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HOW A STATEWIDE STREAM SURVEY CAN AID IN UNDERSTANDING FRESHWATER MUSSEL (BIVALVIA: UNIONIDAE) ECOLOGY: EXAMPLES OF UTILITY AND LIMITATIONS FROM MARYLAND

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ABSTRACT

Gaps in our knowledge of freshwater mussel life history, distribution, and ecology remain even though their study has increased considerably over the past few decades. These studies have traditionally taken place within a population, river, or larger drainage unit, but rarely across a broad landscape, such as a state. Given the imperiled status of a majority of unionid species alternative opportunities to collect valuable data cannot be overlooked. We present results from a statewide biological monitoring program (Maryland Biological Stream Survey) that has incorporated a visual survey for mussels, several example analyses using mussel-bioassessment data, and discuss the utility and limitations of incorporating freshwater mussels into stream assessments. Since 2007, we encountered 11 of the 16 mussel species extant in Maryland during assessments of wadeable streams by using an informal visual survey and recording incidental observations. On several occasions, we have discovered new populations of imperiled mussels or extended a species distribution. The biological and physiochemical data collected at sites coincident with freshwater mussels have allowed us to hypothesize factors potentially limiting species distribution, such as fish-host dynamics, habitat quality, nutrient concentration, and catchment land use. We feel that the addition of a survey effort into a biological monitoring program, invaluable data can be collected that can assist resource managers, malacologists, and researchers answer a variety of questions. Further investigation into the cost-benefits of an appropriate level of sampling effort is needed as this could vary markedly among molluscan faunal regions and by objectives.

KEY WORDS Freshwater mussels, Unionidae, biological monitoring, Maryland Biological Stream Survey

INTRODUCTION

The diversity of freshwater mussels (Bivalvia: Unionidae) in North America is unmatched globally, yet they are among the most imperiled aquatic fauna on the continent (Williams et al., 1993; Bogan, 2008). The high rate of imperilment and extinction in mussels has been linked to habitat and flow alteration, invasive species, loss of host fish, increased siltation, and dam construction (Brim Box & Mossa, 1999; Strayer, 1999a; Vaughn & Taylor, 1999; Watters, 2000). Poor land use practices and pollution have further disrupted freshwater ecosystems ultimately leading to the decline of mussels (Bogan, 1993). This decline has likely had major implications on functioning aquatic ecosystems along with the management, conservation, and restoration of aquatic species. Even though the study of freshwater mussels has increased over the past few decades their conservation still faces several challenges. Foremost, basic life history and distributional information of mussels are lacking for many species (Neves, 1993; Strayer, 2006). Potentially exacerbating this problem is that subjective observations about ecological factors that govern unionid presence typically do not agree with results from quantitative studies (Strayer, 2008). In spite of this, research into the life history and autecology of freshwater invertebrates has declined (Resh & Rosenberg, 2010).

Unionids have long been considered indicators of good water quality (Ortmann, 1909; Neves, 1993), but there is little guidance for resource agencies on ways to utilize them in assessments of stream health (Grabarkewicz & Davis, 2008). The Maryland Biological Stream Survey (MBSS) is a statewide biological monitoring and assessment program administered by the Maryland Department of Natural Resources' Monitoring and Non-Tidal Assessment Division, which has incorporated freshwater mussels into standard operation procedures (Stranko et al., 2007). Objectives of the MBSS are to assess the condition of aquatic resources, identify physiochemical and anthropogenic stressors such as acidification, land alteration, and climate change, and provide an inventory of biodiversity in Maryland's streams (Klauda et al., 1998; Stranko et al., 2005). This is primarily accomplished through a probabilistic design

to make unbiased estimates on the condition of the states' (1st-4th order) wadeable streams (Heimbuch et al., 1999), but has recently included other sampling designs tailored to meet resource management needs.

Such spatially extensive, readily available data sets may be useful in developing empirical models of multiple stressors that can guide future studies with more detailed and costly methods that test mechanistic hypotheses of mussel conservation and ecology (Strayer, 2008). In this study, we present results from the MBSS and offer simple analytical examples that could be conducted with mussel-bioassessment data that can address gaps in freshwater mussel ecology and conservation (National Native Mussel Conservation Committee, 1998; Strayer, 2006). Additionally, we discuss the utility of incorporating mussels into stream monitoring and assessment programs and the limitations that such an endeavor faces.

METHODS

Water chemistry grab samples were collected from the upstream extent of 75-m-long sites during spring (March through April) base-flow conditions and analyzed for pH, acid neutralizing capacity (μeq/L), specific conductance (μS/cm), chloride (mg/L), sulfate (mg/L), total nitrogen (mg/L), ammonia (mg/L), nitrate (mg/L), and total phosphorus (mg/L), using methods described by the U.S. EPA (1986). Water temperature was recorded at 20 minute intervals from June to September with Hobo data-loggers (Onset Corporation) deployed at each site. From these data, we calculated an average of the daily mean temperature (N ≈ 92). Gradient (% slope) was calculated as the change in water surface height between the up and downstream extent of a site using a surveyor's level and metric stadia. Benthic macroinvertebrate samples were collected with a 540 μm D-net from 20, 0.3 m² areas of proportionally available optimal habitat to calculate a benthic macroinvertebrate index of biotic integrity (Stribling et al., 1998).

During summer base-flow conditions, we collected fishes within each site using two-pass depletion with backpack electrofishing units (one anode/3 m of wetted stream width) to calculate a fish index of biotic integrity based on a scale of 1-5 (very poor < 2, poor 2 < 3, fair 3 < 4, and good > 4) (Southerland et al., 2007). From these data, we also calculated the abundance of freshwater mussel host-fishes (Kneeland & Rhymer, 2008; Cummings & Watters, 2010). We visually estimated physical habitat quality using five metrics scored on a 0-20 scale (poor = 0-5, marginal 6-10, suboptimal 11-15, and optimal 16-20): instream habitat, epifaunal substrate, velocity depth diversity, pool-glide quality, and riffle-run quality. Scores of each metric are meant to characterize aspects of habitat important to stream biota. For example, the instream habitat metric relates to the quality and quantity of fish habitat, while the epifaunal substrate metric rates the suitability of benthic macroinvertebrate habitat. Scores for velocity depth diversity and quality of pool-glide and riffle-run habitats are based on the heterogeneity and extent of those habitats. Riffle embeddedness was determined by estimating the percentage of gravel and larger substrates surrounded by fine sediment (< 2 mm). Average stream width (m) was calculated from the wetted width taken at four equally distant transects within the sample reach. Stream flow was measured with a Marsh McBirney FloMate 2000 on a top-setting metric wading rod. Discharge (m³/sec) was then calculated from the cross sectional area of the stream. We hand digitized the catchment upstream from each site based on United States Geological Survey 7.5 minute quarter quad topographic maps using ArcMap 9.3, and calculated drainage area (km²). We then intersected satellite-derived land cover (2001 NLCD; Homer et al., 2007) to catchments and calculated the percent of major land cover types (urban, agriculture, and forest) within catchments.

While at each MBSS site, we searched suitable unionid habitats for ≥ 15 minutes to determine the presence of mussels. Additionally, we searched animal middens when present and noted incidental observations of unionids while sampling other aquatic fauna. When a live mussel was encountered, it was identified and returned to the location of its collection. Representative shells were retained to verify field identifications. A subset of these vouchers were independently verified by the state zoologist and deposited in several museum collections (Delaware Museum of Natural History, Illinois Natural History Survey, and North Carolina Museum of Natural Sciences). The remaining vouchers were housed at MDNR offices in Annapolis, MD and Frostburg, MD for training purposes. Freshwater mussel taxonomy in Maryland follows Turgeon et al. (1998). Annually, field crew managers and leaders receive thorough training in mussel identification along with other taxonomic groups for which data are recorded during MBSS sampling.

We described environmental conditions at sites with *Elliptio fisheriana* (Lea, 1838) to sites where they were apparently absent throughout their range with non-parametric pair-wise comparisons. Continuous variables (water chemistry, habitat, and land use) were compared with Kolmogorov-Smirnov tests and categorical variables (physical habitat metrics and biological multi-metric index scores) with Mann-Whitney U tests. We chose this species as an example as it is restricted to one physiographic region (Coastal Plain); therefore,

conditions should be relatively homogenous (Stribling et al., 1998; Southerland et al., 2007). Absence was presumed if no mussels were detected and present if live or dead specimens were collected. At sites that were sampled on more than one occasion, a species was also assumed present if it was previously encountered. For this study, we defined a species range as the sites within watersheds (Maryland 8-digit) in which we encountered at least one individual of that species. To investigate the role of known and potential fish-hosts on patterns of *E. fisheriana* presence, we calculated the frequency of occurrence for stream fishes collected during MBSS surveys.

RESULTS

From 2007 to 2009, we encountered unionids at 117 of the 595 MBSS sites sampled (20%). At a minimum, 148.75 person-hours were expended searching for mussels. We made 133 observations of live freshwater mussels or dead shell material representing 11 species (Table 1); however most species were encountered infrequently. *Elliptio complanata* (Lightfoot, 1786) was by far the most widely distributed and frequently encountered unionid during MBSS sampling. *Elliptio fisheriana* was the second most encountered species, followed by *Pyganodon cataracta* (Say, 1817), and *Alasmidonta heterodon* (Lea, 1830). The remaining six species were found at < 5 MBSS sites since 2007. Five species, including the state endangered *Alasmidonta varicosa* (Lamarck, 1819) and *Lasmigona subviridis* (Conrad, 1835), have yet to be found during stream assessments.

Freshwater mussel richness in wadeable streams throughout Maryland's 8-digit watersheds was generally low (Figure 1). The most diverse assemblages were generally found in coastal streams on Maryland's Eastern shore, although two Potomac River watersheds also had relatively diverse assemblages for Maryland streams. We rarely found live mussels or spent valves at sites in central and western Maryland. Some notable distributional records resulting from MBSS surveys include the first records of *Elliptio producta* (Conrad, 1836) in the Upper Patuxent River watershed, the range extension of *Alasmidonta undulata* (Say, 1817) in the Patapsco River, and discovery of a relic population of *A. heterodon* in the Upper Choptank River watershed.

Significant differences were found for most physiochemical and biological variables compared between sites with *E. fisheriana* and sites where they were apparently absent (Table 2). When *E. fisheriana* was encountered at MBSS sites, pH, ANC, and nutrient concentrations were higher. These streams were also on average several meters wider, had considerably larger upstream catchments, lower gradient, and greater discharge compared to streams where *E. fisheriana* was not found. Physical habitat metrics were consistently several points higher and often in categories that represented better conditions. Fish and benthic macroinvertebrate community indices were also higher at sites with *E. fisheriana* compared to other sites in their range. Differences observed in the amounts and types of catchment land use were likely representative of prevailing land use patterns than a biological response.

Previously confirmed fish-hosts were frequent to absent at sites where *E. fisheriana* was encountered and uncommon to frequent at sites where they were not encountered (Table 3). Two of these species (Bluegill and Largemouth Bass) are not native, while the other (Johnny Darter) is not native to Maryland's Atlantic Slope. While Largemouth Bass were frequently collected at sites with *E. fisheriana*, only a few bass were typically found at a site. Several native fishes (Tessellated Darter, Pumpkinseed, and Redbreast Sunfish) that are congenerics of *E. fisheriana* host-fish had rates of occurrence as high to nearly double their respective non-native relative. In addition, these native fishes were infrequently collected at sites where *E. fisheriana* was also not found. Other than American Eel, we rarely collected host-fish of congeneric mussels at sites along with *E. fisheriana*.

DISCUSSION

By instituting a simple visual survey at all MBSS sites we have been able to readily collect valuable distributional data concurrent with an array of biological and physiochemical data that address several continuing challenges to freshwater mussel conservation (National Native Mussel Conservation Committee, 1998). Our basic analysis illustrates just one example how the information garnered from stream assessments that include measures of freshwater mussels can be used and lays the foundation for more rigorous hypothesis development. The growing data set will be instrumental in addressing aspects of freshwater mussel management and conservation, such as describing species habitat associations and tolerances to anthropogenic stressors, such as nutrients and urbanization (MDNR, 2005). Within the context of streams on Maryland's Coastal Plain, it appears as though *E. fisheriana* cannot tolerate the conditions of naturally acid, blackwater (i.e. low pH and ANC) or headwater streams, and marginal to poor physical habitat. Our pair-wise comparisons also proved useful in describing broad conditions that typify mussel presence; larger streams with flowing water, and relatively low nutrient concentrations (Watters, 1992; Watters, 2000; Morgan & Kline, 2011). However,

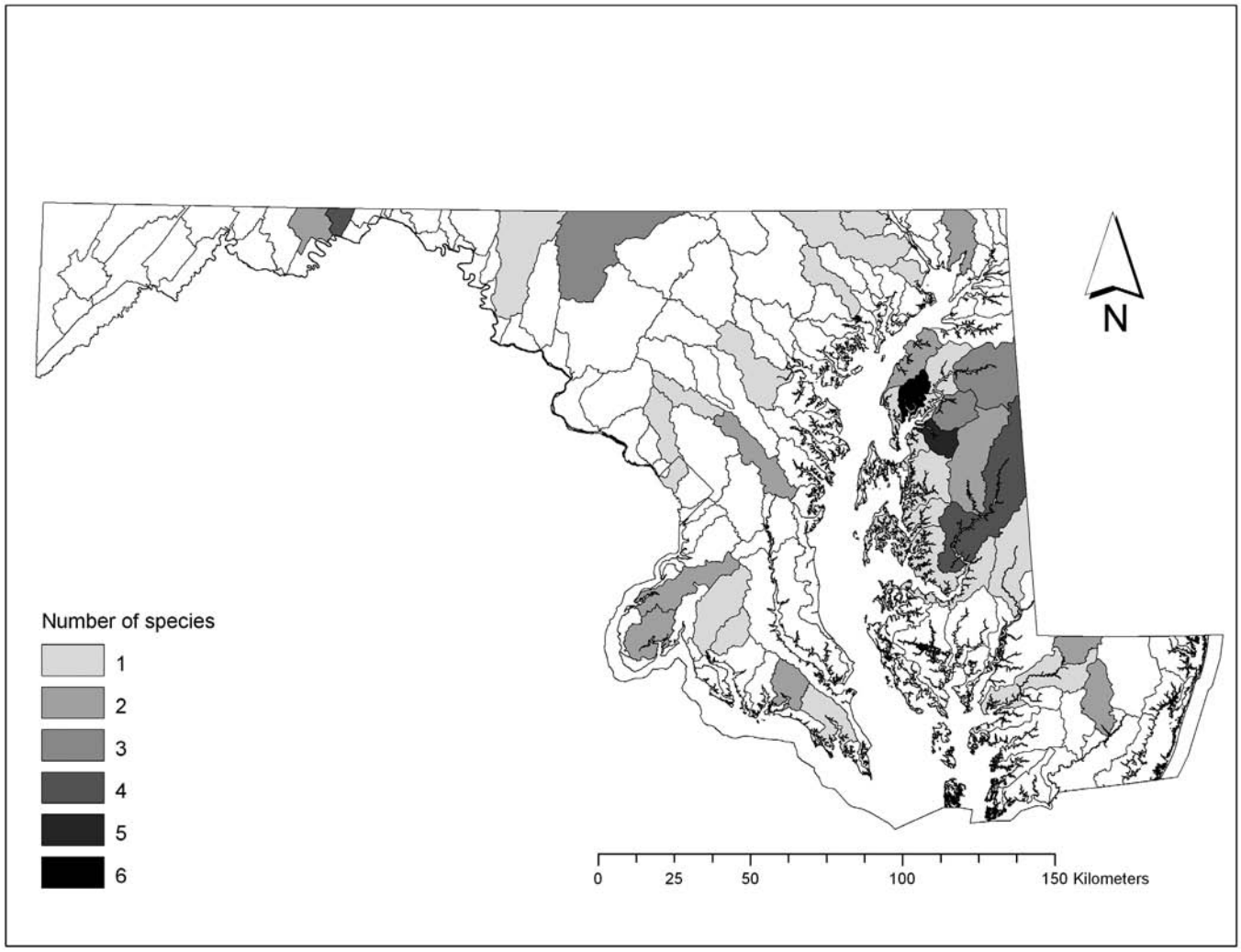

FIGURE 1

Freshwater mussel richness by Maryland 8-digit watershed as observed during the Maryland Biological Stream Survey, 2007-2009.

we recognize that many of these variables are often correlated with one another and must be accounted for to more rigorously hypothesize determinants of distribution. Variables with considerable overlap between sites of mussel absence and presence further illustrate the confounding nature of mussel-habitat associations as they relate to species distribution (Strayer, 2008).

Our findings also indicate that *E. fisheriana* rarely inhabited biologically degraded streams (IBI's ≤ 3), yet were frequently collected in high quality streams (IBI's ≤ 4) (COMAR 26.08.02). This further supports the hypothesis that freshwater mussels are indicators of healthy aquatic ecosystems (Grabarkewicz & Davis, 2008). We feel this highlights a key reason to collect freshwater mussel data as part of a biological monitoring program; regulatory mechanisms (i.e., biocriteria) are in place that react to IBI scores as thresholds of stream and watershed degradation or health (COMAR 26.08.02). In addition, mussel-bioassessment data would be able to support water quality standard (e.g., ammonia and copper) revisions and could be considered for the development of conductivity standards where impacts associated with natural gas extraction are a concern.

The MBSS data set includes over 3,000 randomly selected sites sampled during three state-wide Rounds and approximately 1,000 non-random sites and has been extensively published on using fish and benthic macroinvertebrate as response organisms (e.g. Pinder & Morgan, 1995; Morgan & Cushman, 2005; Stranko et al., 2005; Stranko et al., 2008; Kilian et al., 2010; Hildebrand et al., 2010). Despite the fact that

freshwater mussels are good response organisms for understanding spatial and temporal environmental patterns (Green et al., 1985), only two publications (Mynsberge et al., 2009; Stranko et al., 2010) have included MBSS-mussel observations. While the pitfalls of using readily available, large environmental data sets are known (Anderson et al., 2001; Strayer, 2008) their potential utility should not be ignored. In fact, we have several ongoing studies proposing hypotheses of mussel distribution and tolerances to environmental and anthropogenic stressors that build upon the basic relationships presented in this study (e.g., Haag & Warren, 1998; Nicklin & Balas, 2007). However, since we have no measure of detection and our data are limited to presence-absence we hesitate to test certain hypotheses without confirming the power of our survey methods. Our data may also not be appropriate for making inference about the cause of a species decline (Strayer, 1999b).

Unfortunately, we have no direct way of evaluating the cost-per-unit-effort (site visit). We suspect actual costs were quite low because assessments would have taken place regardless of our mussel survey and the amount of effort per site was relatively minimal. Therefore, some costs (e.g., travel) were independent of the mussel search. Moreover, the importance of recording freshwater mussel observations was recognized at the inception of the MBSS (1995) and visual surveys were conducted during stream assessments in advance of dedicated funding. Considerable effort was made at the onset of the study period to train crew members in freshwater mussel identification in response to concerns over the quality of identifications (discussed in Shea et al., 2011) from prior MBSS Rounds. Annually, time was required to inspect voucher shells, obtain independent confirmation, maintain voucher collections, attend regional identification workshops, examine institutional holdings, and address potential errors in the data. Further investigation into total costs, the cost-benefits of current versus more traditional mussel surveys, and additional survey effort (e.g., timed snorkel searches) are warranted.

It should be noted our technique may not be appropriate in other parts of the country where unionid distribution, diversity, and richness differ due to differences among faunal regions. The inability to detect certain species (e.g. *A. varicosa* and *Ligumia nasuta* (Say, 1817)) was likely due a variety of factors, including their existence in streams primarily outside of the MBSS scope (> 4th order, tidal influence) or in populations with very low abundance. Although not indicated in this study's data, we have recently encountered these species through stream assessments in large river and tidal-fresh habitats as part of other studies while using the same informal visual search. Nonetheless, we realize the current methods may be insufficient to detect and characterize the true mussel richness in some habitats where species with cryptic life history traits reside (Metcalfe-Smith et al., 2001; Tiemann et al., 2009). However, a cursory comparison of our mussel richness data to that collected by the Maryland Natural Heritage Program using timed-snorkel surveys has shown good agreement between most small to medium sized streams and watersheds.

The need for basic information on freshwater mussels remains and is imperative to develop and implement effective management plans, in addition to guide regulatory agencies in the development water quality standards that are more protective of freshwater mussels (Augspurger et al., 2003; Strayer et al., 2004). While the number of resource agencies that currently employ some form of standardized, state-wide unionid survey is increasing (Howells, 2006; Sietman, 2009; Shasteen et al., 2010; Stagliano, 2010), few pair their effort with assessments of water quality and biological condition even though monitoring programs are ubiquitous. To be clear, we are not by any means discrediting the traditional species or watershed centric approach to conducting mussel research, but there are well documented limitations on applying data collected at small scales to populations outside of those studied (Hamilton et al., 1997). We feel the proper context for mussel-bioassessments such as ours is to 1) support new or strengthen existing regulatory mechanisms to be more protective of freshwater mussels, 2) collect relevant landscape and physiochemical data at large spatial scales, and 3) supplement and guide quantitative surveys of imperiled unionids and specious watersheds.

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TABLE 1

Number of Maryland Biological Stream Survey sites sampled (N = 595) where live or dead freshwater mussels were encountered, 2007-2009.

Species	Total	Live	Dead	Proportion of sites
Alasmidonta heterodon (Lea, 1830)	10	2	9	0.02
Alasmidonta undulata (Say, 1817)	3	0	3	< 0.01
Anodonta implicata Say, 1829	2	1	1	< 0.01
Elliptio complanata (Lightfoot, 1786)	76	35	55	0.13
Elliptio fisheriana (Lea, 1838)	17	9	11	0.03
Elliptio producta (Conrad, 1836)	3	2	3	< 0.01
Lampsilis radiata radiata (Gmelin, 1791)	2	0	2	< 0.01
Lampsilis sp.	3	0	3	< 0.01
Leptodea ochracea (Say, 1817)	1	1	0	< 0.01
Pyganodon cataracta (Say, 1817)	15	5	11	0.03
Strophitus undulatus (Say, 1817)	1	0	1	< 0.01

*Due to longstanding problems distinguishing between the non-native *Lampsils cardium* Rafi nesque, 1820, native L. cariosa (Say, 1817), and suspected hybrids between the two in the Potomac River basin, Lampsilis sp. is recorded when an individual resembling either species is encountered.

TABLE 2

Non-parametric comparisons of biological and physiochemical variable medians between Maryland Biological Stream Survey sites where *E. fisheriana* was present (N = 41) and absent (N = 61) in 1st-4th order streams of Maryland, 2007-2009. Sulfate, ammonia, and nitrate concentrations represent the molecules whole weight (e.g., nitrate-nitrogen). Higher physical habitat metric and biological index scores represent superior conditions.

Variable	Present (Median ± SD)	Absent (Median ± SD)	*p*
pH	7.05 ± 0.46	6.45 ± 0.89	<0.001
Conductivity (µS/cm)	56 ± 79	169 ± 116	0.77
Acid neutralizing capacity (µeq/L)	495 ± 350	239 ± 446	0.002
Average daily temperature °C	21.15 ± 1.53	20.90 ± 1.41	0.42
Total phosphorus (mg/L)	0.07 ± 0.03	0.04 ± 0.05	0.02
Total nitrogen (mg/L)	2.26 ± 1.27	2.00 ± 3.20	0.04
Nitrate (mg/L)	1.96 ± 1.32	1.22 ± 2.85	0.02
Ammonia (mg/L)	0.03 ± 0.04	0.03 ± 0.10	0.77
Chloride (mg/L)	14.80 ± 10.01	16.02 ± 24.97	0.52
Sulfate (mg/L)	15.13 ± 7.17	15.02 ± 8.84	0.66
Discharge (m^3/sec)	0.04 ± 0.14	0.01 ± 0.04	<0.001
Average wetted width (m)	4.75 ± 4.15	2.80 ± 1.84	0.001
Fish-IBI	4.33 ± 0.80	3.33 ± 1.23	<0.001
Benthic macroinvertebrate-IBI	4.14 ± 0.82	3.14 ± 1.12	<0.001
Instream habitat quality	14.00 ± 3.17	11.00 ± 4.41	<0.001
Epifaunal substrate quality	13.00 ± 3.78	10.50 ± 4.44	0.001
Velocity-depth diversity	9.00 ± 2.98	7.00 ± 3.61	0.001
Pool-glide score	13.00 ± 3.04	11.00 ± 3.84	0.001
Riffle-run score	11.00 ± 5.85	6.00± 5.98	0.009
Embeddedness (%)	100 ± 30.51	100 ± 28.87	0.54
Urban land use (%)	1.60 ± 2.41	0.90 ± 7.73	0.01
Agricultural land use (%)	68.20 ± 14.85	62.00 ± 25.38	0.09
Forested land use (%)	30.10 ± 14.33	31.80 ± 24.72	0.20
Catchment size (km^2)	19.42 ± 50.76	6.73 ± 19.99	<0.001
Gradient (% slope)	0.09 ± 0.10	0.18 ± 0.19	0.05

TABLE 3

Frequency of occurrence for fishes collected at sites throughout the range of *E. fisheriana* in Maryland. An asterisk (*) indicates a non-native fish species.

		Frequency of occurrence	
		Present	Absent
Confirmed fish hosts		N = 41	N = 54
Etheostoma nigrum	Johnny Darter	0.00	0.00
*Lepomis macrochirus**	Bluegill	0.66	0.57
*Micropterus salmoides**	Largemouth Bass	0.49	0.24
Congenerics of confirmed fish hosts			
Etheostoma olmstedi	Tessellated Darter	0.93	0.48
*Lepomis cyanellus**	Green Sunfish	0.56	0.31
Lepomis gibbosus	Pumpkinseed	0.78	0.54
Lepomis auritus	Redbreast Sunfish	0.56	0.22
Luxilus cornutus	Common Shiner	0.00	0.00
Confirmed fish hosts of congeneric mussels			
Anguilla rostrata	American Eel	0.98	0.74
Dorosoma cepedianum	Gizzard Shad	0.02	0.00
Fundulus diaphanus	Banded Killifish	0.02	0.04
Gambusia holbrooki	Eastern Mosquitofish	0.22	0.11
Perca flavescens	Yellow Perch	0.15	0.09
*Pomoxis nigromaculatus**	Black Crappie	0.17	0.04
*Pomoxis annularis**	White Crappie	0.00	0.04

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REPRODUCTIVE BIOLOGY AND HOST FISHES OF FOUR UNIONIDS FROM THE LAKE PONTCHARTRAIN BASIN, LOUISIANA, U.S.A.

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ABSTRACT

Host fishes, fecundity estimates, and gravid periods were identified for four species of freshwater mussels from the Lake Pontchartrain basin, Louisiana. Two of the mussel species have broad distributions both in the Mississippi River and elsewhere in Louisiana: *Villosa lienosa* (Conrad, 1834) and *Lampsilis teres* (Rafinesque, 1820). The other two species have more restricted distributions: *Quadrula refulgens* (Lea, 1868) and *Lampsilis ornata* (Conrad, 1835). *Lampsilis ornata* is listed as a species of concern in Louisiana. Of the 23 species of fishes tested as potential hosts, we found 4 previously unknown hosts for *Villosa lienosa*: *Lepomis megalotis*, *Lepomis humilis*, *Lepomis microlophus* and *Lepomis cyanellus*, and confirmed 2 previously documented: *Lepomis macrochirus* and *Micropterus salmoides*. *Villosa lienosa* was gravid from April until June and had an estimated fecundity of 38,562 + 3,073 glochidia/female. For *Lampsilis ornata* we established a host relationship with *Luxilus chrysocephalus*, and confirmed *Micropterus salmoides* as a host. *Lampsilis ornata* was gravid from February until April and had a fecundity estimate of 451,214 + 27,239 glochidia/female. *Lampsilis teres* was gravid from April until September and had a fecundity estimate of 407,333 + 24,727 glochidia/female. We confirmed three hosts for *L. teres*: *Micropterus salmoides*, *Pomoxis annularis*, and *Lepomis humilis*, and identified two new hosts: *Lepomis microlophus* and *Notropis venustus*. Only a single *Quadrula refulgens* was found gravid in late June and its fecundity was estimated at 32,450 glochidia and a host was identified as *Pylodictis olivaris*.

KEY WORDS mussels, host fish, Lake Pontchartrain

INTRODUCTION

Freshwater mussels (Unionidae) are among the most endangered aquatic animals in North America (Williams et al., 1993; Neves et al., 1998; Lydeard et al., 2004; Strayer, 2008). Their loss from lotic ecosystems could have considerable effects on ecosystem health and function, because they often provide food resources and physical structure for other macro-invertebrates (Vaughn et al., 2004; Howard & Cuffney, 2003) and are important in lotic nutrient cycling (Vaughn et al., 2008). Within the Lake Pontchartrain Basin of Louisiana there are 32 species of unionids (Stern 1976) with around 17% without identified fish hosts (Oesch, 1995; Howells et al., 1996; Keller & Ruessler, 1997; Watters et al., 2009). Understanding these host relationships is important because host diversity is a strong predictor of mussel diversity (Watters, 1992; Haag & Warren, 1998; Vaughn & Taylor, 2000; Strayer, 2008) and is also an important factor in dispersal, propagation (Newton et al., 2008; Strayer, 2008), and mussel recruitment (Newton et al., 2008).

In vivo host fish determination techniques have been described in several studies (Howard, 1916; Coker et al., 1921; Penn, 1939; Cope, 1959; Hove & Neves, 1994; Watters, 1994; Hove et al., 2000; Yeager & Saylor, 2007). The success of determining the host is based, at least in part, on knowledge of the natural history of the species. The complexity of some unionid life histories makes determination of their hosts difficult. Complicating factors include brooding period length, percentage of population that is gravid, and whether the mussel is a host fish specialist (Farris & Van Hassel, 2007).

The host fish is thus a critical component of the mussel's natural history and is required knowledge for successful conservation. We conducted host fish trails and collected natural history data on four species of mussels: the little spectacle case *Villosa lienosa* (Conrad, 1834), yellow sandshell *Lampsilis teres* (Rafinesque, 1820), purple pimpleback *Quadrula refulgens* (Lea, 1868), and southern pocketbook *Lampsilis ornata* (Conrad, 1835). For each species, we present data on their gravid period, fecundity, and host suitability. These abundant species are found in many of the larger rivers (*Lampsilis teres*, *Lampsilis ornata*, and *Quadrula refulgens*) or in the smallest drainages that support unionid species (*Villosa lienosa*) in the Lake Ponchartrain Basin, Louisiana. The identification of additional host fishes and data on reproductive biology should

aid in future studies detailing important environmental influences on mussels, including a state species of concern, *L. ornata* (Gregory, 2009).

METHODS

Gravid females of all unionids were collected by hand from the Lake Pontchartrain Basin (Fig. 1) in the spring and summer 2008-2010 and transported immediately to the laboratory for host trials. Specimens were collected from the Amite, Tickfaw, and Tangipahoa rivers. All specimens were inspected in the field for engorged marsupia. Females were transported to the lab in aerated coolers. Mussels were isolated in glass aquaria with sand substrate and re-circulating river water.

Glochidia were obtained from gravid females by two methods: 1) direct removal and 2) using expelled glochidial packets. Direct removal involved puncturing the marsupial gills with a 20 gauge needle. Glochidia were then flushed from gills into a Petri dish using a squirt bottle filled with tank water. The second method involved the use of expelled glochidial packets from some of the *Villosa lienosa* individuals. In both cases, the glochidia obtained were held in suspension in beaker of 50 - 100 ml of water with use of a stir rod. Each female had a single 3 ml aliquot of suspended glochidia and water removed for fecundity counts. The 3 ml samples were counted with a dissecting scope at 50X and corrected for the exact volume of water used to keep glochidia in suspension. Viability of the glochidia was tested by exposing a subsample to NaCl. Glochidia were considered viable if > 90% of the subsampled glochidia snapped shut.

The glochidia were then transferred to the fish by direct placement onto the fish's gills. Before infestation with glochidia, fish were anaesthetized with MS-222 (tricane methanesulfonate, trade name Finquel™). The anaesthetized fish had 3 ml of a glochidial water solution injected into their mouths and flushed across the gills. In preliminary experiments with *Lampsilis ornata*, the fish were instead exposed to the glochidia in a heavily aerated beaker. This passive infection method was ineffective and the direct transfer method was therefore used.

Following infestation, the fish were immediately placed into individual 26.5 L aerated tanks. Each tank was kept at 23º C and nitrogenous waste maintained at < 0.2 mg/l through the infestation period. The tank bottom was siphoned twice a week to check for juvenile mussels and to replace existing water with fresh, de-chlorinated water. Between 11-19 L were siphoned each time. All siphoned water was filtered through an 87 µm mesh to retain juveniles or rejected glochidia and filtrates were examined with a 50X dissecting microscope during which all glochidia and juveniles (dead or alive) were counted. Juveniles were characterized by noting movement, or the presence of adductor muscles and a foot. Experiments were terminated after 40 days, if no juveniles were found, or one week after the last juveniles were collected from a tank.

Fish species selected for potential host tests were collected in the same streams as the mussel species. Fishes were sampled in either 1) wadeable streams (Tickfaw and Tangipahoa rivers) with a backpack electrofishing unit (Smith-Root model 15), or 2) from the larger Amite river with an electro-fishing boat, emphasizing fish habitat along banks of the river. The fishes were stored in aerated coolers for transport back to the laboratory. All fishes used in experiments were housed, handled, and disposed off according to departmental and university guidelines. Young individuals of species were preferred for host trails as they were less likely to have developed any immunity to unionid infections. Fishes were held in 3,029 L raceways for no less than a month to prevent accidental introduction of wild glochidia to test tanks. All fishes used in host trails had their gill inspected for glochidial infection before use. Previously known host fishes of mussels were determined from recent literature (Oesch, 1995; Howells et al., 1996; Keller & Ruessler, 1997; Watters et al., 2009).

RESULTS

Twelve gravid *Lampsilis teres*, seven *Lampsilis ornata*, twelve *Villosa lienosa*, and one *Quadrula refulgens* were found. Although *Quadrula refulgens* populations were surveyed for gravid females through two seasons of field work, only a single female was found gravid in late June, and was used to test for host suitability.

Of the twenty-three species of fishes tested as potential hosts (Table 1), we found four previously unknown hosts for *Villosa lienosa*: *Lepomis megalotis*, *Lepomis humilis*, *Lepomis microlophus* and *Lepomis cyanellus*, and confirmed two already documented hosts: *Lepomis macrochirus* and *Micropterus salmoides*. For *Lampsilis ornata*, we established a mussel-host relationship with *Luxilus chrysocephalus*, and confirmed M. salmoides as a host. We confirmed three hosts for *Lampsilis teres*: M. salmoides, *Pomoxis annularis*, and L. humilis, and established two new fish hosts: L. microlophus and Notropis venustus. For *Quadrula refulgens*, a mussel-host-fish relationship was established with *Pylodictis olivaris*.

Brooding period

Lampsilis teres was gravid from April until September and is considered a bradytictic brooder. *Lampsilis*

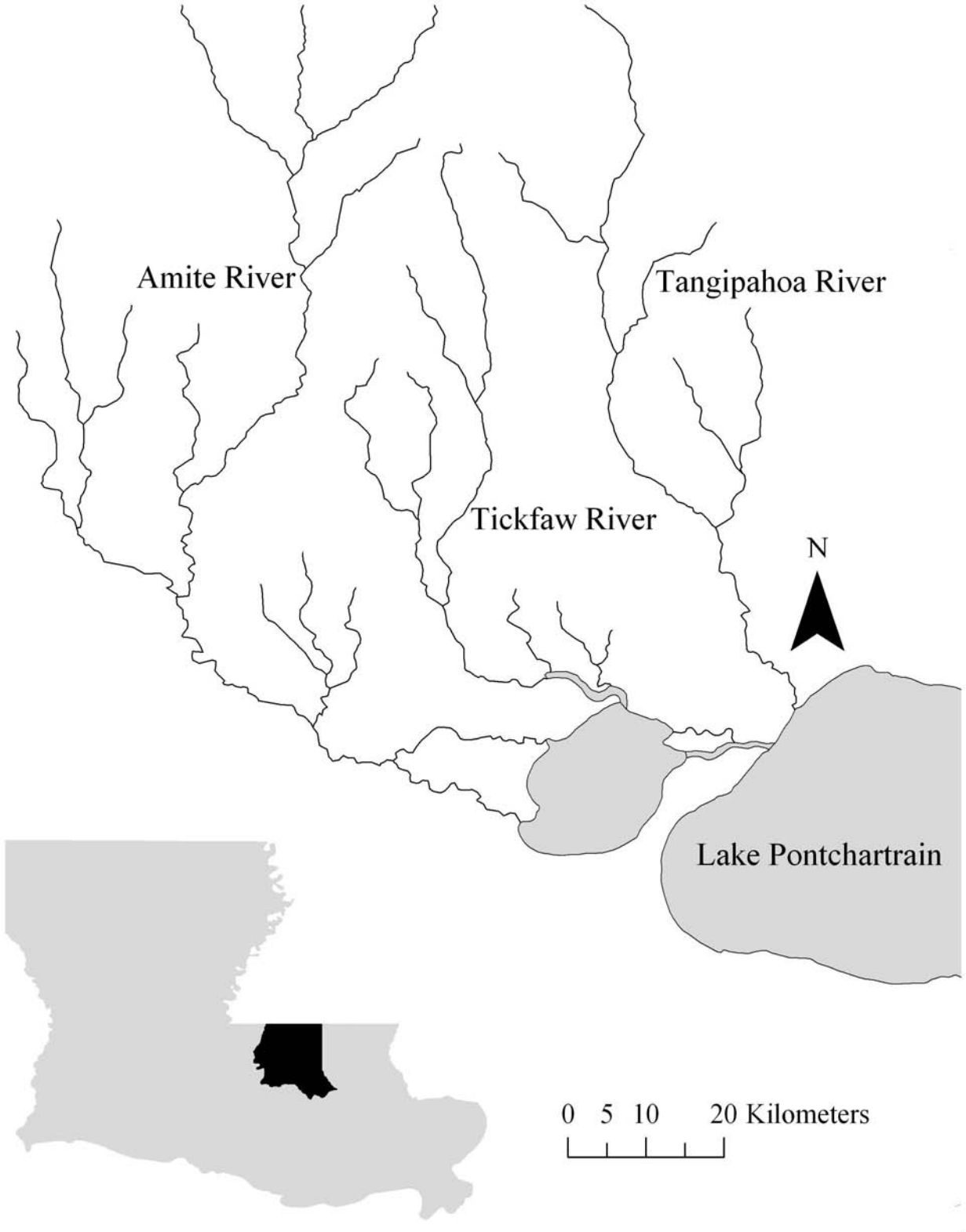

FIGURE 1
Rivers in the Lake Pontchartrain Basin sampled for fishes and mussels for host fish trials.

ornata was gravid from late February until April and is a tachytictic brooder. *Villosa lienosa* was considered in the literature (Keller & Ruessler, 1997) a long term brooder, but was only found gravid in our study from April until June. The single *Quadrula refulgens* gravid female was found in late June, and the species is considered to be a tachytictic brooder.

Fecundity estimates

Lampsilis teres had an average fecundity of 407,333 glochidia with a standard error of +24,727 glochidia for the 12 females that averaged 113.25 mm in shell length. *Lampsilis ornata* had a fecundity of 451,214 +27,239 glochidia for seven females that averaged 89.12 mm. The 12 *Villosa lienosa* averaged 48.15 mm had a fecundity estimate of 38,562 +3,073 glochidia. The single *Quadrula refulgens* was 48.5 mm in length and had an estimated 32,450 glochidia.

DISCUSSION

Understanding the complex reproductive cycle of unionids can be critical to their management and represents a major barrier to their conservation (Yeager & Saylor, 2007). Lack of suitable host fishes may limit the reproductive and dispersal ability of unionids within drainages. We have identified suitable hosts for four species of mussels from the Lake Pontchartrain Basin, Louisiana.

We categorized *Lampsilis teres* as a host generalist because five species, from two families were identified as proper hosts. L. teres is listed in the literature using over a dozen hosts from five families (Keller & Ruessler, 1997; Watters et al., 2009). We classified Villosa lineosa as a specialist on the Centrarchidae family using six species within this family. *Quadrula refulgens* and *Lampsilis ornata* were specialists, with only one and two hosts, respectively. *Lampsilis ornata* was classified as a specialist, because it was tested on 16 species of fish in our study, and 15 species in Haag and Warren's (2003) study. *L. ornata* used only two species as potential hosts from 26 species from nine families used in the two studies. *Micropterus salmoides* was also a host, which was also verified by Haag and Warren (2003), but *Luxilus chrysocephalus*, was a poor host, because it had only nine juveniles metamorphosed. Even though the two hosts are from different families, we believe that *L. ornata* can still be classified as a specialist.

Lampsilis teres and *Villosa lienosa* were gravid for long periods, whereas *Quadrula refulgens* and *Lampsilis ornata* had shorter brooding periods. *Villosa lienosa* is generally considered a long-term brooder, but was only found gravid during the early spring. Several *V. lienosa* aborted glochidia or eggs during transport, or the following day after being housed in the laboratory. Other species did not abort glochidia, suggesting *V. lienosa* are less tolerant of stress. If *V. lienosa* responds to disturbance by aborting glochidia, temperature stress during midsummer could lead to loss of reproduction in the fall.

Only a single gravid female was found of *Quadrula refulgens*, although the species is one of the most abundant mussels in the Amite River, LA. We suggest that this species may be a short term brooder that only broods glochidia for a few weeks. Quadrula species are long lived (Haag, 2009), including Q. refulgens (W. Daniel, unpublished data) and may not reproduce every season. The short brooding period and sporadic reproduction make finding gravid Q. refulgens difficult.

Both of the *Lampsilis* species (*ornata* and *teres*) were relatively fecund compared to *Villosa lienosa* and *Quadrula refulgens*. Thus, we found considerable variation among mussel species in brooding patterns, host selectivity and fecundity. Further studies will allow us to better categorize these mussel species as to their reproductive tactics and life history, and help resource managers better conserve these populations, especially the locally rare *L. ornata*.

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TABLE 1

Fish species tested as a host for each unionid. Numbers represent the total number of live juveniles recovered from host fish. X represents a trial with no juveniles produced. *indicates a previously known host fish.

Family/ Common Name	Scientific Name	*L. ornata*	*V. lienosa*	*L. teres*	*Q. refulgens*
Aphredoderidae					
Pirate Perch	*Aphredoderus sayanus*	X	X	X	
Catostomidae					
Blacktail redhorse	*Moxostoma poecilurum*	X		X	
Centrarchidae					
Bluegill	*Lepomis macrochirus*	X	**119, 76***	X*	X
Green sunfish	*Lepomis cyanellus*	X	**17**	X*	
Largemouth bass	*Micropterus salmoides*	**49, X***	**145***	**154***	
Longear sunfish	*Lepomis megalotis*	X	**266, 176**	X	
Orangespot sunfish	*Lepomis humilis*	X	**72**	**68***	
Shadow bass	*Ambloplites ariommus*		X		
Warmouth	*Lepomis gulosus*	X	X	X*	
White Crappie	*Pomoxis annularis*	X		**28***	
Redear Sunfish	*Lepomis microlophus*		**4**	**60, 37**	
Cyprinidae					
Striped shiner	*Luxilus chrysocephalus*	**9**	X		
Golden shiner	*Notemigonus crysoleucas*	X	X	X	
Blacktail shiner	*Cyprinella venustus*			**12**	
Cyprinodontidae					
Blackstripe topminnow	*Fundulus notatus*	X		X	
Ictaluridae					
Yellow bullhead	*Ameiurus natalis*	X	X	X	X
Tadpole madtom	*Noturus gyrinus*	X	X	X	
Brown madtom	*Noturus phaeus*		X	X	
Flathead catfish	*Pylodictis olivaris*				**28**
Percidae					
Banded darter	*Etheostoma zonale*		X	X	
Blackbanded darter	*Percina nigrofasciata*		X		
Poeciliidae					
Mosquitofish	*Gambusia affinis*	X		X	
Sailfin Molly	*Poecilia laptipinna*	X			

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RECENT MONITORING OF THE FRESHWATER MOLLUSKS OF KINNICONICK CREEK, KENTUCKY, WITH COMMENTS ON POTENTIAL THREATS

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ABSTRACT

This study was conducted to gain a better understanding of the current status of freshwater mollusks in the mainstem of Kinniconick Creek, a small tributary to the Ohio River. Qualitative and quantitative sampling documented 17 species of freshwater mussels and 8 species of freshwater gastropods from mainstem Kinniconick Creek. Declines in freshwater mussel species richness have been observed at several sites since 1983 as well as declines in densities. I discuss potential threats to the mussel fauna posed by excessive particle movement from historical channel alteration, human perturbation, and from changes in precipitation patterns.

KEY WORDS Unionidae, gastropods, snails, Ohio River drainage, drought, monitoring

INTRODUCTION

The freshwater mussel fauna (Mollusca: Bivalvia: Unionidae) of the southeastern United States has undergone dramatic changes as compared to pre-European colonization (Haag, 2009). In Kentucky, declines of freshwater mussels have been attributed to impoundments (Cicerello & Laudermilk, 1997; Sickel & Chandler, 1996), mineral extraction (Anderson et al., 1991; Warren & Haag, 2005) as well as non-point pollution (Houp, 1993). Another mollusk group that has experienced similar impacts, freshwater snails (Mollusca: Gastropoda), has one of the highest imperilment rates of any animal in the United States (Johnson et al., in prep; Neves et al., 1997).

I examined historical and contemporary mussel fauna of Kinniconick Creek in northeastern Kentucky. Kinniconick Creek is a direct tributary to the Ohio River. The stream was systematically inventoried by Warren et al. (1984). Subsequently, the mussel populations have been monitored by the Kentucky State Nature Preserves Commission (KSNPC) which includes quantitative sampling at one site in 1990. No published literature exists on the freshwater gastropod fauna of Kinniconick Creek.

Study Area

Kinniconick Creek drains 517 km2 in Lewis County, Kentucky (Figure 1). The stream has been identified as an aquatic biodiversity hotspot in Kentucky (Cicerello & Abernathy, 2004), and the mainstem has been designated a Reference and Exceptional Value reach (KY DOW, 2008). Much of the watershed is underlain by Mississippian-age oil shales as well as sandstones (Jacobs & Jones, 2004). Lower sections of Kinniconick Creek are underlain with Quaternary alluvium primarily derived from upland sources (Warren et al., 1984), while headwater areas are underlain with Devonian oil shales and limestone (Jacobs & Jones, 2004).

Land use is a mixture of agricultural fields along the floodplains with forest blocks into the uplands. Low density residential development is present throughout the watershed. Local landowners mentioned that in the 1950s, much of the upper portion of Kinniconick Creek was straightened and moved to the valley wall in order to increase farming production in the floodplain. This has resulted in the mobilization of large amounts of material from upland portions of Kinniconick Creek. Upper reaches of Kinniconick Creek are characterized by unstable banks and poor streambed conditions indicative of historical modifications.

METHODS

Qualitative Sampling - Mussels

In 2007 and 2008, I conducted qualitative sampling at fifteen sites (Figure 1) that have been examined in a prior study (Warren et al., 1984). While I examined the same reaches, the exact search areas from previous studies were unknown. Snorkeling, tactile searches, and

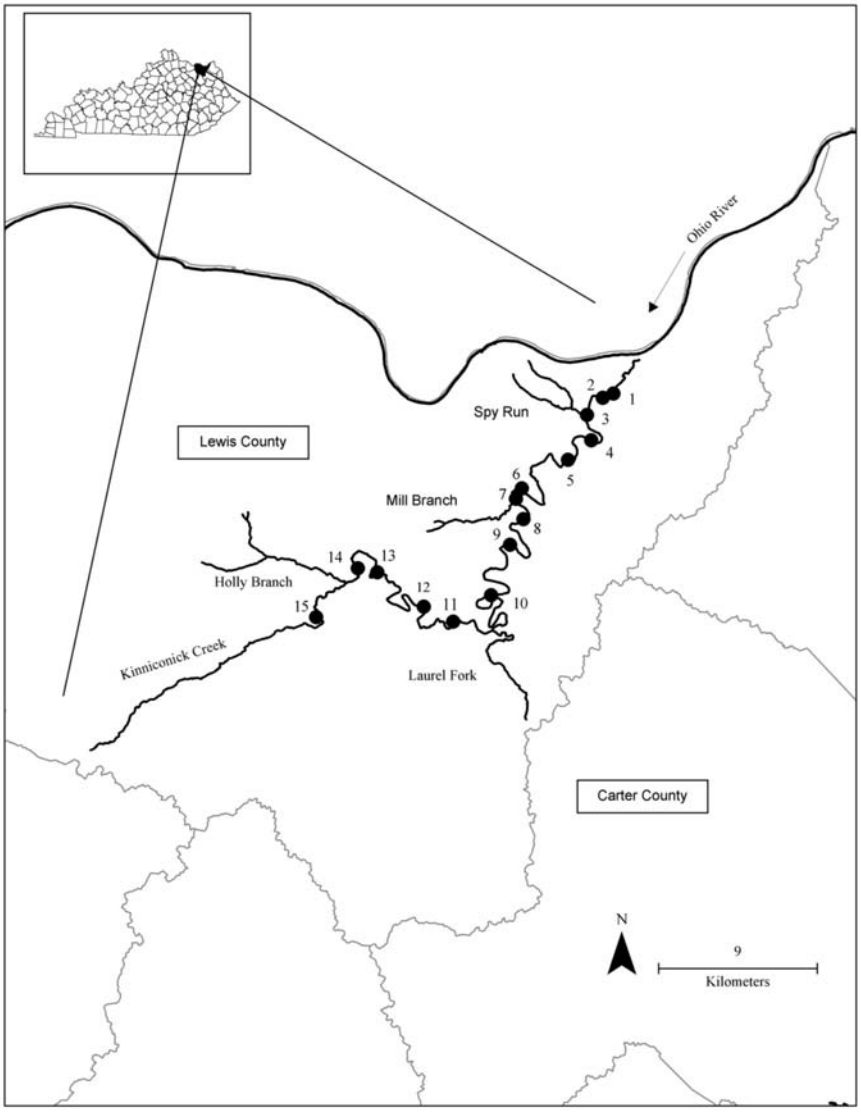

FIGURE 1
Study area and locations of sampling sites. The direction of flow of the Ohio River is indicated by the arrow.

visual searching were used for each site. I constructed a diminishing returns curve for each site to determine when adequate search effort had been expended (Dunn, 2000; Miller & Payne, 1993). To develop the curve, live and recently fresh dead (still containing fresh tissue) mussels were identified and enumerated at 10, 20, 40, and 60 individual intervals. A total of 28.6 person hours were spent in the qualitative sampling phase, with a mean of 1.9 person hours per site. Although exact sampling times of site visits from previous studies are not known, they approximate a minimum of 1 person hour per visit (R. Cicerello, retired KSNPC, per comm. 2007). I visited all sites between May and October; visibility was generally excellent during the study due to drought conditions.

I also examined records from recent sampling efforts at specific sites by KSNPC prior to this study (Table 1). Notable in the mollusk fauna of Kinniconick Creek is *Epioblasma triquetra* (Rafinesque, 1820) (Snuffbox), which has been proposed for listing as an Endangered Species by the United States Fish and Wildlife Service (USFWS, 2010). Additionally, *Simpsonaias ambigua* (Say, 1825) (Salamander Mussel) and *Villosa lienosa* (Conrad, 1834) (Little Spectaclecase) are listed as rare in Kentucky (KSNPC, 2010).

Quantitative Sampling - Mussels

To evaluate trends in demography, quantitative sampling was conducted at one site (the confluence of Mill Branch), which had also been sampled by KSNPC

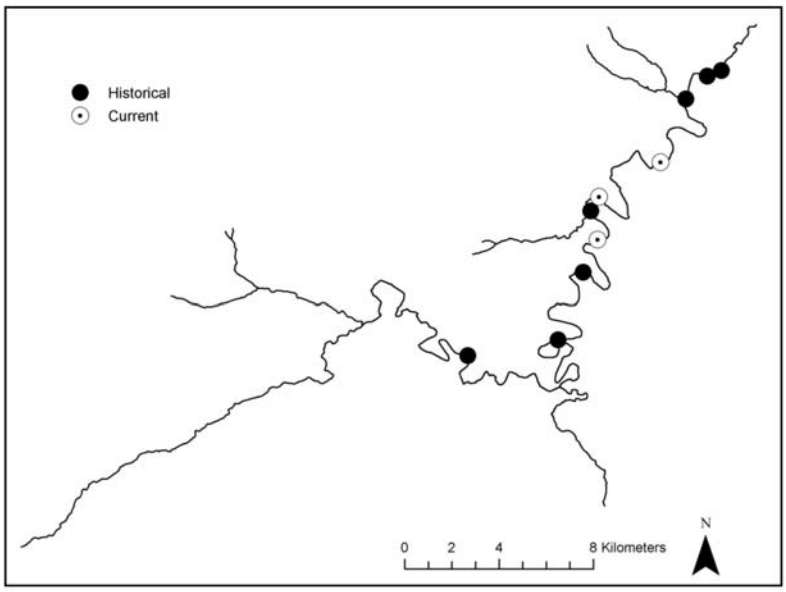

FIGURE 2
Historical versus current distribution of state-listed species in Kinniconick Creek.

in 1990. Prior to sampling, I snorkeled and flagged to delineate the densest portion of the mussel bed and an initial set of quadrats was also used to further delineate the area to focus the sampling. A systematic sampling design with three random starts (Strayer & Smith, 2003) implementing 1 m2 quadrats was used to conduct quantitative sampling over a 16 x 80 m area. A total of 26 quadrats were sampled, which was the number sampled by KSNPC in 1990. Substrates were excavated to a depth of 10-15 cm and sieved through a 1 cm mesh screen. Shells of freshwater mussels collected during this study have been deposited at the Ohio State University Museum of Biological Diversity, Columbus.

Freshwater Snail Sampling

Sampling for freshwater snails was opportunistic and involved collection of available microhabitats at sites after mussel sampling had been completed. The goal was to gather assemblage data on freshwater snails. A hand sieve was used to examine loose substrates such as woody debris or loose sand; other collections were made by hand. Snails were preserved in the field in 70% ethanol and retained for lab identification. References by Basch (1963), Jokinen (1992), Burch (1989), and Wu et al. (1997) were primarily used to confirm specimen identifications of freshwater snails. Voucher specimens are retained at the Kentucky State Nature Preserves Commission in Frankfort.

Taxonomy

Taxonomy generally follows Turgeon et al. (1998) with a few exceptions. *Laevapex* taxonomy follows Walther et al. (2006). *Physa* taxonomy follows Dillon et al. (2002). *Quadrula* taxonomy follows Serb et al. (2003).

Statistical Analyses

To analyze statistical differences in species richness between previous studies and this study, a 2-way Student t-test was conducted. All tests were conducted using Systat software (Version 11) at the 95% level of confidence and screened for normality prior to testing.

RESULTS

I encountered seventeen species and 678 freshwater mussels during this study (Table 2). When comparing the data from the current study to that from 1983, a species richness decline of 50% was observed at 4 sites, with the average richness value declining by 2.2 species per site. Differences in species richness from previous studies were not significantly different ($p > 0.05$). The presence of rare mussel species (Snuffbox, Salamander Mussel, and Little Spectaclecase) all exhibited a dramatic range reduction, from 9 sites occupied historically to 3 sites currently (Figure 2). There were slight increases in species richness at sites 9 and 10 (increased from 7 documented species to 9 species). Four mussels previously documented from either live specimens or shell remains, *Leptodea fragilis* (Rafinesque, 1820), *Ligumia recta* (Lamarck, 1819), *Pleurobema sintoxia* (Rafinesque, 1820), and *Truncilla truncata* Rafinesque, 1820, were not observed during

this study. As with the previous study, species richness was low in the uppermost sites and reached the highest diversity in intermediate reaches. Further, the data show a reduction of freshwater mussel species from lower portion of the stream. The exotic Asiatic Clam, *Corbicula fluminea* (Müller, 1774), was present at all sites.

Quantitative sampling at Site 7 showed a statistically significant decline (3.42 ± 1 mussels/m2 in 1990 versus 0.4 ± 0.23; $p < 0.05$, standard error = 0.119) in density as well as species richness (from 11 to 5 species). In taking the exact same number of quadrats as previous sampling in 1990, the precision of mean estimate was 60%. Because such low densities were observed in 2007-2008, much more quadrat sampling (approximately double the number sampled here) would have been required to approach the 25% precision of the mean value of the 1990 dataset.

Eight species of freshwater snails were located either live or from shell materials (Table 3). The snail fauna did not include any state species of conservation concern. The most common species across all sites were *Helisoma anceps* (Menke, 1830) and *Pleurocera acuta* Rafinesque, 1824. The three upstream most sites supported only pulmonates. Pleurocerids were regularly distributed near the mouth upstream to site 12. Species richness tended to be highest at sites which exhibited the greatest habitat complexity, particularly the presence of floodplains, depositional areas, backwaters, and mixed woody debris. At sites where sedimentation was heavy, snails were typically located only on margins or the undersides of larger rocks.

DISCUSSION

A diverse mussel community remains in Kinniconick Creek although this study suggests some declines in mussel site occupancy and density. Three state-listed mussel species originally reported by Warren et al. (1984) remain extant at a reduced number of sites. Despite the findings of the qualitative phase of the study, it is difficult to evaluate if an actual decline has occurred due to the low statistical power of the sampling methods used in this study (Strayer, 1999a). Metcalfe-Smith et al. (2000) suggest more than 4.5 person-hours is necessary for rare species detection.

Quantitative sampling at Site 7 revealed a pattern of decline in both density and species richness of freshwater mussel. Because the data revealed a variance to mean ratio of 1, the mussels at this site were essentially spatially randomly distributed (Downing & Downing, 1991; Smith, 2006). Furthermore, no juveniles were detected of any species at the quantitative sampling site, which suggests that recruitment may be limited in Kinniconick Creek.

This study showed overall low numbers of freshwater snails in terms of density and species richness. Kinniconick Creek is generally reduced to low-flow pools in mid-summer and as such, the aquatic gastropod assemblage is dominated by pulmonates, which are better adapted to lower dissolved oxygen environments (Lodge et al., 1987). *Pleurocera acuta*, which was regularly distributed across sites typically in very low numbers, occurs in larger densities in streams with higher dissolved oxygen and higher carbonate levels (Houp, 1970). Additionally, Johnson and Brown (1997) determined that adult pleurocerids of *Elimia semicarinata* (Say, 1829) (Pleuroceridae) in Kentucky preferred slower-flowing areas that provided flow refugia, whereas the opposite was true regarding juveniles.

In 2007, Kinniconick Creek was impacted by severe drought, and several sites were reduced to very shallow pools. There have been fourteen drought events in northeastern Kentucky since 1976 that are categorized as extreme drought on the Palmer Drought Severity Index (Palmer, 1965; Figure 3). Three of the drought events, between 1999 and 2007 exceeded -10 on the PDSI (with 2007 being the most severe drought in the basin since 1930). Conversely, the highest five years of extreme rain events on record have been observed since 1976 according to the PDSI, with the 2 highest rain events on record between 1989 and 2004. One serious cause of concern for many aquatic ecosystems is global climate change (Poff et al., 2002; Wrona et al., 2006). Global climate change is thought to threatenfreshwater mussels and fishes in small Nearctic and Palearctic streams (Haag & Warren, 2008; Hastie et al., 2003; Matthews & Marsh- Matthews, 2003). Golladay et al. (2004) and Haag and Warren (2008) measured precipitous declines of freshwater mussels as a result of a severe drought. An indirect stressor associated with low flow periods is recurring die-offs of Asiatic Clams (*Corbicula fluminea*) which are present throughout Kinniconick Creek. Mortality of Asiatic Clams due to decreased dissolved oxygen levels often result in pulses of ammonia (both in the water column and through porewater) which can act to further stress or cause mortality to native mussels (Cherry et al., 2005; Cooper et al., 2005). The interaction between drought periodicity and ammonia loading from Asian Clam turnover is an area that should receive further study.

Changes in substrate fractions towards greater amounts of fine sand with lower amounts of silt and organic components have been shown to promote higher biomass of Asiatic Clams (Cooper, 2007). Excessive sediment was noted as very heavy at several sites during the summer months. The sources for exces-

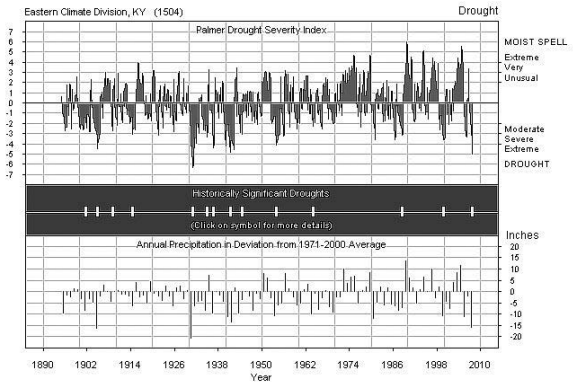

FIGURE 3

Drought and precipitation trends for eastern Kentucky. Graph is available for use at http://kyclim.wku.edu/graphlets/dsg.html.

sive sedimentation in Kinniconick Creek have not been specifically identified, but it likely arises from upland sources as a result of widespread watershed alterations. Headwater sites exhibited headcutting (deeply incised channels that were generally disconnected from the floodplain) which is promoting a condition of greater bed stress and excessive sediment supply. Landowners mentioned efforts in the 1940s and 1950s by the US Soil Service to promote channelization of streams to landowners, ostensibly to assist in crop production and reduce flooding. The aforementioned channelization of the headwater portions of the watershed is a known cause for lowered or disrupted water tables as well as a large contribution of upland sediments in Kentucky streams (A. Parola, University of Louisville, pers. comm., 2009). This particularly applies to substrates during high flow events, as modeled in larger rivers by Morales et al (2006). Virginia Spirea (*Spirea virginiana*), a Federally-Threatened shrub that utilizes cobble bars and stream banks for habitat, has declined over the long-term in several areas in Kinniconick Creek (D. White, KSNPC, pers. comm., 2009). Large woody debris (trees) entering the creek from upstream areas as a result of bank destabilization could be serving to scour the cobble bars that this species thrives on (A. Parola, pers. comm., 2009). The large-scale instability in stream banks and particle movement could be the source of observed declines as water quality is generally very good in Kinniconick Creek. It remains a very rural watershed with less of the influence of impacts typical of more urban areas (stormwater quality, increased impervious surface, etc).

One observation at the higher quality remaining sites in intermediate reaches was the presence of an adjacent area of flow accessible floodplain throughout at least one section of the site in conjunction with larger cobble substrates. Kinniconick Creek maintains a high degree of longitudinal connectivity which is likely an important factor in terms of host fish movement and the long-term maintenance of mussel beds (Newton et al., 2008). Enhanced floodplain connectivity would likely help reduce sheer stress on mussel habitats at the higher quality sites. Low sheer stress has been shown to be an important physical characteristic of robust mussel beds (Howard & Cuffey, 2003; Layzer & Madison, 1995; Peck, 2005; Strayer, 1999b).

Gravel mining for local road maintenance in Laurel Fork and McDowell Fork was observed in the early 1980s by Warren et al. (1984). Instream gravel mining has been reported by Hartfield (1993) as a causal factor in freshwater mussel declines as well as fishes (Cross et al., 1982). Instream mining can alter stream geomorphology, width to depth ratios and stream gradient (Meador & Layher, 1998; Roell, 1999) and result in channel scouring, incision (Kondolf, 1997) and headcutting (Hartfield, 1993; Meador & Layher, 1998). The nature of this activity in Kinniconick Creek requires

further examination. It is possible that much of the local gravel extraction activity is focused on collecting deposits resulting from aforementioned watershed alterations that are being mobilized and redeposited during high flow events, instead of channel excavation and active mining of the stable portions of the channel (A. Parola, pers. comm., 2009). Finally, erosion resulting from all terrain vehicles (ATVs) along streambanks was seen at consecutive sites in the middle portions of the watershed. On one occasion, an individual was encountered riding an ATV directly through a drought-impacted shallow pool containing Snuffbox (*Epioblasma triquetra*) as well as two other state-listed mussel species.

In summary, decreases in overall unionid densities, decreases in site occupancy of rare species, several direct human disturbances to habitat, and potential changes in precipitation patterns are long-term considerations of the freshwater mussels in Kinniconick Creek. Several of these issues are affecting the mussel fauna of other small Ohio River basin streams as well (Fraley & Ahlstedt, 1999). As conservation efforts move forward to protect the remaining high-quality freshwater mussel populations in Kentucky, it will be important to consider the protection of remaining habitats in small watersheds such as the focus of this study, which are susceptible to chronic environmental changes.

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TABLE 1

Previous records from Kinniconick Creek. Numbers in columns refer to the total number of sites a given species has been previously reported.

	Warren et al. (1984)	KSNPC database records (from 1984 to 2007)
Amblema plicata (Say, 1817), Threeridge	2	1
Elliptio crassidens (Lamarck, 1819), Elephantear	1	0
Elliptio dilatata (Rafinesque, 1820), Spike	6	2
Epioblasma triquetra (Rafinesque, 1820) , Snuffbox	6	3
Fusconaia flava (Rafinesque, 1820) , Wabash Pigtoe	5	1
Lampsilis cardium Rafinesque, 1820, Plain Pocketbook	6	1
Lampsilis fasciola Rafinesque, 1820, Wavyrayed Lampmussel	2	0
Lampsilis siliquoidea (Barnes, 1823), Fatmucket	7	1
Lasmigona costata(Rafinesque, 1820), Flutedshell	2	1
Leptodea fragilis(Rafinesque, 1820), Fragile Papershell	1	1
Ligumia recta (Lamarck, 1819), Black Sandshell	0	1*
Pleurobema sintoxia(Rafinesque, 1820), Round Pigtoe	0	1
Potamilus alatus(Say, 1817), Pink Heelsplitter	3	1
Ptychobranchus fasciolaris (Rafinesque, 1820), Kidneyshell	7	2
Pyganodon grandis(Say, 1829), Giant Floater	2	0
Quadrula pustulosa(I. Lea, 1831), Pimpleback	2	0
Quadrula verrucosa (Rafinesque, 1820), Pistolgrip	4	2
Simpsonaias ambigua(Say, 1825), Salamander Mussel	3	0
Strophitus undulatus(Say, 1817), Creeper	4	1
Truncilla truncata Rafinesque, 1820, Deertoe	0	1
Villosa iris (I. Lea, 1829), Rainbow	3	2
Villosa lienosa(Conrad, 1834), Little Spectaclecase	4	3

* reported as weathered dead or relic shell only

TABLE 2

Summary of freshwater mussel observations in Kinniconick Creek by sampling station; species highlighted in bold are Listed as Special Concern, Threatened, or Endangered by Kentucky State Nature Preserves Commission. P = previously reported as live or fresh dead shell; C = present in current study as live or fresh dead.

Station Number	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
No. of person-hours (current study)	1		1.5		2		1		3.5		1		3		3.2		4		3		1.2		1		1.2		1		1	
	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C
Amblema plicata						X*			X	X						X		X	X	X										
Elliptio crassidens									X	X																				
Elliptio dilatata				X*	X	X*		X*	X	X			X	X	X	X	X	X	X	X										
Epioblasma triquetra			X						X				X		X		X	X*	X	X										
Fusconaia flava					X*	X			X	X			X	X	X	X		X	X	X										
Lampsilis cardium				X	X	X*			X	X			X	X	X	X	X	X		X	X									
Lampsilis fasciola					X				X							X		X												
Lampsilis siliquoidea					X				X	X			X		X	X	X	X	X	X			X	X						
Lasmigona costata									X						X					X										
Leptodea fragilis	X*								X																					
Ligumia recta													X*																	
Pleurobema sintoxia																			X											
Potamilus alatus									X						X	X	X	X												
P. fasciolaris					X	X*			X	X		X*	X	X	X	X	X	X	X	X	X		X								
Pyganodon grandis																					X		X	X						
Quadrula pustulosa									X						X															
Quadrula verrucosa													X	X	X	X	X	X	X	X										
Simpsonaias ambigua	X												X					X												
Strophitus undulatus									X	X			X						X				X	X						
Truncilla truncata									X																					
Villosa iris									X					X				X	X	X										
Villosa lienosa					X				X					X	X					X			X							
Total No of Spp – Live or Fresh Dead	1	0	1	1	6	1	0	0	17	8	0	0	9	7	11	9	7	11	10	11	3	0	5	3	0	0	0	0	0	0

* reported as weathered dead or relic shell only

TABLE 3

Freshwater gastropods observed during qualitative searches in Kinniconick Creek, Lewis County, KY in 2007-08. Numbers in top row refer to site numbers.

Order	Family	Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Architaenioglossa	Viviparidae	*Campeloma decisum* (Say, 1817)	X		X	X											
Neotaenioglossa	Pleuroceridae	*Pleurocera acuta* Rafinesque, 1831	X		X	X		X			X			X			
Basommatophora	Ancylidae	*Laevapex fuscus* (C.B. Adams, 1841)												X			
Basommatophora	Lymnaeidae	*Pseudosuccinea columella* (Say, 1817)						X									
Basommatophora	Physidae	*Physa acuta* Draparnaud, 1805														X	
Basommatophora	Physidae	*Physa gyrina* Say, 1821						X					X				
Basommatophora	Planorbidae	*Helisoma anceps* (Menke, 1830)			X			X					X	X	X	X	
Basommatophora	Planorbidae	*Micromenetus dilatatus (*Gould, 1841)			X			X							X	X	
Total			2	0	4	2	0	5	0	0	1	0	2	3	2	3	0

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POPULATION PERFORMANCE CRITERIA TO EVALUATE REINTRODUCTION AND RECOVERY OF TWO ENDANGERED MUSSEL SPECIES, *EPIOBLASMA BREVIDENS* AND *EPIOBLASMA CAPSAEFORMIS* (BIVALVIA: UNIONIDAE)

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ABSTRACT

Genetic and demographic modeling of two endangered mussel species, *Epioblasma brevidens* and *E. capsaeformis*, in the Clinch River, U.S.A., was conducted to determine quantitative criteria to evaluate performance of extant and reintroduced populations. Reintroduction modeling indicated that the initial population size created during a 5 y build-up phase greatly affected final population size at 25 y, being similar to the population size at the end of the build-up phase, especially when expected population growth rate was low (e.g., 1-2%). Excluding age-0 individuals, age-1 juveniles or recruits on average comprised approximately 11% and 15% of a stable population of each species, respectively. Age-class distribution of a stable or growing population was characterized by multiple cohorts, including juvenile recruits, sub-adults, and adults. Molecular genetic and demographic data indicated that the ratio of N_e/N_c was ~5% for both species. Based on this ratio and predicted declines of genetic variation at different population sizes, target total sizes for reintroduced or recovered populations of each species should be ≥10,000 individuals (N_e=500), respectively, and ideally should be comprised of multiple smaller demes spread throughout a river. Because of current barriers to dispersal and the low dispersal capability of some mussel species, reintroductions will play a prominent role in restoring populations in the United States.

KEY WORDS Freshwater mussels, Endangered species, *Epioblasma brevidens*, *Epioblasma capsaeformis*, Genetic and demographic modeling, Population performance criteria

INTRODUCTION

> "There can be no purpose more inspiriting than to begin the age of restoration, re-weaving the wondrous diversity of life that still surrounds us."
> Edward O. Wilson, *The Diversity of Life*

The 19th and 20th centuries were periods of large-scale habitat loss, degradation and fragmentation caused by dam construction and operation, and severe pollution of aquatic ecosystems, with concomitant losses in biodiversity throughout the United States of America (U.S.A.). During this period, freshwater mussel populations declined greatly and are now considered one of the most imperiled groups of animals in the country (Neves et al., 1997). The passage of landmark environmental laws in the U.S.A., such as the Clean Water Act (1972), Endangered Species Act (1973), and Surface Mining Control and Reclamation Act (1977), have helped reduce impacts and raise public awareness toward proper environmental stewardship (Stein et al., 2000; Schwartz, 2008). More than thirty years later, some disturbed aquatic ecosystems are showing signs of improved water quality and physical habitat conditions. However, many mussel species cannot re-colonize previously occupied habitats because dams prevent dispersal of their host fishes. Reintroductions are now needed to restore populations and therefore are recommended in the recovery plans of these endangered species (National Native Mussel Conservation Committee, 1998; USFWS, 2004). Establishing new populations or boosting declining ones meets recovery plan goals and helps to reduce risk to species survival.

The Clinch River in northeastern Tennessee (TN) and southwestern Virginia (VA) of the eastern U.S.A. contains a diverse mussel assemblage of 45 species, with numerous endangered mussel species to include the Cumberlandian combshell (*Epioblasma brevidens* (Lea, 1831)) and oyster mussel (*E. capsaeformis* (Lea, 1834)). Both species are endemic to the Tennessee

and Cumberland river drainages, major tributaries of the Ohio River. These populations are large enough to support translocations of adults and for collecting gravid females to use as broodstock at mussel hatcheries (Jones & Neves, 2011). Releasing translocated and hatchery-reared mussels allows biologists to augment and reintroduce populations to achieve species recovery (USFWS, 2004). If managed properly, populations in the Clinch River can serve as main sources to replenish and rebuild other populations throughout the Tennessee and Cumberland river systems.

While the federal recovery plans for *E. brevidens* and *E. capsaeformis* provide recovery criteria for both species, they are only marginally quantitative because demographic data are lacking to specifically define the criteria. When such data are unavailable, these plans recommend that the information be collected. For example, the plans specify that the demographic structure and effective size of a viable population of each species be determined (USFWS, 2004). The plans further state that, "A viable population is defined as a wild, naturally reproducing population that is large enough to maintain sufficient genetic variation to enable the species to evolve and respond to natural habitat changes without further intervention. Viable populations will therefore be stable and have multiple age classes, including newly recruited juveniles" (USFWS, 2004). Therefore, both demographic and genetic factors must be addressed to determine population viability, to include assessing age-class structure, recruitment level, and effective population size (N_e).

The recovery of *E. brevidens* and *E. capsaeformis* will require that additional self-sustaining populations be established in other rivers by release of translocated and/or hatchery-reared individuals. Ideally, re-introduced populations will be more than self-sustaining, but will grow in size locally and expand to other sites. Thus, the purposes of this study were to determine (1) how many individuals of each species are needed to create a self-sustaining, demographically viable population that is large enough to maintain sufficient genetic variation over time, and (2) practical quantitative criteria to evaluate performance of reintroduced or recovered populations.

METHODS

Predicting decline of genetic diversity

To predict declines in genetic diversity, the program EASYPOP (Balloux, 2001) was used to simulate changes in heterozygosity and allelic diversity over time based on different levels of N_e. Initial measures of allelic diversity and number of polymorphic loci were obtained from Jones et al. (2004). Simulations were conducted assuming random mating among diploid individuals belonging to a single population, and with an equal sex ratio. Number of loci was set to ten, with free recombination between loci and the same mutation scheme and rate ($1x10^{-4}$) for all loci. The selected mutation model was a mixed model with a proportion of both single-step mutation events (90%) and infinite allele mutation events (10%), where the latter mutation scheme allows for equal probability to mutate to any of the possible allelic states (Garza & Williamson, 2001). The number of possible allelic states was set at seventeen for each locus (Jones et al., 2004). Genetic variability of the initial population was set to maximum, meaning that alleles were randomly assigned to individuals. Simulations were conducted for 25 generations and replicated ten times to check for consistency of results.

Census and effective population sizes

Population sizes of *Epioblasma brevidens* and *E. capsaeformis* in the Clinch River, TN were estimated in 2004 by collection of standard, systematic 0.25 m^2 quadrat samples placed along transect lines (Jones & Neves, 2011). Sites sampled during the 2004 census included Wallen Bend [river kilometer (RKM) 309.9], Frost Ford (RKM 291.7) and Swan Island (RKM 277.2), which were selected because they represented the upper, middle and lower boundaries of the study reach, respectively. However, the entire site areas at Wallen Bend and Frost Ford were not sampled in 2004, just the upper ~15% and 63% of each site, respectively. Thus, total population size at each site for both species was estimated by applying the 2004 density estimates to the entire measured site area. This section of river contains robust mussel populations and is the only reach where the abundance of both species is adequate to estimate site-specific census sizes and to collect tissue samples for genetic analyses. In conjunction with 2004 censuses, tissues from 20-30 individuals per site were collected from both species and used to extract DNA and conduct analyses of DNA microsatellites. Contemporary effective population sizes (N_e) were estimated at each site using the linkage disequilibrium (LD) method of Hill (1981). The method is known to be downwardly biased, but the program LDNe corrects the bias and was used to estimate N_e (Waples, 2006; Waples & Do, 2007). The genetic methods used to estimate N_e, including DNA extraction, PCR amplification conditions, size scoring of DNA microsatellites and associated analyses are available in Jones (2009).

Age-structured population models

Age-structured Leslie-matrix population models were implemented in RAMAS Metapop (Akçakaya & Root, 1998) to simulate reintroduction scenarios for *E. brevidens* and *E. capsaeformis*. Modeling was conduct-

ed assuming a single-site management scenario, i.e., a closed population with no immigration and emigration to and from nearby sites, with key parameters summarized in Table 1. Population projections were stochastic (10,000 iterations) and based on a 25 year (y) time horizon.

Maximum age was set in each matrix by the age of the oldest female determined by shell thin-sections, which was 15 y for *E. brevidens* and 10 y for *E. capsaeformis* (Jones & Neves, 2011). Males of each species are known to live longer but were assumed to not limit reproductive longevity of either population. To include the age-0 stage, a total of 16 stages (age classes) were used for *E. brevidens* and 11 stages for *E. capsaeformis*. A life-cycle diagram showing the age stages, survival transitions and recruitment rates of a freshwater mussel species living to a maximum of 10 y is illustrated in Figure 1.

Matrix transition probabilities (i.e., survival rates) from one age class to the next were assumed to be the same for males and females of both species in this study (Table 2). Survival rates were based initially on data collected by Jones and Neves (2011), where rates were determined using collection of dead shells in 0.25 m^2 quadrat samples and from catch-curve analyses of shell length-at-age data. However, the assumptions of either method, especially the latter, are rarely met in field studies and typically give only rough approximations of survival rates (Miranda & Bettoli, 2007). Therefore, survival rates of ages ≥1 were determined by empirical data gathered from the aforementioned study, survival rates of *in-situ* field studies of sub-adult mussels [M. Pinder, Virginia Department of Game and Inland Fisheries (VDGIF), unpublished data], and by examining rates typically reported for other long-lived species (Musick, 1999; Akçakaya et al., 2004). Survival of newly metamorphosed age-0 juvenile mussels is poorly understood but thought by us to fit a Type III survivorship curve. A survival rate of 30% for age-0 juveniles was used based on published (Jones et al., 2005) and unpublished data from laboratory culture studies conducted at the Freshwater Mollusk Conservation Center, Virginia Tech, Blacksburg, and the Aquatic Wildlife Conservation Center, VDGIF, Marion, Virginia. The rate reflects laboratory survival of newly metamorphosed juveniles from age-0 to 1 y old. Specifically, these are age-0 juveniles that upon excysting from fish hosts are considered viable based on observing pedal-feeding locomotion.

Fecundity was implemented in the model as average number of viable juveniles produced per parent individual, to include males. Traditionally, fecundity has been measured as the number of glochidia per gravid female mussel (Haag, 2002; Jones et al., 2004; Jones & Neves, 2002). However, in this study it is used as a composite value representing the net reproductive processes of both males and females, to include gametogenesis, spawning and fertilization, production of glochidia, attachment of glochidia on fish hosts, and ultimately metamorphosis and release of viable juveniles to the river bottom. Since these data are unavailable for most mussel species, it was solved iteratively in the matrix until the desired stable or increasing growth rate (λ) was obtained.

Demographic and environmental stochasticity

Both demographic and environmental stochasticity were included in the model because both sources of variation can alter the risk of population decline and extinction. Demographic stochasticity occurs when populations become very small and random fluctuations in mating and abundance can drive population size to zero. Demographic stochasticity was implemented by sampling abundance of age-1 or older survivors from a binomial probability distribution, and age-0 survivors from a Poisson probability distribution embedded in RAMAS. Fluctuations in environmental conditions, such as droughts and floods, can greatly affect population vital rates (Jones & Neves, 2011). Such environmental stochasticity was incorporated into the model by sampling random values for fecundity rates and survival rates from a lognormal distribution in RAMAS. Field study estimates of standard deviations (SD) for vital rates are sparse for most mussel species and when available, they are typically obscured by measurement error. Thus for both species, the SD (±1) was set at 33% of mean fecundity, 50% of mean survival of age-0 individuals, and 10% of mean survival of age-1 and older individuals. These are estimates of SD based on known characteristics of mussel life history and demography, such as variable recruitment success of juveniles and high annual survival of adults (Haag & Rypel, 2011; Jones & Neves, 2011). Survival and fecundity were assumed to be uncorrelated in the model. Extreme environmental variation such as catastrophes and bonanzas (i.e., a period of very high recruitment and survival) were assumed to be rare and not included in the model.

Initial abundances and ages

Initial abundances for modeling reintroduction of mussels to a site, for example in the upper Clinch River, VA where both species have been extirpated, were based on a predetermined number of mussels to be reintroduced y^{-1} for 5 y. Reintroduced mussels y^{-1} ranged from 24-120 individuals for *E. brevidens* and from 50-400 individuals for *E. capsaeformis* (Table 1). Simulations were conducted based on reintroducing an equal number of individuals ages 4-11 for *E. brevidens* and ages 3-7 for *E. capsaeformis*. These cohorts are abundant

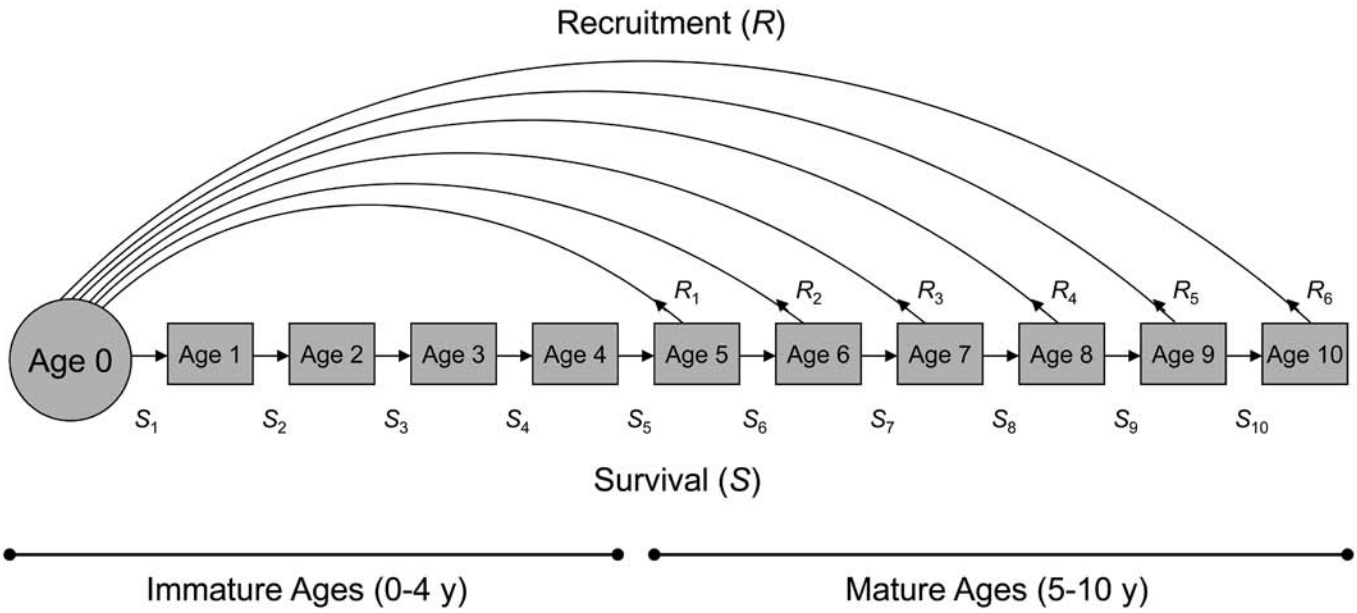

FIGURE 1

A general life-cycle diagram depicting the demography of a freshwater mussel species living to a maximum of 10 y, such as *Epioblasma capsaeformis*. Species living longer can be accommodated in the model by adding age classes, such as five more for *E. brevidens*. Nodes (circle and boxes) represent age-class stages, and arrows between nodes represent transitions (survival) between stages. Recruitment is shown as the number of age-0 individuals produced by adults in mature age classes.

and can be collected easily from the lower Clinch River, TN for reintroduction purposes.

Population growth rate and carrying capacity

Although density-dependent regulation and carrying capacity (*K*) are unknown for mussel populations, it is unrealistic to expect indefinite growth. Thus, a model of exponential population growth with a *ceiling*, set by *K*, was implemented in RAMAS for both species. This strategy allowed exponential population growth at every time step, but if *N*>*K*, then *N* was set equal to *K* (Akçakaya & Root, 1998). Population growth rate was controlled by survival of age-0 individuals. Values above or below the equilibrium survival rate (0.30) allowed the population to increase or decrease. For site reintroduction simulations, *K* was set at 3,000 individuals for *E. brevidens* and 5,000-10,000 individuals for *E. capsaeformis*, depending on population growth rate. These values of *K* represent a density of ~1-2 mussels m^{-2}, which in this study was used as the expected target density at a reintroduction site containing ~2,500-5,000 m^2 of suitable habitat, typical of sites in the upper Clinch River, VA. Because populations of *E. capsaeformis* are known to fluctuate widely and rapidly, three values of *K* were used to allow population growth to occur without being overly influenced by a ceiling value that was set too low, thus allowing for a wider range of demographic possibilities.

Reproductive value

Reproductive value measures the worth of an individual in each age class by the total number of progeny it can be expected to produce, to include its immediate offspring and all future descendants (Fisher, 1930). It is expressed relative to the reproductive value of the first age class, which was set to age-1. Reproductive values were calculated in RAMAS and are a product of the projection matrix.

Reintroduction simulation scenarios

Simulations were conducted by reintroducing equal numbers of individuals per year from targeted age classes. Reintroductions occurred each year for a 5 y population build-up period, which then grew unassisted for the next 20 y. Population growth levels varied from low, intermediate, and high, and were chosen to explore scenarios relevant to the population management of each species (Table 1).

The uncertainty of mean population projections and probability of population decline were assessed for all modeled scenarios. However, not all data were reported because results were very similar for most projections and therefore redundant. Furthermore,

because sample size (N=10,000) of mean trajectories was high, confidence intervals (CI) would be unrealistically narrow. Instead, uncertainty was explored using a small random sub-sample (N=20) of trajectories taken from reintroduction scenarios relevant to the population management of each species.

RESULTS

Effective population size and loss of genetic diversity

Estimates of contemporary N_e ranged from 178 to 223 individuals for *E. brevidens* and from 294 to 2,917 individuals for *E. capsaeformis*, whereas estimates of the census size (N_c) were much higher and ranged from 2,304 to 4,730 individuals and from 3,840 to 176,665 individuals of each species, respectively (Table 3). Estimates of N_e and N_c generally varied congruently among sites for *E. capsaeformis*, where N_e and N_c were highest at Frost Ford and lowest at Swan Island. In contrast, variation of N_e and N_c for E. brevidens was similar among sites. Ratios of N_e/N_c ranged from 0.0389 to 0.0773 for *E. brevidens* and from 0.0093 to 0.0766 for *E. capsaeformis*, with mean values at 0.0572 and 0.0342, respectively (Table 3).

Predicted declines in heterozygosity (H_e) and allelic diversity were greatest at N_e=25, but diminished as N_e increased (Fig. 2). Also, loss of allelic diversity was greater than corresponding declines in H_e. Loss of genetic diversity was minimal for N_e ≥75 out to about 5 generations, which is equivalent to 25 y based on a generation length of 5 y for both species. For example, when N_e=75, mean H_e declined by <5% and mean allelic diversity decreased by ~1.5 alleles, or 8.8%, after 5 generations. The greatest losses occurred when effective population size was at N_e=25, where mean H_e decreased by 10% and mean allelic diversity by ~7.5 alleles, or 44%, after 5 generations. While some loss of genetic diversity was evident for all investigated N_e, losses over longer generation times (≥10) were minimal (<5%) only at N_e=500.

Reintroduction abundance and population restoration success

The number of individuals reintroduced to a site during the 5 y population build-up phase was evaluated under three growth rate scenarios for both species (Fig. 3). Population trajectory patterns were characterized by three stages: (1) a sharp increase in population size during the build-up phase from 0-4 y, (2) followed by a period of disequilibrium when population size briefly declined and fluctuated from 5-14 y, and (3) a period of equilibrium when population size either remained stable or increased steadily from 15-25 y. Following the build-up phase, population size either remained stable or increased at all transplant levels. An important feature of each trajectory was how the number translocated y^{-1} during the build-up phase influenced final population size, and as expected, higher reintroduction numbers resulted in larger final population sizes.

Similarly, reintroduction uncertainty for *E. brevidens* was evaluated under a scenario of transplanting 48 individuals y^{-1} and at a low growth rate (λ=1.0125). The sub-sampled mean was below the modeled mean, but the upper 95% CI contained most of the latter (Fig. 4). Eleven of the sub-sampled population trajectories exhibited an increasing trend and finished greater than the post 5 y build-up population size. None of the sub-sampled trajectories declined to zero, and the minimum at 25 y was 105 individuals. Probability of decline was minimal (<5%) at all reintroduction levels, but slightly higher at 24 individuals y^{-1} (Fig. 5).

Reintroduction uncertainty for *E. capsaeformis* was evaluated under a scenario of transplanting 300 individuals y^{-1} and at a low growth rate (λ=1.025). The sub-sampled mean was generally greater than the modeled mean, but the 95% CIs entirely contained the latter (Fig. 4). Seventeen of the sub-sampled population trajectories exhibited an increasing trend and finished greater than the post 5 y build-up population size. None of the sub-sampled trajectories declined to zero, and the minimum at 25 y was 715 individuals. Probability of decline was minimal (<2.5%) at all transplant levels, but slightly higher at only 50 individuals y^{-1} (Fig. 5).

Although reintroduction uncertainty was evaluated only for the above scenarios, the same standard deviations for vital rates were used in all modeling scenarios. Hence, the uncertainty surrounding all modeling scenarios are quantitatively and qualitatively very similar to the above results. The probability of a 100% decline was extremely low (<1%) for all reintroduction scenarios.

Age class structure and reproductive value

The stable age distributions (SAD) of *E. brevidens* and *E. capsaeformis* demonstrated that as survival of age-0 individuals increased, the proportion of individuals comprising younger age-classes increased (Fig. 6). Although at first glance such small proportional increases of 1-2% or less in the younger age-classes appear minimal, they allowed modeled populations to grow over time. A key feature of the SAD of a population with a positive growth rate was the presence of a high proportion of young individuals. Of course, natural populations rarely resemble the structure of an SAD over short time periods because of uneven recruitment, but if censuses are taken regularly, the mean cohort structure may reflect an SAD. Furthermore, because of

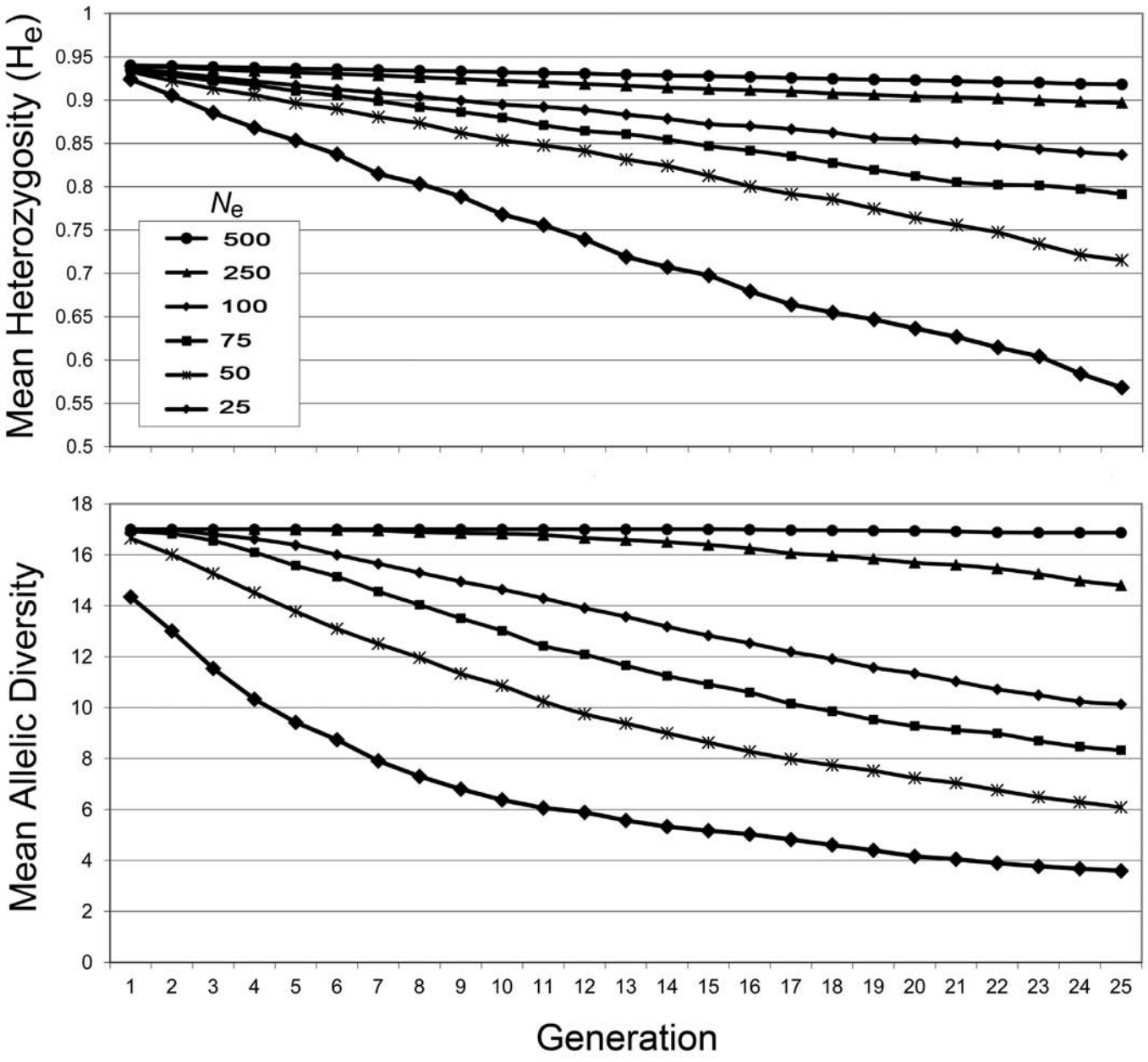

FIGURE 2

Predicted decline in heterozygosity and allelic diversity over time is dependent on effective population size (N_e). Generation length of each species is 5 y.

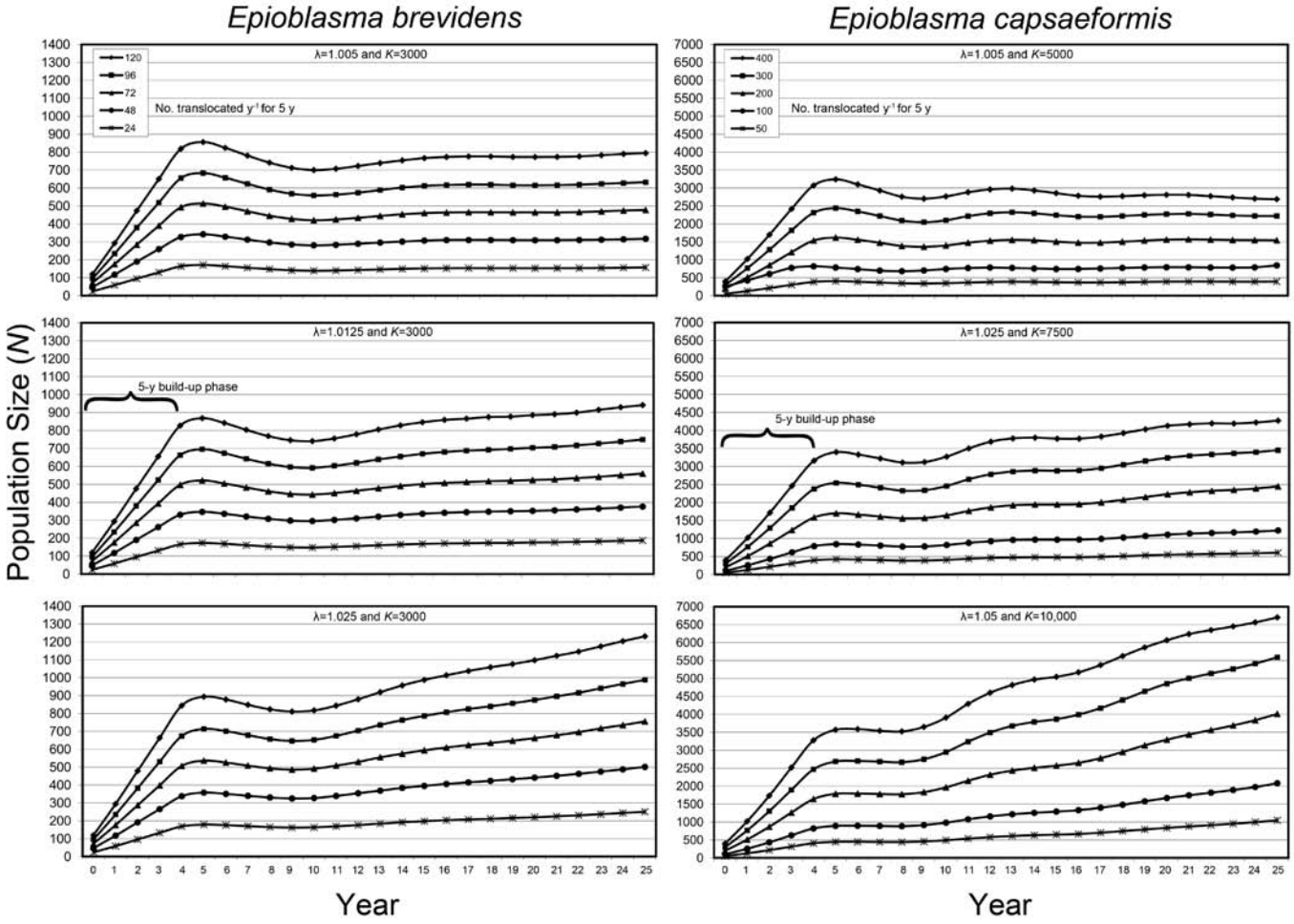

FIGURE 3

Mean population trajectories (10,000 simulations) of *Epioblasma brevidens* and *E. capsaeformis* demonstrate how number of reintroduced adult mussels during a 5 y build-up phase effects population size over a 25 y period. Simulations were conducted using stable, low and moderate growth rates (λ), where K was manipulated only for *E. capsaeformis* (see Methods).

their small sizes (e.g., <5-10 mm) it is difficult to accurately census age-0 juvenile mussels in situ (Jones and Neves 2011). So, in practice, age-1 individuals usually are the youngest age-class in the census. Examining the SAD without age-0 individuals in the distribution allows for a more direct comparison of modeled data with field data. The SAD of an expanding, stable or declining population showed that age-class structure flattened as growth rate declined (Fig. 7). The SAD of an expanding population was characterized by a steep age-class structure with a high proportion and abundance of young individuals, whereas the SAD of a declining population was characterized by a flat age-class structure with a low abundance of young individuals.

For both species, reproductive values were highest for individuals in the 5 y age-class, when maturity is reached (Fig. 6). Reproductive values also were high for age-classes 1-2 years younger or older than 5 y, but declined thereafter, and values were lowest by comparison at the higher growth rates.

DISCUSSION

Effective population size and maintenance of genetic diversity

Effective population size (N_e) is a critical parameter in population biology because it determines the expected rate at which genetic diversity is lost per generation. The census size (N_c) is also important and together these two parameters can be used to evaluate the capacity of a population to maintain genetic diversity over time. Genetic diversity is needed for two primary reasons: (1) so populations can adapt to changing environmental conditions, such as diseases,

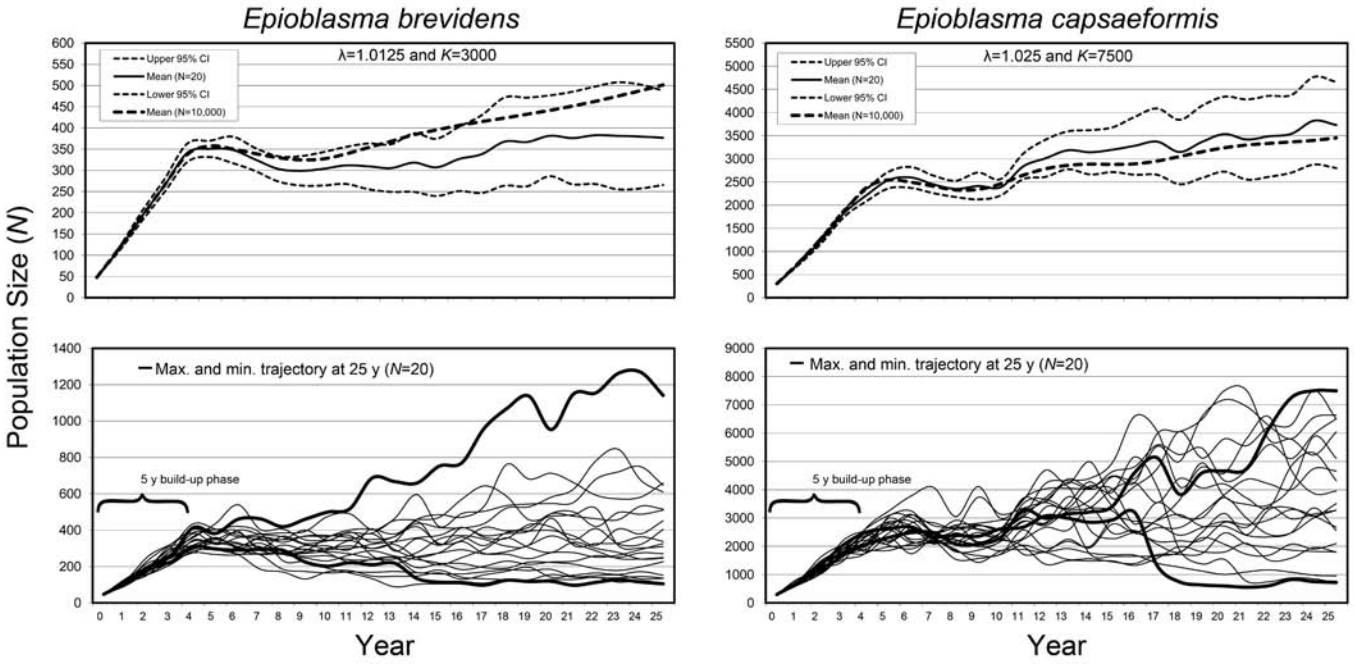

FIGURE 4

The mean of 20 simulated population trajectories (top graph) with 95% confidence intervals (CI), and each corresponding single trajectory (bottom graph) is displayed to show how simulated population size can fluctuate widely over time. Such fluctuations are an inherent outcome of the model and a consequence of the vital rate parameters being treated as stochastic. The figure displays trajectories of reintroductions of either 48 or 300 adults of each species, respectively. The mean trajectories based on 10,000 simulations and modeling scenarios are the same as those given in Fig. 2.

competitors, predators, climate change, habitat alterations and pollution, and (2) low levels have been linked to reductions of population fitness due to inbreeding depression (Frankham, 1996; Reed & Frankham, 2003; Reed, 2005). It is well known that small populations are more susceptible to loss of genetic diversity from genetic drift, and that such loss is the direct result of small and declining population size, which can compromise the ability of populations to respond to environmental change (Frankham et al., 2002).

The recovery plan for *E. brevidens* and *E. capsaeformis* specifies that populations need to be large enough to maintain sufficient genetic variation to be able to adapt to changing environmental conditions (USFWS 2004). Hence, managing for genetic diversity is an identified goal in the recovery plan of these two species. The ratio of N_e/N_c can be used to set a target census size that is sufficient to maintain genetic diversity over time. The results of this study indicate that the ratio of N_e/N_c was low (~5%) for both species, suggesting that a ratio of 5% would be a practical target for either species (Table 4). The genetic modeling conducted in this study suggested that if total N_e=500, then a high proportion (>95%) of molecular diversity could be retained over 25 generations, which is perhaps a realistic management time frame for mussel species with generation lengths of 3-5 y. Currently published guidelines also recommend that N_e=500 to ensure that animal populations retain adaptive potential over long time periods (e.g., >100 generations) (Frankham et al., 2002), which for the mussel species studied here, would require a total N_c=10,000. This census population size could be reached by building up multiple demes spread throughout a river, ideally in a reach unimpeded by dams and that has the natural free-flowing conditions and fish hosts needed to facilitate dispersal among demes. The role of gene flow or connectivity among demes plays a critical role in countering the effects of genetic drift on long-term maintenance of genetic diversity (Palstra & Ruzzante, 2008). The target N_e=500 could be achieved for example by building-up 5-10 local demes with census sizes of 1,000-2,000 individuals per site, which corresponds locally to N_e=50-100. Achieving these recommended or even greater population sizes is feasible and consistent with the known demography of both species at sites in the lower Clinch River, TN (Jones & Neves, 2011).

Estimates of N_e/N_c average approximately 11% for a range of species (Frankham, 1995), but can be much lower (<5%) for species with type III survivorship, which

include some bivalve mollusks and fishes (Hedgecock & Sly, 1990; Hedgecock et al., 1992; Boudry et al., 2002; Turner et al., 2006). Species with low N_e/N_c usually are characterized by life history traits such as high fecundity, high mortality of early life stages, highly variable annual recruitment, low parental care, and a high contribution of offspring to the next generation by relatively few parents. Freshwater mussels are known to exhibit these traits and varying degrees of hermaphroditic reproduction (van der Schalie, 1970), which is essentially a form of inbreeding that can decrease N_e.

Molecular markers are increasingly being used to estimate and monitor N_e in wild populations (Wang, 2005), and are useful for understanding long-term population trends and fluctuations. Severe and sustained declines in molecular variation and N_e may warn of possible declines in adaptive potential and the need to demographically boost or genetically supplement populations as part of a species' conservation program. Jones and Neves (2011) have shown that clear differences exist between the life-history traits and population demography of *E. brevidens* and *E. capsaeformis*, to include life span, population sizes, and recruitment. These differences undoubtedly influence the maintenance of genetic variation of each species. While a total N_e=500 is recommended here to maintain sufficient genetic variation for populations of *E. brevidens* and *E. capsaeformis*, it is critical that molecular and demographic methods be used together to set reintroduction targets and to monitor how populations are progressing over time. Periodic assessments of population size and genetic variation will be required to empirically validate whether targets are being met and sustained. Thus, a practical approach that seeks to maximize both abundance and genetic variation of populations is recommended.

Effect of reintroduction abundance on population restoration success

An important finding of the population reintroduction modeling was that the size of the initial population created during the 5 y build-up phase greatly affected final population size. If the expected growth rate of the reintroduced population was stable or even slightly positive (e.g., 1-2%), then final population size was very similar to size at the end of the build-up phase. In forecasting the expected outcomes of a reintroduction project, assuming a stable or low growth rate is probably the prudent and conservative approach. For example, the modeling results demonstrated that if 72 individuals of *E. brevidens* were transplanted y^{-1} to a site for 5 y, then ~500 individuals would be present at the end of the build-up phase, assuming an annual growth rate of 0.5-1.25% (Fig. 3). Importantly, the final population size at 25 y also would be ~500 individuals or slightly larger depending on the specific growth rate employed. Therefore, it is critical that the intended target census size per site be similar to population size at the end of the build-up phase. Further, the target census size should be large enough to accommodate the N_e that meets established program goals.

Population growth during the build-up phase is enhanced by reintroducing a greater proportion of subadults and younger adults (e.g., ages 4-8) with longer reproductive potential (Fig. 6). When feasible, releasing individuals with high reproductive value will likely be the most effective population reintroduction strategy. For example, translocations of adults proved to be the more effective strategy to restore populations of queen conch (*Strombus gigas* Linneaus, 1758) in over-harvested areas of the Florida Keys, U.S.A., compared to releasing juveniles that had no immediate reproductive output and were susceptible to higher mortality (Delgado et al., 2004).

It is important to emphasize that the population trajectories presented in Fig. 3 are mean values calculated from thousands of stochastic population projections generated by the RAMAS computer program. While such programs are valuable tools in the field of conservation biology, the mean values they provide should be interpreted with caution. The input variables used for most species; e.g., survival and environmental stochasticity, are usually poorly understood. The trajectory of a real population is always singular and influenced by a unique and unpredictable set of variables over a specified time frame, and will ultimately look irregular and more like the individual trajectories presented in Fig. 4. Biologists are aware of how real populations can fluctuate and occasionally do so dramatically, due to stochastic effects from disease, competition, flood, drought, and other factors.

Age class structure and recruitment

Natural populations rarely resemble the cohort structure of a SAD over short time periods, especially when data are from a single census. However, if censuses are taken at regular intervals (e.g., annually), then the mean cohort structure should begin to resemble the SAD. The SAD provides a portrait of the average cohort structure given key input variables, such as survival, fecundity, age at maturation, and maximum age. The SAD can be used to evaluate cohort structure of natural populations and determine whether they are recruiting and surviving at sustainable levels. Populations that are stable or growing will be characterized by a predominance of younger individuals and cohort structure will be skewed to the left, whereas declining and older populations will be characterized by middle to older-aged individuals and cohort structure will be

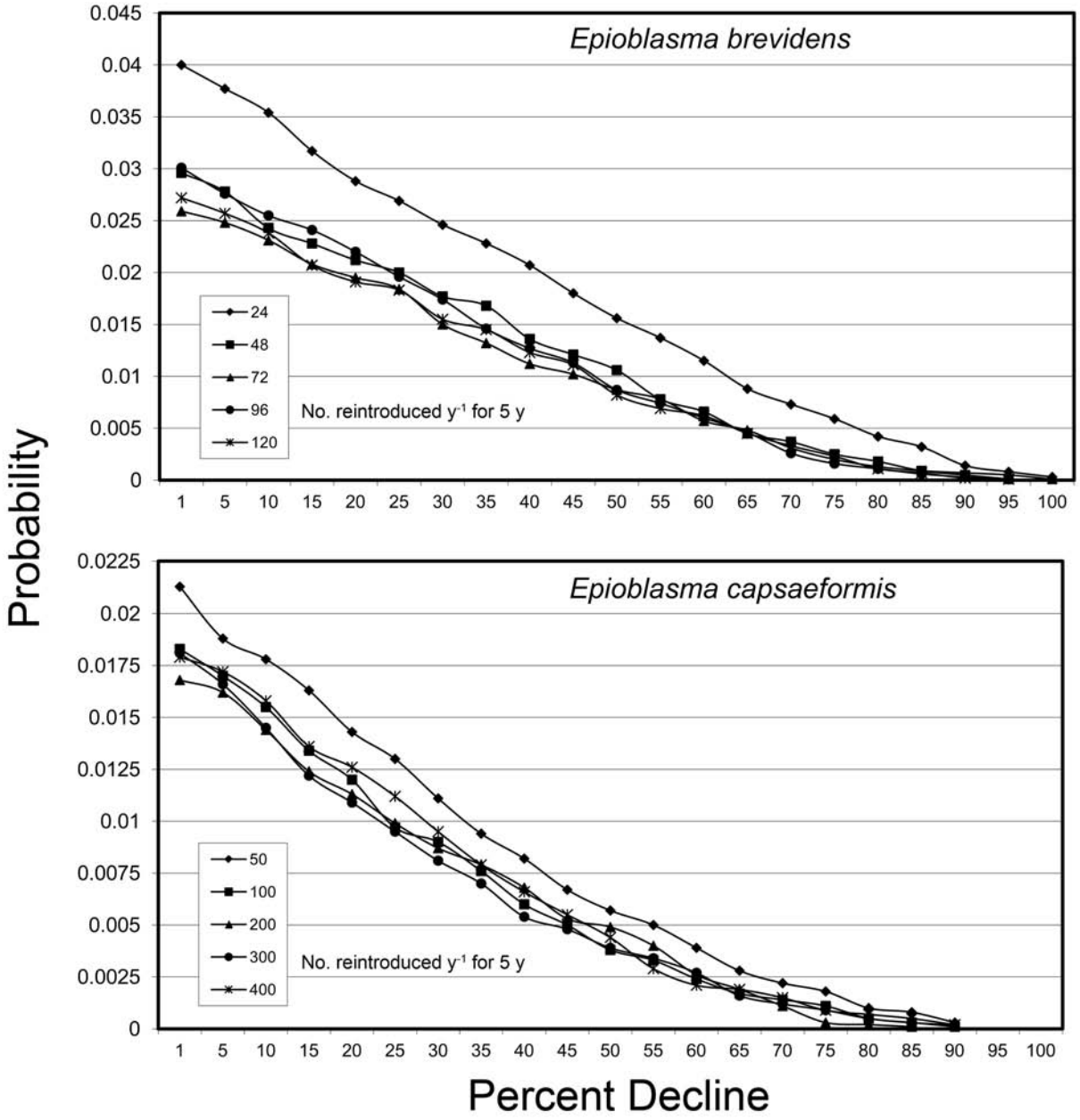

FIGURE 5

Probability of observing a decline from initial abundance over a 25 y period for *Epioblasma brevidens* and *E. capsaeformis*, based on various reintroduction scenarios. All probabilities were computed using the stable growth rate (λ=1.005), which represents the high risk scenario investigated in the study. Probabilities of decline at higher growth rates are lower.

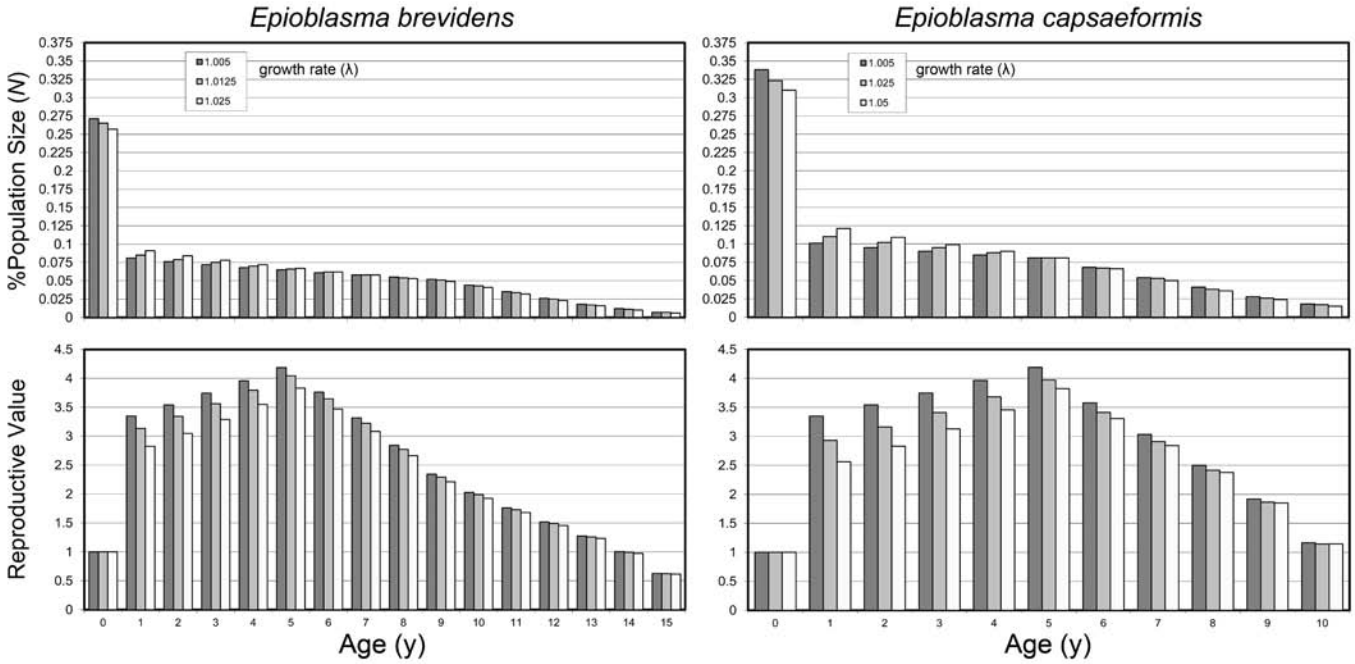

FIGURE 6
Stable-age distributions (SAD) and reproductive values for *Epioblasma brevidens* and *E. capsaeformis*; SADs at higher growth rates were similar to those computed using a stable growth rate, increasing only ~1-2% in younger age-classes (≤5 y).

skewed to the right. Obviously, for a population to grow, the birth rate must exceed the death rate and the longer-lived a species, the less frequently it needs to experience above-average recruitment. Freshwater mussels are typically long-lived (>20 y) animals, and many species do not exhibit high annual recruitment, but rather sporadic recruitment that is occasionally punctuated by exceptional year classes (Payne & Miller, 2000; Strayer et al., 2004). However, shorter-lived species such as *E. capsaeformis* must recruit more often and at greater levels to sustain viable populations, and therefore are more vulnerable to decline and ultimately to extirpation or extinction, especially if population or habitat disturbances are long-lasting (Jones & Neves, 2011).

Two key demographic questions, then, are to determine the cohort structure and annual recruitment levels needed to sustain a stable or growing population of *E. brevidens* and *E. capsaeformis* (USFWS, 2004). The SAD histograms in Fig. 6 show profiles of three cohort structures for each species based on stable, low and moderate growth rates, illustrating that the cohort structure of a stable or growing population should be dominated by immature individuals and young adults. The histograms also indicate that age-0 individuals should make-up about 26-27% of the population for *E. brevidens* and about 31-34% of the population for *E. capsaeformis*, depending on the growth rate examined. These percentages are a product of the Leslie matrices, which were parameterized with input variables to include the age-0 survival rate, which in this study was approximately 30% (Table 2). While these input variables represent areas of uncertainty in the model, the SADs generated for each species are similar to cohort data obtained from field collections. The mean cohort structure (2004-2008) of *E. brevidens* and *E. capsaeformis* in the Clinch River, TN, is currently dominated by younger age groups, indicating that these populations are stable or expanding, respectively (Jones & Neves, 2011). During this period, both populations exhibited strong and weak year-classes, but recruitment was always a measurable feature of the population. Of course, age-frequency histograms produced from real populations in the river are more uneven, but they do match expectations based on the computer-generated SAD. It is difficult to accurately census age-0 individuals in mussel populations because of their small size (typically <10 mm), so age-1 is usually the first age-class assessed as a measure of recruitment. Therefore, if age-0 individuals are removed from the SAD

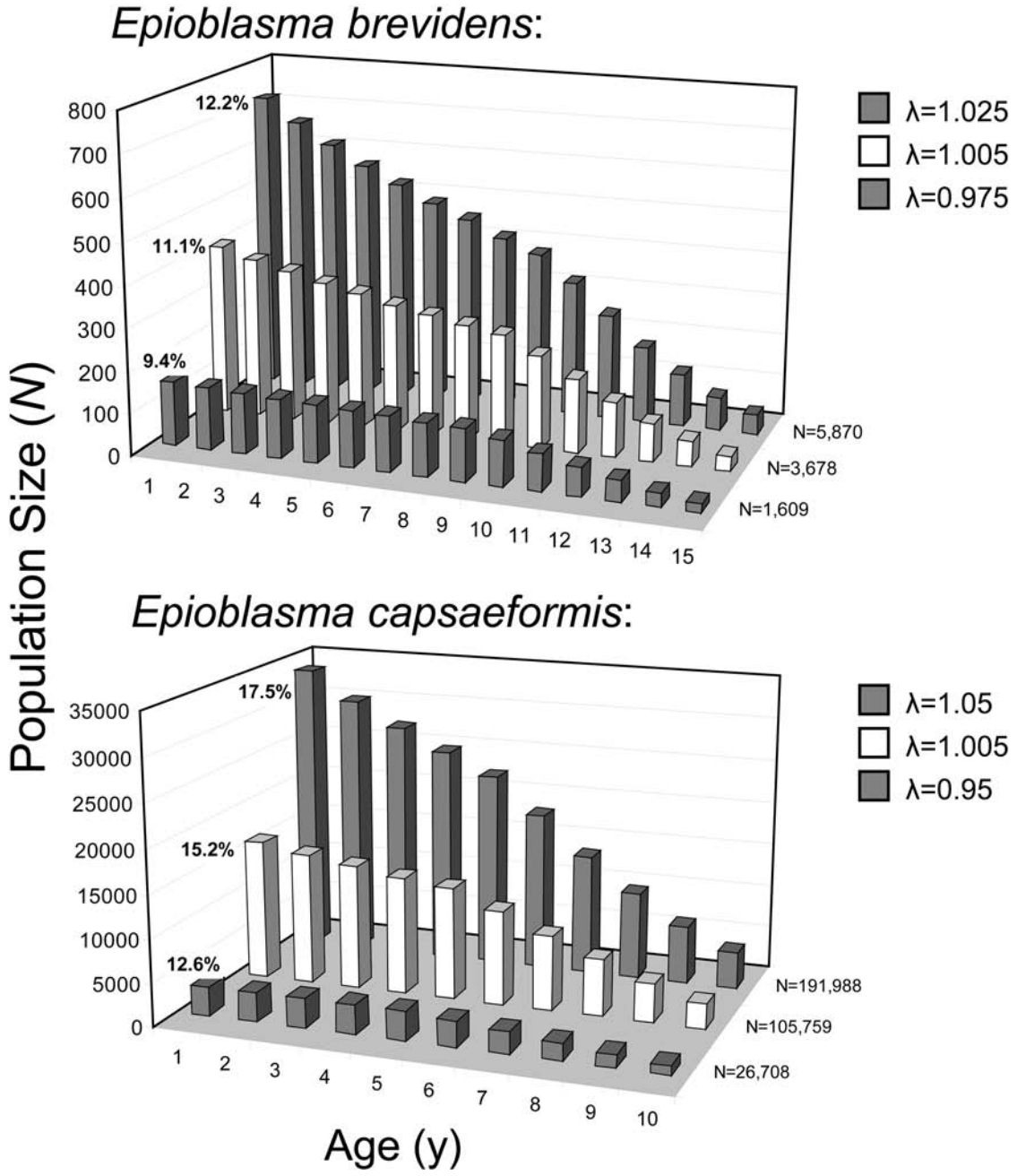

FIGURE 7

Stable-age distributions generated in RAMAS depicting declining (front), stable (middle) and expanding (back) populations of each species. Population sizes (*N*) given on the Z-axis represent mean abundance (10,000 simulations) after 25 y. Age-0 individuals are not shown or included in total *N*. Typically, this cohort is too difficult to sample reliably for freshwater mussels. Instead, age-1 individuals are the first cohort shown along with its percentage of total *N*. Starting population sizes were *N*=4,500 and *N*=152,000 for each species, respectively.

histograms, then age-1 individuals comprise ~11% of a stable population of *E. brevidens* and ~15% of a stable population of *E. capsaeformis* (Fig. 7). These values can be used as criteria to evaluate population performance of these species (Table 4). For example, mean recruitment of age-1 individuals from 2004-2008 for *E. brevidens* was 16.4% (range: 12.0-24.0%), and for *E. capsaeformis*, 28.9% (range: 4.2-56.6%) (Jones & Neves, 2011). These recruitment levels in the river exceed the above criteria and indicate growing populations for both species, a conclusion corroborated by the trend data from 2004-2008 (Jones & Neves, 2011). A study of mussel populations in the Sipsey River, AL of the upper Mobile River basin, found that new recruits comprised an average of 11% of the total population, a figure highly variable among species, sites, and years (Haag, 2002; USFWS, 2004). Haag (2002) further demonstrated, using stochastic stage-based matrix models, that mean recruitment must be 5-12% depending on the species to maintain a stable or increasing population. These recruitment levels are generally lower than the projected values for *E. brevidens* and *E. capsaeformis*, but were estimates derived from longer-lived mussel species. Maximum age or mean age-at-death of a species or population is a life history trait that plays an important role in governing sustainable recruitment; namely, long-lived species can recruit less frequently and at lower levels than short-lived species (Haag & Rypel, 2011; Jones & Neves, 2011).

Addressing modeling uncertainty

In this study, the two areas of modeling uncertainty that deserve further consideration are: (1) predicting declines of genetic diversity based on effective population size, and (2) the species-specific demographic input variables used for the Leslie-matrices. First, the simulations conducted in EASYPOP to predict declines of genetic diversity did not account for effects of hermaphroditic reproduction, fluctuating population size, or overlapping generations due to extended life span. The first two demographic factors would act to increase the rate of loss of genetic variation, while the last demographic factor would act to decrease the rate of loss of genetic variation. The program can simulate effects of different levels of hermaphroditic mating, but the incidence or rate of hermaphroditism is unknown for either *E. brevidens* or *E. capsaeformis*. Until studies are conducted to examine rates of hermaphroditic reproduction across a range of mussel taxonomic groups, modeling its effect on maintenance of genetic diversity will remain too speculative to be of predictive value. In addition, population size is held constant during program simulations; therefore, effects of fluctuating population size on genetic diversity are not considered, which would be important for species such as *E. capsaeformis*, especially at small population sizes. Also not accounted for in the model was increased life span and overlapping generations, which would act to decrease the loss of genetic diversity. Thus, for species such as *E. brevidens* that exhibit longer life span and perhaps a more stable population size over time, such species would contain a greater number of overlapping generations, and therefore a higher ratio of N_e/N_c and capacity to retain genetic variation over time. Again, the mean ratio of N_e/N_c for *E. brevidens* was slightly higher than that for *E. capsaeformis* (Table 3). Other areas of modeling uncertainty include the mutation rate for molecular markers used in simulations, which in this study was based on a commonly reported rate for microsatellites in the literature, but higher or lower rates would slow down or accelerate loss of genetic variation, respectively.

The input variables used to parameterize each species Leslie-matrix are another source of uncertainty, including: (1) survival of age-0 individuals and other cohorts, (2) maximum age, (3) average age or size at maturation, (4) average fecundity of females, and (5) effects of density-dependence. The survival rates used in this study were derived using a combination of empirical data, anecdotal observations, and professional judgment. Survival rate of individuals ≥1 y old likely is high (>90% y^{-1}) for mussel species early in life (e.g., ages 1-5), but then decreases as mussels become reproductively active, due to predation, physiological stress of reproduction and other factors. The shape and slope of a species or population survival curve will vary and be influenced by both environmental conditions and longevity. However, the estimated survival rate of age-0 individuals is the least certain. Although set at 30% for each species in this study (Table 1), field and laboratory studies are needed to better quantify the mean rate and variance of these parameters.

Maximum age of *E. brevidens* and *E. capsaeformis* in the Leslie-matrices was set at 15 and 10 y, respectively, based on ages of collected females. It is possible that maximum age of the former species was set too low. Males of the species in the Clinch River can live to at least 28 y, suggesting that females also live longer than 15 y (Jones and Neves 2011). Increasing maximum age in either species' matrix would change modeling results. Importantly, it would act to decrease the recruitment rate needed to maintain stable or growing populations. Thus, the maximum ages used here provide higher, but arguably more conservative estimates of recruitment for reintroduction and recovery purposes. Additional sampling and thin-sectioning of shells could possibly identify the presence of older females in the population of both species, but setting the maximum age based on older, perhaps se-

nescent individuals may not reflect average population dynamics for the species. Age at maturation was set at 5 y for both species, but favorable environmental conditions could enhance growth and allow some individuals in the population to mature at younger ages, perhaps in 3 or 4 y. Accounting for a proportion of earlier maturing individuals (<5 y) would increase population recruitment and growth.

Density-dependent factors are not well understood for freshwater mussels, but population growth cannot go unchecked indefinitely. Limiting factors such as competition for physical space, fish hosts, food, predation and other factors will eventually limit population growth. However, most mussel species occur at sufficiently low densities that density-dependent factors likely would not affect population growth. Hence, setting carrying capacity (K) or a population ceiling for most species may be arbitrary, but likely one that is useful to prevent unrealistically high trajectories from occurring during simulations. Time series data on population sizes across a range of sites could help inform such decisions. In this study, population ceilings were set at sufficiently high levels as to minimally influence mean trajectories, and were based on time-series data from multiple sites in the Clinch River (Ahlstedt et al., 2005: Jones & Neves, 2011).

Finally, as more data become available, the modeling assumption of a closed population at restoration sites should be re-evaluated for species utilizing host fishes with higher dispersal capabilities, such as *E. brevidens*. It is likely that a percentage of local fish hosts infested with glochidia from released mussels would disperse away from the site. However, an equal number of infested fish hosts would not disperse to the site because nearby or adjacent sites would lack established populations. Therefore, the site emigration rate would likely exceed the immigration rate depending on the dispersal ability of the fish hosts. The net effect would be to decrease juvenile recruitment and the local population growth rate.

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TABLE 1

Summary of the Leslie matrix model parameters used in RAMAS to simulate population growth and reintroduction of the Cumberlandian combshell (*Epioblasma brevidens*) and oyster mussel *(E. capsaeformis*). Simulations were conducted using an exponential growth model, where standard deviation (SD) represents environmental variation and was sampled from a log-normal distribution.

		E. brevidens	*E. capsaeformis*
Parameter	Description	Value (SD)	Value (SD)
Age at first reproduction	Males and females	5 years	5 years
Population growth rate (λ)	Stable population	$\lambda \approx 1.005$	$\lambda \approx 1.005$
	Low growth	$\lambda \approx 1.0125$	$\lambda \approx 1.025$
	Moderate growth	$\lambda \approx 1.025$	$\lambda \approx 1.05$
Survival rate (S) of Age-0 juveniles controls λ	Equals stable population	0.30 (0.15)	0.30 (0.15)
	Equals low growth	0.323 (0.16)	0.35 (0.17)
	Equals moderate growth	0.363 (0.18)	0.42 (0.21)
Initial population size (N)	Reintroduced population (number released y^{-1} for 5 y)	24 48 72 96 120	50 100 200 300 400
Ages of adults (y)	Reintroduced	4-11	3-7
Carrying capacity (K)	Reintroduced population	3,000	5,000 7,500 10,000
Type of density dependence		Ceiling (=K)	Ceiling (=K)

TABLE 2

Age-structured Leslie matrices of survival and fecundity values used to simulate population growth and reintroduction of Cumberlandian combshell (*Epioblasma brevidens*) and oyster mussel *(E. capsaeformis*). The three different survival values of juvenile mussels in the first column (0-1*) correspond to stable, low and moderate population growth simulated in the study (see Table 1).

Epioblasma brevidens:

Immature Age Classes (0-4) Mature Age Classes (5-15)

	0-1*	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15
0-1						0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63
1-2	0.300 0.323 0.363															
2-3		0.95														
3-4			0.95													
4-5				0.95												
5-6					0.95											
6-7						0.95										
7-8							0.95									
8-9								0.95								
9-10									0.95							
10-11										0.85						
11-12											0.80					
12-13												0.75				
13-14													0.70			
14-15														0.65		
15															0.60	0.00

Epioblasma capsaeformis:

Immature Age Classes (0-4) Mature Age Classes (5-10)

	0-1*	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10
0-1						1.17	1.17	1.17	1.17	1.17	1.17
1-2	0.30 0.35 0.42										
2-3		0.95									
3-4			0.95								
4-5				0.95							
5-6					0.95						
6-7						0.85					
7-8							0.80				
8-9								0.75			
9-10									0.70		
10										0.65	0.00

TABLE 3

Effective population sizes (N_e) and census sizes (N_c)) for *Epioblasma brevidens* and *E. capsaeformis* in the Clinch River, TN at Wallen Bend (WB), Frost Ford (FF) and Swan Island (SI). The 95% confidence intervals are given in parentheses. Sampling was conducted in 2004.

Species	Site	N_e	N_c	N_e/N_c
Epioblasma brevidens	WB	223 (49; Infinity)	4,023 (3*; 8,495)	0.0554
	FF	184 (65; Infinity)	4,730 (3*; 9,988)	0.0389
	SI	178 (57; Infinity)	2,304 (541; 4,067)	0.0773
				Mean=0.0572
Epioblasma capsaeformis	WB	350 (124; Infinity)	37,615 (23,298; 51,798)	0.0093
	FF	2,917 (128; Infinity)	176,665 (140,670; 212,472)	0.0168
	SI	294 (94; Infinity)	3,840 (1,401; 6,278)	0.0766
				Mean=0.0342

*The lower confidence interval was set based on the number of mussels collected in quadrat samples at each site.

TABLE 4

Proposed population performance criteria to evaluate reintroduction and recovery of two endangered mussel species. Values are intended as overall targets to evaluate a contiguous riverine population comprised of multiple demes.

Species	Total N_e	Total N_c	Mean recruitment y^{-1} of age-1 juveniles	Mean age-class structure
Epioblasma brevidens	≥500	≥10,000	≥11%	• Age-classes ranging from 1-12+ yrs. • Age-classes 1-4 comprise ~40% of *N*
Epioblasma capsaeformis	≥500	≥10,000	≥15%	• Age-classes ranging from 1-8+ yrs. • Age-classes 1-4 comprise ~50% of *N*

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A QUALITATIVE FRESHWATER MUSSEL (BIVALVIA: UNIONIDAE) SURVEY OF THE LAMINE AND BLACKWATER RIVER BASINS, MISSOURI

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ABSTRACT

From 2003 to 2006 freshwater mussels (Bivalvia: Unionidae) were qualitatively surveyed in the Lamine River basin, a Missouri River tributary in west central Missouri. Timed searches (average time/site = 1.9 hr) were conducted to ascertain the distribution, diversity and abundance of unionids in the basin. A total of 45 sites were sampled and 5287 individuals from 27 species were observed, including *Ligumia recta*, a Missouri Species of Conservation Concern. The invasive *Corbicula fluminea* was observed live at nearly all sampling locations throughout the basin. Overall average Catch per Unit Effort (CPUE, live individuals/person hr) was 54.7 and ranged from 0 to 417.6. *Amblema plicata* was the most abundant species, with 2989 individuals recovered at 34 sites, representing 56.5% of the live mussels collected. *Leptodea fragilis* and *Potamilus alatus* were the most widely distributed species, each occurring at 36 sites. The Lamine basin unionid fauna (30 historic, 27 extant species) is more diverse than that of prairie streams in the Missouri River system and is similar to Ozark rivers. Given the anthropogenic impacts occurring in the basin, the Lamine River basin has a diverse freshwater mussel fauna. A number of species rich mussel assemblages were observed in the mainstem Lamine River. Continuing with management objectives to maintain water quality, improve aquatic habitat, and work with private landowners to stabilize streambanks and improve riparian zones will be necessary to maintain the diversity of freshwater mussels in the Lamine River basin.

KEY WORDS Freshwater Mussels, Qualitative Survey, Lamine River, Blackwater River, Missouri

INTRODUCTION

With less than 25% of the fauna considered stable (Williams et al., 1993), native freshwater mussels (Mollusca: Bivalvia: Unionidae and Margaritiferidae) are one of the most endangered groups of animals in North America (Stein et al. 2000). In Missouri, 10 species are listed as state endangered, 9 of which are also either federally endangered or candidate species; 19 other species are considered Missouri Species of Conservation Concern (SOCC). With 42% of the statewide fauna considered to be SOCC, freshwater mussels rank second only to crayfish in terms of imperilment in Missouri (MDC 2011). Documenting the distribution and diversity of freshwater mussels is a key aspect of their conservation (NNMCC 1998, MDC 2008).

Previous survey efforts in the Lamine River basin have documented 30 species, including 2 SOCC: *Anodonta suborbiculata* Say, 1831, and *Ligumia recta* (Lamarck, 1819) (Utterback, 1915–1916, 1917; Oesch, 1995) (Table 1). Utterback (1915–1916, 1917) documented 21 species from the Blackwater River portion of the basin, but unfortunately included few specific details about collection locations or species distributions. Oesch (1995) reported 30 species from 10 locations in the basin, adding 9 species to the fauna that had not been previously reported: *Fusconaia flava* (Rafinesque, 1820), Obliquaria reflexa Rafinesque, 1820, Pleurobema sintoxia (Rafinesque, 1820), *Potamilus alatus* (Say, 1817), *Potamilus ohiensis* (Rafinesque, 1820), *Quadrula pustulosa* (Lea, 1831), *Truncilla donaciformis* (Lea, 1828), *Truncilla truncata* Rafinesque, 1820, and *Venustaconcha ellipsiformis* (Conrad, 1836). Other than these limited survey efforts, little was known of the diversity and distribution of freshwater mussels in the Lamine River basin. This survey was conducted to document the distribution, diversity and abundance of unionid mollusks, in particular SOCC, in the Lamine River Basin.

The Lamine River is the 3rd largest free-flowing river in Missouri (Brown et al., 1992), and together with

its largest tributary (Blackwater River), the basin drains approximately 6863 km2 of the Central Plains Aquatic Subregion (Sowa et al., 2005) in west central Missouri (Figures 1 and 2). This subregion was largely glaciated during the Pleistocene Epoch, and is characterized by low, rolling plains. Surface runoff is the primary source of water to typical streams within the subregion, and stream discharge fluctuates widely from extremely low base flow conditions to relatively high peak discharges following rain events (Sowa et al., 2005, 2007). The Lamine River is an Ozark border stream (Pflieger, 1989), and is unique because it straddles the border between the largely glaciated Central Dissected Till Plains and unglaciated sections of the Ozarks (Sowa et al., 2005). Tributary streams from the west tend to be of a lower gradient and primarily turbid, with sand and silt substrates, while tributaries from the south and east tend to be clear with gravel substrates similar to Ozark streams (Sowa et al., 2005).

Historically the basin was dominated by tallgrass prairie to the west, transitioning to oak and mixed-hardwood forested areas in the east (Sowa et al., 2005). Presently, landuse in the basin is largely agricultural, either row crops or pasture, with only a few remnants of native prairie remaining (MDNR, 2008). There are a number of sizeable communities in the basin, each of which has numerous permitted point source discharges. Threats and impacts to the basin's mussel fauna include point source pollution discharges, channelization, head cutting, nonpoint source runoff, gravel mining operations, and invasive species (Brown et al., 1992). Brown et al. (1992) considered aquatic habitat quality to be fair throughout the Lamine River portion of the basin, however lack of riparian corridor and areas of intensive streambank erosion were prevalent in select areas. Fortunately, approximately 92% of the mainstem Lamine River remains unmodified (Brown et al., 1992). In contrast, many streams in the Blackwater River portion of the basin, including the Blackwater River mainstem itself, have been extensively channelized (S.E. McMurray, pers. obs.).

METHODS

Freshwater mussels were qualitatively sampled by experienced personnel with timed searches at 45 locations from 2003 to 2006 (total search time = 86.1 person hr, average time/site = 1.9 person hr) (Fig. 1 and 2, Appendix A). Timed, qualitative searches were conducted to maximize species richness and optimize our ability to detect rare species (Strayer et al., 1997; Vaughn et al., 1997). Search time at each location was dependent upon stream size and the amount of area that could be searched. Sampling locations were chosen in the field based on availability and quality of habitat (e.g., stable substrates, suitable flow) and signs of mussel assemblages (e.g., shell material on gravel bars, live animals observed), and were accessed via public or private accesses, bridge crossings, or boat. These sites included new as well as previously surveyed locations. Additional collections of shell material, previously unreported, were made between 1995 and 1999 by Missouri Department of Conservation (MDC) staff.

Depending upon water clarity and depth mussels were surveyed visually with snorkeling or view scopes or with tactile searches, in all available habitats. All mussels were identified, counted, and returned to the substrate; shell material was also collected from each location. Length measurements (anterior to posterior margins) were made from all mussels collected from 4 assemblages in the Lamine River (locations 4, 10, 22, 27), 1 assemblage in Muddy Creek (location 28), and 1 assemblage in Spring Fork (location 37) (Figures 1 and 2). Nomenclature largely follows Turgeon et al. (1998), except where accepted taxonomic changes have occurred. Conservation status follows Williams et al. (1993) and the Global Rank and State Rank of each species observed follow MDC (2011) and NatureServe (2010). The Global Rank is an assessment of global imperilment primarily based on the number of occurrences worldwide, and range from G1 (Critically Imperiled) to G5 (Secure) (MDC, 2011; NatureServe, 2010). The State Rank is a measure of imperilment primarily based on the number of occurrences of a species in Missouri, and as with Global Ranks ranges from S1 (Critically Imperiled) to S5 (Secure) (MDC 2011).

RESULTS

We observed 5287 individuals representing 27 species at the 45 locations surveyed in the basin (Appendix A). Average Catch per Unit Effort (CPUE, live individuals/person hr) for all survey locations was 54.7, ranging from 0 to 417.6. *Amblema plicata* was by far the most dominant species collected with 2989 individuals occurring at 34 of 45 sites (75.5%), representing 56.5% of live mussels collected (Table 2). *Leptodea fragilis* and *Potamilus alatus* were the most commonly encountered species, each occurring at 36 locations. Including *A. plicata*, 12 species had relative abundance values greater than 1.0%. A majority of the species observed (n=15) had relative abundance values less than or equal to 1.0% (Table 2). The invasive species *Corbicula fluminea* (Müller, 1774) was observed live at nearly all sampling locations throughout the basin, but counts of individuals were not made.

At the 6 locations where length measurements were collected the most dominant species observed,

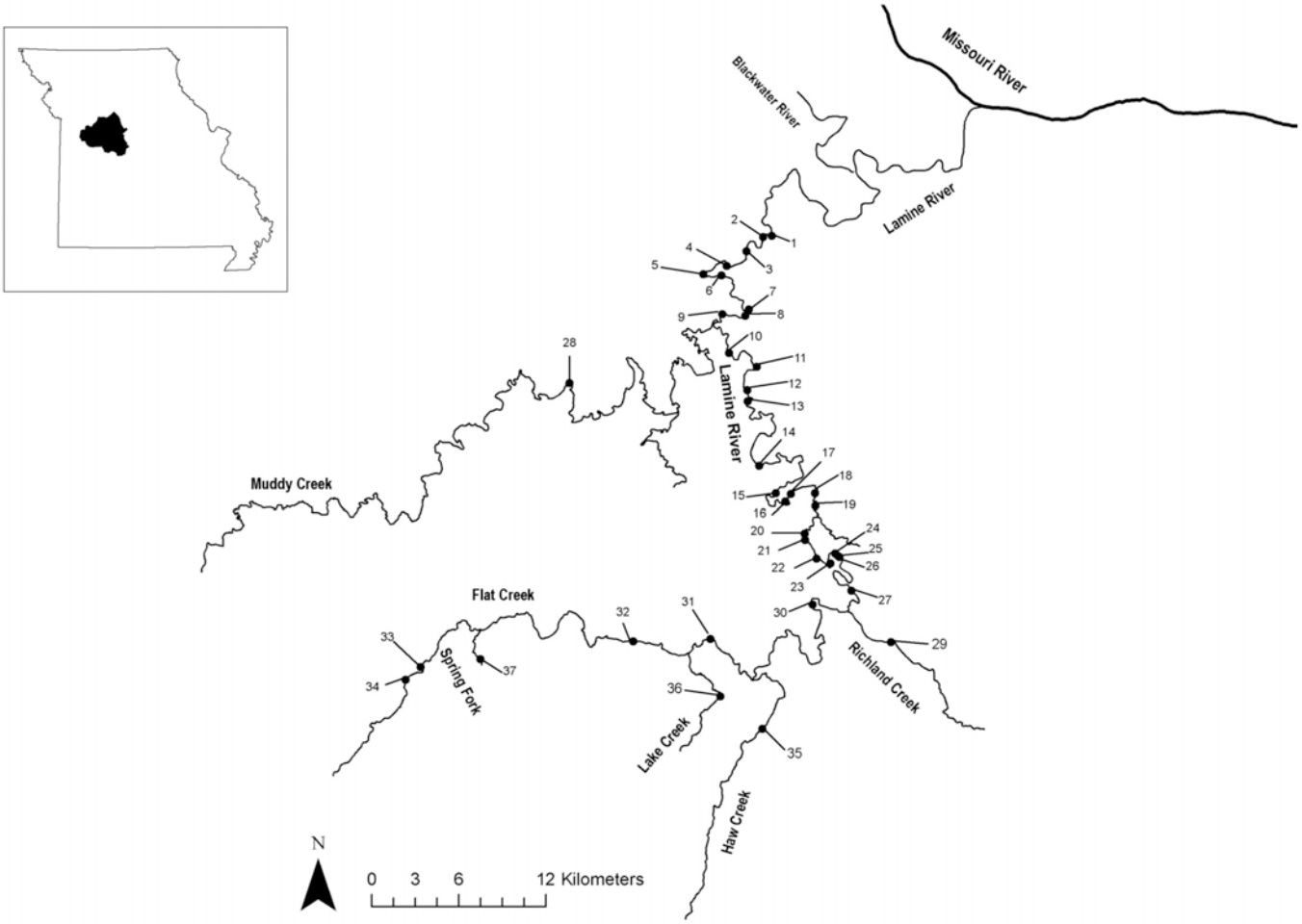

FIGURE 1

Qualitative freshwater mussel survey sites in the Lamine River, Missouri, 2003 – 2006. Inset shows the location of the basin in Missouri.

Amblema plicata, ranged from 25 – 156 mm in shell length (n = 146, $\bar{x}$ = 108.4 ± 32.3 mm). With the exception of *Lampsilis cardium* (n = 28, 57 – 159 mm, $\bar{x}$ = 126. 4 ± 30.0), *Obliquaria reflexa* (n = 37, 27 – 82 mm, $\bar{x}$ = 64.4 ± 12.2), and *Quadrula quadrula* (n = 70, 29 – 127 mm, $\bar{x}$ = 97.329 ± 23.9) the most abundant species observed were largely represented by larger, and therefore older, individuals (Figure 3).

Nearly all of the 27 species observed during this survey effort were found in the Lamine River mainstem, with 3 species (*Ligumia recta*, *Ellipsaria lineolata* and *Venustaconcha ellipsiformis*) restricted to the mainstem of that river. *Megalonaias nervosa* (Rafinesque, 1820) was only found live in the Lamine River mainstem, but was represented by shell material from a single location in Muddy Creek, a Lamine River tributary. *Ligumia subrostrata* (Say, 1831), *Pyganodon grandis* (Say, 1829), and *Toxolasma parvum* (Barnes, 1823) were only represented by shell material in the mainstem Lamine River, but were found live in other portions of the basin. *Uniomerus tetralasmus* (Say, 1831) was the only species that did not occur in the Lamine River mainstem; it was restricted to the South Fork Blackwater River and Flat Creek.

Most of the species observed in the present survey were S4 or S5 species (Apparently Secure or Secure, respectively) (MDC 2011). A single Missouri SOCC, *Ligumia recta*, was represented by a total of 4 live individuals at 4 locations in the Lamine River mainstem. Weathered and subfossil shell material was collected at an additional 9 locations also in the Lamine River mainstem. Globally, *L. recta* is a G5 (Secure) species, but is an S2 (Imperiled) species in Missouri (MDC 2011, NatureServe 2010.

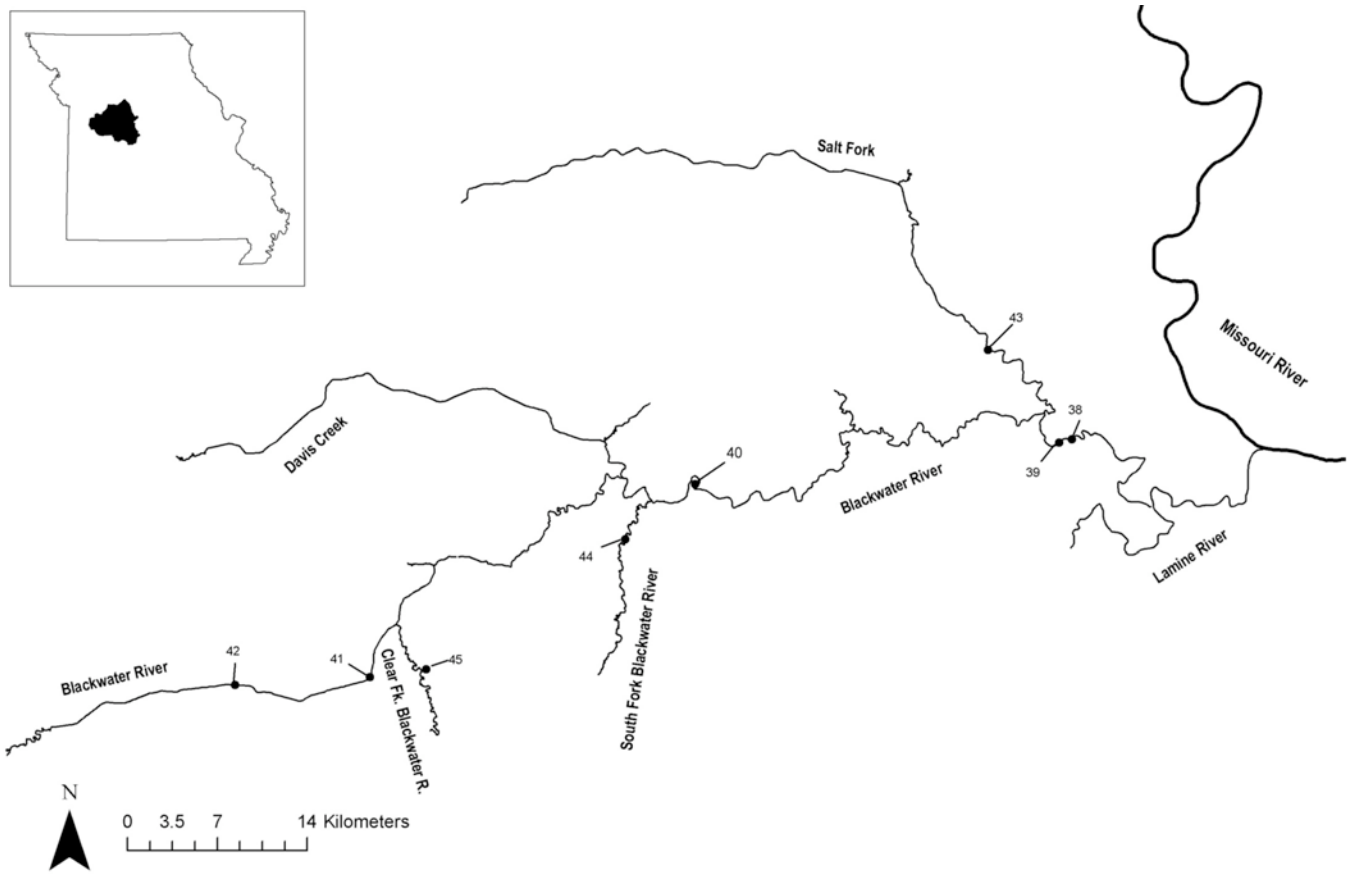

FIGURE 2
Qualitative freshwater mussel survey sites in the Blackwater River, Missouri, 2003 – 2006. Inset shows the location of the basin in Missouri.

DISCUSSION

With a fauna of 30 species, freshwater mussel diversity in the Lamine River basin is similar to Ozark streams in the Missouri River system, such as the Sac River (39 species; Hutson & Barnhart, 2004; MDC, unpubl.), Pomme de Terre River (31 species, Hutson & Barnhart, 2004), and Gasconade River (43 species; Buchanan, 1994; Bruenderman et al., 2001; MDC, unpubl. data). In contrast, the Lamine River basin is much more diverse than prairie rivers in the Missouri River system such as the Platte River (12 species, MDC, unpubl. data) and Grand River (19 species, MDC, unpubl. data). This is reflective of the ichthyofauna of these systems, with Ozark rivers being more diverse than their prairie counterparts (Pflieger, 1997).

The dominant species in the Lamine River basin, *Amblema plicata*, is relatively common and widely distributed in the Midwest (Cummings & Mayer, 1992) and in Missouri (Oesch, 1995). *Amblema plicata* is a habitat generalist, appears to be tolerant of a wide range of water quality, and therefore may become a dominant species in many river systems (Oesch, 1995). *Amblema plicata* has been found to be the dominant species in other river systems with varying degrees of impacts similar to those observed in the Lamine River basin (i.e., high sediment loads, hydromodification). Ahlstedt & Jenkinson (1991) reported that A. plicata represented >54% of the mussels collected in the lower St. Francis River (Missouri and Arkansas). Hutson & Barnhart (2004) reported that *A. plicata* represented 43% of the mussels collected in the Pomme de Terre River (Missouri). Wentz et al. (2009) reported that *A. plicata* represented >55% of the mussels collected in the Tyronza River (Arkansas).

While qualitative visual or tactile searches without excavation tend to oversample large or sculptured species and underestimate smaller species and individuals (Obermeyer, 1998), Christian et al. (2005) concluded that visual and tactile searches by experienced personnel could reveal recruitment when it was

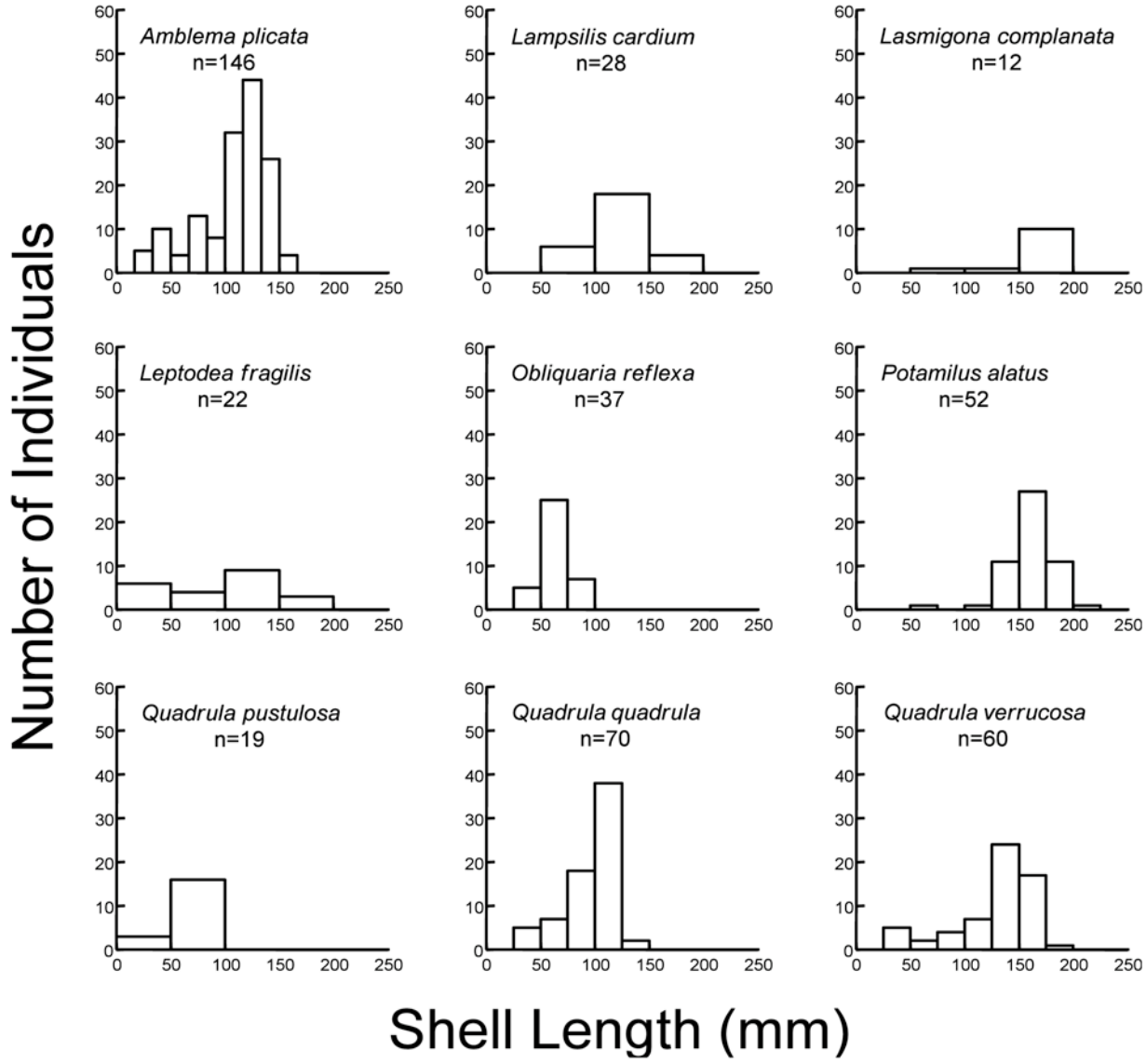

FIGURE 3
Size frequencies of 9 of the most abundant species collected from 6 locations in the Lamine River basin, Missouri, 2003 – 2006.

occurring. Few small juveniles (< 20 mm total shell length) were observed in the samples that were measured, and our size frequency distributions indicated unimodal recruitment patterns in the 9 most abundant species in the basin. This paucity of juveniles could be due to a lack of recent recruitment. However, given the intrinsic variability in freshwater mussel recruitment, even sporadic patterns of recruitment can sufficiently maintain populations (Neves & Widlak, 1987; Payne et al., 1997).

Three species previously reported to occur in the basin were not observed in the present survey. Utterback (1915–1916, 1917) reported *Cyclonaias tuberculata* (Rafinesque, 1820) and *Lasmigona costata* (Rafinesque, 1820) as “fairly abundant” and *Anodonta suborbiculata* as “scarce”. Based on Utterback (1915–1916, 1917) Oesch (1995) also reported each of these species from the Blackwater River portion of the basin prior to 1920, but no more recent collections were noted. These species have apparently been extirpated from the basin, presumably due to the extensive modification of the Blackwater River.

Corbicula fluminea was common and abundant throughout the basin, and it has been demonstrated

that increased ammonia levels following large-scale die-offs of C. fluminea are detrimental to native mussels (Cooper et al., 2005). No Dreissena polymorpha Pallas, 1769, were observed in the Lamine River basin. However, D. polymorpha occurs in the Missouri River basin and several reservoirs in Missouri (MDC, unpubl. data). Private watercraft can move freely between the Lamine and Missouri rivers, and other infested waterbodies, and therefore could aid in the dispersal of this invasive species into the Lamine River system.

Notwithstanding the anthropogenic impacts occurring in the basin, the Lamine River basin has a diverse freshwater mussel fauna, and a number of species rich mussel assemblages were observed in the mainstem Lamine River. Continuing with management objectives proposed by Brown et al. (1992) to maintain water quality, improve aquatic habitat, and work with private landowners to stabilize streambanks and improve riparian zones will be necessary to maintain the diversity of freshwater mussels in the Lamine River basin.

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TABLE 1

Freshwater mussel (Bivalvia: Unionidae) species reported from the Lamine River basin, Missouri, from Utterback (1915–1916, 1917, "Blackwater River Basin"), Oesch (1995), and present survey.

Scientific Name	Global Rank/ State Rank[A]	Utterback (1915–1916, 1917)	Oesch (1995)	Present Survey
Amblema plicata	G5/S5	×	×	×
Anodonta suborbiculata[B]	G5/S2	×	×	
Cyclonaias tuberculata	G3/S4	×	×	
Ellipsaria lineolata	G4-5/S4	×	×	×
Elliptio dilatata	G5/S4	×	×	×
Fusconaia flava	G5/S4		×	×
Lampsilis cardium	G5/S4-5	×	×	×
Lampsilis siliquoidea	G5/S4-5	×	×	×
Lampsilis teres	G5/S4	×	×	×
Lasmigona c. complanata	G5/S4	×	×	×
Lasmigona costata	G5/S4	×	×	
Leptodea fragilis	G5/S4	×	×	×
Ligumia recta[B]	G5/S2	×	×	×
Ligumia subrostrata	G5/S4	×	×	×
Megalonaias nervosa	G5/S4	×	×	×
Obliquaria reflexa	G5/S4		×	×
Pleurobema sintoxia	G4-5/S4		×	×
Potamilus alatus	G5/S5		×	×
Potamilus ohiensis	G5/S4		×	×

TABLE 1
(cont.)

Pyganodon grandis	G5/S5	×	×	×
Quadrula pustulosa pustulosa	G5/S4		×	×
Quadrula quadrula	G5/S4	×	×	×
Quadrula verrucosa	G4-5/S4-5	×	×	×
Strophitus undulatus	G5/S4	×	×	×
Toxolasma parvus	G5/S4	×	×	×
Truncilla donaciformis	G5/S4		×	×
Truncilla truncata	G5/S4		×	×
Uniomerus tetralasmus	G5/S4	×	×	×
Utterbackia imbecillis	G5/S4	×	×	×
Venustaconcha ellipsiformis	G4/S4		×	×
Corbiculidae				
Corbicula fluminea				×
Total Native Species (30)		21	30	27[C]

[A] Source: MDC (2008), NatureServe (2010)
[B] Missouri Species of Conservation Concern (MDC 2011)
[C] Includes previously unreported shell collections made by Missouri Department of Conservation staff, from 1995 – 1999

TABLE 2

Number collected, number of occurrences (live and dead) and percentage of sites, and relative abundance of freshwater mussels collected in the Lamine River basin, Missouri presented in order from highest to lowest relative abundance.

Species	Number Collected Live	Number of Occurrences (%)	Relative Abundance (%)
Amblema plicata	2989	34 (75.5)	56.5
Potamilus alatus	431	36 (80.0)	8.2
Quadrula quadrula	385	32 (71.1)	7.3
Lampsilis siliquoidea	278	10 (22.2)	5.3
Quadrula verrucosa	183	33 (73.3)	3.5
Lampsilis cardium	169	32 (71.1)	3.2
Obliquaria reflexa	162	27 (60.0)	3.1
Quadrula p. pustulosa	159	31 (68.9)	3.0
Leptodea fragilis	85	36 (80.0)	1.6
Lasmigona c. complanata	80	29 (64.4)	1.5
Ligumia subrostrata	77	9 (20.0)	1.5
Megalonaias nervosa	61	12 (26.7)	1.2
Elliptio dilatata	52	16 (35.6)	1.0
Pyganodon grandis	35	9 (20.0)	0.7
Truncilla donaciformis	22	28 (62.2)	0.4
Utterbackia imbecillis	22	13 (28.9)	0.4
Truncilla truncata	21	21 (46.7)	0.4
Lampsilis teres	18	24 (53.3)	0.3
Pleurobema sintoxia	18	11 (24.4)	0.3
Potamilus ohiensis	12	22 (48.9)	0.2

TABLE 2
(cont.)

Ellipsaria lineolata	8	8 (17.8)	0.2
Fusconaia flava	6	11 (24.4)	0.1
Strophitus undulatus	6	9 (20.0)	0.1
Ligumia recta	4	13 (28.9)	0.1
Toxolasma parvus	2	8 (17.8)	0.0
Uniomerus tetralasmus	1	2 (0.04)	0.0
Venustaconcha ellipsiformis	1	3 (0.07)	0.0

APPENDIX A

Number and collecting location CPUE (live individuals/hr) of freshwater mussels collected from the Lamine River basin, Missouri. For shell material, FD = Fresh Dead, WD = Weathered Dead, and SF = Subfossil.

	Collecting Location											
Genus/Species	1	2	3	4	5	6	7	8	9	10	11	12
Amblema plicata	WD	29	81	94	84	WD	74	763	SF	74	19	433
Ellipsaria lineolata				1		WD	1	2			SF	
Elliptio dilatata					FD	WD	SF	1			1	
Fusconaia flava								1			1	
Lampsilis cardium		2	WD	9	6	FD	10	6	SF	7	5	8
Lampsilis siliquoidea												
Lampsilis teres		1	WD	1				1				2
Lasmigona c. complanata			1	1			2	11		3	1	6
Leptodea fragilis	WD		2	9	1	WD	2	6	1	6	1	2
Ligumia recta					WD	WD	1	FD				SF
Ligumia subrostrata												
Megalonaias nervosa		5		WD				WD	SF		8	8
Obliquaria reflexa	WD	3	8	18	WD	WD		28		8	WD	5
Pleurobema sintoxia						WD		WD				1
Potamilus alatus	1	1	9	7	8	WD	12	101		9	2	12
Potamilus ohiensis						FD	WD	1			SF	FD
Pyganodon grandis			WD									
Quadrula pustulosa	WD	4	1	7	2	WD	3	10	WD	4	3	10
Quadrula quadrula	WD	6	18	26	6	WD	16	20	WD	6	3	27
Quadrula verrucosa					1	WD	1	1				
Strophitus undulatus												
Toxolasma parvus	5		9	4	8	WD	1	5		6	8	6
Truncilla donaciformis	WD	WD	1	1	FD	WD	WD	WD		4	1	1
Truncilla truncata				WD	FD		WD	2		4	WD	1
Uniomerus tetralasmus												
Utterbackia imbecillis			WD				1					FD
Venustaconcha ellipsiformis												
Live Totals:	6	51	130	178	116	0	124	959	1	131	53	522
Person Hours:	1.0	2.0	2	4	1.67	0.83	1.33	2.92	0.5	1.17	2.0	1.25
CPUE:	6	25.5	65	44.5	69.6	0	93	328.8	2	112.3	26.5	417.6

APPENDIX A
(cont)

Genus/Species	13	14	15	16	17	18	19	20	21	22	23	24
Amblema plicata	163	79	5	1	WD	2	126	6	154	48	14	5
Ellipsaria lineolata	2						1			1		
Elliptio dilatata	3	47	WD	SF	WD	SF				WD		SF
Fusconaia flava	1	3	WD		WD	SF						WD
Lampsilis cardium	8	19	1	FD	FD	SF	18	5	1	5	9	
Lampsilis siliquoidea		1										WD
Lampsilis teres	1	6	WD	WD			WD	1	1			WD
Lasmigona c. complanata	2	2				1	6	1	11	2	4	1
Leptodea fragilis	2	2	FD	FD		1		1	14	6	3	WD
Ligumia recta		2	SF	SF			1			SF		SF
Ligumia subrostrata												SF
Megalonaias nervosa	9	29	SF			SF						WD
Obliquaria reflexa	8	7	FD	FD	WD	SF	18	1	15	3	1	
Pleurobema sintoxia	6	10	WD			WD				1		SF
Potamilus alatus	11	31	1	WD	WD	2	61	3	19	43	8	4
Potamilus ohiensis	1	WD	1	1	WD		1			FD		WD
Pyganodon grandis									FD			
Quadrula pustulosa	22	26	WD	FD		SF	5		1	WD	1	WD
Quadrula quadrula	32	10	FD	3		SF	25	1	27	7		WD
Quadrula verrucosa		3										
Strophitus undulatus			WD	FD								
Toxolasma parvus	10	20	WD	1		SF	3	6	3	12	3	2
Truncilla donaciformis	1	WD	FD	FD				FD	4	3	FD	FD
Truncilla truncata	2	1	FD	1					3	3		WD
Uniomerus tetralasmus												
Utterbackia imbecillis			FD	FD					FD	WD	FD	WD
Venustaconcha ellipsiformis										WD		
Live Totals:	284	298	8	7	0	6	265	25	253	134	43	12
Person Hours:	3.75	4.67	1.07	1.0	0.5	1.0	2.0	1.67	2.67	3.12	0.67	0.83
CPUE:	75.7	63.6	7.5	7	0	6.0	132.5	15.0	94.9	43.0	64.5	14.4

APPENDIX A
(cont)

Genus/Species	25	26	27	27	28	29	30	31	32	33	34	35
Amblema plicata	146	16	19	38	5		39	7	WD	465		
Ellipsaria lineolata												
Elliptio dilatata				WD			WD		SF			
Fusconaia flava					WD		SF					
Lampsilis cardium	1	6	6	17	3	FD	2	3	1	10		
Lampsilis siliquoidea							WD	2	WD	236	WD	
Lampsilis teres			SF	2	1		WD		WD			
Lasmigona c. complanata	2		3	5	1		3	1	1	7		
Leptodea fragilis	2	5	4	10	FD		1	1	WD	1		
Ligumia recta		WD	WD									
Ligumia subrostrata							WD	2	FD	69		
Megalonaias nervosa	2											
Obliquaria reflexa	3	1	3	31			1					
Pleurobema sintoxia				WD			WD					
Potamilus alatus	26	2	7	14	WD		30	1	WD	6		
Potamilus ohiensis	2		WD	4			WD					
Pyganodon grandis								1	FD	28		
Quadrula pustulosa	5		5	12	9		1	13	SF	10		
Quadrula quadrula	1		11	50			8		1	76		
Quadrula verrucosa				WD	FD		WD		WD			
Strophitus undulatus				WD	FD		WD	2				
Toxolasma parvus	5	4	5	6	41		5	SF	SF	3		
Truncilla donaciformis		2	3	1	WD		WD					
Truncilla truncata		1	1	1	WD		1		WD			
Uniomerus tetralasmus										1		
Utterbackia imbecillis							WD	FD	WD	21		
Venustaconcha ellipsiformis		1	SF									
Live Totals:	195	38	67	191	60	0	91	33	3	933	0	0
Person Hours:	0.53	1.42	4.0	1.5	3	0.67	2	3.25	3.0	6.67	0.75	0.53
CPUE:	365.6	26.8	16.8	127.3	20	0	45.5	10.2	1	140.0	0	0

APPENDIX A
(cont)

Genus/Species	36	37	38	39	40	41	42	43	44	45	Live Totals
Amblema plicata		WD									2989
Ellipsaria lineolata											8
Elliptio dilatata											52
Fusconaia flava		SF									6
Lampsilis cardium		1									169
Lampsilis siliquoidea		39						SF		SF	278
Lampsilis teres			SF			WD	1	FD	WD	WD	18
Lasmigona c. complanata		1	WD					1	SF	WD	80
Leptodea fragilis		1	FD	SF		WD		1		WD	85
Ligumia recta											4
Ligumia subrostrata		WD					1		1	4	77
Megalonaias nervosa											61
Obliquaria reflexa		WD									162
Pleurobema sintoxia											18
Potamilus alatus		WD	FD			WD		WD			431
Potamilus ohiensis			WD			1	SF	FD	WD		12
Pyganodon grandis		5	WD						WD	1	35
Quadrula pustulosa		5									159
Quadrula quadrula				WD		WD	WD	5			385
Quadrula verrucosa											6
Strophitus undulatus									FD	FD	2
Toxolasma parvus		WD		WD						2	183
Truncilla donaciformis		WD	FD					WD			22
Truncilla truncata		WD									21
Uniomerus tetralasmus									FD		1
Utterbackia imbecillis											22
Venustaconcha ellipsiformis											1
Live Totals:	0	52	0	0	0	1	2	7	1	7	5287
Person Hours:	0.53	1.33	0.67	0.83	3.0	1.33	1.33	2.2	2.0	2.0	86.1
CPUE:	0	39.0	0	0	0	0.8	1.5	3.2	0.5	3.5	

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ASSESSING ACCUMULATION AND SUBLETHAL EFFECTS OF LEAD IN A UNIONID MUSSEL

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ABSTRACT

Lead (Pb) contamination of the environment remains a global problem. Previous studies have demonstrated that Pb deposited onto roadside sediments from the past use of leaded gasoline in vehicles may be mobilized into rivers and streams, thereby resulting in exposure to aquatic biota. The aims of this study were to conduct a 28-day laboratory toxicity test with Pb and adult Eastern Elliptio (*Elliptio complanata*; family Unionidae) mussels to determine uptake kinetics and to assess several potential non-lethal biomarkers of Pb exposure. Mussels were collected from a relatively uncontaminated reference site and exposed to a control and eight concentrations of Pb (as lead nitrate) ranging from 1 to 251 µg/L, as a static renewal test. There were five replicates per treatment with one mussel per replicate. The hemolymph of mussels from four of the replicates was repeatedly sampled (days 7, 14, 21, and 28) for analysis of Pb and ion (Na+, K+, Cl-, Ca2+) concentrations. The mussels in the fifth replicate per treatment were only sampled on day 28 and served as a comparison to the repeatedly sampled mussels. The accumulation of Pb in mussel tissue was also evaluated during the study. No mussels died during the test. We found that measured concentrations of Pb in mussel hemolymph suggested regulation of the heavy metal up to 66 µg/L by day 14, whereas concentrations in tissue proved to be strongly correlated ($R2 = 0.98$; $p < 0.0001$) throughout the 28-day exposure, displaying concentration dependent uptake. The concentration of Pb in mussel hemolymph, which can be sampled and measured non-lethally, is a suitable marker of recent Pb exposure in mussels. In contrast, none of the ion concentrations measured in the hemolymph from the repeatedly sampled mussels was significantly changed with increasing concentrations of Pb, whereas the mussels from the fifth replicate sampled only on day 28 showed altered calcium concentrations. The activity of δ-aminolevulinic acid dehydratase (ALAD), a demonstrated Pb-specific biomarker in vertebrates and some invertebrates, which was also evaluated as a potential endpoint in an initial evaluation for this study, proved to be an unsuitable biomarker in *Elliptio complanata*, with no detectable activity observed. This finding was in contrast to a second freshwater, but non-unionid bivalve tested, the Asian Clam *Corbicula fluminea*, which had detectable ALAD activity.

KEY WORDS ALAD, Bioavailability, Biomarkers, *Elliptio complanata*, Lead, Unionidae

INTRODUCTION

Lead (Pb) contamination is a global environmental problem. Many studies have demonstrated excess levels of Pb in roadside sediments (Latimer et al., 1990; Mielke, 1999; Sutherland & Tolosa, 2000; Sutherland, 2003; Weiss et al., 2006) and other ecosystem compartments associated with the past use of leaded gasoline. Most of the Pb in sediment is found in the small grain fraction (< 63 µm), which is more likely to be re-suspended or eroded into rivers and streams adjacent to roads (Angelo et al., 2007; Sutherland & Tolosa, 2000; Weiss et al., 2006). Native freshwater mussels belonging to the family Unionidae are suspension and deposit-feeding, long-lived (10-100 yr) organisms that reside burrowed in sediments of streams and rivers (McMahon & Bogan, 2001) and, therefore, may be among the groups of aquatic organisms adversely affected by persistent, low-level exposure to Pb in our surface waters.

Unionids are one of the most imperiled faunal groups in the world, especially in North America, where almost 70% of the nearly 300 native species are considered vulnerable to extinction or are already extinct

(Bogan, 1993; Williams et al., 1993; Graf & Cummings, 2007). Unionids are also recognized as one of the most sensitive groups of organisms that have been tested to date with certain contaminants like ammonia and copper, compared to other commonly tested aquatic organisms like fish and crustaceans (Augspurger et al., 2003; March et al., 2007). Mussels, being suspension and deposit feeders, are exposed to a wide variety of contaminants, including Pb, throughout their life (Cope et al., 2008), and are considered to be good sentinels for assessing environmental conditions (Metcalfe-Smith et al., 1996; Gundacker, 2000; Dobrowolski & Skowrońska, 2002; Yap et al., 2004). A recent study found that while the Na+,K+-ATPase enzyme was present and inhibited by Pb in the unionid mussel, Eastern Elliptio, *Elliptio complanata* (Lightfoot, 1786), results were variable at environmentally relevant concentrations (Mosher et al., 2010) and, therefore, recommended that more specific, non-lethal biomarkers should be assessed. As there is mechanistic understanding for Ca-Pb interactions (Grosell et al., 2006), it is possible that the presence of Pb would interfere with the transport and uptake of this and other ions. Changes in Ca2+ and other ion concentrations (Dietz, 1985) could affect pH and result in reduced shell formation.

Because unionids are such an imperiled fauna, it is critical to develop non-lethal sampling techniques and associated biomarkers of toxicant exposure and effect (Newton & Cope, 2006), when available. Fortunately, the extraction of hemolymph (the circulatory fluid) has been shown to be a suitable non-lethal sampling approach for assessing health and condition of mussels (Gustafson et al., 2005a). Therefore, this study utilized the repeated, non-lethal sampling of mussel hemolymph to evaluate the adverse effects of Pb. The specific aims of this study were to conduct a 28-day laboratory toxicity test with Pb and adult *Elliptio complanata* to determine uptake kinetics and to assess potential non-lethal biomarkers of Pb exposure and effect in mussel hemolymph, focusing on Pb and ion (Na+, K+, Cl-, Ca2+) concentrations. The accumulation of Pb in mussel tissue, in relation to the non-lethal measurements, was also evaluated during the study. One of the classic biomarkers of Pb exposure in mammals, fish, and some invertebrates is δ-aminolevulinic acid dehydratase (ALAD) activity (Schmitt et al., 2002; Ahamed et al., 2005; Schmitt et al., 2005; Aisemberg et al., 2005), but it has not been demonstrated in unionid mussels. Because of the positive results with ALAD and the non-unionid freshwater bivalve the Asian Clam, *Corbicula fluminea* (Pallas, 1769), reported by Company et al. (2008), an additional aim of this study was to determine if ALAD activity is present in the hemolymph of *Elliptio complanata* and assess its use as a potential Pb biomarker.

MATERIALS AND METHODS

Collection, Transport, and Holding of Mussels

A total of 53 adult *Elliptio complanata* were collected from a rural, forested, and relatively uncontaminated section of the Eno River near Hillsborough, North Carolina, USA (NCDENR 2009). Test mussels averaged 77 mm in total length, ranging from 68 to 88 mm, and had a mean wet weight of 69 g, ranging from 40 to 98 g. Upon collection, mussels were promptly placed in ice chests to maintain their temperature near the 21°C river water, covered with damp mesh bags to prevent desiccation and temperature change, and transported directly to the laboratory (30 min transport time). Upon arrival at the laboratory, each mussel was scrubbed with a soft-bristle brush and rinsed with deionized water. Five mussels were randomly chosen for baseline measurements of the test endpoints (Pb and ion (Na+, K+, Cl-, Ca2+) concentrations in hemolymph, Pb concentrations in body tissue) for comparative purposes. These five mussels were each weighed, measured for total length, gently pried open, had ~ 1 mL of hemolymph extracted from the anterior adductor muscle, and were then bagged and stored frozen (-20°C) for Pb analysis. The hemolymph was divided into two cryotubes, with one frozen at -80°C for analysis of ion concentrations, and the other at -20°C for Pb analysis. The remaining mussels were placed into individual 3-L glass aquaria. The aquaria each contained 2 L of ASTM soft water (ASTM 1993) that was gently aerated (5-15 bubbles/s) by a central aeration unit (Sweet Water Air Pump SL24 Aquatic Eco-Systems, Inc., Apopka, FL, USA). For the toxicity test, there were nine target Pb treatment concentrations (0, 1.95, 3.9, 7.8, 15.6, 31.25, 62.5, 125 and 500 µg/L), and five replicates per treatment with one mussel per replicate. The 45 test mussels were then acclimated to test conditions for 72 h prior to initiation. Immediately prior to the start of the test on day 0, the mussel in each aquarium was fed 20 mL of a suspension containing 2 mL of Instant Algae® Shellfish Diet and 1 mL *Nannochloropsis* concentrate (Reed Mariculture, Campbell, CA, USA) in 1 L of deionized water. The mussel in each jar was allowed to siphon and feed for 2 h, after which a complete water renewal and toxicant spiking commenced. Mussel feeding and water and toxicant renewals were conducted three times per week during the 28-day test in this same manner.

Experimental Procedures

Pb Exposure Study

All laboratory methods followed ASTM guidelines for conducting laboratory toxicity tests with freshwater mussels (ASTM 2006), with modifications for testing adult mussels. Water samples (5 mL) were taken from three of the five replicate test aquaria per treat-

ment concentration every 0, 48, and 72 h post-renewal throughout the 28-day exposure for verification of Pb concentration. These samples were stored preserved (75 µL of concentrated trace metal grade nitric acid) until analysis. Alkalinity, hardness, pH, temperature, and dissolved oxygen were all measured in each aquarium before test initiation and then three times per week thereafter. Water temperature and dissolved oxygen were measured with a calibrated multi-probe (YSI Model 556 MPS, Yellow Springs Instruments, Yellow Springs, OH, USA). Water pH was measured with a calibrated Beckman Model Φ 240 meter (Beckman Instruments, Fullerton, CA, USA). Alkalinity and hardness were measured by standard titrametric methods (APHA et al. 1995). Physiochemical characteristics of test water averaged 21.0°C (range 20.9 – 21.29) for temperature, dissolved oxygen 8.3 mg/L (range 7.9 – 8.7), pH 8.0 (range 7.8 – 8.1), alkalinity 30 mg/L as CaCO3 (range 28 – 32), and hardness 42 mg/L (range 40 – 44).

On day 0 of the test, each mussel was removed from its aquarium, gently pried open, a 25 gauge syringe was used to withdraw 0.25 mL of hemolymph from the anterior adductor muscle, and was then immediately returned to the aquarium. Hemolymph was composited from the first four mussels (replicates) from each test concentration, including the control, to achieve 1 mL total volume. Hemolymph was then divided into aliquots of 0.5 mL for ion (Na+, K+, Cl-, Ca2+) analysis stored at -80°C, and 0.5 mL for Pb analysis stored at -20°C. The sampling of hemolymph from these same first four mussel replicates of each treatment was repeated weekly on days 7, 14, 21 and 28 in the same manner. The fifth and final mussel (replicate) for each test concentration was not sampled until the end of the experiment (d 28) as a control for the repeated, weekly hemolymph sampling. Water and toxicant renewals were conducted three times per week. Before each renewal, the mussel in each aquarium was fed and allowed to siphon for 2 h, as previously described. Each aquarium was then siphoned and renewed (~ 90%) with fresh ASTM soft water. Aquaria were then spiked with Pb from a concentrated stock solution (1,000 mg/L) prepared from lead nitrate to generate the final target Pb concentrations.

Analytical Procedures

All samples of mussel hemolymph, body tissue, and test water were analyzed for Pb concentrations with standard methods at RTI International (Research Triangle Park, NC, USA) according to good laboratory practices and strict quality assurance protocols. Briefly, mussel tissues were lyophilized and homogenized, with a nominal weight of 250 mg aliquoted and heated with a mixture of concentrated nitric and hydrochloric acids. Hydrogen peroxide was added to aid in the decomposition of organic material. The hemolymph samples had a 0.2 mL aliquot transferred to an acid washed 15 mL plastic centrifuge tube, and 4.8 mL of a 2.5% HCl-2.5% HNO3 acid extraction solution was added to each sample and vortex mixed. Samples were placed in a water bath at 60°C for 30 min, then vortex mixed, allowed to cool to room temperature, and centrifuged for 30 min at 2,800 RPM. A 3 mL aliquot of the supernatant liquid was removed for analysis. Samples were then analyzed by magnetic sector inductively coupled mass spectrometry (Thermo Element 2 Magnetic Sector ICP-MS). The average percent recovery of Pb from spiked mussel tissue samples was 101%, and ranged from 99-103%. Recovery of Pb in samples of hemolymph averaged 97% and ranged from 87-102%.

All hemolymph samples were analyzed for ion concentrations at the Analytical Service Laboratory in the Department of Soil Sciences at North Carolina State University (Raleigh, NC, USA) according to standard methods, good laboratory practices and strict quality assurance procedures with two Dionex Ion Chromatographs (DX-500 and 4000, Dionex Corporation, Sunnyvale, CA, USA). Using an autosampler (Dionex AS-50) fitted with two injection valves, the samples were simultaneously analyzed for anions and cations. Chloride analysis was performed using a separation column AS 22 and conductivity detection for the cations analysis was done on a Dionex CS 12A column. The concentration of each analyte was determined by comparing the peak area in the chromatograms (using Dionex Software Peak Net 5.21) to those generated with standard solutions.

Because ALAD has recently been found in the freshwater *Corbicula fluminea* (Company et al. 2008), an initial assessment with *Corbicula fluminea* was conducted alongside *Elliptio complanata* for the presence and relative detectability of ALAD among the bivalve species. To ensure data quality and validation for ALAD analysis in the previously untested mussel tissues, the fathead minnow, *Pimephales promelas*, was selected as the test organism to serve as a positive control in the ALAD analysis because this fish has been shown to provide consistent ALAD responses (Spokas et al. 2006). In the initial assessment of ALAD detectability, three *Elliptio complanata*, two *Corbicula fluminea* (collected from the same stream) and one *Pimephales promelas* were analyzed. The bivalves were each weighed, measured, and hemolymph, gill, mantle, foot and viscera samples were taken for analysis and stored at 80°C. All laboratory materials (e.g., glass pipettes, cryotubes) used for hemolymph and fish blood collection were heparinized before use to minimize clotting. The ALAD assay in this assessment utilized methods

to increase sensitivity in order to detect the presence of ALAD in each species. It was modified from that of Schmitt et al. (2005) for use with mussel hemolymph and tissue using a microplate assay. Mussel tissue was sonicated to minimize clotting. In detail, mussel tissues and fish blood samples were removed from the -80°C freezer and placed in a 4°C refrigerator to thaw, along with one cryotube of a porphobilinogen (PBG) stock solution (221 µM PBG). Six centrifuge tubes were labeled for each sample: blank A, B and C, and aminolevulinic acid (ALA) A, B and C. To each blank tube, 50 µL of assay buffer (0.2% Triton X-100 in 0.1M phosphate buffer (pH 6.2)) was added. To each ALA tube, 50 µL of ALA buffer (670 µg ALA·HCl/mL) was added. 75 µL of assay buffer were added to six of the seven PBG standard curve tubes, along with the controls. Blood samples were pipetted and weighed, and an equal volume (10 µg = 10 µL) of deionized water was added. Blood dilutions and hemolymph samples were then both sonicated for 10 min. PBG serial dilution was prepared from the thawed stock solution, 150 µL was pipetted into the empty tube, and a 1:1 serial dilution of the remaining six tubes was prepared transferring 75 µL at a time and vortexing. Then 200 µL of each sonicated sample was added per tube for that sample, vortexed for five seconds, and incubated for 4 h in a 37°C water bath. Modified Ehrlich's reagent was prepared by weighing out and mixing the appropriate amount of p-dimethylamino benzaldehyde to Ehrlich's reagent (e.g. 0.545 g to 30 mL Ehrlich's reagent) for the number of samples being run per batch. After removing the samples from the water bath, the reaction was terminated by the addition of 200 µL stop solution (TCA/n-ethylmaleimide solution) to each tube. Samples were vortexed and centrifuged at 1,000 x g for 10 min. A 100 µL aliquot of supernatant from each sample was pipetted into a 96-well plate, and 100 µL of modified Ehrlich's reagent was added to each well. The plate was placed on a plate shaker for 15 min of color development, and the absorbance was read on a FusionTM Universal Microplate Analyzer (A153600 Meriden, CT, USA) at 540 nm. To normalize the ALAD results among the different tissues and organisms, the Bradford Protein assay (IBI-Shelton Scientific, Peosta, IL, USA), a kit containing 0.5 mg/mL bovine serum albumin (BSA), 0.15 M NaCl and a Bradford Reagent consisting of Coomassie blue, a dye that binds protein, was used to determine the protein concentration in samples by generating a BSA linear standard curve plotting absorbance at 595 nm (Spectronic® Genesys™, Milton Roy Company, Rochester, NY, USA) versus protein concentration. ALAD activity was then reported as uM PBG/min/mg protein.

Statistical Analysis

Data were analyzed with StatCrunch software (www.statcrunch.com, Department of Statistics, University of South Carolina, Columbia, SC, USA). One way Analysis of Variance and Simple Linear Regressions were performed with statistical significance determined at $\alpha = 0.05$ for all tests.

RESULTS

During the 28-d test with Pb, the average measured Pb concentration in samples of test water (n = 3) at time 0 and immediately following each renewal was 73% of the target concentration. Concentrations decreased with time in each replicate to an average of 10.5% of the target by 48 h and 7.4% by 72 h before the next renewal. The average daily exposure concentration was calculated as the weekly average of 3 time 0 h (T0), 3 time 48 h (T48) and 1 time 72 h (T72) measurement(s), to be 0.9, 1.3, 3.2, 6.4, 10.5, 25.9, 66.3 and 250.8 µg Pb/L, and were the values used hereafter to denote the actual measured Pb treatment groups.

No mussels died during the test. The average Pb concentration in mussel tissue at the end of the 28-d study was strongly correlated to exposure concentrations (Fig. 1) with an R2 = 0.98 and $p < 0.0001$. Because the tissue samples from the replicates for a treatment were composited for analysis, variation is not reported.

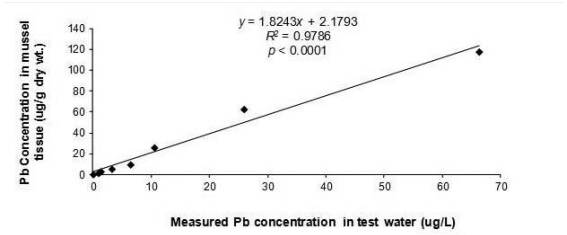

FIGURE 1

Relation between waterborne Pb exposure concentrations from control to 251 µg/L and concentrations of Pb in mussel tissue at the end of the 28-d laboratory test.

Concentrations of Pb in hemolymph of the repeatedly sampled mussels (replicates 1 – 4), which were composited per treatment group to obtain sufficient volume, had several different trends over the 28-d study depending on their Pb exposure. These results are summarized for measured exposures of 0 – 66 µg/L (Fig. 2A) to show better endpoint resolution, and for all concentrations from 0 to 251 µg/L (Fig. 2B). For Pb exposures of ≤ 6 µg/L, mussels slowly increased concentrations of Pb in their hemolymph over time, never exceeding three times their exposure concentration. For exposures of 11 – 66 µg/L, concentrations plateau around d 14 with the 11 µg/L treatment group at 3.6 times its exposure concentration and the 26 and 66 µg/L exposures at 1.4 and 1.2 times exposure concentrations, respectively. However, for the greatest exposure concentration of 251 µg/L, hemolymph concentrations never plateau, but appeared to bioconcentrate with rapid, linear accumulation, as shown by the best fit line with an R2 = 0.98, and p = 0.0009, to five times the exposure concentration. The replicate 5 mussels (i.e., those not sampled until d 28) had Pb hemolymph concentrations similar to their corresponding treatment group replicates 1 through 4 mussels on day 28, except for the greatest exposure which had a hemolymph concentration greater than 1,700 µg/L. This was over 500 µg Pb/L above the concentration of the repeatedly sampled mussels in that treatment group.

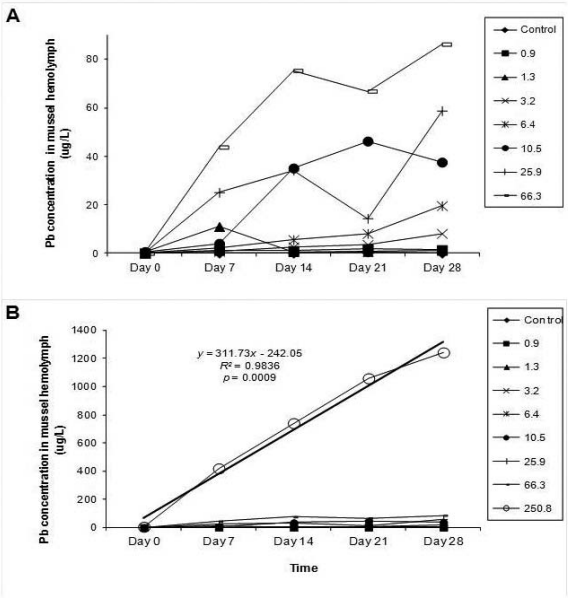

FIGURE 2

Concentrations of Pb in hemolymph of the repeatedly sampled mussels (replicates 1 – 4) over the (A) low range (0 – 66 µg Pb/L; provided for endpoint resolution) and (B) full range (0 – 251 µg Pb/L) of exposures at each time point sampled through the 28-d laboratory test. Because the tissue samples from the replicates for a treatment were composited for analysis, variation is not reported.

None of the ion (Na+, K+, Cl-, Ca2+) concentrations measured in the hemolymph from the repeatedly sampled mussels was significantly changed with increasing concentrations of Pb, whereas the mussels from the fifth replicate sampled only on day 28 showed altered calcium concentrations. The Ca2+ concentrations in hemolymph from the non-repeatedly sampled replicate 5 mussels were found to be below the lower 95% confidence interval (CI) of 12.85 mg/dL, which was derived from our five baseline mussels, for low Pb exposures of 1 – 3 µg/L, and above the CI (16.23 mg/dL) for high Pb exposures of 11, 66 and 251 µg/L (Table 1). Because the control treatment remained within the CI, this suggests that Pb exposure altered Ca2+ within the hemolymph.

TABLE 1

Hemolymph calcium (Ca2+) concentrations (in mg/dL) of each treatment group of the non-repeatedly sampled replicate 5 mussels on d 28 of the test compared to the 95% confidence interval (CI) of the baseline measurements taken before test initiation.

Measured Pb in Test Water (µg/L)	Hemolymph Calcium (mg/dL)	Baseline 95% CI (12.85 – 16.23)
Control	15.5	=
0.9	12.4	<
1.3	12	<
3.2	12.1	<
6.4	13.3	=
10.5	19.2	>
25.9	15.8	=
66.3	18.4	>
250.8	16.9	>

Results from the ALAD comparison assay showed significant activity (180 uM PBG/min/mg protein) in the positive control fish, Pimephales promelas ($p < 0.0001$) compared to the negative controls. However, no significant ALAD activity was detected at any time in Elliptio complanata, neither in hemolymph nor in tissue (gill, mantle, foot or viscera). In contrast, when comparing species for activity, the non-unionid Corbicula fluminea had significant ALAD activity ($p = 0.0002 – 0.0098$), producing levels of 0.72 – 0.93 uM PBG/min/mg protein, or an average of 46.7 ng PBG/min/mg protein at 37°C compared to the results of Company et al., (2008) of 1.5 ng PBG/min/mg protein at room temperature. Therefore, ALAD activity in Elliptio complanata was not considered a viable biomarker for the unionid tested and was not measured in mussels from the 28 d Pb exposure study.

DISCUSSION

We found that *Elliptio complanata*, a freshwater mussel of the family Unionidae, accumulated waterborne Pb extremely rapidly by ventilation across the gills in our laboratory study, a finding that was supported by rapidly declining concentrations of Pb measured in test water following each renewal, and concomitant increasing Pb concentrations in mussel hemolymph and body tissue throughout the test. To place the accumulation of Pb by mussels in this study into an environmentally relevant perspective, we found that the lowest exposure concentration of 0.9 µg/L for 28 d resulted in an average tissue concentration of 1.5 µg/g dry weight, which is similar to the average tissue concentration (1.6 µg/g dry wt.) measured in *Elliptio complanata* (n =240) sampled from natural populations at 40 stream sites across North Carolina (Mosher, 2008). The fact that mid-range Pb exposures resulted in a plateau of Pb concentration in the mussel hemolymph by d 14, whereas the greatest exposure concentrations resulted in rapid accumulation throughout the exposure duration, suggests that metabolic regulation of Pb was occurring in the mussels. Because the concentrations of Pb in test water were being depleted just as rapidly by the end of the experiment as they were in the beginning, it is unlikely that the mussels reduced uptake appreciably over time. This suggests that either the mussels started transporting the lead from hemolymph into tissue and/or shell, or they started eliminating it more efficiently in lysosomes through urine and pseudo-feces (Amiard et al., 1995; Marigómez et al., 2002), where it would then be bound and settle on the floor of the aquaria.

Calcium concentrations in hemolymph of the non-repeatedly sampled mussels (replicate 5 from each treatment) appeared to be altered by Pb exposure. When comparing the 95% CI reference values for Ca2+ levels in *Elliptio complanata* (13.1 – 23.7 mg/dL) generated by Gustafson et al. (2005b), the lowest three Pb

exposures in this test also resulted in a decrease below this lower limit. Because this observation was based only on a single mussel per concentration, this relation is uncertain and requires additional research. However in another 28-day Pb exposure study by Mosher et al. (2010) in which mussels were sampled terminally (rather than repeatedly) at the same 0, 7, 14, 21, and 28 day time intervals, they found similar results with significant increases in Ca2+ at the greatest Pb exposure of 245 µg/L. While no trends were determined between Pb exposure and ion levels in hemolymph of the mussels repeatedly sampled in this study, this may have been due to the damaging effects of repeated puncturing of the anterior adductor muscle during sampling rather than to the exposure to Pb. Even though repeated hemolymph sampling of three times over seven months has been determined to be non-lethal and pose no apparent physiological harm (Gustafson et al., 2005a), the repeated sampling of five times over one month may have been causing additional stress, as well as possibly allowing direct transport of ions into and out of the adductor muscle via the tracts left by the 25 gauge needle. By the end of the experiment, some of the adductor muscles had visible holes in the side from tearing, as a result of weakening from multiple punctures with little time for recovery. We believe that five repeated sampling periods of test mussels for hemolymph within 28 d was too aggressive, causing tissue damage in some cases, and, therefore, potentially masking any trends in ion levels with Pb exposure concentrations. A future study with an experimental design that would provide for greater numbers of non-repeatedly sampled mussels to be analyzed would be recommended to determine if Ca2+ concentrations are unequivocally adversely affected by Pb exposure.

For the potential development and application of an assay to rapidly and sub-lethally assess Pb exposure in native freshwater mussels, we found that simple measurements of Pb concentration in mussel hemolymph were highly indicative of real-time and recent exposures. This type of application could be easily applied to field monitoring situations in which mussels could be sampled and replaced in their habitat without damaging populations. In addition, we found that Ca2+ concentrations in hemolymph may be useful in determining the overall health impact of a mussel population to Pb exposure, given enough individuals were sampled to reduce variability. Moreover, additional research is needed in assessing Ca2+ concentrations in response to other environmental stressors before such a monitoring assessment and link could be made.

Finally, our results indicated that while we confirmed the presence of ALAD in *Corbicula fluminea* (e.g., Company et al., 2008), it does not appear to be a suitable biomarker in the unionid mussel *Elliptio complanata* due to the lack of detectable activity at baseline metabolic conditions. Mollusks can contain either hemocyanin or hemoglobin for oxygen transport within the hemolymph, or have no respiratory proteins at all (Mangum et al., 1987; Alyakrinskaya, 2003), depending on the genera. While the absence of iron hemoglobin in hemolymph does not necessarily negate its presence in other tissues of bivalves (Alyakrinskaya, 2003), we found no evidence of ALAD activity in *Elliptio complanata* hemolymph, gill, mantle, foot or visceral tissue.

Conclusion

Overall, we found that measurements of Pb concentration in mussel hemolymph were highly indicative of real-time and recent exposures, and may provide a suitable marker to rapidly and sub-lethally assess Pb exposure and toxicity in native freshwater mussels in both laboratory and stream settings. Ca2+ concentrations in hemolymph could potentially be adversely affected by Pb exposure in non-repeatedly sampled mussels, although further assessment is needed to confirm this relationship. Concentrations of Pb measured in body tissue were strongly correlated with the full range (0-251µg/L) of Pb exposure concentrations. Thus, freshwater mussels appear to accumulate Pb in a concentration dependent manner and begin to actively regulate Pb uptake by d 14 of exposure, based on measured hemolymph concentrations.

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THERMAL TOLERANCES OF FRESHWATER MUSSELS AND THEIR HOST FISHES: SPECIES INTERACTIONS IN A CHANGING CLIMATE

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ABSTRACT

Rising environmental temperatures result from changes in land use and global climate and can cause significant shifts in the composition and distribution of species within communities. In freshwater systems, the larval life stage, glochidia, of Unionida mussels develops as an obligate parasite on host fish gills or fins before transforming into the juvenile stage and dropping to the sediment to complete the life cycle. Because of the relationship between freshwater mussels and their often specific host fish species, mussels are not only limited by their own variable thermal tolerances, but also by those of their host fish. Our intent was to compile data from available literature regarding thermal sensitivities of eight species of freshwater mussels and their host fishes, to determine if the community structure of these systems is at risk from rising environmental temperatures. Mussels were both more and less thermally sensitive than specific host fish species (2.9 ºC mean absolute difference between mussel and host; range = 0 – 6.8 ºC). In 62% of mussel-host fish comparisons, freshwater mussels were more thermally tolerant than their hosts (3.4 ºC mean difference; range = 0.2 – 6.8 ºC), suggesting that some mussels are effectively more stenothermic than tolerance criteria indicate, which may pose additional environmental risk. Further analysis revealed that variation in mussel thermal tolerance could not be attributed to mussel acclimation temperature, species, life stage, or mean host fish thermal tolerance, suggesting that mussel thermal tolerance is controlled by multiple interacting and complex factors. Our findings in this meta-analysis suggest that thermal effects of anthropogenic landscape alteration and climate change may be compounded for freshwater mussels via their obligate life cycle interaction with fish and highlight the importance of considering global change effects in a community context.

KEY WORDS Host fish, Stream community, Thermal tolerance, Unionidae

INTRODUCTION

Stream and river temperatures have been increasing, with a mean temperature increase of 0.009 – 0.077 °C per year in United States waters (Kaushal et al., 2010). Rising environmental temperatures can cause significant shifts in the composition and distribution of species within communities (Smith et al., 2006). Aquatic systems are much more constrained than are terrestrial systems in the ways in which organisms can respond to warming, and therefore, thermal effects may be more pronounced (Shuter & Post, 1990). Because of this, and also because changes in temperature unrelated to climate change in stream ecosystems have been well documented (Feller, 1981; Hewlett & Fortson, 1982), aquatic ecosystems are ideal model systems to study the ecological consequences of climate change.

Freshwater mussels (Order Unionida) fulfill their considerable role in the aquatic community by converting particulate matter from the water column into a food source for other organisms (Vaughn et al., 2004; Howard & Cuffey, 2006). The freshwater mussel family Unionidae is suffering a high rate of extinction; nearly 70% of North America's 297 species are extinct or vulnerable to extinction (Bogan, 1993; Williams et al., 1993; Graf & Cummings, 2007). The most notable

cause of decline in freshwater mussels is habitat degradation; other impacts include water withdrawal for industry, pollution, and urbanization (Bogan, 2008).

Freshwater mussels are a threatened taxon due in part to their unique life history strategies. They rely on host fish to complete their life cycle with a larval life stage, glochidia, that must infest the gills or fins of host fish as obligate parasites before transforming into the juvenile life stage and dropping to the sediment to continue development into benthic-dwelling adults (e.g., Watters, 2007).

Because of the relationship between mussels and their host fishes, freshwater mussels are not only potentially affected by their own variable thermal tolerance limits, but also by those of their host fish (Biro et al., 2007; Daufresne & Boet, 2007; Schmutz et al., 2007; Steingraeber et al., 2007). Although species interactions could be important in the ability of species to respond to climate change (Walther et al., 2002), this dynamic remains poorly explored for freshwater mussels (Spooner et al., 2011). Because some unionid mussels are host specific and may have different environmental requirements than their hosts, they represent an ideal case to explore the extent to which species interactions can and will mediate responses to climate change. The freshwater mussel-host fish relationship is a fitting model to explore both climate change in an aquatic context and interspecies relationships in the context of global change.

To elucidate the linkage between climate change and freshwater mussel survival, we collected representative thermal tolerance data for eight species of mussels as well as their host fishes. We then used these data to compare the thermal tolerances of these two groups of interacting organisms and propose scenarios of population and functional changes related to rising environmental temperatures.

METHODS

We compiled thermal tolerance data for glochidia of eight freshwater mussel species and seven species of juvenile freshwater mussels (Pandolfo et al., 2010). The mussel species represent two tribes (Lampsilini, Quadrulini) from the Ambleminae subfamily and one tribe (Anodontini) of the Unioninae subfamily: Fatmucket (*Lampsilis siliquoidea* (Barnes, 1823)), Pink Heelsplitter (*Potamilus alatus* (Say, 1817)), Black Sandshell (*Ligumia recta* (Lamarck, 1819)), Butterfly (*Ellipsaria lineolata* (Rafinesque, 1820)), Eastern Creekshell (*Villosa delumbis* (Conrad, 1834)), Washboard (*Megalonaias nervosa* (Rafinesque, 1820)), White Heelsplitter (*Lasmigona complanata* (Barnes, 1823)), and Brook Floater (*Alasmidonta varicosa* (Lamarck, 1819)) (Turgeon et al., 1998).

Thermal tolerances for the freshwater mussels were designated by median lethal temperatures (LT50s) (Pandolfo et al., 2010). Host fish were identified for the eight species of freshwater mussels according to the Ohio State University Mussel/Host database (Cummings & Watters, 2002) and by personal communication with propagation experts; only findings that observed juvenile metamorphosis in nature or in laboratory studies were included (Table 1). Thermal tolerance data for host fish species were collected from several sources. Lethal threshold temperatures (incipient lethal temperature; ILT) from the Environmental Protection Agency's Water Quality Criteria (1972) and Wismer & Christie (1987) were used when available, as these data coincided most directly with the LT50 measure used for freshwater mussels. For species where no lethal threshold was available, critical thermal maximum temperatures (CTmax), using loss of equilibrium as an endpoint, were derived from Beitinger et al. (2000) and Wismer & Christie (1987). For species where ILT or CTmax were not available, upper thermal tolerance limit (UTTL) data were applied from Eaton et al. (1995).

Upper thermal tolerances for host fish were plotted with freshwater mussel LT50s against acclimation temperature for each freshwater mussel species (Figure 1). In most instances, fish thermal tolerance increased linearly with increasing acclimation temperature, providing a reasonable indication of the upper thermal threshold for a species. However, the freshwater mussel thermal tolerances were not linearly related to acclimation temperature (Pandolfo et al., 2010). Because there was no significant effect of acclimation on freshwater mussel thermal tolerances, we were unable to conduct statistical comparisons on the linear regressions. Therefore, we qualitatively compared mussel thermal tolerances with fish thermal tolerances. If mussel tolerance was generally less than corresponding fish thermal tolerance (i.e., plotted points fell to the left), then the mussels were considered less thermally tolerant than the fish hosts and vice versa.

We also compared mean fish ILTs to mean mussel LT50s for the species with suitable data. To coincide with freshwater mussel LT50s, only fish ILTs for acclimation temperatures within the range of 22 – 27 °C were used. We conducted 29 species-specific comparisons between thermal tolerance means (e.g., mean Fatmucket versus mean Largemouth Bass, *Micropterus salmoides*), with comparisons possible for 6 of the 8 mussel species (Fatmucket, Black Sandshell, White Heelsplitter, Washboard, Brook Floater, and Eastern Creekshell). For each comparison, relative and absolute differences were calculated.

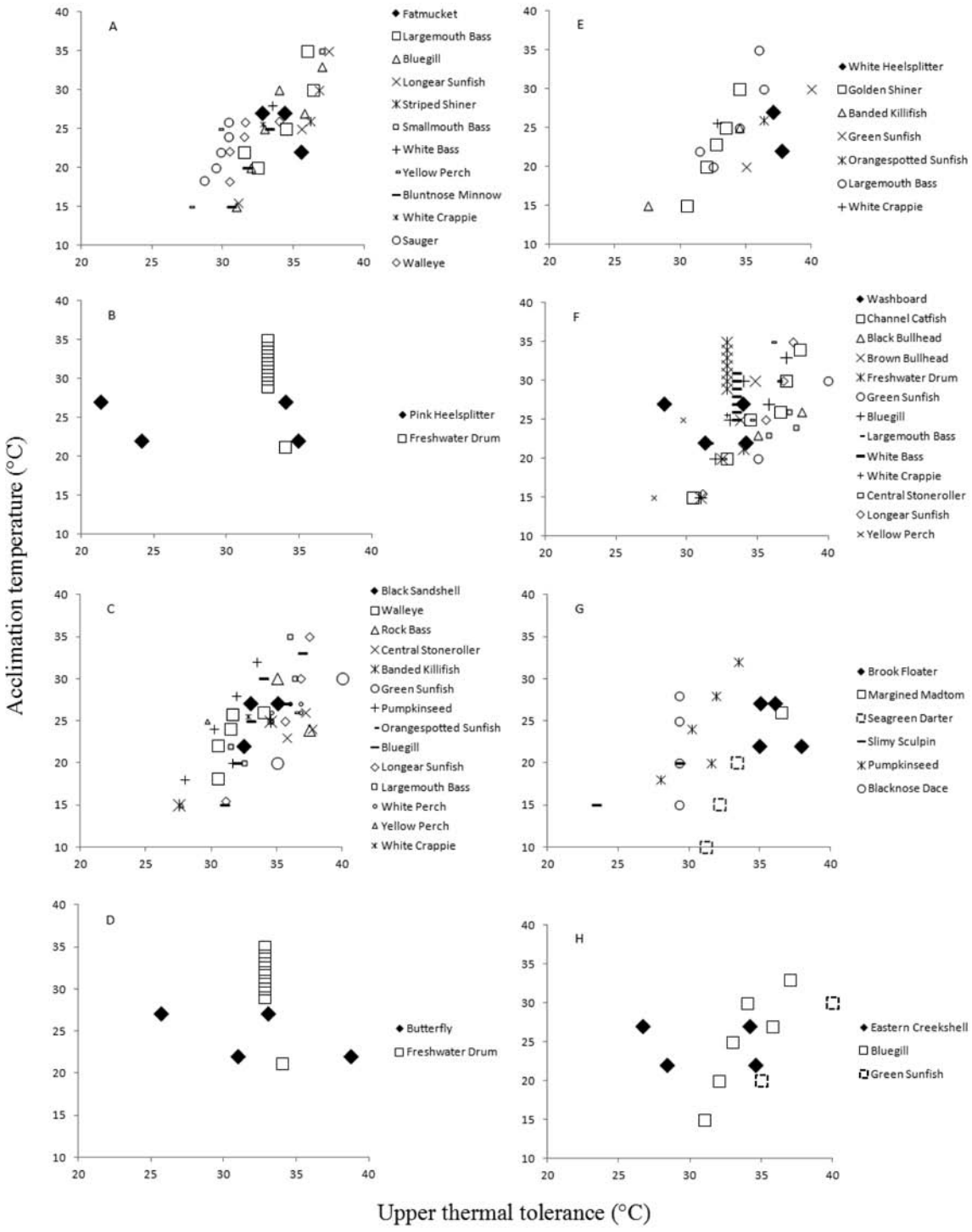

FIGURE 1

Upper thermal tolerances of eight species of freshwater mussels and their host fish. Each mussel species is graphed in a separate panel with its host fish: Fatmucket (A), Pink Heelsplitter (B), Black Sandshell (C), Butterfly (D), White Heelsplitter (E), Washboard (F), Brook Floater (G), and Eastern Creekshell (H). Freshwater mussels are denoted by the large diamond (◆), fish used to transform mussels from Pandolfo et al. (2010) are denoted by the large square (□).

For the same subset of data that was used to examine quantitative differences in mussel and fish thermal tolerances (six mussel species), a fixed-effects generalized linear model was used to assess the effects of mussel acclimation temperature, species, life stage, and host fish thermal tolerance on freshwater mussel thermal tolerances (SAS PROC GLM, version 9.2, SAS Institute Inc., Cary, North Carolina). Host fish thermal tolerance was incorporated into the model as a mean among fish species of host fish tolerance for each mussel species. Because host fish thermal tolerance was determined by mussel species, species and host fish thermal tolerance were confounded variables (i.e., one mean fish tolerance datum for each mussel species). To reduce covariate redundancy, species was omitted from the model and host fish thermal tolerance was retained to represent the effect of host fish thermal tolerance among mussel species.

RESULTS

LT50s were available for glochidia and juvenile freshwater mussels at two acclimation temperatures (Table 2) (Pandolfo et al., 2010). For both life stages, the overall LT50s ranged from 21.4 °C to 42.6 °C with a mean of 33.1 °C. Fish thermal tolerance values ranged from 23.5 °C to 38.1 °C with a mean of 33.1 °C (Table 1). Fish thermal tolerance varied according to acclimation temperature, as well as the method used to determine the tolerance value.

Relative thermal tolerance between freshwater mussels and their corresponding host fish varied among mussel species, and for some mussels, it varied among host fish species. Fatmucket appeared more thermally tolerant than Sauger (*Sander canadensis*) and Yellow Perch (*Perca flavescens*). Pink Heelsplitter and Butterfly shared the same host fish, Freshwater Drum (*Aplodinotus grunniens*), which had limited thermal tolerance data available. Both Pink Heelsplitter and Butterfly had a wider LT50 range than Freshwater Drum's UTTL, though more data are needed. Black Sandshell appeared more thermally tolerant than Yellow Perch, and more sensitive than Orangespotted Sunfish (*Lepomis humilis*) and Central Stoneroller (*Campostoma anomalum*). White Heelsplitter was more thermally tolerant than Largemouth Bass (*Micropterus salmoides*), Golden Shiner (*Notemigonus crysoleucas*), White Crappie (*Pomoxis annularis*), and Banded Killifish (*Fundulus diaphanus*). Washboard was less thermally tolerant than Green Sunfish (*Lepomis cyanellus*), Black Bullhead (*Ameiurus melas*), and Central Stoneroller. Brook Floater was more thermally tolerant than Slimy Sculpin (*Cottus cognatus*), Blacknose Dace (*Rhinicthys atratulus*), and Pumpkinseed (*Lepomis gibbosus*). Eastern Creekshell in this study (Pandolfo et al., 2010) were transformed by a hybrid Bluegill-Green Sunfish (*Lepomis macrochirus x L. cyanellus*); therefore, thermal tolerance data were considered for both species, because data were not available for the hybrid. Eastern Creekshell appeared to be similarly tolerant to both Bluegill and Green Sunfish; but, it remains unclear where the hybrid's thermal tolerance would occur.

Among mussel–fish relationships for which comparable thermal tolerance data were available, the mean of absolute differences between tolerances for mussels and corresponding host fish was 2.9 °C (n = 29, range = 0 – 6.8 ºC). Mussels were more thermally tolerant than their host fish in 18 of 29 comparisons (62%), and among those, the mean difference was 3.4 ºC (range = 0.2 – 6.8 ºC). Fatmucket, Black Sandshell, White Heelsplitter, and Brook Floater were more tolerant than their hosts in the majority of comparisons. Fatmucket was more thermally tolerant than Largemouth Bass, Yellow Perch, Bluntnose Minnow, Sauger, and Walleye and less tolerant than Bluegill and Longear Sunfish. Black Sandshell was more tolerant than Walleye, Banded Killifish, Pumpkinseed, Bluegill, Longear Sunfish, Largemouth Bass, and Yellow Perch and less tolerant than only Rock Bass. White Heelsplitter was more thermally tolerant in all three comparisons to Golden Shiner, Banded Killifish, and Largemouth Bass. Brook Floater was also more tolerant in both comparisons to Pumpkinseed and Blacknose Dace. Eastern Creekshell was only compared with Bluegill, and it was less tolerant than that species. Only Washboard demonstrated a strong trend of lower thermal tolerance than the majority of its hosts. Channel Catfish, Black Bullhead, Brown Bullhead, Bluegill, Largemouth Bass, and Longear Sunfish were all more thermally tolerant than Washboard, and the mussel was only more tolerant than Yellow Perch. In those cases where the fish host is more thermally tolerant than the mussel, tolerance differed by a mean of 2.2 ºC (range = 0.1 – 3.6 ºC).

Variation among mussel thermal tolerances could not be significantly attributed to mussel acclimation temperature, life stage, or mean host fish thermal tolerance. Though host fish thermal tolerance accounted for the largest source of variation in the model, the effect was not significant ($p = 0.098$). Acclimation temperature was also not a significant factor ($p = 0.275$), nor was mussel life stage ($p = 0.773$). Acclimation temperature and life stage were not expected to be significant effects, based on related previous analyses of the data (Pandolfo et al., 2010).

DISCUSSION

Although we cannot conclude that host fish thermal tolerance significantly affects freshwater mussel

thermal tolerance, host fish thermal tolerance was the most explanatory variable in our model. Despite the limited sample sizes and power of our analysis, we found a nearly significant effect ($p < 0.10$). As additional data become available for meta-analyses such as these, we suspect that a significant relationship may be revealed, reflecting the intrinsic species interactions involved in mussel thermal tolerance that varies among mussel species. The qualitative comparisons presented here demonstrated that, for the species examined, freshwater mussels generally have a thermal tolerance that is similar to or slightly greater than the thermal tolerance of their host fishes. In that prevalent case where a fish host is more stenothermic than the parasitizing mussel, the effective thermal tolerance of the mussel is reduced by the obligate relationship with the fish. However, these results and conclusion are based on an examination of acute thermal thresholds which may not adequately express the complexity of potential climate change scenarios.

As a response to global climate change, decreasing mussel survival may be a function of not only first order temperature or flow effects, but also of changing interactions with their host fishes (Spooner et al., 2011). Mussel population dynamics can also be impacted if increased water temperatures decrease the infestation success of glochidia on the host fish or if too few mussels are recruited to reproductive maturity to maintain the population. The mussels examined in our comparative study are dependent on predominantly coolwater and warmwater assemblage species as their hosts (Stefan et al., 1995), and therefore, we can potentially classify these mussels based on the classification of their hosts. Though not included in our study, mussel species exist that occupy cold headwater streams that are thermally buffered, relative to coolwater or warmwater stream habitats, and therefore, they parasitize coldwater fish as hosts (Bogan, 2002). These mussels are most likely to be adversely affected by global climate change and stream warming. It is also possible that mussels or fish that appear more heat tolerant may actually be more at risk from climate change because heat tolerant species may be living closer to their thermal limits (Tomanek & Somero, 1999). Evidence exists that some fish species are already encountering temperatures at their upper lethal limit in North America (Eaton et al., 1995; Caissie, 2006).

The bulk of aquatic thermal tolerance testing to date has been conducted on fish (e.g., Beitinger et al., 2000). From such studies, we have gained insight on the effects of temperature on basic physiological processes (van Dijk et al., 1999; Widmer et al., 2006; Fontaine et al., 2007). Increases in environmental temperature have also been shown to adversely affect fish assemblages (e.g., Keleher & Rahel, 1996; Peterson & Kwak, 1999; Flebbe et al., 2006). One long term study found that an increase of 1.5 ºC in the average water temperature in the Upper Rhone River caused southern fish species to displace northern fish species (Daufresne et al., 2004). The increase of southern warmwater fish into the range of the northern cooler water fish was consistent with predictions based on latitudinal, altitudinal, and stream order gradient hypotheses (Brown, 1971; Vannote et al., 1980).

Studies with mollusks have found, as in those with fish, that increases in temperature can affect various physiological functions, including immune condition (Chen et al., 2007), filtration rate (Shulte, 1975; Han et al., 2008), oxygen consumption (Newell et al., 1977; Han et al., 2008), excretion rates (Han et al., 2008), and growth (Han et al., 2008). To a degree, increased energy input (e.g., through filtration) may compensate for increased metabolic demands, but there appears to be a thermal limit above which the positive relationship between temperature and physiological function plateaus or becomes negative due to increasing energetic costs (Schulte, 1975; Newell et al., 1977). Rising temperatures have been associated with alterations in reproduction in the marine bivalve *Macoma balthica* (Philippart et al., 2003) and increased spawning in marine *Perna canaliculus* and *Mytilus galloprovincialis* (Petes et al., 2007). In addition to the findings on sublethal effects of thermal stress, a number of studies have addressed acute thermal limits (Ansell et al., 1980; Iwanyzki & McCauley, 1993; Urban, 1994; Pandolfo et al., 2010). Laboratory tests have shown that viability of glochidia can vary widely even at a common temperature among species belonging to the same tribe (Cope et al., 2008). Laboratory tests also show that increasing temperature causes a decrease in glochidial viability (Jansen et al., 2001; Zimmerman & Neves, 2002; Akiyama & Iwakuma, 2007).

The obligate parasite-host relationship between freshwater mussels and fish provides an insightful example of how the loss of one species in a community can initiate cascading effects for additional species. These cascades may lead to chains of extinction among any number of species that interact in a critical manner. In perhaps the clearest case of coextinction in the literature, severe reductions in populations of the Eel Grass *Zostera marina* drove the host-specific Eelgrass Limpet, *Lottia alveus*, to extinction (Carlton et al., 1991). Changes in environmental temperatures can also cause asynchrony in species interactions. Increased temperature caused the bivalve *Macoma balthica* to adjust its reproductive schedule which led to asynchrony with the presence of phytoplankton and shrimp necessary for juvenile survival (Philippart et al.,

2003). For freshwater mussels, asynchrony with the presence of host fish could lead to a collapse of mismatched populations.

A number of scenarios warrant consideration to examine the interactions of freshwater mussels with host fishes in the context of climate change (Figure 2). The thermal tolerance of freshwater mussels can potentially be higher, lower, or similar to their host fish. Each of these possibilities may lead to very different outcomes, each with distinct implications for conservation and management of freshwater mussels. If freshwater mussels and their host fishes have similar thermal tolerances, then no species interaction effects are expected to compound any adverse effects from climate change. This does not imply that climate change does not pose a risk to mussels or their hosts, but that they are expected to respond in similar manners, and therefore their relationship can be conserved. However, even if host fish remain within range of freshwater mussels, glochidia may not transform successfully outside an optimal temperature range (Roberts & Barnhart, 1999).

An important consideration is that freshwater mussels are more constrained in their mobility than are their host fish. Freshwater mussels do not have the option to relocate, and must be able to tolerate local environmental conditions to survive (Golladay et al., 2004). As temperature increases, some fish species may shift their distribution as a response, with warmwater species moving into cooler habitats, or relocating to lower order streams. Because freshwater fish are able to detect differences in water temperature and relocate to cooler water when available, the fish may more easily alter their distribution outside of the range of the freshwater mussels that rely on them (Kaya et al., 1977; Headrick & Carline, 1993; Schaefer et al., 2003). In this scenario, the thermal tolerance criteria for mussels do not indicate their effective vulnerability to temperature rise unless those thresholds for host fish are also considered. If this occurs, mussels may be able to parasitize other more tolerant fish species as alternate hosts. However, most mussel species specialize with one or only a few fish species as hosts, but specificity varies among species (Haag & Warren, 2003). In general, freshwater mussels become locally adapted to their host fish and experience greater transformation success with fish in their native habitat than with fish from other areas (Rogers et al., 2001).

Another scenario is that if the host fish have thermal tolerances greater than the dependent mussels, the fish will not need to relocate to cooler habitat. The possibility remains in this scenario that through typical fish movement, mussels may be dispersed to cooler habitats where they will be more suited for survival. However, if this is not the case, mussel populations may decline due to decreased glochidial infestation success or thermal mortality of mussels of all life stages, despite the presence of their host fish. If mussel populations become too small and fragmented, sperm may not reach females during the spawning season, and such populations will be unable to contribute genetically (Downing et al., 1993; Strayer et al., 2004; McLain & Ross, 2005).

Organisms can adapt to environmental changes in two ways: changes within individuals (phenotypic plasticity) or evolutionary changes (Berteaux et al., 2004). However, freshwater mussel adaptation may be limited due to their extended life span, as species with long generation times respond relatively slowly to environmental changes (Berteaux et al., 2004; Rowe, 2008). In addition, recruitment does not necessarily occur annually; for instance, a population study of the freshwater mussel Ebonyshell, *Fusconaia ebena* (Lea, 1831), found successful recruitment only once every 5 to 10 years (Payne & Miller, 2000). Thus, the population dynamics of freshwater mussels are complex, and populations may exhibit negative growth and highly variable recruitment, while long-lived individuals thrive (Strayer et al., 2004).

Aquatic species may also have to cope with the shifting distributions of more thermally tolerant non-indigenous species (Stachowicz et al., 2002; Carveth et al., 2006), and land-use changes can combine with climate change effects to the detriment of aquatic organisms (Peterson & Kwak, 1999). Environmental temperature rise may result in unexpected changes in ecosystems as regime shifts occur (Hsieh et al., 2005), and the many factors involved in climate change may interact in a synergistic fashion (Portner et al., 2005). In fact, alterations in flow regime as a result of changing precipitation patterns may be at least as threatening to aquatic species as increasing temperatures (Peterson & Kwak, 1999).

Our analysis highlights the importance of considering global change effects in a community context, but additional research is required to fully understand and plan for climate change and the thermal tolerance dynamics of freshwater mussels and their host fish. More data are needed on thermal tolerances of specific host fish-mussel pairs, the transformation success rate with alternate hosts, and local and broad-scale influences of flow and land cover before it is possible to determine which of the proposed scenario outcomes is most plausible among freshwater mussel species (Figure 2). Surveys of mussel assemblage structure along temperature gradients would provide critical information, as

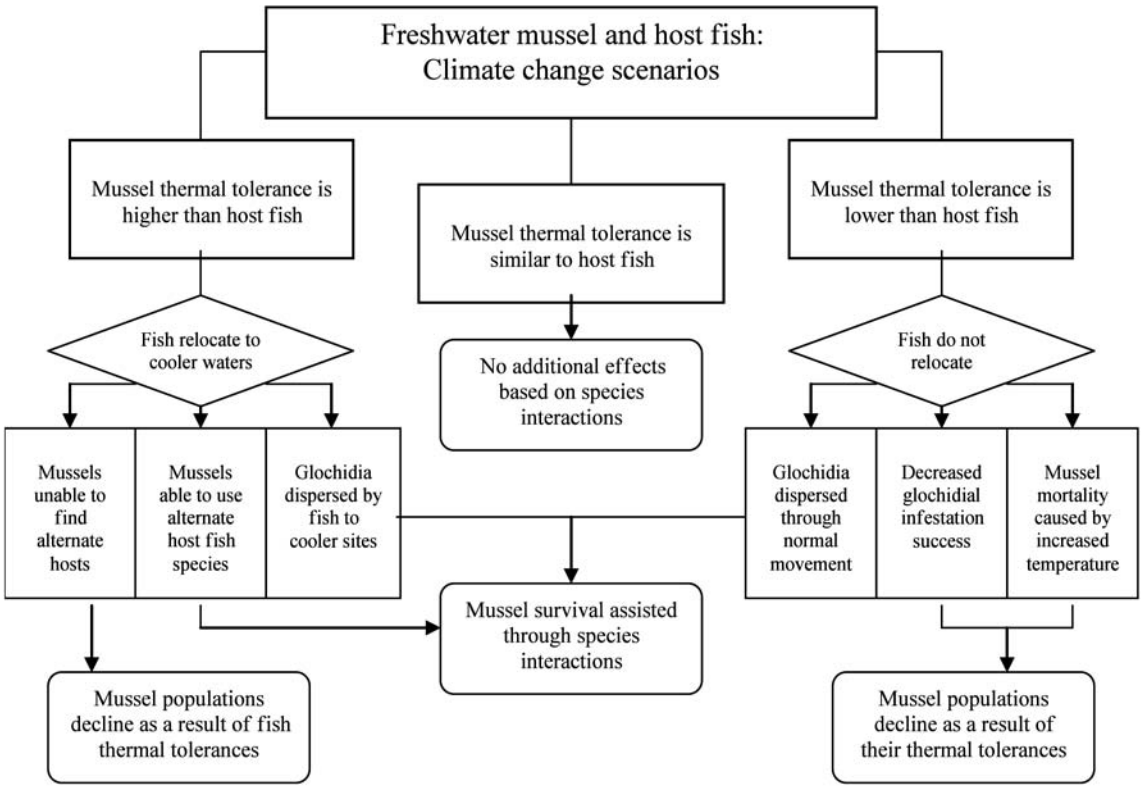

FIGURE 2

Flow diagram of potential interaction scenarios for freshwater mussels and their host fishes in the context of climate change.

would additional laboratory investigations of infestation success of glochidia on multiple fish species in relation to environmental temperature changes.

Research on climate change effects cannot be conducted for every species and community; therefore the focus must be on species with a disproportionately important function in their ecosystems (Bale et al., 2002). We further propose that freshwater mussels are a crucial fauna to study in the context of global change, not only because they are one of the most endangered aquatic faunal groups in North America, but also because of their unique life history strategies. Unionids provide a means for measuring the importance of species interactions as a component of climate change using a sensitive model species in aquatic systems—if freshwater mussels will not be our aquatic climate change canary, which fauna will?

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TABLE 1

Thermal tolerance data compiled from literature for freshwater fish species that serve as hosts for freshwater mussels. All temperatures are ºC; acclimation temperature is in parentheses. ILT=incipient lethal temperature, CTmax=critical thermal maximum, UTTL=upper thermal tolerance limit.

Freshwater mussel	Host fish	Fish species name	Thermal tolerance	Method	Source
Fatmucket	Largemouth Bass[1]	*Micropterus salmoides*	32.5 (20)	ILT	EPA 1972
(*Lampsilis siliquoidea*)			31.5 (22)	ILT	EPA 1972
			34.5 (25)	ILT	EPA 1972
			36.4 (30)	ILT	EPA 1972
			36 (35)	ILT	Wismer and Christie 1987
	Bluegill	*Lepomis macrochirus*	31 (15)	ILT	Wismer and Christie 1987
			32 (20)	ILT	Wismer and Christie 1987
			33 (25)	ILT	Wismer and Christie 1987
			35.8 (27)	ILT	Wismer and Christie 1987
			34 (30)	ILT	Wismer and Christie 1987
			37 (33)	ILT	Wismer and Christie 1987
	Longear Sunfish	*Lepomis megalotis*	31.1 (15.5)	ILT	Wismer and Christie 1987
			35.6 (25)	ILT	EPA 1972
			36.8 (30)	ILT	EPA 1972
			37.5 (35)	ILT	EPA 1972
	Striped Shiner	*Luxilus chrysocephalis*	36.2 (26)	CTmax	Beitinger et al. 2000
	Smallmouth Bass	*Micropterus dolomieu*	37 (35)	ILT	Wismer and Christie 1987
	White Bass	*Morone chrysops*	33.5 (28)	UTTL	Eaton et al. 1995
	Yellow Perch	*Perca flavescens*	27.7 (15)	ILT	EPA 1972
			29.7 (25)	ILT	EPA 1972
	Bluntnose Minnow	*Pimephales notatus*	30.6 (15)	ILT	EPA 1972
			31.7 (20)	ILT	EPA 1972
			33.3 (25)	ILT	EPA 1972
	White Crappie	*Pomoxis annularis*	32.8 (25.6)	UTTL	Eaton et al. 1995
	Sauger	*Sander canadensis*	28.7 (18.3)	ILT	Wismer and Christie 1987
			29.5 (19.9)	ILT	Wismer and Christie 1987
			29.9 (22)	ILT	Wismer and Christie 1987
			30.4 (23.9)	ILT	Wismer and Christie 1987
			30.4 (25.8)	ILT	Wismer and Christie 1987
	Walleye	*Stizostedion (Sander) vitreum*	30.5 (18.2)	ILT	Wismer and Christie 1987
			30.5 (22.1)	ILT	Wismer and Christie 1987
			31.5 (24)	ILT	Wismer and Christie 1987
			31.6 (25.8)	ILT	Wismer and Christie 1987
			34 (26)	ILT	Wismer and Christie 1987
Pink Heelsplitter	Freshwater Drum[1]	*Aplodinotus grunniens*	34 (21.2)	CTmax	Wismer and Christie 1987
(*Potamilus alatus*)			32.8 (29-35)	ILT	Wismer and Christie 1987
Black Sandshell	Walleye[1]	*Stizostedion (Sander) vitreum*	30.5 (18.2)	ILT	Wismer and Christie 1987
(*Ligumia recta*)			30.5 (22.1)	ILT	Wismer and Christie 1987
			31.5 (24)	ILT	Wismer and Christie 1987
			31.6 (25.8)	ILT	Wismer and Christie 1987
			34 (26)	ILT	Wismer and Christie 1987
	Rock Bass	*Ambloplites rupestris*	37.5 (23.9)	ILT	Wismer and Christie 1987
			35 (30)	ILT	Wismer and Christie 1987
	Central Stoneroller	*Campostoma anomalum*	35.8 (23)	CTmax	Beitinger et al. 2000
			37.7 (24)	CTmax	Beitinger et al. 2000
			37.2 (26)	CTmax	Beitinger et al. 2000
	Banded Killifish	*Fundulus diaphanus*	27.5 (15)	ILT	EPA 1972
			34.5 (25)	ILT	Wismer and Christie 1987
	Green Sunfish	*Lepomis cyanellus*	35 (20)	ILT	Wismer and Christie 1987
			40 (30)	ILT	Wismer and Christie 1987
	Pumpkinseed	*Lepomis gibbosus*	28 (18)	ILT	Wismer and Christie 1987
			31.6 (20)	ILT	Wismer and Christie 1987
			30.2 (24)	ILT	Wismer and Christie 1987
			31.9 (28)	ILT	Wismer and Christie 1987
			33.5 (32)	ILT	Wismer and Christie 1987

TABLE 1
(cont.)

Freshwater mussel	Host fish	Fish species name	Thermal tolerance	Method	Source
Black Sandshell	Orangespotted Sunfish	*Lepomis humilis*	36.4 (26)	CTmax	Beitinger et al. 2000
(*Ligumia recta*)	Bluegill	*Lepomis macrochirus*	31 (15)	ILT	Wismer and Christie 1987
continued			32 (20)	ILT	Wismer and Christie 1987
			33 (25)	ILT	Wismer and Christie 1987
			35.8 (27)	ILT	Wismer and Christie 1987
			34 (30)	ILT	Wismer and Christie 1987
			37 (33)	ILT	Wismer and Christie 1987
	Longear Sunfish	*Lepomis megalotis*	31.1 (15.5)	ILT	Wismer and Christie 1987
			35.6 (25)	ILT	EPA 1972
			36.8 (30)	ILT	EPA 1972
			37.5 (35)	ILT	EPA 1972
	Largemouth Bass	*Micropterus salmoides*	32.5 (20)	ILT	EPA 1972
			31.5 (22)	ILT	EPA 1972
			34.5 (25)	ILT	EPA 1972
			36.4 (30)	ILT	EPA 1972
			36 (35)	ILT	Wismer and Christie 1987
	White Perch	*Morone americana*	34.6 (25-26)	ILT	Wismer and Christie 1987
			36.8 (26-27)	ILT	Wismer and Christie 1987
			36 (27)	ILT	Wismer and Christie 1987
	Yellow Perch	*Perca flavescens*	27.7 (15)	ILT	EPA 1972
			29.7 (25)	ILT	EPA 1972
	White Crappie	*Pomoxis annularis*	32.8 (25.6)	UTTL	Eaton et al. 1995
Butterfly	Freshwater Drum[1]	*Aplodinotus grunniens*	34 (21.2)	CTmax	Wismer and Christie 1987
(*Ellipsaria lineolata*)			32.8 (29-35)	ILT	Wismer and Christie 1987
White Heelsplitter	Golden Shiner[1]	*Notemigonus crysoleucas*	30.5 (15)	ILT	EPA 1972
(*Lasmigona complanata*)			32 (20)	ILT	EPA 1972
			32.7 (22.8)	ILT	Wismer and Christie 1987
			33.5 (25)	ILT	EPA 1972
			34.5 (30)	ILT	EPA 1972
	Banded Killifish	*Fundulus diaphanus*	27.5 (15)	ILT	EPA 1972
			34.5 (25)	ILT	Wismer and Christie 1987
	Green Sunfish	*Lepomis cyanellus*	35 (20)	ILT	Wismer and Christie 1987
			40 (30)	ILT	Wismer and Christie 1987
	Orangespotted Sunfish	*Lepomis humilis*	36.4 (26)	CTmax	Beitinger et al. 2000
	Largemouth Bass	*Micropterus salmoides*	32.5 (20)	ILT	EPA 1972
			31.5 (22)	ILT	EPA 1972
			34.5 (25)	ILT	EPA 1972
			36.4 (30)	ILT	EPA 1972
			36 (35)	ILT	Wismer and Christie 1987
	White Crappie	*Pomoxis annularis*	32.8 (25.6)	UTTL	Eaton et al. 1995
Washboard	Channel Catfish[1]	*Ictalurus punctatus*	30.4 (15)	ILT	EPA 1972
(*Megalonaias nervosa*)			32.8 (20)	ILT	EPA 1972
			34.5 (25)	ILT	EPA 1972
			36.6 (26)	ILT	Wismer and Christie 1987
			37 (30)	ILT	EPA 1972
			38 (34)	ILT	Wismer and Christie 1987
	Black Bullhead	*Ameiurus melas*	35 (23)	ILT	Wismer and Christie 1987
			38.1 (26)	CTmax	Beitinger et al. 2000
	Brown Bullhead	*Ameiurus nebulosus*	31 (15)	ILT	EPA 1972
			32.5 (20)	ILT	EPA 1972
			33.8 (25)	ILT	EPA 1972
			34.8 (30)	ILT	EPA 1972
			41 (35)	ILT	Wismer and Christie 1987
	Freshwater Drum	*Aplodinotus grunniens*	34 (21.2)	CTmax	Wismer and Christie 1987
			32.8 (29-35)	ILT	Wismer and Christie 1987
	Green Sunfish	*Lepomis cyanellus*	35 (20)	ILT	Wismer and Christie 1987
			40 (30)	ILT	Wismer and Christie 1987
	Bluegill	*Lepomis macrochirus*	31 (15)	ILT	Wismer and Christie 1987
			32 (20)	ILT	Wismer and Christie 1987
			33 (25)	ILT	Wismer and Christie 1987
			35.8 (27)	ILT	Wismer and Christie 1987
			34 (30)	ILT	Wismer and Christie 1987
			37 (33)	ILT	Wismer and Christie 1987

TABLE 1
(cont.)

	Host fish	Fish species name	Thermal tolerance	Method	Source
Washboard	Largemouth Bass	*Micropterus salmoides*	32.5 (20)	ILT	EPA 1972
(*Megalonaias nervosa*)			31.5 (22)	ILT	EPA 1972
continued			34.5 (25)	ILT	EPA 1972
			36.4 (30)	ILT	EPA 1972
			36 (35)	ILT	Wismer and Christie 1987
	White Bass	*Morone chrysops*	33.5 (25-31)	UTTL	Eaton et al. 1995
	White Crappie	*Pomoxis annularis*	32.8 (25.6)	UTTL	Eaton et al. 1995
	Central Stoneroller	*Campostoma anomalum*	35.8 (23)	CTmax	Beitinger et al. 2000
			37.7 (24)	CTmax	Beitinger et al. 2000
			37.2 (26)	CTmax	Beitinger et al. 2000
	Longear Sunfish	*Lepomis megalotis*	31.1 (15.5)	ILT	Wismer and Christie 1987
			35.6 (25)	ILT	EPA 1972
			36.8 (30)	ILT	EPA 1972
			37.5 (35)	ILT	EPA 1972
	Yellow Perch	*Perca flavescens*	27.7 (15)	ILT	EPA 1972
			29.7 (25)	ILT	EPA 1972
Brook Floater	Margined Madtom[1]	*Noturus insignis* [2]	36.5 (26)	CTmax	Beitinger et al. 2000
(*Alasmidonta varicosa*)	Seagreen Darter[1]	*Etheostoma thalassinum* [3]	31.2 (10)	CTmax	Beitinger et al. 2000
			32.2 (15)	CTmax	Wismer and Christie 1987
			33.4 (20)	CTmax	Beitinger et al. 2000
	Slimy Sculpin	*Cottus cognatus*	23.5 (15)	ILT	Wismer and Christie 1987
			29.4 (20)	CTmax	Wismer and Christie 1987
	Pumpkinseed	*Lepomis gibbosus*	28 (18)	ILT	Wismer and Christie 1987
			31.6 (20)	ILT	Wismer and Christie 1987
			30.2 (24)	ILT	Wismer and Christie 1987
			31.9 (28)	ILT	Wismer and Christie 1987
			33.5 (32)	ILT	Wismer and Christie 1987
	Blacknose Dace	*Rhinicthys atratulus*	29.3 (15)	ILT	Wismer and Christie 1987
			29.3 (20)	ILT	EPA 1972
			29.3 (25)	ILT	EPA 1972
			29.3 (28)	ILT	EPA 1972
Eastern Creekshell	Hybrid Bluegill[1]	*Lepomis machrochirus cyanellus*			
(*Villosa delumbis*)	Bluegill	*Lepomis macrochirus*	31 (15)	ILT	Wismer and Christie 1987
			32 (20)	ILT	Wismer and Christie 1987
			33 (25)	ILT	Wismer and Christie 1987
			35.8 (27)	ILT	Wismer and Christie 1987
			34 (30)	ILT	Wismer and Christie 1987
			37 (33)	ILT	Wismer and Christie 1987
	Green Sunfish	*Lepomis cyanellus*	35 (20)	ILT	Wismer and Christie 1987
			40 (30)	ILT	Wismer and Christie 1987

[1]Fish used to transform juvenile mussels from Pandolfo et al. (2010).

[2]Thermal data for Slender Madtom (*Noturus exilis*).

[3]Thermal data for Greenside Darter (*Etheostoma blennioides*).

TABLE 2

Freshwater mussel thermal tolerance data. LT50s for glochidia (24 h) and juvenile (96 h) mussels at 22 ºC and 27 ºC acclimation temperatures (Pandolfo et al. 2010). All LT50s reported as ºC.

	LT50			
	Glochidia		Juveniles	
Species	22 °C Acclimation	27 °C Acclimation	22 °C Acclimation	27 °C Acclimation
Lampsilis siliquoidea		32.8	35.6	34.4
Potamilus alatus	24.2	21.4	35.0	34.1
Ligumia recta	42.6	33.0	32.5	35.1
Ellipsaria lineolata	31.0	25.7	38.8	33.1
Lasmigona complanata	37.8	37.1		
Megalonaias nervosa	31.3	28.4	34.2	34.0
Alasmidonta varicosa	38.0	36.1	35.0	35.1
Villosa delumbis	28.4	26.7	34.6	34.2

WALKERANA The Journal of the Freshwater Mollusk Conservation Society

OUR PURPOSE

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

1) Advocate conservation of freshwater molluscan resources;

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

3) Promote science-based management of freshwater mollusks;

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

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

OUR HISTORY

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

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