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Genetic and environmental implications of reintroducing laboratory-raised unionid mussels to the wild

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Abstract: The reintroduction of endangered species is a potentially useful conservation strategy, which in the case of freshwater unionid mussels, must be preceded by the successful laboratory rearing of juvenile mussels on their host fishes. However, an understanding of the genetic and environmental implications of reintroductions of artificially propagated mussels is required. Unfortunately, there is a dearth of information on these issues with respect to freshwater mussels. In general, regarding the genetic effects of reintroductions, small founder populations may lead to low heterozygosity (reduced genetic variability) in the reintroduced populations, which can make them more susceptible to extinction. Captive breeding programs may also alter the genetic composition of species through artificial selection, whether intentional or unintentional. Captive breeding may also affect an individual's interactions with conspecifics or predators by altering behaviour. Genetic problems in reintroduced populations also have the potential to affect wild populations, particularly by reducing variability among populations of the same species and eliminating local adaptation. There is also the possibility that diseases, parasites, or exotic species may be spread when populations are relocated or augmented. Recommendations related to the minimization of these impacts are presented for freshwater mussels, with the recognition that many of the issues will require additional study.

Résumé : La réintroduction d'espèces en péril peut être une stratégie utile de conservation; dans le cas des moules d'eau douce (unionidés), il faut au préalable réussir l'élevage sur leurs poissons hôtes des jeunes moules produites en laboratoire. Il est aussi nécessaire d'avoir une compréhension des conséquences génétiques et environnementales des réintroductions de moules reproduites artificiellement. Il y a malheureusement une pénurie de tels renseignements dans le cas des moules d'eau douce. En général, les effets génétiques des réintroductions, soit les petites populations fondatrices, mènent à une hétérozygotie faible (variabilité génétique réduite) dans les populations réintroduites qui peut les rendre plus sujettes à l'extinction. Les programmes d'élevage en captivité peuvent aussi modifier la composition génétique de l'espèce par sélection artificielle délibérée ou non. De plus, l'élevage en captivité altère potentiellement le comportement et ainsi affecte les interactions des individus avec les autres de même espèce et avec leurs prédateurs. Les problèmes génétiques des populations réintroduites peuvent affecter aussi les populations sauvages, en particulier en réduisant la variabilité au sein des populations de même espèce et en éliminant les adaptations locales. Il y a finalement la possibilité de répandre des maladies, des parasites ou des espèces exotiques lors des déplacements et des consolidations des populations. Nous formulons des recommandations pour minimiser ces impacts chez les moules d'eau douce, tout en reconnaissant que plusieurs des questions requièrent des études additionnelles.

[Traduit par la Rédaction]

Introduction

It has been reported that the extinction rate of the freshwater fauna in North America is approximately five times that of terrestrial fauna and three times greater than the rate for marine fauna (Ricciardi and Rasmussen 1999). Among these taxa, freshwater mussels (family Unionidae) rank high, as they are also among the most endangered groups of organisms in the world (Bogan 1993; Ricciardi et al. 1998; Lydeard et al. 2004). Indeed, estimates indicate that almost

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50% of the freshwater bivalves in North America are either extinct or near extinction (Bogan 1993), or more precisely, 72% of native freshwater mussels in North America are listed as extinct, endangered, threatened, or of special concern (Williams et al. 1993). These declines have been attributed to a number of factors, including (1) habitat degradation caused by changes in land use, i.e., channel or stream alterations, dams and impoundments, and external inputs of silt or fine particulate matter, nutrients, and toxins (e.g., McMahon 1991; Bogan 1993); (2) commercial shell harvest, both historical for the button trade and contemporary for the cultured pearl industry (e.g., McMahon 1991; Bogan 1993); (3) introduced species including zebra mussels (e.g., Ricciardi et al. 1998; Strayer and Malcom 2007); and (4) the loss of suitable host fish (e.g., Staton et al. 2003). Considerable efforts, including population augmentation through artificial propagation and translocation of adults (see below), are, therefore, under way to address these declines and where possible to help in species and ecosystem recovery (e.g., US Fish and Wildlife Service 2003; Environment Canada 2006).

The goal of the Canadian Species at Risk Act (SARA) and Environment Canada's Recovery of Nationally Endangered Wildlife (RENEW) program is the recovery of nationally endangered species and their habitats (Environment Canada 2006). In the case of freshwater unionid mussels, recovery involves three linked components: (1) the biology of the mussels, (2) the host fish that facilitate the development and dispersal of mussel larvae, and (3) mussel habitats that have been degraded through alterations in uses of land and water (e.g., Staton et al. 2003). Considerable efforts have been devoted to the biology and identification of host fishes for freshwater mussel species at risk (Table 1; cf., Hoggarth 1992), including the activities at the University of Guelph, where recent successes in the laboratory rearing of juvenile mussels could lead to their reintroduction into riverine habitats in southwestern Ontario. However, before a reintroduction or repatriation can take place a number of important genetic (e.g., founder effect, genetic drift, and inbreeding depression; Lacy 1987; Leberg 1990; Jones et al. 2006) and environmental (e.g., behavioural changes, disease transmission, introduced species; Snyder et al. 1996; Bohlin et al. 2002; Cope et al. 2003) issues must be resolved.

Unfortunately, there is a dearth of information on both the genetic and the environmental implications of mussel reintroduction, which has left policy makers with little scientifically based information to inform their decisions. This is despite the fact that significant reintroductions have occurred in the USA (US Fish and Wildlife Service 2003) and that there is a clear need to identify the key literature, approaches, and examples from other systems related to the issue. It is the purpose of this study, therefore, to examine the genetic and environmental implications of reintroducing freshwater mussels to the wild. Wherever possible, we have endeavoured to include molluscan and non-molluscan examples from freshwater systems, although in many cases the former are not available. It is anticipated that examples of other freshwater organisms may serve as a model for freshwater mussels. The ultimate goal of the study is to provide an overview of the considerations required to make reintroductions successful while doing no harm to the species or the environment.

Terminology

A variety of terms have been used to describe the status of endangered species and the different conservation methods used to augment or reintroduce their populations to the wild. We have adopted the SARA terminology related to the status (e.g., extinct, extirpated, threatened, of special concern) of freshwater mussels (Environment Canada 2006) (Table 2), and we use conservation terms consistent with definitions from the World Conservation Union (IUCN 1998) and Fischer and Lindenmayer (2000) (Table 2).

Relocation describes any movement of an individual or populations from one area to another, and is used in the broadest sense. The term of most relevance here is reintroduction, which is the introduction of individuals or a population into an area that is a part of the species' historical range but from which it has been extirpated. A translocation is a natural or artificial movement of an individual or a population from one part of its current range to another. For example, zebra mussel adults can translocate to new areas by detaching from their byssal attachment (Ackerman et al. 1994). Augmentation is used to describe the addition of individuals to an existing population of the same species to increase the local population size.

Life history of unionids

Freshwater unionids have a unique and complex life history, which begins with the fertilization of eggs retained in the marsupium (modified gills) of the female mussel by sperm released into the water column by males, and continues with the development of a parasitic larval stage (glochidium) (Wächtler et al. 2001). When the glochidia are released, they must attach to a vertebrate host (the salamander mussel, *Simpsonaias ambigua*, uses the mudpuppy, *Necturus maculosus*, as a host, whereas all other mussels use fish hosts) (McMahon 1991). Various mussel species have evolved different strategies of ensuring that this occurs, including the display of lures or the release of worm-like aggregates of glochidia (conglutinates) to which hosts are attracted (Wächtler et al. 2001). When the juveniles are developmentally mature they detach from the host and settle to the substrate. If settlement occurs over a suitable substrate the juvenile mussel will become established and spend the first few years of its life completely burrowed (McMahon 1991).

This complex life cycle is advantageous because it provides a dispersal mechanism for juvenile mussels, and it also provides glochidia with a source of food while they are attached (McMahon 1991). However, it renders unionids dependent on the presence of their host fish, and as a result, unionids are susceptible to environmental perturbations that affect them and (or) their hosts.

The environmental requirements of freshwater mussels include a number of chemical, physical, and biological factors (e.g., Dillon 2000). Given the ecological and biological diversity of the taxon it is difficult to define the optimal conditions for their growth (McMahon 1991; Dillon 2000). In general, one of the most critical factors is a reasonable level of dissolved calcium (often associated with high water hardness, pH, alkalinity, and conductivity), which is needed for

Table 1. Freshwater mussel species at risk in Canada, their COSEWIC status, and success of laboratory rearing (Environment Canada 2006; K. McNichols, J.D. Ackerman, and G.L. Mackie, unpublished data).

Mussel species	SARA schedule 1 status	Laboratory-raised juveniles
Alasmidonta heterodon (dwarf wedgemussel)	Extirpated (New Brunswick)	N/A
Epioblasma torulosa rangiana (northern riffleshell)	Endangered (Ontario)	Yes
Epioblasma triquetra (snuffbox)	Endangered (Ontario)	Yes
Lampsilis fasciola (wavy-rayed lampmussel)	Endangered (Ontario)	Yes
Ligumia nasuta (eastern pondmussel)	Endangered (Ontario)*	N/A
Obovaria subrotunda (round hickorynut)	Endangered (Ontario)	Yes
Pleurobema sintoxia (round pigtoe)	Endangered (Ontario)	N/A
Ptychobranchus fasciolaris (kidneyshell)	Endangered (Ontario)	Yes
Simpsonaias ambigua (salamander mussel)	Endangered (Ontario)	N/A
Villosa fabalis (rayed bean)	Endangered (Ontario)	Yes
Villosa iris (rainbow mussel)	Endangered (Ontario)*	N/A
Quadrula quadrula (mapleleaf mussel)	Endangered (Saskatchewan)* (Nelson designatable unit)	N/A
	Threatened (Great Lakes) (Western St. Lawrence designatable unit)	
Lampsilis cariosa (yellow lampmussel)	Special Concern (New Brunswick, Nova Scotia)	N/A
Gonidea angulata (Rocky Mountain ridged mussel)	Special Concern (British Columbia)	N/A

*Proposed for SARA 1 status.

shell formation and growth. Other water quality variables include moderate to high levels of dissolved oxygen and seston for suspension feeding, which are both affected by water velocity. Water velocity also can influence the quantity, quality, and stability of sediments and benthic substrates in which freshwater mussels are found, through hydraulics and through the delivery of dissolved oxygen and reduction of ammonium (McMahon 1991). The regional and local geomorphology are also important, as they affect stream order, stream morphometry (including depth), in-stream habitat, and substrate stability for lotic species, and the equivalent relationships of lake morphometry, etc., for lentic species (Dillon 2000). Considerable efforts have been directed to the definition of essential habitats for freshwater mussel species at risk (e.g., Morris 2006), which remains difficult given the limited ability to characterize the niche of individual species (cf. Green 1971).

Before reintroduction

As in all endeavours, a decision has to be made as to what is being conserved, whether it is a species, subspecies, or population (Philippart 1995), as this will determine the practices used for reintroduction (e.g., the source of founding individuals). Different "units" have been described as priorities for conservation strategies (Table 2), such as an evolutionarily significant unit (ESU), which is based on conserving phylogeny and allele frequencies (Moritz 1994). Alternatively, there are management units (MUs), which are based on the conservation of divergent allele frequencies and which do not consider phylogenetic relationships (Moritz 1994). Conservation units (CUs) are simply populations or groups of populations that are important to be conserved, which may incorporate the two aforementioned units (Geist and Kuehn 2005). Designatable units (DUs) are used by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) to increase the consistency with which it assigns status. COSEWIC's (2006) "usual approach to assigning status is, first, to examine the species as a whole and then, if deemed appropriate, to examine the status of designatable units below the species level. In cases where particular designatable units are strongly suspected of being at risk, or where they are so different in distribution or conservation status that an overall assessment would not capture the conservation concerns, COSEWIC will assess single designatable units below the species level. Status may be assigned to subspecies, varieties, or geographically or genetically distinct populations which may be recognized in cases where a single status designation for a species is not sufficient to accurately portray probabilities of extinction within the species."

The simplest unit to conserve may appear to be the species; however, the taxonomy of unionids is not always clear, as they have often been classified by their shells and external morphology, which can be highly variable and subject to environmental influences (Mulvey et al. 1997). In addition, the genetic relationships among freshwater mussels are often not well understood (Mulvey et al. 1997; Geist and Kuehn 2005). Therefore, the utmost care must be taken to reintroduce the correct unit of conservation, particularly in augmentations.

Another possible conservation unit is a metapopulation, which is a group of populations connected by dispersal of individuals between patches (Hanski 1998; Young 1999). There are different ways of considering metapopulations in reintroductions. Individuals from the source population may be used as founders for a reintroduction within the range of the metapopulation, as dispersal among populations may decrease their genetic variability. Alternatively, establishing several small populations in different patches may help establish a metapopulation that could have more persistence than a single population (Young 1999).

Genetic implications

There are a number of genetic implications pertaining to reintroduced populations, including the founder effect, ge-

Table 2. Terminology related to the status, conservation method, and designation of freshwater unionid mussels.

Term	Definition
Status (Environment Canada 2006)	
Extinct species	A species that no longer exists in captivity or the wild
Extirpated species	A species that no longer exists at a specific location in the wild, but exists elsewhere in the wild
Endangered species	A species that is facing imminent extirpation or extinction
Threatened species	A species that is likely to become endangered if nothing is done to reverse the factors leading to its extirpation or extinction
Special concern species	A species that may become a threatened or an endangered species because of a combination of biological characteristics and identified threats
Conservation methods (IUCN 1998; Fischer and Lindenmayer 2000)	
Translocation	The natural or artificial movement of an individual or a population from one part of its current range to another (e.g., Ackerman et al. 1994)
Relocation	Any movement of an individual or populations from one area to another, and used in the broadest sense
Reintroduction	The introduction of individuals or a population into an area that is a part of its historical range, but from which it has been extirpated
Augmentation	The addition of individuals to an existing population of the same species (e.g., supportive breeding; Ryman 1991)
Conservation units	
Evolutionarily significant unit (ESU)	Based on conserving phylogeny and allele frequencies (Moritz 1994)
Management units (MUs)	Based on the conservation of divergent allele frequencies, and do not consider phylogenetic relationships (Moritz 1994)
Conservation units (CUs)	Populations or groups of populations that are important to be conserved and may incorporate the two aforementioned units (Geist and Kuehn 2005)
Designatable units (DUs)	The introduction of individuals or a population into an area that is a part of its historical range, but from which it has been extirpated (COSEWIC 2006)
Metapopulation	A group of populations that are connected by dispersal of individuals between patches (Hanski 1998; Young 1999)

netic drift, and inbreeding depression (Lacy 1987; Leberg 1990; Jones et al. 2006). All these effects may result in the loss of heterozygosity (i.e., genetic variability) along with a potential decline in growth and (or) reproductive rate and an increased susceptibility to environmental change (Leberg 1990; Earnhardt 1999). This situation can be exacerbated if founder populations have low heterozygosity and (or) possess deleterious alleles, which could have negative impacts on wild populations of the same or closely related species if there is interbreeding between the populations (Philippart 1995). Wild populations could also be affected negatively if there is a large influx of exogenous genes, decreasing variability among populations and breaking down local adaptation (i.e., outbreeding depression; Waples 1991). One way of avoiding this problem is through supportive breeding, which is to reintroduce juveniles produced by females that have been obtained locally (Ryman 1991). The genetic implications of reintroducing a species can range from minimal to profound, and may have the potential to impact the source, the founder, and the wild populations. Because of their wide-reaching impacts, the genetic implications of any kind of relocation must be considered carefully before any action is taken.

Introduced populations

As indicated above, many of the genetic problems associated with the introduced population stem from the use of a small number of individuals to create the new population — namely, (1) founder effect, (2) genetic drift, and (3) inbreeding depression.

The genetic variability of a population will be reduced if it is founded with a small number of individuals (i.e., founder effect) that lack the variability of the original population (Leberg 1990) or possess a high variance in reproductive success (Boudry et al. 2002). When there are only a small number of founders, the genetic variability is very limited and the population is less able to adapt to change, and more susceptible to stochastic events (Earnhardt 1999).

Genetic drift is the random process whereby alleles are lost from (or fixed in) a small population, and it is one of the most powerful forms of genetic change in computer simulations (Lacy 1987). Genetic drift is almost inevitable in captive populations, but it is also relatively easy to prevent by importing individuals from the wild (Lacy 1987).

One of the problems with inbreeding is the loss of heterozygosity, which can lead to the expression of deleterious recessive alleles that are normally hidden (i.e., inbreeding depression; Storfer 1999). The loss of heterozygosity can lead to decreased growth rate, survival, and fecundity and potentially lead the population to extinction (Leberg 1990).

To the best of our knowledge, there have not been any studies examining the genetic effects of reintroducing a small group of freshwater mussels. However, these effects have been observed in other taxa when small groups have been reintroduced to an area or when a small number have been used for captive breeding.

For example, farmed Chinese sucker (*Myxocyrianus asiaticus* Bleeker) were found to have low genetic variability compared with an historic wild group (Wan and Fang 2002). Computer simulations have been used to predict genetic effects, as it is not always easy to monitor populations in the wild. One such model by Waples and Do (1994) predicted that as long as salmonid population sizes remained high after augmentation, the risk of inbreeding negatively affecting the population was marginal (Waples and Do 1994). However, Laikre (1999) found that hereditary diseases were extremely common as a result of inbreeding in captive mammal populations.

A high degree of heterozygosity does not guarantee that a population will have a high growth rate (Leberg 1993), nor does a high degree of homozygosity associated with inbreeding necessarily lead to extinction. Some species may continue to grow and produce viable offspring. For example, a source population of 46 beavers (*Castor fiber*) reintroduced into 11 sites in Sweden grew to approximately 100 000 several decades later (Ellegren et al. 1993). Recent modeling efforts have suggested that whereas inbreeding depression decreases time to extinction, it is the initial rate of growth of the population (or relationship to carrying capacity) that explains some of these observed differences related to the effects of inbreeding (Brook et al. 2002; Thévenon and Couvet 2002).

Interestingly, it has been argued that because inbreeding exposes deleterious alleles to natural selection, a short period of inbreeding may increase the fitness of the population in the long term because those alleles will be removed from the gene pool (Waples and Do 1994). However, even if a population with low genetic variability is growing and seems to be successful, it is more susceptible to stochastic events, and there is a lower probability that it will be able to adapt to environmental change or new selection pressures (Taylor et al. 2005).

Given that founder effects, genetic drift, and inbreeding are due to starting a population with a small number of individuals, it is recommended that reintroduced populations begin with as large a founder population as possible. In some cases, it may not be realistic to start a breeding population in captivity with a large number of founders. However, it has also been found that even a small amount of migration is sufficient to counteract these genetic problems, and introducing a small number of wild individuals to the captive population every generation can retain the genetic variability in a species (Lacy 1987). This may be possible through the use of glochidia from a large number of females that are returned to the wild with a portion of their glochidia intact.

Artificial selection

Despite the best of intentions, selection is a continual process that does not stop simply because an animal is in captivity (Snyder et al. 1996). However, selective pressures are quite different in the captive environment, and many selective pressures may be relaxed from what is experienced in the wild, causing directional change in genetic makeup (Waples 1991). Traits such as tameness can be strongly selected for, and behaviours that are learned are quickly lost in captivity (Snyder et al. 1996). Moreover, a recent report indicates that inducible defences begin to break down after eight generations in captivity (Kraaijeveld-Smit et al. 2006). This may prove to be an important issue for unionid mussels if their ability to lure fish is affected.

Artificial selection is a particular problem in farmed species, as they are deliberately selected for enhanced growth and early sexual maturity (Kallio-Nyberg and Koljonen 1997). However, even when the goal is to keep the captive population as genetically close to the wild as possible, changes still occur. For example, phenotypes that may be eliminated in the wild can survive through vulnerable young stages, allowing genes to be passed on that might not be passed on in the wild (Einum and Fleming 2001). Heath et al. (2003) noted a change in the size of salmon eggs over a few generations of captive breeding, with a trend towards smaller eggs. Small eggs produce small juveniles, which tend to have lower fitness in the wild, but with these selection pressures reduced in captivity, females produced small eggs to increase their fecundity (Heath et al. 2003). This trait is heritable and if it is passed on to wild populations, females that have small eggs may have lower fitness because juveniles from small eggs may not survive (Heath et al. 2003). It is quite possible that artificial glochidial infestation of fish could lead to similar consequences.

Negative effects on source populations are generally easy to avoid, as long as the source population remains large. Therefore, there is a balance between using an appropriate-sized founder population to avoid the effects mentioned above and a large enough source population that it can quickly recover from the loss of individuals (Young 1999). Individuals should not be selected on the presence or absence of a specific trait, as this would be a form of artificial selection if the trait is genetically based and would alter the genetic composition of both the source and founder populations (Leberg 1990). To avoid negative effects on a source population of freshwater mussels, it is recommended that after glochidia are taken from a source population, the adult females are returned to the wild. Alternatively, a method employed at the University of Guelph facility is to remove only half of the glochidia (those in one gill) from the adult females and then return the females to the wild so that their reproductive cycle may continue naturally using the remaining glochidia.

There may be genetic impacts on the wild population following a population augmentation as a result of the issues discussed above. Augmenting small populations with individuals from another population can introduce much-needed genetic variability to the gene pool and counteract the effects of inbreeding depression or genetic drift (Hindar et al. 1991; Ryman 1991). However, it can also change the composition of the wild gene pool enough that local adaptations, which may have taken many generations to develop, are lost and fitness is decreased, resulting in outbreeding depression (Hindar et al. 1991; Waples 1991).

Although this contingency has not been studied in freshwater mussels, the potential exists that it could occur. Local adaptation was found in the marine mussel *Mytilus trossulus*, which was not expected, since the free-swimming veliger larval stage is expected to disperse over large distances (Yanick et al. 2003). Specifically, mussels from areas nearly 150 km apart, which were raised in cages, had higher survival and growth rates when they were relocated to local areas, whereas mussels from farther away did not perform as well (Yanick et al. 2003). This indicates that outbreeding depression could be of concern when augmenting freshwater mussel populations.

Similarly, the performance of indigenous fish is usually better than that of introduced fish in the wild (Hindar et al. 1991). Regardless of whether local adaptation may be lost, hybridization between the two groups is usually detrimental to the wild population (Philippart 1995). Indeed, there is potential for significant interbreeding between wild and sea-ranched fish, which could pass on traits selected for in captivity to wild populations (Gausen and Moen 1991; Petersson and Jarvi 1997; Kallio-Nyberg and Koljonen 1997).

As indicated above, supportive breeding involves removing individuals from a population, breeding them in captivity, and releasing the juveniles into the same population (Ryman 1991). Survival of juveniles is usually higher in captivity, and this method provides a rapid increase in the population without introducing any exogenous genes and the risk of outbreeding depression. However, this method is not without its problems. Of particular concern is that by choosing only a small number of individuals from the population, the variance in family size increases, resulting in a decrease in the effective population size (Ryman 1991; Laikre and Ryman 1996). It is relevant to note that marine bivalves are known to have relatively small effective population size (e.g., Boudry et al. 2002). It is not known whether the genetics of freshwater mussels is similar in this context.

It takes only one generation of breeding to introduce deleterious alleles or disrupt local adaptation and many generations to undo the damage. Therefore, the benefits of population augmentation must be weighed against the risks.

Environmental implications

In terms of the environmental implications of reintroductions, behavioural changes are common in many taxa and have been observed to affect both reproductive success and predator avoidance. Introducing a large number of new individuals can also have negative impacts on native populations or the environment, if the species is affected by density-dependent factors (Bohlin et al. 2002). For example, disease (or parasites) transmission from populations bred in captivity to wild populations is common and can be devastating for wild animals, as diseases may be spread to populations that have no immunity (Snyder et al. 1996). However, this is unlikely when reintroducing mussel species at risk to areas where they have been extirpated but the habitat has been rehabilitated.

In the case of the reintroduction of freshwater mussels, the accidental spread of dreissenid mussels is a real risk and precautions must be taken to guard against this possibility (Cope et al. 2003).

Clearly, the environmental implications of species reintroductions are just as important as the aforementioned genetic considerations. Individuals interact with other individuals, other species, and their environment in many different ways, and reintroducing individuals to an area can affect any or all of these interactions.

Behaviour

Behaviour can be altered in many ways by captive rearing, and these alterations can have wide-reaching implications. To the best of our knowledge, there are no data related to the behaviour of freshwater mussels (e.g., valve gaping, burrowing activity, clearance or filtering rates, locomotion, mantle displaying) and captive rearing. However, given the relative importance of this concern, it is appropriate to examine how behaviours have been altered in other taxa.

Reproductive behaviour can change in a captive environment, as has been seen in a number of captively bred salmonids. Specifically, females' nesting and mate signaling can be disrupted (Petersson and Jarvi 1997) and differences in homing ability, spawning location, and spawning time may diminish lifetime reproductive success (Gausen and Moen 1991; Fleming and Petersson 2001). Aggression and, potentially, mating events may also differ between wild and hatchery-raised fish, although it is not known whether this is due to genetics or the environment (Berejikian et al. 1996; Petersson and Jarvi 2000).

Differences in predator avoidance have been noted in hatchery-reared fish (Berejikian 1995; Dellefors and Johnsson 1995; Johnsson et al. 2001) and some of the differences could be attributed to genetics (Berejikian 1995; Ferno and Jarvi 1998). As noted above, the breakdown in response to predators can occur in eight generations of captive breeding (Kraaijeveld-Smit et al. 2006). The risk of changing behaviour due to imprinting in captivity is greater when there are behaviours that are learned (Snyder et al. 1996).

Given that behaviour is altered in many species that are kept in captivity, it is difficult to completely replicate wild conditions. As a result, it is recommended that if individuals are to be reintroduced into the wild, captive environments should be kept as realistic and complex as possible (Philippart 1995), and individuals should be kept in captivity for as short a time as possible (Snyder et al. 1996). In the case of freshwater mussels, it might be desirable to vary females among years, which will require knowledge of the population structure.

Density-dependent effects

A sudden increase in population size may have negative impacts on either an introduced or an augmented population if the species is affected by density-dependent factors. Density dependence may affect fecundity, survival, or migration of a population (Bohlin et al. 2002).

In some cases, increases in population size can be beneficial to populations. The Allee effect describes a situation in which reproduction is reduced because of a small population size, usually because of inbreeding, demographic stochasticity, or a reduction in cooperative interactions (Courchamp et al. 1999). Although the Allee effect has been studied in highly social animals, Stoner and Ray-Culp (2000) found that it also affected populations of queen conch (*Strombus gigas*). Spawning and mating behaviour were not observed in conch populations that were below certain size thresholds (Stoner and Ray-Culp 2000).

The role of density dependence was considered in a translocation of four species of freshwater mussels (*Quadrula pustulosa pustulosa*, *Elliptio dilatata*, *Lampsilis higginsii*, and *Lampsilis cardium*) in Minnesota and Wisconsin (Cope et al. 2003). In some cases, the density of mussels (per square metre) after translocation was double or triple the original density, but this did not affect the survival of the mussels, which was quite high after translocation (Cope et al. 2003). It is not known whether the Allee effect is important in terms of sexual reproduction, but data suggest that population densities of 10 individuals·m^{-2} enhance reproductive success in *Eliptio complanata* in lakes (Downing et al. 1993). In this context, a reintroduction program may have a positive influence on reproductive success. Conversely, density-dependent factors may lead to increased competition. This has been noted in fish populations (Bohlin et al. 2002), but it is not known whether similar competition exists for unionid mussels.

Diseases and exotic species

Diseases and parasites can be easily spread between populations, especially between reintroduced populations raised in captivity and those that have always been in the wild (Cunningham 1996). Disease and parasites affect native populations of the same or closely related species by causing death, increasing susceptibility to predation or other diseases, lowering reproduction, or a combination of all the above (Cunningham 1996). In relocating freshwater mussels, another relevant problem is the spread of invasive species such as dreissenid mussels when adult freshwater mussels are translocated from a river or lake where dreissenid mussels are present.

Dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis* in North America) are invasive species that compete with unionids and colonize their shells, which affects their movement and feeding ability and may ultimately lead to their extirpation (Nalepa 1994; Cope et al. 2003; Strayer and Malcom 2007). To the best of our knowledge, there are no examples of dreissenids being relocated along with other mussel species, as dreissenids are well recognized as a risk to native mussel populations. Relocation efforts often include plans to prevent spread of these invasive species, or to avoid areas where there is the potential for colonization by dreissenids.

Relocations of four mussel species in Minnesota and Wisconsin involved washing individuals with a wire brush prior to relocation (Cope et al. 2003). Similar precautions were also used in the relocation of two Canadian mussel species at risk in Lake St. Clair (Metcalfe-Smith et al. 2005). Other recommendations involve holding scrubbed mussels in quarantine for 30 days to monitor the presence of zebra mussels prior to reintroducing adult native freshwater mussels to a new area (Biggins et al. 2001). In addition, releasing juveniles or glochidia reared in captivity ensures that there has been no contact with zebra mussels.

Preventing the spread of disease in freshwater mussels is also of concern, particularly if a small population with low genetic variability is to be augmented with new individuals, which may infect the population with diseases to which it has no resistance (Snyder et al. 1996). It is recommended that captive populations are held in single-species facilities within their natural range of conditions to avoid the exchange of exotic pathogens, although it is not recommended that they are held in completely sterile conditions (Snyder et al. 1996). Captive animals should also be subjected to the natural parasite load of the species, which could help ensure survival once the animals are introduced to the wild (Cunningham 1996). However, a recent analysis of salmon aquaculture indicates that density-dependent factors in salmon cages that foster parasitic sea lice (*Lepeophtheirus salmonis* and *Caligus* spp.) can lead to the transmission of sea lice to wild salmon (Krkošek et al. 2006). It is important to take precautions to avoid the spread of parasites, pathogens, and exotic species, as these could seriously impact both the introduced and native populations.

Other considerations

The age structure of the reintroduced population must be determined, including whether adults, juveniles, or glochidia should be released, or a combination of all the above. For example, it may be relatively simple to infest fish with large numbers of glochidia, which can be released (US Fish and Wildlife Service 2003; Kelner and Mussel Coordination Team 2005), but it may be difficult to assess the success of this approach directly. This is due in part to the fact that successful infestation, whether natural or artificial, may be affected by the immunological response of fish to prior glochidiosis, as is evident in the higher rates of glochidiosis in young age classes of host fish (Hastie and Young 2001). Recent evidence indicates that this resistance to infection by glochidia may persist for a year or more (Dodd et al. 2006). In addition, infection by glochidia from one species may offer cross-resistance to infection by glochidia from other species (Dodd et al. 2005). In addition, the dispersal of glochidia on infested fish may be somewhat dependent on the mobility of the fish species chosen (McLain and Ross 2005). This is clearly an area of great concern in captive breeding programs as well as an opportunity for further research.

Recently, juvenile *Lampsilis higginsi* (Higgins' eye pearly mussel) were raised on fish kept in cages in the field (e.g., US Fish and Wildlife Service 2003), which provides an opportunity to measure transformation rates. Alternatively, adult freshwater mussels tend to have high survival rates when they are relocated, but not as many can be moved and the process is more labour-intensive (Kelner and Mussel Coordination Team 2005). Mussel relocation projects have incorporated various strategies, even within the same project (Biggins et al. 2001; Kelner and Mussel Coordination Team 2005). Apparently, translocation of adults within the same river system usually leads to high survival rates after the translocation (Cope et al. 2003; Havlik 2005; Jones et al. 2005). Mathematical models can also provide guidance on which life stage should be augmented. For example, model populations of Griffon vultures (*Gyps fulvus*) containing only juveniles tended to become extinct during the first 40 years after reintroduction more often than model populations composed only of adults (Sarrazin and Legendre 2000; Robert et al. 2004). For this long-lived species, it was recommended that adults be released, as reintroduced adult populations had much higher success rates, but results could be different for short-lived species (Sarrazin and Legendre 2000). Freshwater mussels would be considered long-lived species in this context.

The sex ratio of an introduced population should also be considered. Linklater (2003) used the Trivers–Willard model to determine the sex ratio of reintroduced populations. This model is used to predict the sex of offspring when resources are limited and reproductive success varies between males and females (Linklater 2003). In relocations, males often have lower survival, but if the species is polygynous, they also have greater opportunity to have more offspring. Therefore, Linklater (2003) recommends that more males should be introduced when resources allow for a "soft release", which includes pre-release training and post-release monitoring and care. However, more females should be released when there are only enough resources for a "hard release" (i.e., no training or monitoring) (Linklater 2003). In this way, the probability of a successful reintroduction is balanced with the ability to produce a large number of offspring. The sex ratio that is used should correspond to the reproductive strategy of the species such that the effective population size is maximized. It remains to be determined whether the initial stages of a reintroduction program should be biased towards larger numbers of female mussels.

One of the critical questions that needs to be addressed is how many individuals should be used to start a captive population or start a new wild population. Given the problems associated with introducing small populations (see above), one might wish to identify a lower limit for the founder population. Unfortunately, such a limit does not appear to exist, although a founding population of 50 individuals was suggested by Leberg (1990) as sufficient to preserve allelic diversity. Other suggestions range as high as 500 founders to preserve enough genetic variation for adaptation to occur, but a high number of founders does not guarantee a successful reintroduction (Lande 1988). Lande (1988) discusses cases where reintroductions of more than 500 individuals have failed, primarily due to demographic reasons.

Griffith et al. (1989) predicted that the probability of a successful translocation increased with the number of individuals released, with an inflection point indicating that there was a minimum number needed to ensure success. However, the relationship was asymptotic, suggesting that there is also a point at which adding more individuals does not increase the probability of success (Griffith et al. 1989). The inflection point varied among species, again indicating that there is no set lower limit for all species.

It is often recommended that the greatest number of founders possible should be used, as long as it does not interfere with the source population (Leberg 1990; Philippart 1995; Fischer and Lindenmayer 2000). However, if the species is endangered, this may be a very small number.

Successful reintroductions

Different relocation projects use different definitions of success, which makes comparisons difficult. For example, Serfass et al. (1993) were most interested in whether a reintroduced river otter (*Lutra canadensis*) population was persistent and reproducing and made no attempt to measure genetic variability. The same criteria were used for a reintroduction of natterjack toads (*Bufo calamita*; Denton et al. 1997). This also is the case in many freshwater mussel relocations (Cope et al. 2003; Havlik 2005). When populations are augmented, success can also be determined by the ability of the introduced individuals to contribute to the genetics of the native population (Leberg and Ellsworth 1999; Ellsworth et al. 1994).

One of the most important factors in the overall success of a reintroduction project is whether the original factors that caused the decline of the species had been corrected (IUCN 1998; Sarrazin and Barbault 1996; Fischer and Lindenmayer 2000). However, the success of reintroductions of birds and mammals is also influenced by the habitat quality of the reintroduction site, the number of animals released, and the legal status of the animal (i.e., threatened or endangered, game species) (Griffith et al. 1989; Wolf et al. 1996). Similar factors will likely be relevant for freshwater mussels, as will commercial harvesting and the continued problem of invasive species. Releases into the core of the species' historical range have also been found to be more successful than releases into the periphery (Wolf et al. 1996). There are five basic criteria used by COSEWIC in Canada to assess status of a species: (1) declining total population; (2) small distribution, and a decline or fluctuation; (3) small total population size and a decline; (4) very small population and restricted distribution; and (5) quantitative analysis. If it can be demonstrated that a population is increasing in size, the species' distribution (e.g., extent of occurrence or area of occupancy) is increasing, and (or) populations are having smaller fluctuations, the species' status can be downlisted and one can argue that a successful reintroduction has occurred.

In terms of habitat or site selection, substrate composition and geographical location seem to be of particular importance for freshwater mussels. For example, Dunn et al. (2000) analyzed the results of freshwater mussel translocations and found that success was affected by the stability of the substratum, regardless of water velocity, with greater stability leading to greater mussel recovery. Substrate stability was also a factor in the success of the translocation of mussels in Minnesota and Wisconsin, with the lowest mussel survival occurring at sites with sandy sediment (Cope et al. 2003).

If possible, individuals should be relocated to sites that are close to the site of the source population to account for local adaptation (Maitland and Evans 1986). Young (1999) also suggests that sites for fish reintroduction should be geographically close to the source population, as well as to sites having a similar ecology. Although this does not guarantee success, the chances of fishes possessing the necessary adaptations or the ability to adapt to the new site are higher (Young 1999). Sites chosen for relocation also need to have all of the habitats required for completion of the life cycle (Maitland and Evans 1986). The release area should have characteristics that allow populations to be established over the long term and should allow for natural dispersal of individuals (Wynhoff 1998), which for freshwater mussels means that the necessary fish hosts must be present at the new site.

When captive rearing is to be used for reintroduction of a species, it has been suggested that animals whose behaviour is instinctive may be more successfully reintroduced than those whose behaviour is learned (Snyder et al. 1996). In amphibians, natural behaviour has been retained after generations of captive breeding, and the release of tadpoles allows for imprinting on the release pond and the development of

Table 3. Implications of the reintroduction of freshwater mussels to the wild.

Type of implication	Consequences	Recommendations
Genetic implications		
Genetic constitution of introduced populations		
Founder effect	Reduced genetic variability	Use glochidia from a large number of female mussels
Genetic drift	Loss of genetic variability	Use glochidia from a large number of female mussels
Inbreeding depression	Reduced genetic variability, expression of deleterious alleles	Use glochidia from a large number of female mussels
Potential for artificial selection in breeding program		
Change in behaviour	Lower fitness in wild	Minimize time in captivity Vary females among years Simulate field-like conditions in lab
Change in trait	Lower fitness in wild	Minimize time in captivity Vary females among years Simulate field-like conditions in lab Use wide variety of fish hosts
Potential for artificial selection in source population		
Removal of individuals	Loss of genetic variability, artificial selection, inbreeding depression	Return females to wild with portion of glochidia intact
Potential for artificial selection in wild populations		
Influx of individuals	Outbreeding depression	Use supportive breeding Maintain local population structure
Interbreeding/hydridization	Introgression, sterility	Maintain local population structure Minimize introduction of foreign genotypes
Environmental implications		
Behavioural changes		
Reproductive interactions, timing, mantle displaying	Reproductive failure	Minimize time in captivity Vary females among years Simulate field-like conditions in lab Use wide variety of fish hosts
Density-dependent effects		
Loss of Allee effect	Reproductive failure	Increase population density in wild
Introductions of diseases and exotic species		
Disease transmission	Catastrophic loss, reproductive failure	Minimize time in captivity Maintain strict hygiene Minimize cross-contamination of mussels from different sources
Parasite transmission	Catastrophic loss, reproductive failure	Minimize time in captivity Maintain strict hygiene Minimize cross-contamination of mussels from different sources
Exotic species	Catastrophic loss, reproductive failure	Minimize time in captivity Maintain strict hygiene Minimize cross-contamination of mussels from different sources
Other considerations		
Sex ratio	Reproductive failure	Maintain reintroduction programs over several years
Age structure	Reproductive failure	Maintain reintroduction programs over several years

behavioural patterns (Bloxam and Tonge 1995). In the same way, freshwater mussels may be ideal candidates for this kind of conservation program.

Recommendations and conclusions

We have reviewed the genetic and environmental implications of reintroducing freshwater mussel species to the wild (Table 3). Genetic problems such as inbreeding and genetic drift may arise from a small founder population, resulting in the loss of genetic variability in the new population (Lacy 1987; Leberg 1990). Genetic problems in the introduced individuals may be passed to the native population and also cause outbreeding depression, where the introduction of exogenous genes leads to the breakdown of local adaptations (Waples 1991). These genetic problems have not been observed in freshwater mussels, perhaps because the genetics of freshwater mussels is not well studied, but they have been noted in other species, and should, therefore, be of concern. Clear goals are necessary, and steps should be taken to preserve the genetic variability of the group or unit to be conserved (Philippart 1995; Jones et al. 2006).

It will be critical to examine the ecological and evolutionary factors that affect populations in the field. This will inform practical decisions on the location, phenology, and availability of gravid females and their glochidia, as well as the optimum size and time for release of juveniles. In addition, it will provide direction on host-fish relationships that will be required for the development of breeding programs based on the population history of the area.

Rearing animals in captivity can lead to artificial selection owing to different or relaxed selection pressures compared with those in the wild (Waples 1991). This has not been observed in freshwater mussels, but to the best of our knowledge, it has not been studied. It has been recommended that captive environments, as well as microhabitats, are kept as realistic and as similar to the natural environment as possible (Philippart 1995).

Interactions among organisms have the potential to change after reintroduction if captive breeding alters reproductive behaviour, predator avoidance, and (or) aggression (Dellefors and Johnsson 1995; Berejikian et al. 1996; Petersson and Jarvi 1997 among others). This has been shown in different fish species and is of particular concern when populations are to be augmented.

The potential to spread disease, parasites, or invasive species such as dreissenid mussels must also be considered before a reintroduction takes place, and precautions must be taken to avoid such spread (Cunningham 1996; Cope et al. 2003). Animals may need to be placed in quarantine prior to release. It may also be relevant to ensure captive individuals have a normal parasite load if they are to be introduced to the wild.

One of the most critical questions to be addressed is the number of individuals to be reintroduced, and there seems to be no set lower limit to guarantee success (Griffith et al. 1989). It has been recommended that as many individuals as possible are used as a founder population, and this may vary among species.

There is some evidence of success pertaining to the translocation of mussel species in North America; however, these efforts have mostly involved moving adults from one part of a river system to another, and there are no long-term results as yet. There is little understanding of the genetic effects in freshwater mussels, as this is still a field that is not well studied or understood (Geist and Kuehn 2005). It is reasonable to assume that freshwater mussels will be affected by small population sizes.

Additional research is needed to determine the long-term survival and reproductive success of reintroduced freshwater mussels along with other species in the area. Reintroductions thus far lack clear and consistent methodology and have different definitions of success and variable reporting of results. In order for scientists and managers to learn about past reintroductions, and what has made them a success or a failure, reintroductions need to be treated more as scientific experiments, and results must be reported with greater consistency. Clearly, there is much to be learned about the implications of freshwater mussel reintroductions. However, using the lessons learned from other taxa, it is clear that reintroductions can be an important conservation tool, but they must be undertaken with caution.

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