Genetics, demography and modeling of freshwater mussel (Bivalvia: Unionidae) populations in the Clinch River, U.S.A.

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ABSTRACT

Genetic variation was examined in two endangered mussel species, *Epioblasma brevidens* and *E. capsaeformis*, and a common species *Lampsilis fasciola*, in the Clinch River, TN, by screening mitochondrial DNA (mtDNA) sequences and nuclear DNA microsatellites. These species use fish hosts with varying dispersal capabilities, ranging from low, moderate, and high, respectively. Patterns of mtDNA polymorphism exhibited different trends for long-term population sizes for each species during the Holocene (~10,000 ya to present); namely, *E. brevidens* has declined over time, *E. capsaeformis* has remained stable, and *L. fasciola* has expanded. Long-term effective population size (N_e) was smallest in *E. brevidens*, intermediate in *E. capsaeformis*, and highest in *L. fasciola*. Moderately diverged mtDNA lineages, perhaps indicative of secondary contact, were observed in *E. brevidens* and *E. capsaeformis*. High levels of gene flow (*Nm*) were estimated among demes of *L. fasciola* using traditional *F*-statistics and likelihood estimates of *Nm*, whereas such metrics were lower in *E. brevidens* and *E. capsaeformis*. Data are consistent with population dynamics and life history traits of each species and their fish hosts.

Age, shell growth, and population demography of *Epioblasma brevidens*, *E. capsaeformis*, and *Lampsilis fasciola* were studied from 2004-2007 in a 32-km reach of the Clinch River, TN. Observed maximum age and length of *E. brevidens* was 28 y and 71.5 mm for males and 11 y and 56.6 mm for females; of *E. capsaeformis*, 12 y and 54.6 mm for males and 9 y and 48.6 mm for females; and of *L. fasciola*, 45 y and 91.3 mm for males and 13 y and 62.6 mm for females. For all three species, observed maximum age and length was greater among males than females. Estimated population size in this river reach was approximately 43,000 individuals for *E. brevidens*, 579,000 individuals for *E. capsaeformis*, and 30,000 individuals for *L. fasciola*. Mean recruitment y^{-1} of 1 y-old *E. brevidens* ranged from 7.1% to 20%, of *E. capsaeformis* from 4.0% to 32.4%, and of *L. fasciola* from 5.8% to 25.6%. Population growth rate y^{-1} was 24.9% for *E. brevidens*, 34.6% for *E. capsaeformis*, and -22.4% for *L. fasciola*. Mortality rates of females were higher than for males of *E. capsaeformis* and *L. fasciola*, but not *E. brevidens*. Juvenile mussels were collected but temporally and spatially variable in occurrence, and a significant component of the age-class structure of all three species. Recruitment was very high during 2006-2007 for *E. capsaeformis* and other species, likely due to low river discharges in the spring-summer of 2005-2007. Surplus individuals of *E. brevidens* and *E. capsaeformis* are currently available to conduct translocations for restoration purposes.

Population modeling of *Epioblasma brevidens* and *E. capsaeformis* in the Clinch River was conducted to determine suitable harvest levels for translocation of sub-adults and adults, and to determine quantitative criteria for evaluating performance and recovery of extant and reintroduced populations. For both species, the recommended annual harvest was <1% of local population size to minimize risk of decline. Reintroduction

modeling indicated that size of the initial population created during a 5 y build-up phase greatly affected final population size at 25 y, being similar to size at the end of the build-up phase, especially when expected 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. The age-class distribution of a stable or growing population was characterized by multiple cohorts, to include 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 sizes for reintroduced or recovered populations of each species should be ≥5,000 individuals (N_e=250) and ≥10,000 individuals (N_e=500), respectively, and should be comprised of multiple smaller demes spread throughout a river. Populations of both species are currently large enough to sustain harvest for translocation and reintroduction purposes, offering an effective species recovery strategy.

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*All data, tables, and figures contained in this dissertation are original and were collected and created by the author, respectively, unless otherwise indicated.

CHAPTER 1

HISTORICAL DEMOGRAPHY AND GENE FLOW OF THREE MUSSEL SPECIES (BIVALVIA: UNIONIDAE) IN THE CLINCH RIVER, U.S.A.: GENETIC EVIDENCE FOR POPULATION EXPANSION AND CONTRACTION DURING THE HOLOCENE

ABSTRACT

Patterns of intraspecific genetic variation are shaped by contemporary population processes and by historical events that have occurred over millennial time-scales. Vicariance biogeography and paleo-climate change control long-term population trends, historical isolation and incidence of secondary contact throughout a species range, whereas life history traits interact with environmental variables to affect current dispersal rates and fluctuations in population size. Genetic variation was examined in two cognate endangered mussel species, *Epioblasma brevidens* and *E. capsaeformis*, and a common species, *Lampsilis fasciola*, in the Clinch River, TN, U.S.A, by screening mitochondrial DNA (mtDNA) sequences and nuclear DNA microsatellites. These species use specific primary fish hosts with varying dispersal capabilities, ranging from low, moderate, and high, respectively. Patterns of mtDNA polymorphism exhibited different trends for long-term population sizes for each species during the Holocene (~10,000 ya to present); namely, *E. brevidens* has declined over time, *E. capsaeformis* has remained stable, and *L. fasciola* has expanded. Long-term effective population size (N_e) was smallest in *E. brevidens*, intermediate in *E. capsaeformis*, and highest in *L. fasciola*. Moderately diverged mtDNA lineages, perhaps indicative of secondary contact, were observed in *E. brevidens* and *E. capsaeformis*. High levels of gene flow (*Nm*) were estimated among demes of *L. fasciola* using traditional *F*-statistics and likelihood estimates of *Nm*, whereas such metrics were lower in *E. brevidens* and *E. capsaeformis*. Data are consistent with known population dynamics and life history traits of these species and their fish hosts.

KEY WORDS: Freshwater mussels, historical population trends, fish hosts, N_e, and *Nm*

INTRODUCTION

The freshwater pearlymussels of North America (United States and Canada) are extremely diverse, with native species comprising nearly 300 of the approximately 840 species recognized worldwide (Williams et al. 1992; Graf and Cummings 2007). This fauna is recognized as globally significant and serves an important role in the function and maintenance of healthy freshwater ecosystems (Vaughn and Hakenkamp 2001; Lydeard et al. 2004). However, due to anthropogenic impacts, a severe decline of these bivalve mollusks has been well documented (Neves et al., 1997; Lydeard et al., 2004). Scientists are only beginning to understand the many unusual features of their reproductive biology and to appreciate the complexity of their conservation needs (Strayer et al. 2004). Currently, freshwater mussels and snails are the most endangered group of animals in North America (NA) (Neves et al. 1997). Many efforts are ongoing to protect and restore these faunal groups, including research to understand their conservation genetic needs and priorities (Jones et al. 2006a). Recent intraspecific genetic studies of mussels have revealed a range of complexity and diversity of molecular markers, phenotypes, and life history traits (Kelly and Rhymer 2005; Grobler et al. 2006; Jones et al. 2006b; Serb 2006; Elderkin et al. 2007; Zanatta and Murphy 2007). The factors responsible for shaping these patterns of genetic diversity are both historical (e.g., vicariance biogeography and paleo-climate change) and contemporary (e.g., recent gene flow and genetic drift) in nature.

Modern forms of freshwater mussels begin to appear in sedimentary strata of the Eocene (58-37 mya) epoch (Watters 2001). For example, fossilized shells of the genus

Lampsilis have been collected in Eocene shale of the Green River formation, located in the interior basins of western United States (Surdam and Wolfbauer, 1975). Some lineages are perhaps older, such as *Margaritifera margaritifera* (Linnaeus, 1758), which occurs in NA and Europe, last linked during the Eocene by the Greenland land-bridge (Bauer and Wachtler 2001). Hence, it is likely that many extant mussel lineages have existed for millions of years, and thus have been exposed to a broad range of palaeo-climatic conditions. However, it is the Pleistocene ice ages (1.65 mya – 10,000 ya) that likely have had the greatest effect on the current distribution and patterns of genetic diversity of extant species, especially those occupying temperate latitudes (Pielou 1991; Hewitt 1996). This epoch was characterized by extreme oscillation of global climate, resulting in the formation, expansion and contraction of large continental ice sheets. Glaciers advanced and contracted repeatedly over millennia, creating glacial cycles that dramatically affected continental biota (Lessa et al. 2003). The Croll-Milankovitch theory proposes that one of the major controlling mechanisms of these cycles was variation in the earth's orbit around the sun; the orbit (eccentricity) has a 100-kyr cycle, the axial tilt (obliquity) a 41-kyr cycle, and the wobble (precession) a 19-23-kyr cycle (Hewitt 2000). These orbital variations changed the Earth's insolation and the solar energy it received. Ice age cycles contained both warm periods (inter-glacials) and cold periods (glacials). Glacials typically spanned ~100,000 y and inter-glacials ~10,000 y, with as many as nine cycles spanning the Pleistocene. During the last glacial cycle – the Wisconsin – the maximum extent of the Laurentide ice sheet of NA peaked ~20,000 ya, with its southern boundary reaching into the upper Midwestern (40°N) United States, a time when global sea-level was >120 m below present levels (Cronin et al. 1981; Pielou 1991). Species

responses to these climatic oscillations obviously were varied, with some species expanding their ranges during inter-glacials, while those of others contracted, and vice-versa (Hewitt 1996).

The upland portions of the Tennessee and Cumberland rivers in the southeastern United States are termed the Cumberlandian Region and serve as major drainage networks for the southern Appalachian Mountains (Figure 1). These highland areas contain the most diverse temperate aquatic fauna in the world. The drainage is characterized by clear, high-gradient streams with gravel substrates. Various hypotheses have been proposed to explain this diversity, including vicariance biogeography and dispersal (Mayden 1985, 1988; Near and Keck 2005). However, because the region was not glaciated during the Pleistocene, it likely served as the refugium and putative center of origin for numerous aquatic species. Many mussel and fish species are endemic to the region and occupy various habitat niches (Etnier and Starnes 1993; Parmalee and Bogan 1998). The main tenet of the vicariance hypothesis is that the current distributions of related taxa reflect the fragmentation of once widespread ancestral biotas that pre-date the Pleistocene (Mayden 1985, 1988; Bermingham and Avise, 1986). The vicariance hypothesis predicts that fragmented populations should be divergent at molecular markers and perhaps exhibit adaptation to local environments (co-adaptation). Conversely, the dispersal hypothesis proposes that certain regions served as the center of origin and/or refugium for species and that they subsequently dispersed into unoccupied regions during different times throughout the Pleistocene, and occasionally earlier epochs (Near and Keck 2005). The dispersal hypothesis predicts that populations should be closely related.

Both models are useful to explain how populations can become isolated, diverged from one another, and sometimes re-connected (=secondary contact).

The larval stage (glochidium) of most freshwater mussel species is an obligate parasite on fish. Glochidia require specific fish hosts in order to transform to juveniles and disperse into new habitats. Hence, dispersal rates and gene flow of mussels are highly dependent on the dispersal of fish hosts. The mussel species of interest in this study are two Cumberlandian endemic and endangered species, the Cumberland combshell (*Epioblasma brevidens*) (Lea, 1831) and oyster mussel (*E. capsaeformis*) (Lea, 1834), and a third widespread species, the wavy-rayed lampmussel (*Lampsilis fasciola*) Rafinesque, 1820 (Figure 1). The last species is distributed throughout rivers of the Cumberlandian region, Ohio River drainage and Lake Erie basin. Currently, all three species inhabit the Clinch River in eastern Tennessee (TN) and southwestern Virginia (VA), U.S.A, which is the primary study area of this investigation. These mussel species express marked differences in life history characteristics, especially in dispersal of their primary fish hosts. The *E. capsaeformis* utilizes small darters in the genus *Etheostoma* as its primary hosts (Yeager and Saylor 1995; Jones et al 2006b). Small darters (50-60 mm total length) of various species have been shown to disperse relatively short distances (100-200 m) per year (Roberts and Angermeier 2007). The *E. brevidens* uses the logperch (*Percina caprodes*) as its primary fish host (Yeager and Saylor 1995), a relatively large (120 mm), mobile darter. By comparison, *L. fasciola* has even greater dispersal capabilities, utilizing highly mobile black basses (*Micropterus* spp.) as its primary fish hosts. Basses can move more than 1 km in a single day (Lyons and Kanehl 2002). These primary fish hosts presumably have dispersal abilities that can be broadly

categorized as low, medium, and high, respectively. In addition to dispersal, these mussel species exhibit differences in population growth rates, fecundity, population sizes, and longevity (Chapter 2). While *E. capsaeformis* can quickly achieve high densities and large local subpopulation (deme) sizes, it is a relatively short-lived species (typically <10 years), and prone to population declines and local extirpation (see Chapter 2). In comparison, *E. brevidens* and *L. fasciola* are longer-lived and characterized by smaller but seemingly more stable populations.

It is against the background of these contrasting historical and contemporary forces that patterns of genetic diversity were examined for *E. brevidens*, *E. capsaeformis*, and *L. fasciola* in the Clinch River. The main questions of interest in this study were to examine how presumed dispersal of fish hosts influenced genetic structure of these mussel species and how mussel populations might have responded to the end of the last glacial cycle. Two specific hypotheses were tested. First, mussel species of *high*-dispersal capability should migrate readily among local demes and exhibit minimal population structure. Such species should colonize available habitat quickly, expand their range during an inter-glacial, and display a concordant demographic expansion signature in their DNA polymorphism. Second, *low*-dispersal mussel species should migrate less frequently among local demes and exhibit pronounced population genetic structure. Such species should colonize habitat slowly, not easily expand their range during an inter-glacial, and exhibit a pattern of DNA polymorphism indicative of stability or decline. To test these hypotheses, mitochondrial DNA sequences were analyzed for signatures of long-term population trends (i.e., expansion, stability or decline), nuclear DNA microsatellites were analyzed for population genetic structure and gene flow (*Nm*), and

both marker types were used to provide estimates of long-term effective population size (N_e). Such data can provide insights into historical demography and population structure of mussel species. The inferences gained might also help to predict responses to climate change and therefore aid in conservation planning.

MATERIALS AND METHODS

Tissue sampling, DNA extraction and PCR amplification

Samples of fresh mantle tissue from individuals of *E. brevidens*, *E. capsaeformis* and *L. fasciola* were collected in 2004 from the following three locations in the Clinch River, Hancock County, TN (RKM=river kilometer): Wallen Bend (WB) (RKM 309.6), Frost Ford (FF) (RKM 291.7), and Swan Island (SI) (RKM 277.2) (Table 1). These sites were selected because they represent the furthest upstream, an equidistant midpoint, and downstream locations in the river where adequate sample sizes could be obtained for all three species. A small piece of mantle tissue (20-30 mg) was collected non-lethally from ~20-30 live mussels from each species/site (Naimo et al., 1998). Tissues were preserved in 95% ethanol and stored at -20°C prior to DNA extraction. Total genomic DNA was isolated from ~20 mg of frozen mantle tissue using the Purgene DNA extraction kit (Gentra Systems, Inc., Minneapolis, MN). DNA concentration was determined by flourescence assay (Hoefer TKO 1000 Flourometer, Hoefer Scientific Instruments, San Francisco, CA), and its quality visually inspected in a 0.8% agarose gel.

Sequences of the two regions of mitochondrial DNA (mtDNA) were amplified by polymerase chain reaction (PCR) using primers and conditions reported in Serb et al. (2003) for the first subunit of NADH dehydrogenase (*ND1*), and Merritt et al. (1998) for *cytochrome-b*. Primer sequences for *ND1* were: forward: 5'-TGGCAGAAAAGTGCATC AGATTAAAGC-3'; and reverse: 5'-CCTGCTTGGAAGGCAAGTGTACT-3'. The PCR reaction mixture for *ND1* consisted of 100 ng of genomic DNA, 1x PCR buffer, 4.0 mM $MgCl_2$, 0.4 mM dNTPs, 1.0 μM each primer, and 1.5 U Ampli*Taq* Gold DNA polymerase, with ddH_2O added to a total volume of 25 μL. The thermal cycling profile consisted of an initial 95°C for 8 min; followed by 35 cycles of: 94°C for 40 sec, 50°C for 60 sec and 72°C for 90 sec; with a final extension step at 72°C for 2 min; and a final hold at 4°C. Primer sequences for *cytochrome-b* were: forward: 5'- AAGAAGTATCA TTGCGGTTG -3'; and reverse: 5'- TGTGGGGCGACTGGTATTACTAA -3'. The PCR amplification conditions for *cytochrome-b* consisted of 25 ng of genomic DNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μM each primer, and 1.5 U Ampli*Taq* Gold DNA polymerase in a total volume of 20 μL. The thermal PCR cycling profile was: 94 °C for 2 min; followed by 40 cycles of: 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min; a final extension at 72 °C for 6 min; and a final hold at 4 °C.

All DNA sequence PCR products were sequenced with a Big Dye Terminator Cycle Sequencing kit with Ampli*Taq* DNA polymerase (Applied Biosystems, Foster City, CA). Cycle sequence reactions were purified using a Qiagen DNA Purification Kit (Qiagen, Carlsbad, CA), and subjected to electrophoresis and sequencing using an Applied Biosystems (ABI) 3100 automated sequencer.

Microsatellite loci and primers were developed and characterized using DNA of *E. capsaeformis* (Jones et al., 2004) and *L. abrupta* (Eackles and King 2002) and were screened in all sampled individuals using similar marker sets (Table 1). PCR amplification protocols followed Eackles and King (2002) and consisted of 100 ng of genomic DNA, 1x PCR buffer, 2 mM $MgCl_2$, 0.25 mM dNTPs, 0.5 μM each primer, and 1.0 U Ampli*Taq* DNA polymerase (Perkin-Elmer Applied Biosystems (ABI), Perkin-Elmer Corp.; Foster City, CA) in a total volume of 20 μL. PCR thermal cycling conditions were: 94 °C for 2 min; followed by 35 cycles of 94 °C for 40 sec, 58 °C for 40 sec, and 72 °C for 1 min; followed by a final extension at 72 °C for 1 min; and a hold at 4 °C (Eackles and King 2002). All amplification reactions were conducted separately for each locus; however, many PCR products were pooled for multiplex fragment analysis. Fragment lengths were resolved on an ABI3100 automated sequencer and results stored as GENESCAN files; GENEMAPPER software (ABI) was used to score the results.

Assessment of molecular genetic diversity

DNA sequences were edited and aligned using the program SEQUENCHER (version 3.0, Gene Codes Corporation). The mtDNA sequences of the *cytochrome-b* and *ND1* regions were combined for all analyses in a total evidence approach (Kluge, 1989), enhancing estimation of genetic diversity parameters and of phylogenetic relationships. Standard measures of intraspecific mtDNA genetic diversity were assessed for each species, both for their respective localized demes and for their global population (data from all demes were combined) (see Nei and Kumar 2000 for equations), including: (1)

haplotype diversity (*h*), i.e., the probability that two randomly chosen mtDNA sequences are different in a sample; (2) number of polymorphic or segregating sites (*s*); (3) mean number of nucleotide differences among sequences (*k*); and (4) nucleotide diversity (π), i.e., the probability that two randomly chosen homologous nucleotides are different in the sample. Haplotype clades were defined by the presence of a presumably diagnostic nucleotide(s). Parameter estimates and associated variances were calculated using DNAsp 4.0 software (Rozas et al. 2003).

Genetic variation across 9-10 DNA microsatellite loci for each species and respective demes was quantified in terms of observed heterozygosity, average expected heterozygosity, allelic diversity (mean number of alleles per locus), and number of private alleles; i.e., those occurring at only one of the investigated site locations. Population genetic statistics were calculated using FSTAT v. 2.9.3.2 (Goudet 1995). Demes of each species were screened for linkage disequilibrium (LD) between all pairs of loci and for deviations from Hardy-Weinberg equilibrium (HWE) at each locus using the program ARLEQUIN, ver. 3.0 (Excoffier et al. 2005). Significance of LD pairwise tests was determined using the likelihood-ratio test (Slatkin and Excoffier 1996), and HWE using the exact test with a Markov chain (Guo and Thompson 1992). The *p*-values for pairwise LD values were Bonferroni-corrected for multiple tests (Rice 1989). Microsatellite data sets were tested for genotyping errors caused by null alleles, stuttering and short allele dominance using a Monte Carlo simulation of expected allele size differences using MICROCHECKER (Van Oosterhout et al. 2004).

DNA mutation rates

Estimates of long-term N_e and divergence times, requires an estimate of the nucleotide mutation rate (μ). The mutation rate of $\mu=1.0 \times 10^{-7}$ nucleotide changes per site^{-1} year^{-1} was applied to the mtDNA analyses conducted in this study, and was obtained from exponential decay rate curves for protein-coding genes of avian and primate taxa (Ho et al. 2005). For freshwater mussels, rates of *cytochrome-b* and *ND1* are higher when compared to other genes (e.g., *16S* and *COI*) (Lydeard et al. 1996; Roe et al. 2001; Jones et al. 2006), so the above rate was selected from near the upper-end of the curves. This rate is an order of magnitude faster than the whole mtDNA genome substitution rate of 2% per million y ($\mu=2.0 \times 10^{-8}$) first estimated from primates (Brown et al. 1979). However, estimates of short-term (<1-2 Myr) mutation rates have been shown to be demonstrably higher (10X or greater) when taken from analyses of pedigrees or intra-specific variation (Parsons et al. 1997; Lambert et al. 2002; Ho et al. 2005), compared to rates inferred from phylogenetic (species-level) studies.

Mutation rates of DNA microsatellites are not as well understood, but are considered to be much higher than those for mtDNA sequences (Goldstein and Schlotterer 1999). No direct estimates are available for these markers in freshwater mussels, so a generally accepted mutation rate of 1×10^{-4} y^{-1} was used for the microsatellites analyzed in this study, established from the mean rate observed among organisms in other studies (Lai and Sun 2003). This mutation rate was used by Kelly and Rhymer (2005) in their study of the tidewater mucket *L. cariosa*, thereby providing a direct comparison between this and other mussel studies.

Phylogenetic analysis and divergence times

A linearized neighbor-joining (NJ) tree was constructed using MEGA 3.1 to determine genealogical relationships and divergence levels of mtDNA haplotypes of *E. brevidens*, *E. capsaeformis*, and *L. fasciola* (Takezaki et al. 1995; Kumar et al. 2004) The model of sequence evolution used for the NJ analyses was the Kimura 2-parameter model (K2P) (Kimura 1980), determined by the program MODELTEST 3.6 (Posada and Crandall 1998). Bootstrap analysis (1000 replicates) was conducted to assess support for the individual nodes of the phylogenetic tree (Felsenstein 1985). Statistical parsimony networks of haplotypes were constructed using a 95% connection limit in TCS software version 1.21 (Clement et al. 2000).

Mean divergence times or time to most recent common ancestor (t_{MRCA}) of mtDNA haplotypes of each species, including each clade, were estimated using Bayesian analysis in the program BEAST (Drummond and Rambaut 2003). The rate of nucleotide substitution was allowed to vary among tree branches using a relaxed molecular clock model, with variants drawn from an uncorrelated lognormal distribution. Nucleotide substitutions were modeled using a General Time Reversible model and site heterogeneity using the Gamma + Invariant Sites option. Codon positions were partitioned in the model using a (1+2) +3 approach, where both the substitution model and among-site rate heterogeneity model were unlinked across codon positions. Coalescent tree priors were used to model population growth of the mtDNA sequence data sets; *constant size* for both *Epioblasma* species and *expansion growth* for *L. fasciola*.

The program was initiated with a burn-in of 100,000 cycles, with parameter values sampled every 1000 steps from 10,000,000 Markov chain Monte Carlo (MCMC) steps. Chain convergence to a stable distribution was determined by visual inspection of posterior-probability plots in the sub-program Tracer available in BEAST.

Historical demographic analyses

Using mtDNA sequence data, patterns of historical population expansion, decline or stability were tested employing three independent analytical approaches: exponential population growth rate (g) (Kuhner et al. 1998), F_S test of selection (Fu 1997), and mismatch distributions (Rogers and Harpending 1992).

The population growth parameter g was estimated using the software LAMARC (http://evolution.gs.washington.edu/lamarc/index.html), which uses a maximum likelihood (ML) coalescent-based approach with a Metropolis-Hastings Markov chain Monte Carlo (MCMC) algorithm to sample simulated genealogies and to estimate parameters. Watterson's θ and a g value of 1 were used to initiate analyses. The initial search strategy was set at 10 short chains, sampling every 20 genealogies for 20,000 steps, while the final search strategy was set at 10 long chains, sampling every 20 genealogies for 400,000 steps. Heating was set at high, with four temperatures (1.0, 1.1, 1.2 and 1.3) to ensure a wide search of ML tree space. The program was run several times to ensure consistent estimation of g. Positive values of g signify historical population growth or expansion, whereas negative values indicate population decline. The program provides approximate

95% confidence intervals (CI) for *g* values, which were considered positive or negative, respectively, if zero was not contained within the CI's.

The F_S statistic is a non-coalescent based method that tests for an excess of 'young', or minimally diverged haplotypes compared to expected patterns of DNA polymorphism under a model of neutrality (Fu 1997). It is intended as a test of selection (e.g., selective sweeps), but can be used to test for demographic processes leading to similar patterns. Large negative F_S values indicate population expansion; values and their significance (α=0.05) were calculated using 1000 permutations in ARLEQUIN.

Simulation studies have demonstrated that mismatch distributions (observed frequency distributions of pairwise differences between sequences) exhibit multimodal or ragged distributions for non-expanding, demographically stationary populations, and smoother or unimodal distributions for expanding populations (Slatkin and Hudson 1991; Rogers and Harpending 1992). As implemented in ARLEQUIN, a model of population expansion was tested using a nonlinear least squares approach to estimate model parameters: $\theta_0=2\mu N_0$ (θ before expansion), $\theta_1=2\mu N_1$ (θ after expansion) (Rogers and Harpending 1992). Significance of test results was determined using a goodness-of-fit test of observed versus expected mismatch distribution under a model of expansion.

Effective population size

Long-term effective population sizes (N_e) were assessed among species using several estimates of genetic diversity (θ), calculated from both mtDNA sequences and nuclear DNA microsatellites. Long-term N_e is an estimate of the equilibrium or average

N_e over a lengthy period of time (1000s of generations), and is determined by the cumulative forces of mutation and genetic drift over many generations (Hedrick 2005). For maternally inherited mtDNA, the female effective population size is $\theta=2N_{e(f)}\mu$, and for nuclear DNA, effective population size is $\theta=4N_e\mu$ (Kimura and Crow 1964), where μ is the nucleotide substitution rate per generation (see below for derivation of μ per generation). Current and historical trends in long-term N_e were measured independently using both marker types and different methods to estimate θ. Current mtDNA genetic diversity ($\theta_{current}$) was taken from nucleotide diversity (π) (Nei and Kumar, 2000), and historical genetic diversity ($\theta_{historical}$) was estimated for both marker types using the previously described ML-based coalescent approach in LAMARC.

Mean age at first reproduction was used as the generation time (T_g), and is 5 y for *E. brevidens* and *E. capsaeformis*, and 4 y for *L. fasciola* (Jones and Neves 2008). The above per year mutation rates were converted to a per generation rate by dividing by T_g. Female effective population size $N_{e(f)}$ was converted to total N_e by multiplying by two, assuming an equal sex ratio for each species.

Analysis of population genetic structure and gene flow

Population genetic structure – within-deme versus among-deme genetic diversity, genetic differentiation (F_{ST}), gene flow (Nm), and migration rates (m) – for each species was assessed among demes using nuclear DNA microsatellites and a suite of traditional and ML-based analyses. The relative contributions of within and among-deme genetic diversity to total genetic diversity were estimated using analysis of molecular variance

(AMOVA) (Excoffier et al. 1992; Michalakis & Excoffier 1996), and differentiation between deme pairs was quantified using Weir and Cockerham's (1984) multilocus analogue of the F_{ST} test statistic (Wright 1931), which assumes an infinite allele model. Values for F_{ST} range from 0 (no differentiation) to 1 (complete differentiation); values <0.05 reflect low levels of genetic differentiation, values from 0.05-0.15 reflect moderate to high levels, and values >0.15 reflect very high levels (Wright 1978; Balloux & Lugon-Moulin 2002). Both AMOVA and F_{ST} statistics were calculated using ARLEQUIN software, as well as a non-parametric permutation method (10,000 permutations) to test whether obtained values were significantly different from zero. Indirect measures of gene flow (*Nm*=effective number of migrants per generation) were obtained by following Wright's (1931) approximation of $Nm \approx 0.25(1/F_{ST} - 1)$. This method assumes that population allele frequencies are in equilibrium with respect to genetic drift, migration and equal population sizes, and thus reflects longer-term gene flow over many generations.

Assignment tests (AT) were used to determine recent population structure; i.e., the last 1-3 generations. The AT utilizes data from DNA microsatellites to estimate the probability that an individual's multi-locus genotype originated from a particular investigated population (Paetkau et al. 1995, Rannala and Mountain 1997). The AT was implemented using Geneclass2 (Piry et al. 2004) as described by Rannala and Mountain (1997), which estimates population posterior probabilities of allele frequencies given the observed allele frequency data. Population posterior probabilities then are used to estimate the probability that an individual belongs to a respective population. A Chi-

square (χ^2) test was used to test whether level of population assignment was greater than could be expected by chance.

Direction and amount of *Nm* between demes were estimated using LAMARC, employing the ML coalescent-based approach and MCMC algorithm to estimate parameters. This approach allows for more biologically realistic scenarios of population structure, such as non-symmetrical migration and unequal population sizes. The search strategy was set using default settings with heating set to high. LAMARC provides a likelihood-based estimate of the immigration parameter *M*, expressed as $M=m/u$, where *m* is the directional immigration rate per generation and *u* is the neutral mutation rate per allele per generation, which was 0.0005 for *E. brevidens* and *E. capsaeformis*, and 0.0004 for *L. fasciola*. In other words, *M* is the immigration rate from donor population to recipient population, where migration rate is scaled to the mutation rate by using $m = M\mu$. To express migration in terms of 4*Nm*, the parameter *M* was multiplied by the recipient population's value of θ. The resulting value was divided by four to compare to the traditional F_{ST}-based approach for estimating *Nm*.

RESULTS

Genetic variation of mitochondrial DNA sequences

Comparison of mitochondrial DNA sequences among investigated species revealed that intraspecific genetic variation was least in *E. brevidens*, intermediate in *E. capsaeformis*, and greatest in *L. fasciola*. Analysis of combined DNA sequences for the

cytochrome-b and *ND1* regions identified 6 haplotypes in *E. brevidens*, 17 haplotypes in *E. capsaeformis*, and 33 haplotypes in *L. fasciola* (Table 2). Segregating sites(*s*) were least abundant in *E. brevidens* (*n*=9), moderately abundant in *E. capsaeformis* (*n*=17), and most numerous in *L. fasciola* (*n*=42) (Table 3). Nucleotide site variation was highest in the aligned site matrix of each species at *cytochrome-b* (1.4-4.2%), followed by *ND1* (0.5-3.3%), corroborating the finding of Jones et al. (2006b); namely, that *cytochrome-b* is the more variable gene region among species of *Epioblasma*. Specific patterns of intraspecific mtDNA variation were observed for each species.

Observed haplotype diversity was moderate to high in *E. brevidens* (global *h*=0.62), with reduced levels observed at SI, where *h*=0.48 (Table 3). Nearly all haplotypes were shared among demes. Haplotype *Ebrev01* was most frequent and comprised >70% of the sample, but a divergent and less frequent lineage (clade 2) comprised of two closely-related haplotypes was observed, with each characterized by 5-6 seemingly fixed nucleotides (Table 2). Global nucleotide diversity was low at π=0.00177, with a mean *k* of 2.2 nucleotide differences among sequences. Overall, nucleotide variation was moderate, occurred at similar levels among demes, and likely adequately assessed for the species in the Clinch River.

In comparison, observed haplotype diversity was higher in *E. capsaeformis* (global *h*=0.70), with levels ranging from *h*=0.65 (WB) to *h*=0.81 (FF). Many haplotypes were shared among demes; however, each deme contained several unique haplotypes. Haplotypes *Ecap01* and *Ecap05* were most frequent and together comprised >76% of the sample. The remaining haplotypes occurred infrequently and were characterized by singletons or other uncommon polymorphisms. Three distinct clades were observed

among haplotypes, with clade 3 being most divergent. Clades were characterized by 2-4 fixed nucleotides. Globally, nucleotide diversity was low at π=0.00208, with a mean *k* of 2.6 nucleotide differences among sequences. Observed nucleotide site variation was not evenly partitioned among sampled demes, with the SI deme containing greater variation than demes sampled upstream. Total sample size was large (*N*=90) and likely captured most of the nucleotide variation occurring at the investigated gene regions for this species in the river. Unequal partitioning of genetic variation among sites underscores the need for adequate among-deme and within-deme sampling to fully characterize variation.

Haplotype diversity was highest in *L. fasciola* (global *h*=0.95), with high levels observed in all three demes. Of the 65 samples analyzed, slightly more than 50% of haplotypes were unique, suggesting that a substantial amount of genetic variation in the population may have remained unsampled. Thus, sample size likely was inadequate to capture the "true" level of genetic variation contained in *L. fasciola* in the Clinch River. Many haplotypes were shared among demes; however, each deme contained numerous unique haplotypes. Haplotype *Lfasc01* was most frequent, but only comprised 17% of the sample. The remaining haplotypes occurred infrequently and were characterized primarily by singletons and other uncommon polymorphisms. Three distinct clades were observed among haplotypes; however, clades 1 and 2 were closely related and separated by only one nucleotide. Haplotypes belonging to clade 3 were most divergent, but were rare to uncommon in the total sample. Global nucleotide diversity was still moderately low at π=0.00306, with a mean *k* of 3.6 nucleotide differences among sequences, but higher than variation observed in *E. brevidens* and *E. capsaeformis*.

Genetic variation of DNA microsatellites

Genetic variation at microsatellite loci exhibited a similar pattern to that observed for mtDNA; i.e., genetic diversity generally was lowest in *E. brevidens*, intermediate in *E. capsaeformis*, and highest in *L. fasciola* (Table 4). However, levels of genetic variation between *E. capsaeformis*, and *L. fasciola* were comparable, with both species exhibiting high expected heterozygosity (H_e) and allelic diversity (see Appendix). This result may reflect regeneration of allelic variation by *E. capsaeformis* following past population fluctuations. Because DNA microsatellites are known to have high mutation rates [typically 10^{-3} to 10^{-4} in many taxa, Goldstein and Schlotterer (1999)], it is conceivable that the genetic history of these markers for *E. capsaeformis* has been obscured by regeneration of allelic diversity. For example, Mank and Avise (2003) expressed concern that high mutation rates and subsequent homoplasy of microsatellites were obscuring genetic differences between species of North Atlantic eels (Anguillidae).

Demes of *E. brevidens* contained no pairs of alleles with significant deviations from LE. Significant deviations (p<0.001; α=0.05) from HWE were observed at *Ecap01*, *Ecap07*, and *Lab206* at WB, *Ecap01* at FF, and *Ecap01* and *Ecap09* at SI. For all three species, HWE disequilibria were from either heterozygote excesses or deficiencies and distributed randomly at a small number of loci and demes. Further, no evidence was found for genotyping errors or large-allele drop-out, although increased homozygosity at some loci suggested the possible presence of null alleles, or inbreeding due to small population size and hermaphroditic reproduction (van der Schalie 1970). Global-observed heterozygosity (H_0=0.68) and expected heterozygosity (H_e=0.68) were high, with average

allelic diversity at 10.5/locus, and similar levels observed among demes (Table 4). Each deme contained a small number of alleles not detected at the other sampled sites, or so-called private alleles, with 2-7 observed/site. Global θ was 0.559 and slightly lower as measured per site.

Demes of *E. capsaeformis* contained no pairs of alleles with significant deviations from LE. Significant deviations (p<0.01; α=0.05) from HWE, exhibiting either heterozygote excess or deficiency, were observed at *Ecap06* and *Ecap09* at WB, *Ecap01* and *Ecap02* at FF, and *Ecap01* at SI. Global-observed heterozygosity (H_0=0.81) and expected heterozygosity (H_e=0.85) were very high, with average allelic diversity at 15.2/locus. Similar levels of gene diversity were observed among demes; however, each contained 5-13 private alleles. Global θ was 5.37 and lower when measured per site.

Following the Bonferroni correction, demes of *L. fasciola* contained one allele pair (*Ecap02*, *Lab206*) at FF that exhibited significant deviation from LE. Significant deviations (p<0.01; α=0.05) from HWE, exhibiting either heterozygote excess or deficiency, were observed at *Ecap04, Ecap06*, *Ecap07*, and *Lab211* at WB, *Ecap02*, *Ecap04*, *Ecap06* and *Ecap07* at FF, and *Ecap02*, *Ecap06*, *Ecap07* at SI. Global observed heterozygosity (H_0=0.71) and expected heterozygosity (H_e=0.84) were very high, with average allelic diversity at 17.7/locus. Similar levels of gene diversity were observed among demes, with each site containing 12-15 private alleles. Global θ was 17.98, the highest observed among the three investigated species, and again lower per site.

Phylogenetic analyses and diversification times

The main result of the phylogenetic analyses was that each mussel species exhibited branching patterns indicative of different long-term population trajectories (Figures 2-3). The numerous haplotypes (N=33) resolved for *L. fasciola* were characterized by short branch lengths, minimal divergence at 1-4 nucleotides, and weak bootstrap support for presumed clades (Table 1). In phylogenetic appraisals, numerous closely related haplotypes, shallow phylogenetic tree structure, "star-like" parsimony network, and unimodal "wave-like" mismatch distribution are indicators of population expansion (Slatkin and Hudson 1991; Rogers and Harpending 1992). A much smaller number of haplotypes (N=6) were resolved for *E. brevidens*, but characterized by longer branch lengths and stronger bootstrap support for the clades. A reduced number of haplotypes coupled with divergent lineages is an indicator of long-term population decline, secondary contact, and perhaps past bottlenecks. An intermediate number of haplotypes (N=17) was resolved for *E. capsaeformis*, which were characterized by a branching pattern similar to that of *E. brevidens*. However, clades and associated haplotypes displayed a more complex tree topology, exhibiting weak to moderate divergence at 2-6 fixed nucleotides, perhaps indicating long-term population stability. For all three species, divergence times (t_{MRCA}) originated in the late Pleistocene and Holocene for clades comprised of closely related haplotypes, and to the late Pleistocene for more distantly related haplotypes (Figure 2).

Statistical parsimony analysis of mtDNA haplotypes produced networks ranging from simple (i.e., *E. brevidens*) to more complex (i.e., *L. fasciola*) (Figure 3). The

number of inferred mutational steps connecting haplotypes to the ancestral haplotype ranged from 1-6 in *E. brevidens* and 1-10 in *E. capsaeformis*. In contrast, *L. fasciola* contained numerous, closely related haplotypes just 1-2 steps from the ancestral haplotype. A few haplotypes, such as those belonging to clade 3, were characterized by longer pathways of 7-8 steps. The pathway and high number of steps to *Lfasc24* may be erroneous and due to homoplasy; hence, alternative pathways are shown (Figure 3).

Historical demographic trends

Historical demographic analyses corroborated the results of the phylogeny; namely, that the pattern of mtDNA genetic variation for *L. fasciola* was consistent with population *expansion*, that of *E. capsaeformis* with population *stability*, and that of *E. brevidens* with population *decline*. First, for *L. fasciola*, the population growth parameter (g=1987) was very high, the 95% CI's did not contain zero, and the Fu's F_S test statistic was negative (-26.17) and highly significant (Table 5). Second, the mismatch distribution for this species was very wave-like and unimodal, and the exponential expansion model indicated a rapid increase in population size from θ_0=1.44 to θ_{final}=105.12 (Figure 4). Taken together, these results support the hypothesis of significant demographic expansion of *L. fasciola* in the Clinch River. Results for *E. capsaeformis* were more ambiguous. The population growth parameter (g=575) was positive and hence indicated growth, but the 95% CI's contained zero; thus, less certainty existed regarding the direction of population growth. Similarly, the Fu's F_S test statistic was negative (-4.82) but not significant. The mismatch distribution for this species was ragged and

multimodal, and the exponential expansion model indicated only a slight increase in population size from θ_0=2.51 to θ_{final}=2.76, suggesting demographic stability in *E. capsaeformis*. The population growth parameter (g=-540) for *E. brevidens* was negative and indicated decline; however, the data should be interpreted cautiously since the 95% CI's contained zero. The Fu's F_S test statistic was positive (2.32) and hence did not indicate population expansion. The mismatch distribution for this species was bimodal, and the exponential expansion model indicated only a slight increase in population size from θ_0=0 to θ_{final}=1.75, again suggesting a pattern of demographic decline and perhaps secondary contact in *E. brevidens*.

Long-term effective population sizes

Long-term effective population sizes derived from global θ values demonstrated that N_e was largest in *L. fasciola*, intermediate in *E. capsaeformis*, and smallest in *E. brevidens* (Table 6). These globally derived N_e values assumed that migration and gene-flow have occurred among demes over multi-generational time-scales, hence justifying combining data from all three sites. For *L. fasciola*, historical N_e was much larger than current N_e. The high mtDNA variation observed for this species is under-represented by the estimator θ_π, whereas the ML-based estimator of θ from both marker types is a better reflection of this species' genetic diversity. As reported above, ~50% of sampled mtDNA haplotypes were unique, suggesting that a high level of genetic variation remains un-sampled. The pattern of nucleotide polymorphism displayed by *L. fasciola* signifies that high levels were maintained over long time-periods; i.e., perhaps thousands of

generations. It is known that ML-based estimators of θ utilize more information in DNA and are better at assessing unsampled and historical variation (Felsenstein 1992). Historical N_e for *E. capsaeformis* was more than twice as high as current N_e, but error due to under-sampling is unlikely to be a factor contributing to the discrepancy. It appears that historical variation in population size for this species was higher than what currently exists. In comparison, historical N_e for *E. brevidens* was slightly less than current N_e.

Estimates of global N_e derived from DNA microsatellites typically were lower than those derived from mtDNA sequences, with one exception; current N_e from mtDNA was the lowest for *L. fasciola* (Table 6). As expected, site-specific estimates of N_e from DNA microsatellites were lower than global estimates, but again highest for *L. fasciola* (Figure 5). In most instances, estimates of N_e from mtDNA and DNA microsatellites were of similar magnitude for each species.

Population genetic structure among demes

The AMOVA analysis showed small but significant genetic structuring among sampled demes of *E. capsaeformis*, with 1% of total variation due to differences among demes (Table 7). Although variation was minimal, it was highly significant ($p<0.001$) and due primarily to variation at three loci, *Ecap01*, *Ecap04*, and *Lab213*. Based on F_{ST}, significant differentiation was detected between the demes at WB and FF ($p=0.0039$), and between WB and SI ($p<0.0001$) (Figure 5). In contrast, populations of *E. brevidens* and *L. fasciola* did not exhibit significant genetic structuring. For these two species, AMOVA revealed that only 0.5% and 0.3% of total variation respectively, was due to differences

among demes, and none of the pairwise F_{ST} comparisons were significant. However, even though the level of genetic differentiation in *E. brevidens* supported a null hypothesis of panmixia among demes, the AMOVA and F_{ST} values for this species were greater than those observed for *L. fasciola*. These results should be viewed in the context of the spatial scale of this study, as only about 15-30 river kilometers separated the demes. Demes separated by greater distances in the river likely would exhibit greater differentiation.

Gene flow and migration rates between demes

Indirect estimates of gene flow based on Wright's F_{ST} resulted in pairwise estimates of *Nm* ranging from 16 to 62 effective migrants per generation among demes of *E. capsaeformis* (Figure 5); when compared to *E. brevidens* and *L. fasciola*, less overall gene flow was evident for this species. For example, the level of gene flow between WB and FF, and between WB and SI, was much less than that observed for the other two species. In addition, for the two sites separated by the greatest distance (~32 RKM), WB and SI, gene flow was reduced for all three species compared to that measured among most of the other demes. Generally, pairwise *Nm* values were moderate to high among demes of all three species, based on traditional analysis of *Nm*. Several pairwise comparisons for *E. brevidens* (e.g., WB to FF) and *L. fasciola* (e.g., WB to FF and FF to SI) essentially indicated panmixia or "infinite" gene flow among demes.

The ML-based estimates of directional gene flow and respective 95% CI's resulted in pairwise estimates of *Nm* ranging from ~2-3 effective immigrants per

generation among demes of *E. capsaeformis* and *E. brevidens*, whereas *Nm* values for *L. fasciola* were more than twice as high. Elevated *Nm* values for *L. fasciola* were due to higher N_e, not an increase in migration rates (*m*), which were ~1% for all three species. However, *Nm* values for all three species were much less than values obtained from the traditional F_{ST}-based method. For example, traditional *Nm* values among demes for *E. brevidens* ranged between 31 and "infinity", whereas ML-based estimates were much lower, ranging between, 1.88-3.31. All pairwise, ML-based directional estimates of *Nm* were relatively symmetrical, meaning the 95% CI's around both respective *Nm* values overlapped between demes.

Assignment tests correctly assigned individuals of *E. capsaeformis* to their original deme with greater frequency than would be expected by chance ($p<0.05$), but not individuals of *E. brevidens* and *L. fasciola*, also suggesting higher levels of migration and gene flow from neighboring demes for the latter two species (Figure 6).

DISCUSSION

Historical demography, dispersal and vicariance

The historical demographic analyses presented in this study infer the long-term population decline, stability, and expansion of *E. brevidens*, *E. capsaeformis*, and *L. fasciola* respectively, in the Clinch River. Concordantly, Peacock et al. (2005) used data from prehistoric Native American shell middens to show that species of *Epioblasma* have declined in NA over the past 5000 years, presumably due to increases in human impacts,

possibly from human population expansion following the advent of maize agriculture or long-term ecological or climatic changes unrelated to human activities. These patterns lead one to speculate on mechanisms influencing distribution and abundance of mussel species within and outside of the Cumberlandian Region. The region is considered the center of origin or glacial refugium for many species, especially those belonging to the genus *Epioblasma* (Johnson 1978). Thus, the question can be re-cast as a dichotomy – are such demographic controls inherently intrinsic (i.e., dispersal or other aspects of species biology) or extrinsic (i.e., vicariance) in nature? Further, could fish host usage be predictive of the pattern of genetic polymorphism?

Ichthyologists consider both dispersal and vicariance to be important factors explaining the distribution of interior highland fishes in NA (Mayden 1988). Since mussels rely on fish to host their glochidia, these hypotheses logically apply to mussels as well. However, which factor plays the greater role in controlling distribution probably depends on the particular mussel species and fish host utilized. Recent genetic studies of intraspecific variation of mussels suggest that species utilizing hosts of abundance and high dispersal ability display correspondingly high levels of genetic variation and little population structure over large distances in streams and rivers. Both Berg et al. (1998) and Elderkin et al. (2007) found minimal genetic structure for *Quadrula quadrula* and *Amblema plicata*, respectively, over large geographic distances (>1000 km). These two mussel species are widely distributed throughout the Mississippi River basin and utilize highly mobile host fishes, such as catfishes and basses. Further, these mussel species contain genetic variation indicative of a population expansion. The parsimony network of mtDNA haplotypes of *A. plicata* presented by Elderkin et al. (2007) shows numerous

(*N*=36) closely-related haplotypes in a "star-like" configuration, suggesting that expansion has occurred for the species. In contrast, other recent studies have reported significant population genetic structure for species utilizing hosts with low dispersal (e.g., darters, sculpins, and minnows). For example, analysis of DNA microsatellites by Zanatta and Murphy (2007) found significant genetic structure for demes of *Epioblasma torulosa rangiana* in the Allegheny River separated by only 15 km; this congener uses small darters as hosts. Grobler et al. (2006) reported significant genetic structure among populations of *Lexingtonia dolabelloides*, a species that uses small minnows as hosts, in the Duck River in central Tennessee, compared to those in the headwaters of the Tennessee River system in Virginia (i.e., the Clinch and Holston rivers). These studies and mine have shown that while some mussel species have experienced population expansion, others have not. Thus, dispersal likely is the main factor controlling the distribution of mussel species with high dispersal ability. The widespread and ubiquitous distribution of such mussel species throughout the Mississippi River basin strongly supports dispersal as the driving mechanism. Historically, the basin was an open, interconnected network of rivers with few barriers to dispersal for many aquatic species.

Vicariant events such as stream capture and water-level fluctuations of inland and coastal waterways certainly have played a role in shaping distribution of mussel species, especially those with low dispersal ability. The distribution of species belonging to the genus *Epioblasma* provides an excellent example. These species are considered the most endangered group of mussels in NA, with more than half of the species now extinct (Johnson 1978). Of the 17 recognized species, approximately half (8 spp.) were distributed both inside and outside of the Cumberlandian Region (Figure 1), including: *E.*

flexuosa, *E. florentina*, *E. obliquata*, *E. personata*, *E. propinqua*, *E. torulosa*, *E. triquetra*, and *E. turgidula* (Johnson 1978). Two species, *E. penita* and *E. othcaloogensis*, are restricted to the Mobile River basin, which drains directly into the Gulf of Mexico. The species *E. florentina curtisii*, *E. turgidula* and *E. triquetra* are the only members of the group distributed west of the Mississippi River, primarily in the Ozark highlands of Arkansas and Missouri, with the last species being the most widely distributed member of the genus, occurring in the upper Mississippi, Ohio and Great Lakes (i.e., lakes Erie and Michigan) drainage basins. The species *E. obliquata*, *E. torulosa*, *E. flexuosa*, *E. propinqua* and *E. personata* are primarily found in the Ohio River basin, but the first two also are known from the lower Great Lakes.

Dispersal during the early Holocene (~10,000 ya - present) provides the best explanation for how *E. obliquata*, *E. torulosa* and *E. triquetra* and other species expanded northward and occupied the recently glaciated Great Lake basins (Graf 1997, 2002). As the glaciers retreated in NA, the melt-water created outlets draining directly into the interior basin. These historical drainage outlets, such as the Wabash-Maumee route that connected Lake Erie to the lower Ohio River, allowed mussels and their fish hosts to colonize the Great Lakes (Ortmann 1924). Zanatta and Murphy (2007) reported reduced mtDNA polymorphism for *E. torulosa* inhabiting the Sydenham River (Lake Erie basin) compared to populations in the southern portion of its range (such as the Allegheny River, western Pennsylvania). Additionally, Burdick and White (2007), Elderkin et al. (2007), and Zanatta et al. (2007) reported less genetic variation for populations of *Fusconaia flava*, *A. plicata* and *L. fasciola*, respectively, inhabiting northern portions of their ranges, demonstrating a general pattern of reduced genetic

variation following post-Pleistocene colonization of glaciated regions. A gradient of low to high genetic variation for northern versus southern populations of NA fishes already has been established (Bernatchez and Wilson 1998).

How *E. florentina curtisii*, *E. triquetra* and *E. turgidula* invaded the western Mississippi River basin also is intriguing. Are these populations evidence of a once-widespread ancestral fauna fragmented over time by vicariant events, or another example of dispersal out of the Cumberlandian Region? Support for vicariance would be strengthened if divergent genomes existed in these western populations. However, the recent study by Zanatta and Murphy (2008) showed that while the population of *E. triquetra* occupying the St. Francis River, Missouri (MO), south of the Ozark Crest, does contain unique mtDNA haplotypes, divergence is very low (<0.5%) when compared to other populations. Further, a population north of the Ozark Crest in the Bourbeuse River, MO, lacks such genetic uniqueness (Zanatta and Murphy 2008). Finally, to explain how *E. penita* and *E. othcaloogensis* colonized the Mobile River basin, one must assume that their occurrence in the drainage is the result of a vicariant event, such as stream capture. These species are clearly analogues of two Cumberlandian endemic species, *E. brevidens* and *E. haysiana*, respectively (Johnson 1978). Because of these and other shared faunal elements between the two basins, and the strange north-westerly course taken by the lower Tennessee River, various stream capture scenarios have been hypothesized by authors for over 100 years (Mills and Kaye 2001).

Available genetic data lend more support to the dispersal hypothesis to explain distributional and genetic patterns of *Epioblasma* spp. and other species in the interior highlands, but, as ichthyologists similarly have concluded, vicariance is needed

occasionally to explain the distributional patterns of some species. The shallow levels of genetic divergence observed among many mussel populations suggest that such patterns of nucleotide polymorphism are of late Pleistocene and Holocene origin. Molecular clock calibrations based on earlier time periods, such as mid-to-early Pleistocene or earlier epochs, seem unlikely because calculated mutation rates would be very slow ($<1 \times 10^{-8}$) and hence require extremely high N_e, which is incongruent with other evidence, such as N_e derived from multiple nuclear microsatellite markers (Chapters 1 and 3), current population sizes of extant species (Chapter 2), and mussel life history strategies (e.g., Type III survivorship). The mtDNA mutation rates calibrated from some pedigree and intra-specific variation studies are high (Ho et al. 2005), which supports the hypothesis that many of the numerous singleton and infrequent polymorphisms documented in recent intra-specific mussel studies arose during the the late Pleistocene and Holocene.

Responses of high-dispersal vs. low-dispersal mussel species to glacial cycles

Stream networks provide environments conducive to repeated expansion and contraction of populations following ice age cycles. Presumably during glacial cycles, the range of some species contracted into separate refugia, thereby promoting genome divergence, and then expanded and mixed again during interglacials. Such dynamics likely have acted to promote genome reorganization and genetic polymorphism found within terrestrial species (Hewitt 1996). How aquatic species have responded to glacial cycles is less clear. Some authors have proposed that dispersal of freshwater fishes is enhanced during glacial periods because water level reductions create more continuous

shallow high-gradient stream conditions between disjunct geographical regions (Bermingham and Avise 1986; Mayden 1988; Near and Keck 2005). Big-river environments, such as the present-day Mississippi River, likely are major barriers to dispersal of fishes preferring shallow highland stream habitats, such as many species of darters and minnows, although these conditions likely promote dispersal of larger, more mobile fishes. Hence, interglacial cycles may act to ultimately isolate many mussel species relying on low dispersal hosts. In any case, multi-modal mismatch distributions can provide evidence for secondary contact. However, simulations conducted by Slatkin and Hudson (1991) showed that for populations maintaining constant population size, bimodal sequence distributions are common. The multi-modal ragged mismatch distributions observed in *E. brevidens* and *E. capsaeformis* alternatively can be explained by stable population size over time. A stable population will fluctuate in size over time, and as it does, genetic variation will be lost or reduced in frequency through genetic drift. During periods of population growth, haplotypes will increase randomly in frequency due to differential reproductive success of individuals. Such dynamics presumably lead to the uneven mismatch distributions seen in many natural populations. Discernment between the effects of stable population size and secondary contact may be difficult, but genomes of moderate divergence of 1-2% or more may be indicative of secondary contact.

Long-term effective population size and maintenance of genetic diversity

Perhaps the most surprising result of this study was the high level of genetic variation observed at both mtDNA and microsatellite DNA markers for *L. fasciola*,

variation which seemingly was contrary to the relatively small sizes of demes that currently reside in gravel shoals of the Clinch River. Long-term N_e of this species was incongruent with a recent (2004-2007) estimate of census size (N_c) of ~30,000 individuals inhabiting the investigated reach, RKM 277.2-309.6 (Chapter 2). However, local abundance of this species is similar throughout the 320-RKM section of the free-flowing upper river, indicating that total population size is about 300,000 individuals (Ahlstedt 1991). This high level of genetic diversity and large long-term N_e are consistent when viewed in context; that over millennia this species has expanded its range, grown in population size, and maintained connectivity among demes over a wide geographic area due to high dispersal of its fish hosts, *Micropterus* spp. Individuals in the river can live to >40 y and the population contains numerous overlapping generations, where abundance has remained stable and not fluctuated greatly over the last 30 y (Chapter 2; Ahlstedt 1991). Thus when acting together, long lifespan, reduced variance in reproductive success, and high migration among demes likely have facilitated maintenance of genetic diversity and high N_e of *L. fasciola*. Prior to impoundment of tributary rivers in the upper Tennessee River system during the early 20th century, the population of this species likely was comprised of individuals residing in the Clinch River, and also in adjacent rivers such as the Powell, Holston, French Broad, and others. A scenario of a large contiguous population of *L. fasciola* in the upper Tennessee River system is supported by studies of other species with high genetic variation and little genetic structure over large geographic distances which parasitize mobile fish hosts (Berg et al. 1998; Elderkin et al. 2007). Similarly, Zanatta et al. (2007) recorded low divergence ammong populations of *L. fasciola* inhabiting rivers draining the Great Lakes of southern Ontario.

In contrast, populations of *E. brevidens* and *E. capsaeformis* have maintained less genetic variation and lower N_e over time, perhaps due to less connectivity among demes, fluctuating population sizes, shorter lifespan and other aspects of their life histories. From the same river reach, demographic estimates of N_c for both species are ~30,000 and >500,000 individuals, respectively (Chapter 2). Abundance of these two species is either greatly reduced or zero in other sections of the upper river. Thus, the ratio of long-term effective size to current total census population size (N_e/N_c) for these two species was ~1-2% or less. For many wild animal populations, the ratio of N_e/N_c is on average approximately 10% (Frankham et al., 2002). The ratios reported here are lower than for many other species, but comparable to some marine bivalve mollusks (Hedgecock et al. 1992), and will change with fluctuations in census and effective population size over time. Low N_e relative to census size likely is the result of high variance in reproductive success, at least in part due to hermaphroditic reproduction (van der Schalie, 1970), low fertilization success between males and females, low infestation success of glochidia on host fish, and variable recruitment due to stochastic environmental processes. In addition, site-specific estimates of contemporary N_e and N_e/N_c for *E. brevidens* and *E. capsaeformis* based on a recently developed linkage disequilibrium method (see Chapter 3 for methods and results) were with one exception very similar to the site-specific long-term estimates reported in Figure 5. This method does not require an estimate of the mutation rate to calculate effective size. Hence, these results support the assertion that mutation rates used in this study for both DNA microsatellites and mtDNA were of the right magnitude. In this study however, N_e estimated from mtDNA was considerably higher than N_e estimated from nuclear DNA microsatellites. Possible explanations for this

discrepancy include: (1) the mtDNA mutation rate was too slow, (2) the nuclear DNA microsatellite mutation rate was too fast, and (3) the level of homoplasy of microsatelite loci was high and thus reduced estimates of genetic diversity (θ) (Estoup et al. 2002 and Li et al. 2002 provide reviews on microsatellite homoplasy and mutation).

Population structure and gene flow

Traditional measures of population genetic structure (e.g., AMOVA and F_{ST}) rejected the null hypothesis of panmixia for investigated demes of *E. capsaeformis*, but not for *E. brevidens* and *L. fasciola*. However, ML-based estimates of directional *Nm* clearly showed that gene flow was restricted among demes of *E. brevidens*. Available criteria suggest that $Nm \leq 5$ and $m < 0.1$ between populations or demes can lead to demographic independence (Waples and Gaggiotti 2006; Palsboll et al. 2006). The values obtained for *E. brevidens* were at or below these criterion values, indicating possible demographic independence among demes. Indirect estimates of *Nm* from F_{ST} have been criticized for having high variance and for being based on the biologically unrealistic assumptions of the island model (Whitlock and McCauley 1999). Many populations inhabiting rivers are better characterized using a stepping-stone model (Kimura and Weiss 1964), and genetic differentiation under such a model is even greater for the same number of migrants entering a deme each generation (Whitlock and McCauley 1999). Hence, indirect estimates of *Nm* taken under conditions of isolation-by-distance are likely to lead to overestimates of gene flow. Indirect methods also do not allow for estimating asymmetric migration among demes, which is certainly common in mussel populations

inhabiting rivers. The ML-based method seemed to provide better estimates of *Nm*, to include the directionality of gene flow and migration rates. Berg et al. (2007) proposed that mussel species common to large rivers exhibit relatively high amounts of within-population genetic variation and little differentiation over large geographical distances, whereas mussel species typical of small streams show lower within-population genetic variation and greater among-population differentiation. The two *Epioblasma* species exhibited restricted gene flow and reduced genetic variation among demes when compared to *L. fasciola*, further corroborating the central argument of this study; namely, that fish host-mediated dispersal and biological processes influencing N_e likely drive much of the underlying genetic structure of unionids.

Summary and conclusions

Patterns of distribution, abundance and genetic variation of NA freshwater mussel populations are the result of both historical and ecological processes operating over millennial to contemporary time-scales. The current distributions of some species are restricted to specific geographic areas such as the Cumberlandian region. While the reasons for this confinement are thought to be mainly ecological in nature, historical events and conditions may not have allowed some species to escape refugia, especially those of more recent origin and with limited dispersal capability. It is clear that many animal and plant species have greatly expanded their range to colonize habitats previously rendered inaccessible or inhospitable during glacial cycles (Pielou 1991; Hewitt 1996; Graf 1997, 2002). Although both dispersal and vicariance hypotheses are

needed to explain these patterns in mussels, fish host-mediated dispersal likely is the primary mechanism. Recent studies representing a range of species have shown that mussel populations exhibit low to moderate levels of intraspecific divergence and high-levels of shared genetic variation over broad geographic areas within the Mississippi River valley, and based on the results of this study, it is likely that much of this variation originated during the late Pleistocene and Holocene, findings which are incompatible with the predictions of the pre-Pleistocene vicariance hypothesis.

Available evidence for population expansion and secondary contact suggests that mixing of divergent populations has occurred naturally for terrestrial species in NA and Europe (Hewitt 1996), with similar evidence of post-glacial expansion and secondary contact beginning to emerge for mussels in the Mississippi River system (Elderkin et al. 2007; Elderkin et al. 2008). Mussels have an inherent capacity to colonize and adapt to novel environments over time; witness the rapid (~60 y) faunal changes, colonization and high densities occurring in the impounded reach of the lower Tennessee River in Kentucky Lake, KY (Sickell et al. 2007). Hence, the genetic and demographic structure of mussel populations primarily is controlled by dispersal and abundance of fish hosts, and life history traits such as lifespan. Therefore, the results of this study and others suggest:

- Long-term demographic trends for mussel populations are variable but can be broadly characterized by patterns of *expansion*, *stability* or *decline* and *secondary contact* based on data from their distribution, abundance and DNA polymorphism.

● Mussel species exhibiting a pattern of DNA polymorphism consistent with population *expansion* will utilize high-dispersal fish hosts and have larger long-term N_e, while those exhibiting a pattern of *stability* or *decline* will utilize low-dispersal fish hosts and have smaller long-term N_e.

● Mussel species exhibiting larger long-term N_e and high-dispersal are likely to be characterized by panmictic population structure over large geographic distances, while those exhibiting smaller long-term N_e and low-dispersal are characterized by meta-population structure over small geographic distances.

● The low divergence of mtDNA sequence variation reported in recent mussel studies coupled with a high short-term mtDNA mutation rate, indicates that considerable extant population genetic variation, e.g., singletons and uncommon polymorphisms, likely originated during the late Pleistocene and Holocene.

● The relatively recent origin of such intra-specific genetic variation and high gene-flow among demes implies that many mussel populations may not be highly co-adapted to local environmental conditions; hence, those occupying contiguous regional river networks may be genetically and ecologically exchangeable.

● Divergent DNA lineages within and among populations may have originated during earlier time periods and in different geographic areas, perhaps indicating

increased isolation, secondary contact, introgression or cryptic biodiversity, and warrant careful consideration in conservation planning.

Biologists are accustomed to recording population change over years or decades, and therefore our understanding of population demography over centuries or millenia is limited. Hence, the distribution and abundance of many mussel species appears to have changed minimally based on current collection records and those of 18th and 19th century naturalists. However, this view is likely mistaken for most species when considered even over short geological time frames such as the late Pleistocene or Holocene. The construction of dams and other barriers has constrained the current distributions of many species, preventing typical population responses to shifts in water quality and habitat related to climate change or other disturbances. Extinction of additional species characterized by low abundance and dispersal is nearly certain, unless natural resource managers are aggressive in expanding their ranges and population sizes. A major conclusion of population genetics and conservation biology over the last few decades is that maintenance of population genetic variation and population viability is enhanced by increased migration and N_e among demes or subpopulations (Beissinger and McCullough, 2002). For many mussel species, such dynamics no longer can occur naturally and hence will require active management by biologists to reintroduce individuals to establish new populations and augment depauperate ones. The few high quality, free-flowing rivers in NA should be viewed as refugia for mussels, to be managed to sustain their abundance and genetic diversity.

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Table 1. Sample sizes for mitochondrial DNA sequences and DNA microsatellite loci for investigated species; --- = locus not analyzed for a species.

		mtDNA		nDNA										
				Microsatellite Loci										
Species	Total sample size	*ND1*	*cyto-chrome-b*	*Ecap01*	*Ecap02*	*Ecap04*	*Ecap05*	*Ecap06*	*Ecap07*	*Ecap08*	*Ecap09*	*Lab206*	*Lab211*	*Lab213*
E. brevidens	79	77	77	77	79	79	79	77	79	78	78	78	---	79
E. capsaeformis	90	90	90	90	90	90	90	90	90	90	90	89	---	88
L. fasciola	65	65	65	---	65	65	65	64	65	65	---	65	65	65

Table 2. Observed haplotypes and polymorphic sites (**bold**) in a combined analysis of *cytochrome-b* (361 bp) and *ND1* (813*-888 bp) mitochondrial DNA sequences of *Epioblasma brevidens*, *E. capsaeformis* and *Lampsilis fasciola* from the Clinch River, Hancock County, Tennessee.

Polymorphic Sites

E. brevidens:

			cytochrome-b					*ND-1*			
			1	2	2	3	3	2	5	6	8
			8	5	8	5	5	7	4	5	0
Haplotype	N	Clade	7	0	6	0	2	6	8	3	7
Ebrev01	41	1	C	G	T	C	C	C	A	C	C
Ebrev02	2	1	•	•	**C**	•	•	•	•	•	•
Ebrev03	1	1	•	•	•	**T**	•	•	•	•	•
Ebrev04	7	1	•	•	•	•	•	•	**G**	•	•
Ebrev05	10	2	**T**	**A**	•	•	•	**T**	•	**T**	**T**
Ebrev06	10	2	**T**	**A**	•	•	**T**	**T**	•	**T**	**T**

E. capsaeformis:

			cytochrome-b					*ND-1*										
				1	1	2	3		1	2	3	4	5	5	6	6	7	7	7
			3	7	7	7	4	8	2	6	8	2	5	6	3	7	1	2	9
Haplotype	N	Clade	5	5	8	5	5	1	9	3	6	5	2	1	3	4	6	0	2
Ecap01	39	1	T	C	C	A	G	G	A	T	G	A	A	G	G	T	T	C	T
Ecap02	1	1	•	•	•	•	•	•	•	•	•	•	•	•	**A**	•	•	•	•
Ecap03	2	1	•	•	•	•	**A**	•	•	•	•	•	•	•	•	•	•	•	•
Ecap04	1	1	•	•	•	•	•	•	•	•	•	•	**G**	•	•	**C**	•	•	•
Ecap05	30	2	•	•	•	•	•	•	•	**C**	•	•	•	•	•	**C**	•	•	•
Ecap06	1	2	•	**T**	•	•	•	•	•	**C**	•	•	•	•	•	**C**	•	•	•
Ecap07	1	2	•	•	•	•	•	**A**	•	**C**	•	•	•	•	•	**C**	•	•	•
Ecap08	1	2	•	•	•	•	•	•	**G**	**C**	•	•	•	•	•	**C**	•	•	•
Ecap09	1	2	•	•	•	•	•	•	•	**C**	•	•	•	•	•	**C**	•	•	**C**
Ecap10	1	2	•	•	•	•	**A**	•	•	**C**	•	•	•	•	•	**C**	•	•	•
Ecap11	3	3	**C**	•	**T**	**G**	•	•	•	•	•	**G**	•	**A**	•	•	**C**	•	•
Ecap12	1	3	**C**	•	**T**	**G**	**A**	•	•	•	•	**G**	•	**A**	•	•	**C**	•	•
Ecap13	1	3	**C**	•	**T**	•	•	•	•	•	•	**G**	•	**A**	•	•	**C**	•	•
Ecap14	1	3	**C**	•	**T**	**G**	•	•	•	•	**A**	**G**	•	**A**	•	•	•	•	•
Ecap15	1	3	**C**	•	**T**	**G**	•	•	•	•	**A**	**G**	**G**	**A**	•	•	•	•	•
Ecap16	1	3	**C**	•	**T**	•	•	•	•	•	**A**	**G**	•	**A**	•	•	•	**T**	•
Ecap17	1	3	**C**	•	**T**	**G**	•	•	•	•	**A**	**G**	•	**A**	•	•	•	**T**	•

Table 2. Continued

L. fasciola:

cytochrome-b

Haplotype	N	Clade	91	106	121	199	201	208	226	244	250	271	283	287	310	322	341
Lfasc01	11	1	C	T	C	C	**C**	T	T	C	G	G	C	T	C	T	C
Lfasc02	1	1	•	•	•	•	•	**C**	•	•	•	•	•	•	•	•	•
Lfasc03	1	1	•	•	•	**T**	•	•	•	•	•	•	•	•	•	•	•
Lfasc04	2	1	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc05	1	1	•	•	•	•	•	•	•	**T**	•	•	•	•	•	•	•
Lfasc06	1	1	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc07	1	1	•	•	•	•	•	•	•	•	•	•	**T**	•	•	•	•
Lfasc08	1	1	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc09	1	1	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc10	1	1	**T**	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc11	5	1	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc12	1	1	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc13	1	1	•	•	•	•	•	•	•	•	•	•	•	**C**	•	•	•
Lfasc14	1	1	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc15	1	1	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc16	7	2	•	•	•	•	**T**	•	•	•	•	•	•	•	•	•	•
Lfasc17	1	2	•	•	•	•	**T**	•	•	•	•	•	•	•	•	•	•
Lfasc18	2	2	•	•	•	•	**T**	•	•	•	•	•	•	•	•	•	•
Lfasc19	1	2	•	•	•	•	**T**	•	•	•	•	•	•	•	•	•	•
Lfasc20	3	2	•	•	•	•	**T**	•	•	•	•	•	•	•	•	•	•
Lfasc21	2	2	•	•	•	•	**T**	•	•	•	•	•	•	•	•	•	**T**
Lfasc22	2	2	•	•	•	•	**T**	•	•	•	**A**	•	•	•	•	•	**T**
Lfasc23	1	2	•	•	•	•	**T**	•	•	•	•	•	•	•	•	•	**T**
Lfasc24	1	2	•	•	•	•	**T**	•	•	•	•	•	•	•	•	•	•
Lfasc25	3	2	•	•	•	•	**T**	•	•	•	•	•	•	•	**T**	•	•
Lfasc26	2	2	•	•	**T**	•	**T**	•	•	•	•	•	•	•	•	•	•

ND-1

Haplotype	33	36	50	72	105	114	121	213	231	402	411	423	436	462	467	468	476	507	555	564	582	600	606	645	688	692	762
Lfasc01	C	T	T	C	T	G	G	A	G	T	T	G	A	C	T	A	T	A	C	G	G	T	T	T	C	T	G
Lfasc02	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc03	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc04	•	•	•	•	•	•	•	**G**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc05	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc06	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	**C**	•	•	•	•	•	•	•	•	•	•
Lfasc07	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	**A**	•	•	•	•	•	•	•
Lfasc08	•	•	•	•	•	•	**A**	•	•	•	•	•	•	•	•	•	•	•	•	•	**A**	•	•	•	•	•	•
Lfasc09	•	•	•	•	•	•	•	•	•	•	•	**A**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	**A**
Lfasc10	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	**A**
Lfasc11	•	•	•	•	**C**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc12	•	•	•	•	**C**	•	•	•	•	•	•	•	•	•	•	•	•	•	**T**	•	•	•	•	•	•	•	•
Lfasc13	•	•	•	•	**C**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc14	•	•	•	•	**C**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	**T**	•	•
Lfasc15	•	•	•	•	•	•	•	•	**A**	•	•	•	**T**	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc16	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc17	•	•	•	•	•	•	•	•	•	•	•	•	•	**A**	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc18	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	**C**	•
Lfasc19	•	•	•	•	•	•	•	•	•	•	•	•	•	•	**C**	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc20	•	•	•	•	•	•	•	•	•	•	**C**	•	•	•	•	•	•	•	**T**	•	•	•	•	•	•	•	•
Lfasc21	•	**C**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc22	•	**C**	•	**T**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc23	•	**C**	•	**T**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc24	•	•	•	•	•	•	•	•	**A**	•	•	•	•	•	•	•	•	•	•	•	•	•	•	**C**	•	•	•
Lfasc25	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	**C**	•	•	•
Lfasc26	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	**C**	•	•	•

Table 2. Continued

L. fasciola:

cytochrome-b

Haplotype	N	Clade	91	106	121	199	201	208	226	244	250	271	283	287	310	322	341
Lfasc27	1	2	•	•	•	•	**T**	•	•	•	•	•	•	•	•	•	•
Lfasc28	3	2	•	•	•	•	**T**	•	•	•	•	•	•	•	•	•	•
Lfasc29	1	2	•	•	•	•	**T**	•	•	•	•	**A**	•	•	•	•	•
Lfasc30	1	2	•	**C**	•	•	**T**	•	**C**	•	•	•	•	•	•	**C**	•
Lfasc31	1	3	•	•	•	•	**T**	•	**C**	•	•	•	•	•	•	**C**	•
Lfasc32	1	3	•	•	•	•	**T**	•	**C**	•	•	•	•	•	•	•	•
Lfasc33	2	3	•	•	•	•	**T**	•	**C**	•	•	•	•	•	•	**C**	•

ND-1

Haplotype	33	36	50	72	105	114	121	131	231	402	411	423	436	462	467	468	476	507	555	564	582	600	606	645	688	692	762
Lfasc27	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	**G**	•	•	•	•	•	•	•	**C**	•	•	•
Lfasc28	**T**	•	•	•	•	•	•	•	**A**	•	•	•	•	•	•	•	•	•	•	•	•	•	**C**	•	•	•	•
Lfasc29	**T**	•	**C**	•	•	•	•	•	**A**	•	•	•	•	•	•	•	•	•	•	•	•	•	**C**	•	•	•	•
Lfasc30	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Lfasc31	•	•	•	•	•	**A**	•	•	**A**	**C**	•	•	•	•	•	•	•	•	•	•	•	**G**	•	•	•	•	•
Lfasc32	•	•	•	•	•	**A**	•	•	**A**	**C**	•	•	•	•	•	•	•	**G**	•	•	•	•	•	•	•	•	•
Lfasc33	•	•	•	•	•	**A**	•	•	**A**	**C**	•	•	•	•	•	•	•	**G**	•	•	•	•	•	•	•	•	•

Table 3. Summary of observed mitochondrial DNA sequence variation (± standard deviation) for three species of freshwater mussels. Global values were obtained by pooling and analyzing data together from all three sites.

Species	Population	Sample size (*n*)	No. haplotypes (unique)	Haplotype diversity (*h*)	No. segregating sites (*s*)	Mean no. nucleotide differences (*k*), range	Nucleotide diversity (π)
E. brevidens	Wallen Bend	22	6 (1)	0.64	9	2.2, 0-7	0.00179
	Frost Ford	28	4 (0)	0.68	7	2.6, 0-7	0.00206
	Swan Island	21	4 (0)	0.48	7	1.8, 0-6	0.00181
	Global	71	6	0.62 ± 0.055	9	2.4, 0-7	0.00189 ± 0.001
E. capsaeformis	Wallen Bend	30	7 (4)	0.65	13	2.0, 0-9	0.00163
	Frost Ford	30	7 (5)	0.66	12	2.0, 0-9	0.00160
	Swan Island	30	10 (3)	0.81	12	3.7, 0-9	0.00296
	Global	90	17	0.70 ± 0.035	17	2.6, 0-10	0.00208 ± 0.001
L. fasciola	Wallen Bend	28	19 (8)	0.96	27	3.4, 0-9	0.00288
	Frost Ford	17	14 (4)	0.98	23	4.5, 0-10	0.00382
	Swan Island	20	14 (9)	0.93	22	3.0, 0-9	0.00257
	Global	65	33	0.95 ± 0.015	42	3.6, 0-10	0.00306 ± 0.002

Table 4. Summary of observed microsatellite DNA loci variation (± standard deviation) for three species of freshwater mussels. Theta (θ) followed by approximate 95% confidence intervals was estimated from simulations based on the maximum likelihood coalescent approach of Kuhner et al. (1998). "---"= data not applicable. Global values were obtained by pooling and analyzing data together from all three sites.

Species	Population	Sample size (n)	Observed hetero-zygosity	Expected hetero-zygosity	Average allelic diversity	No. of private alleles	θ
E. brevidens	Wallen Bend	23	0.6957	0.7657	9.0	7	0.490 (0.436, 0.540)
	Frost Ford	28	0.7172	0.7638	8.1	4	0.227 (0.209, 0.261)
	Swan Island	28	0.6410	0.7655	8.4	2	0.423 (0.388, 0.460)
	Global	79	0.6840	0.7675	10.5 ± 5.7	---	0.559 (0.524, 0.589)
E. capsaeformis	Wallen Bend	30	0.8133	0.8485	11.3	5	0.304 (0.277, 0.336)
	Frost Ford	30	0.7961	0.8452	12.8	13	0.656 (0.599, 0.719)
	Swan Island	30	0.8163	0.8335	12.2	9	0.400 (0.365, 0.439)
	Global	90	0.8087	0.8479	15.2 ± 5.9	---	5.370 (5.100, 5.660)
L. fasciola	Wallen Bend	28	0.6894	0.8347	14.1	18	0.784 (0.719, 0.867)
	Frost Ford	17	0.7279	0.8719	12.2	12	3.025 (2.689, 3.149)
	Swan Island	20	0.7500	0.8295	12.1	15	1.065 (0.961, 1.193)
	Global	65	0.7184	0.8446	17.7 ± 6.2	---	17.980 (16.89, 19.14)

Table 5. Estimates of genetic diversity (θ) obtained from nucleotide diversity (π) and from simulations using the ML-coalescent based approach of Kuhner et al. (1998), representing current (± standard deviation) and historical levels (95% confidence intervals), respectively. Also reported are estimates of historical population growth derived from ML-coalescent (g) and non-coalescent based methods (F_S).

				Fu's F_S	
Species	$\theta_{\pi(\text{current})}$	$\theta_{\text{historical}}$ (95% CI)	g (95% CI)	F_S	P
E. brevidens	0.00189 ± 0.00104	0.00110 (0.00084, 0.00238)	-540 (-1940, 1072)	2.32	>0.10
E. capsaeformis	0.00208 ± 0.00112	0.00512 (0.00319, 0.00798)	575 (-138, 2058)	-4.82	>0.10
L. fasciola	0.00306 ± 0.00157	0.05089 (0.02496, 0.17359)	1987 (1148, 3847)	-26.17	<0.001

Table 6. Long-term effective population sizes (N_e) estimated from genetic diversity (θ), derived from analysis of mitochondrial DNA sequences and nuclear DNA microsatellites. Estimates of θ are from nucleotide diversity π (current) and from simulations (historical) based on the ML-coalescent approach of Kuhner et al. (1998).

	mitochondrial DNA		nuclear DNA
Species	$N_{e\,(current)}$	$N_{e\,(historical)}$	$N_{e\,(historical)}$
Epioblasma brevidens	3,780	2,200	280
Epioblasma capsaeformis	4,160	10,240	2,685
Lampsilis fasciola	7,650	127,225	11,237

A mutation rate of 1.0 X 10^{-7} nucleotide changes site^{-1} year^{-1} for mitochondrial DNA and 1 X 10^{-4} changes allele^{-1} year^{-1} for microsatellite DNA was used to estimate N_e. The generation time (T_g) for *E. brevidens* and *E. capsaeformis* is 5 y and 4 y for *L. fasciola*.

Table 7. Results of analysis of molecular variance (AMOVA) for *Epioblasma brevidens*, *E. capsaeformis* and *Lampsilis fasciola* in the Clinch River, TN. Estimates were obtained from analyses of DNA microsatellite loci.

Source of variation	d.f.	Sum of squares	Percentage of variation	Fixation index (F_{st})	*p*-value
E. brevidens:					
Among populations	2	9.40	0.48	0.00476	p=0.175
Within populations	155	582.21	99.52		
Total	157	591.61	100.00		
E. capsaeformis:					
Among populations	2	13.34	0.98	0.00981	p<0.001
Within populations	177	740.28	99.02		
Total	179	753.62	100.00		
L. fasciola:					
Among populations	2	8.58	0.33	0.00328	p=0.516
Within populations	127	478.38	99.67		
Total	129	486.96	100.00		

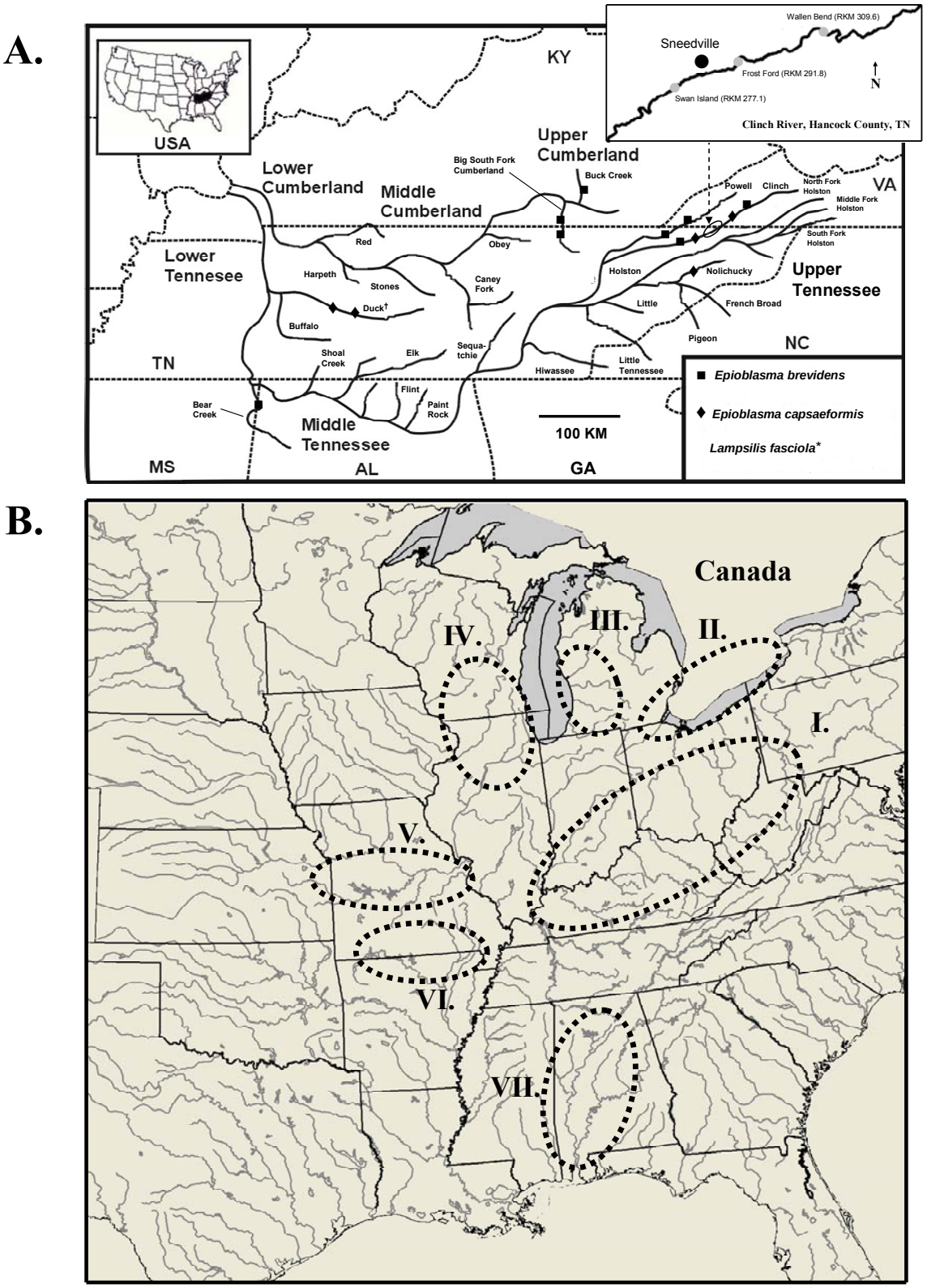
A.
USA
KY
VA
NC
TN
MS
AL
GA
Lower Cumberland
Middle Cumberland
Upper Cumberland
Lower Tennessee
Middle Tennessee
Upper Tennessee
Big South Fork Cumberland
Buck Creek
Red
Obey
Harpeth
Stones
Caney Fork
Duck†
Buffalo
Shoal Creek
Elk
Sequa-tchie
Flint
Paint Rock
Bear Creek
Hiwassee
Little Tennessee
Holston
Nolichucky
Little
French Broad
Pigeon
Powell
Clinch
North Fork Holston
Middle Fork Holston
South Fork Holston
100 KM
Epioblasma brevidens
Epioblasma capsaeformis
Lampsilis fasciola*
Wallen Bend (RKM 309.6)
Sneedville
Frost Ford (RKM 291.8)
Swan Island (RKM 277.1)
N
Clinch River, Hancock County, TN
B.
Canada
I.
II.
III.
IV.
V.
VI.
VII.

Figure 1.

Figure 1. **A.** Current distributions of *Epioblasma brevidens* ■ and *E. capsaeformis* ♦ in the Cumberlandian physiographic region, USA, are shown. Both species are endemic to the region and historically occurred in many rivers in the Cumberland and Tennessee river basins. †The population of *E. capsaeformis* in the Duck River is considered a separate species from populations in the Clinch and Nolichucky rivers (Jones et al. 2006). *****_Lampsilis fasciola_ currently is widely distributed throughout Tennessee, Cumberland, Ohio and Lake Erie drainage basins. **B.** The distribution of *Epioblasma* spp. outside of the Cumberlandian Region is in seven drainage regions: I. Ohio River drainage: *E. flexuosa*, *E. obliquata*, *E. personata*, *E. propinqua*, *E. torulosa*, and *E. triquetra*; II. Lake Erie basin: *E. obliquata*, *E. torulosa*, and *E. triquetra*; III. Lake Michigan basin: *E. torulosa* and *E. triquetra*; IV. Upper Mississippi River drainage: *E. triquetra*; V. Lower Missouri River drainage north of the Ozark Crest: *E. triquetra*; VI. Upper White River drainage south of the Ozark Crest: *E. florentina*, *E. triquetra* and *E. turgidula*; and VII. Mobile River drainage: *E. penita* and *E. othcaloogensis*.

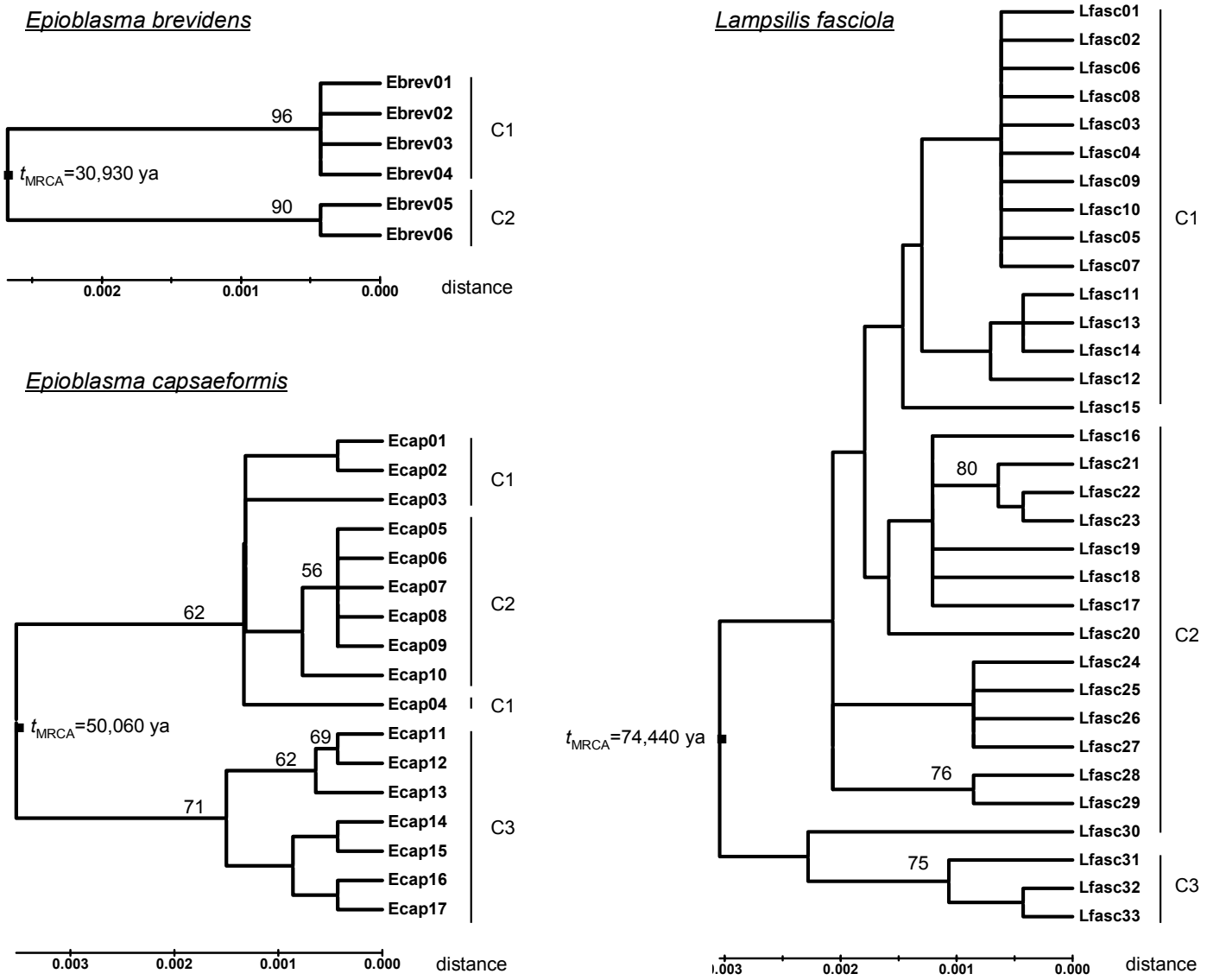
Epioblasma brevidens
Ebrev01
Ebrev02
Ebrev03
Ebrev04
Ebrev05
Ebrev06
96
90
C1
C2
t_{MRCA}=30,930 ya
0.002
0.001
0.000
distance
Epioblasma capsaeformis
Ecap01
Ecap02
Ecap03
Ecap05
Ecap06
Ecap07
Ecap08
Ecap09
Ecap10
Ecap04
Ecap11
Ecap12
Ecap13
Ecap14
Ecap15
Ecap16
Ecap17
62
56
69
62
71
C1
C2
C1
C3
t_{MRCA}=50,060 ya
0.003
0.002
0.001
0.000
distance
Lampsilis fasciola
Lfasc01
Lfasc02
Lfasc06
Lfasc08
Lfasc03
Lfasc04
Lfasc09
Lfasc10
Lfasc05
Lfasc07
Lfasc11
Lfasc13
Lfasc14
Lfasc12
Lfasc15
Lfasc16
Lfasc21
Lfasc22
Lfasc23
Lfasc19
Lfasc18
Lfasc17
Lfasc20
Lfasc24
Lfasc25
Lfasc26
Lfasc27
Lfasc28
Lfasc29
Lfasc30
Lfasc31
Lfasc32
Lfasc33
80
76
75
C1
C2
C3
t_{MRCA}=74,440 ya
0.003
0.002
0.001
0.000
distance

Figure 2.

Figure 2. Unrooted linearized neighbour-joining trees of observed mitochondrial DNA haplotypes. Relationships among haplotypes were inferred from combined mitochondrial DNA regions of *cytochrome-b* (361 bp) and *ND1* (888 bp); the Kimura 2-parameter model of nucleotide substitution was used to construct the tree. Numbers at branches are bootstrap support (1000 replicates); only values >50% shown. The estimated time to most recent common ancestor (t_{MRCA}) for haplotypes of each species, was inferred using a relaxed molecular clock Bayesian method (see Methods). The estimate of t_{MRCA} 95% highest posterior density (HPD) interval for *E. brevidens* was 3,142 to 92,380 ya, for *E. capsaeformis* 14,170 to 102,000 ya and for *L. fasciola* 22,200 to 171,400 ya. Estimates of t_{MRCA} for clades of each species were: *E. brevidens*, Clade 1(C1)=10,500 ya (95% HPD interval 708 to 35,250 ya) and Clade 2(C2)=6,468 ya (95% HPD interval 208 to 21,700 ya); *E. capsaeformis*, C1=9,084 ya (95% HPD interval 1,728 to 19,720 ya), C2=7,883 ya (95% HPD interval 1,628 to 17,320 ya) and Clade 3(C3)=14,670 ya (95% HPD interval 2,614 to 35,500 ya); *L. fasciola*, C1=22,530 ya (95% HPD interval 7,167 to 44,850 ya), C2=25,890 ya (95% HPD interval 9,138 to 48,440 ya) and C3=66,700 ya (95% HPD interval 17,570 to 150,200 ya).

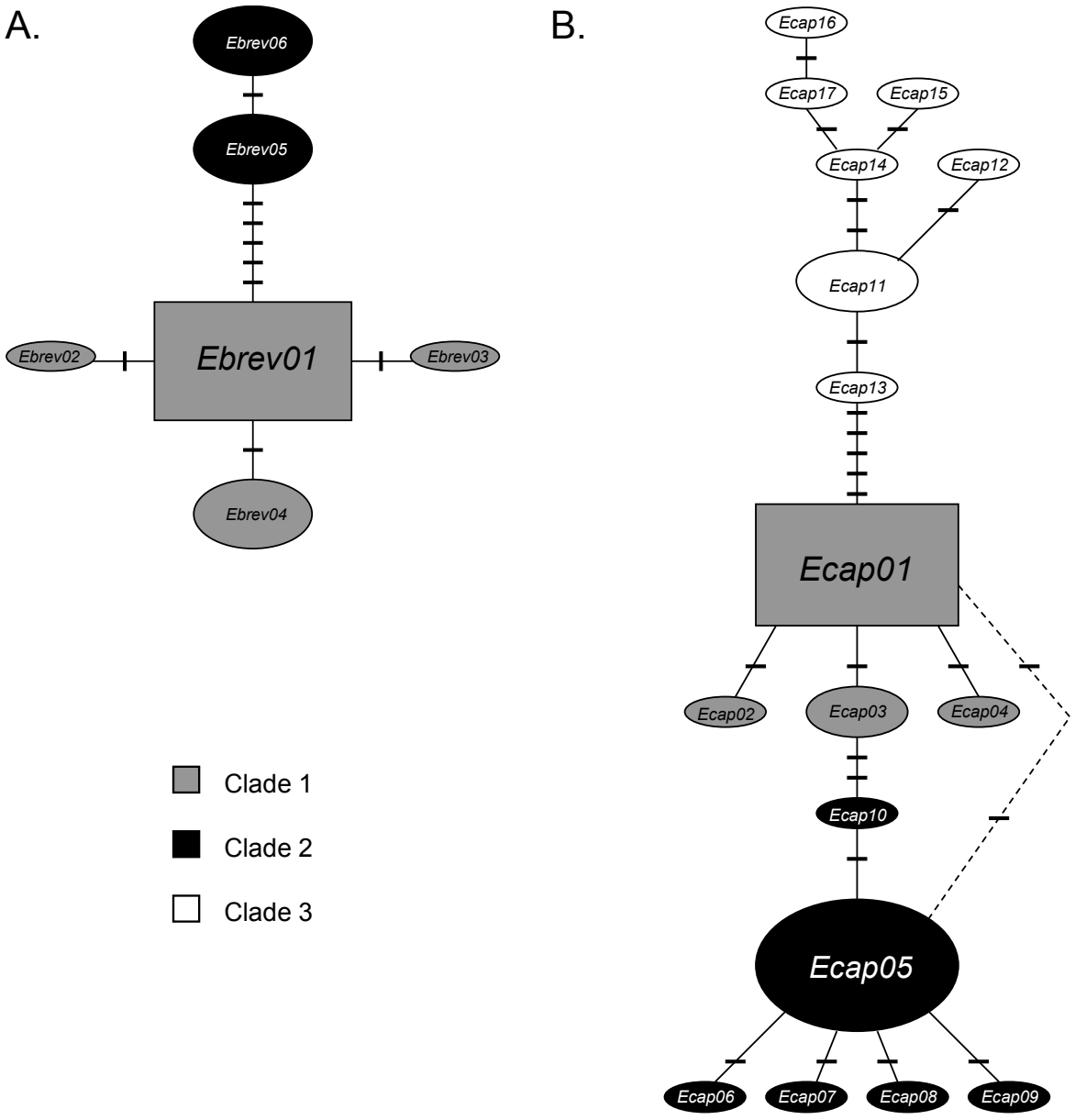
A.
Ebrev06
Ebrev05
Ebrev01
Ebrev02
Ebrev03
Ebrev04
Clade 1
Clade 2
Clade 3
B.
Ecap16
Ecap17
Ecap15
Ecap14
Ecap12
Ecap11
Ecap13
Ecap01
Ecap02
Ecap03
Ecap04
Ecap10
Ecap05
Ecap06
Ecap07
Ecap08
Ecap09

Figure 3.

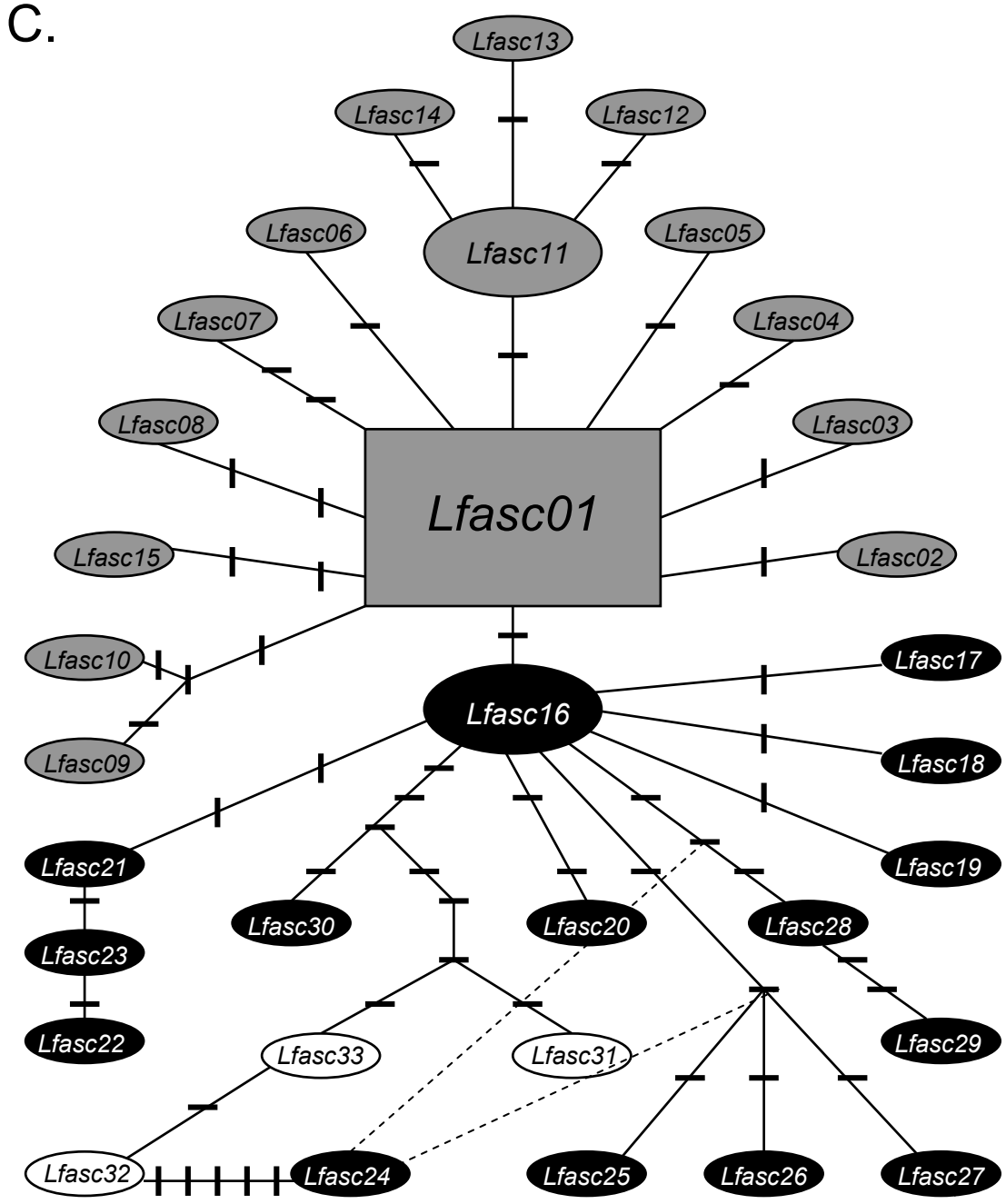

Figure 3. Continued.

Figure 3. Maximum parsimony haplotype networks for **A.** *E. brevidens*, **B.** *Epioblasma capsaeformis*, and **C.** *L. fasciola*. Geometric shapes represent haplotypes and size is proportional to its observed frequency in the study. Networks were constructed using combined sequences from the mitochondrial *cytochrome-b* and *ND1* regions. Inferred ancestral haplotypes are displayed in square boxes; dash marks along branches equal number of steps from ancestral haplotype, and broken lines are alternative pathways.

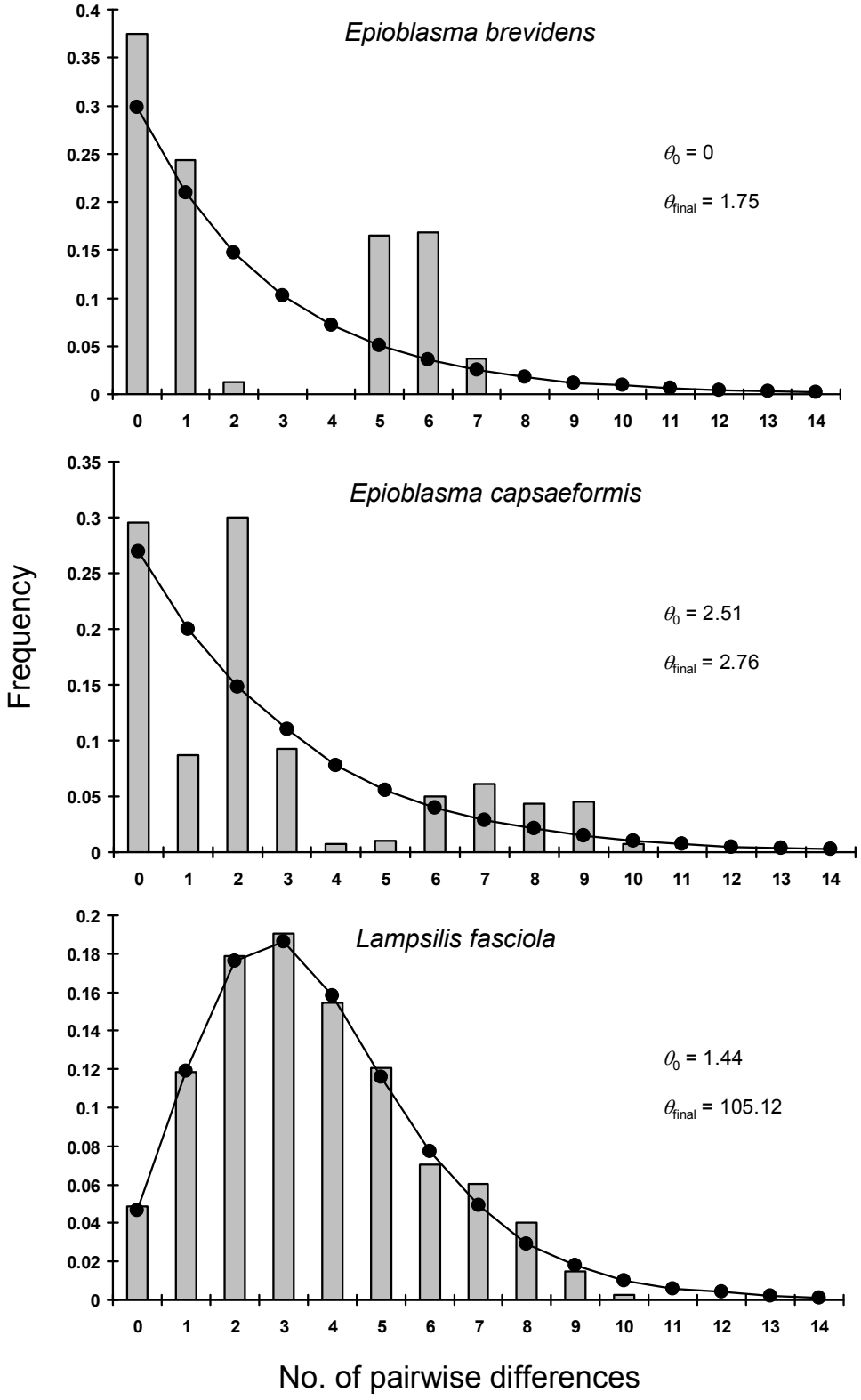
Epioblasma brevidens
θ0 = 0
θfinal = 1.75
Epioblasma capsaeformis
θ0 = 2.51
θfinal = 2.76
Lampsilis fasciola
θ0 = 1.44
θfinal = 105.12
Frequency
No. of pairwise differences

Figure 4.

Figure 4. Mismatch distributions of investigated mussel species collected in the Clinch River, TN. Analyses were conducted using combined mitochondrial DNA sequences of *cytochrome-b* and *ND1*. Grey bars are the observed frequencies of pairwise differences among sequences, and lines are the expected frequencies under a model of population expansion.

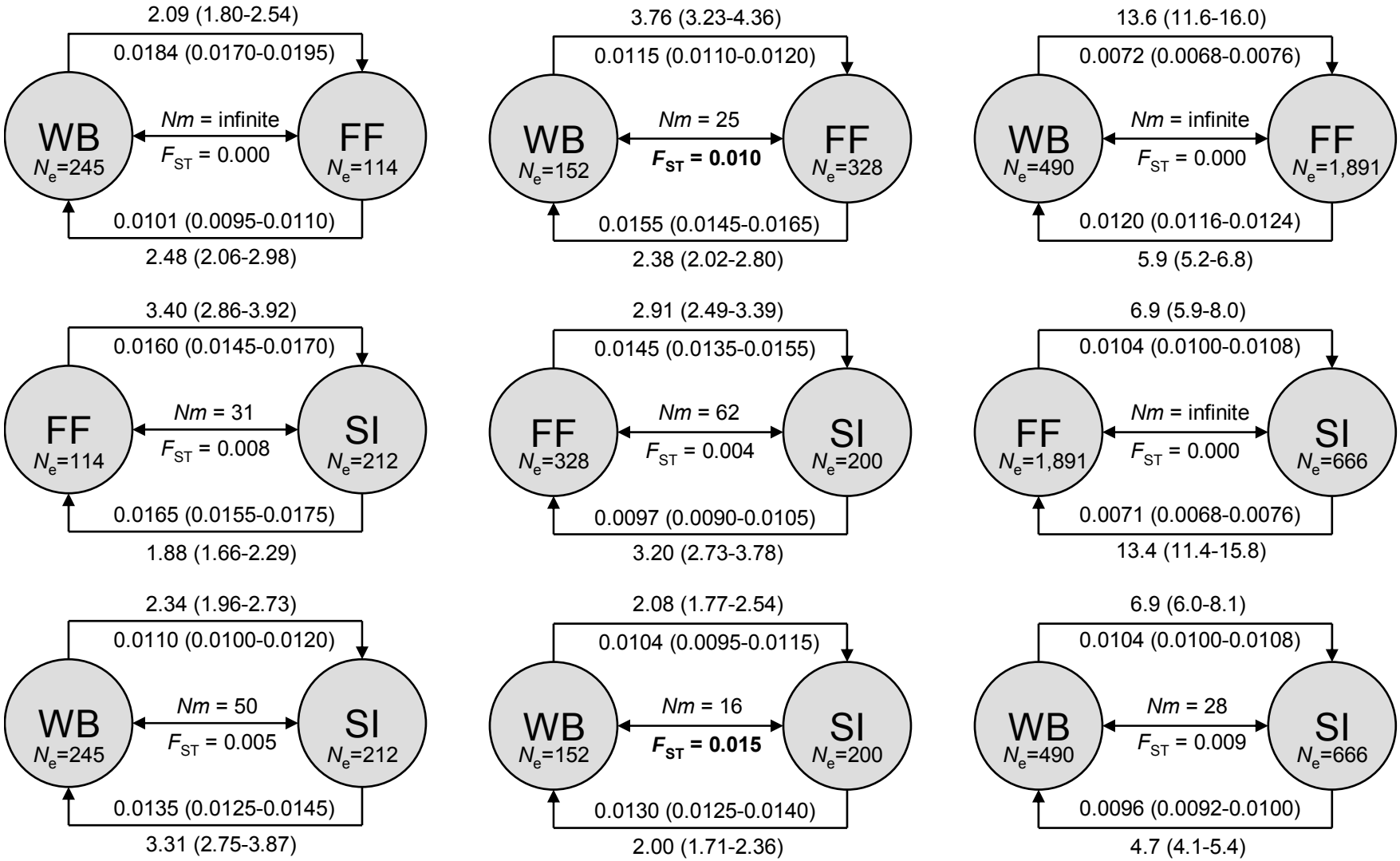
E. brevidens:
2.09 (1.80-2.54)
0.0184 (0.0170-0.0195)
WB
Ne=245
Nm = infinite
FST = 0.000
FF
Ne=114
0.0101 (0.0095-0.0110)
2.48 (2.06-2.98)
3.40 (2.86-3.92)
0.0160 (0.0145-0.0170)
FF
Ne=114
Nm = 31
FST = 0.008
SI
Ne=212
0.0165 (0.0155-0.0175)
1.88 (1.66-2.29)
2.34 (1.96-2.73)
0.0110 (0.0100-0.0120)
WB
Ne=245
Nm = 50
FST = 0.005
SI
Ne=212
0.0135 (0.0125-0.0145)
3.31 (2.75-3.87)
E. capsaeformis:
3.76 (3.23-4.36)
0.0115 (0.0110-0.0120)
WB
Ne=152
Nm = 25
FST = 0.010
FF
Ne=328
0.0155 (0.0145-0.0165)
2.38 (2.02-2.80)
2.91 (2.49-3.39)
0.0145 (0.0135-0.0155)
FF
Ne=328
Nm = 62
FST = 0.004
SI
Ne=200
0.0097 (0.0090-0.0105)
3.20 (2.73-3.78)
2.08 (1.77-2.54)
0.0104 (0.0095-0.0115)
WB
Ne=152
Nm = 16
FST = 0.015
SI
Ne=200
0.0130 (0.0125-0.0140)
2.00 (1.71-2.36)
L. fasciola:
13.6 (11.6-16.0)
0.0072 (0.0068-0.0076)
WB
Ne=490
Nm = infinite
FST = 0.000
FF
Ne=1,891
0.0120 (0.0116-0.0124)
5.9 (5.2-6.8)
6.9 (5.9-8.0)
0.0104 (0.0100-0.0108)
FF
Ne=1,891
Nm = infinite
FST = 0.000
SI
Ne=666
0.0071 (0.0068-0.0076)
13.4 (11.4-15.8)
6.9 (6.0-8.1)
0.0104 (0.0100-0.0108)
WB
Ne=490
Nm = 28
FST = 0.009
SI
Ne=666
0.0096 (0.0092-0.0100)
4.7 (4.1-5.4)

Figure 5.

Figure 5. Pairwise estimates of equilibrium (historical) gene flow (*Nm*) and F_{ST} for three species in the Clinch River, TN, respectively, are shown above and below bi-directional arrow lines between populations. Bold values are statistically significant ($p<0.01$). Abbreviations are Wallen Bend (WB), Frost Ford (FF), and Swan Island (SI). Directional maximum likelihood estimates of historical *Nm* and *m* (with 95% confidence intervals) are shown on the outside and inside along arrow lines, respectively. Values within circles are estimated long-term effective population sizes (N_e) based on the genetic diversity (θ) values presented in Table 4.

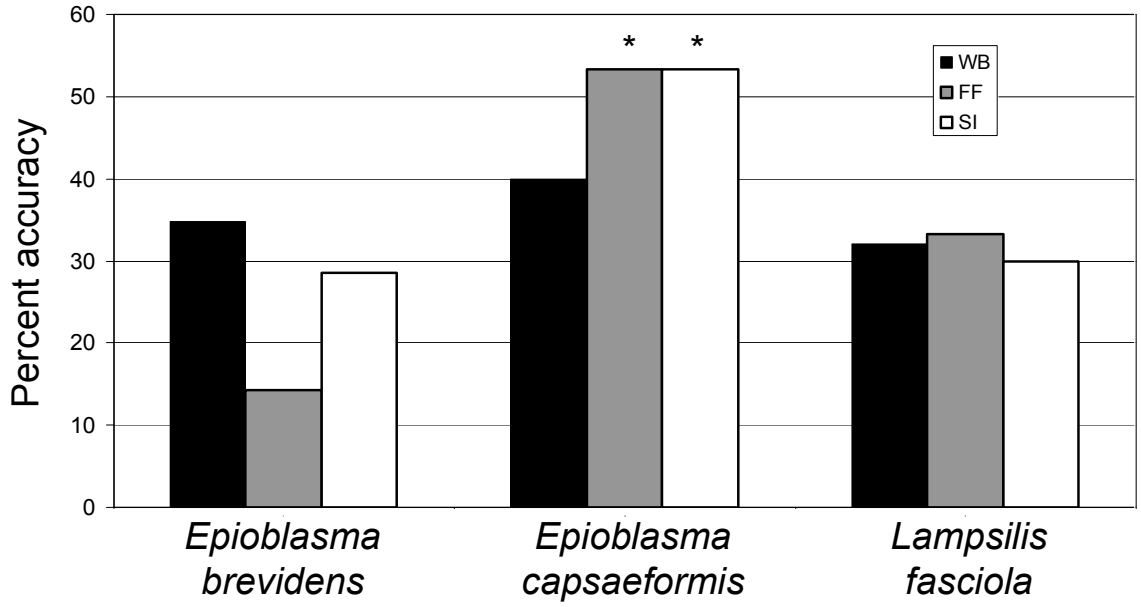

Figure 6. Percentage of individuals of each species correctly assigned to their collection site. Abbreviations are Wallen Bend (WB), Frost Ford (FF), and Swan Island (SI). *Represents significance at $\alpha = 0.05$ based on a χ^2 test of whether individuals were assigned more frequently to their own population than would be expected by chance if there were no differences among populations.

APPENDIX

Allele frequencies, number of alleles and hetrozygosities of DNA microsatellite loci for each species at three sites in the Clinch River, TN. Allele sizes are given in number of base pairs to include the primer flanking regions. --- = allele not present in sample.

Epioblasma brevidens:

Locus	Allele	Wallen Bend	Frost Ford	Swan Island	All Sites
Ecap01	162	39.13	32.14	26.92	32.47
	164	4.35	8.93	---	4.55
	166	8.70	21.43	11.54	14.29
	168	6.52	---	3.85	3.25
	170	15.22	5.36	17.31	12.34
	172	21.74	26.79	36.54	28.57
	174	4.35	5.36	3.85	4.55
	# alleles	**7**	**6**	**6**	**7**
	H_e	**0.7778**	**0.7792**	**0.7624**	**0.7772**
Ecap02	118	65.22	60.71	44.64	56.33
	124	34.78	39.29	55.36	43.67
	# alleles	**2**	**2**	**2**	**2**
	H_e	**0.4638**	**0.4857**	**0.5032**	**0.4951**
Ecap04	96	2.17	1.79	---	1.27
	98	15.22	12.50	7.14	11.39
	102	21.74	26.79	16.07	21.52
	104	13.04	10.71	8.93	10.76
	106	6.52	10.71	10.71	9.49
	112	4.35	1.79	5.36	3.80
	114	17.39	7.14	14.29	12.66
	116	2.17	---	---	0.63
	118	10.87	19.64	26.79	19.62
	120	6.52	7.14	10.71	8.23
	122	---	1.79	---	0.63
	# alleles	**10**	**10**	**8**	**11**
	H_e	**0.8783**	**0.8552**	**0.8584**	**0.8626**
Ecap05	167	6.52	5.36	---	3.80
	175	2.17	---	---	0.63
	181	6.52	1.79	3.57	3.80
	183	2.17	3.57	8.93	5.06
	187	2.17	---	---	0.63
	189	13.04	10.71	32.14	18.99
	191	41.30	39.29	26.79	35.44

	193	10.87	14.29	12.50	12.66
	195	6.52	16.07	7.14	10.13
	197	---	---	7.14	2.53
	199	---	3.57	---	1.27
	201	2.17	5.36	---	2.53
	203	6.52	---	1.79	2.53
	# alleles	**11**	**9**	**8**	**13**
	H_e	**0.799**	**0.7935**	**0.8039**	**0.8096**

Ecap06	250	10.87	17.86	17.31	15.58
	260	8.70	5.36	7.69	7.14
	262	---	1.79	---	0.65
	266	2.17	---	1.92	1.30
	274	4.35	1.79	---	1.95
	280	---	---	1.92	0.65
	284	---	1.79	1.92	1.30
	286	---	---	1.92	0.65
	288	4.35	1.79	1.92	2.60
	290	10.87	16.07	9.62	12.34
	292	6.52	5.36	3.85	5.19
	294	6.52	---	5.77	3.90
	296	2.17	---	3.85	1.95
	298	6.52	5.36	9.62	7.14
	300	6.52	8.93	3.85	6.49
	302	4.35	1.79	9.62	5.19
	304	4.35	7.14	5.77	5.84
	306	8.70	5.36	1.92	5.19
	308	6.52	10.71	5.77	7.79
	310	4.35	8.93	5.77	6.49
	312	2.17	---	---	0.65
	# alleles	**17**	**15**	**18**	**21**
	H_e	**0.9498**	**0.913**	**0.9344**	**0.9268**

Ecap07	109	4.35	---	1.79	1.90
	111	28.26	26.79	30.36	28.48
	113	2.17	3.57	1.79	2.53
	115	2.17	7.14	3.57	4.43
	117	4.35	---	1.79	1.90
	119	6.52	8.93	10.71	8.86
	121	2.17	8.93	3.57	5.06
	123	---	---	3.57	1.27
	125	19.57	12.50	23.21	18.35
	127	2.17	3.57	3.57	3.16
	129	---	3.57	5.36	3.16
	131	17.39	21.43	7.14	15.19
	133	2.17	---	---	0.63
	135	---	3.57	---	1.27
	143	8.70	---	1.79	3.16
	145	---	---	1.79	0.63
	# alleles	**12**	**10**	**14**	**16**
	H_e	**0.8522**	**0.8558**	**0.8429**	**0.8504**

Ecap08	143	54.35	67.86	53.70	58.97
	145	39.13	28.57	42.59	36.54
	147	6.52	3.57	3.70	4.49
	# alleles	**3**	**3**	**3**	**3**
	H_e	**0.5594**	**0.4649**	**0.5388**	**0.52**

Ecap09	126	---	---	1.79	0.64
	132	21.74	11.11	17.86	16.67
	134	17.39	20.37	28.57	22.44
	136	21.74	16.67	14.29	17.31
	138	17.39	16.67	8.93	14.10
	142	6.52	5.56	8.93	7.05
	146	4.35	5.56	5.36	5.13
	148	6.52	7.41	1.79	5.13
	154	---	5.56	---	1.92
	156	4.35	9.26	10.71	8.33
	158	---	1.85	---	0.64
	166	---	---	1.79	0.64
	# alleles	**8**	**10**	**10**	**12**
	H_e	**0.8512**	**0.8833**	**0.85**	**0.8599**

Lab206	188	2.17	---	1.85	1.28
	192	2.17	---	---	0.64
	200	52.17	39.29	44.44	44.87
	204	13.04	16.07	27.78	19.23
	208	2.17	---	1.85	1.28
	212	4.35	5.36	---	3.21
	216	10.87	28.57	12.96	17.95
	220	2.17	---	---	0.64
	224	6.52	7.14	7.41	7.05
	228	4.35	3.57	3.70	3.85
	# alleles	**10**	**6**	**7**	**10**
	H_e	**0.7043**	**0.7422**	**0.7142**	**0.7262**

Lab213	142	10.87	14.29	19.64	15.19
	150	36.96	19.64	26.79	27.22
	154	4.35	3.57	7.14	5.06
	158	6.52	5.36	5.36	5.70
	162	6.52	5.36	---	3.80
	166	4.35	19.64	14.29	13.29
	170	10.87	7.14	8.93	8.86
	174	2.17	3.57	3.57	3.16
	192	15.22	19.64	14.29	16.46
	196	2.17	1.79	---	1.27
	# alleles	**10**	**10**	**8**	**10**
	H_e	**0.8213**	**0.8656**	**0.8468**	**0.8472**

Epioblasma capsaeformis:

Locus	Allele	Wallen Bend	Frost Ford	Swan Island	All Sites
Ecap01	146	13.33	10.00	6.67	10.00
	148	1.67	1.67	3.33	2.22
	152	---	1.67	8.33	3.33
	154	5.00	---	1.67	2.22
	156	---	3.33	3.33	2.22
	158	3.33	11.67	5.00	6.67
	160	13.33	3.33	5.00	7.22
	162	5.00	1.67	8.33	5.00
	164	10.00	1.67	3.33	5.00
	166	8.33	5.00	8.33	7.22
	168	11.67	11.67	5.00	9.44
	170	8.33	8.33	10.00	8.89
	172	5.00	6.67	5.00	5.56
	174	---	8.33	5.00	4.44
	176	5.00	6.67	3.33	5.00
	178	8.33	3.33	5.00	5.56
	180	1.67	3.33	1.67	2.22
	182	---	1.67	8.33	3.33
	184	---	3.33	3.33	2.22
	186	---	1.67	---	0.56
	188	---	1.67	---	0.56
	190	---	1.67	---	0.56
	196	---	1.67	---	0.56
	# alleles	**14**	**22**	**19**	**23**
	H_e	**0.9237**	**0.9452**	**0.9525**	**0.9431**
Ecap02	107	6.67	8.33	6.67	7.22
	115	---	1.67	---	0.56
	121	26.67	11.67	11.67	16.67
	123	10.00	11.67	3.33	8.33
	125	23.33	43.33	58.33	41.67
	129	28.33	15.00	11.67	18.33
	131	5.00	6.67	6.67	6.11
	133	---	1.67	1.67	1.11
	# alleles	**6**	**8**	**7**	**8**
	H_e	**0.7904**	**0.7633**	**0.6328**	**0.7531**
Ecap04	92	1.67	---	1.67	1.11
	94	---	3.33	---	1.11
	98	1.67	---	---	0.56
	100	---	1.67	---	0.56
	102	3.33	6.67	3.33	4.44
	104	15.00	11.67	6.67	11.11
	106	26.67	50.00	48.33	41.67
	108	25.00	11.67	15.00	17.22
	110	16.67	5.00	16.67	12.78
	114	6.67	8.33	5.00	6.67

	116	1.67	---	---	0.56
	122	1.67	---	3.33	1.67
	124	---	1.67	---	0.56
	# alleles	**10**	**9**	**8**	**13**
	H_e	**0.8232**	**0.7192**	**0.7186**	**0.7652**

Ecap05	175	---	1.67	---	0.56
	177	1.67	1.67	8.33	3.89
	181	---	---	3.33	1.11
	183	1.67	1.67	5.00	2.78
	185	5.00	8.33	3.33	5.56
	187	---	1.67	---	0.56
	189	6.67	10.00	5.00	7.22
	191	33.33	23.33	15.00	23.89
	193	5.00	5.00	13.33	7.78
	195	1.67	6.67	3.33	3.89
	197	3.33	3.33	5.00	3.89
	199	10.00	1.67	5.00	5.56
	201	5.00	5.00	---	3.33
	203	---	5.00	6.67	3.89
	205	5.00	5.00	3.33	4.44
	207	5.00	3.33	1.67	3.33
	209	1.67	1.67	5.00	2.78
	211	3.33	3.33	6.67	4.44
	213	1.67	1.67	---	1.11
	215	6.67	1.67	---	2.78
	217	---	1.67	3.33	1.67
	219	---	3.33	---	1.11
	223	3.33	3.33	6.67	4.44
	# alleles	**17**	**22**	**17**	**23**
	H_e	**0.8672**	**0.9215**	**0.9367**	**0.9133**

Ecap06	215	---	5.00	---	1.67
	217	8.33	11.67	10.00	10.00
	219	6.67	---	---	2.22
	225	---	---	1.67	0.56
	229	1.67	---	---	0.56
	231	5.00	5.00	1.67	3.89
	235	40.00	25.00	31.67	32.22
	237	1.67	5.00	1.67	2.78
	239	31.67	36.67	36.67	35.00
	241	5.00	11.67	16.67	11.11
	# alleles	**8**	**7**	**7**	**10**
	H_e	**0.735**	**0.7814**	**0.739**	**0.7524**

Ecap07	98	---	---	3.33	1.11
	104	---	5.00	---	1.67
	108	3.33	1.67	6.67	3.89
	110	1.67	6.67	6.67	5.00
	112	11.67	3.33	6.67	7.22
	114	8.33	5.00	11.67	8.33

	116	5.00	6.67	8.33	6.67
	118	10.00	---	6.67	5.56
	120	6.67	8.33	1.67	5.56
	122	10.00	8.33	5.00	7.78
	124	13.33	10.00	8.33	10.56
	126	6.67	10.00	13.33	10.00
	128	6.67	8.33	3.33	6.11
	130	3.33	11.67	3.33	6.11
	132	3.33	3.33	1.67	2.78
	134	---	3.33	1.67	1.67
	136	1.67	---	---	0.56
	138	5.00	3.33	---	2.78
	142	---	---	3.33	1.11
	144	---	---	3.33	1.11
	146	---	---	3.33	1.11
	148	3.33	1.67	1.67	2.22
	150	---	3.33	---	1.11
	# alleles	**16**	**17**	**19**	**23**
	H_e	**0.9339**	**0.9412**	**0.9424**	**0.9405**

Ecap08	129	11.67	8.33	1.67	7.22
	133	1.67	3.33	---	1.67
	135	---	3.33	3.33	2.22
	137	6.67	8.33	3.33	6.11
	139	3.33	15.00	3.33	7.22
	141	6.67	---	8.33	5.00
	143	35.00	38.33	35.00	36.11
	145	18.33	15.00	26.67	20.00
	147	10.00	5.00	5.00	6.67
	149	---	---	1.67	0.56
	151	6.67	3.33	11.67	7.22
	# alleles	**9**	**9**	**10**	**11**
	H_e	**0.8192**	**0.8017**	**0.7927**	**0.807**

Ecap09	134	5.00	1.67	---	2.22
	136	13.33	6.67	15.00	11.67
	138	8.33	8.33	3.33	6.67
	140	26.67	35.00	23.33	28.33
	142	1.67	5.00	1.67	2.78
	144	11.67	8.33	16.67	12.22
	146	20.00	20.00	15.00	18.33
	148	---	8.33	1.67	3.33
	150	6.67	3.33	13.33	7.78
	152	6.67	1.67	8.33	5.56
	154	---	1.67	1.67	1.11
	# alleles	**9**	**11**	**10**	**11**
	H_e	**0.8531**	**0.8215**	**0.8605**	**0.8462**

Lab206	188	15.00	1.72	6.67	7.87
	192	---	---	1.67	0.56
	196	---	---	1.67	0.56

	200	---	1.72	---	0.56
	204	6.67	5.17	6.67	6.18
	208	8.33	17.24	11.67	12.36
	212	11.67	10.34	6.67	9.55
	216	1.67	1.72	---	1.12
	220	35.00	22.41	31.67	29.78
	224	6.67	15.52	11.67	11.24
	228	5.00	13.79	16.67	11.80
	232	6.67	8.62	1.67	5.62
	236	3.33	1.72	3.33	2.81
	# alleles	**10**	**11**	**11**	**13**
	H_e	**0.8311**	**0.8699**	**0.8435**	**0.851**

Lab213	138	1.72	---	---	0.57
	142	10.34	8.33	6.90	8.52
	146	8.62	6.67	15.52	10.23
	150	---	1.67	3.45	1.70
	154	---	---	3.45	1.14
	158	---	1.67	1.72	1.14
	162	3.45	---	3.45	2.27
	166	3.45	1.67	3.45	2.84
	170	1.72	---	10.34	3.98
	174	12.07	3.33	---	5.11
	178	15.52	15.00	5.17	11.93
	182	12.07	15.00	13.79	13.64
	186	3.45	10.00	12.07	8.52
	190	15.52	20.00	8.62	14.77
	194	8.62	13.33	10.34	10.80
	198	1.72	---	1.72	1.14
	210	1.72	3.33	---	1.70
	# alleles	**14**	**12**	**14**	**17**
	H_e	**0.908**	**0.8876**	**0.9165**	**0.9073**

Lampsilis fasciola:

Locus	Allele	Wallen Bend	Frost Ford	Swan Island	All Sites
Ecap02	107	76.79	64.71	75.00	73.08
	109	1.79	11.76	---	3.85
	113	3.57	---	---	1.54
	121	1.79	5.88	5.00	3.85
	123	---	---	2.50	0.77
	125	3.57	---	2.50	2.31
	129	12.50	11.76	12.50	12.31
	131	---	5.88	---	1.54
	133	---	---	2.50	0.77
	# alleles	**6**	**5**	**6**	**9**
	H_e	**0.3987**	**0.5633**	**0.4282**	**0.4502**

Ecap04	94	3.57	17.65	15.00	10.77
	96	3.57	8.82	5.00	5.38
	98	1.79	---	---	0.77
	100	21.43	11.76	17.50	17.69
	102	26.79	26.47	45.00	32.31
	104	12.50	2.94	---	6.15
	106	3.57	5.88	---	3.08
	108	1.79	8.82	12.50	6.92
	110	1.79	2.94	---	1.54
	112	1.79	2.94	---	1.54
	114	1.79	---	---	0.77
	120	12.50	11.76	5.00	10.00
	122	7.14	---	---	3.08
	# alleles	**13**	**10**	**6**	**13**
	H_e	**0.8558**	**0.8752**	**0.7423**	**0.8352**

Ecap05	202	1.79	2.94	5.00	3.08
	204	3.57	---	2.50	2.31
	206	7.14	5.88	5.00	6.15
	208	3.57	---	2.50	2.31
	212	10.71	2.94	12.50	9.23
	214	8.93	17.65	5.00	10.00
	216	1.79	2.94	2.50	2.31
	218	7.14	2.94	7.50	6.15
	220	25.00	14.71	5.00	16.15
	222	5.36	5.88	2.50	4.62
	224	7.14	2.94	7.50	6.15
	226	1.79	11.76	17.50	9.23
	228	5.36	8.82	7.50	6.92
	230	1.79	5.88	2.50	3.08
	232	3.57	5.88	5.00	4.62
	238	---	---	2.50	0.77
	244	3.57	5.88	---	3.08
	248	1.79	---	---	0.77
	256	---	---	5.00	1.54
	258	---	2.94	2.50	1.54
	# alleles	**17**	**15**	**18**	**21**
	H_e	**0.9065**	**0.9305**	**0.941**	**0.9286**

Ecap06	224	3.57	21.88	10.00	10.16
	228	1.79	3.13	---	1.56
	234	5.36	---	2.50	3.13
	236	3.57	9.38	10.00	7.03
	238	8.93	15.63	7.50	10.16
	240	1.79	---	---	0.78
	242	1.79	---	---	0.78
	244	---	---	2.50	0.78
	248	1.79	---	---	0.78
	250	41.07	28.13	32.50	35.16
	252	21.43	21.88	35.00	25.78
	254	8.93	---	---	3.91
	# alleles	**11**	**6**	**7**	**12**

	H_e	**0.7766**	**0.8165**	**0.7641**	**0.7875**
Ecap07	107	3.57	2.94	---	2.31
	111	---	2.94	---	0.77
	119	1.79	---	2.50	1.54
	123	3.57	2.94	7.50	4.62
	125	1.79	---	---	0.77
	127	3.57	2.94	---	2.31
	129	3.57	---	7.50	3.85
	131	1.79	---	2.50	1.54
	133	1.79	---	7.50	3.08
	137	1.79	2.94	---	1.54
	139	10.71	8.82	10.00	10.00
	141	7.14	14.71	7.50	9.23
	143	5.36	14.71	7.50	8.46
	145	10.71	17.65	22.50	16.15
	147	5.36	---	---	2.31
	149	1.79	---	2.50	1.54
	151	---	---	7.50	2.31
	153	---	8.82	---	2.31
	155	3.57	2.94	2.50	3.08
	157	14.29	2.94	2.50	7.69
	159	7.14	5.88	5.00	6.15
	161	1.79	---	5.00	2.31
	163	3.57	---	---	1.54
	165	1.79	---	---	0.77
	171	3.57	2.94	---	2.31
	173	---	5.88	---	1.54
	# alleles	**22**	**15**	**15**	**26**
	H_e	**0.9461**	**0.9234**	**0.9205**	**0.9349**
Ecap08	137	---	5.88	---	1.56
	143	14.81	11.76	17.50	14.84
	145	22.22	11.76	10.00	15.63
	147	3.70	2.94	10.00	5.47
	149	1.85	---	---	0.78
	151	---	---	2.50	0.78
	153	16.67	17.65	10.00	14.84
	155	---	2.94	12.50	4.69
	157	14.81	14.71	5.00	11.72
	159	3.70	2.94	2.50	3.13
	161	1.85	5.88	7.50	4.69
	163	7.41	---	7.50	5.47
	165	9.26	8.82	5.00	7.81
	167	1.85	2.94	7.50	3.91
	169	1.85	2.94	2.50	2.34
	171	---	5.88	---	1.56
	179	---	2.94	---	0.78
	# alleles	**12**	**14**	**13**	**17**
	H_e	**0.877**	**0.9234**	**0.9231**	**0.9047**

Lab111	206	3.57	---	---	1.54
	222	3.57	---	2.50	2.31
	226	3.57	2.94	2.50	3.08
	230	5.36	5.88	---	3.85
	234	3.57	2.94	5.00	3.85
	238	8.93	2.94	2.50	5.38
	242	---	---	7.50	2.31
	246	3.57	8.82	15.00	8.46
	250	7.14	8.82	7.50	7.69
	254	10.71	11.76	5.00	9.23
	258	10.71	11.76	15.00	12.31
	262	10.71	8.82	7.50	9.23
	266	8.93	8.82	17.50	11.54
	270	7.14	14.71	7.50	9.23
	274	8.93	5.88	---	5.38
	278	---	---	2.50	0.77
	282	---	2.94	---	0.77
	286	1.79	2.94	---	1.54
	294	---	---	2.50	0.77
	298	1.79	---	---	0.77
	# alleles	**16**	**14**	**14**	**20**
	H_e	**0.9383**	**0.9358**	**0.9167**	**0.9286**

Lab206	184	---	2.94	---	0.77
	192	8.93	5.88	10.00	8.46
	204	1.79	8.82	2.50	3.85
	208	---	2.94	2.50	1.54
	212	8.93	2.94	5.00	6.15
	216	1.79	8.82	10.00	6.15
	220	17.86	11.76	2.50	11.54
	224	23.21	14.71	20.00	20.00
	228	14.29	17.65	22.50	17.69
	232	8.93	11.76	7.50	9.23
	236	10.71	5.88	5.00	7.69
	244	3.57	5.88	5.00	4.62
	252	---	---	2.50	0.77
	260	---	---	2.50	0.77
	276	---	---	2.50	0.77
	# alleles	**10**	**12**	**14**	**15**
	H_e	**0.8721**	**0.918**	**0.8949**	**0.889**

Lab213	152	1.79	2.94	---	1.54
	156	1.79	2.94	2.50	2.31
	160	---	2.94	---	0.77
	172	1.79	2.94	---	1.54
	176	---	2.94	5.00	2.31
	180	---	---	2.50	0.77
	184	7.14	2.94	---	3.85
	188	1.79	---	---	0.77
	192	7.14	5.88	2.50	5.38
	196	---	---	12.50	3.85
	200	7.14	5.88	10.00	7.69

	204	5.36	8.82	10.00	7.69
	208	7.14	---	5.00	4.62
	212	16.07	11.76	15.00	14.62
	216	5.36	5.88	2.50	4.62
	220	8.93	5.88	---	5.38
	224	8.93	11.76	7.50	9.23
	228	3.57	5.88	7.50	5.38
	232	3.57	---	10.00	4.62
	236	1.79	2.94	---	1.54
	240	3.57	---	---	1.54
	244	1.79	5.88	---	2.31
	252	3.57	---	2.50	2.31
	256	---	---	2.50	0.77
	260	---	2.94	---	0.77
	276	1.79	5.88	2.50	3.08
	304	---	2.94	---	0.77
	# alleles	**20**	**19**	**16**	**27**
	H_e	**0.9416**	**0.9608**	**0.9346**	**0.9431**

CHAPTER 2

AGE, GROWTH, AND POPULATION DEMOGRAPHY OF THREE FRESHWATER MUSSEL SPECIES (BIVALVIA: UNIONIDAE) IN THE CLINCH RIVER, U.S.A.

ABSTRACT

Age, shell growth, and population demography of two endangered mussel species, *Epioblasma brevidens* and *E. capsaeformis*, and a third non-listed species, *Lampsilis fasciola*, were studied from 2004-2007 in a 32-km reach of the Clinch River, TN. Observed maximum age and length of *E. brevidens* was 28 y and 71.5 mm for males and 11 y and 56.6 mm for females; of *E. capsaeformis*, 12 y and 54.6 mm for males and 9 y and 48.6 mm for females; and of *L. fasciola*, 45 y and 91.3 mm for males and 13 y and 62.6 mm for females. For all three species, observed maximum age and length was greater in males than females. Estimated population size in this river reach was 43,000 individuals for *E. brevidens*, 579,000 individuals for *E. capsaeformis*, and 30,000 individuals for *L. fasciola*. Mean recruitment y^{-1} of 1 y-old's ranged from 7.1% to 20% for *E. brevidens*, from 4.0% to 32.4% for *E. capsaeformis*, and from 5.8% to 25.6%. for *L. fasciola*. Population growth rate was 24.9% for *E. brevidens*, 34.6% for *E. capsaeformis*, and -22.4% for *L. fasciola*. Mortality rates of females were higher than those for males of *E. capsaeformis* and *L. fasciola*, but not of *E. brevidens*. Juvenile mussels were detectable but temporally and spatially variable in occurrence, and a significant component of the age-class structure of all three species. Recruitment was high during 2006-2007 for *E. capsaeformis* and other species, perhaps due to low river discharges in the spring-summer of 2005-2007. Surplus individuals of *E. brevidens* and *E. capsaeformis* are currently available to conduct translocations for restoration purposes.

Key words: freshwater mussels, *Epioblasma brevidens*, *E. capsaeformis*, *Lampsilis fasciola*, age, growth, population demography, juvenile recruitment.

INTRODUCTION

The decline of freshwater mussels is a global issue stemming from factors related to habitat loss and degradation of river and lake ecosystems (Neves et al., 1997; Lydeard et al., 2004). The management and restoration of mussels will require a thorough understanding of species life history and population biology (National Native Mussel Conservation Committee, 1998). The larvae (glochidia) of most mussel species require specific fish hosts in order to transform into juveniles and disperse into new habitats. Vital rates such as population growth, recruitment, and mortality are poorly understood for most species, but the studies that have been conducted have shown a wide range of population responses under various ecological conditions (Negus, 1966; Neves and Widlak, 1987; Hastie et al., 2000b; Payne and Miller, 2000; Haag, 2002; Villella et al., 2004; Howard and Cuffey, 2006). Most species are characterized by sporadic recruitment, high longevity (>20 years), and complex life histories. The lack of information on mussel population biology hinders the ability of managers to effectively develop and implement species recovery plans. Population dynamics data serve would critical management needs, including: (1) risk assessment of site-specific impacts, (2) identifying biotic and abiotic factors influencing population trends, (3) prioritizing imperiled species for recovery, and (4) identifying actions to restore and maintain demographically healthy populations of endangered species. The tools of conservation biology used to guide management activities, such as habitat assessment and restoration programs, population viability analysis (PVA), geographic information systems (GIS), and hatchery supplementation programs, are dependent on inputs of demographic data.

The population dynamics of mussel species will vary considerably over space and time, and are influenced greatly by life history traits such as longevity, somatic growth and fish host usage. Quantification of these vital rates and population demographic characteristics, such as age-class structure and density, is essential to monitoring efforts aimed at measuring effects of stream discharge, water temperature, habitat quality, fish host availability, sedimentation, nutrient levels, municipal wastewater discharge, and a suite of contaminants and other variables. Hence, a major challenge for natural resource managers will be to identify, quantify, and distinguish natural population fluctuations from changes caused by anthropogenic disturbances. This challenge can be met by studying the dynamics of healthy populations to serve as a baseline against which to judge the performance of populations in need of restoration and management.

The Clinch River in Hancock County, Tennessee, U.S.A. contains a diverse mussel assemblage of >40 species (Ahlstedt, 1991). Although this assemblage has been monitored for density and richness for 25 years, from 1979-2004 (Ahlstedt et al., 2005), no attempts were made to assess vital rates of these species, and only a few studies characterized age-class structure (Scott, 1994; Rogers et al., 2001; Jones and Neves, 2002; Jones et al., 2004). Populations of most species occurring in this river reach have been recruiting regularly and are considered sustainable, especially in the last 10 years. Thus, this reach offers an excellent opportunity to collect demographic data to establish species-level baselines. Three species were selected to study age, shell growth, and population demography, the two endangered species *Epioblasma brevidens* and *E. capsaeformis* and a third non-listed species *L. fasciola*. These species were selected because they exhibit differences in life history characteristics and population dynamics;

namely, in population growth rates and sizes, longevity, and dispersal abilities of primary fish hosts. For example, *E. capsaeformis* can achieve large local population sizes, but it is a relatively short-lived species (typically <10 years) with presumably poor dispersal capabilities. It utilizes small darter species in the subgenus *Nothonotus* as its primary fish hosts; e.g., redline darter *Etheostoma rufilineatum*. By comparison, *E. brevidens* is longer-lived, characterized by smaller population sizes and moderate dispersal capabilities, utilizing a relatively mobile, large darter as its primary fish host; i.e., logperch *Percina caprodes*. Similarly, *L. fasciola* rarely achieves large local population sizes, but has even greater dispersal ability than the two endangered species, utilizing black basses *Micropterus* spp. as its primary fish hosts. These different life history traits likely influence the ability of these species to maintain abundance and colonize habitats.

The purpose of this study was to collect demographic data to be able to implement scientifically defensible management actions, such as harvest and translocation of adults, to restore mussel populations in the Clinch River and in other rivers targeted for population restoration in the Tennessee River system. The intent also was to establish demographic baselines for extant species in the river, against which to judge future population performance and evaluate restoration success in other rivers. The objectives of this study were to compare population demography of *E. brevidens*, *E. capsaeformis* and *L. fasciola*, in terms of: (1) shell growth, maximum size and age, (2) vital rates of population growth, recruitment and mortality, (3) age-class structure and population size, (4) spatial and temporal variation of these parameters, and (5) assess whether these parameters are influenced by ecological variables. Although the study was focused on three species, the data collected and approach used can be applied to other species.

MATERIALS AND METHODS

Study area and site selection

The study area is a 32-km reach of the Clinch River from river kilometers (RKM) 277.1 to 309.6, Hancock, County, Tennessee, U.S.A. The reach is located in northeastern Tennessee just south of the Virginia border, in the Valley and Ridge physiographic province of the southern Appalachian Mountains. Since riverine mussels like to burrow into the stream bottom to feed, reproduce and avoid predation, they prefer stable shoals comprised of gravel and sand substrates. This type of habitat is abundant in the river, but typically interspersed with longer, slower-flowing pools (>1 RKM) containing poorer quality habitat. A typical gravel shoal is about 100-200 m long, but occasionally longer. The river is fourth-order throughout Hancock County and features moderate gradient riffle-run fluvial morphology. Because of high richness (>40 species) and endemism of the mussel species, the river is of national significance to conservation of mussel resources in the United States (Ahlstedt 1991, Neves et al. 1997). Furthermore, most endangered and non-listed mussel species currently exhibit recruitment of juveniles in this reach, presumably indicative of a "healthy", relatively undisturbed assemblage. Freshwater mussels were sampled at 13 sites, selected from those previously surveyed by Ahlstedt (1991). These sites represent all major shoals in the study reach and are summarized by locations, characteristics and sampling information (Table 1). A few small sites <1000 m^2 were not sampled because of difficult access, but the contribution of these mussel assemblages to total population abundance is considered minimal.

Age and growth

Shells of *Epioblasma brevidens*, *E. capsaeformis*, and *Lampsilis fasciola* were collected from the study area at various site locations from 2004-2006. A few shells of *L. fasciola* were obtained at sites in Virginia during the current or earlier collecting periods in the last 10 years (y). Shells of various lengths were collected to represent the population size-class structure of each species in the river. Thin-sections of shells were prepared following procedures described by Clark (1980) and Neves and Moyer (1988), using a Buehler Isomet low-speed saw unit with a diamond-impregnated blade (Buehler, Evanston, Illinois). Shells were cut from the center of the umbo to the ventral margin. Cut valves were glued (2-Ton Clear Epoxy, Illinois Tool Works, Devcon, Massachusetts) to petrographic microslides (27 × 46 mm), vacuum-sealed into a petrographic chuck, attached to the cutting arm of the saw, and sectioned at a thickness of 280 μm (Neves and Moyer 1988). Thin-sections of shells were examined under 40X magnification. Internal growth lines were considered true annuli if they were continuous from the umbo region to the outer surface of the shell. It was assumed, based on previous shell-aging in the rivers of southwest Virginia (Neves and Moyer 1988), that one annulus was formed each year. The assumption of annual shell ring deposition in freshwater mussels has been further validated in more than a dozen species in North America (Veinott and Cornett, 1996; Haag and Commens, 2008). Lengths for 1 to 3 y-old individuals, and occasionally older age classes, were obtained by back-calculating length-at-age based on internal annuli of 5-10 older shells (Bruenderman and Neves 1993) because small shells <3 y old and older, larger shells were difficult to collect from the river.

Live mussels collected from 2004-2007 were aged using predicted length-at-age as computed by a von Bertalanffy growth curve (VBGC) (von Bertalanffy 1938). The VBGC is written as:

$$L_t = L_\infty [1\text{-}e^{-k(t\text{-}t0)}]$$

where, L_∞ (L-infinity) is a theoretical maximum (asymptotic) length, k is a growth coefficient indicating how quickly L_∞ is approached, t is time or age in years, t_0 is the time in years when length would theoretically be equal to zero, and e is the natural log exponent. All three species are sexually dimorphic; thus, age and growth analyses were conducted separately for males and females. Sex ratio was determined using frequency of ≥2 y-old individuals because 1y-olds are not clearly dimorphic. The latter were randomly split using a 50:50 ratio unless the sex was discernable.

Population demography

Population demographic characteristics, such as population density and abundance, and age-class frequency, were estimated at various sites in the river from 2004-2007. All sampling was conducted in late summer or early fall when water levels were low and juvenile mussels had reached sizes (>10 mm) suitable for collection. Three sites, upper Wallen Bend (WB), upper Frost Ford (FF) and Swan Island (SI), were sampled consecutively each year to examine population change over time. These sites were selected because they represent the upper (RKM 309.9), middle (RKM 291.7) and

lower (RKM 277.2) boundaries of the study reach, and each have different habitat and location characteristics that are suitable for long-term monitoring. Data were collected by systematic, 0.25 m^2 quadrat samples placed along transect lines. Both quadrats and transects were evenly spaced throughout the entire shoal area. Total square area (m^2) of mussel beds was determined by multiplying mean river width, measured at 10 m intervals, by total length of the reach (Table 1). Small, exposed gravel bars and islands not containing mussels but within the immediate shoal area were measured and removed from analysis. Site dimensions (length and width) were measured using a standard 100 m measuring tape. Upstream and downstream limits of the bed were determined by visually inspecting for substrate composition (e.g., an abrupt change from suitable gravel substrate to unsuitable bedrock or soft sediments), water depth, flow velocity, and absence of mussels. All 0.25 m^2 quadrats were excavated to hardpan, or to approximately 20 cm in depth. Mussels were measured for length (nearest 0.1 mm) using digital calipers and replaced at their approximate position of collection. Mean population size at each site was estimated by multiplying mussel density (m^{-2}) by total site area.

Age frequencies of live mussels were determined using predicted length-at-age from the VBGC. Since the number of individuals collected was greater for *E. capsaeformis* and to maintain sampling consistency among sites each year, age frequencies for this species were estimated only from data collected at WB, FF and SI. However, because the number of individuals collected was much less for *E. brevidens* and *L. fasciola*, mean age frequencies were estimated from all sites sampled during 2004-2007, and total age frequencies y^{-1} from all sites sampled in a single year.

Population processes, such as growth rate and mortality rate, were estimated using standard demographic procedures described in Morris and Doak (2002) and Miranda and Bettoli (2007), respectively. For samples collected at yearly intervals during the current study, growth rate was computed at each time step using a discrete time population growth equation:

$$\lambda_t = (N_{t+1} / N_t)$$

where, λ_t is the annual population growth rate, N_t is the number of individuals (or density) in the population at year t, and N_{t+1} is number of individuals in the next year. The natural log ($\log_e$) of λ values was used to compute the arithmetic mean, standard error, and associated 95% confidence intervals (CI), and then transformed back using inverse $\log_e$. A time series of mussel density data (1979 to 2004) for the Clinch River, Hancock County, TN, was obtained from Ahlstedt et al. (2005). These data were collected by random 0.25 m^2 quadrat sampling at three sites (RKM 305.1, 295.6, and 277.1) in areas of each shoal containing high mussel densities to facilitate long-term monitoring. Although these data differed in sample design, they were collected using a consistent procedure and hence were suitable to provide estimates of annual population growth rate over a longer 25 y time period. Since these sites were sampled about every 5 y and occasionally at unequal intervals, the linear regression method proposed by Dennis et al. (1991) was used to estimate mean λ and associated 95% CIs; see Morris and Doak (2002) for a description of the method. The linear regression method assumes that censuses are

uncorrelated from one interval to the next. The Durbin-Watson *d* statistic was used to test for strength of temporal autocorrelation in the data.

A catch-curve regression analysis of number-at-age was conducted to estimate total mortality for each species:

$$\ln(N_t) = \ln(N_0) - Z(t)$$

where, N_t is the number in a year class at time t, N_0 is the original number in a year class, and Z is the instantaneous rate of mortality (Miranda and Bettoli, 2007). This procedure is computationally analogous to simple linear regression [$y=a - b(x)$], where the slope (b) is equivalent to Z. The instantaneous rate (Z) was converted to an interval or annual (y^{-1}) mortality rate (A), where $A=1-e^{-Z}$. Because the catch-curve procedure requires a large sample size encompassing a range of age and size classes to obtain a reliable estimate of mortality, estimates were obtained with both sexes combined and then separately to obtain sex-specific rates. The assumptions of catch-curve regression to estimate mortality are: (1) constant recruitment, (2) equal survival among year classes, (3) constant survival from year to year, (4) constant natural mortality each year and among all year classes, and (5) catch-curves are fitted to samples representative of the true age structure of the population (Miranda and Bettoli, 2007). An independent estimate of mortality was obtained by comparing density of dead shells to live individuals collected in quadrat samples. All empty shells were assumed to have died within a 1-year time period unless exhibiting signs of long-term erosion and dissolution; i.e., chalky and brittle nacre.

Since females of each species display a highly visible mantle-lure when releasing glochidia, the density (m^{-2}) of displaying females was quantified using quadrat sampling. A quadrat (N=12 per site) consisted of two 15.24 m long weighted lines evenly spaced 1.52 m wide, and systematically positioned along transect lines to provide even coverage of the site area. The long axis of the quadrat was oriented in the direction of flow, allowing a biologist to snorkel upstream between the lines to count female mussels.

Recruitment was defined in this study as the percentage of 1 y old individuals relative to the census size per site. Age-0 individuals typically are too small (<10 mm) to be reliably sampled. For all three species, mean recruitment percentage was calculated using data from all sites sampled in a year. The approximate lower end (lower bound) of the size range of 1 y old individuals and the predicted lengths of 2 y olds (upper bound) were used to categorize 1 y olds of each species (see Appendix). Basic correlation analyses were conducted to test for stock-recruitment relationships between number of displaying female mussels (stock) from 2004 to 2006 and number of 1 y old mussels (recruits) in subsequent years from 2005 to 2007. These analyses were conducted using only data from WB, FF, and SI where both data types were available.

Data analyses

Statistical tests were as follows: simple linear regression to test for significance of trends in the time series data, a general linear model analysis of variance (ANOVA) to test for pairwise differences in the time series, and Pearson correlation coefficient to test for positive or negative correlation between random variables. Statistical analyses were

conducted using MINITAB Statistical Software (Minitab, Inc., State College, Pennsylvania). Parameters of the von Bertalanffy growth equations of shell length and catch-curve mortality analyses and associated significance tests were estimated using Fisheries Analyses and Simulation Tools software (FAST 2.0) (Auburn University, Alabama).

RESULTS

Age and growth

Observed maximum age and length of *Epioblasma brevidens* was 28 y and 71.5 mm for males, and 11 y and 56.6 mm for females (Table 2). Predicted maximum length (L_∞) was 73.3 mm for males and 54.9 mm for females, and the growth coefficient (k) for each sex was 0.085 y^{-1} and 0.217 y^{-1}, respectively (Figure 1).

Observed maximum age and length of *E. capsaeformis* was 12 y and 54.6 mm for males, and 9 y and 48.6 mm for females. Predicted maximum length was 39.3 mm for males and 49.5 mm for females, and the growth coefficient for each was 0.439 y^{-1} and 0.276 y^{-1}, respectively. The oldest male (12 y) of *E. capsaeformis* was not included in computing the growth curve for males because the length of this individual upwardly biased the predicted length. The growth curve including this individual fit the data poorly (R^2=0.85) when compared to only using ages 0-10 y (R^2=0.99); therefore, this data point was considered an outlier and excluded from the growth curve analysis.

Observed maximum age and length of *L. fasciola* was 45 y and 91.3 mm for males, and 13 y and 62.6 mm for females. Predicted maximum length was 78.1 mm for males and 58.0 mm for females, and the growth co-efficient for each was 0.172 y^{-1} and 0.331 y^{-1}, respectively. Maximum age and length of females in this study did not match those of Scott (1994), who reported a maximum age and length of 24 y and 89.8 mm for shells collected in the upper Clinch River, VA. The sample size of thin-sectioned shells in the study by Scott (1994) was larger (N=91) and taken from four sites. Thus, maximum age and length of female *L. fasciola* in the TN reach of the river must be similar.

For all three species, observed maximum age and length was greater in males than females. Although based on current shell lengths, average lengths of mature females of *E. capsaeformis* were greater than males in the river. Predicted and observed shell growth for all three species and both sexes was highest through ages 0-5 y and then began to decrease thereafter (see Appendix for predicted growth increments). Differences in growth among species and sexes were reflected in VBGC parameter estimates of the growth coefficient k and L_∞. For example, females of *E. brevidens* and *L. fasciola* exhibited higher k and lower L_∞ than in males. The lowest observed k (0.085) was for males of *E. brevidens*, where the growth curve was more linear and gradually approached L_∞. Bauer (1992) and Hastie et al. (2000a) showed that these parameters were inversely correlated among European populations of *Margaritifera margaritifera*, where populations comprised mainly of small, short-lived adults displayed a higher relative mean growth rate compared to populations of larger, older individuals. A similar growth pattern generally held between sexes and species in this study, with shorter-lived, smaller females and species exhibiting higher k and lower L_∞. Exceptions include: (1) higher k

and lower L_∞ in males of *E. capsaeformis*, as males of this species typically are smaller (<42.0 mm) than females, which tends to increase *k* relative to females, and (2) the high *k* observed in females of *L. fasciola*. This high *k* value likely is anomalous and due to a lack of older and larger shells in the samples. Again, Scott (1994) found older, larger individuals and thus lower and perhaps more typical *k* values (0.16-0.21) for females of this species. However, the results of this study and others generally illustrate the interconnectedness between maximum age and size with growth rate; namely, that as the former two increase, the latter will decrease.

Population size and density

Estimates of total population size for all three species should be viewed as conservative, as marginal habitat areas were not surveyed. Individuals of each species are known to occur in such habitat but at very low density. Total population size of *E. brevidens* in the investigated reach was estimated at 43,426 individuals, with moderate to large differences observed among sites (Table 3). Local population size ranged from a low of 660 individuals at Briery Creek (RKM 280.8) to a high of 9,030 individuals at FF (RKM 291.8), with 95% CI greater than ±50% of the means. At sites monitored consecutively during 2004-2007, population sizes were stable from 2004-2006, with moderate increases detected during 2006-2007, although trends were not significant (Figure 2). Density was consistent among sites and sample years, ranging between 0.2-0.6 m^{-2} and always <1 m^{-2}. Likewise, the species occurred at densities ranging from 0.1-1.1 m^{-2} at twelve long-term monitoring sites sampled from 1979-2004 in Tennessee and

Virginia (Ahlstedt et al. 2005). During this 25-y period, most estimates of density were below 1 m^{-2} (Figure 3).

Total population size of *E. capsaeformis* was estimated at 579,141 individuals, with large differences observed among sites (Table 3). Local population size ranged from a low of 4,637 individuals at Sneedville (RKM 287.6) to a high of 330,097 individuals at FF, with 95% CIs typically less than ±50% of the means. At sites monitored consecutively, population sizes appeared stable during 2004-2006, and then increased greatly during 2006-2007 due to high juvenile recruitment (Figure 2). During this time period, the species density tripled at FF and reached a remarkable 21.9 m^{-2} in 2007, perhaps the highest recorded density of an endangered mussel species in North America. Similarly, density increased more than three-fold at WB and SI, but absolute density was much less than FF.

Total population size of *L. fasciola* was estimated at 29,532 individuals, with relatively modest differences observed among sites (Table 3). Local population size ranged from a low of 660 individuals at Briery Creek to a high of 5,700 individuals at FF, with 95% CIs typically ±60-100% of the means. At sites monitored consecutively, population sizes appeared stable during 2004-2007, with slight but insignificant decreases over time (Figure 2). Density of this species also was consistent among sites and sample years, ranging between 0.13-0.53 m^{-2} but always <1 m^{-2}. In 2005 at SI, no individuals of this species were collected in quadrat samples; thus, the estimate of 0.0 m^{-2} shown in Figure 2. The species obviously was present at the site but was not detected in quadrat samples because it occurred at a low density. It was found at densities <0.75 m^{-2} at long-term (1979-2004) monitoring sites sampled by Ahlstedt et al. (2005) (Figure 3).

Population age structure

Both mean frequency (two-dimensional histograms) and total frequency y^{-1} (three-dimensional histograms) of sampled individuals by age-class are shown in Figure 4. Mean age-class frequencies from 2004-2007 of males and females are given separately and were biased toward males for all three species. Total age-class frequencies y^{-1}, are displayed with sexes combined. Trends for each species during this period were for higher frequency of younger individuals and lower frequency of older individuals – a pattern expected for populations that are regularly recruiting. Sample sizes ranged from 15 to 33 individuals for *E. brevidens*, 105 to 311 for *E. capsaeformis*, and 16 to 26 for *L. fasciola*. Middle-aged (4-8 y) and older individuals (>9 y) dominated the samples taken from 2004-2006, whereas sub-adults (1-3 y) were prevalent in 2007, especially for *E. capsaeformis*. This species showed strong signs of recruitment of 1-y old individuals beginning in 2006, with an exceptionally large increase observed in 2007. Older individuals (>10 y) of *L. fasciola* were not observed, despite the high maximum age of males based on shell aging. Older individuals undoubtedly existed in the population, but their occurrence seemingly was uncommon and not detected in the study. In this reach, the population of this species currently appears to contain mostly younger individuals.

Number of displaying female mussels

Estimated number of displaying females of *E. brevidens* ranged from zero at WB in 2005 to a high of 818 at FF in 2004 (Figure 5). Among-site variation was high but

similar in magnitude, with estimates typically in the hundreds of individuals. No significant trends over time were detected. The number of displaying females relative to total sexually mature site^{-1} (≥5 y) was 0-100% y^{-1} over the study period (Table 4). No correlation (p=0.148) was observed between number displaying and site population size.

Estimated number of displaying females of *E. capsaeformis* ranged from a low of 165 at SI in 2006 to a high of 19,958 at FF in 2005. Among-site variation was high and dissimilar in magnitude, with estimates ranging from hundreds to many thousands of individuals. For example, the number of displaying females was much higher at FF, where estimates ranged from about 10,000-20,000 y^{-1}. Significant differences between some sample years were observed at WB and FF (Figure 5). The number of displaying females relative to total sexually mature site^{-1} (≥5 y) was 27-100% y^{-1}. A significant positive correlation (Pearson correlation=0.864, p=0.003) was observed between number displaying and site population size.

Estimated number of displaying females of *L. fasciola* ranged from zero at WB in 2005 to a high of 214 at FF in 2005. Among-site variation was high but similar in magnitude, with estimates ranging from dozens to about 200 female mussels. No significant trends were detected. Number of displaying females relative to total sexually mature site^{-1} (≥4 y) was 0-8% y^{-1}, and there was no correlation (p=0.407) between number displaying and site population size.

Juvenile recruitment

Recruitment of 1 y-old *E. brevidens* ranged from a low of 7.1% in 2006 to a high of 20% in 2004 (Figure 6). Variation within sample years was high, with 95% CIs typically exceeding yearly means by 100%. Mean recruitment of 1 y-olds estimated across sample years (2004-2007) was 15.7%. During this period, recruitment appeared consistent and no significant trends were detected. There was no significant correlation (p=0.968) between number of displaying females and number of recruits.

Recruitment of 1 y old *E. capsaeformis* ranged from a low of 4.0% in 2004 to a high of 32.4% in 2007. Variation within sample years was high, with 95% CIs typically 50% of yearly means. Mean recruitment of 1 y olds measured across all sample years was 19.2%, with a significant increase detected from 2004 to 2007. No significant correlation (p=0.067) was observed between number of displaying females and number of recruits at the 0.05 α-level. However, at a slightly higher α-level (e.g., 0.075), the relationship would be significant.

Recruitment of 1 y old *L. fasciola* ranged from a low of 5.8% in 2005 to a high of 25.6% in 2007. Variation within sample years also was high, with 95% CIs exceeding yearly means by 100%. Mean recruitment of 1 y olds measured across all sample years was 15.3%, with no significant trends detected. There was no significant correlation (p=0.339) between number of displaying females and number of recruits.

The mean number and total frequency of 1 y olds presented earlier in Figure 4 can be viewed in the context of recruitment. For example, total frequency of 1 y old *E. capsaeformis* was low (≤5) in 2004 and 2005, increased to >30 in 2006, and then jumped

to nearly 150 in 2007, whereas total frequencies of 1 y old *E. brevidens* and *L. fasciola* were more consistent and of similar magnitude, ≤ 8 (Figure 4). Hence, the data underlying Figures 4, 6, and 7 for all three species essentially are the same and mirror each other, but analyzed and presented differently to show different population characteristics and processes.

Population growth and mortality rates

Population growth rate of *E. brevidens* was 6.3% from 1979-2004, based on data collected by Ahlstedt et al. (2005), and 24.9% from 2004-2007 based on data obtained in the current study (Table 2). For all three species, no temporal auto-correlation was detected among censuses. From catch-curve regression analyses, mean annual mortality rate was 12.5% for males and 7% for females (Figure 7), and 14.8% with sexes combined (Table 2). The combined estimate was provided for all three species to supplement estimates obtained for each sex, due to lower sample sizes of males and females when analyzed separately (Figure 7). Mean mortality rate was 5.9% based on dead shells (sexes combined) obtained from quadrats (Table 2).

Population growth rate of *E. capsaeformis* was 12.5% from 1979-2004 and 34.6% from 2004-2007. Annual mortality rate was 21.0% for males and 44.0% for females, 27.9% for both sexes combined, and 7.9% based on dead shells. These rates for both parameters were the highest observed among all three species.

Population growth rate of *L. fasciola* was 14.3% from 1979-2004 and -22.4% from 2004-2007, the only declining growth rate observed among the investigated species.

Annual mortality rate was 16.0% for males and 21.0% for females, 24.2% for both sexes combined, and 5.9% based on dead shells. The mortality rate of females was higher than for males of *E. capsaeformis* and *L. fasciola*, but not for *E. brevidens*; however, female mortality of the latter was not significant (Figure 7).

DISCUSSION

Influence of life history traits on mussel population dynamics

Life history traits such as body size and lifespan are intrinsic to individuals within species and populations, and their expression imposes significant constraints on the structure and function of populations. For example, the shorter-lived *E. capsaeformis* must compensate for its relatively brief lifespan with higher population growth rate (r) and abundance. Unfortunately, such demographic data are limited for most mussel populations, but available studies on fishes can provide insights, especially since mussels utilize them as hosts. The classic paper by Beverton and Holt (1959) showed that populations of marine fishes comprised of smaller-sized, shorter-lived adults typically exhibited accelerated body growth (=higher k) and a compensative increase in r; those that contained larger-sized, longer-lived adults have lower k and r. Further, as k increased, the instantaneous mortality rate (Z) increased (Beverton and Holt 1959). Therefore, to a certain extent, population-level performance, as measured in growth and mortality, is bounded by factors intrinsic to individual-level performance, such as maximum age, size, age-at-maturity and fecundity. Life history traits in fishes have been

fundamental determinants of population performance and their investigation has been central to understanding ecology and resource management (Winemiller and Rose 1992).

The influence of age and growth on population dynamics of the European pearl mussel *Margaritifera margaritifera* was described in a series of papers by Bauer (1992 and references therein); as life span and maximum size increased, *k* declined. Generally, an increase in *k* was correlated with a reduction in life span, maximum size, and lower latitude populations (Bauer 1991). Somatic growth was positively influenced by extrinsic factors such as increased temperature and habitat productivity, such that maximum size and age correspondingly declined (Bauer 1992). In the early 20th century, some Bavarian populations had reached extremely high densities, where hundreds m^{-2} were known to have occurred over 20-30 km of stream length (Bauer 1991). However, as individual life span was very high (50-100 y), the inherent capacity of these populations to recruit and rebound from declines due to habitat degradation likely was limited and too slow. The observed pattern and rate of decline for many *M. margaritifera* populations was gradual and extended over a long trajectory, but one that ultimately led to extirpation. This fate is shared with many long-lived species of North American mussels; for example, where populations trapped in reservoir environments or other altered lotic conditions are cutoff from their hosts and are non-reproducing – such populations can persist for decades but ultimately die out.

The relationship between individual-performance and population-performance largely has been unexplored for North American freshwater mussels. The handful of studies reporting von Bertalanffy growth parameters loosely support the above hypotheses (Table 5), but lack the corroborative population-level data to draw inferences

between individual-level and population-level performance. For example, species belonging to the subfamily Ambleminae usually are longer-lived (16-61 y) and exhibit reduced *k* (0.01-0.19) when compared to species belonging to the subfamily Lampsilinae, which usually are shorter-lived (11-32 y), exhibit higher *k* (0.07-0.40), but vary widely among species. Further, most lampsilines are sexually dimorphic and exhibit differences in age and growth between sexes; e.g., females of *L. fasciola* and *Lemiox rimosus* have reduced age and size but higher *k* compared to males (Table 5). Most amblemines are not sexually dimorphic, making broader taxonomic-level comparisons difficult. These results suggest that mussel species, populations, and even sexes expressing the life history traits of short life span and high growth may warrant special conservation consideration. They are likely to have higher natural mortality and lower capacity to withstand long-term impacts, despite the possible advantages of higher *r*. Many of the shorter-lived lampsiline species, such as those in the genera *Epioblasma*, *Lemiox*, and *Villosa*, express these traits and therefore may be more vulnerable to local extirpation and ultimately extinction. In fact, in the Clinch River upstream of Norris Reservoir, three species of *Epioblasma* and two species of *Villosa* are now either extinct or extirpated from the river, respectively, and several species belonging to these genera are locally extirpated from reaches in the upper river in Virginia.

A general understanding of a species' life history and demographic characteristics provides the basis for effective population management. Recent life-history theory has placed less emphasis on categorizing species as purely *r*- and *K*-strategists; however, dominant themes of the theory such as density-dependent regulation, resource availability, and environmental variability are still prominent considerations in current

demographic and system modeling (Reznick et al. 2002). Although freshwater mussels exhibit traits of both *r*-strategists (high fecundity, low survival of newly metamorphosed juveniles, and no parental care) and *K*-strategists (increased longevity and high adult survival) (Villella et al. 2004), a wide continuum and mix of traits are expressed among species. Relative to other mussel species, *E. capsaeformis* expresses traits more characteristic of an *r*-strategist as described by MacArthur and Wilson (1967) and Pianka (1970), such as shorter life-span, higher population growth rate (r_{max}), smaller body size, and a population size that is more variable and non-equilibrium over time. Thus, even though classical life history theory cannot neatly categorize the demographic traits of most species, especially among a diverse group of invertebrates such as freshwater mussels, it still offers heuristic and predictive values that biologists can utilize to prioritize species for conservation.

Influence of ecological processes on mussel population dynamics

There are a range of familiar ecological processes that likely play a role in regulating mussel populations, such as drought, flood, seasonal stream temperatures, predation from muskrats, fishes and other animals, fish host availability, and habitat quality. How these extrinsic factors regulate populations needs further study, but clearly, many negative effects already have been documented (Hastie et al. 2001; Neves et al. 1997; Neves and Odom 1989). For example, losses from muskrat predation can be severe, exceeding 20% of population size in just a few years and hindering recovery of endangered mussels (Neves and Odom 1989). The positive influences on mussel

populations are less understood; certainly, clean water is a basic requirement, but given a healthy environment, what natural ecological processes are most responsible for their regulation? Are the important factors more abiotic (e.g., temperature, stream discharge) or biotic (e.g., inter-specific competition, disease) in nature? In a regulatory setting, how can one distinguish fluctuations due to natural vs. anthropogenic factors? Thus, it is important that sustained quantitative monitoring occur for populations of conservation concern to answer such questions. Two key questions that should always be considered are: (1) is the population of interest increasing or declining? and (2) how much statistical uncertainty exists in the assessment? As these are answered, the ecological and anthropogenic correlates can be sought.

Observed variation in population size and density among study species was striking. The shorter-lived *E. capsaeformis* achieved a much greater population size and density in a short 4 y time period, being 10 to 20-fold greater in abundance than *E. brevidens* and *L. fasciola*, and it was the only investigated species to significantly increase in population size from 2004-2007. Density of this species was high but locally quite variable, especially at FF where density and population size nearly tripled in 1 y, suggesting that population dynamics of this species are governed at a finer spatial-scale and more influenced by features of local habitat or fish fauna. In contrast, populations of the longer-lived *E. brevidens* and *L. fasciola* have remained seemingly stable over time, showing either slight increases or decreases, respectively. The density of each of these two species was lower, less variable, and similar among sites, suggesting that their dynamics are governed over larger spatial-scales. These results demonstrate that population change for some mussel species can be extremely rapid under the right

ecological conditions, whereas the pace of population growth for others can be insignificant under the same conditions. It is likely that *E. capsaeformis* is an outlier in this regard, but the paradigm of mussels being predominately long-lived and slow-growing needs to be reconsidered species by species, with careful attention given to their maximum age and growth rate.

The high recruitment and large increase in population size of *E. capsaeformis* during 2006-2007 are likely due in part to the moderate to low flows (discharge) that occurred in the spring-summer of 2005 and 2006 (Figure 8). First, it is possible that low discharge in the river during this critical period acted to facilitate mussel-host interactions and therefore to increase infestation of glochidia onto the host. The broad homogeneous shoal at FF provides ideal flow conditions for females to display and release their glochidia, being more laminar and less turbulent, and hence conducive to its small-body size and weight when in the hyporheic zone. During these conditions, water clarity is improved and the small darter (Percidae) hosts of *E. capsaeformis* are likely to be dispersed on shoals to feed, reproduce and interact with mussels rather than searching refuge due to high, turbulent flow. Discharge was <2000 cubic feet sec^{-1} (cfs) in late May and June during this period, followed in the summer months by low discharge and only a few minor spates of 2000-4000 cfs. The vast majority of female *E. capsaeformis* will display their mantle-lure to attract fish hosts typically from spring to early-summer (April-June) (Jones et al. 2005). While a few may display through July and into early August, the bulk and peak of the event occurs in this narrow time-frame. The host presumably is attracted initially to the color of the whitish-blue mantle-pad, and then, to the micro-lures that seemingly mimic cercae of larval-stage aquatic insects (Jones et al.

2006). Thus, moderate to low discharge conditions are probably important in aiding this complex interaction between the female mussel and host. Second, low discharge and reduced spates during the summer period likely facilitate the settlement, byssal-thread attachment, feeding, and growth of young juveniles, thus increasing their survival. Once glochidia have attached and encysted on a host, they usually take 2-4 wk to transform and excyst in the spring-summer water temperatures (21-24 °C) of the river (Jones et al. 2005). In contrast, discharge was much higher in 2003-2004, with major spates occurring throughout the spring and summer; recruitment of 1 y old juveniles was low in each following year. These discharge dynamics are likely favorable to *E. brevidens*, *L. fasciola* and other species as well, but the females of these two species display their mantle lures over a wider time-frame, to include the fall. Thus, infestation of glochidia on hosts and juvenile recruitment of these species may not be as affected by the spring-summer discharge period.

The effect of discharge on recruitment and settlement of juveniles has been hypothesized for some time (Cocker et al. 1921; Neves and Widlak 1987). Recent studies have shown that high and low discharge can strongly influence survival of both adults and juveniles. Recruitment of juvenile *Margaritifera falcata* in a California stream was more successful during low discharge years, with a 60% decline in recruitment observed during high discharge years (Howard and Cuffey 2006). In the summer of 2000, a severe drought in the southeastern United States caused record low flows in the Flint River basin (Golladay et al. 2004). These authors observed population increases of common mussel species and no change in the imperiled species at stream sites that maintained flow. Payne and Miller (2000) documented two very successful recruitment years in 1981 and

1990 for *Fusconaia ebena* in the lower Ohio River. These cohorts dominated the age-class structure for a decade or longer. They attributed this success to a rapid spring water rise that aggregated spawning *Alosa chrysochloris* – the only known host – over the mussel beds, which presumably enhanced glochidial infestation, and also to a quick return to normal flow conditions that allowed successful settlement of juveniles. Of course, record low discharge (100-150 cfs) was observed for several months in the Clinch River during the summers of 2005 and 2007, and as shown, recruitment of *E. capsaeformis* and other species was strong during these historic drought events. Studies thus point out that recruitment can be surprisingly robust under low discharge conditions. Some mortality of adults of this and other species was observed in the de-watered margins of shoals during this period, but high recruitment more than offset the losses. However, Haag and Warren (2008) caution that the effects of drought and low discharge can be context-specific, with high mortality occurring for mussel populations occupying small streams where dessication is more severe.

Flood conditions seem to have the opposite effect, inhibiting recruitment and inducing mortality in adults. In February 1998, a major flood occurred in the River Kerry of north-western Scotland, killing an estimated 50,000 *M. margaritifera* or 4-8% of the total population (Hastie et al. 2001). Large-scale channel destruction resulted in severe scouring and loss of mussel beds. Gangloff and Feminella (2007) studied mussel abundance and richness in relation to stream geomorphology at 24 sites in eight southern Appalachian streams and concluded that habitat conditions during floods, rather than during summer base-flow, was the limiting factor. Finally, other ecological factors such

as seasonal temperatures also may influence mussel population dynamics, especially as they relate to the brooding and release of glochidia (Jones et al. 2005).

Vital rates and age-structure of healthy mussel populations

The Clinch River, TN contains one of the best examples of a diverse assemblage of mussels in North America, with multiple cohorts and yearly recruitment for most species and a corresponding complexity of population responses. The river offers the opportunity to establish long-term monitoring in order to understand typical dynamics of numerous species. This knowledge is critical for establishing species-level baselines to gauge restoration efforts. So then, what should the recruitment rate, mortality rate and age structure of a healthy population look like? For a population to grow, obviously, the average survival rate must exceed the average mortality rate over some defined time period, but the intervening distribution of age-classes can accommodate a wide variety of population structures, especially for long-lived species, thus challenging design of population models and monitoring protocols to capture a full range of responses.

Mean age structure of *E. capsaeformis* was dominated by younger age classes, and annual recruitment averaged 19.2%. The annual mortality rates estimated by the linear catch-curves do not account for age-specific mortality, which is clearly lower for ages 1-5 compared to ages 6-10 (Figure 7). Therefore, based on expected survivorship taken from a stable age distribution and accounting for age-specific mortality, mean annual recruitment of 1 y-olds is about 15% (Figure 9). The recruitment pattern for this species was irregular and pulsed, characterized by boom and bust years, with the high

recruitment seen in 2006-2007 perhaps atypical and related to low discharge conditions. Of course, age structure will vary with changing conditions, but for short-lived species such as *E. capsaeformis*, recruitment will on average need to be ≥15% to maintain population stability and growth. The population is currently exceeding this replacement rate and hence growing. Sample sizes of live *E. brevidens* and *L. fasciola* were low, with no obvious patterns of age-specific mortality. No individuals >10 y of *L. fasciola* were collected, therefore the catch-curve estimates of male and female mortality of this species are unrealistically high. However, given the longer lifespan of these two species, average annual mortality is ~10-12%, suggesting that similar recruitment rates are needed to maintain homeostasis.

Mortality rates estimated from collected dead shells were <10% for all three species. This more conservative method provided independent verification for rates derived from catch or survivorship curves, and supports the claim that rates derived from the latter for *L. fasciola* were too high. However for *E. capsaeformis*, the mean mortality estimate based on the dead shell method is probably too low for two reasons. First, the shell of this species is small, thin, light-weight and thus susceptible to being washed away into pool areas during high flow events. In the spring, hundreds of fresh-dead female shells can be seen lying on the large shoals (e.g., FF) shortly after the peak of the display period, and they are nearly all gone 2-3 mo later. This mortality may be related to physiological stress involved with displaying for an extended time period (>2 wk). Second, the thin shell erodes and breaksdown quickly, perhaps <1 y based on field observations, and hence its detection in quadrat samples would be under-represented. The

general observed pattern of higher mortality and lower maximum age of females may be related to stress of brooding and releasing glochidia and vulnerability to predation.

Strayer et al. (2004) posed the following question; if mussel species are reproductively viable for decades, does their recruitment occur during most years or only rarely and under just the right combination of conditions? The results of this study and others of presumably healthy mussel populations can begin to answer this question. As shown here, it is obvious that short-lived (<15-20 y) species will need to sustain higher levels of recruitment or they will decline and die out. It is important to note that despite its capacity for fast population growth and to achieve high abundance, the short-lived *E. capsaeformis* is more susceptible to extirpation, whereas longer-lived (>20-30 y) species can afford to recruit less frequently and at lower levels in order to maintain their populations. Recruitment success will be driven by environmental conditions such as discharge; under the right conditions, recruitment can be very high and under the wrong conditions, very low. Therefore, patterns of recruitment success for many species will be characterized by both good and bad years. Some populations may recruit at low levels (e.g., <5%) for years and then occasionally under favorable conditions are punctuated with a large cohort, such as seen in *F. ebena* in the lower Ohio River (Payne and Miller, 2000). Villella and Smith (2004) studied the population dynamics of three Atlantic slope mussel species from 1996-2000 in the Cacapon River, West Virginia, a tributary of the Potomac River, and showed that recruitment rates for *Elliptio complanata* and *Lampsilis cariosa* were low (1-4%), while those of *E. fisheriana* were periodically high (15-23%). Haag (2002) studied the population dynamics of numerous Gulf slope mussel species from 1999-2001 (this study is ongoing – W.R. Haag, U.S. Forest Service pers. comm.) in

the Sipsey River, AL, a tributary of the Tombigbee River, and showed that recruitment rates of *Elliptio arca*, *Fusconaia cerina*, *Pleurobema decisum*, *Quadrula asperata*, and other species were highly variable along taxonomic, spatial and temporal scales, ranging from nearly 0 to 60%. Thus, highly variable recruitment is likely a normal pattern for most riverine species, which inevitably will lead to unequal and disparate age-class structures. However, recent studies conducted on healthy populations have shown that while juvenile mussels can be temporally and spatially variable in occurrence, they are a detectable and significant feature of the age-class structure. Importantly, these studies provide a quantitative basis to evaluate the performance of populations that are being restored or those in decline.

Summary and conclusions

Density and size-class data of mussels now have been collected in the Clinch River, TN, for 28 y. Populations of most species have maintained abundance or seen positive growth during this period. A few of the short-term, summer brooding species of the genera *Fusconaia*, *Pleurobema*, and *Lexingtonia* appear to have declined but continue to recruit in the river. Many of the nearly 40 species in this reach of river occur at a density of <1 m^{-2} and thus are difficult to accurately assess for recruitment and age-class structure. Nonetheless, they have persisted for decades, again emphasizing the importance of long-term monitoring and adequate time series data to understand demographic trends of long-lived and uncommon species. A comprehensive approach was used in this study to assess population demographics of three mussel species,

incorporating information from *intrinsic* factors such as maximum lifespan, maximum size, shell growth rate, and fish host usage and *extrinsic* factors such as river discharge and habitat quality. The results of this study allowed for the following conclusions:

- Maximum shell length and age of *E. capsaeformis* were lower than for *E. brevidens* and *L. fasciola*, indicating this shorter-lived species has comparatively higher vital rates.
- Population growth, recruitment, and mortality rates of *E. capsaeformis* were correspondingly higher than those of *E. brevidens* and *L. fasciola*.
- Juveniles, sub-adults, and young to middle-aged adults dominated the age-class structure of all three species, indicating young populations and active recruitment for each.
- Recruitment success of *E. capsaeformis* was temporally variable and likely influenced by low stream discharge in spring and early summer.
- Density of *E. brevidens* and *L. fasciola* remained stable and at similar but low levels (<1 m^{-2}) among sites over a 28 y period. In contrast, density of *E. capsaeformis* was temporally and spatially variable, exhibiting a significant increase over time and wide-ranging levels among sites (0.0-21.9 m^{-2}).
- Density of *E. capsaeformis* was much higher at larger sites, such as Frost Ford, indicating that population dynamics of this species are controlled at finer spatial scales. In contrast, density of *E. brevidens* and *L. fasciola* were similar among sites, indicating dynamics of these species are controlled at larger spatial scales.

A surplus of adult *E. brevidens* and *E. capsaeformis* is currently available at investigated sites in the Clinch River, TN to conduct translocations for restoration purposes. Because of the current abundance and positive growth rate of these species in the river, conducting translocations to restoration sites is feasible. Appropriate numbers of adult mussels to be harvested site^{-1} y^{-1} were determined using a stochastic harvest model, where the recommended harvest was ≤1% of local population size y^{-1} (Chapter 3).

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Table 1. Location, dimensions, and sampling information for sampled sites in the Clinch River, TN, *Data sampled in 2004 for these sites were obtained from Ostby (2005) and Ahlstedt et al. (2005), respectively.

Site location name	River Kilometer (River Mile)	Latitude	Longitude	Site Dimensions			No. (*n*) 0.25 m^2 quadrats y^{-1}	Year (s) sampled
				Length (m)	Width (m)	Area (m^2)		
Wallen Bend (upper)	309.6 (192.4)	36° 34' 44.85"	83° 00' 10.59"	43	74	3,182	60	2004-2007
Wallen Bend (lower)	309.5 (192.3)	36° 34' 50.24"	83° 00' 20.82"	410	41.3	16,933	120	2007
Kyles Ford*	305.1 (189.6)	36° 33' 43.54"	83° 02' 23.48"	200	75	15,000	146	2004
Webb Island	301.7 (187.5)	36° 33' 04.22"	83° 03' 49.14"	143	32	4,576	60	2006
Brooks Island (upper)*	295.6 (183.7)	36° 32' 12.23"	83° 07' 19.19"	80	45	3,600	26	2004
Brooks Island (lower)	295.3 (183.5)	36° 32' 12.50"	83° 07' 34.88"	120	50	6,000	60	2005
Little E Island	293.7 (182.5)	36° 32' 27.77"	83° 08' 47.92"	160	70	11,200	60	2005
Frost Ford (upper)	291.8 (181.3)	36° 31' 56.20"	83° 09' 2.41"	215	70	15,050	60	2004-2007
Frost Ford (lower)	291.3 (181.0)	36° 31' 48.88"	83° 09' 3.13"	172	50	8,600	72	2007
Falls Branch Shoal	288.7 (179.4)	36° 31' 18.68"	83° 11' 39.6"	127	42	5,334	40	2006
Sneedville	287.6 (178.7)	36° 31' 11.06"	83° 11' 25.07"	56	36	2,016	40	2006
Briery Creek Shoal	280.8 (174.5)	36° 29' 53.62"	83° 15' 26.52"	150	44	6,600	40	2006
Swan Island	277.1 (172.2)	36° 28' 23.84"	83° 17' 23.71"	128	45	5,760	60	2004-2007

Table 2. Summary of key life history and population parameters for investigated species in the Clinch River, TN. All data are from the current study (2004-2007) except those collected from 1979-2004 by Ahlstedt et al. (2005). The population mortality rates were calculated by combining data from both sexes and using two independent methods: (1) dead shells collected from quadrats, and (2) catch-curve regression analysis. Sex-specific mortality rates are reported in Figure 6.

	Maximum observed age (y)		Maximum observed length (mm)		Total number of individuals ≥2 y old (2004-2007)		Population growth rate λ (±95% CI) expressed as annual net growth		Population mortality rate (±95% CI)	
Species	Males	Females	Males	Females	Males	Females	1979-2004	2004-2007	Dead shells	Catch-curve
Epioblasma brevidens	28	15	71.5	56.6	44	34	6.3% (±11.3%)	24.9% (±95.3%)	5.9% (±11.8%)	14.8% (±5.0%)
Epioblasma capsaeformis	12	9	54.6	48.6	271	243	12.5% (±22.5%)	34.6% (±132.6%)	7.9% (±6.0%)	27.9% (±17.4%)
Lampsilis fasciola	45	13	91.3	62.6	47	25	14.3% (±26.0%)	-22.4% (±71.1%)	5.9% (±7.0%)	24.2% (±12.8%)

Table 3. Estimates of population size for investigated mussel species in the Clinch River, TN; only data from 2007 were used to calculate population sizes for sites sampled in multiple years.

SITE	Year (s) sampled	Number of Individuals (± 95% CI)		
		Epioblasma capsaeformis	*Epioblasma brevidens*	*Lampsilis fasciola*
Wallen Bend (upper)	2004-2007	16,122 ± 4,143	1,061 ± 897	849 ± 810
Wallen Bend (lower)	2007	38,946 ± 14,841	3,951 ± 4,032	5,644 ± 4,755
Kyles Ford	2004	35,400 ± 8,232	7,800 ± 3822	5,700 ± 3,528
Webb Island	2006	6,711 ± 3,069	1,220 ± 1,165	1,525 ± 1,291
Brooks Island (upper)	2004	9,415 ± 4,681	3,877 ± 3,342	1,108 ± 1,504
Brooks Island (lower)	2005	13,600 ± 7,527	1,200 ± 1,334	1,600 ± 1,527
Little E Island	2005	59,901 ± 13,893	1,007 ± 1,388	3,523 ± 2,521
Frost Ford (upper)	2004-2007	330,097 ± 75,188	9,030 ± 5,482	3,010 ± 3,346
Frost Ford (lower)	2007	32,967 ± 8,155	3,822 ± 2,514	956 ± 1,314
Falls Branch	2006	15,469 ± 7,026	5,334 ± 2,902	3,200 ± 2,393
Sneedville	2006	4,637 ± 1,781	1,008 ± 838	605 ± 875
Briery Creek	2006	8,580 ± 5,039	660 ± 1,295	660 ± 1,295
Swan Island	2004-2007	7,296 ± 3,305	3,456 ± 2,357	1,152 ± 1,281
	TOTAL	**579,141 ± 156,880**	**43,426 ± 31,368**	**29,532 ± 26,440**

Table 4. Percentage of displaying female mussels relative to the number of sexually mature females per site is reported with number of females observed displaying (in parentheses). Percentages given should be considered rough approximations, as the 95% CIs are large (>50-100% of mean values) for both sexually mature females (CIs not given) and displaying females (see Figure 4 for CIs). Females of *Epioblasma brevidens* and *E. capsaeformis* were considered mature at ≥5 y and *Lampsilis fasciola* at ≥4 y. ND = not enough data to make a calculation because no mature females were collected in the quadrat samples.

		Sites		
Species	Sample year	Wallen Bend	Frost Ford	Swan Island
Epioblasma brevidens	2004	ND (104)	82% (818)	100% (781)
	2005	0% (0)	12% (486)	ND (227)
	2006	43% (91)	32% (324)	100% (391)
Epioblasma capsaeformis	2004	100% (2111)	66% (9980)	100% (500)
	2005	100% (850)	59% (19,958)	27% (309)
	2006	100% (422)	65% (13,608)	ND (165)
Lampsilis fasciola	2004	8% (35)	4% (82)	4% (31)
	2005	0% (0)	ND (214)	ND (41)
	2006	ND (46)	ND (107)	ND (0)

Table 5. Observed maximum age (A_{max}) and the estimated von Bertalanffy growth parameters of asymptotic length (L_∞) and growth constant (k) of North American (U.S.A.) mussel species reported from other studies. *The study by Scott (1994) includes additional estimates of A_{max} and L_∞, but only the maximum values are reported here; thus, associated k values are at the lower-end but likely more representative estimates.

Subfamily and species	Location	A_{max}	L_∞	k	Study
Ambleminae					
Amblema plicata	Ouachita River, Arkansas	25 y	87 mm	0.13	Christian et al. 2000
	White River, Arkansas	25 y	58 mm	0.19	
Elliptio dilitata	Clinch River, Virginia	61 y	98 mm	0.10	Scott 1994*
Fusconaia cor	Clinch River, Virginia	20 y	65 mm	0.12	Kitchel 1985
Fusconaia cuneolus	Clinch River, Virginia	32 y	82.4 mm	0.13	Brunderman and Neves 1993
Fusconaia ebena	White River, Arkansas	27 y	116 mm	0.13	Christian et al. 2000
	Black River, Arkansas	51 y	58 mm	0.18	
Megalonaias nervosa	St. Francis River, Arkansas	41 y	218 mm	0.04	
	Cache River, Arkansas	42 y	185 mm	0.01	
Quadrula quadrula	Ozark Reservoir, Arkansas	16 y	20 mm	0.10	
	Dardanelle Reservoir, Arkansas	24 y	49 mm	0.13	
Lampsilinae					
Actinonaias pectorosa	Clinch River, Virginia	21 y	146 mm	0.08	Scott 1994
Cyprogenia stegaria	Clinch River, Tennessee	26 y	53 mm	0.15	Jones and Neves 2002

Dromus dromas	Clinch River, Tennessee	25 y	70 mm	0.12	Jones et al. 2004
Lampsilis fasciola (♂)	Clinch River, Virginia	32 y	87 mm	0.13	Scott 1994
Lampsilis fasciola (♀)		24 y	78 mm	0.16	
Lemiox rimosus (♂)	Clinch River, Tennessee	15 y	78 mm	0.07	Jones et al. 2008
Lemiox rimosus (♀)		11 y	35 mm	0.31	
Lemiox rimosus (♂)	Duck River, Tennessee	15 y	56 mm	0.24	
Lemiox rimosus (♀)		11 y	38 mm	0.40	
Medionidus conradicus	Clinch River, Virginia	24 y	61 mm	0.19	Scott 1994
Villosa iris		25 y	79 mm	0.09	

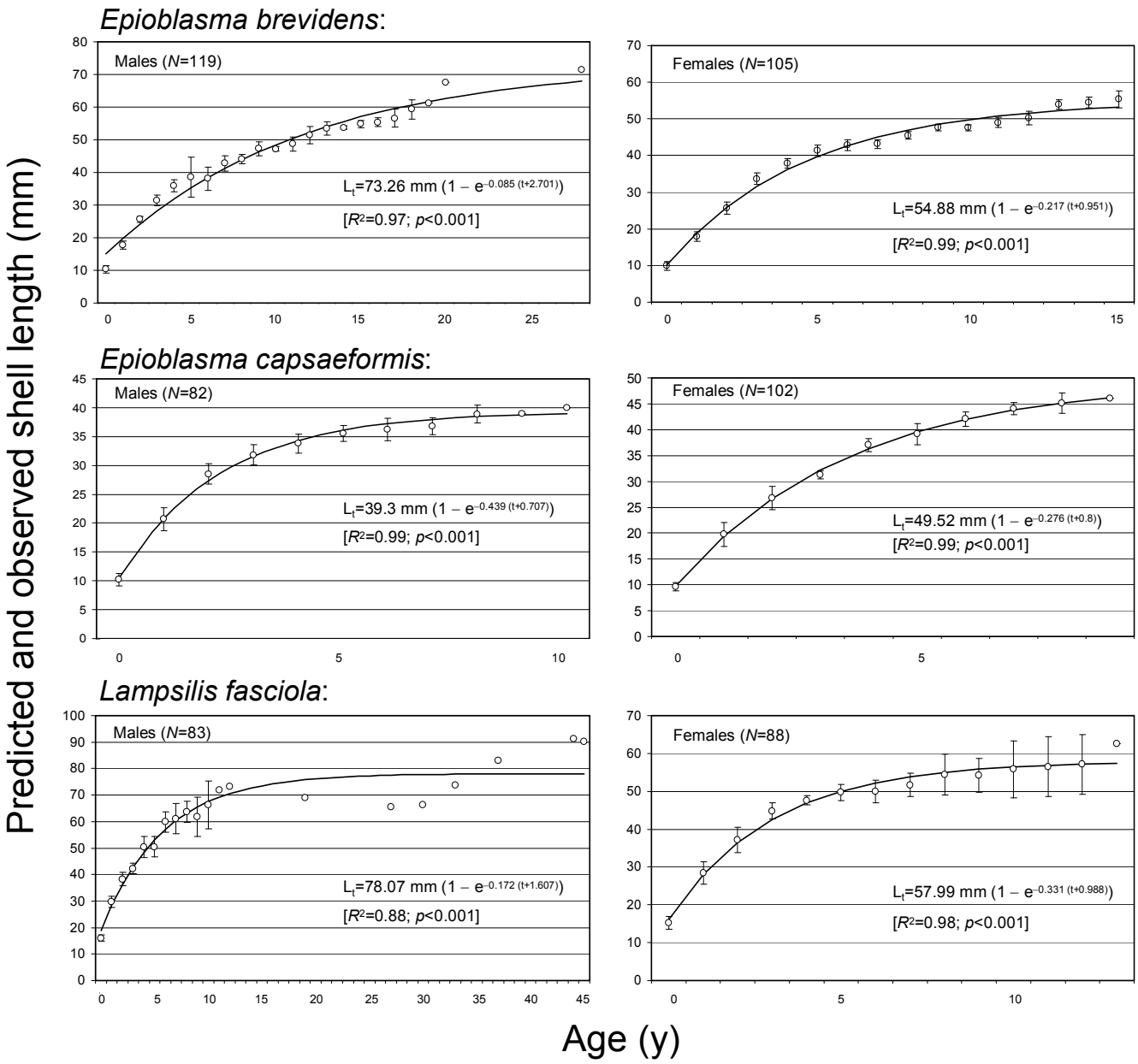

Figure 1. Estimated von Bertalanffy growth curves of predicted length-at-age (solid line) for investigated species in the Clinch River, TN, U.S.A. Mean observed length-at-age is shown by open circles along with 95% confidence intervals. Circles without confidence intervals denote only one observation (n=1) at that age.

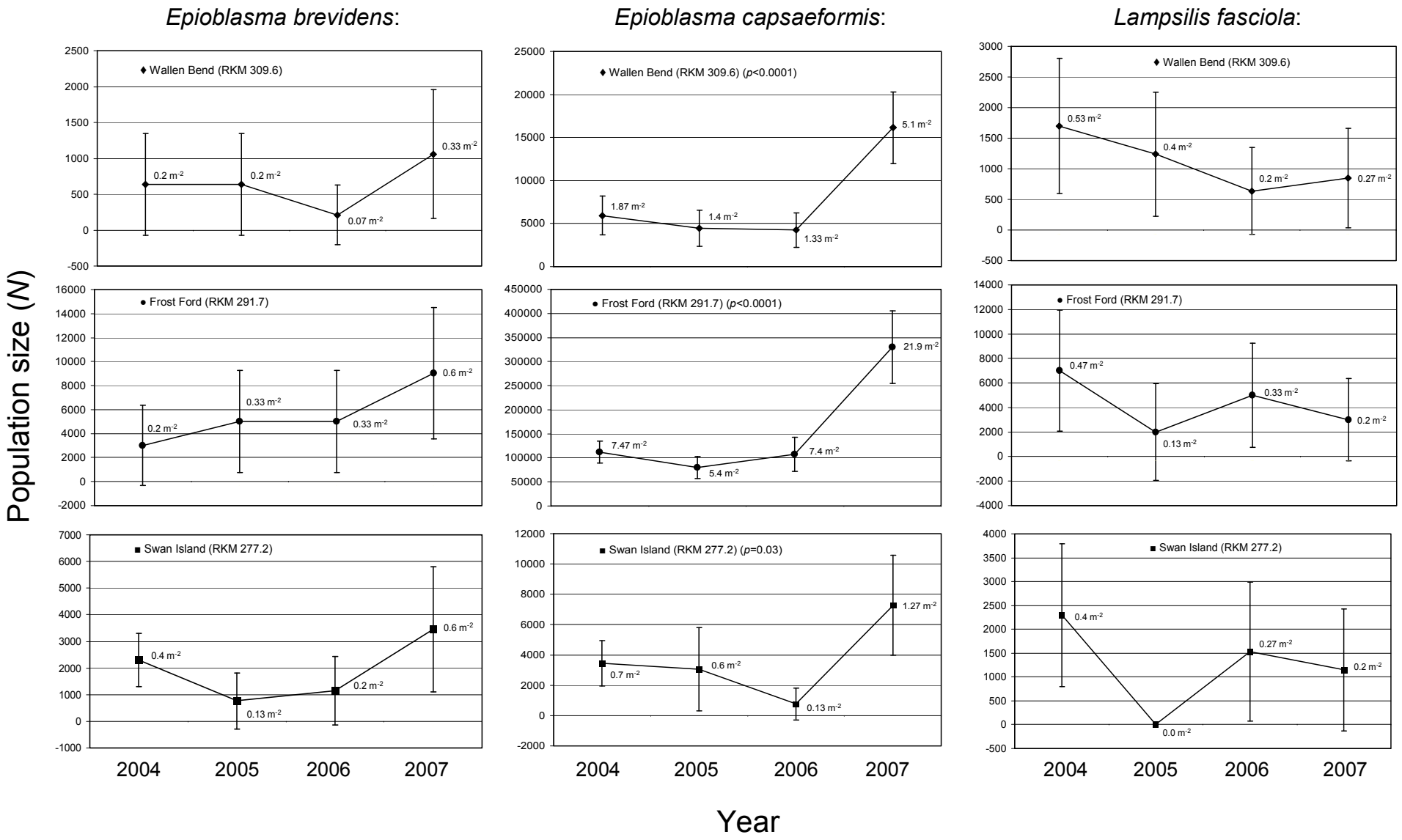
Epioblasma brevidens:
Epioblasma capsaeformis:
Lampsilis fasciola:
Population size (*N*)
♦ Wallen Bend (RKM 309.6)
0.2 m-2
0.2 m-2
0.07 m-2
0.33 m-2
♦ Wallen Bend (RKM 309.6) (*p*<0.0001)
1.87 m-2
1.4 m-2
1.33 m-2
5.1 m-2
♦ Wallen Bend (RKM 309.6)
0.53 m-2
0.4 m-2
0.2 m-2
0.27 m-2
● Frost Ford (RKM 291.7)
0.2 m-2
0.33 m-2
0.33 m-2
0.6 m-2
● Frost Ford (RKM 291.7) (*p*<0.0001)
7.47 m-2
5.4 m-2
7.4 m-2
21.9 m-2
● Frost Ford (RKM 291.7)
0.47 m-2
0.13 m-2
0.33 m-2
0.2 m-2
■ Swan Island (RKM 277.2)
0.4 m-2
0.13 m-2
0.2 m-2
0.6 m-2
■ Swan Island (RKM 277.2) (*p*=0.03)
0.7 m-2
0.6 m-2
0.13 m-2
1.27 m-2
■ Swan Island (RKM 277.2)
0.4 m-2
0.0 m-2
0.27 m-2
0.2 m-2
2004
2005
2006
2007
Year

Figure 2.

Figure 2. Estimates of population size for investigated species at three sites in the Clinch River, TN, sampled consecutively from 2004-2007. Error bars represent 95% confidence intervals. The corresponding site density is shown next to population size. Reported *p*-values indicate that a significant increase in population size occurred for *Epioblasma capsaeformis* in 2007, compared to previous sampled years.

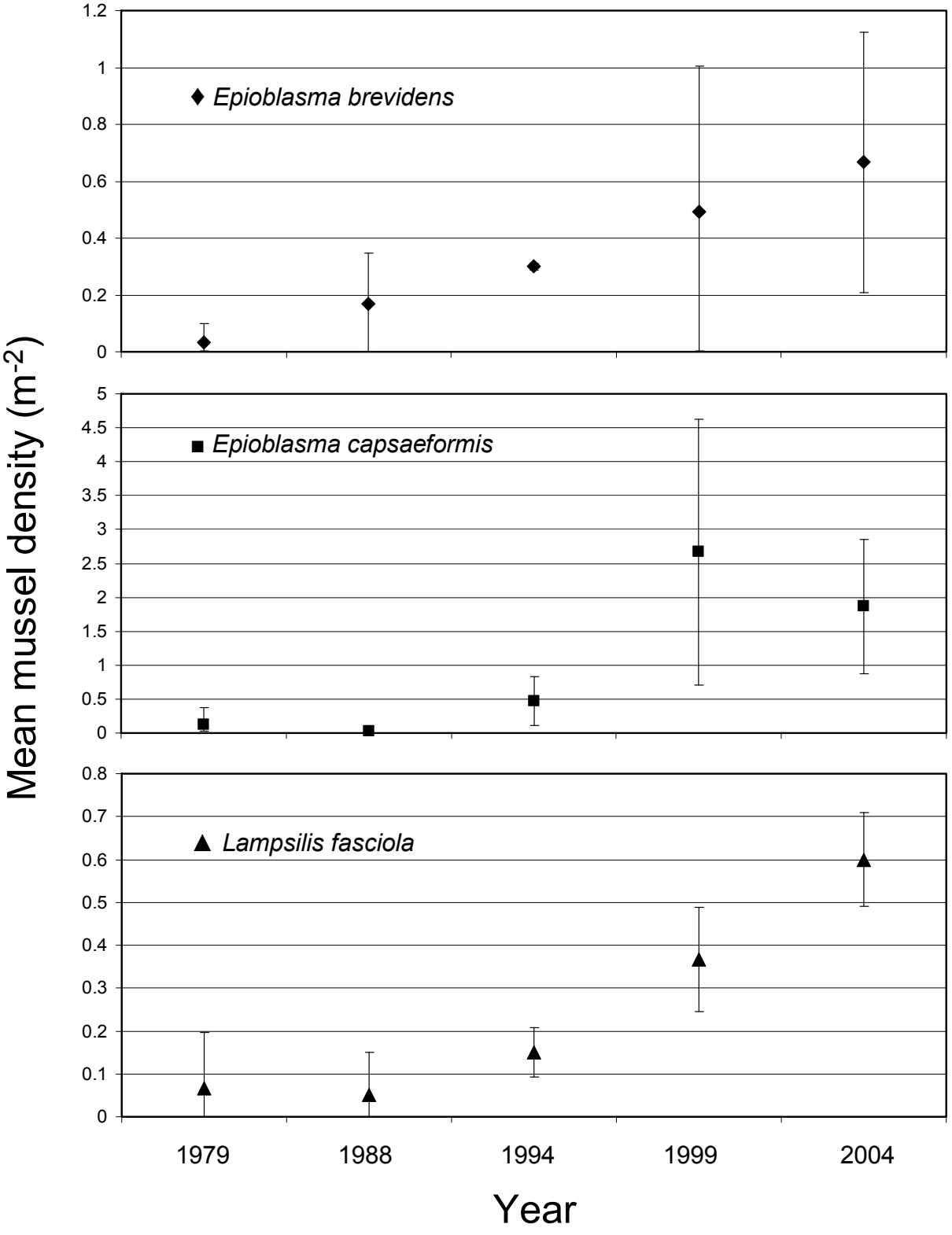
Mean mussel density (m-2)
♦ Epioblasma brevidens
■ Epioblasma capsaeformis
▲ Lampsilis fasciola
1.2
1
0.8
0.6
0.4
0.2
0
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1979
1988
1994
1999
2004
Year

Figure 3.

Figure 3. Mean density for investigated species over a 25 y period (1979-2004) estimated from three sites [Swan Island (RKM 277.1), Brooks Island (RKM 295.6), and Kyles Ford (RKM 305.1)] in the Clinch River, TN; data are from Ahlstedt et al. (2005). Error bars represent 95% confidence intervals.

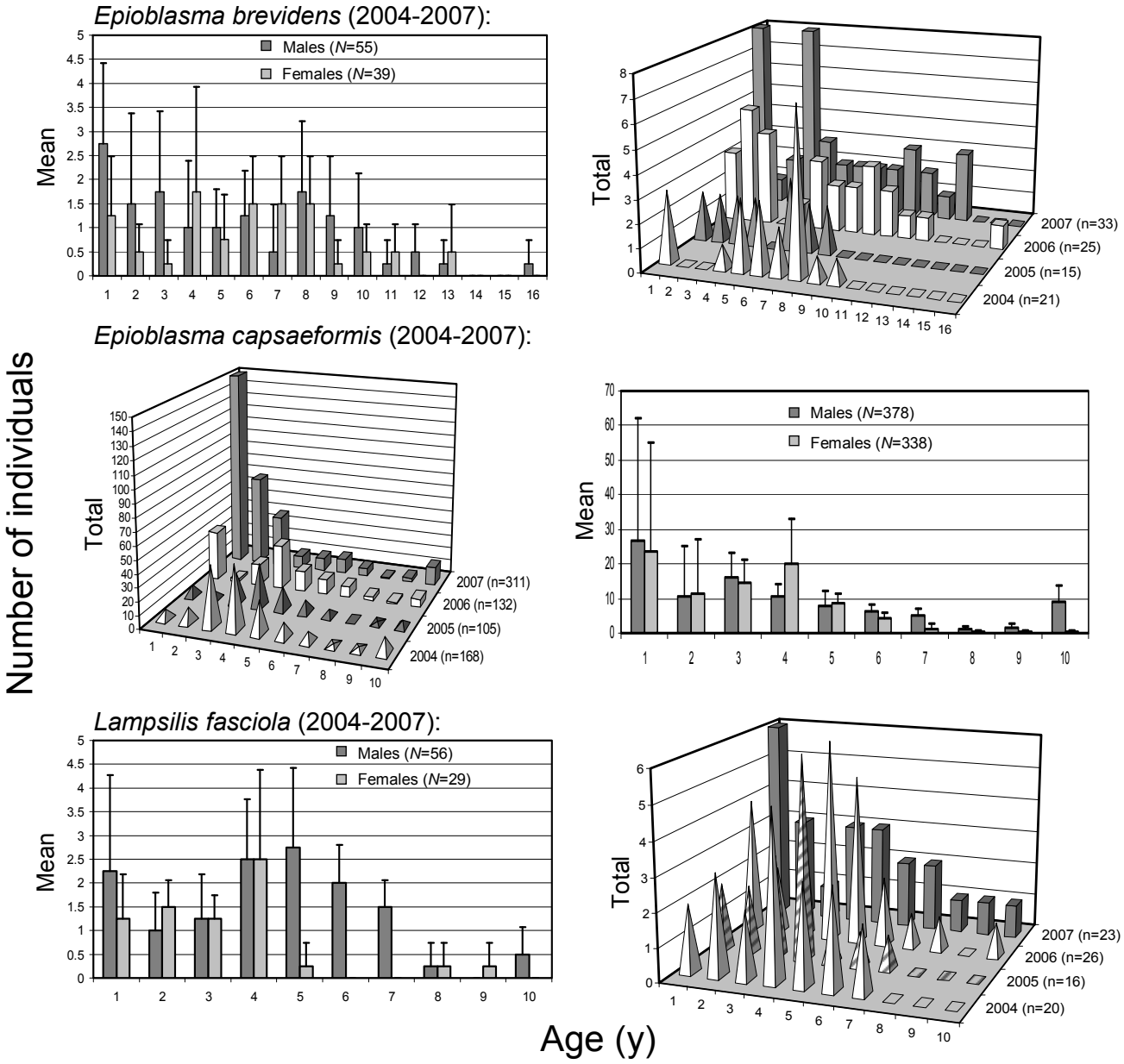
Epioblasma brevidens (2004-2007):
Males (*N*=55)
Females (*N*=39)
Mean
Total
2007 (n=33)
2006 (n=25)
2005 (n=15)
2004 (n=21)
Epioblasma capsaeformis (2004-2007):
Males (*N*=378)
Females (*N*=338)
Mean
Total
2007 (n=311)
2006 (n=132)
2005 (n=105)
2004 (n=168)
Lampsilis fasciola (2004-2007):
Males (*N*=56)
Females (*N*=29)
Mean
Total
2007 (n=23)
2006 (n=26)
2005 (n=16)
2004 (n=20)
Number of individuals
Age (y)

Figure 4.

Figure 4. Population age histograms for investigated species in the Clinch River show (1) mean frequencies for males and females, where error bars represent 95% confidence intervals, and (2) total frequencies for each year sampled. Because sample sizes were small for *Epioblasma brevidens* and *Lampsilis fasciola*, mean frequencies were estimated from all sites sampled during 2004-2007 and total frequencies year^{-1} from all sites sampled in a single year. In contrast, sample sizes were much larger for *E. capsaeformis*; therefore, to maintain sampling consistency among sites year^{-1}, age frequencies were estimated only from data collected at WB, FF and SI (RKM 309.9, 291.7, and 277.2, respectively). No quantitative differences exist between differently shaped and colored frequency bars in 3-dimensional histograms, which were used only to help visualize population structure of sample years.

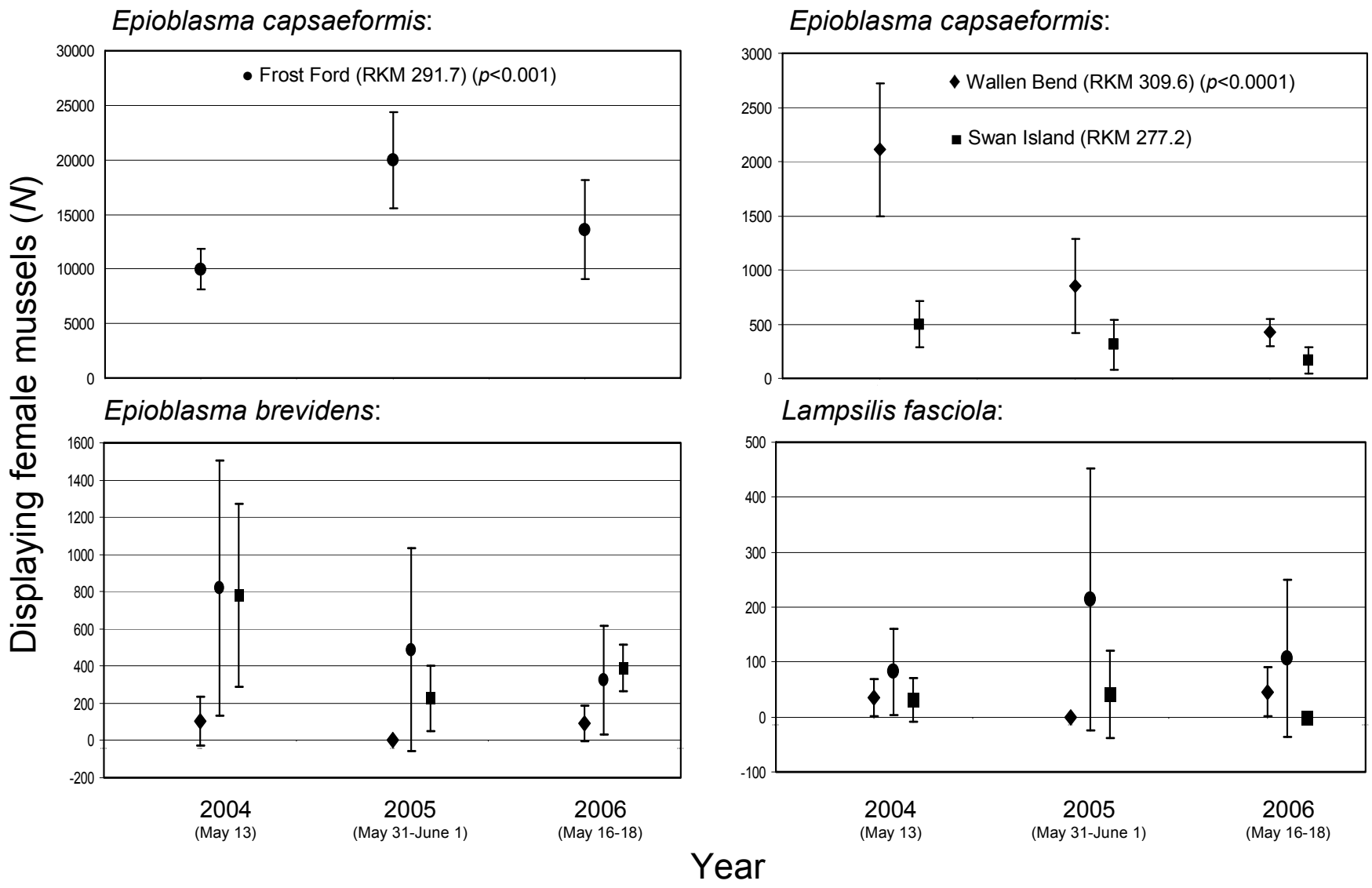
Epioblasma capsaeformis:
● Frost Ford (RKM 291.7) (p<0.001)
Epioblasma capsaeformis:
♦ Wallen Bend (RKM 309.6) (p<0.0001)
■ Swan Island (RKM 277.2)
Epioblasma brevidens:
Lampsilis fasciola:
Displaying female mussels (N)
30000
25000
20000
15000
10000
5000
0
3000
2500
2000
1500
1000
500
1600
1400
1200
800
600
400
300
200
100
-200
-100
2004
(May 13)
2005
(May 31-June 1)
2006
(May 16-18)
Year

Figure 5.

Figure 5. Estimated number of displaying female mussels for investigated species at three sites in the Clinch River, TN, sampled consecutively from 2004-2007. Error bars represent 95% confidence intervals. Reported *p*-values indicate either significant increases or decreases in number of displaying females of *Epioblasma capsaeformis* at FF (2005 only) or WB, respectively, compared to previous sample years. Sample dates are when data were collected, but also represent the approximate peak of the display period for female *E. capsaeformis* (Jones et al. 2005), which typically coincides with receding water level (<1100 cfs) in late spring (mid-May to early June).

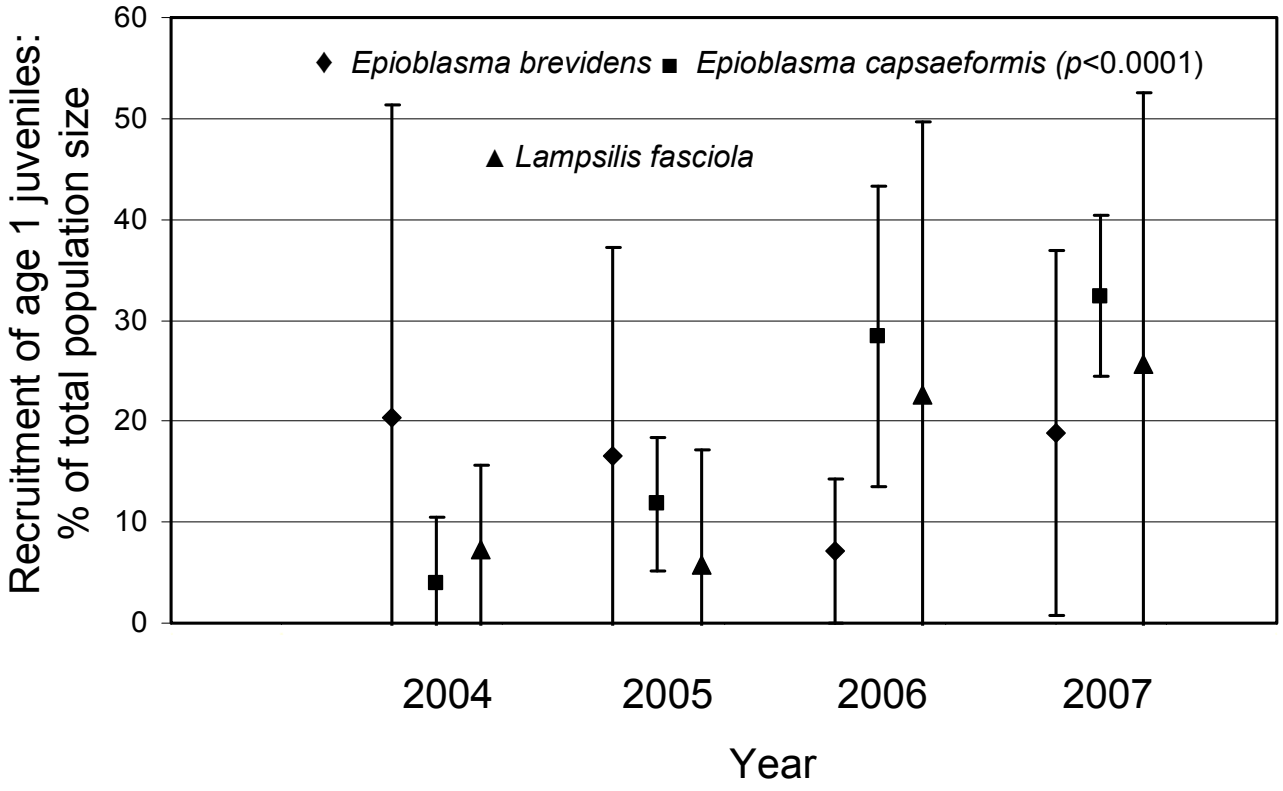

Figure 6. Recruitment of 1 y-old juveniles for investigated mussel species sampled across all sites y^{-1} in the Clinch River, TN. Error bars represent 95% confidence intervals. Reported *p*-value indicates a significant increase in juvenile recruitment from 2004-2007 for *Epioblasma capsaeformis*.

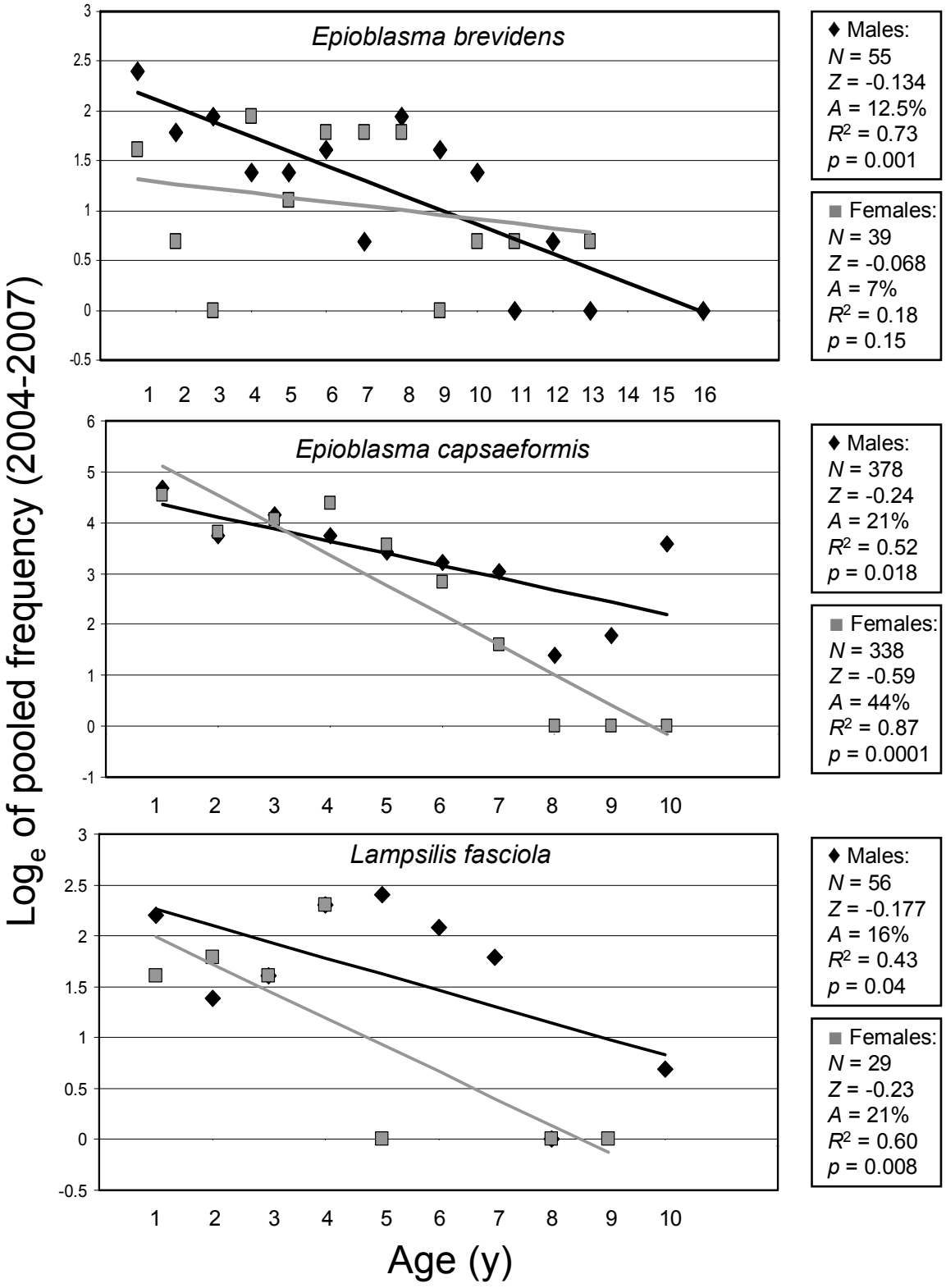
Epioblasma brevidens
♦ Males:
N = 55
Z = -0.134
A = 12.5%
R2 = 0.73
p = 0.001
■ Females:
N = 39
Z = -0.068
A = 7%
R2 = 0.18
p = 0.15
Epioblasma capsaeformis
♦ Males:
N = 378
Z = -0.24
A = 21%
R2 = 0.52
p = 0.018
■ Females:
N = 338
Z = -0.59
A = 44%
R2 = 0.87
p = 0.0001
Lampsilis fasciola
♦ Males:
N = 56
Z = -0.177
A = 16%
R2 = 0.43
p = 0.04
■ Females:
N = 29
Z = -0.23
A = 21%
R2 = 0.60
p = 0.008
Loge of pooled frequency (2004-2007)
Age (y)

Figure 7.

Figure 7. Sex-specific mortality rates of investigated species were estimated from catch-curve linear regression analyses, where *N* is sample size, *Z*=instantaneous mortality rate, and *A*=annual mortality rate. Frequency data are pooled across years and sites; however, data for *E. capsaeformis* are pooled from just WB, FF, and SI.

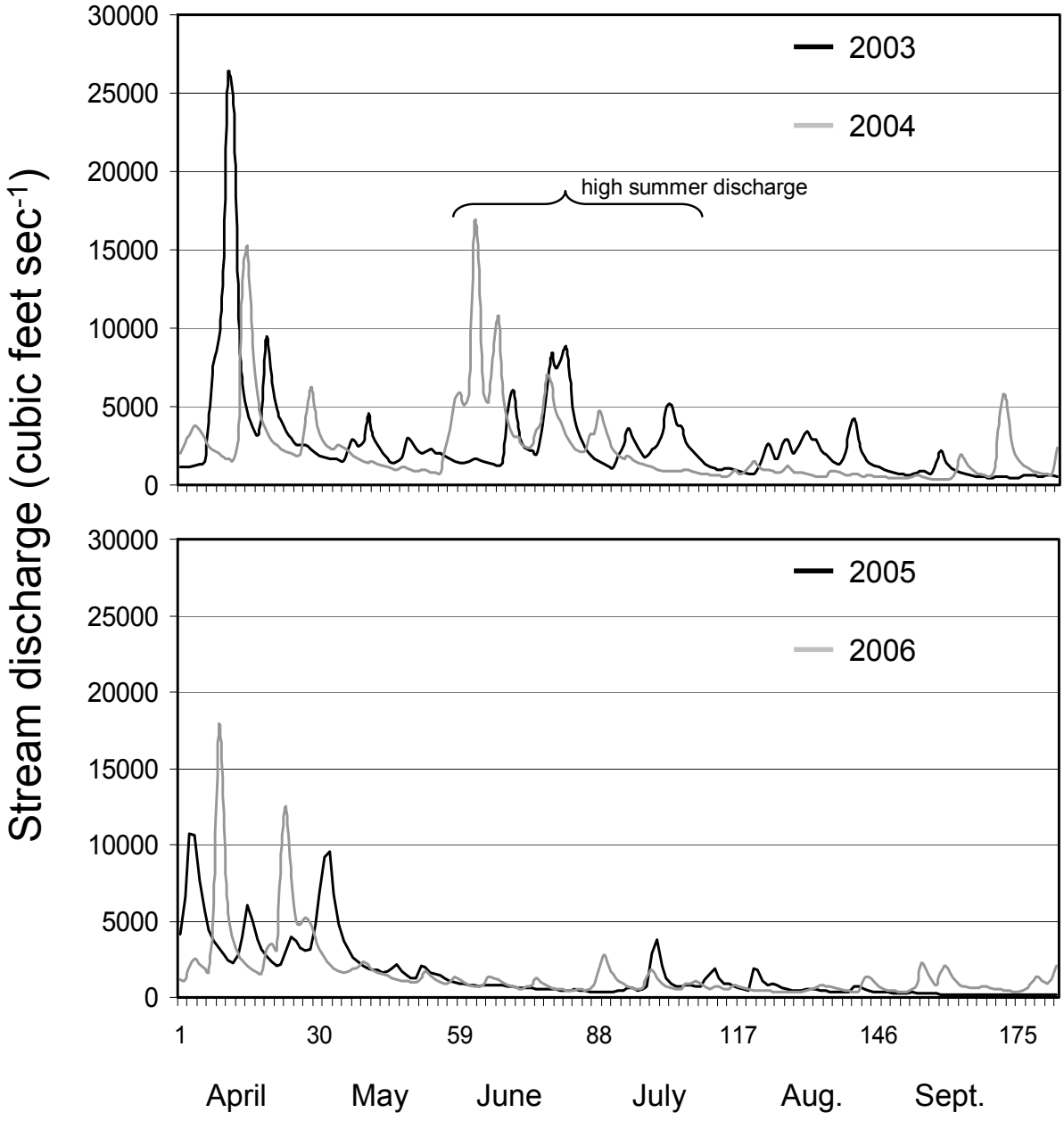

Figure 8. Daily stream discharge in the Clinch River (~RKM 244.4), Claiborne Co., TN taken at U.S. Geological Survey stream gauge #0352800 located upstream of Tazewell, TN.

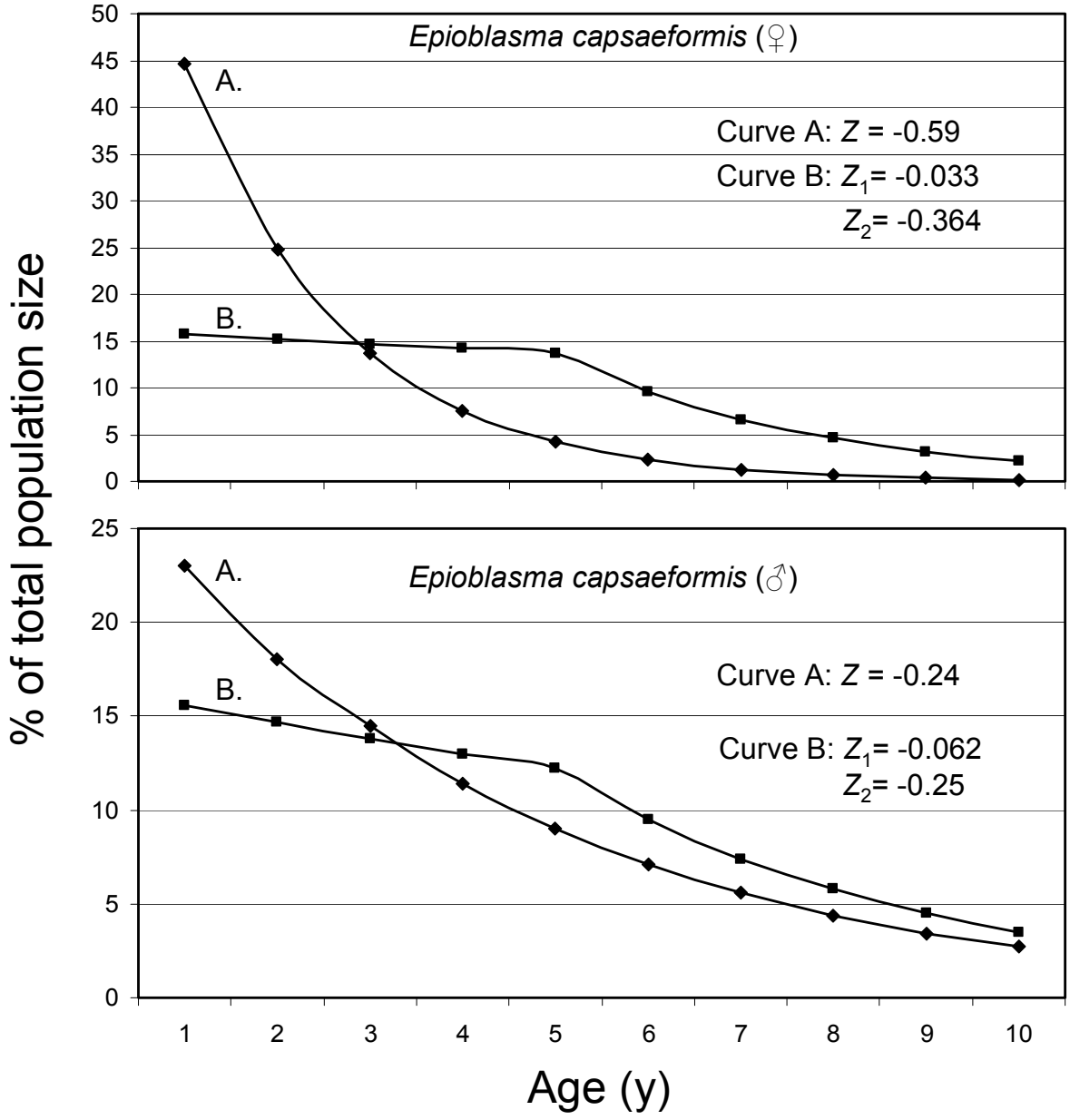

Figure 9. Sex-specific survivorship curves were computed using $N_t=N_0e^{-Zt}$, where N_t is the number in a age-class at time t, N_0 original number in a age-class, and Z is the instantaneous mortality rate. Curve A for each sex was computed for ages 1-10 using Z reported in Figure 6, and curve B was computed using two age-specific rates, Z_1 for ages 1-5 and Z_2 for ages 6-10.

APPENDIX

Observed mean (standard error) lengths and predicted shell lengths based on analysis of internal annuli of *Epioblasma brevidens*, *E. capsaeformis*, and *Lampsilis fasciola* from the Clinch River, Tennessee.

Age and growth key for ***Epioblasma brevidens***:

Internal annulus (age)	Sex	No. of indiv-iduals	Observed length (mm) Mean (SE)	Range	Predicted length (mm)	Growth increment (mm)
0	Male	10	10.4 (0.58)	8.4-13.2	15.1	15.1
	Female	10	9.9 (0.60)	7.5-12.8	10.2	10.2
1	Male	10	17.7 (0.61)	15.0-20.7	19.8	4.7
	Female	11	17.9 (0.68)	14.0-21.7	18.9	8.7
2	Male	10	25.7 (0.48)	22.7-27.8	24.2	4.4
	Female	10	25.6 (0.85)	22.3-32.3	25.9	6.0
3	Male	12	31.4 (0.81)	28.5-37.9	28.2	4.0
	Female	13	33.7 (0.77)	30.3-38.8	31.6	5.7
4	Male	14	36.0 (0.94)	30.8-43.1	31.9	3.7
	Female	12	37.9 (0.68)	33.8-40.8	36.1	4.5
5	Male	5	38.6 (3.10)	32.2-47.3	35.3	3.4
	Female	5	41.3 (0.81)	39.1-43.2	39.8	3.7

6	Male	6	38.1 (1.80)	33.7-43.6	38.4	3.1
	Female	7	42.8 (0.74)	39.8-45.2	42.7	2.9
7	Male	4	42.8 (1.17)	40.4-46.0	41.2	2.8
	Female	5	43.2 (0.62)	41.3-44.8	45.1	2.4
8	Male	6	44.0 (0.73)	40.8-45.7	43.8	2.6
	Female	4	45.5 (0.54)	44.8-47.1	47.0	1.9
9	Male	4	47.3 (1.10)	44.7-50.0	46.2	2.4
	Female	5	47.7 (0.48)	46.7-49.0	48.5	1.5
10	Male	4	47.2 (0.39)	46.3-48.0	48.4	2.2
	Female	5	47.6 (0.45)	45.9-48.2	49.8	1.3
11	Male	4	48.7 (1.09)	45.9-50.9	50.5	2.1
	Female	5	49.0 (0.68)	47.0-50.6	50.8	1.0
12	Male	4	51.4 (1.36)	49.0-54.8	52.3	1.8
	Female	4	50.2 (0.92)	49.1-53.0	51.6	0.8
13	Male	5	53.4 (1.05)	50.0-55.7	54.0	1.7
	Female	4	53.9 (0.67)	52.3-55.0	52.2	0.6
14	Male	4	53.7 (0.26)	53.1-54.3	55.6	1.6
	Female	3	54.5 (0.78)	53.4-56.0	52.7	0.5
15	Male	4	54.8 (0.55)	53.8-56.2	57.1	1.5
	Female	2	55.4 (1.18)	54.3-56.6	53.2	0.5
16	Male	4	55.4 (0.67)	54.3-57.1	58.4	1.3
17	Male	4	56.6 (1.43)	53.0-60.0	59.6	1.2

18	Male	2	59.3 (1.55)	57.8-60.8	60.7	1.1
19	Male	1	61.3	---	61.7	1.0
20	Male	1	67.6	---	62.7	1.0
21	Male	0	---	---	63.5	0.8
22	Male	0	---	---	64.3	0.8
23	Male	0	---	---	65.0	0.7
24	Male	0	---	---	65.7	0.7
25	Male	0	---	---	66.3	0.6
26	Male	0	---	---	66.9	0.6
27	Male	0	---	---	67.4	0.5
28	Male	1	71.5	---	67.9	0.5

Age and growth key for ***Epioblasma capsaeformis***:

Internal annulus (age)	Sex	No. of indiv-iduals	Observed length (mm)		Predicted length (mm)	Growth increment (mm)
			Mean (SE)	Range		
0	Male	10	10.2 (0.56)	8.0-12.9	10.5	10.5
	Female	10	9.7 (0.39)	7.7-11.5	9.8	9.8
1	Male	10	20.7 (1.0)	15.7-25.4	20.7	10.2
	Female	10	19.7 (1.2)	16.1-27.0	19.4	9.6
2	Male	10	28.5 (0.90)	24.2-32.5	27.3	6.6
	Female	10	26.8 (1.1)	21.1-31.8	26.6	7.2

3	Male Female	10 10	31.8 (0.90) 31.3 (0.41)	28.5-35.6 28.6-33.4	31.6 32.1	4.3 5.5
4	Male Female	10 10	33.8 (0.83) 37.0 (0.68)	30.6-37.6 32.9-40.3	34.3 36.3	2.7 4.2
5	Male Female	10 13	35.6 (0.70) 39.1	32.8-39.9 35.8-48.6	36.1 39.5	1.8 3.2
6	Male Female	7 23	36.2 (0.98) 42.0 (0.71)	35.5-39.0 35.9-47.3	37.2 41.9	1.1 2.4
7	Male Female	10 11	36.8 (0.77) 44.1 (1.0)	33.9-40.0 39.9-48.0	38.0 43.8	0.8 1.9
8	Male Female	3 4	38.9 (0.78) 45.1 (0.36)	38.1-40.5 44.7-46.2	38.5 45.1	0.5 1.3
9	Male Female	1 1	39.5 46.0	39.0 46.0	38.8 46.2	0.3 1.1
10	Male	1	40.0	40.0	39.0	0.2

Age and growth key for ***Lampsilis fasciola***:

Internal annulus (age)	Sex	No. of indiv-iduals	Observed length (mm) Mean (SE)	Range	Predicted length (mm)	Growth increment (mm)
0	Male Female	10 10	16.0 (0.57) 15.2 (0.82)	13.5-19.3 10.3-17.3	18.9 16.2	18.9 16.2

1	Male	10	29.6 (1.07)	24.6-34.2	28.3	9.4
	Female	10	28.4 (1.49)	21.2-34.8	28.0	11.8
2	Male	12	38.3 (1.28)	31.8-44.9	36.2	7.9
	Female	11	37.1 (1.68)	26.6-46.3	36.4	8.4
3	Male	10	42.3 (1.06)	37.6-49.6	42.8	6.6
	Female	10	44.8 (1.11)	40.6-50.5	42.5	6.1
4	Male	10	50.4 (2.08)	34.6-58.0	48.4	5.6
	Female	10	47.6 (0.62)	45.1-51.2	46.9	4.4
5	Male	5	50.5 (1.97)	47.3-55.7	53.1	4.7
	Female	10	49.7 (1.09)	45.5-56.5	50.0	3.1
6	Male	5	59.9 (1.96)	53.0-64.3	57.0	3.9
	Female	7	50.0 (1.52)	45.5-55.4	52.3	2.3
7	Male	5	61.1 (2.92)	50.4-65.9	60.4	3.4
	Female	6	51.7 (1.58)	47.7-56.1	53.9	1.6
8	Male	3	63.6 (2.02)	60.3-67.3	63.2	2.8
	Female	4	54.4 (2.75)	49.5-61.0	55.0	1.1
9	Male	3	61.8 (3.77)	54.8-67.8	65.5	2.3
	Female	3	54.2 (2.28)	51.5-58.8	55.9	0.9
10	Male	2	66.4 (4.61)	61.8-71.0	67.5	2.0
	Female	2	55.8 (3.85)	52.0-59.7	56.5	0.6
11	Male	1	72.0	---	69.2	1.7
	Female	2	56.5 (4.05)	52.5-60.6	56.9	0.4
12	Male	1	73.1	---	70.6	1.4

	Female	2	57.1 (4.02)	53.2-61.2	57.2	0.3
13	Male	0	---	---	71.7	1.1
	Female	1	62.6	---	57.4	0.2
14	Male	0	---	---	72.7	1.0
15	Male	0	---	---	73.6	0.9
16	Male	0	---	---	74.3	0.7
17	Male	0	---	---	74.9	0.6
18	Male	0	---	---	75.4	0.5
19	Male	1	69.0	---	75.8	0.4
20	Male	0	---	---	76.2	0.4
21	Male	0	---	---	76.5	0.3
22	Male	0	---	---	76.7	0.2
23	Male	0	---	---	76.9	0.2
24	Male	0	---	---	77.1	0.2
25	Male	0	---	---	77.3	0.2
26	Male	0	---	---	77.4	0.1
27	Male	1	65.6	---	77.5	0.1
28	Male	0	---	---	77.6	0.1
29	Male	0	---	---	77.7	0.1
30	Male	1	66.3	---	77.7	0.0
31	Male	0	---	---	77.8	0.1
32	Male	0	---	---	77.8	0.0
33	Male	1	73.7	---	77.9	0.1

34	Male	0	---	---	77.9	0.0
35	Male	0	---	---	77.9	0.0
36	Male	0	---	---	78.0	0.1
37	Male	1	83.0	---	78.0	0.0
38	Male	0	---	---	78.0	0.0
39	Male	0	---	---	78.0	0.0
40	Male	0	---	---	78.0	0.0
41	Male	0	---	---	78.0	0.0
42	Male	0	---	---	78.0	0.0
43	Male	0	---	---	78.0	0.0
44	Male	1	91.3	---	78.0	0.0
45	Male	0	90.2	---	78.0	0.0

CHAPTER 3

POPULATION MODELING OF TWO ENDANGERED MUSSEL SPECIES IN THE CLINCH RIVER, U.S.A.: QUANTITATIVE CRITERIA TO EVALUATE HARVEST AND REINTRODUCTION OF *EPIOBLASMA BREVIDENS* AND *EPIOBLASMA CAPSAEFORMIS*

ABSTRACT

Population modeling of two endangered mussel species, *Epioblasma brevidens* and *E. capsaeformis*, in the Clinch River, U.S.A., was conducted to determine suitable harvest levels for translocation of sub-adults and adults, and quantitative criteria to evaluate performance and recovery of extant and reintroduced populations. For both species, the recommended annual harvest was <1% of local population size to minimize risk of decline. Reintroduction modeling indicated that size of the initial population created during a 5 y build-up phase greatly affected final population size at 25 y, being similar to size at the end of the build-up phase, especially when expected 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 ≥5,000 individuals (N_e=250) and ≥10,000 individuals (N_e=500), respectively, and ideally should be comprised of multiple smaller demes spread throughout a river. Demes of both species in the river are currently large enough to sustain harvest for translocation and reintroduction purposes, offering an effective biological recovery strategy.

Key words: freshwater mussels, endangered, *Epioblasma brevidens*, *E. capsaeformis*, population modeling, harvest, reintroduction, quantitative recovery 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 intense overexploitation, habitat loss and degradation, and severe pollution of aquatic ecosystems, with concomitant losses in biodiversity throughout the United States. The passage of landmark environmental laws 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 stewardship of our natural heritage (Stein et al. 2000; Schwartz 2008). Thirty years later, some disturbed ecosystems are healing; they show signs of improved water quality and physical habitat conditions and are ready to have species once lost, returned home. Hence, as we begin the 21st century, natural resource managers are charged with restoring these plant and animal communities. Freshwater mussel populations have declined greatly in past decades and are considered one of the most imperiled groups of animals in the country (Neves et al., 1997). Many species cannot re-colonize previously occupied habitats because of man-made barriers such as dams that prevent dispersal of host fishes. Reintroductions are 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. Unfortunately, source populations of adults for translocation and for use as broodstock for propagation of juveniles are now fragmented and rarely demographically robust. However, the Clinch River in northeastern Tennessee (TN) contains a diverse mussel assemblage of >40 species, with several endangered mussel species such as the Cumberland combshell (*Epioblasma brevidens*) and oyster mussel (*E. capsaeformis*) that currently contain large enough populations to support translocations of adults (Chapter 2). In the Tennessee River system, only the Duck River in west-central TN contains populations of endangered mussels suitable for such a purpose. If managed properly, populations in these two rivers can serve as main sources to replenish and rebuild other populations throughout the system for decades to come.

Although the federal recovery plans for *E. brevidens* and *E. capsaeformis* provide recovery criteria for both species, generally they are marginally quantitative because demographic data are lacking to specifically define criteria. When such data are unavailable, these plans recommend that such information be collected. For example, the plans recommend that the demographic structure and effective size of a viable population of each species be determined as specific recovery tasks (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 the release of adults and the propagation of juveniles. Ideally, re-introduced populations will be more than self-sustaining, to grow in size locally and expand to other sites. Thus, the purpose of this study was to address two main questions: (1) how many individuals of either species can be harvested from a local population without causing the source population to decline, and (2) how many individuals are needed to create a self-sustaining, demographically viable population that is large enough to maintain sufficient genetic variation over time? Answering these questions will provide the quantitative criteria needed to guide harvest and translocation of adults, and to evaluate population reestablishment and sustainability.

METHODS

MAINTENANCE OF GENETIC DIVERSITY

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 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 and 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 (Chapter 2). Sites sampled were Wallen Bend, Frost Ford, and Swan Island, which were selected because they represented the upper [river kilometer (RKM) 309.9], middle (RKM 291.7) and lower (RKM 277.2) boundaries of the study reach, respectively. 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 and Do 2007).

POPULATION MODELING AND MANAGEMENT

Age-structured population models

Age-structured Leslie-matrix population models were implemented in RAMAS Metapop (Akçakaya and Root 1998) to simulate harvest and reintroduction scenarios for *E. brevidens* and *E. capsaeformis* in the Clinch River. Modeling was conducted assuming a single-site management scenario, i.e., immigration and emigration to and from nearby sites were in equilibrium, 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* (Chapter 2). 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*.

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 in Chapter 2, 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 and Bettoli 2007). Therefore, survival rates of ages ≥1 were determined by empirical data gathered in Chapter 2, survival rates of *in situ* field studies of sub-adult mussels [N. Eckert, 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 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 average survival of newly metamorphosed juveniles that excysted from fish hosts and 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 and Neves, 2002). However, here it is 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 a population to extinction. 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 drought and flood, can greatly affect population vital rates. 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 inherently 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 best guess 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 (Chapter 2). Survival and fecundity were assumed to be uncorrelated in the model. Extreme

environmental variation such as catastrophes and bonanzas were assumed to be rare and not included in the model.

Initial abundances and ages

Initial abundances for modeling harvest were based on population censuses taken at numerous sites in the Clinch River, TN from 2004-2007 (Chapter 2). Excluding age-0 individuals, average population size for *E. brevidens* was ~3,000 individuals, and ~100,000 individuals for *E. capsaeformis* at several of the largest sites in the river. Population sizes were chosen to represent current abundances at sites where each species likely would be harvested. Harvest simulations were initiated using a stable age distribution (SAD) that included age-0 individuals. Starting sizes were 4,200 and 152,000 individuals, respectively. Simulations were conducted based on harvesting an equal number of individuals ages 4-11 for *E. brevidens* and ages 3-7 for *E. capsaeformis*.

Initial abundances for modeling reintroduction of mussels to a site, for example in the upper Clinch River, VA, were based on a predetermined number of mussels to be translocated y^{-1} for 5 y. Translocated mussels y^{-1} ranged from 24-120 individuals for *E. brevidens* and from 50-400 individuals for *E. capsaeformis* (Table 1). Simulations were based on translocating an equal number of individuals of each harvested age class. The harvest and reintroduction strategies in this study assume that abundance and age structure of each species will be maintained through time at levels similar to those currently encountered on the Tennessee portion of the river.

Population growth rate and carrying capacity

Although density-dependent regulation and carrying capacity (*K*) are unknown parameters 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 and 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 harvest simulations, *K* was set at 10,000 individuals for *E. brevidens* and 350,000 individuals for *E. capsaeformis*, based on current maximum population sizes recorded at target sites in the Clinch River, TN (Chapter 2). For 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 set too low for 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 were a product of the projection matrix.

Harvest and reintroduction simulation scenarios

To examine the effect of harvesting individuals of *E. brevidens* and *E. capsaeformis* from a locally large population (deme) in the Clinch River, TN, simulations were conducted to assess how different harvest-levels and growth rates might affect population trajectories. Individuals were viewed as being "harvested" because they were permanently removed from the deme, with the intent of being translocated to another reach of river for reintroduction purposes; therefore, harvest-levels matched reintroduction-levels in the study (Table 1). Simulations were conducted by harvesting or transplanting equal numbers of individuals per year from targeted age classes. Harvest occurred each year for a 25 y period, whereas transplants only occurred each year for a 5 y population build-up period, which then grew unassisted for the next 20 y. Harvest, transplant, and population growth levels were varied from low, intermediate, and high, and were chosen to explore scenarios relevant to population management of each species.

Declining population trajectories were not modeled because they represent scenarios incompatible with harvest management of an endangered species.

Uncertainty of mean population projections and the probability of decline were assessed for all modeled scenarios. However, not all data were reported here because they were very similar for most projections and therefore redundant. Furthermore, since sample size (N=10,000) of mean trajectories was extremely high, confidence intervals (CI) would be unrealistically narrow. Instead, uncertainty was explored using a small random sub-sample (N=20) of trajectories taken from harvest and restoration scenarios most relevant to population management.

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 considerably 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, but also lowest at Swan Island. 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 over time were greatest at N_e=25, but diminished as N_e increased (Figure 2). Also, loss of allelic diversity was greater than corresponding declines in H_e. Loss of genetic diversity was minimal for $N_e \geq 75$ 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 obviously occurred when effective population size was lowest 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 (<5-10%) only at N_e=250.

Effect of harvest on population size

The effect of harvest on population size over time was evaluated under stable, low and moderate population growth rates for both species (Figure 3). For *E. brevidens*, population size declined at all harvest levels when growth was stable (λ=1.005), but declined minimally when harvest was 24 individuals y^{-1}. At low growth (λ=1.0125), population size declined at the two highest harvest levels of 72 and 96 individuals y^{-1}. At moderate growth (λ=1.025), population size increased at all harvest levels except at 96 individuals y^{-1}, where population size remained essentially unchanged over time. Harvest uncertainty for *E. brevidens* was evaluated at a harvest level of 48 individuals y^{-1} and at the low growth rate (λ=1.0125). These harvest settings represented a likely site-specific management scenario to harvest individuals of the species from the river. The sub-

sampled mean was well below the modeled mean, but the upper 95% CI contained most of the latter (Figure 5). More than half of the sub-sampled population trajectories generally exhibited a declining trend and finished below the initial population size. Although none of the sub-sampled trajectories declined to zero, one fell to a low of 94 individuals. The probability or risk of decline increased substantially at higher harvest levels. For example, there was a 5% chance of a 95% probability of decline in population size at a harvest level of 48 individuals y^{-1}, compared to a 15% chance of a 95% probability of decline in population size at a harvest level of 96 individuals y^{-1} (Figure 6).

For *E. capsaeformis*, small increases in population size were observed initially at all harvest levels when growth was stable (λ=1.005), but then after 15 y population size declined slightly, especially at the higher harvest levels of 300 and 400 individuals y^{-1}. At both low (λ=1.025) and moderate (λ=1.05) growth, population size increased substantially despite annual harvest, and all mean trajectories were very similar over time. Uncertainty was evaluated under a scenario of harvesting 300 individuals y^{-1} and a low growth rate (λ=1.025). The sub-sampled mean was below the modeled mean, but both exhibited similar trajectories and the upper 95% CI entirely contained the latter (Figure 5). More than half of the sub-sampled population trajectories exhibited an increasing trend and finished above the initial population size. None of the sub-sampled trajectories declined to zero, and only one fell slightly below 50,000 individuals. The low probability of decline was nearly identical at all harvest levels, including no harvest, indicating that harvest had a negligible effect on population trajectories (Figure 6).

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 (Figure 4). 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. However, 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 transplant 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 (Figure 6). 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} (Figure 6).

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 (Figure 6). 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} (Figure 6).

Although harvest and restoration uncertainty were evaluated only for the above scenarios, the same standard deviations for vital rates were used in all modeling scenarios. Hence, these results are quantitatively and qualitatively very similar to the above results. With the exception of the two highest harvest scenarios for *E. brevidens*, the probability of a 100% decline was extremely low (<1%) for all other harvest or restoration 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 (Figure 7). 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 their small sizes

(e.g., <5-10 mm) it is difficult to accurately census age-0 juvenile mussels *in situ*. So in practice, age-1 individuals usually are the youngest age-class in the census. A look at 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 (Figure 8). 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 (Figure 7). Reproductive values also were high for age-classes a couple of 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. Equally important is the census size (N_c) 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, 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 and Frankham 2003; Reed 2005). It is 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 combined 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 stated 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 reasonable target for either (Table 4). Current guidelines 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 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 and Ruzzante 2008). However, the genetic modeling conducted in this study suggested that if total N_e=250,

then a high proportion (~90%) of molecular diversity could be retained over 25 generations, which is perhaps a more realistic management time frame for mussel species with generation lengths of 3-5 y, and additionally, would require a smaller total population size of N_c=5,000. The smaller effective population size of N_e=250 is recommended here as an overall target for species such as *E. brevidens* that are longer-lived, occur at lower densities, and have more stable populations over time. For example, a target size of N_e=250 for *E. brevidens* could be achieved by enhancing or building-up 5-10 local demes with census sizes of 500-1000 individuals per site, which corresponds to local sizes of N_e=25-50. The larger size of N_e=500 is recommended as the overall target for *E. capsaeformis* because it is a shorter-lived species and has more variable population size over time, because extreme fluctuations in population size can greatly reduce N_e. This target N_e for *E. capsaeformis* could be achieved by enhancing or building-up 5-7 local demes with census sizes of 1500-2000 individuals per site, which corresponds locally to N_e=75-100. Furthre, these census sizes represent a stocking 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; therefore, sites with greater suitable habitat (m^2) will require larger census sizes to maintain similar densities. Achieving these recommended or even greater population sizes is feasible and consistent with the known demography of both species at sites in the Clinch River, TN.

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 and 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 1966, 1970), which is essentially an extreme form of inbreeding that decreases N_e.

The consequences of losing genetic diversity in freshwater mussel populations and other invertebrate species are poorly understood. Since the reproductive biology of many mollusks typically involves some level of self-fertilization (Dillon 2000), rates of natural inbreeding are presumably high compared to other species. Evidence of inbreeding depression, as measured by decreased fitness at quantitative traits, is lacking for mussels; however, no studies have been conducted to directly address the question. Recent studies have shown severe declines in genetic diversity of mussel populations in the Duck River, TN, and Big South Fork Cumberland River, TN and KY that have been through bottlenecks (Grobler et al. 2006; Jones et al. 2006). Follow-up field surveys have documented that these populations have rebounded in recent years and are currently exhibiting strong recruitment (Ahlstedt et al. 2004). Hence, it is unclear whether these mussel populations are or will be affected by inbreeding depression, or if loss of genetic diversity has reduced adaptive potential. In addition, conducting studies to test for inbreeding depression in mussels would be difficult and time consuming. Furthermore, molecular measures of genetic variation are not good predictors of adaptive potential (Frankham et al. 2002). Variation at neutral molecular markers decreases with population size following a sigmoid function (Frankham 1996), but heritability (h^2) of quantitative traits decreases with population size only at very small population sizes (Willi et al.

2006). Thus, while loss of quantitative trait heritability or adaptive potential is considered a serious consequence of declining population size (Frankham et al. 2002), the mean correlation (r=0.22) across a range of studies linking molecular variation and quantitative variation is weak (Reed and Frankham 2001). Importantly, key quantitative measures for life history traits and heritability, which are considered the most important traits for assessing fitness, showed no correlation to molecular variation (Reed and Frankham 2001). These studies and others referenced therein suggest that molecular variation may not serve as an adequate proxy to assess loss of adaptive potential, unless losses are severe and sustained.

While trends in molecular variation may not correlate well with quantitative variation, loss of molecular variation should arguably still be monitored in populations of endangered mussel species. 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, and recognizing management units. 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. This study has shown that clear differences exist between the life history traits and population demography of *E. brevidens* and *E. capsaeformis*, to include fish host-driven dispersal, life span, population sizes, and recruitment. These differences undoubtedly influence maintenance of genetic variation and N_e of each species. A total N_e=250-500 is probably large enough to maintain sufficient genetic variation for populations of *E. brevidens* and *E. capsaeformis*, especially at fitness-related quantitative loci over time-scales that are appropriate for

management. However, for rarer, less abundant mussel species, such targets will be impractical, and will have to be set based on reintroduction strategies that account for current population size and genetic variation (Jones et al. 2006). 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. A practical approach that seeks to maximize both abundance and genetic variation of populations is recommended.

Effects of harvest on population abundance

Population growth and density of *E. brevidens* has remained surprisingly constant over a 28 y period (1979-2007) in the Clinch River, TN, where density has ranged from approximately 0.1-1.0 m^{-2} per site (Ahlstedt et al. 2005; Chapter 2). Trend data during this period have shown minor, non-significant fluctuations in density, indicating that the population has remained stable in the TN portion of the river for decades. During this time period, the species has not exhibited rapid population growth, perhaps due to aspects of its life history, such as a longer life span and use of an uncommon primary fish host, the logperch (*Percina caprodes*) (Yeager and Saylor, 1995). Hence for *E. brevidens*, the modeled population trajectories based on stable or low growth rates probably are best suited for evaluating effects of harvest, which showed that an annual harvest of about 40-50 individuals or greater could hinder population growth or cause decline (Figure 3). To reiterate, modeled harvest scenarios were based on a starting population size of 4,500

individuals (Table 1). Given the inherent uncertainty in estimating population size – especially for an endangered species that occurs at low densities (e.g., <1 m^{-2}) – and the logistical difficulties associated with regularly conducted censuses, it is important that harvest levels for translocation purposes be set that are unlikely to cause decline. Thus for *E. brevidens*, it is recommended from the results of this study that annual harvest be <1% of population size at the local site.

In contrast, growth and density of *E. capsaeformis* have increased remarkably over the same period, where density typically was <1 m^{-2} at sites from the late 1970's into the mid-1990's, and then exploded in about a 10 y period from 1999-2007, to a density greatly exceeding 1 m^{-2} at most sites and a high of 21.9 m^{-2} recorded at Frost Ford in 2007 (Ahlstedt et al. 2005; Chapter 2). Concordantly, trend data showed a significant increase in population density, as the species exhibited rapid population growth (>30% y^{-1}) during this period. This rapid growth may be attributable partly to its use of darter hosts in the genus *Etheostoma* (Yeager and Saylor 1995), which are common in the river, to possibly accommodate for its shorter life span, and favorable environmental conditions such as low flows in the spring and summer. Therefore, simulations based on low to moderate growth rates are justified for evaluating effects of harvest on this species (Figure 3). Again, harvest scenarios for this species were based on a starting population size of 152,000 individuals (Table 1). A harvest strategy of 200-300 individuals y^{-1} would be appropriate at sites with a local size of 50,000-100,000 individuals or greater. Such harvest levels are logistically feasible and would not cause decline, as long as environmental conditions remained favorable for continued population recruitment and growth. For example, given the above abundance of 152,000 individuals and a growth

rate of 2.5%, an annual harvest of 300 individuals would diminish growth by <0.5%, allowing the population to still grow by 2% per year. Hence, an occasional harvest of >300 individuals could be justified if logistics allowed larger translocations. Thus for *E. capsaeformis*, it is also recommended that annual harvest be <1% of population size at the site. Even at this low harvest level and given the current abundance of the species in the river, several hundred to more than a thousand individuals per year could be collected for translocation purposes with no detrimental effect on the entire population.

It is important to emphasize that the population trajectories presented in Figure 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. They simply represent a likely outcome given a set of input variables. 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 Figure 5. Biologists are aware of how real populations can fluctuate and occasionally do so dramatically, due to stochastic effects from disease, competition, flood, drought, etc. Even in healthy ecosystems, populations can decline naturally over time due to a variety of factors. Therefore, biologists should plan and conduct translocations to restore mussel populations based on quantitative field data. Collection of quantitative data will ensure that populations are regularly monitored to track trends, and that harvest levels are adjusted accordingly so that translocation efforts do not contribute to population decline.

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 translocated 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% (Figure 4). Importantly, notice that the final population size at 25 y also would be ~500 individuals or slightly larger depending on the exact growth rate employed. It is critical that target census size match or be similar to the population size at the end of the build-up phase.

Population growth during the build-up phase is enhanced by transplanting a greater proportion of sub-adults and younger adults (e.g., ages 4-8) with longer reproductive potential (Figure 7). In practice however, population size at the end of the build-up phase will be constrained by the number and age of individuals that can be collected and transplanted each year. For species such as *E. brevidens* that occur at lower densities, the number that can be collected annually per site and made available for translocation will be constrained by absolute abundance and by agency policies to protect local demes. 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*) 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).

The target census size should be large enough to accommodate the N_e that meets established program goals. For a species such as *E. brevidens*, the number of individuals available for translocations may be limited, so setting a realistic site or deme-specific census size of say, 500 would be a feasible reintroduction target. It would give a N_e of 25, assuming the ratio of N_e/N_c was 5%. Hence, additional and perhaps larger demes would be needed to meet a goal of establishing a total N_e=250. Similar reintroduction scenarios and targets could be planned for *E. capsaeformis*, but adjusted accordingly to take advantage of its greater abundance in the river.

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 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 and 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.

Two key 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). These tasks are identified in the recovery plan of both species because the information is critical to evaluating population performance and recovery. The SAD histograms in Figure 7 show profiles of three cohort structures for each species based on stable, low and moderate growth rates, illustrating that 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 here 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 (2004-2007) 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 (Chapter 2). During this period, both populations exhibited strong and weak year-classes, but recruitment was always a measurable feature of the population. Of course, histograms produced from 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 histograms, then age-1 individuals comprise ~11% of a stable population of *E. brevidens* and ~15% of a stable population of *E. capsaeformis* (Figure 8). 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 2004-2007 for *E. brevidens* was 15.7% (range: 7.1-20.3%), and for *E. capsaeformis*, 19.1% (range: 4.0-32.4%) (Chapter 2). These recruitment levels in the river exceed the above criteria and indicate that both populations should have increased in size during this period, a conclusion already corroborated by trend data of the populations. 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.

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 quantitative 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 hinder or accelerate loss of genetic variation, respectively.

The input variables used to parameterize each species Leslie-matrix are another important source of uncertainty, especially for: (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 obviously vary and be influenced by both environmental conditions and longevity. However, the known 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 and other species. Quantifying the age-0 survival rate would be facilitated by estimates of natural juvenile excystment from fish hosts *in situ*. Numerous studies have shown that natural infestation levels of glochidia on fish hosts (e.g., minnows, darters) are low, typically 1-10 attached glochidia per fish for a range of mussel and fish host species inspected, and additionally, infested fishes usually represent a small proportion (typically <20%) of the total number of fishes examined (Neves and Widlak, 1988; Weaver et al. 1991; Bruenderman and Neves, 1993; Hove and Neves, 1994; Weiss and Layzer, 1995; Layzer et al. 2003). The number of *Epioblasma* glochidia infested per fish host (perhaps >50-100 glochidia per infestation) may be higher than other mussel species because species of *Epioblasma* have specialized shell morphologies and mantle-lures that can capture and hold their hosts (similar to a Venus fly-trap) to facilitate infestation of glochidia (Jones 2004; Jones et al. 2006: N. King, Virginia Polytechnic Institute and State University, pers. obs. 2007; Barnhart et al. 2008). Host capture seemingly is a rare event by female mussels. This behavior has been observed infrequently by biologists working regularly in the Clinch River; e.g., only six documentations have been recorded from 1998-2008. Therefore, these studies and anecdotal observations of host capture by *Epioblasma* females suggest that glochidial infestations on host fishes occur at low levels, indicating that survival of metamorphosed juveniles may be higher than expected. The modeling results showed that if survival of age-0 individuals was 30% and population growth was stable, age-0 individuals would comprise 27.1% (*N*=1,220) of the *E. brevidens* population and 33.8% (*N*=51,376) of the *E. capsaeformis* population (Figure 7). Further, on average the age-0 cohort should

represent the largest percentage of the total population cohort size (Table 1). If host capture is the primary infestation mechanism, *in situ* production of age-0 individuals would result from a substantial number of infestation events. In the two cases presented here, perhaps hundreds (~500) occur per site y^{-1} for *E. capsaeformis*, and a dozen or less occur per site y^{-1} for *E. brevidens*. Since the latter species uses a large darter as its main host (*Percina caprodes*), it is capable of producing more juveniles per infested fish. While the above scenarios are mostly hypothetical, they demonstrate the complexity and need for additional research of age-0 production and survival rates, inquiries critical to establishing quantitative restoration criteria for these and other freshwater mussels.

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 (Chapter 2), suggesting that females also live longer than 15 y. 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 senescent 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. Fecundity or mean number of offspring produced per female or male/female pair also is poorly understood for mussels, to include how cross-fertilization or hermaphroditic modes of reproduction influence fecundity. In this study, fecundity was simply averaged across all mature individuals in the population and set to levels to achieve a stable or growing population. More realistic modeling scenarios of fecundity would require a better quantitative understanding of the mussel-fish host infestation process as discussed above.

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: Chapter 2).

Conclusions and recommendations

Populations of some endangered mussel species are now large enough to sustain harvest for translocation purposes, including the two species evaluated in this study. Translocations offer a cost-effective strategy to recover these and other populations in the United States. Water quality and habitat conditions are improving in some rivers and streams throughout the country, providing an opportunity to reintroduce species to additional sites and thus facilitate their recovery. In this regard, the results of this study provide the following conclusions and recommendations to help guide harvest and reintroduction of *E. brevidens* and *E. capsaeformis* in the Tennessee River system:

- Harvest of each species should be <1% of local population (deme) size in the Clinch River, TN.
- Reintroduced populations and demes should ideally be large enough at the end of the proposed build-up phase to meet expected N_e and N_c restoration targets and goals.
- Excluding age-0 individuals, age-1 juveniles or recruits should comprise ~11% and ~15% of a stable population of each species, respectively.
- The age-class distribution of a stable or growing population should contain multiple cohorts (juvenile recruits, sub-adults, and adults), but be dominated by younger cohorts.
- Molecular genetic and demographic data suggest that the target ratio of N_e/N_c should be ~5% for both species.

- Therefore to prevent declines of genetic variation, target population sizes of reintroduced or recovering populations of each species should be ≥5,000 individuals (N_e=250) and ≥10,000 individuals (N_e=500), respectively, and be comprised of multiple smaller demes spread throughout a river, and in close enough proximity to facilitate fish host mediated migration among demes.

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. Hence, as efforts to reintroduce and augment mussel populations continue to focus on developing propagation technology, release of cultured juveniles, and translocation of adults, reintroduction programs would benefit by using an experimental approach to test and achieve specific restoration hypotheses, objectives and goals. Merely evaluating release outcomes through rudimentary follow-up monitoring limits knowledge gained, i.e., only through *post hoc* interpretation of collection results (Seddon et al. 2007). Since specific quantitative criteria to evaluate population performance are sparse or lacking for most endangered species, the science and practice of reintroduction biology for mussels could be improved by conducting releases as planned experiments, designed to test the effectiveness of various release strategies and viability of different initial population sizes. Studies are needed to compare: (1) survival of released adults versus that of juveniles, (2) site abundance and density needed to promote sustainable recruitment and population growth, (3) influence of habitat factors on survival and growth of released adults and juveniles, such as examining effects of substratum and flow conditions, and (4) total population size and deme sizes needed to maintain sufficient

genetic variation over time. Modeling approaches can give important insights into developing performance criteria and factors that may influence viability, but ultimately, populations must be evaluated empirically using properly designed monitoring programs. Reintroduction and augmentation programs will face many trade-offs between costs and effectiveness of different release strategies and methods (Haight et al. 2000). Thus, only through carefully designed studies with adequate monitoring to compare reintroduction methods will optimal strategies be quantitatively validated.

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Table 1. Summary of matrix model parameters used in RAMAS to simulate population growth, harvest and reintroduction of the Cumberland combshell (*Epioblasma brevidens*) and oyster mussel (*E. capsaeformis*) in the Clinch River. Simulations were conducted using an exponential growth model, where standard deviation (SD) represents environmental variation and was sampled from a log-normal distribution.

Parameter	Description	*E. brevidens* Value (SD)	*E. capsaeformis* 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)	Harvested population	4,500	152,000
	Reintroduced population (number y^{-1} for 5 y)	24 48 72 96 120	50 100 200 300 400
Ages of adults (y)	Harvested or reintroduced	4-11	3-7
Carrying capacity (K)	Harvested population	15,000	350,000
	Reintroduced population	3,000	5,000 7,500 10,000
Type of density dependence		Ceiling (=K)	Ceiling (=K)

Table 2. Age-structured matrices of survival and fecundity values used to simulate population growth, harvest and restoration of Cumberland combshell (*Epioblasma brevidens*) and oyster mussel (*E. capsaeformis*) in the Clinch River. The three different survival values of juvenile mussels in the first column (0-1*) correspond to stable, low and moderate growth rates 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

Table 2. Continued.

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 (0; 8,495)	0.0554
	FF	184 (65; Infinity)	4,730 (0; 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

Table 4. Proposed restoration and recovery criteria used to evaluate populations of the 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	≥250	≥5,000	≥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*

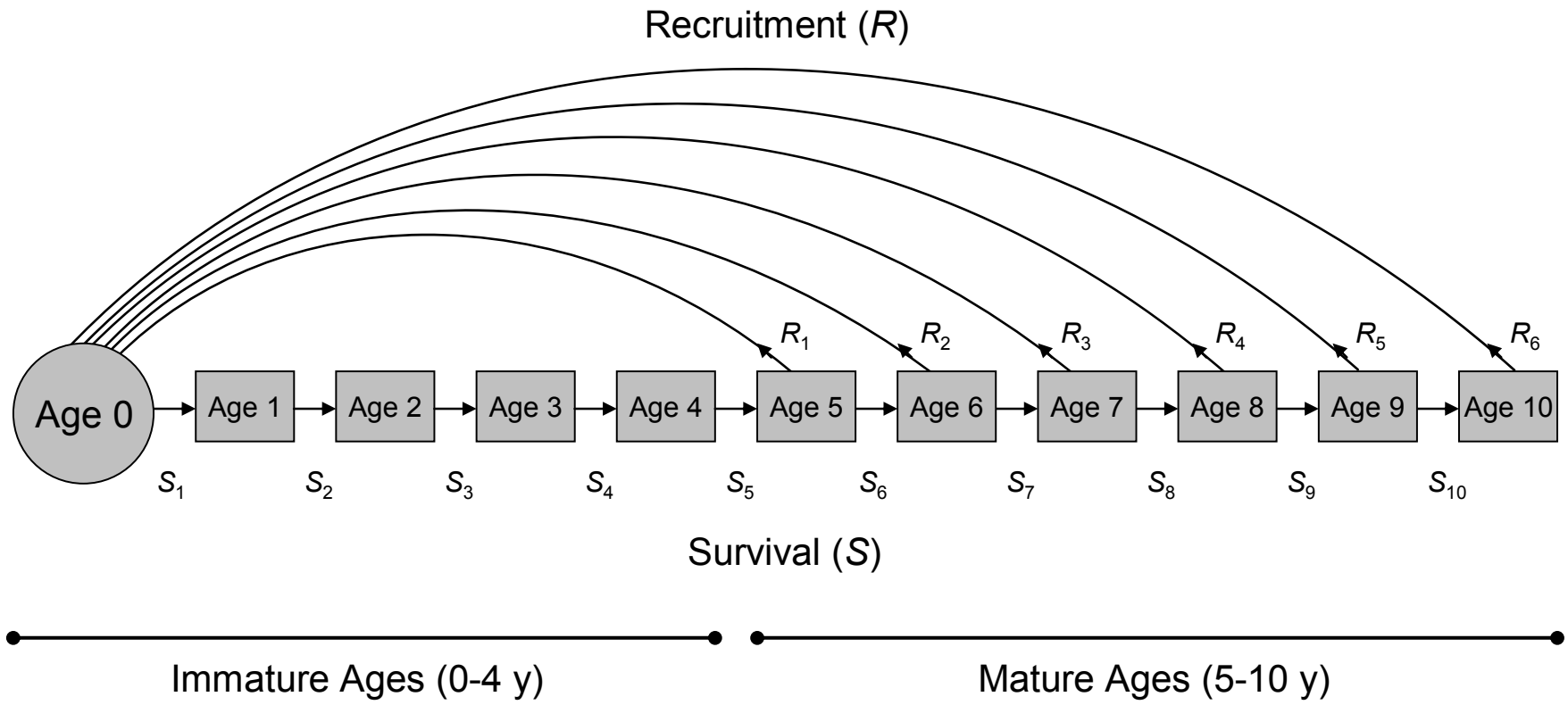
Recruitment (R)
R1
R2
R3
R4
R5
R6
Age 0
Age 1
Age 2
Age 3
Age 4
Age 5
Age 6
Age 7
Age 8
Age 9
Age 10
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
Survival (S)
Immature Ages (0-4 y)
Mature Ages (5-10 y)

Figure 1.

Figure 1. A general life-cycle diagram is shown 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.

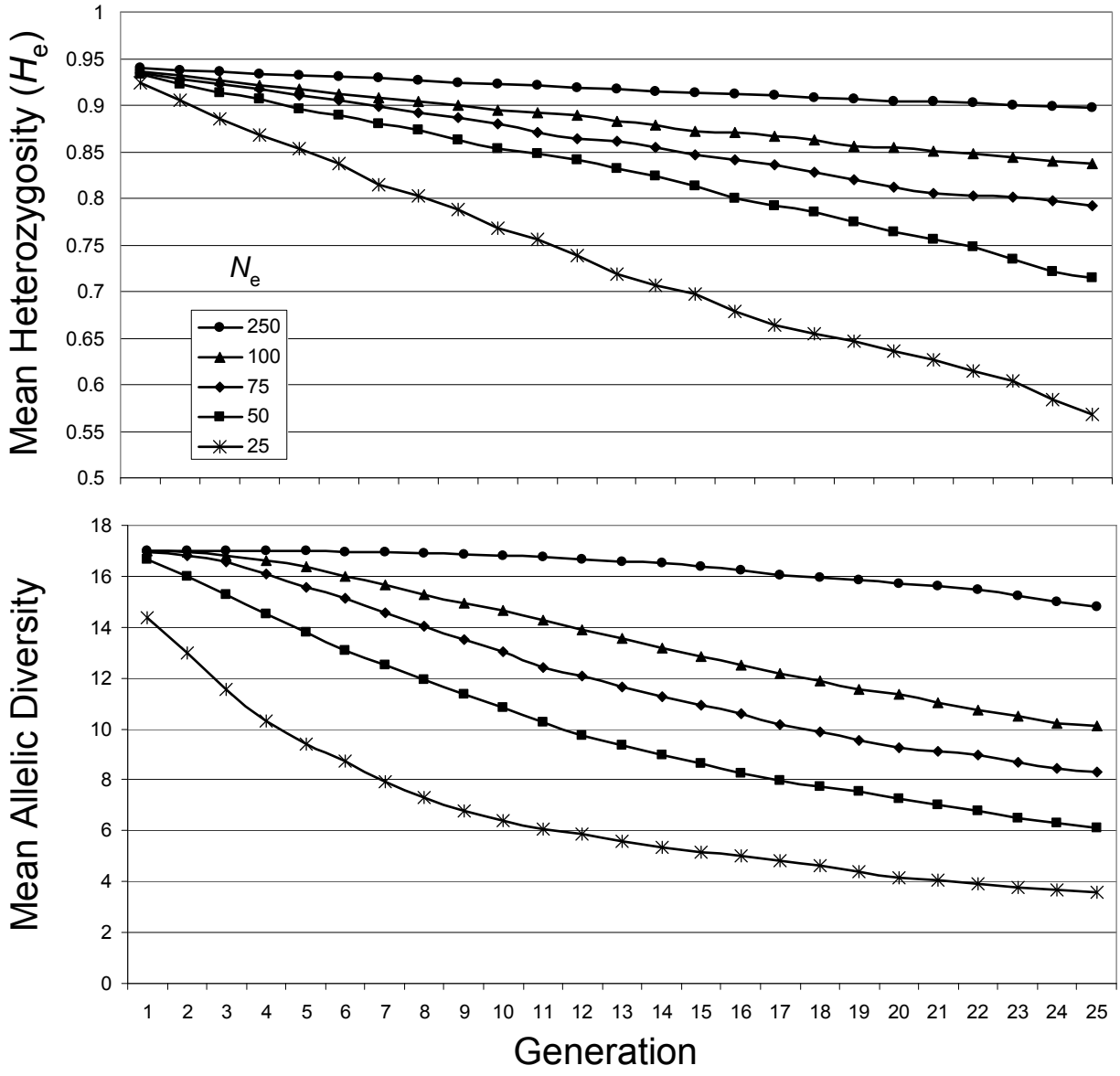

Figure 2. Predicted decline in heterozygosity and allelic diversity over time is dependent on effective population size (N_e). Generation length of each species is 5 y.

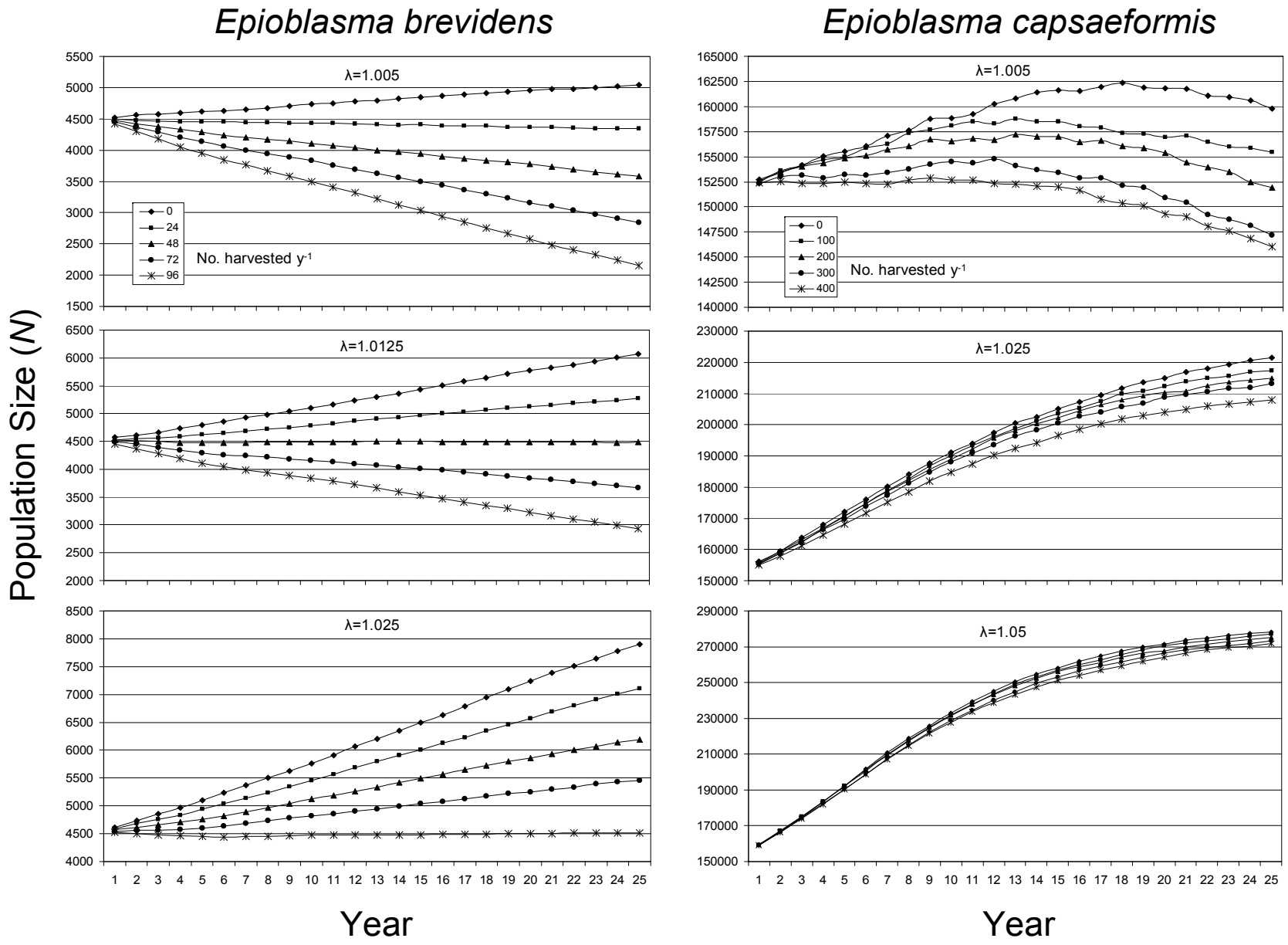
Epioblasma brevidens
Epioblasma capsaeformis
Population Size (N)
λ=1.005
λ=1.0125
λ=1.025
λ=1.005
λ=1.025
λ=1.05
0
24
48
72
96
No. harvested y-1
0
100
200
300
400
No. harvested y-1
Year
Year

Figure 3.

Figure 3. Mean population trajectories (10,000 simulations) of *Epioblasma brevidens* and *E. capsaeformis* demonstrate effect of different harvest intensities y^{-1} over a 25 y period. Simulations were conducted using stable, low and moderate population growth rates (λ), and K was set at 15,000 and 350,000 individuals, respectively.

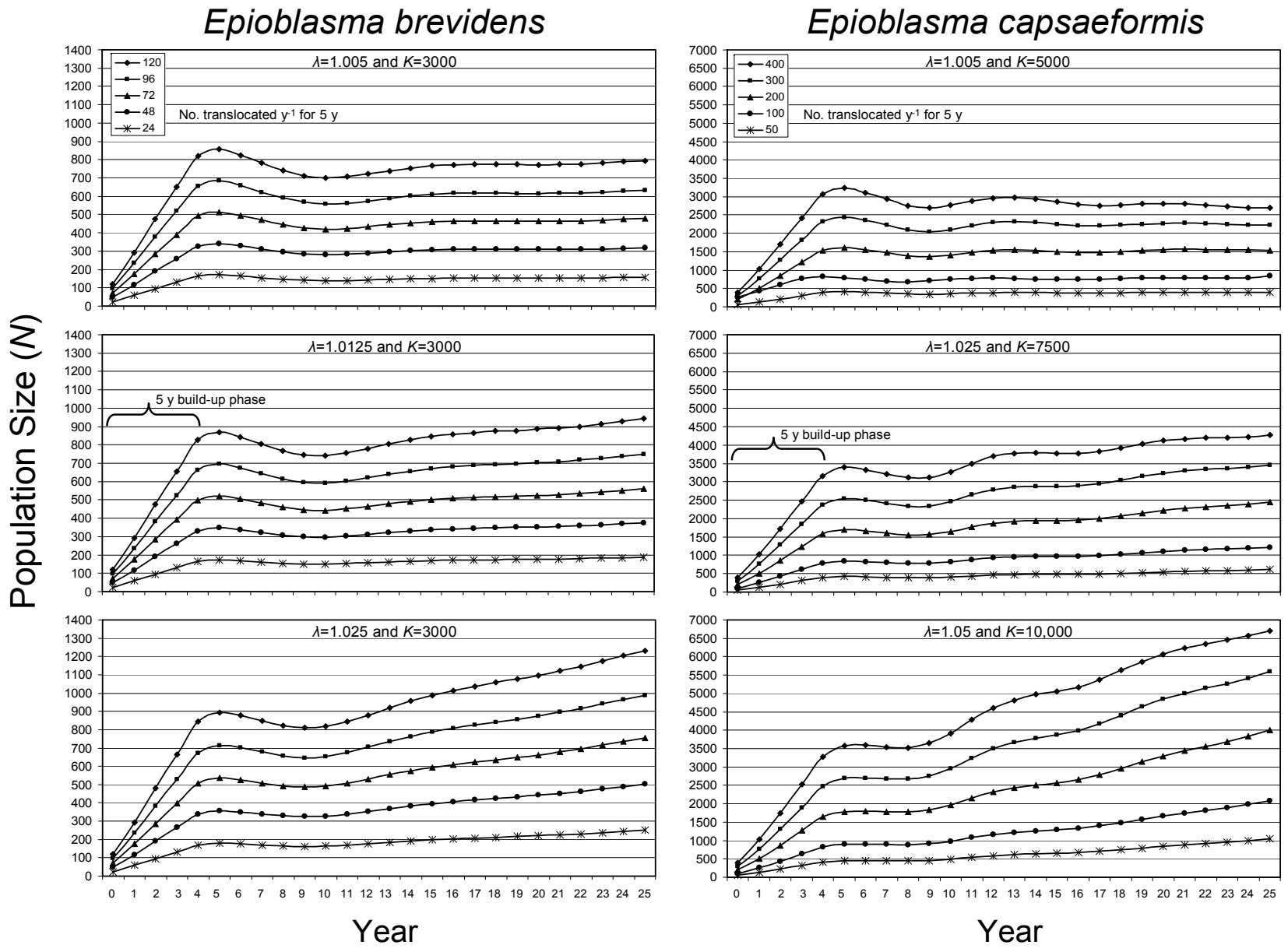
Epioblasma brevidens
Epioblasma capsaeformis
Population Size (N)
Year
Year
λ=1.005 and K=3000
No. translocated y-1 for 5 y
120
96
72
48
24
λ=1.005 and K=5000
400
300
200
100
50
λ=1.0125 and K=3000
5 y build-up phase
λ=1.025 and K=7500
λ=1.025 and K=3000
λ=1.05 and K=10,000

Figure 4.

Figure 4. Mean population trajectories (10,000 simulations) of *Epioblasma brevidens* and *E. capsaeformis* demonstrate how number of translocated adult mussels during a 5 y build-up phase affect population size over a 25 y period. Simulations were conducted using stable, low and moderate growth rates (λ), where K was variable only for *E. capsaeformis* (see Methods).

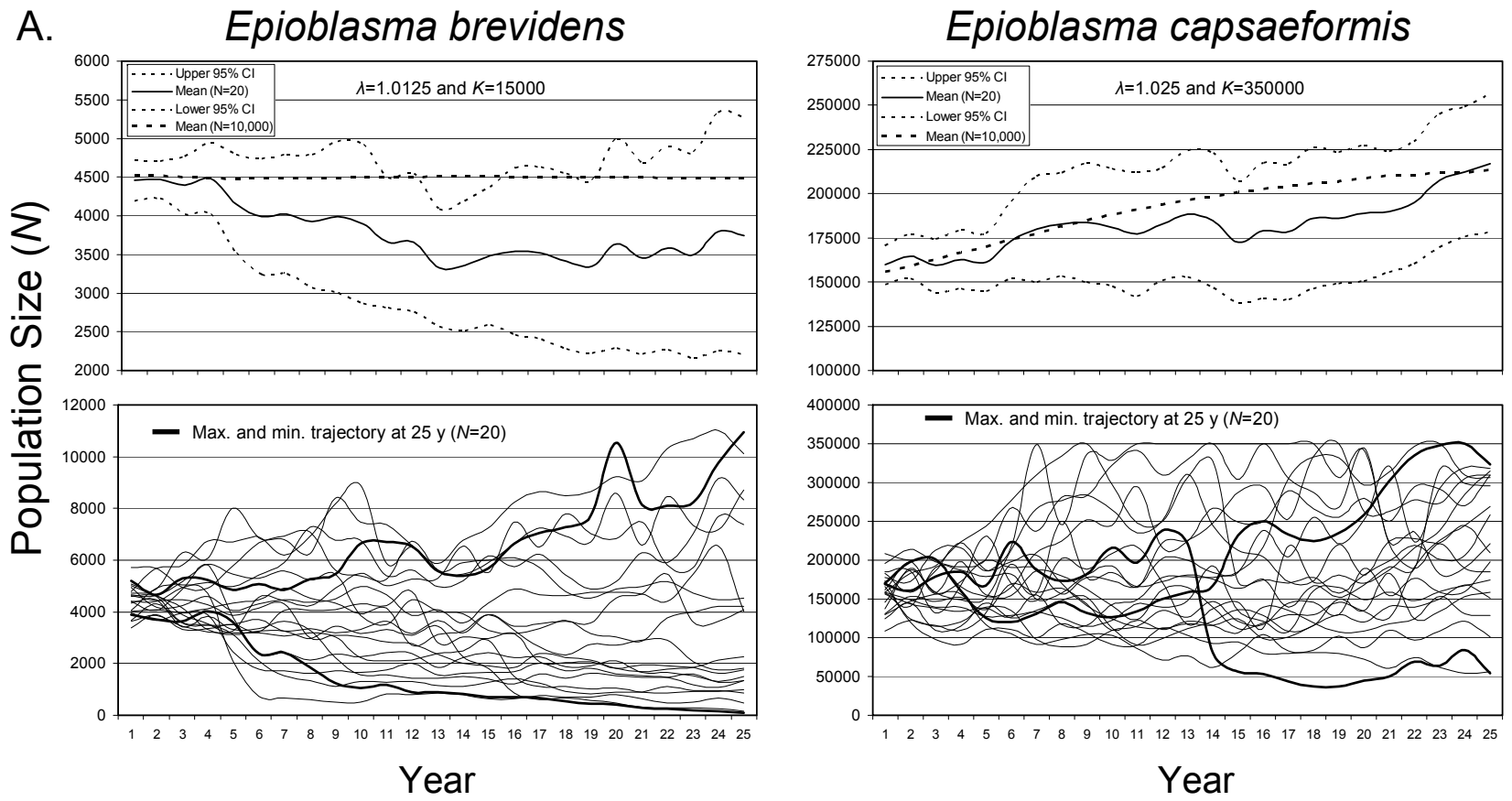

Figure 5 (Panel A).

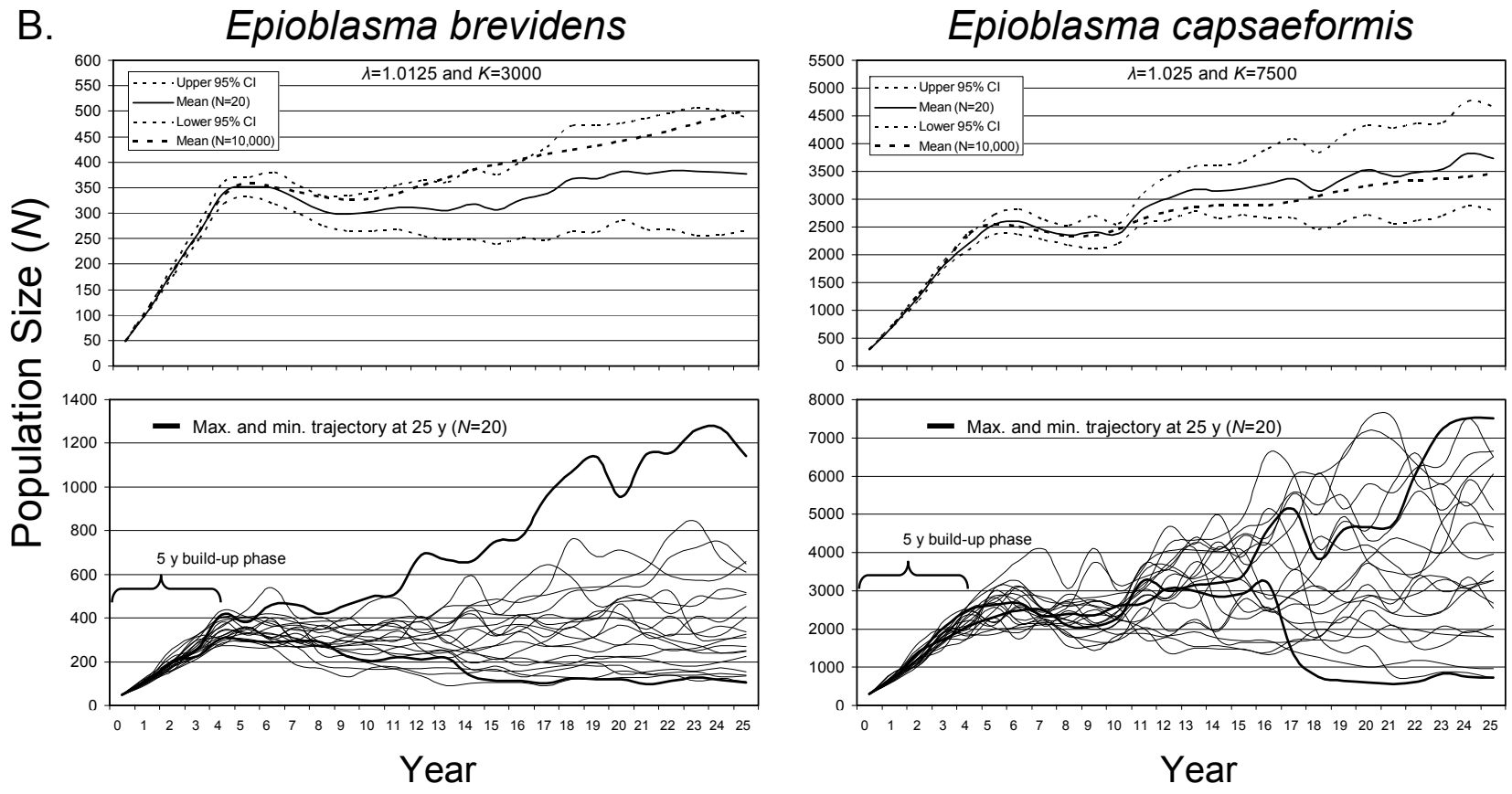

Figure 5 (Panel B).

Figure 5. 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 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 top panel (A) and the bottom panel (B) represent trajectories affected by either harvest or translocations 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 already given in Figures 3 and 4.

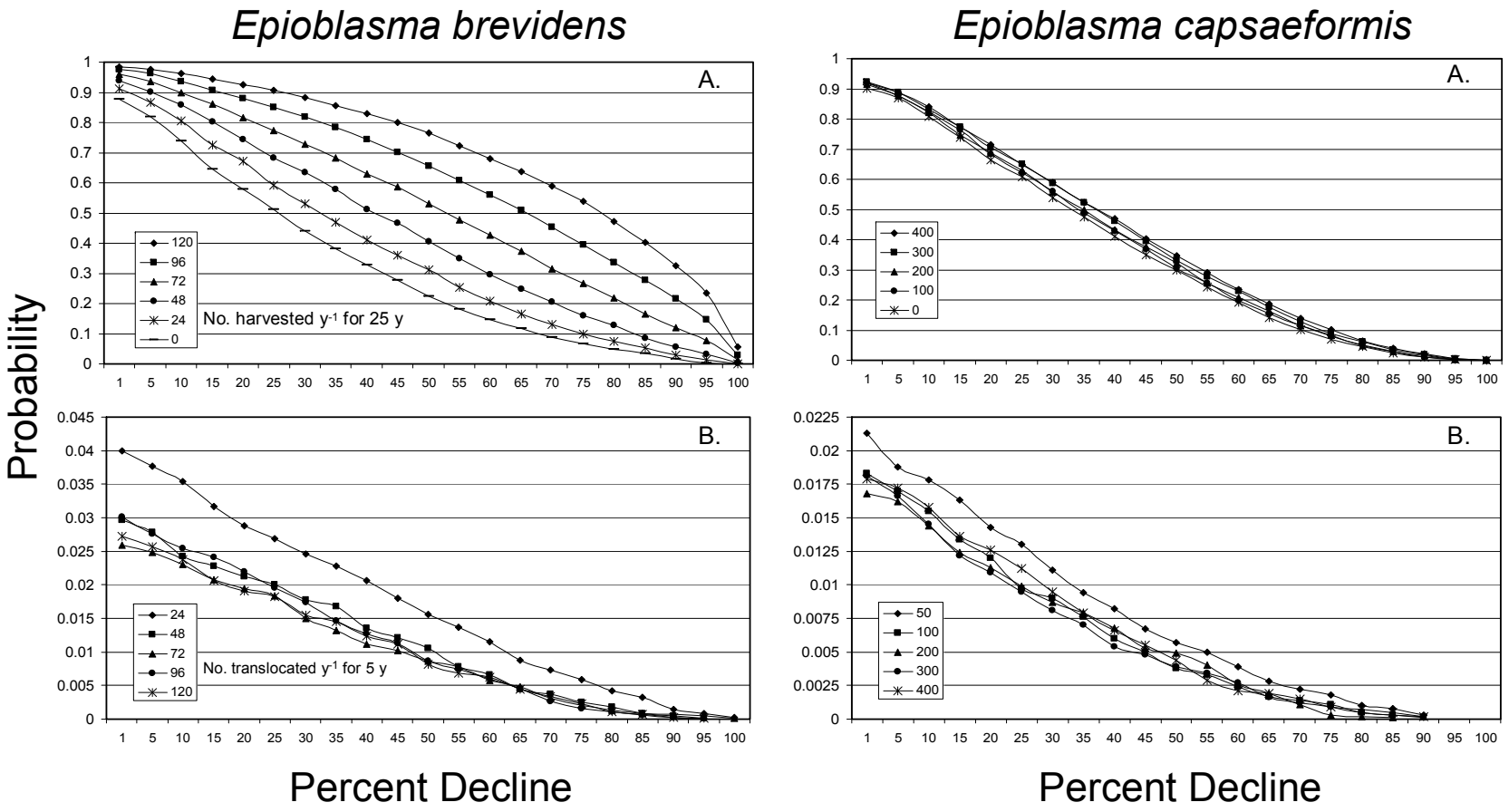

Figure 6. Probability of observing a decline from initial abundance over a 25 y period for *Epioblasma brevidens* and *E. capsaeformis*, based on various harvest (A) and translocation scenarios (B). All probabilities were computed using the stable growth rate (λ=1.005), which represents the high risk scenario investigated in the study. Probabilities of decline at higher growth rates are lower.

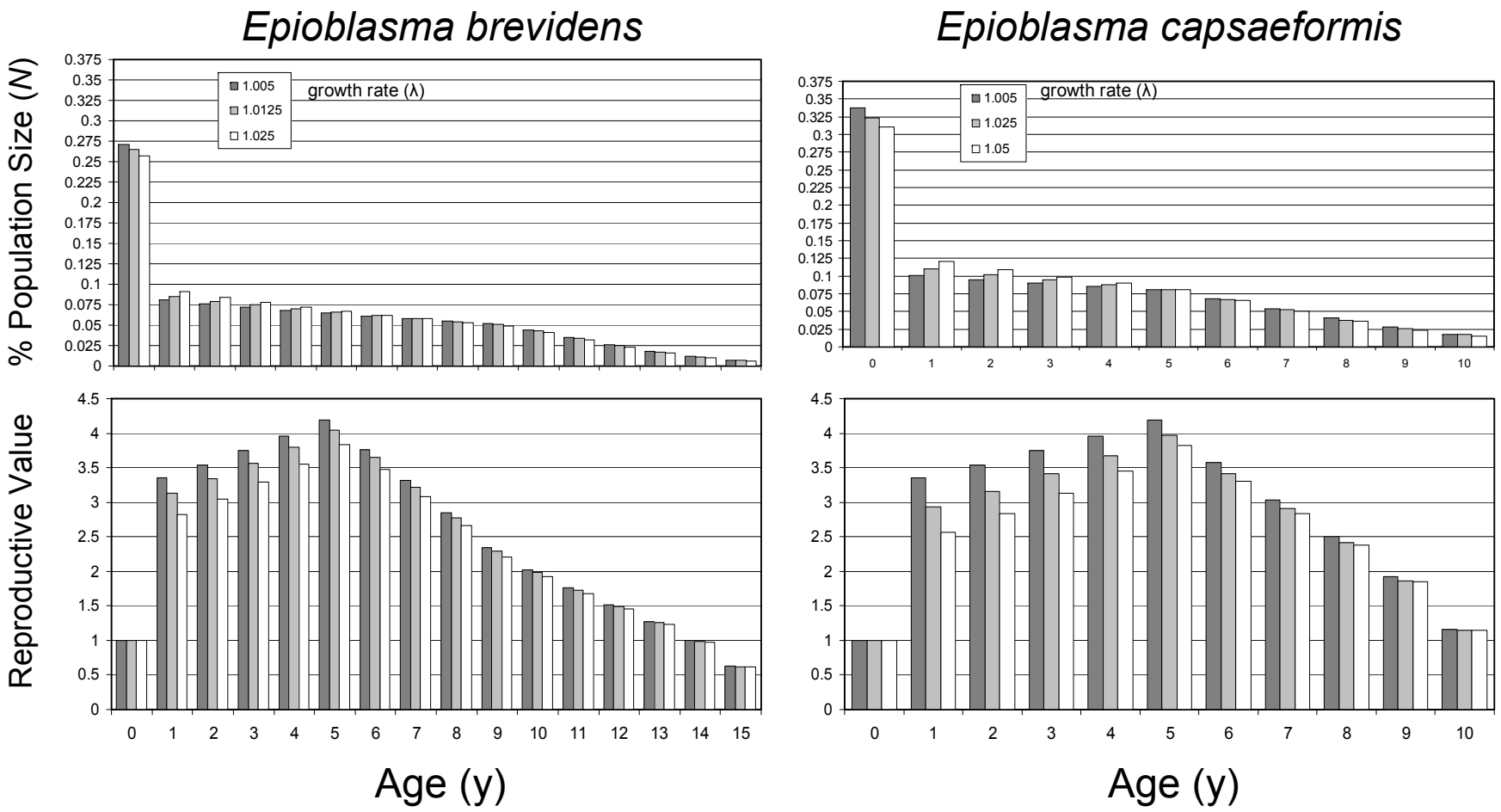

Figure 7. Stable-age distributions (SAD) and reproductive values for *Epioblasma brevidens* and *E. capsaeformis*; SADs at higher growth rates were similar to those computed using a stable growth rate, increasing only ~1-2% in younger age-classes (≤5 y).

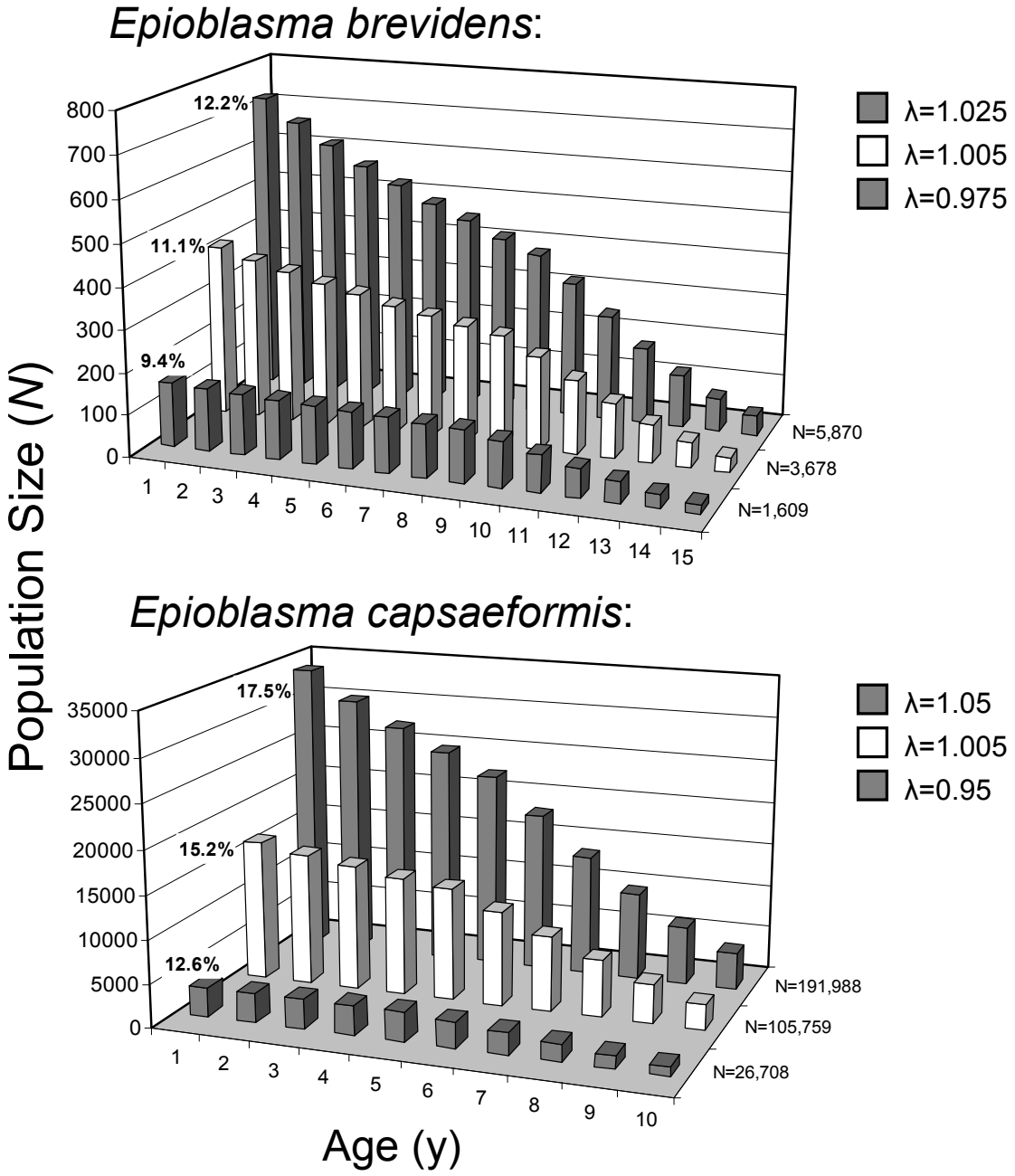
Epioblasma brevidens:
Population Size (N)
800
700
600
500
400
300
200
100
0
12.2%
11.1%
9.4%
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
N=5,870
N=3,678
N=1,609
λ=1.025
λ=1.005
λ=0.975
Epioblasma capsaeformis:
35000
30000
25000
20000
15000
10000
5000
0
17.5%
15.2%
12.6%
1 2 3 4 5 6 7 8 9 10
Age (y)
N=191,988
N=105,759
N=26,708
λ=1.05
λ=1.005
λ=0.95

Figure 8.

Figure 8. Stable-age distributions generated in RAMAS depicting declining (front), stable (middle) and expanding (back) populations of each species were generated without harvest. 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. 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.