to become dislodged and potentially stranded in unsuitable areas.

Longitudinal Movements and Detection

Twenty individuals were detected outside of the study area, including one detected in the relocation area in August 2015 but located ~50 m downstream of the relocation area in October 2015 (individual not found in September 2015). While considered sessile organisms, mussels, including *L. cardium*, are known to move >10 m / week during warmer periods (Newton et al. 2015). Relocated mussels have been reported to move at greater rates perhaps to seek more suitable

habitat (Bolden and Brown 2002; Peck et al. 2014). However, as time elapses, the movement differences can become non-significant (Peck et al. 2014).

Seventeen individuals were not detected during our study post-release. There are several possible reasons, including predation, tag failure, or mussels moving or being swept beyond our monitoring area. PIT tags decrease burrowing rates, thus increasing the time needed to burrow into the substrate, and thereby increasing the risk of predation or dislodgement during flooding (Wilson et al. 2011). Peck et al. (2014) suggested relocated mussels can be highly susceptible to mammalian predation as a result of increased vulnerability during extremely low water levels. We did not sample the riparian areas for shell middens so we cannot comment on predation. During our July 2013 monitoring event, one tagged *L. cardium* was found while snorkeling but the tag failed to register in the PIT tag reader. We assumed the glass case was compromised post-release. Lastly, we cannot rule out some animals moved upstream of the study area as witnessed by both Bolden and Brown (2002) and Peck et al. (2014). As noted above, mussels can move vast distances in a short period. Future studies could sample riparian areas for middens, as well as sampling buffer areas upstream and downstream of the relocation area, to increase detection rates and strengthen apparent survival rates.

Conservation Implications

The goal of relocation is to collect and relocate mussels in a cost-effective manner while ensuring high survival of the relocated individuals without jeopardizing the resident fauna (Havlik 1997). We recommend at least three years of post-release monitoring to assess apparent survival rates, similar to the recommendations of others (e.g., Cope and Waller 1995; Havlik 1997; Villella et al. 2004). Monitoring for three years not only increases the chances to document reproductive success but also increases the chances of detecting individuals (Cope and Waller 1995; Havlik 1997). Ten individuals went undetected the first two years following relocation only to be found at least once during the third year. Data such as these could affect survival estimates because of individual detection issues (Nichols 1992; Villella et al. 2004). Detecting unaccounted individuals refines survivorship estimates and provides a better estimate of the relocation success (Layzer and Gordon 1993; Cope and Waller 1995; Villella et al. 2004).

Future studies could address the effects of initial mortality by collecting hemolymph during initial relocation and some defined time-period after (e.g., 2 months post-relocation) to examine body condition and measure physiological responses to relocation. In addition, testing for effects of different placement methods (e.g., burying mussel vs. depositing them on the streambed surface) on relocation survival is important. These studies could help explain potential stress related factors that might cause a reduction in apparent survival rates post-relocation. Lastly, if earthen causeways are needed, relocations areas should be placed outside the direct zone of influence to negate any possible effects of the impounded waters or subsequent dam removal. Natural resource agencies should work with construction companies on the timing of construction activities to increase survival of relocated animals. One example is being on site for rescue operations as a causeway is removed.

Future construction relocation work similar to our project should be considered in an objective manner and not a method to circumvent protective conservation legislation (Havlik 1997; Cosgrove and Hastie 2001). Relocations can be simple but are often labor-intensive and time-consuming and require various permits, especially when dealing with threatened and endangered species (Havlik 1997; Miller and Payne 2006). However, by following the steps outlined here and by others (e.g., Dunn and Sietman 1997; Dunn et al. 2000), short-distance mussel relocation can be a viable minimization tool for protecting freshwater mussels during bridge construction projects.

ACKNOWLEDGMENTS

This study was funded in by the Illinois State Toll Highway Authority under the guidance of B. Wagner. C. Butkus, T. Dallas, S. Douglass, A. Haskett, C. Henry, E. Kessler, K. Low, E. Moen, J. Ross, C. Schmidt, J. Sherwood, A. Stites, A. Stodola, M. Van Der Bosch, and R. Vinsel of the INHS assisted in collecting. J. Laake, the creator of the RMark package, provided insightful discussions and assistance with constructing the multifactored models. D. Kane with the Boone County Conservation District and M. Holan and T. Hartley from the Winnebago County Forest Preserve District assisted with obtaining site permits and access to their property. J. Jarvis of the INHS created the study area map. All work was conducted under permits from the Winnebago and Boone County Forest Preserve Districts.

LITERATURE CITED

Bertrand, W. A., R. L. Hite, and D. M. Day. 1996. Biological Stream Characterization (BSC): Biological assessment of Illinois stream quality through 1993. Biological Streams Characterization Work Group, Springfield, Illinois. 44 pp.

Bolden, S. R., and K. M. Brown. 2002. Role of stream, habitat, and density in predicting translocation success in the threatened Louisiana pearlshell, *Margaritifera hembeli* (Conrad). Journal of the North American Benthological Society 21:89-96.

Burnham, K. P., and D. R. Anderson. 1998. Model selection and inference: a practical information-theoretical approach. Springer-Verlag, New York. 488 pp.

Cope, W. G., and D. L. Waller. 1995. Evaluation of freshwater mussel relocation as a conservation and management strategy. Regulated Rivers: Research and Management 11:147-155.

Cope, W. G., M. C. Hove, D. L. Waller, D. J. Hornbach, M. R. Bartsch, L. A. Cunningham, H. L. Dunn, and A. R. Kapuscinski. 2003. Evaluation of relocation of unionid mussels to in situ refugia. Journal of Molluscan Studies 69:27-34.

Cosgrove, P. J., and L. C. Hastie. 2001. Conservation of threatened freshwater pearl mussel populations: river management, mussel translocation and conflict resolution. Biological Conservation 99:183-190.

Dunn, H. L. 1993. Survival of unionids four years after relocation. Pages 93-99 *in* K. S. Cummings, A. C. Buchanan, and L. M. Koch, editors. Conservation and Management of Freshwater Mussels. Proceedings of a UMRCC Symposium, 12-14 October 1992, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois. 189 pp.

Dunn, H. L., and B. E. Sietman. 1997. Guidelines used in four geographically diverse unionid translocations. Pages 176-183 *in* K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo, editors. Conservation and management of freshwater mussels II: Initiatives for the future. Proceedings of a UMRCC Symposium, 16-18 October 1995, St. Louis Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois. 293 pp.

Dunn, H. L., B. E. Sietman, and D. E. Kelner. 2000. Evaluation of recent unionid (Bivalvia) relocations and suggestions for future relocations and reintroductions. Pages 169-183 *in* R. A. Tankersley, D. I. Warmolts, G. T. Watters, B. J. Armitage, P. D. Johnson, and R. S. Butler, editors. Freshwater Mollusk Symposia Proceedings. Part II. Proceedings of the First Freshwater Mollusk Conservation Society Symposium. Ohio Biological Survey Special Publication, Columbus. 274 pp.

Gasner, A. M., T. J. Newton, and R. Haro. 2015. Effects of elevated water temperature on physiological responses in adult freshwater mussels. Freshwater Biology 60:1705-1716.

Hamilton, H., J. Brim Box, and R. M. Dorazio. 1997. Effects of habitat suitability on the survival of relocated freshwater mussels. Regulated Rivers: Research and Management 13:537-541.

Havlik, M. E. 1997. Are unionid translocations a viable mitigation technique? The Wolf River, Wisconsin, experience, 1992 – 1995. Pages 184-195 *in* K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo, editors. Conservation and management of freshwater mussels II: Initiatives for the future. Proceedings of a UMRCC Symposium, 16-18 October 1995, St. Louis Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois. 293 pp.

Hua, D., Y. Jiao, R. Neves, and J. Jess. 2015. Use of PIT tags to assess individual heterogeneity of laboratory-reared juveniles of the endangered Cumberlandian combshell (*Epioblasma brevidens*) in a mark–recapture study. Ecology and Evolution 5:1076–1087.

Islam, A., H. F. Hamid, and F. Li. 2014. Bridge condition assessment from dynamic response collected using wireless sensor networks. Proc. SPIE 9064, Health Monitoring of Structural and Biological Systems 2014, 90640B (March 9, 2014); doi:10.1117/12.2044265

Kurth, J., C. Loftin, J. Zydlewski, and J. Rhymer. 2007. PIT tags increase effectiveness of freshwater mussel recaptures. Journal of the North American Benthological Society 26:253-260.

Laake, J. L. 2013. RMark: An R interface for analysis of capture-recapture data with MARK. Alaska Fisheries Science Center Processed Report 2013-01. 25 pp.

Layzer, J. B., and M. E. Gordon. 1993. Reintroduction of mussels into the Upper Duck River, Tennessee. Pages 89–92 *in* K.S. Cummings, A.C. Buchanan, and L.M. Koch, editors. Conservation and Management of Freshwater Mussels. Proceedings of a UMRCC Symposium, 12-14 October 1992, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois. 189 pp.

Lo, S. 2014. In flux: America's transportation infrastructure. Defense Transportation Journal 2014(February):18-19.

Lydeard, C., R. H. Cowie, W. F. Ponder, A. E. Bogan, P. Bouchet, S. A. Clark, K. S. Cummings, T. J. Frest, O. Gargominy, D. G. Herbert, R. Hershler, K. E. Perez, B. Roth, M. Seddon, E. E. Strong, and F. G. Thompson. 2004. The global decline of nonmarine mollusks. Bioscience 54:321-330.

Miller, A. C., and B. S. Payne. 2006. A retrospective analysis of a large-scale endangered species translocation project. Environmental Practice 8:115-124.

Newton, T. J., S. J. Zigler, and B. R. Gray. 2015. Mortality, movement and behaviour of native mussels during a planned water-level drawdown in the Upper Mississippi River. Freshwater Biology 60:1-15.

Nichols, J. D. 1992. Capture-recapture models. BioScience 42:94-102.

Oblad, B. R. 1980. An experiment in relocating endangered and rare naiad mollusks from a proposed bridge construction site at Sylvan Sough, Mississippi River near Moline, Illinois. Pages 211-222 *in* J. Rasmussen, editor. Proceedings of the UMRCC Symposium on Upper Mississippi River Bivalve Mollusks. Upper Mississippi River Conservation Committee, Rock Island, Illinois. 270 pp.

Page, L. M., K. S. Cummings, C. A. Mayer, S. L. Post, and M. E. Retzer. 1992. Biologically significant Illinois streams. An evaluation of the streams of Illinois based on aquatic biodiversity. Illinois Natural History Survey, Center for Biodiversity Technical Report 1992(1). 485 pp.

Peck, A. J., J. L. Harris, J. L. Farris, and A. D. Christian. 2014. Survival and horizontal movement of the freshwater mussel *Potamilus capax* (Green, 1832) following relocation within a Mississippi Delta stream system. American Midland Naturalist 172:76-90.

R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. url - https://www.R-project.org/

Shasteen, D. K., S. A. Bales, and A. P. Stodola. 2013. Freshwater mussels of the Rock River tributaries: Pecatonica, Kishwaukee, and Green river basins in Illinois. Illinois Natural History Survey Technical Report 2013(13):1-27.

Sheehan, R. J., R. J. Neves, and H. E. Kitchel. 1989. Fate of freshwater mussels transplanted to formerly polluted reaches of the Clinch and North Fork Holston rivers, Virginia. Journal of Freshwater Ecology 5:139-149.

Strayer, D. L., J. A. Downing, W. R. Haag, T. L. King, J. B. Layzer, T. J. Newton, and S. J. Nichols. 2004. Changing perspective on pearlymussels, North America's most imperiled animals. BioScience 54:429-439.

Trdan, R. J., and W. R. Hoeh. 1993. Relocation of two state-listed freshwater mussel species (*Epioblasma torulosa rangiana* and *Epioblasma triquetra*) in Michigan. Pages 100-105 *in* K. S. Cummings, A. C. Buchanan, and L. M. Koch, editors. Conservation and Management of Freshwater Mussels. Proceedings of a UMRCC Symposium, 12-14 October 1992, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, Illinois. 189 pp.

Vaughn, C. C., C. L. Atkinson, and J. P. Julian. 2015. Drought-induced changes in flow regimes lead to long-term losses in mussel-provided ecosystem services. Ecology and Evolution 5:1291-1305.

Villella, R. F., D. R. Smith, and D. P. Lemarie. 2004. Estimating survival and recruitment in a freshwater mussels population using mark-recapture techniques. American Midland Naturalist 151:114-133.

Wickham, H. 2009. Ggplot2: Elegant graphics for data analysis. Springer, New York, New York.

Williams, J. D., M. L. Warren, Jr., K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18(9):6-22.

Wilson, C. D., G. Arnott, N. Reid, and D. Roberts. 2011. The pitfall with PIT tags: marking freshwater bivalves for translocation induces short-term behavioural costs. Animal Behaviour 81:341-346.