Vol. 12: 1–9, 2010
doi: 10.3354/esr00286

ENDANGERED SPECIES RESEARCH
Endang Species Res

Published online June 4

OPEN ACCESS

REVIEW

Captive breeding of the endangered freshwater pearl mussel *Margaritifera margaritifera*

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ABSTRACT: Freshwater pearl mussels (Unionidae: Bivalvia) rank among the most endangered aquatic invertebrates, and this has recently prompted a number of initiatives designed to propagate the species through captive breeding. Yet there are few guidelines to aid in freshwater mussel culture for conservation, and few or no results on the fate of released juveniles. We reviewed various *ex situ* strategies for freshwater mussel conservation with emphasis on the freshwater pearl mussel *Margaritifera margaritifera* (L.), one of the most critically endangered unionids. Captive breeding could help safeguard critically endangered populations, but current rearing methods need to be optimised. Areas in particular need of research include the collection and storage of viable glochidia, the development of efficient rearing systems, and the formulation of algal diets. Likewise, the degree of host specificity warrants further investigation, as this will largely dictate the success of reintroduction programmes. Finally, we note that more information is needed on the degree of genetic structuring and post-release survival before translocation programmes can be recommended. As with other conservation projects, captive breeding of the freshwater pearl mussel cannot compensate for loss of critical habitats and is likely to be most efficient in combination with *in situ* conservation, not in isolation.

KEY WORDS: Freshwater pearl mussel · *Margaritifera margaritifera* · Captive breeding · Host specificity · Juvenile culture

INTRODUCTION

Freshwater pearl mussels (Unionacea) are among the most endangered aquatic organisms in the world (IUCN 1996, Strayer et al. 2004). With a maximum life span in excess of 100 yr, some pearl mussels also rank among the slowest-growing and longest-living known invertebrates (Ziuganov et al. 2000, Anthony et al. 2001), which makes their conservation particularly problematic (Cosgrove & Hastie 2001, Hastie et al. 2003).

The accelerated decline of many freshwater mussels has recently prompted a flurry of initiatives designed to propagate and restore the species in Europe (Buddensiek 1995, Beasley & Roberts 1999, Hastie & Young 2003a, Preston et al. 2007) and elsewhere (Strayer et al. 2004, Barnhart 2006). In the UK, unprecedented steps have recently been taken to safeguard entire *Margaritifera margaritifera* (L.) populations by collecting adults from the wild and bringing them into captivity in the hope of establishing living gene banks and aiding the recovery of self-sustaining populations (Taylor 2007). Yet, there is a paucity of data on critical life stages, the relative merits of different conservation strategies, and the fate of cultured juveniles.

Given that resources allocated to mussel conservation are always likely to be limited, it is essential to weigh up and prioritise the different options available to freshwater managers and wildlife officials (Araujo & Ramos 2001). There are relatively few published studies on conservation of freshwater mussels compared to other freshwater mussel topics (Fig. 1), despite the fact that these species are increasingly imperiled. Whilst the *in situ* requirements of different freshwater mussel species have already been discussed by others (Neves

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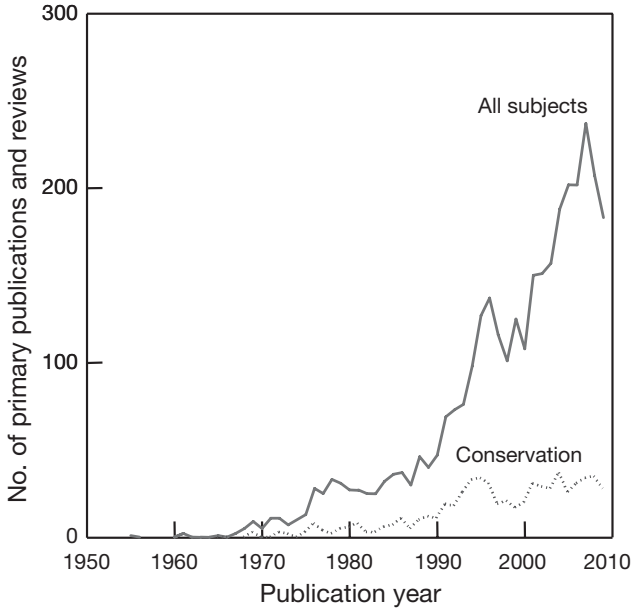

Fig. 1. Trends in the total number of primary publications and reviews on freshwater mussels (All subjects) and those that deal specifically with conservation issues (conservation). Data are from the ISI Web of Science (Conservation) (www.isiknowledge.com). While research effort on freshwater mussels has grown exponentially over the last 2 decades, relatively little of it has been directed towards addressing the conservation needs of mussels despite the fact that they are becoming increasingly imperiled

& Widlak 1987, Layzer & Madison 1995, Valovirta 1998, Hastie et al. 2000, Brainwood et al. 2008, Geist 2010), few guidelines exist for *ex situ* conservation. Here, we critically review various strategies for *ex situ* conservation of the freshwater pearl mussel, examine the main gaps in knowledge, and indicate those areas in most need of research. Although we have largely focused our attention on the freshwater pearl mussel, we have also drawn information from other freshwater mussels, where appropriate. Our objectives were 2-fold: (1) to illustrate the range of options available for the artificial propagation of freshwater mussels, and (2) to weigh the main advantages and limitations of different captive breeding strategies for conservation.

STRATEGIES FOR *EX SITU* CONSERVATION

The conservation of *Margaritifera margaritifera* faces several challenges, not least being the low rates of recruitment in natural populations. This is offset by a long reproductive lifespan and high fecundity, although freshwater pearl mussels do not reach sexual maturity until an age of 10 to 20 yr (Bauer 1987a, Skinner et al. 2003). *Ex situ* conservation of freshwater pearl mussels involves some or all of the following steps (Fig. 2): (1) fertilisation of females in captivity, (2) infection and encystment of glochidia in suitable fish hosts, (3) stocking of infected fish into existing or historical mussel rivers, (4) harvesting and rearing of excysted larvae, and (5) release of captive-reared juvenile mussels. Historically, *ex situ* conservation projects have, on the whole, been uncoordinated and poorly planned, with results difficult to quantify due to the slow turnover of this species (Hastie & Young 2003a).

Fertilisation of females in captivity

Mussel fertilisation rates are known to be influenced by the spatial distribution of broodstock (Downing et al. 1993), and the aim of aggregating adult mussels in captivity is to achieve higher fertilisation rates and greater production of glochidia. In common with other freshwater bivalves, sexes in the freshwater pearl mussel are separate (dioecious) and reproduction takes place after 10 to 20 yr, typically in February or March (Young & Williams 1984a,b, Skinner et al. 2003). Males release sperm into the water; the sperm is carried downstream and inhaled by females to fertilise their eggs, which are kept in modified marsupia in the gills (Smith 1979, Skinner et al. 2003). Fertilisation often occurs synchronously within a population, and appears also to be linked to water temperature (Ross 1992, Buddensiek 1995, Hastie & Young 2003b), as in other species of freshwater mussel (Watters & O'Dee 1999). At low densities, females can turn hermaphroditic, but whether this results in self-fertilisation is not clear (Bauer 1987b, Hanstén et al. 1997).

It is as yet unclear how many adults are required to achieve a reproductively viable population in captivity. In Wales, the Freshwater Pearl Mussel Recovery Group advocated in 2005 the collection from the wild of all adult mussels in the most critically endangered populations (those consisting of fewer than 100 mussels), and the rearing in captivity of at least 50 adult mussels from each of the other populations (Taylor 2007).

Adult mussels have been kept in flow-through systems fed with river water or in re-circulating systems. In flow-through systems, mussel broodstock can be maintained in salmonid hatchery troughs supplied with filtered river water (30 µm) to reduce sediment loads, and covered with sand and gravel (Hastie & Young 2003a, Preston et al. 2007). Very little is known about the diet requirements of adult *Margaritifera margaritifera*, although information from other freshwater bivalves suggests that they probably feed on freshwater algae within the 15 to 40 µm range (Winkel & Davids 1982). In the wild, Mandal et al. (2007) found varying proportions of blue-green algae, green algae and diatoms in the gut of the freshwater mussel *Lamel-*

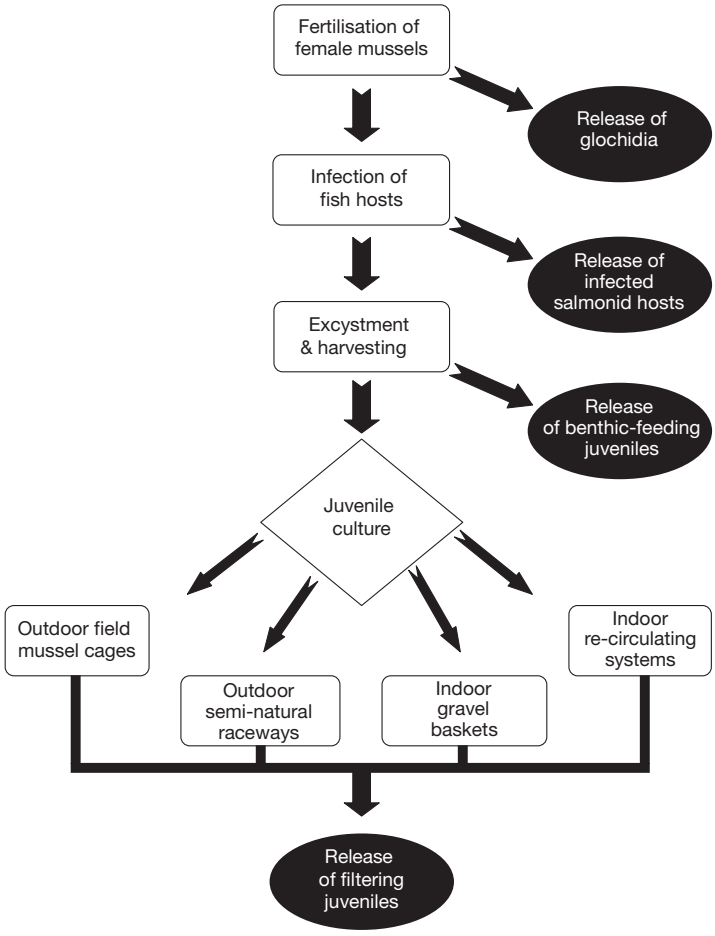

Fig. 2. *Ex situ* conservation strategies for the propagation of the freshwater pearl mussel *Margaritifera margaritifera*. See 'Strategies for *ex situ* conservation' for details on each strategy

lidens marginalis. Mussels kept in recirculating systems need to be fed with a suitable algal diet, but it is unclear whether supplemental feeding is needed in flow-through systems, or what effects, if any, different diet may have on reproduction and gamete quality. Recent research on stable isotope composition of mussel shells (Geist et al. 2005) may assist in the formulation of suitable diets for captive mussels.

Infection of fish hosts and host specificity

Although glochidia of most unionid mussels can readily attach to the tissue and gill filaments of various fish species (Strayer et al. 2004), metamorphosis and full larval development is normally only possible on a few host species (Dodd et al. 2006). In the case of *Margaritifera margaritifera*, each female can release between 1 million and 4 million glochidia, which drift downstream and die within 24 to 48 h if they cannot attach to a suitable fish host (Hastie & Young 2003b), although in some cases they can remain infective for up to 6 d (Ziuganov et al. 1994, Skinner et al. 2003). Margaritiferids appear to be highly host-specific, being closely linked to non-migratory brown trout *Salmo trutta* and migratory fishes — salmonids in the case of *M. margaritifera*, and acipenserids in the case of *M. auricularia* (Altaba 1990, Ziuganov et al. 1994, Bauer 2000). The Atlantic salmon *Salmo salar* is thought to be the primary fish host for *M. margaritifera* across its range (Ziuganov 2005), although brook trout *Salvelinus fontinalis* in eastern North America and brown trout in Europe can also act as suitable hosts (Young & Williams 1984b, Bauer 1987a, 2000, Cunjak & McGladdery 1991, Hastie & Young 2001, 2003b, Morales et al. 2004). Artic charr *Salvelinus alpinus* may also act as a viable fish host in northern Europe (Bauer 1987a), but this has not yet been confirmed (Hastie & Young 2001). Walker (2007) noted that, although rare, *S. alpinus* coexist in rivers with *M. margaritifera* in Scotland, providing the opportunity for glochidia to encyst on this species.

What seems clear is that *Margaritifera margaritifera* cannot metamorphose in the gills of Pacific salmonids (Young & Williams 1984a, Bauer 2000, Skinner et al. 2003, Ziuganov 2005). Earlier accounts on the susceptibility of Pacific salmonids to *M. margaritifera* in western North America (Meyers & Millemann 1977) are now believed to refer to the closely related species *M. falcata* (Stone et al. 2004), and may explain the contradictory results. Table 1 summarises the known hosts of *M. margaritifera* across its range. The extent to which freshwater pearl mussels show intraspecific variation in host specificity is not known and warrants further study, as this may dictate the success of reintroduction programmes.

Encystment of glochidia

Perhaps the simplest way to achieve host encystment of glochidia is by making gravid mussels cohabit with juvenile salmonids in hatchery troughs (Treasurer et al. 2006). Typically 0+ salmonid fry are used (either Atlantic salmon or brown trout) to maximise encystment, as older salmonid parr may show acquired immunity from previous exposures (Treasurer et al. 2006). Rearing salmonids and mussels together appears to result in high encystment rates (Treasurer et al. 2006), and it is possible that the release of glochidia in *Margaritifera margaritifera* is facilitated by the close

Table 1. Geographic variation in the salmonid hosts of the freshwater pearl mussel *Margaritifera margaritifera*. (?): Host species uncertain

Country	Salmonid host	Source
Austria	*Salmo trutta*	Lahnsteiner & Jagsch (2005)
Belgium	*S. trutta*	Araujo & Ramos (2001)
Czech Republic	*S. trutta*	Hruska (1999)
Estonia	*Salmo salar, S. trutta*	Geist et al. (2006)
Finland	*S. salar, S. trutta*	Araujo & Ramos (2001)
France	*S. salar, S. trutta*	Araujo & Ramos (2001)
Germany	*S. trutta, Salvelinus alpinus*?	Bauer (1987a), Bauer & Vogel (1987), Buddensiek (1995)
Great Britain	*S. salar, S. trutta, S. alpinus*?	Young & Williams (1983), Bauer (1987a), Hastie & Young (2001, 2003a)
Ireland	*S. salar, S. trutta*	Beasley & Roberts (1999) Preston et al. (2007)
Latvia	*S. trutta*	Rudzite (2004)
Luxembourg	*S. trutta*	Araujo & Ramos (2001)
Norway	*S. salar, S. trutta*	Wächtler et al. (2000)
Portugal	*S. salar, S. trutta*	Reis (2003)
Russia	*S. salar, S. trutta*	Ziuganov et al. (1994)
Spain	*S. salar, S. trutta*	Alvarez-Claudio et al. (2000), Morales et al. (2004)
Sweden	*S. salar, S. trutta*	Erikson et al. (1998)
USA (northeast)	*S. salar, Salvelinus fontinalis, S. trutta?*	Cunjak & McGladdery (1991)

proximity of suitable fish hosts, as shown in other freshwater mussels (Haag & Warren 2000). Research on the role of fish hosts in triggering *M. margaritifera* spatting would seem warranted in order to optimise captive breeding programmes.

As an alternative to the cohabitation method, the outflow of tanks housing gravid mussels can be diverted into fish tanks housing hatchery-reared juvenile salmonids (Hastie & Young 2003a, Preston et al. 2007). Hastie & Young (2001, 2003a) showed that large numbers of Atlantic salmon and brown trout could be infected in this way, with glochidia loads ranging between 10 and 800 glochidia per fish. More recently, Preston et al. (2007) used the same approach to infect large numbers of juvenile brown trout, with low (~1%) host mortalities.

In captivity, released glochidia which do not find their way into fish hosts can often be observed as a white, dense cloud in or around the adult female. This can be collected, diluted if necessary and either poured directly into hatchery tanks, or be given as a bath to batches of fish in small volumes of water to achieve infection. Spatting can also be induced in captivity, when it does not occur naturally. To induce glochidia release, gravid females are first placed in chilled de-chlorinated tap water. The release of glochidia is usually observed within 1 h as water rises to room temperature (Meyers & Millemann 1977). Induction of spatting is believed to be caused by thermal shock and respiratory stress, resulting in the forced release of glochidia from the modified gill marsupia to reduce oxygen demand; more oxygen becomes available to the female after expelling the brooding glochidia (Hastie & Young 2003b). Glochidia are then examined for viability, with cilia movement and 'winking' of valves as viability criteria; various salt concentrations can also be used to elicit an open/close response to determine glochidia viability (Meyers & Millemann 1977). Only glochidia spawned on the same day are normally used. The use of induced glochidia allows better control over exposure concentrations, but it is not known to what extent this method compromises glochidia viability compared to the viability of those obtained from naturally spawned mussels. Indeed, spat induced by thermal shock have sometimes been found to consist of immature, non-viable glochidia.

Stocking of infected fish hosts

The release of artificially infected hosts into rivers has a long history (Buddensiek 1995, Valovirta 1998, Hruska 2001, Preston et al. 2007), although results have been difficult to quantify. In Germany, and more recently also in the British Isles, there have been large releases of infected salmonid hosts, but evidence for recruitment of second-generation juvenile mussels is lacking (Hastie & Young 2003a). In theory, the release of artificially infected hosts makes conservation sense, as the maturing glochidia would fall from the host and populate the rivers in a 'natural' way, and would also reduce the costs and time associated with an extended period of juvenile mussel rearing in captivity. Moreover, artificial infection typically results in glochidia loads many times higher than those commonly found in the wild (Karna & Millemann 1978, Hruska 2001),

which may aid in the propagation of freshwater mussels. However, mortality of hatchery-reared salmonids is usually very high immediately following stocking (Aprahamian et al. 2003), and most excysted glochidia do not seem to find a suitable substrate in which to continue their development (Buddensiek 1995, Hastie & Young 2003a).

Harvesting and rearing of excysted (post-parasitic) juvenile mussels

An alternative to the release of infected fish hosts carrying glochidia is the captive-rearing of juvenile mussels through the post-parasitic stage. This is expected to offer greater control over the survival and growth of mussels (Treasurer et al. 2006, Preston et al. 2007), but it represents a long-term programme that requires a committed facility and staff, as several years will pass between infecting the fish hosts and the production of juvenile mussels for restoration.

It takes around 10 mo for glochidia to develop on suitable salmonid fish hosts, but 95% of glochidia die before reaching this stage (Hastie & Young 2003a). After completing development, glochidia excyst from host tissue, fall away and must be collected, typically in plankton nets placed directly over outflow pipes (Buddensiek 1995). Juveniles can then be transferred to outgrow tanks and maintained for the next few years, until they are large enough to survive in the wild or taken into the next rearing phase. Some knowledge on the timing of excystment is advantageous to optimise the collection of mussel seed in the following spring (Hastie & Young 2003a). Hruska (1992) first proposed the concept of 'degree days' required to reach excystment, and concluded that a period of 15°C water temperature was required for the last few weeks. At captive breeding facilities in Wales, juveniles have excysted following an average of 2381 degree days during the period 2005 to 2008 (range = 2229–2619 degree days). By keeping a record of degree days, 150 µm mesh plankton nets can be placed over outflow pipes in anticipation of juvenile excystment, and the feeding regimes of host fish reduced to make it easier to harvest the post-parasitic juveniles. Post-parasitic juvenile mussels begin to pedal-feed on algae and organic matter as soon as they fall from the fish host, and will therefore require suitable substrate for their initial development (Geist & Auerswald 2007). The transition from benthic to filter feeding represents a critical period for survival in captive breeding programmes (Hastie & Young 2003a), as the early juvenile stages appear to be very vulnerable to disturbance and have narrow substrate requirements (Young & Williams 1983). Several factors are critical for their survival and growth, including substrate type, silt content, water quality and an adequate supply of nutrients (Skinner et al. 2003, Geist et al. 2006). Barnhart (2006) found that occasional handling improved juvenile survival in North American freshwater mussels, possibly due to the removal of silt and debris. Predation and competition by microfauna may also play an important role in early juvenile mortality (Zimmerman et al. 2003). Several methods have been employed in the culture of juvenile freshwater pearl mussels, including the use of outdoor mussel cages, semi-natural stream channels, salmonid hatching baskets and recirculation systems (Fig. 2).

Mussel cages

The use of mussel cages to rear excysted juvenile *Margaritifera margaritifera* in the wild was pioneered by Buddensiek (1995). Mortality amongst post-parasitic juveniles was found to be around 70% during the first months (June to December), but decreased after the first winter. Only animals larger than 900 µm had a 50% chance of surviving to their second growing period, and all juveniles less than 700 µm in size died during the June to December period. Therefore, initial size appeared to be a critical factor for survival of juvenile mussels. In a similar study in Scotland, Hastie & Young (2003a) reported a 3% survival rate after 12 mo of cage rearing in the wild. In comparison, juvenile *M. margaritifera* kept in similar mussel cages at a hatchery attained a 7% survival rate after 10 mo. Thus, while mussel cages may offer some advantages for the culture of juvenile mussels under more natural conditions, current methods would need to be optimised and scaled up for conservation purposes. In this sense, an upwelling 'mussel-silo' cage system has recently been developed in North America to rear juvenile mussels in flowing waters with reduced risk of siltation (Barnhart 2006).

Semi-natural stream channels

Preston et al. (2007) recently assessed the merits of using hatchery raceways covered with gravel to serve as semi-natural stream channels for the rearing of encysted salmonids. Excysted mussels were allowed to fall in the substrate and complete their development, and analysis of gravel core samples approximately 1 yr after the introduction of encysted hosts showed relatively high densities of juvenile mussels, up to 13 200 mussels in 1 cohort. This study was the first in the UK to culture and maintain large numbers of juvenile pearl mussels for restoration purposes, although similar methods have been used in the United States with

other freshwater mussels (Williams et al. 1993, Beaty & Neves 2004). The advantages of this method include that it capitalises on high encystment loads of artificially infected hosts, and allows glochidia to excyst under more controlled substrate and flow conditions. However, it is as yet unclear whether this method can be scaled up for long-term propagation, how long mussels should be kept in stream channels, or what precautions are needed to harvest delicate juveniles from the natural substrate.

Salmonid hatching baskets

The use of hatching baskets represents the most widespread method of culturing freshwater pearl mussels during the early stages (Hastie & Young 2003a, Skinner et al. 2003). Excysted juvenile mussels are collected in outflow mesh screens and transferred to indoor salmonid hatchery troughs fitted with hatching baskets covered with a 1 to 2 mm layer of fine gravel (150 to 500 µm). Filtered river water upwells through each gravel basket, helping to reduce silt loads, while algae and organic matter enrich the gravel and provide nutrition for the juveniles. Post-parasitic mussels can be reared in this way for 12 to 18 mo, until they are large enough to be transferred to larger facilities or released into the wild (Hastie & Young 2003a). Survival of juvenile mussels reared by this method appeared to have been high during the first few months post-excystment (Taylor 2007), but this was followed by high mortalities during the second year. As with other rearing systems, little is known about causes of juvenile mussel mortality in captivity, although predation by flatworms, mechanical damage and silting up are thought to be important at the post-parasitic stage (Zimmerman et al. 2003, Barnhart 2006).

Recirculation systems

Recirculation systems offer greater control over environmental variables than typical flow-through facilities, and these have been tried successfully for culturing various species of freshwater mussels in North America (Jones & Neves 2002, Jones et al. 2004, 2005, Barnhart 2006), but not yet in *Margaritifera margaritifera*. Mussel recirculating systems typically consist of nested chambers with a downwelling flow at a rate of ca. 400 l h^{-1} (Barnhart 2006). Substrate is required in recirculating systems for growth and survival, although this can perhaps make juvenile mussels more vulnerable to flatworm predation (Zimmerman et al. 2003). Supplemental feeding of unicellular green algae has also been found necessary (Barnhart 2006), but little is known about optimal algal diets. For example, survival in captivity of juveniles of the dromedary pearly mussel *Dromus dromas* was 30% after 2 wk when fed the green alga *Nannochloropsis oculata* (Jones et al. 2004). Growth and survival of juvenile freshwater mussels appears to be higher in flow-through than in recirculating systems (Jones & Neves 2002), possibly due to diet imbalance. Early survival and growth are also higher when juvenile bivalves are reared on natural sediments rather than on commercial shellfish diets (Naimo et al. 2000), emphasising that for many species the formulation of algal diets constitutes one of the greatest challenges for captive rearing.

Stocking of juvenile mussels

Some attempts have been made to release glochidia directly into upstream tributaries to infect wild hosts, although there are no results available to ascertain the success of this strategy (Geist & Kuehn 2005). On the other hand, releases of cultured post-parasitic freshwater pearl mussels have not yet occurred, as these have not been cultured in sufficient numbers. The aim of the captive breeding of unionid mussels is to release individuals back into rivers at some point in the future. The success of the programme will therefore ultimately depend on the ability of captive-bred individuals to survive and reproduce in the natural environment, not on the success of the rearing programme itself. However, it is unknown whether captive populations will adapt to the natural environment, and how juvenile mussels will fare compared to wild populations; this is an area where research is urgently needed (Hoftyzer et al. 2008).

CONCLUSIONS

As with other unionid mussels, the conservation of *Margaritifera margaritifera* is problematic and exacerbated by the continuation of many practices that actively contribute to their decline (Strayer 2008). The problems of silt pollution, unsympathetic riparian management, habitat fragmentation and declining host populations need to be addressed whilst there are still sufficient numbers of reproductively viable adult mussels. In common with other freshwater mussels (Berg et al. 2007, Elderkin et al. 2007, Zanatta & Murphy 2007), *M. margaritifera* shows a significant degree of population structuring (Machordom et al. 2003), even at small spatial scales (Geist & Kuehn 2005, Bouza et al. 2007). Areas colonised by *M. margaritifera* since the last glacial maxima display high genetic diversity (Geist & Kuehn 2008, Geist et al. 2009), and this may be indica-

tive of locally adapted populations, as seen in their salmonid hosts (Garcia de Leaniz et al. 2007), and should be taken into account when developing *ex situ* conservation programmes for the species (Geist & Kuehn 2005). For example, translocations of mussels between watersheds or introduction of artificially reared individuals may result in gene introgression and the breakdown of local adaptations, further compromising the conservation of depleted populations. Given what has been learned over the last few decades about the genetic risks of fish stocking (reviewed by Cross et al. 2007), the artificial propagation of freshwater mussels should take into account the genetic variation, effective population size and number and extent of neighbouring mussel conservation units. It can be argued that until the situation in rivers improves, the conservation of this species will depend on captive breeding. There may simply be too few individuals to maintain self-sustaining populations, particularly in the face of sudden pollution events, massive floods or other catastrophes. However, it can also be argued that unless the underlying threats facing the species are also addressed, captive breeding alone is unlikely to save endangered freshwater mussels from extinction. Indeed, relying on captive breeding alone is dangerous and is what Meffe (1992) termed 'techno-arrogance' and 'half-way technologies', i.e. when resources are simply diverted from habitat protection to artificial propagation, and technology is used for treating the symptoms rather than the causes of decline. Captive breeding cannot be a substitute for habitat restoration (Christian & Harris 2008), and single-species approaches are unlikely to work with pearl mussels, as these can conflict with the conservation of other species (see Geist & Kuehn 2008). Instead, success is most likely to come from multi-faceted projects which take a holistic, integral approach to conservation and rely on 4 underlying principles: (1) legal protection and policing, (2) public awareness, (3) habitat restoration and (4) artificial breeding.

Acknowledgements. We thank S. Consuegra and 3 anonymous referees for making useful comments on the manuscript.

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Editorial responsibility: Paul Snelgrove, St. Johns, Canada

Submitted: February 16, 2009; Accepted: April 12, 2010
Proofs received from author(s): May 27, 2010