

## GENETIC MANAGEMENT GUIDELINES FOR CAPTIVE PROPAGATION OF FRESHWATER MUSSELS (UNIONOIDEA)

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**ABSTRACT** Although the greatest global diversity of freshwater mussels (~300 species) resides in the United States, the superfamily Unionoidea is also the most imperiled taxon of animals in the nation. Thirty-five species are considered extinct, 70 species are listed as endangered or threatened, and approximately 100 more are species of conservation concern. To prevent additional species losses, biologists have developed methods for propagating juvenile mussels for release into the wild to restore or augment populations. Since 1997, mussel propagation facilities in the United States have released over 1 million juveniles of more than a dozen imperiled species, and survival of these juveniles in the wild has been documented. With the expectation of continued growth of these programs, agencies and facilities involved with mussel propagation must seriously consider the genetic implications of releasing captive-reared progeny. We propose 10 guidelines to help maintain the genetic resources of cultured and wild populations. Preservation of genetic diversity will require robust genetic analysis of source populations to define conservation units for valid species, subspecies, and unique populations. Hatchery protocols must be implemented that minimize risks of artificial selection and other genetic hazards affecting adaptive traits of progeny subsequently released to the wild. We advocate a pragmatic, adaptive approach to species recovery that incorporates the principles of conservation genetics into breeding programs, and prioritizes the immediate demographic needs of critically endangered mussel species.

**KEY WORDS:** freshwater mussels, genetic guidelines, conservation units, artificial propagation, imperiled species.

### INTRODUCTION

North America contains the greatest diversity of freshwater mussels in the world, approximately 300 species. However, the superfamily Unionoidea is the most imperiled group of animals in the United States, with 213 species (72%) considered endangered, threatened, or of special concern (Williams et al. 1993, Neves 1999). Already, approximately 35 species, or 12% of the North American mussel fauna, have become extinct in the last 100 y (Neves et al. 1997), an extinction rate comparable to estimated faunal losses in tropical rainforests (Ricciardi & Rasmussen 1999). For example, the Tennessee River basin historically was home to 102 species of mussels, and hence is the putative center of mussel diversity in North America (Parmalee & Bogan 1998). Of those original 102 species, 12 are extinct, 26 are listed as endangered under the Endangered Species Act, 20 are extirpated from the basin, and only about 30 species have stable populations (Parmalee & Bogan 1998). Most of the endangerment is caused by habitat loss and degradation caused by dams, sedimentation, water pollution, dredging and other anthropogenic factors (Neves et al. 1997, Neves 1999). Without immediate efforts to recover the 70 federally listed and numerous other imperiled species in United States watersheds, the extinction of additional species is likely. With this goal in mind, a committee of experts prepared a National Strategy for the Conservation of Native Freshwater Mussels to coordinate a nationwide conservation program (National Native Mussel Conservation Committee, 1998). This document elaborates on the genetic concerns expressed in the national strategy.

Propagation and culture of endangered mussel species typically

is recommended in recovery plans (e.g., US Fish and Wildlife Service (USFWS) 2004), to augment population sizes and to reintroduce species to sites within their historical ranges. A joint policy concerning controlled propagation was adopted by USFWS and the National Marine Fisheries Service (NMFS) to provide guidance and consistency for implementation of species recovery activities involving captive propagation (USFWS and NMFS, 2000). This policy recognizes controlled propagation as a useful tool for establishing new, self-sustaining populations; for supplementing or enhancing wild populations; and for holding offspring of listed species for part of their development if suitable natural conditions do not exist (USFWS and NMFS, 2000). Over the last 10 y, propagation technology has been developed at the Freshwater Mollusk Conservation Center at Virginia Polytechnic Institute and State University (Virginia Tech) and at other facilities in the United States to produce endangered juvenile mussels for this purpose (Neves 2004). Currently, 15 federal and state facilities propagate freshwater mussels in the Southeast and Midwest: Alabama Department of Conservation and Natural Resources, Kentucky Department of Fish and Wildlife Resources, Mammoth Cave National Park (Kentucky), University of Minnesota, North Carolina State University, Ohio State University, Southeast Aquarium Research Institute (Georgia), Southwest Missouri State University (SWMSU), Tennessee Tech University, USFWS Genoa National Fish Hatchery (Wisconsin), USFWS Mammoth Springs National Fish Hatchery (Arkansas), USFWS Warm Springs National Fish Hatchery (Georgia), USFWS White Sulphur Springs National Fish Hatchery (West Virginia), Virginia Department of Game and Inland Fisheries, and Virginia Tech University. These facilities have conducted critical life history studies on freshwater mussels (e.g., Jones & Neves 2002; Neves 2004) and, during the past several years, have released over 1 million juveniles of more than a dozen endangered species into rivers throughout the eastern United States. Survival of laboratory-reared juveniles 1–3 y of age after

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release already has been documented. For example, researchers at SWMSU produced thousands of juvenile Neosho mucket, *Lampsilis rafinesqueana* (Frierson, 1927), and reintroduced them in 2000 into historical habitat in the Fall and Verdigris rivers, Kansas. Biologists recovered 28 juveniles of this species at release sites in 2002 (C. Barnhart, SWMU, pers. comm. 2003). The endangered Higgin's-eye pearl mussel, *Lampsilis higginsii* (I. Lea 1857), and endangered oyster mussel, *Epioblasma capsaeformis* (I. Lea, 1834), have been propagated, outplanted, and recovered at release sites in the upper Mississippi River, Wisconsin, and Clinch River, Tennessee, respectively (R. Gordon, USFWS, Genoa National Fish Hatchery, pers. comm. 2002, Jones & Neves, unpubl. data, 2004). Therefore, propagation of mussels offers state and federal hatcheries an opportunity to expand their mission and assume an important role in conservation of biological diversity in the United States.

Federal and state biologists are optimistic about using propagation technology as a recovery tool for endangered mussels, and as mitigation for mussel populations killed by toxic spills or other anthropogenic impacts. However, as these programs mature and become more successful, the genetic implications of releasing captive-reared progeny to natal or other rivers must be considered. Propagation programs will be challenged to increase population sizes, whereas simultaneously trying to avoid negative consequences of altering the genetic resources of populations (Miller & Kapuscinski 2003). Because little is known about conservation genetics of mussels, researchers and natural resource managers will have to apply the science developed by professionals involved in conservation genetics of fishes, marine bivalves, and other organisms (Lannan 1980a, Lannan 1980b, Meffe 1986, Gaffney et al. 1993, Waples 1999, Hallerman 2003).

In this paper, we discuss application of the principles of conservation genetics to protect genetic resources of mussel populations. Our intent is to identify and justify basic, practical, genetic guidelines for their captive propagation. Readers should be aware that the current state-of-knowledge concerning mussel propagation technology is still in its infancy. Hence, some of the population genetic concerns presented are based upon theoretical principles. Key biological information often is lacking for mussels (e.g., population genetic structure, degree and distribution of adaptive genetic variation, numbers of juveniles needed to demographically boost and effectively restore populations, robust estimates of juvenile mortality in the laboratory and field, and effective and minimal viable population sizes). Therefore, questions concerning effects of artificial propagation technology on variation of adaptive genetic traits (e.g., life history traits) are yet unanswered. Propagation programs must take an adaptive approach to management of mussel resources, one that readily learns from results and applies best available science to conservation goals. Ten guidelines are discussed in this paper and are primarily aimed at avoiding genetic hazards associated with implementation of hatchery supplementation programs (Table 1). The intent of this paper is to remind mussel culturists of basic genetic guidelines and protocols to help protect genetic resources of propagated mussel species. We anticipate that as propagation technology advances, more sophisticated genetic management guidelines and plans will be needed to advise hatchery managers.

#### *Life History of Freshwater Mussels*

Development of conservation strategies unique to mussels must be grounded in an understanding of their life histories, population

genetic structure and population dynamics. Mussels are suspension-feeders that live most of their lives embedded in the gravel, sand or mud substrates in rivers or lakes. They are generally long-lived animals that exhibit slow to moderate population recruitment rates. Many species commonly live for more than 20 y, with some living more than 150 y (Ziuganov et al. 1998). Eggs of female mussels are fertilized internally by sperm released by males into the water and taken in by females during siphoning. The sexes are separate in most species, but some species are hermaphrodites (van der Schalie 1966, 1970). The embryos then develop in the gills of the female until becoming mature parasitic larvae (glochidia). Once the glochidia are mature, the female releases them into the water, where they must attach and encyst on the gills, fins or epidermis of a suitable host fish for metamorphosis to the juvenile stage. Glochidia of most mussel species require specific fish hosts to transform into juveniles and disperse into new habitats. To maximize attachment of glochidia to host fish, some mussel species produce glochidia in packets (conglutinates) or have mantle-tissue modified into the shape of lures that closely resemble prey items (Fig. 1). Female mantle tissue and conglutinates can mimic insect larvae and pupae, leeches, flatworms, and even other fish, all of which seem to attract host fish closer for possible infestation by glochidia (Parmalee & Bogan 1998). Metamorphosis typically requires 2–3 wk, depending on seasonal water temperatures. Once this parasitic transformation is complete, juveniles ex cyst and drop from the fish host to begin their lives on the bottom of a river or lake. The juvenile must settle into suitable substrate to have a high likelihood of survival.

Basic life history data, estimates of population size, and assessments of population genetic structure are lacking or sparse for many endangered mussel species. However, this information is critical for making sound management decisions during captive propagation of species. Thus, it is imperative that natural resource managers and administrators recognize that meeting many of the guidelines discussed in this paper will require that studies be conducted to assess population size, population genetic structure and life history parameters prior to implementation of propagation activities for some species, especially when multiple populations of a species exist and augmentation is an intended recovery strategy. In the remainder of the paper, we discuss the genetic issues that should be of concern to mussel culturists, each followed by its recommended guideline.

#### *Addressing Causes of Decline and Extinction*

The decline of mussel species throughout North America in the 20th century is attributed to degradation of habitat from various factors, including channelization, damming, mining, pollution, residential development, silting of rivers, and more recently, competition with the exotic zebra mussel *Dreissena polymorpha* (Pallas 1771). Dams change the flow, temperature and dissolved oxygen regimes of free-flowing rivers, such that the reproductive cycle of freshwater mussels is disrupted; gametogenesis is inhibited and fish hosts that prefer shallow, free-flowing river habitat are extirpated from impounded reaches. Thus, dams prevent or inhibit dispersal of mussels, limiting their ability to recolonize historic habitats and sustain natal ranges. Pollution and siltation of rivers degrades benthic habitats and interferes with osmoregulation, feeding and survival of adults and juveniles. Zebra mussels attach to the shells of native mussels and directly interfere with feeding, respiration and reproduction, causing a decline in physiological condi-

TABLE 1.

Summary and justification for the 10 genetic guidelines recommended in this paper for captive propagation of freshwater mussels.

	Summary	Justification
Guideline 1:	Threats to population persistence should be identified and, when feasible, corrected prior to implementing captive propagation for a species.	Increases availability of suitable habitat for population restoration
Guideline 2:	Each mussel species targeted for recovery using propagation technology should have a recovery plan that defines: (1) necessity of genetic characterization of remaining populations; (2) number of populations to be augmented or reintroduced to effectively recover the species; (3) appropriate locations for release of juvenile mussels; (4) number of juveniles to be released per year at a site; (5) number of gravid females to be collected per year for broodstock and (6) field and laboratory protocols to minimize genetic risks incurred by recovery activities.	Promotes implementation of hatchery activities using approved plans designed to protect genetic resources of populations
Guideline 3:	Collection of gravid female mussels for an augmentation ideally should come from the natal river, or from the closest genetically similar viable population, and that for restoring species into historical river habitat from the closest adjacent river system.	Maintains within- and among-population genetic variation
Guideline 4:	Establish an appropriate number of gravid females to be collected each year for propagation from a small population, as well as protocols to monitor survival and recruitment of artificially propagated juveniles.	Minimizes over-collection of broodstock from small populations
Guideline 5:	Maintain the largest possible genetically effective population size ( $N_e$ ) of propagated juvenile mussels by collecting an appropriate number of adult females each year to use as broodstock, and when feasible, rotate broodstock periodically.	Maintains within-population genetic variation
Guideline 6:	To avoid declines in population fitness due to outbreeding depression, populations that qualify as evolutionarily significant units (ESUs), subspecies, or closely related species should not be mixed.	Maintains among-population genetic variation
Guideline 7:	Reduce domestication selection during propagation and culture of juvenile mussels by mimicking natural life history processes, such as fish hosts, diet, temperature regimes, and habitat of a targeted species as closely as possible in the hatchery.	Increases progeny fitness and survival when released to the wild
Guideline 8:	Protocols are needed to prevent mixing of species or other management units through inadvertent exchanges of juveniles on laboratory equipment.	Maintains among population genetic variation
Guideline 9:	Release an appropriate number of juvenile mussels from an appropriate number of parents at release sites to maximize effective population size ( $N_e$ ), and at an early life stage to maximize survival in the wild, and to minimize the effects of domestication selection.	Maintains within population genetic variation and reduces domestication selection
Guideline 10:	Monitoring, evaluation, and database management should be regarded as an integral part of any augmentation or restoration program, followed as appropriate with modification of program goals and operations procedures to promote program effectiveness.	Promotes program effectiveness and adaptive management

tion and eventual death (Haag et al. 1993, Neves 1999). Both habitat degradation and nonindigenous species accelerate native mussel population declines by negatively affecting vital rates, notably reproduction, recruitment, survival and dispersal. Identifying threats to population persistence in species targeted for recovery is an important step in determining the feasibility and necessity of captive propagation. Only when the causes of decline are identified and corrected can conservationists effectively implement augmentations and reintroductions to remedy small population problems (Caughley 1994) and re-establish populations within historical ranges. Hence, propagation programs should be viewed as a re-

covery tool that is integrated within larger ecosystem management programs of habitat protection and restoration. Propagation of endangered mussel species is a supplement rather than a substitute for addressing factors responsible for population declines.

*Guideline 1:* Threats to population persistence should be identified and, when feasible, corrected prior to implementing captive propagation for a species.

#### *Propagation and Recovery Goals*

Because species conservation units are identified by genetic studies, the focus of recovery efforts for some species will shift to



**Figure 1.** (A & B) Phenotypically variable mantle displays of the wavy-rayed lampmussel *Lampsilis fasciola* mimic prey of predatory fish hosts, (C) Conglutinates of kidneyshell *Ptychobranchnus fasciolaris* mimic the larval stage of blackflies (Simuliidae) to attract darter hosts, and (D) Conglutinates of fluted kidneyshell *Ptychobranchnus subtentum* mimic the pupal stage of blackflies (length of conglutinates is 3–5 mm). Photographs by Jess Jones.

implementation of a captive propagation program. Hatcheries will be used to produce and release sufficient numbers of juvenile mussels of suitable physiological and genetic quality to alleviate the immediate threat of extinction for an endangered mussel species, and to demographically boost a population to the point where it is self-sustaining. Species should be prioritized for recovery based on their risk of extinction using analytic tools such as population viability analysis (PVA) (Beissinger & McCullough 2002). Accomplishing these goals will require restoration, augmentation and protection of viable populations of targeted species and their habitats, and continued research into their life history and population dynamics. *Restoration* is the re-establishment of populations into historical habitats from which the species has been extirpated, whereas *augmentation* is the rehabilitation of demographically depressed populations with translocated adults or hatchery-reared progeny. To achieve these goals, propagation programs will need to adopt straightforward guidelines to help protect genetic resources of species prior to initiating captive propagation activities.

Criteria for down-listing endangered species to threatened and ultimately to recovered status are stated in federal recovery plans (e.g., USFWS 1984, 2004) and are useful for developing propagation goals. These plans provide basic biological information pertinent to the recovery of a species. In addition to biological requirements, recovery plans typically require the existence of 3–6

(e.g., 6 for *Epioblasma capsaeformis*), and sometimes more, distinct viable populations of a species for down-listing from endangered to threatened (USFWS, 2004). Plans define a viable population as a wild, naturally reproducing population that is large enough to maintain sufficient genetic variation to enable the species to adapt and respond to natural habitat changes without further intervention (USFWS 2004). Populations are considered distinct when they are sufficiently separated such that a single mortality event would not eliminate or reduce more than one population.

*Guideline 2:* Each mussel species targeted for recovery using propagation technology should have a recovery plan that defines: (1) necessity of genetic characterization of remaining populations; (2) number of populations to be augmented or reintroduced to effectively recover the species; (3) appropriate locations for release of juvenile mussels; (4) number of juveniles to be released per year at each site; (5) number of gravid females to be collected per year for broodstock and (6) field and laboratory protocols to minimize genetic risks incurred by recovery activities.

#### *Genetic Hazards and Risks*

Hatchery and field activities associated with captive propagation programs pose genetic hazards for a targeted population. A hazard is an adverse genetic consequence of hatchery activities on

a population, and a risk is the probability that a hazard will occur (Busack & Currens 1995). Four types of genetic hazards have been identified: (1) extinction; (2) loss of within-population genetic variation; (3) loss of between-population genetic variation and (4) domestication selection (Busack & Currens 1995). The risk is generally low for causing the extinction of a species (Type 1 Hazard) by recovery activities of a hatchery program; however, the over-collection of broodstock warrants further consideration, to be discussed in the next section. The loss of within-population genetic variation (Type 2 Hazard) is generally caused by propagation of progeny from a limited number of parental broodstock. Loss of within-population genetic variation is accelerated when only a few adults are used as broodstock to produce progeny for release back into the natal population or when there is high variance of reproductive success among breeders (Hallerman 2003). The loss of between-population variation (Type 3 Hazard) is caused when genetic distinctiveness is reduced or lost because of mixing populations that otherwise would not interbreed naturally through migration. Because scientists are still uncertain of the effects of losing genetic variation on mussel population fitness, cognizant hatchery personnel should attempt to minimize human-caused losses of genetic variation (Hard 1995, Waples 1999). Domestication selection (Type 4 Hazard) is the consequence of any change in the selection regimen experienced by a cultured population, relative to what it would have experienced in the wild (Waples 1999). Hatcheries can alter selection regimes in several ways (discussed in detail later). Therefore, personnel involved with the design and implementation of hatchery supplementation programs need to recognize genetic hazards and understand how to avoid or minimize risks associated with propagation activities of targeted species, as discussed in the sections that follow.

#### *Selection of Broodstock Source Populations*

Gravid female mussels typically are collected directly from their natal river for use as hatchery broodstock. Populations in close proximity to one another within a river basin are typically best suited for use as broodstock to restore or augment adjacent populations with propagated juveniles. Hence when possible, collection of gravid females for augmenting a population should come from the natal river. Restoration of a species into an historical stream of occurrence should use broodstock from the closest adjacent watershed based on stream distance and with the most similar genetic and ecological characteristics. Source populations should be similar to the recipient population based on: (1) genetic lineage; (2) life history patterns; (3) ecology of the originating environment and (4) physiographic division (Miller & Kapuscinski 2003). In regards to the last factor, the close proximity of populations does not preclude the need for genetic analysis, especially for mussel species that have limited dispersal capabilities and occur in smaller headwater streams, such as some *Epioblasma* and *Pleurobema* spp. that use darters and minnows as hosts. Fine-scale geographic patterns of genetic variation may exist for these species. In such cases, the desire to preserve native population genetic structure (to avoid Type 3 Hazard) must be carefully balanced with the need to augment the population with progeny from a population in another stream. Further, viable populations of many endangered species are few, and some species are reduced to a single population. In these cases, the need for among-population genetic analysis will be limited or not necessary, and selection of source populations for translocation or captive propagation generally can be based on geography alone or criteria to prevent extinction.

*Guideline 3:* Collection of gravid female mussels for an augmentation ideally should come from the natal river, or from the closest genetically similar viable population, for restoring species into an historical river, from the closest adjacent river system.

Collection of an excessive number of adult female mussels for broodstock from a population can effectively “mine” natural populations by removing reproductive individuals from their source population and potentially contribute to decline (Type 1 Hazard, Miller & Kapuscinski 2003). This can happen when the survival of hatchery-reared progeny is less than survival of those produced naturally. For critically endangered species comprised of a single small population, it may be necessary to establish a maximum number of females to be collected each year for use as broodstock. This practice can help prevent over-collection of gravid females from a population and allow for some level of annual *in situ* reproduction to occur. For example, the population of endangered tan riffleshell *Epioblasma florentina walkeri* (Wilson & Clark 1914) in the Clinch River watershed occurs only in a 1,200 m reach of a tributary stream. The population size has been estimated at  $n = 2,000$  (Rogers et al. 2001). However, based on field observations of the number of gravid females releasing glochidia each year in the spring (J. Jones, unpublished data), the actual number of breeding females is much smaller. In such cases, establishing an appropriate number of gravid females to be collected each year for broodstock from a small population is a prudent measure to ensure continuation of annual *in situ* population reproduction. In addition, it is important to monitor the success of propagation efforts, to determine whether recruitment of hatchery-reared juveniles exceeds that of naturally produced juveniles, and that artificial propagation truly contributes to an increase of the targeted population.

*Guideline 4:* Establish an appropriate number of gravid females to be collected each year for propagation from a small population as well as protocols to monitor survival and recruitment of artificially propagated juveniles.

#### *Maintaining Genetic Resources of Cultured Mussel Species*

The American conservationist Aldo Leopold (1949) once stated that the art of successful tinkering requires that we first save all of the parts. Leopold’s advice certainly is applicable to conservation of genetic resources of propagated species; however, heeding this advice will require that culturists have detailed knowledge of the genetic composition of populations and an understanding of the effective population size ( $N_e$ ) needed to maintain appropriate levels of genetic diversity. Genetic studies will be needed to elucidate the genetic structure of populations, especially to determine the presence and proportions of rare alleles in populations. Once this information is available, an appropriate broodstock effective population size ( $N_e$ ) can be determined to maintain genetic variation.

The effective population size ( $N_e$ ) is defined as: the size of an *idealized population* that would lose genetic diversity at the same rate as the actual population under consideration (Kimura 1983). An idealized population assumes: (1) no migration; (2) distinct, nonoverlapping generations; (3) number of breeding adults is the same in all generations and (4) all individuals are potential breeders (Kimura 1983). Furthermore, it is assumed that all individuals in an idealized population randomly mate, and the population is closed in all succeeding generations; other simplifying conditions exist as well for an idealized population. Obviously, riverine populations of mussels do not meet these conditions, but the behavior

of how genes are transmitted from generation to generation in an idealized population provides useful theoretical predictions about how real populations can lose genetic diversity. For example, if a real population loses genetic diversity at the same rate as an idealized population of 100, then the  $N_e$  of the real population is 100, even if it contains 1,000 individuals (Frankham et al. 2002). For many wild populations, the estimated ratio of effective population size to census population size ( $N_e/N_c$ ) is approximately 10% (Frankham et al. 2002). Hence, the actual number of breeding adults in a natural or captive population contributing their offspring to the next generation is considerably less than the census size of a wild or broodstock population.

Populations of imperiled mussel species often are small and susceptible to loss of genetic variation through ecological, demographic and anthropogenic factors, to include artificial propagation. Furthermore, once these populations become small, genetic variation typically is further eroded by nonselective forces, such as inbreeding and genetic drift. Random genetic drift occurs at a rate inversely proportional to the genetically effective population size ( $N_e$ ) (Kimura & Crow 1963). Importantly, loss of within-population genetic variation (Type 2 Hazard) can result in a reduced capacity of populations to adapt to changing environments, which is manifested as a decrease in fitness of individuals within a population (Meffe 1986). Because management for a large  $N_e$  is necessary to avoid inbreeding and loss of genetic variation, what, then, are guidelines that mussel culturists and biologists can follow to accomplish these goals? Popular management guidelines—such as the “50/500 rule,” which recommends an  $N_e$  of 50 to prevent inbreeding depression and 500 to prevent long-term erosion of genetic variability by genetic drift (Frankel & Soule 1981)—are helpful but often impractical for critically endangered mussel species. Therefore, a long-term strategy is needed to increase  $N_e$  over many year-classes, especially for small populations. In addition, because little is known of mussel reproductive biology (i.e., fertilization success rates) equal sex ratios may have to be assumed. For example, if 10 gravid females are collected as broodstock, it might be assumed that each female was fertilized by one male, and therefore,  $N_e = 20$ . However, it is likely that  $N_e$  is much lower in natural populations of some species because of hermaphroditic reproduction and low fertilization success between males and females. A target sample of 20–25 randomly collected animals can contain ~98% of the expected heterozygosity of a wild population (Lacy 1994), and could be achieved for even small populations over 1–5 y. Accordingly, multiple gravid female mussels should be collected annually from various sites to represent a range of river locations, habitats and subpopulations within the source population. Larger ( $n > 5,000$ ) populations of an endangered mussel species are likely to contain considerably higher amounts of genetic variation; therefore, collection of a greater number of gravid females per year is necessary to increase  $N_e$  and genetic diversity of propagated cohorts over time. Other researchers have recommended collecting a minimum of 50–200 individuals to serve as broodstock (Ryman & Stahl 1980, Allendorf & Ryman 1987). Such a strategy helps ensure that any rare alleles (e.g., those at a frequency of <5%) occurring in a population are adequately represented in the broodstock and subsequent progeny. Thus, for larger populations where collection of gravid females can easily be accomplished, it is recommended that >50 individuals be targeted over time to augment or re-establish populations. All females should be tagged prior to their release back to the river or if held in a hatchery as captive broodstock. This will prevent excessive

use and over-representation of the genomes of a limited number of females (see discussion of Ryman & Laikre [1991] effect below). In addition, tagged mussels can be tracked in the field and hatchery for survival and subsequent gravidity. In the future, factorial mating designs (in which males and/or females are mated with multiple members of the other sex) might be used to increase genetically effective population size of hatchery-produced progeny. Thus, with time, we hope to gain the ability to implement direct matings and thereby minimize loss of within-population variation.

*Guideline 5:* Maintain the largest possible genetically effective population size ( $N_e$ ) of propagated juvenile mussels by collecting an appropriate number of adult females each year to use as broodstock and, when feasible, rotate broodstock periodically.

Because the effect of loss of genetic diversity in mussel populations is unknown, management of effective population size and genetic variation for mussel species should be a primary concern to biologists and culturists. However, technical constraints confronting propagation of some endangered mussel species dictate that these genetic concerns will be difficult to accommodate initially. Some species are now sufficiently rare, that obtaining even a few gravid females per year for propagation is difficult (Rogers et al. 2001, Jones et al. 2004). The high fecundity and output of glochidia by individual females provides an opportunity to produce many more juveniles than would have survived in nature; such recovery opportunities should be exploited to alleviate demographic and environmental threats to persistence of small populations. Thus, in the initial stages of recovery for some endangered mussel species, increasing population density to alleviate immediate threats to population persistence will have to be weighed against managing for increasing genetically effective population size and genetic diversity. Clearly, there is a need to balance our capacity to produce and release numerous progeny while trying to maintain genetic diversity of populations (Type 2 and 3 Hazards).

#### *Outbreeding Depression*

Outbreeding depression is a decrease in fitness of progeny upon breakup of coadapted gene complexes resulting from mating of distantly related individuals (Dobzhansky 1937). Although untested in freshwater mussels, outbreeding depression has posed a threat to population viability in some species of marine bivalve mollusks (Lannan 1980a, Lannan 1980b, Gaffney et al. 1993, Boudry et al. 2002). We hypothesize that mussel species and populations that have limited dispersal capabilities and that are subject to local environmental selection pressures may have developed coadapted gene complexes for adaptation to such environments, to include local host fish communities. For example, recent research on fish host specificity has demonstrated that glochidia obtained from allopatric mussel populations can exhibit significant among-population variation in transformation success when exposed to local fish host communities (Rogers et al. 2001, Eckert 2003, Jones et al. 2006). Other factors, such as differences in various life history parameters (e.g., spawning seasonality), population demographic parameters, physiological response to water quality (e.g., differences in local geochemistry) and other potentially adaptive traits should be assessed by biologists. Thus, we suspect that some populations of freshwater mussels may be vulnerable to outbreeding depression, and mixing distinct populations may disrupt genetic adaptation to local environmental conditions.

*Guideline 6:* To avoid declines in population fitness caused by outbreeding depression, allopatric populations that qualify as evo-

lutionarily significant units (ESUs), subspecies, or closely related species should not be mixed.

#### **Domestication Selection**

Domestication selection (Type 4 Hazard) causes genetic changes in captive-held populations. In the captive rearing environment, artificial selective forces can replace those of natural selection. Domestication selection occurs because a different set of progeny survive in the hatchery than would have survived in the wild. Genetic changes can affect morphological, physiological, or behavioral traits and lead to decreased performance and survival of captive-reared progeny in natural environments. Because mussel propagation is still in its infancy, domestication selection has not been documented in the rearing of a mussel species; however, it has in the rearing of fishes (Miller & Kapuscinski 2003) and marine bivalves in hatcheries (see annotated bibliography by Moore & Seeb 2001). For example, many salmon hatcheries producing fish to augment wild populations are careful to collect breeders from different time-periods through the spawning run of a particular stock. This field-collection practice allows genetic representation of breeders that collectively spawn from early to late in the run. Similar practices may be necessary for some species of mussels to prevent artificial selection. For example, females of the endangered oyster mussel *Epioblasma capsaeformis* in the Clinch River, Tennessee, typically begin displaying their mantle-pad lure and releasing glochidia to host fish in April and continue into June (Jones et al. 2005). Some individual females display early in the spring, whereas others display much later. These differences in the timing of release of glochidia by *E. capsaeformis* may be genetically controlled and suggest that gravid females should be collected at different times throughout the glochidial release period. If, for example, time of glochidial release is under genetic control, then excessive propagation and release of juvenile mussels from females collected in the early spring could shift forward the glochidial release period of a targeted population relative to that of the wild population.

Research is needed to determine how domestication selection could alter the genetics of captive-reared juvenile mussels through stages in the propagation process, including investigation of the following: (1) most appropriate time of year to remove glochidia from the female mussel to maximize maturity of glochidia (Jones et al. 2005); (2) use of marginally-suited host fish for transforming glochidia to the juvenile stage; (3) appropriate diet, optimum substratum, exposure to disease and rearing temperatures and (4) length of culture period in captivity before release to the wild. To minimize domestication selection, we must clearly understand natural regimes and requirements for fish host usage, grow-out temperature, substrates, growth rates, light regimen, diet and size of juveniles at release relative to naturally-produced juveniles, thereby mimicking the ecology and habitat of a species as closely as possible throughout the propagation process (Maynard et al. 1995, Flagg & Nash 1999).

*Guideline 7:* Reduce domestication selection during propagation and culture of juvenile mussels by mimicking natural life history processes, such as fish hosts, diet, temperature regimes, and habitat of a targeted species as closely as possible in the hatchery.

#### **Laboratory Protocols to Prevent Mixing of Mussel Species**

The establishment of laboratory protocols to prevent the inadvertent mixing of species or other management units is important

to protect the integrity of genetic resources. Most propagation facilities rearing juvenile mussels for augmentation or restoration are cultivating multiple species and populations from different drainages. For example, at the Freshwater Mollusk Conservation Center at Virginia Tech University, juveniles of 6–9 endangered mussel species are produced each year, representing species from several major river drainages. In these situations, separate tank systems are required for holding host fish and for grow-out of juveniles from different drainages. Because juvenile mussels are small (~200–1,000  $\mu\text{m}$ ) for the first 60 days of life and can easily attach to laboratory equipment used for handling juveniles, such as sieves, siphons and Petri dishes, these items also should be kept separate for each lot and disinfected regularly. All hatchery personnel should be trained in field and laboratory protocols to reduce the risk of unintentional mixing of cultured populations.

*Guideline 8:* Protocols to prevent mixing of species or other management units through inadvertent exchanges of juveniles on laboratory equipment are needed to protect genetic resources of freshwater mussel populations.

#### **Release of Propagated Juveniles**

A suite of factors should be considered before juvenile mussels are released to the wild. Such planning is especially important for critically endangered populations with small effective population sizes ( $N_e$ ). Small populations (e.g.,  $n = 500$ – $1,000$  and  $N_e < 50$ – $100$ ) warrant special attention if they serve as a source for augmentation or reintroduction. First, production and release of thousands of juveniles from a small number of adult females into a small ( $n < 1,000$ ) recipient population can significantly decrease  $N_e$ , because of unequal contributions of progeny from only a few progenitors (Ryman & Laikre 1991). Therefore, a target number of offspring should be established for release into a small population prior to augmentation. Excess progeny could be released at adjacent shoals or at other acceptable sites. Second, selection of suitable release sites should be based on at least the following criteria: (1) biological requirements of the species, such as presence of fish hosts; (2) habitat quality and (3) thorough assessment of localized and upstream threats to release sites. Third, juveniles should be released at the earliest life-stage possible that will maximize survival in the wild. There is a trade-off between how long juveniles are reared in the hatchery, to increase survival rate relative to juveniles reared naturally and continued exposure to the hatchery environment and the associated extent of domestication selection (Miller & Kapuscinski 2003). Exposure to natural environmental patterns and selective forces at an early life stage may prove most beneficial to ensure fitness in the wild of hatchery-reared juveniles. Fourth, juveniles should be released under moderate-to-low flow conditions to allow settlement at the selected site on the river bottom, and at the appropriate time of year (spring-summer). Fifth, release methods and sites should be selected to increase the range and connectivity of localized demes and populations. For example, juveniles could be released at suitable sites between known locations of upstream and downstream demes. Sixth, as propagation technology improves and juveniles are grown to larger sizes, juveniles should be marked with a tag or chemical stain to facilitate monitoring efforts (see Eads & Layzer 2002).

The possibility of releasing host fish infested with glochidia would allow natural dispersal and colonization of habitats otherwise excluded by only releasing hatchery-reared juveniles, spread risk of mortality at localized stream reaches, and may minimize

future inbreeding. However, this practice risks loss of juveniles after settlement into unfavorable areas, and makes monitoring of survival success difficult. Under some circumstances, such as in small streams, this strategy may be more effective than site specific releases of cultured juveniles.

*Guideline 9:* Release an appropriate number of juvenile mussels from an appropriate number of parents at release sites to maximize effective population size ( $N_e$ ), and at an early life stage to maximize survival in the wild, and to minimize the effects of domestication selection.

#### **Monitoring and Adaptive Management**

Captive propagation of mussels is a new recovery option, and is as much an art as a well-established science at this time. Success must be measured not in terms of how many juveniles are outplanted, but rather in terms of how many juveniles recruited into or established a spawning population. Furthermore, data on (1) number of gravid females used to produce juveniles; (2) locations where females were collected; (3) number of juveniles released per site and river location; (4) juvenile characteristics (e.g., age, size and condition) and (5) river conditions at the time of release, should be recorded and submitted to the responsible natural resource agency. Standard data sheets should be prepared and used for all releases. It is critical that protocols to monitor survival and recruitment of artificially propagated juveniles are established and implemented, and project data are collected in an appropriate agency database. Ultimately, success will be measured in terms of the establishment of self-sustaining populations. Hence, monitoring should be regarded as an integral part of any captive propagation and release program.

Because of the many unknowns in mussel biology and uncertainties in long-term effects, hatchery programs may be experimental in nature, but should be integrated into an adaptive management program, with careful attention to monitoring and re-evaluation of goals and protocols. Under the adaptive management paradigm, results of monitoring are used, as appropriate, to modify management goals and operations procedures so that, over time, learning occurs and the overall program becomes more effective

(Holling 1978). Adaptive management has proven useful for management of Pacific salmonids (Hilborn & Winton 1993, Walters et al. 1993), and we acknowledge that it is essential for captive propagation and outplanting of imperiled mollusks.

*Guideline 10:* Monitoring, evaluation, and database management should be regarded as an integral part of any augmentation or restoration program, followed as appropriate with modification of program goals and operations procedures to promote program effectiveness.

#### **Concluding Remarks**

We advocate application of the principles of conservation genetics to species recovery efforts for freshwater mussels. However, these principles should be recognized as guidelines, and not as goals per se (Neves et al. 1997). Propagation technology and techniques will continue to develop as a recovery tool for a greater suite of species, and to hopefully prevent further extirpations and extinctions. Propagation may effectively alleviate problems associated with small populations, and has the potential to re-establish populations extirpated by known and ameliorated causes. Although propagation offers a wealth of benefits for conservation and restoration, managers of propagation facilities must recognize how each stage in the propagation process can affect the genetic integrity of mussel populations targeted for recovery. A conservation program of sound aquaculture practices, knowledge of the faunal group and application of conservation genetic principles will provide the tools needed to recover and restore species now threatened with extinction.

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