

Population Performance Criteria to Evaluate Reintroduction and Recovery of Two Endangered Mussel Species, *Epioblasma brevidens* and *Epioblasma capsaeformis* (Bivalvia: Unionidae)

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

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ABSTRACT

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

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

INTRODUCTION

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

The 19th and 20th centuries were periods of large-scale habitat loss, degradation and fragmentation caused by dam construction and operation, and severe pollution of aquatic ecosystems, with concomitant losses in biodiversity throughout the United States of America (U.S.A.). During this period, freshwater mussel populations declined greatly and are now considered one of the most imperiled groups of animals in the country (Neves et al., 1997). The passage of landmark environmental laws in the U.S.A., such as the Clean Water Act (1972), Endangered Species Act (1973), and Surface Mining Control and Reclamation Act (1977), have helped reduce impacts and raise public awareness toward proper environmental stewardship (Stein et al.,

2000; Schwartz, 2008). More than thirty years later, some disturbed aquatic ecosystems are showing signs of improved water quality and physical habitat conditions. However, many mussel species cannot re-colonize previously occupied habitats because dams prevent dispersal of their host fishes. Reintroductions are now needed to restore populations and therefore are recommended in the recovery plans of these endangered species (National Native Mussel Conservation Committee, 1998; USFWS, 2004). Establishing new populations or boosting declining ones meets recovery plan goals and helps to reduce risk to species survival.

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

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

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

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

METHODS

Predicting decline of genetic diversity

To predict declines in genetic diversity, the program EASYPOP (Balloux, 2001) was used to simulate changes in heterozygosity and allelic diversity over time based on different levels of N_e . Initial measures of allelic diversity and number of polymorphic loci were obtained from Jones et al. (2004). Simulations were

conducted assuming random mating among diploid individuals belonging to a single population, and with an equal sex ratio. Number of loci was set to ten, with free recombination between loci and the same mutation scheme and rate (1×10^{-4}) for all loci. The selected mutation model was a mixed model with a proportion of both single-step mutation events (90%) and infinite allele mutation events (10%), where the latter mutation scheme allows for equal probability to mutate to any of the possible allelic states (Garza & Williamson, 2001). The number of possible allelic states was set at seventeen for each locus (Jones et al., 2004). Genetic variability of the initial population was set to maximum, meaning that alleles were randomly assigned to individuals. Simulations were conducted for 25 generations and replicated ten times to check for consistency of results.

Census and effective population sizes

Population sizes of *Epioblasma brevidens* and *E. capsaeformis* in the Clinch River, TN were estimated in 2004 by collection of standard, systematic 0.25 m² quadrat samples placed along transect lines (Jones & Neves, 2011). Sites sampled during the 2004 census included Wallen Bend [river kilometer (RKM) 309.9], Frost Ford (RKM 291.7) and Swan Island (RKM 277.2), which were selected because they represented the upper, middle and lower boundaries of the study reach, respectively. However, the entire site areas at Wallen Bend and Frost Ford were not sampled in 2004, just the upper ~15% and 63% of each site, respectively. Thus, total population size at each site for both species was estimated by applying the 2004 density estimates to the entire measured site area. This section of river contains robust mussel populations and is the only reach where the abundance of both species is adequate to estimate site-specific census sizes and to collect tissue samples for genetic analyses. In conjunction with 2004 censuses, tissues from 20–30 individuals per site were collected from both species and used to extract DNA and conduct analyses of DNA microsatellites. Contemporary effective population sizes (N_e) were estimated at each site using the linkage disequilibrium (LD) method of Hill (1981). The method is known to be downwardly biased, but the program LDNe corrects the bias and was used to estimate N_e (Waples, 2006; Waples & Do, 2007). The genetic methods used to estimate N_e , including DNA extraction, PCR amplification conditions, size scoring of DNA microsatellites and associated analyses are available in Jones (2009).

Age-structured population models

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

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

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

Matrix transition probabilities (i.e., survival rates) from one age class to the next were assumed to be the same for males and females of both species in this study (Table 2). Survival rates were based initially on data collected by Jones and Neves (2011), where rates were determined using collection of dead shells in 0.25 m² quadrat samples and from catch-curve analyses of shell length-at-age data. However, the assumptions of either method, especially the latter, are rarely met in field studies and typically give only rough approximations of survival rates (Miranda & Bettoli, 2007). Therefore, survival rates of ages ≥ 1 were determined by empirical data gathered from the aforementioned study, survival rates of *in-situ* field studies of sub-adult mussels [M. Pinder, Virginia Department of Game and Inland Fisheries (VDGIF), unpublished data], and by examining rates typically reported for other long-lived species (Musick, 1999; Akçakaya et al., 2004). Survival of newly metamorphosed age-0 juvenile mussels is poorly understood but thought by us to fit a Type III survivorship curve. A survival rate of 30% for age-0 juveniles was used based on published (Jones et al., 2005) and unpublished data from laboratory culture studies conducted at the Freshwater Mollusk Conservation Center, Virginia Tech, Blacksburg, and the Aquatic Wildlife Conservation Center, VDGIF, Marion, Virginia. The rate reflects laboratory survival of newly metamorphosed juveniles from age-0 to 1 y old. Specifically, these are age-0 juveniles that upon excysting from fish hosts are considered viable based on observing pedal-feeding locomotion.

Fecundity was implemented in the model as average number of viable juveniles produced per parent individual, to include males. Traditionally, fecundity has been measured as the number of glochidia per gravid female mussel (Haag, 2002; Jones et al., 2004; Jones & Neves, 2002). However, in this study it is used as

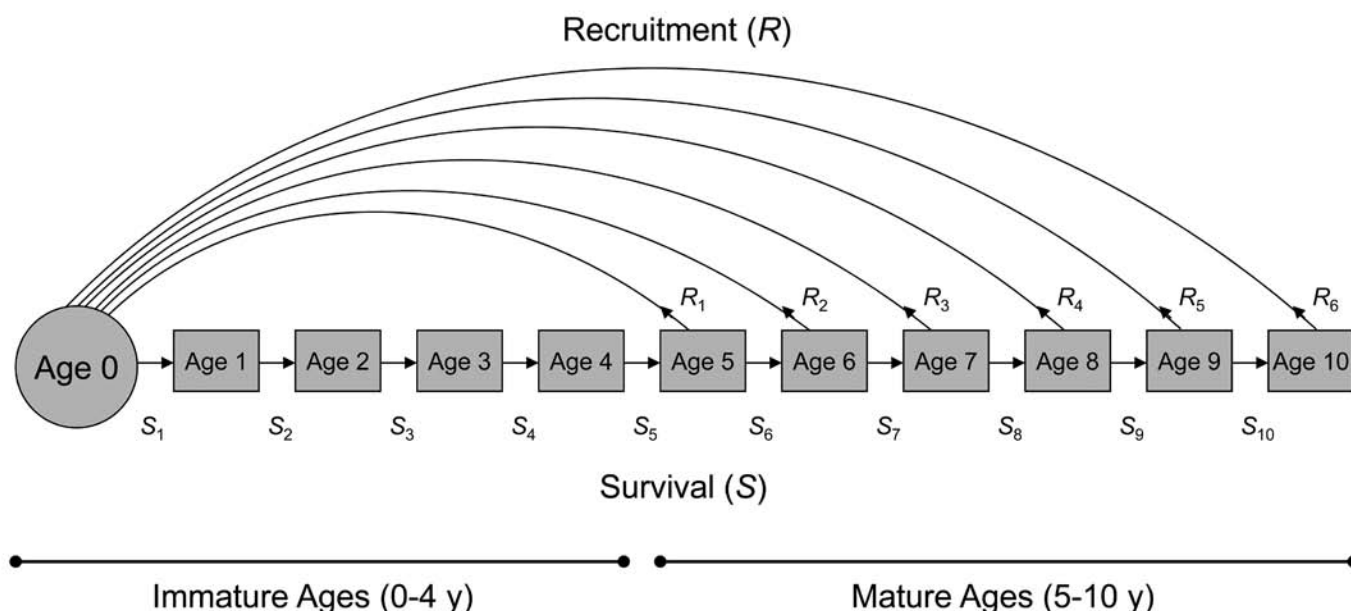
a composite value representing the net reproductive processes of both males and females, to include gametogenesis, spawning and fertilization, production of glochidia, attachment of glochidia on fish hosts, and ultimately metamorphosis and release of viable juveniles to the river bottom. Since these data are unavailable for most mussel species, it was solved iteratively in the matrix until the desired stable or increasing growth rate (λ) was obtained.

Demographic and environmental stochasticity

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

Initial abundances and ages

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

**FIGURE 1**

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

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

Population growth rate and carrying capacity

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

demographic possibilities.

Reproductive value

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

Reintroduction simulation scenarios

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

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

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

RESULTS

Effective population size and loss of genetic diversity

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

Predicted declines in heterozygosity (H_e) and allelic diversity were greatest at $N_e=25$, but diminished as N_e increased (Fig. 2). Also, loss of allelic diversity was greater than corresponding declines in H_e . Loss of genetic diversity was minimal for $N_e \geq 75$ 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 occurred when effective population size was at $N_e=25$, where mean H_e decreased by 10% and mean allelic diversity by ~7.5 alleles, or 44%, after 5 generations. While some loss of genetic diversity was evident for all investigated N_e , losses over longer generation times (≥ 10) were minimal (<5%) only at $N_e=500$.

Reintroduction abundance and population restoration success

The number of individuals reintroduced to a site during the 5 y population build-up phase was evaluated under three growth rate scenarios for both species (Fig. 3). Population trajectory patterns were characterized by three stages: (1) a sharp increase in population size during the build-up phase from 0-4 y, (2) followed by a period of disequilibrium when population size briefly declined and fluctuated from 5-14 y, and (3) a period of equilibrium when population size either remained stable or increased steadily from 15-25 y. Following the build-up phase, population size either remained stable or

increased at all transplant levels. An important feature of each trajectory was how the number translocated y^{-1} during the build-up phase influenced final population size, and as expected, higher reintroduction numbers resulted in larger final population sizes.

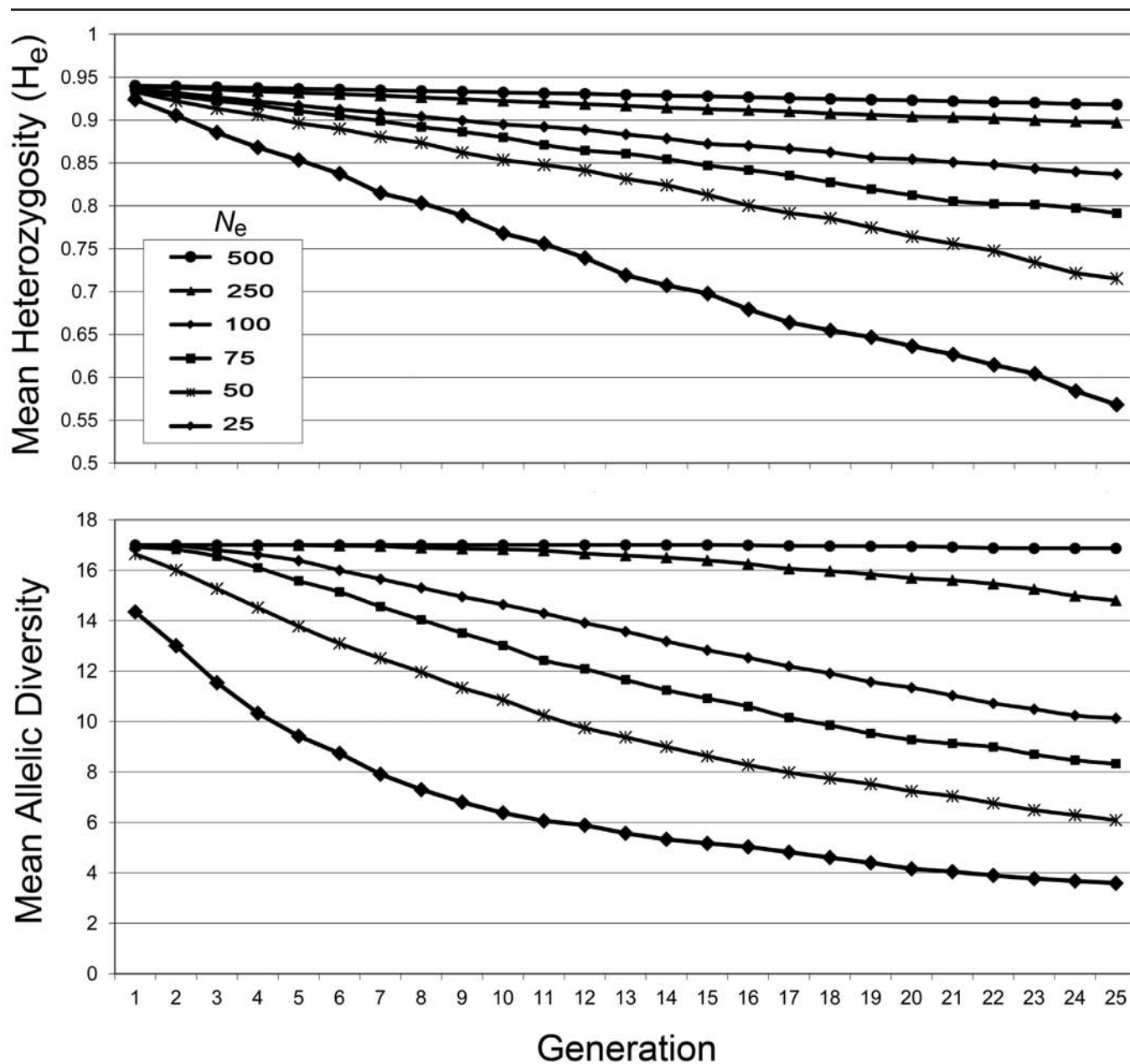
Similarly, reintroduction uncertainty for *E. brevidens* was evaluated under a scenario of transplanting 48 individuals y^{-1} and at a low growth rate ($\lambda=1.0125$). The sub-sampled mean was below the modeled mean, but the upper 95% CI contained most of the latter (Fig. 4). Eleven of the sub-sampled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

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

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

Age class structure and reproductive value

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

**FIGURE 2**

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

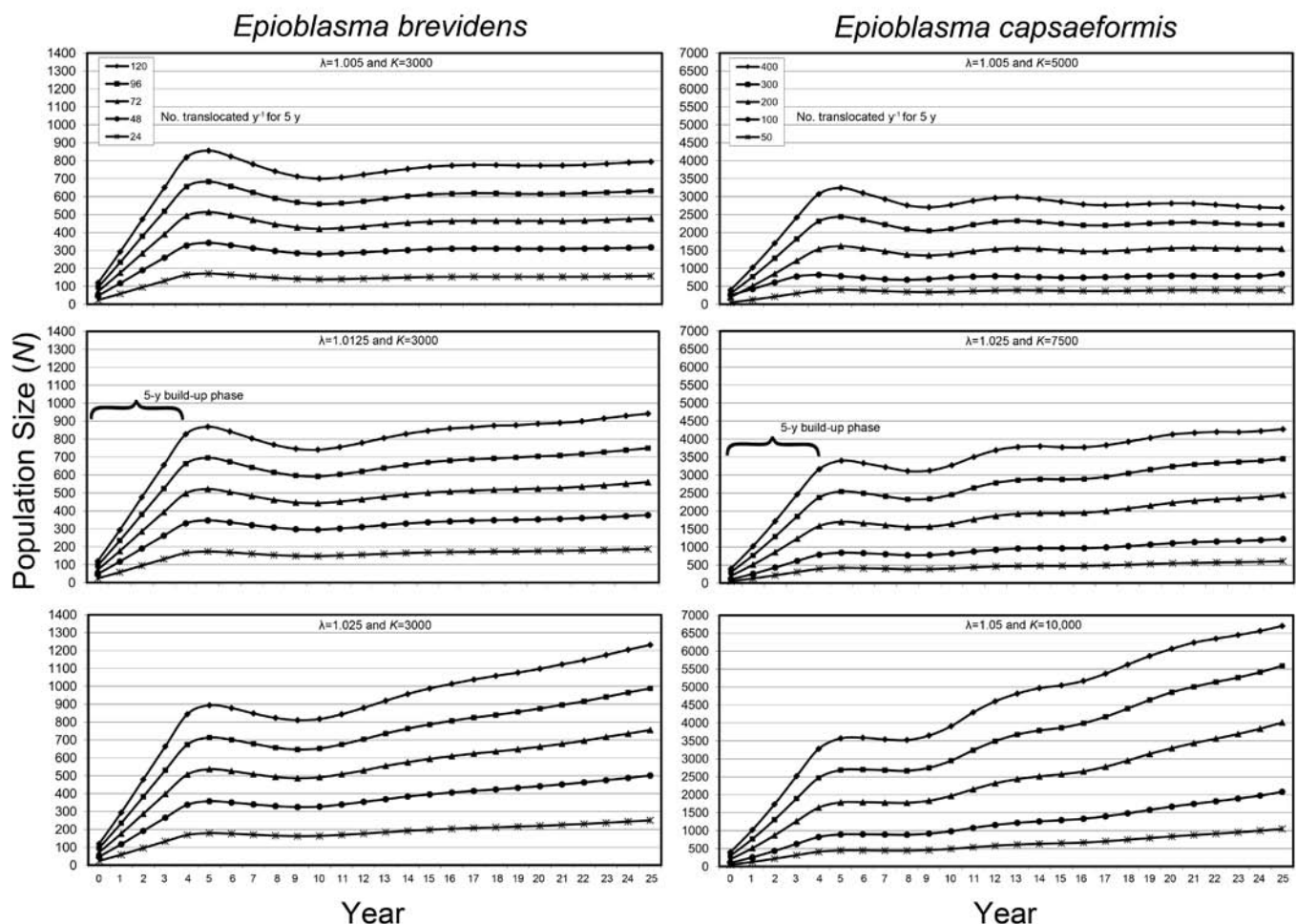


FIGURE 3

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

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

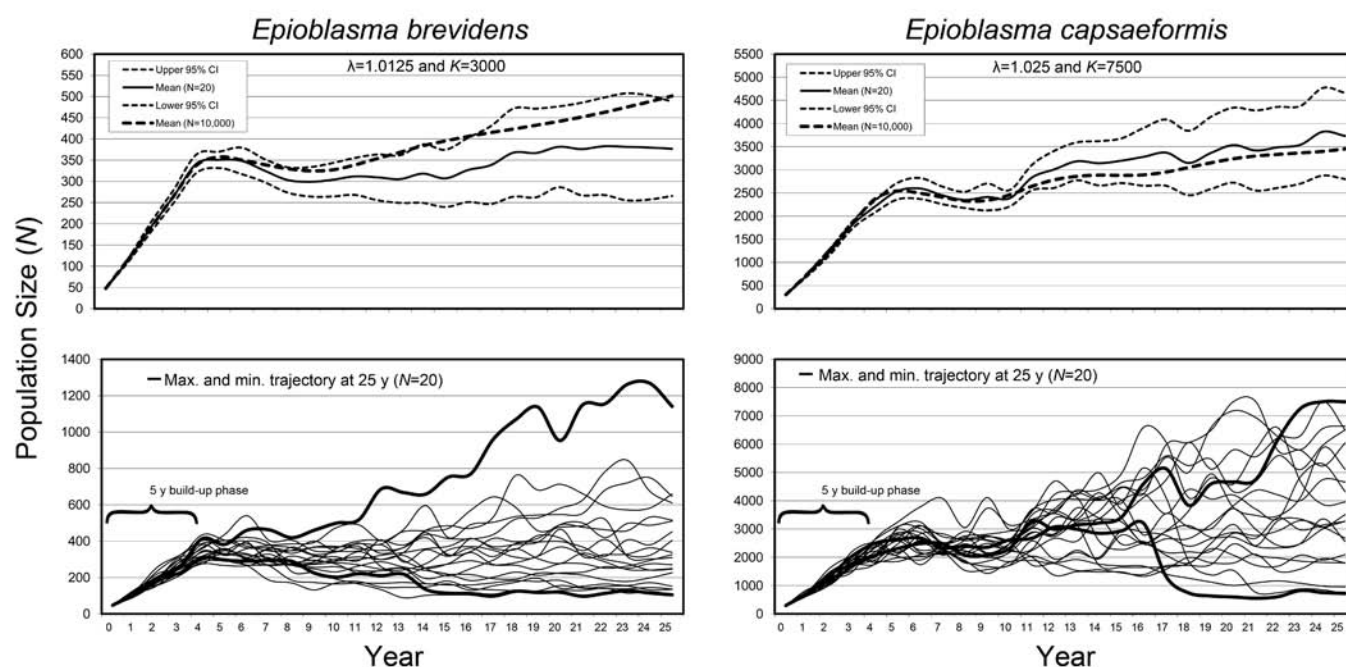
For both species, reproductive values were highest for individuals in the 5 y age-class, when maturity is reached (Fig. 6). Reproductive values also were high

for age-classes 1-2 years younger or older than 5 y, but declined thereafter, and values were lowest by comparison at the higher growth rates.

DISCUSSION

Effective population size and maintenance of genetic diversity

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

**FIGURE 4**

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

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

The recovery plan for *E. brevidens* and *E. capsaeformis* specifies that populations need to be large enough to maintain sufficient genetic variation to be able to adapt to changing environmental conditions (USFWS 2004). Hence, managing for genetic diversity is an identified goal in the recovery plan of these two species. The ratio of N_e/N_c can be used to set a target census size that is sufficient to maintain genetic diversity over time. The results of this study indicate that the ratio of N_e/N_c was low ($\sim 5\%$) for both species, suggesting that a ratio of 5% would be a practical target for either species (Table 4). The genetic modeling conducted in this study suggested that if total $N_e=500$, then a high proportion ($>95\%$) of molecular diversity could be retained over 25 generations, which is perhaps a

realistic management time frame for mussel species with generation lengths of 3-5 y. Currently published guidelines also recommend that $N_e=500$ to ensure that animal populations retain adaptive potential over long time periods (e.g., >100 generations) (Frankham et al., 2002), which for the mussel species studied here, would require a total $N_c=10,000$. This census population size could be reached by building up multiple demes spread throughout a river, ideally in a reach unimpeded by dams and that has the natural free-flowing conditions and fish hosts needed to facilitate dispersal among demes. The role of gene flow or connectivity among demes plays a critical role in countering the effects of genetic drift on long-term maintenance of genetic diversity (Palstra & Ruzzante, 2008). The target $N_e=500$ could be achieved for example by building-up 5-10 local demes with census sizes of 1,000-2,000 individuals per site, which corresponds locally to $N_e=50-100$. Achieving these recommended or even greater population sizes is feasible and consistent with the known demography of both species at sites in the lower Clinch River, TN (Jones & Neves, 2011).

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

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

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

Effect of reintroduction abundance on population restoration success

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

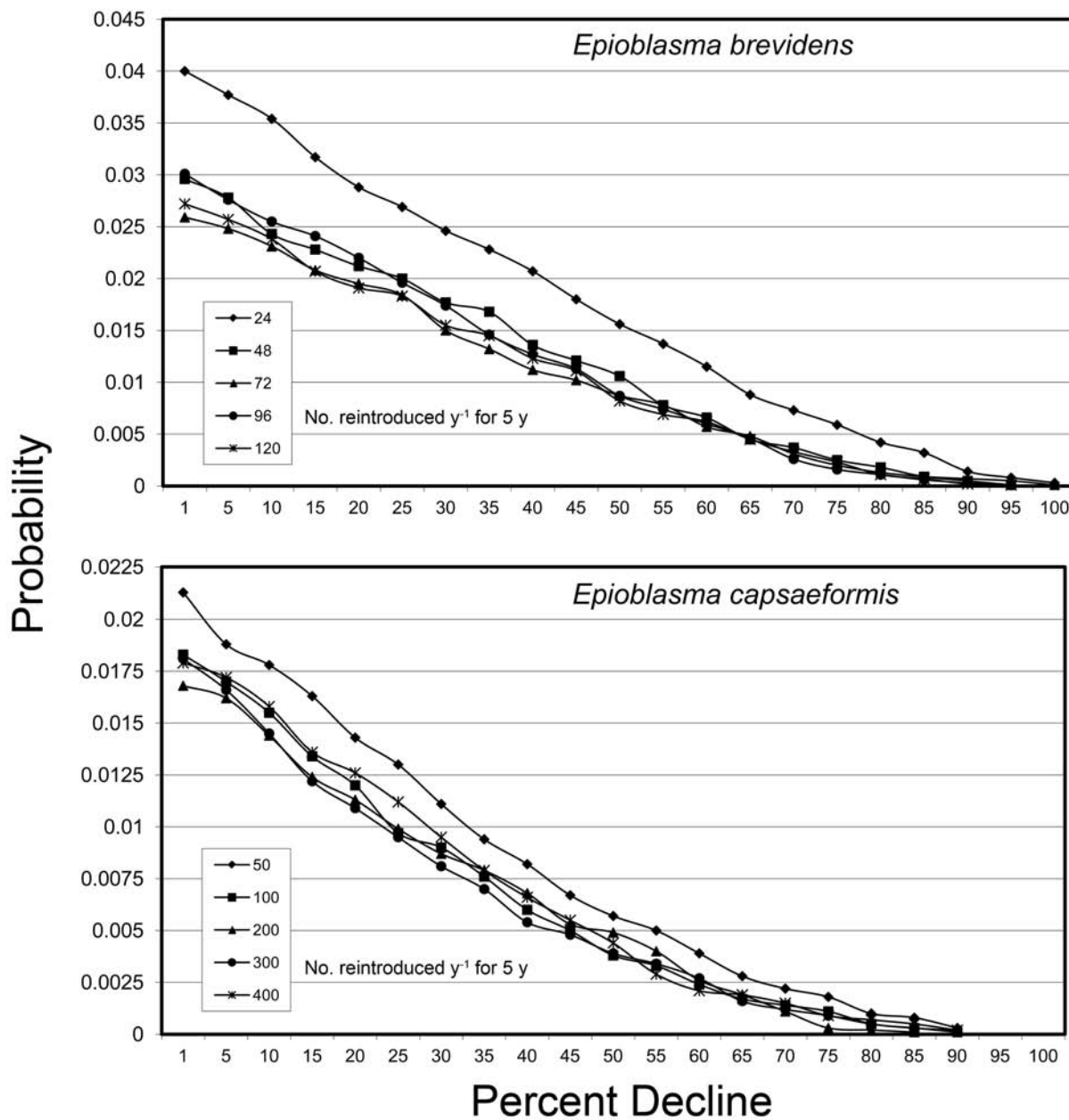
als or slightly larger depending on the specific growth rate employed. Therefore, it is critical that the intended target census size per site be similar to population size at the end of the build-up phase. Further, the target census size should be large enough to accommodate the N_e that meets established program goals.

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

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

Age class structure and recruitment

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

**FIGURE 5**

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

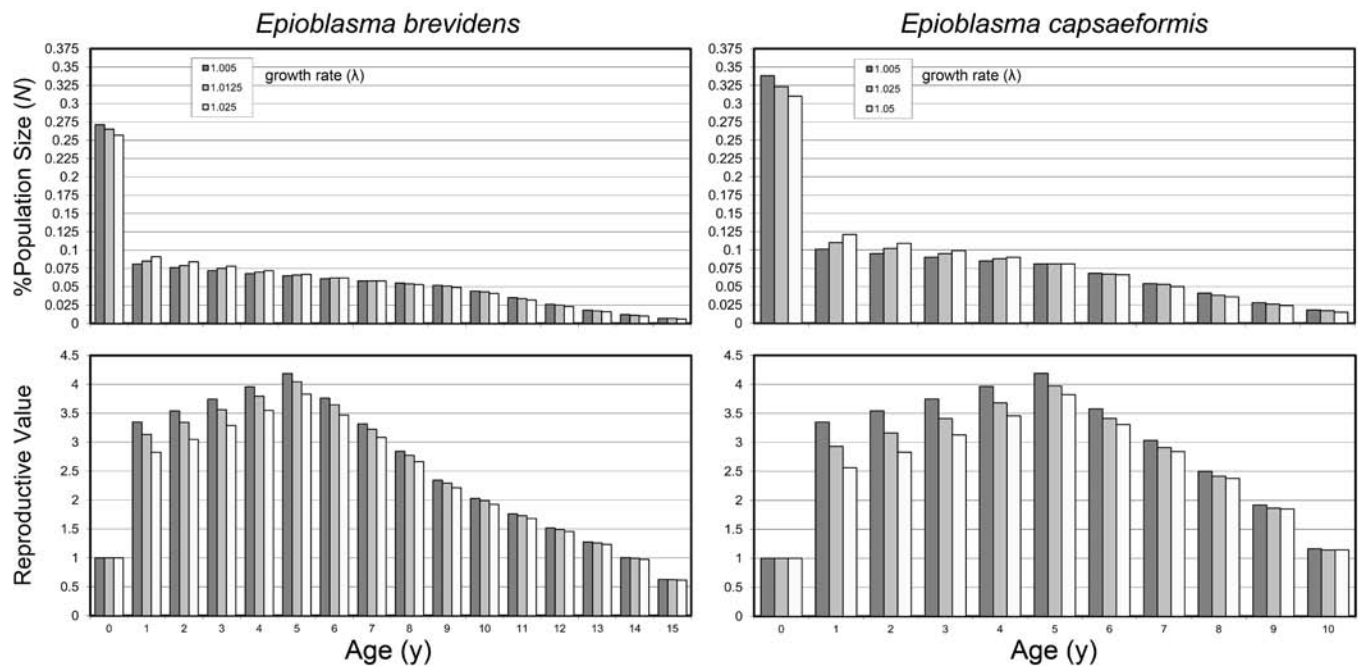


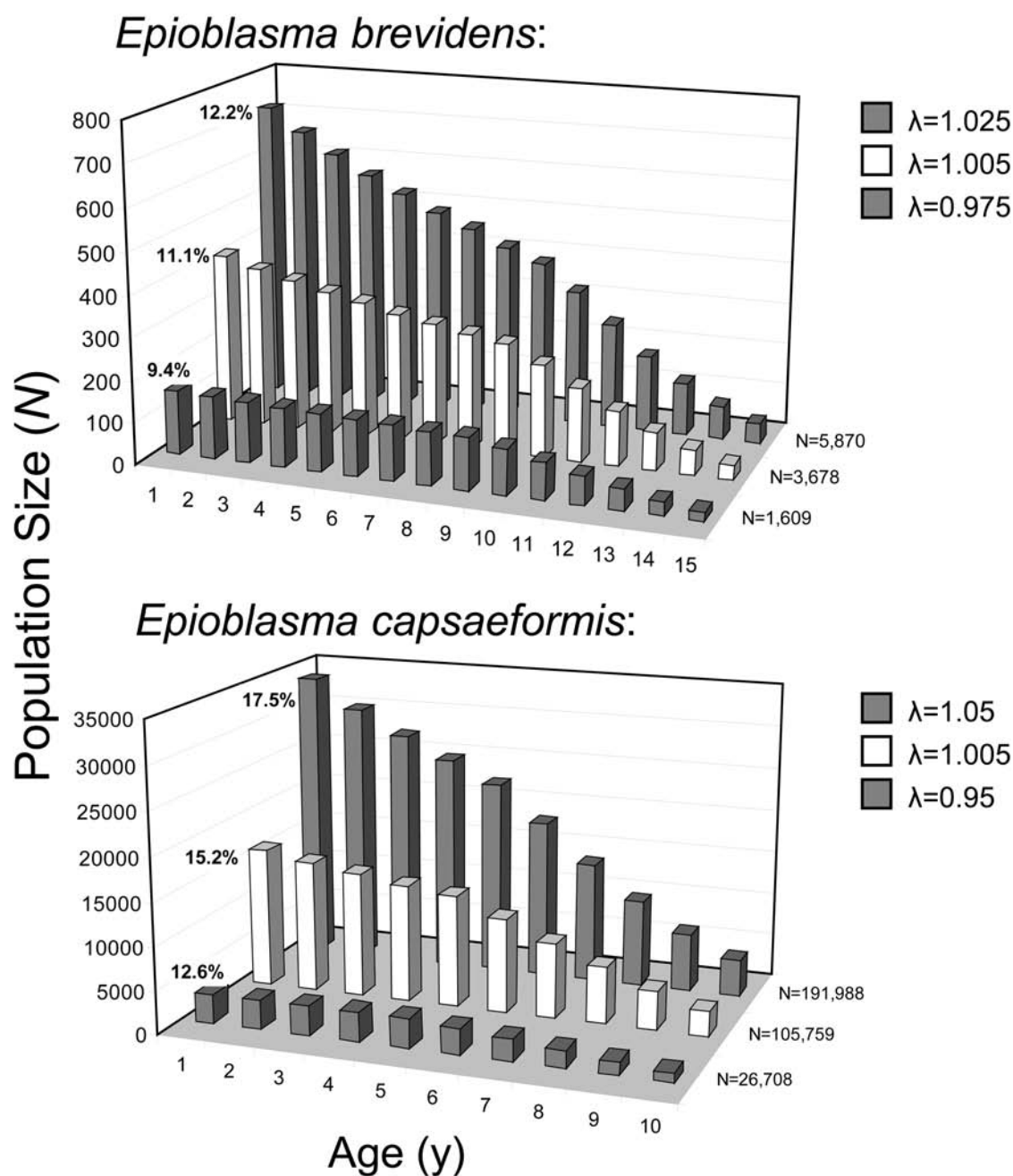
FIGURE 6

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

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

Two key demographic questions, then, are to determine the cohort structure and annual recruitment levels needed to sustain a stable or growing population of *E. brevidens* and *E. capsaeformis* (USFWS, 2004). The SAD histograms in Fig. 6 show profiles of three cohort structures for each species based on stable, low and moderate growth rates, illustrating that the cohort structure of a stable or growing population should be dominated by immature individuals and young adults. The histograms also indicate that age-0 individuals

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

**FIGURE 7**

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

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

Addressing modeling uncertainty

In this study, the two areas of modeling uncertainty that deserve further consideration are: (1) predicting declines of genetic diversity based on effective population size, and (2) the species-specific demographic input variables used for the Leslie-matrices. First, the simulations conducted in EASYPOP to predict declines of genetic diversity did not account for effects of hermaphroditic reproduction, fluctuating population size, or overlapping generations due to extended life span. The first two demographic factors would act to increase the rate of loss of genetic variation, while the last demographic factor would act to decrease the rate of loss of genetic variation. The program can simulate effects of different levels of hermaphroditic mating, but the incidence or rate of hermaphroditism is unknown for either *E. brevidens* or *E. capsaeformis*. Until studies are conducted to examine rates of hermaphroditic reproduction across a range of mussel taxonomic groups, modeling its effect on maintenance of genetic diversity will remain too speculative to be of predictive value. In addition, population size is held constant during program simulations; therefore, effects of fluctuating population size on genetic diversity are not considered, which would be important for species such as

E. capsaeformis, especially at small population sizes. Also not accounted for in the model was increased life span and overlapping generations, which would act to decrease the loss of genetic diversity. Thus, for species such as *E. brevidens* that exhibit longer life span and perhaps a more stable population size over time, such species would contain a greater number of overlapping generations, and therefore a higher ratio of N_e/N_c and capacity to retain genetic variation over time. Again, the mean ratio of N_e/N_c for *E. brevidens* was slightly higher than that for *E. capsaeformis* (Table 3). Other areas of modeling uncertainty include the mutation rate for molecular markers used in simulations, which in this study was based on a commonly reported rate for microsatellites in the literature, but higher or lower rates would slow down or accelerate loss of genetic variation, respectively.

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

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

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

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

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

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TABLE 1

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

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

TABLE 2

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

Epioblasma brevidens:

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

Epioblasma capsaeformis:

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

TABLE 3

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

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

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

TABLE 4

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

Species	Total N_e	Total N_c	Mean recruitment y^{-1} of age-1 juveniles	Mean age-class structure
<i>Epioblasma brevidens</i>	≥ 500	$\geq 10,000$	$\geq 11\%$	<ul style="list-style-type: none"> • Age-classes ranging from 1-12+ yrs. • Age-classes 1-4 comprise ~40% of N
<i>Epioblasma capsaeformis</i>	≥ 500	$\geq 10,000$	$\geq 15\%$	<ul style="list-style-type: none"> • Age-classes ranging from 1-8+ yrs. • Age-classes 1-4 comprise ~50% of N