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Multiple paternity promotes genetic diversity in captive breeding of a freshwater mussel

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ABSTRACT

Captive breeding can be an important supplement to habitat restoration in the conservation of threatened species. Careful choice of breeding regimes and genetic monitoring are essential to maintain genetic integrity and genetic diversity of target populations. Many species of freshwater mussels are threatened globally, and captive breeding has been employed many places. The genetic consequences of those breeding programmes are however largely unknown, as are the mating patterns that determine offspring genetic diversity. We explored changes in genetic diversity from adult freshwater pearl mussels to offspring in two alternative breeding approaches. Genetic diversity was measured at 15 microsatellite markers and mating patterns were reconstructed by parentage analysis. In the first approach, fertilisation took place among broodstock mussels in captivity, while in the second approach, fertilisation took place in the wild. In the population fertilised in captivity, female contribution was extremely skewed. This resulted in substantial loss of genetic diversity, but many sires within female broods (multiple paternity) limited the loss of genetic diversity. Fertilisation in nature largely maintained genetic diversity in the offspring, compared to sampled adult mussels, despite a skewed female contribution. Genetic diversity was maintained because a high level of multiple paternity allowed the number of sires to largely exceed the number of dams. Our results show the potential of genetic monitoring to improve stocking of freshwater mussels. We show different success in maintaining genetic diversity between the two breeding approaches and emphasise that the choice of methods should consider conditions for fertilisation in nature and reproductive contribution of mussels in captivity.

1. Introduction

Freshwater mussels are among the most endangered groups of animals worldwide (Strayer et al., 2004; Lopes-Lima et al., 2017). Causes for the dramatic decline of many species during the last century include overharvesting, water pollution and habitat degradation (Strayer et al., 2004; Lopes-Lima et al., 2017). Conservation programmes typically focus on the restoration of suitable habitat. At the same time, captive breeding programmes have been established many places worldwide to maintain imperilled populations or to re-establish lost populations (Gum et al., 2011; Jones et al., 2006; Patterson et al., 2018). Captive breeding is generally considered the last resort, because it is cost and labour intense and carries the risk of altering the gene pool of a population (Fraser, 2008; Snyder et al., 1996; Laikre et al., 2010). Therefore, captive breeding programmes are

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typically limited to populations or species that are in severe risk of extinction, such as many freshwater mussels (Jones et al., 2006; Fraser, 2008). Freshwater mussels are long-lived and produce large numbers of parasitic larvae that develop into juveniles while attached to the gills of a fish host. The early life-stages of freshwater mussels have a high mortality and are typically also the most sensitive to habitat degradation (Lopes-Lima et al., 2017). Captive breeding of freshwater mussels is potentially highly efficient when juvenile mussels can be reared beyond this critical early phase of their life-cycle (Gum et al., 2011; Jones et al., 2006).

A major challenge in captive breeding is to maintain the genetic integrity and diversity of target populations (Lynch and O'Hely, 2001; Fraser, 2008). This requires a careful breeding scheme and genetic monitoring (Laikre et al., 2010). The genetic integrity of populations is maintained by avoiding crossing among genetically distinct populations. Maintaining the genetic diversity of populations depends primarily on the number of female and male broodstock individuals and variation in reproductive success within sexes. A skewed distribution, with few broodstock individuals contributing large proportions of offspring, may strongly reduce offspring genetic diversity (Fraser, 2008; Laikre et al., 2010). In freshwater mussel populations with limited natural recruitment, reintroduced captive-bred offspring can constitute a large part of a generation. If those offspring descend from a small set of broodstock mussels or if offspring numbers are strongly skewed among broodstock mussels, this will significantly alter the gene pool of the population, and likely reduce effective population size and genetic diversity (Ryman-Laikre effect; Ryman and Laikre, 1991). Despite increasing efforts of captive breeding of freshwater mussels, and existing genetic management guidelines for captive breeding of freshwater mussels (Hoftyzer et al., 2008; Jones et al., 2006), only few studies have evaluated how breeding schemes affect genetic diversity (Wilson et al., 2012; Kyle et al., 2016).

The freshwater pearl mussel (*Margaritifera margaritifera*) is one of currently 16 recognized species of freshwater mussels in Europe (Lopes-Lima et al., 2017). Freshwater pearl mussels were historically distributed across large parts of Europe but have decreased dramatically during the last century (Lopes-Lima et al., 2017). Today, the species is listed as endangered in the IUCN red list (IUCN, 2017). Many of the remaining populations lack successful recruitment, as indicated by the absence of smaller, i.e. younger mussels. Juvenile freshwater pearl mussels live buried in the river bed and require sufficiently oxygenated substratum. Such substratum is lost many places because of eutrophication and siltation, preventing successful recruitment of freshwater pearl mussels (Geist and Auerswald, 2007; Geist, 2010; Lopes-Lima et al., 2017). More than 95% of the remaining populations in central and southern Europe lack recent recruitment, typically for 30–50 years, and are thus functionally extinct (Young et al., 2001; Geist, 2010). While habitat degradation is considered the largest threat to current populations, overexploitation strongly contributed to the historic decline and pollution and loss of the fish host are further known threats to freshwater pearl mussels (Lopes-Lima et al., 2017). Captive breeding programmes for freshwater pearl mussels have been established in many European countries, including the Czech Republic, France, Germany, Northern Ireland, Norway, Luxembourg, Spain and the UK (Gum et al., 2011; Larsen, 2015). This study was carried out at a hatchery for freshwater pearl mussels in Norway. Norway holds a large part of the remaining populations in Europe, but about one third of the populations were estimated to lack recent recruitment (Larsen, 2010). A national captive breeding programme was established in 2011, with a central hatchery in Austevoll (Hordaland county, Norway). Different methods have been practiced in captive breeding of freshwater pearl mussels, including the collection of glochidia (larvae) and naturally infested host fishes (Gum et al., 2011). However, the most established methods are the collection of gravid female mussels and the collection of adult male and female broodstock mussels with fertilisation taking place in captivity (Jones et al., 2006; Preston et al., 2007; Gum et al., 2011; Larsen, 2015). The former method is also most commonly used in captive breeding of many species of freshwater mussels in North America (Jones et al., 2006; Patterson et al., 2018).

In this study, we tested the genetic consequences of two approaches in captive breeding of freshwater pearl mussels. In the first approach, fertilisation took place in captivity, with males and females interacting freely in a common tank. In the second approach, females were collected after fertilisation had taken place in the river. How those alternative approaches affect offspring genetic diversity depends on the number of males and females contributing offspring and variation in the number of offspring within sexes. Freshwater pearl mussels are sperm casters, with males releasing sperm into the water and females retaining eggs (Young and Williams, 1984). This may promote offspring genetic diversity, because many males can fertilise a single female (Anthony and Blumstein, 2000; Hyde et al., 2008; Wacker et al., 2018). Alternatively, spermcast mating may decrease offspring genetic diversity under fertilisation in nature if not all females receive sufficient sperm to fertilise all eggs (Levitan, 1998). This would lead to an increase in variation in female reproductive success and thus reduce offspring genetic diversity. Under high densities in captive conditions, individuals of both sexes may contribute relatively evenly, increasing offspring genetic diversity. Freshwater pearl mussels may reproduce as hermaphrodites (Grande et al., 2001) and a previous study suggested that this is more common under low population densities (Bauer, 1987). Hermaphroditic reproduction and potential self-fertilisation may strongly affect genetic diversity and we analysed whether hermaphroditism occurred in the two breeding approaches. We analysed changes in genetic diversity from broodstock mussels to offspring under the two breeding approaches, using 15 microsatellite markers.

2. Methods

2.1. Fertilisation in hatchery

Adult mussels were collected from a natural population in River Utvik (Trøndelag county) and brought to the hatchery. The River Utvik population consists of less than 200 individuals and lacks recent recruitment (Larsen, 2017). The natural host of

the population is landlocked brown trout (*Salmo trutta*). On 19 August 2012, 33 adult mussels (shell length [mm]: range = 81–110; mean = 98; SD = 8) were collected.

River Utvik mussels were placed non-systematically in a channel (440 × 40 cm) with unidirectional water flow. This resulted in small distances between mussels, which ensured that females could be fertilised by sperm from males positioned upstream. The outgoing water was directed into a tank with suitable host fishes, allowing mussel larvae to infest a host and to develop into juvenile mussels. Juvenile (0+) domesticated brown trout (Tinnhølen, Hordaland county) were used as host. Domesticated fish were used to reduce the risk of introducing diseases into the hatchery.

Utvik brood mussels were collected in autumn 2012 but did not release larvae that year, which is in line with an observed low proportion of gravid mussels in the river and likely explained by eutrophication and siltation (Larsen, 2017). Utvik mussels reproduced successfully in the hatchery the first time in autumn 2013 and we studied offspring genetic diversity from this first year of reproduction in captivity. Infestation was successful and about 9000 juvenile mussels detached from the fish in spring 2014 and were transferred to channels with suitable substrate for further development. Juvenile mortality was exceptionally high in the hatchery in summer 2014 because of problems with water quality, and ca. 260 juveniles were alive at the time of genetic sampling. On 28–29 October 2015, 194 offspring (about two years old) were collected for genetic analysis. Genetic samples from all adults were taken on 4 June 2015 by gently collecting material from the visceral mass with a cotton swab (Karlsson et al., 2013). Cotton swabs were stored in individual tubes containing 600 μl lysis buffer (QIAGEN ™).

2.2. Fertilisation in nature

Adult mussels were collected from a natural population in River Slørdal (Trøndelag county) on 20 August 2015, after fertilisation was expected to have taken place naturally in the river. The collected mussels released larvae in early to mid September and in populations in mid-Norway gravidity typically lasts 28–38 days (Larsen, 2012). A total of 52 mussels (shell length [mm]: range = 97–146; mean = 120; SD = 12) were collected without knowledge of sex or whether females were gravid. The River Slørdal population consists of ca. 3600 adult individuals, but recent recruitment is only sporadic (Esplund and Julien, 2016). The primary natural host of the mussel population is expected to be Atlantic salmon (G.H. Johnsen, unpublished data).

River Slørdal mussels were placed in a 1 × 1 m tank with circulating permanent water flow (25–35 cm/s). The outgoing water was directed into a tank with suitable host fishes. Both domesticated Atlantic salmon (Bjoreio, Hordaland county) and domesticated brown trout (Botsvann, Aust-Agder county) were used as host fishes and were successfully infested by the River Slørdal mussels. Mussels released larvae and infested host fishes in September 2015. About 4000 juvenile mussels detached from the fish in spring 2016 and were transferred to channels for further development. A total of 200 juveniles were sampled for genetic analysis, half of which were sampled on 3 June 2016 and 22 September 2016 respectively. DNA samples of adults were taken on 22 September 2016, with the same methods as used for River Utvik mussels.

2.3. Genetic analysis

For juveniles, DNA was extracted from the whole animal and for adults from cotton swabs using DNEASY tissue kit (QIAGEN). The mussels were genotyped at 15 loci: MarMa3050, MarMa3621, MarMa4277, MarMa4322, MarMa2671, MarMa4143, MarMa5280 (Geist et al., 2003) and Mm2201, Mm2230, Mm2235, Mm2240, Mm2207, Mm2210, Mm2233, Mm2236 (Garlie, 2010). The former set of seven loci is part of a set of nine loci (Geist and Kuehn, 2005; Geist et al., 2003) that has been widely used in population genetics studies (e.g. Geist and Kuehn, 2005; Karlsson et al. 2014; Zanatta et al., 2018). The latter set of eight loci (Garlie, 2010) was included to increase statistical power, specifically in parentage analysis.

PCR was carried out in two multiplexes (Karlsson et al., 2016). The PCR protocol was as follows: 2 μl DNA, 4 μl Qiagen multiplex mastermix, 0.8 μl primermix and 1.6 μl RNase free water (Karlsson et al., 2016). The PCR was run on a Quattro Cycler (VWR) in the following conditions: denaturation for 15 min at 95 °C, followed by 30 cycles of 57 °C for 90 s and 72 °C for 60 s, and a final step of 60 °C for 30 min (Karlsson et al., 2016). The PCR products were separated and visualised on an ABI 3130xl DNA analyser (Applied Biosystems) and sized using GENEMAPPER ver. 3.7 (Applied Biosystems).

3. Assignment of parentage

3.1. Fertilisation in hatchery

We used CERVUS to assign genotyped offspring to dams and sires among the broodstock mussels. We used the option of parental pair analysis with unknown sex and an estimated mistyping rate of 0.01. Following standard procedures in CERVUS, we accepted assignment of parentage with a critical Delta value (difference in likelihood-ratio between the first and second most likely candidate pairs) set for a confidence level of 95%. Female freshwater pearl mussels may reproduce as hermaphrodites under certain conditions (Bauer, 1987) and we allowed for self-fertilisation in assignment of parentage. We inferred the sex of broodstock mussels from parentage. One single female was observed in the hatchery tank to release a large quantity of larvae and parentage analysis revealed an accordingly high contribution by one broodstock mussel. We assumed those to be identical and assigned the individual female. Most other broodstock mussels could then be sexed from mating patterns because both females and males produced offspring with multiple mates. A small proportion of reproducing

broodstock mussels could not be sexed because they had only produced genotyped offspring with a single mate (see Results). Those procedures resulted in the assignment of a single juvenile to two males. While this may have resulted from hermaphroditic reproduction, an erroneous assignment appeared more likely to explain this single incidence and we conservatively excluded the juvenile from analysis. The statistical power of the genetic markers was not sufficient to assign all genotyped offspring to parent pairs with the required certainty (see Results) and a low rate (5%) of erroneous assignments was expected from the above analysis.

3.2. Fertilisation in nature

While all dams were known to be present among the broodstock mussels, relatively few sires were expected to be represented among the collected adults. Fertilisation had taken place in the river, in a population of several thousand individuals and the number of potential sires was therefore very high in relation to the number of collected adults. We used CERVUS to assign offspring to dams, and to sires if they were among the collected adults. For offspring for which the father had not been collected, we reconstructed paternal genotypes with COLONY (Wang, 2004). The sex of the adults contributing offspring was inferred from parentage. This allowed to estimate the number of sires across and within female broods and the distribution of number of offspring among sires. The reconstruction of maternal half-sibling groups was highly robust, allowing to estimate the number of sires contributing to each female brood with high certainty (Wacker et al., 2018). There was however considerable uncertainty in the reconstruction of paternal half-sibling groups, i.e. whether females shared sires, which may have affected the effective number of male breeders (see Results). Details of the applied methods are described in Wacker et al. (2018).

3.3. Analysis of genetic diversity

We tested for the assumptions of Hardy-Weinberg and linkage disequilibrium with significance tests adjusted with Bonferroni correction in GENEPOP 4.7 (Rousset, 2008). Expected heterozygosity and allelic richness were calculated in CERVUS (Kalinowski et al., 2007) and FSTAT (Goudet, 1995) respectively. The effect of offspring sample size on the number of detected alleles was graphically explored by jackknife analysis with the jackmsatpop function in the R package PopGenKit (Paquette, 2012). Differences in allelic richness and expected heterozygosity between broodstock mussels and offspring were tested with the non-parametric Wilcoxon sign-rank test for paired data.

The effective number of breeders (N_{eb}) for genotyped offspring was estimated from demographic data. N_{eb} was calculated from variation in the number of offspring among males and females assigned parentage. We first estimated N_{eb} for males and females separately as: $N_{eb} = (N\ k\ -\ 1)/(k\ -\ 1\ +\ Vk/k)$ with N = number of breeders, k = average number of offspring and Vk = variance in number of offspring (Crow and Kimura, 1970; Caballero, 1994). The number of male and female offspring were scaled respectively for an average number of offspring of two. The total number of effective breeders was then calculated as $N_{eb} = 4\ N_{eb}[male]\ N_{eb}[female]/(N_{eb}[male]\ +\ N_{eb}[female])$ (Crow and Kimura, 1970; Caballero, 1994). To explore how the number of sampled offspring affected the effective number of breeders, we used repeated subsampling without replacement, estimating the effective number of breeders for each subsample with the above methods (Perrier et al., 2014). For each sample size, 1000 iterations were run.

4. Results

We did not detect significant deviation from Hardy-Weinberg equilibrium for any locus or significant deviation from linkage disequilibrium for any pair of loci. Details on genotyping success and marker variability are reported in Table S1.

4.1. Fertilisation in hatchery

Genotyping was successful at all loci for all broodstock mussels and for 179 out of 194 offspring. Twelve out of 15 offspring for which genotyping failed were dead at the time of collection.

Offspring of hatchery fertilised *River Utvik* mussels had reduced genetic diversity compared to broodstock mussels. Expected heterozygosity across loci was lowered by 11%, from 0.483 in adults to 0.428 in offspring (Fig. 1; Wilcoxon sign-rank test: $P = 0.059$, $N = 15$). Allelic richness was lowered by 23%, from an average of 3.93 alleles in adults to 3.03 alleles in offspring (Fig. 2; Wilcoxon sign-rank test: $P = 0.008$, $N = 15$). The average number of alleles per locus in the complete offspring sample was 3.40 ($N = 179$). Rarefaction curves for the number of alleles in dependence of sample size are shown in Fig. S1a.

Offspring genetic diversity was reduced because few females reproduced and because of strong variation in female contribution. Parentage was successfully assigned for 150 out of 179 offspring, identifying a total of 26 parents (out of 33 broodstock mussels). For the remaining 29 offspring, two or more pairs had too similar likelihoods to be the parents to assign parentage with required certainty (95% correct assignment). The sex was inferred for 23 out of 26 contributing broodstock mussels (see Methods), revealing a sex ratio of 5:18 (presumably females:males). We did not find evidence for hermaphroditic reproduction. Female offspring contribution was highly variable. Females contributed between one and 110 offspring (mean = 29.6; CV = 1.53; Fig. 3), with a single female contributing 74% of the successfully assigned offspring. Each female

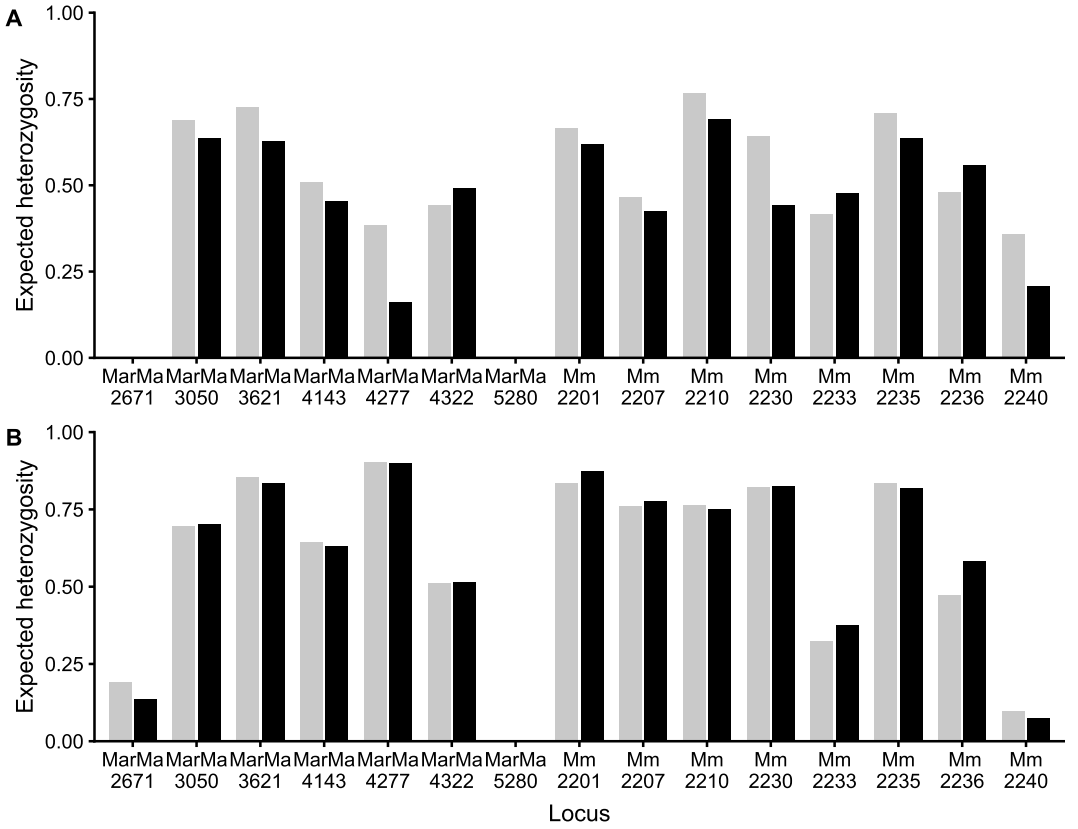

Fig. 1. Expected heterozygosity in adult (grey bars) and offspring (black bars) freshwater pearl mussels at 15 microsatellite loci. For adults from (A) River Utvik (N = 33), fertilisation took place in captivity (mussel hatchery). For adults from (B) River Slørdal (N = 52), fertilisation took place in the river. Offspring were sampled and genotyped as juveniles, after the parasitic stage (River Utvik: N = 179; River Slørdal: N = 198).

produced offspring with multiple sires, with one to four sires for the females contributing few offspring and 15 sires for the female contributing a large part of offspring. The 18 male broodstock mussels contributed between one and 28 offspring (mean = 8.2; CV = 0.86; Fig. 3).

The effective number of breeders contributing to the 150 successfully assigned offspring was 5.7. The effective number of female breeders was only 1.6, while the effective number of male breeders was 14.1. Subsampling of offspring suggested that the 150 assigned offspring were sufficient to estimate the effective number of breeders for all offspring produced (Fig. S2a).

4.2. Fertilisation in nature

Genotyping was successful for all 52 adults and for 198 out of 200 offspring. Details on genotyping success and marker variability are reported in Table S1.

Offspring of *River Slørdal* mussels had high genetic diversity, with no reduction in heterozygosity and moderate reduction in allelic richness compared to adults. Mean expected heterozygosity across loci was 0.586 in adults and 0.580 in offspring (Fig. 1; Wilcoxon sign-rank test: P = 0.95, N = 15). Mean allelic richness was lowered by 8%, from 8.52 alleles in adults to 7.87 alleles in offspring (Fig. 2; Wilcoxon sign-rank test: P = 0.021, N = 15). The average number of alleles per locus in the complete offspring sample was 9.53 (N = 198). Rarefaction curves for the number of alleles in dependence of sample size are shown in Fig. S1b.

Offspring genetic diversity was high because each female produced offspring with many (mostly not collected) males. Multiple paternity generated high offspring genetic diversity despite variable female offspring contribution. The 198 offspring were assigned to 20 mothers, which contributed between one and 43 offspring (mean = 9.9; CV = 1.1; Fig. 3). The number of offspring differed significantly among females (df = 19, $\chi^2 = 232.5$, P < 0.001). A total of 77 males were reconstructed by COLONY to have sired the sampled offspring (Fig. 3). While each male typically contributed only a single offspring within the brood of one female, most males produced offspring with more than one female. Males contributed between one and five offspring (mean = 2.6; CV = 0.4; Fig. 3). The fathers of 13 offspring were detected among the collected adult mussels, with seven males contributing between one and five offspring each. We did not find evidence for hermaphroditic reproduction.

The effective number of breeders contributing to the 198 offspring was 41.0. The effective number of female and male breeders was 11.2 and 116.5 respectively. Subsampling of offspring suggested that the 198 assigned offspring were sufficient

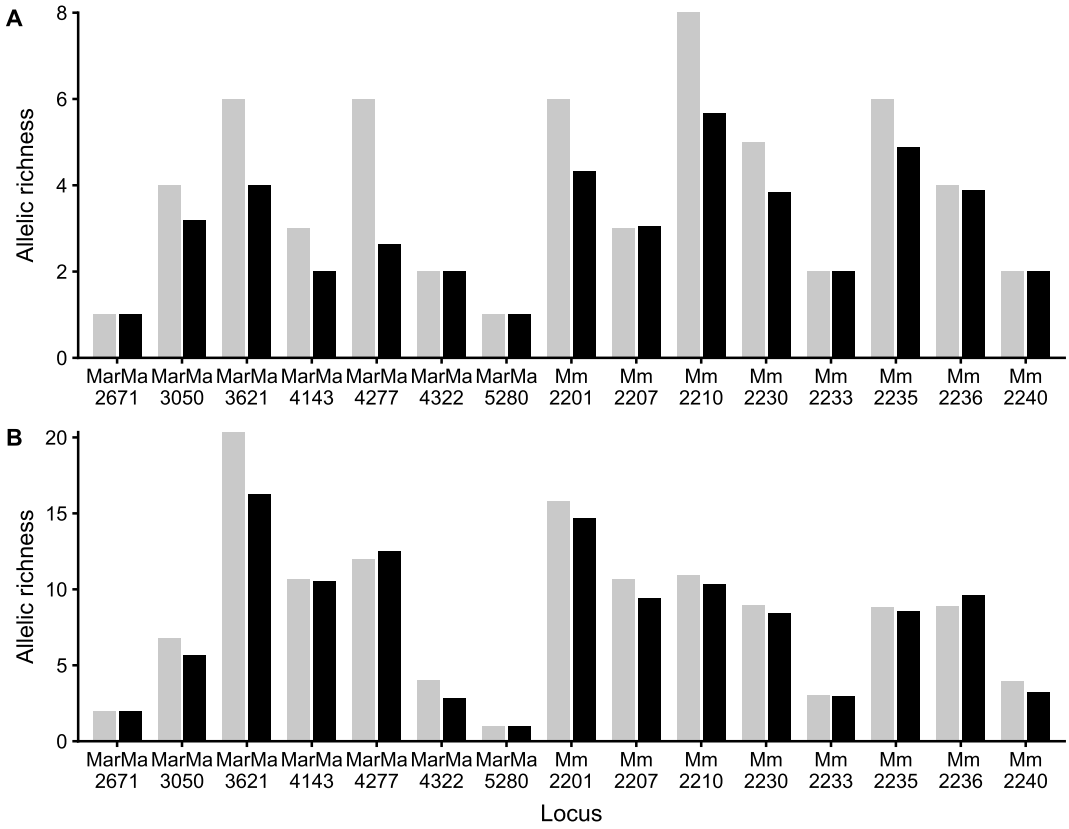

Fig. 2. Allelic richness in adult (grey bars) and offspring (black bars) freshwater pearl mussels at 15 microsatellite loci. For adults from (A) River Utvik (N = 33), fertilisation took place in captivity (mussel hatchery). For adults from (B) River Slørdal (N = 52), fertilisation took place in the river. Offspring were sampled and genotyped as juveniles, after the parasitic stage (River Utvik: N = 179; River Slørdal: N = 198).

to estimate the effective number of breeders contributing to all offspring (Fig. S2b). The effective number of male breeders was in contrast limited by sample size of genotyped offspring. Also, uncertainty in the identification of paternal half-sibling groups (i.e. offspring sharing a father) in our analysis may have affected the distribution of offspring among males and thereby the effective number of male breeders. However, the effective number of breeders was close to its theoretical maximum under the given female contribution (Fig. S2b), and therefore robust to deviations in the effective number of male breeders.

5. Discussion

Our results highlight the importance of knowledge of mating systems in captive breeding. High levels of multiple paternity promoted offspring genetic diversity under both fertilisation in nature and fertilisation in captivity. Despite variable success among females fertilised in the wild, genetic diversity was largely maintained through the genetic contribution of many sires within and across female broods. Hatchery fertilised mussel were collected from a population under environmental stress, manifested as a low proportion of gravid mussels observed in the field (Larsen, 2017). While the hatchery fertilised mussels were kept in captivity for one year before production, we cannot rule out that the poor condition of the mussels remained and was the reason for the low proportion of mussels producing offspring and the strongly skewed contribution. While this resulted in reduced offspring genetic diversity, the loss of genetic diversity was limited by the contribution from a much larger part of broodstock mussels as sires.

In freshwater mussels exhibiting high levels of multiple paternity, collecting gravid mussels or glochidia (Gum et al., 2011) may be a highly efficient approach to maximise offspring genetic diversity. This requires that conditions for fertilisation in nature are good and that the collected gravid mussels do not release premature glochidia, which may be induced by stress under collection (P.J., personal observation). This approach also requires detailed knowledge on the timing of fertilisation in nature, which varies among and within populations. When fertilisation takes place in captivity, conditions should allow for fertilisation by multiple males. Multiple paternity strongly limited loss of genetic diversity in offspring of the studied population with fertilisation in captivity, where female contribution was extremely skewed. Observations on the release of glochidia in other populations under cultivation suggest a more balanced female contribution (P.J., personal observation), such as found in this study for the population that was fertilised in the wild. Multiple paternity is expected to also be an

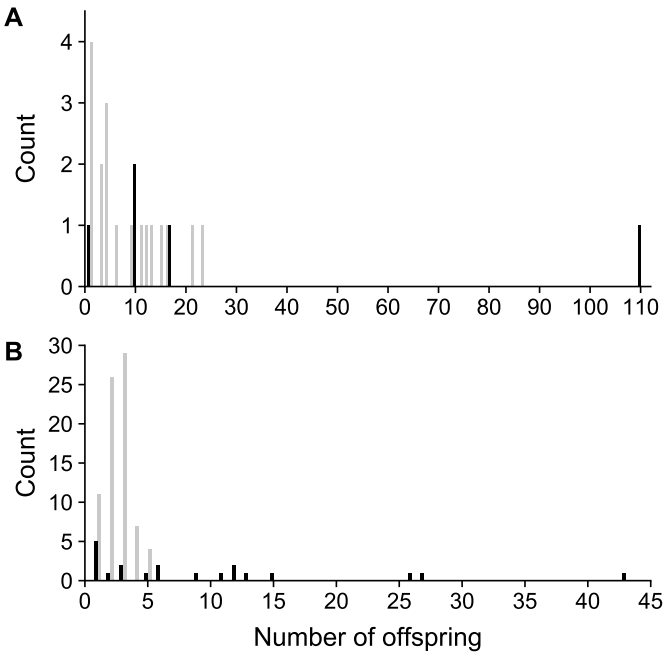

Fig. 3. Distribution of number of genotyped offspring for male (grey bars) and female (black bars) freshwater pearl mussels from (A) River Utvik (fertilisation in captivity) and (B) River Slørdal (fertilisation in nature). Adults that did not contribute to the genotyped offspring are not shown.

important factor for offspring genetic diversity under fertilisation in captivity in populations that do not suffer from environmental stress and show a more moderate skew in female contribution.

Future studies should examine intra- and inter-population variation in the mating system of freshwater mussels. For example, multiple paternity may not be equally pronounced in small or fragmented populations and for such populations, collecting brood mussels for fertilisation in the hatchery might be a better approach. While captive breeding programs have been established for many species of freshwater mussels worldwide, including the diverse freshwater mussel fauna of North America, knowledge of their mating system is largely lacking (Jones et al., 2006; Wacker et al., 2018). It is therefore unknown how multiple paternity and other mating system characteristics shape genetic diversity in captive breeding of other freshwater mussel species (Wacker et al., 2018). Genetic monitoring has previously been reported for captive breeding of freshwater pearl mussels in Northern Ireland (Wilson et al., 2012; Kyle et al., 2016). Fertilisation in captivity among ca 90–140 broodstock mussels resulted in only moderate loss of genetic diversity (Wilson et al., 2012; Kyle et al., 2016). The mating patterns that had produced the observed offspring genetic diversity were however unknown.

Multiple paternity may generally promote genetic diversity in captive breeding of animals with multiple mating or external fertilisation, as predicted by theory (Anthony and Blumstein, 2000; Sugg and Chesser, 1994; Pearse and Anderson, 2009). In our study, multiple paternity allowed (i) breeding males to largely outnumber breeding females and (ii) reproductive success to be less variable in males than females, increasing the effective number of breeders. Multiple paternity may however not be beneficial when the sex ratio of breeders is close to equal and female reproductive success is close to uniform. With an even sex ratio of breeders and no variation in female reproductive success, the effective number of breeders is also maximised under monogamous mating (Fisher, 1930). Multiple paternity may in that scenario decrease the effective number of breeders when random processes or sperm competition lead to variation in male reproductive success. The latter has been found in captive breeding of salmonids, where eggs are often fertilised in vitro by mixed sperm from multiple males, which may differ in quantity and quality of sperm (Wedekind et al., 2007).

This study provides important insights into genetic diversity in captive breeding of freshwater mussels but is not intended to evaluate the Norwegian captive breeding program. Only a single year and two populations were studied, and breeding methods have been refined since the study was carried out. As discussed above, the River Utvik population was untypical in a strongly skewed sex ratio of reproducing broodstock mussels, likely induced by environmental stress. This is in line with a low occurrence of gravid mussels in the wild population (Larsen, 2017). Notably, release of sperm is more common in the wild population, which may indicate that females suffer more from environmental stress than males. Freshwater pearl mussels may reproduce as hermaphrodites (Bauer, 1987; Grande et al., 2001), and an alternative explanation is that mussels preferentially invested into male gametes under environmental stress. We did however not find evidence for simultaneous hermaphroditism or self-fertilisation, as individual mussels contributed as either female or male to the sampled offspring. In contrast to self-fertilisation, simultaneous hermaphroditism may increase offspring genetic diversity in captive breeding, by increasing the number of contributing dams and sires and by reducing variation in offspring contribution within sexes.

Hermaphroditic reproduction may affect genetic diversity in captive breeding of other populations and future studies should address the occurrence and effects of hermaphroditism in freshwater pearl mussels.

Fertilisation in captivity may also have been affected by the use of stream channels with unidirectional water flow, because any female mussels upstream would not get fertilised or fertilised by fewer males. Stream channels have now been replaced by tanks with circulating water, allowing fertilisation of mussels to take place regardless of their position in the tank. Our results show that it is important to monitor the relative contribution from brood mussels to achieve a better representation of the population in the released mussels. Brood mussels with a large contribution could for example be excluded from production of the next cohort. In addition to adjusting the relative contribution of individual mussels or rotation of broodstock mussels, we suggest that a moderate number of mussels are being stocked from many years instead of stocking a large number of mussels from only one or a few years of production.

We focussed on the comparison of genetic diversity in collected adults with produced offspring and did not evaluate to what extent the collected adults reflected population genetic diversity. Rarefaction curves (Fig. S1) suggested that significant proportions of genetic diversity (number of alleles) were missed when sampling 33 and 52 mussels respectively. Also, we evaluated mating patterns and offspring genetic diversity from offspring collected at two years age, i.e. after the parasitic stage and subsequent growth of juveniles. Mortality rates are high during the first two years (Bauer, 1987; Jansen et al., 2001), with potentially strong domestication selection under infestation of fish, the parasitic stage or juvenile survival (Williams and Hoffman, 2009; Christie et al., 2012). From our data we are not able to exclude that selection substantially affected the inferred mating patterns and offspring genetic diversity. Observations of a single hatchery fertilised female releasing large numbers of larvae do however match our results from parentage analysis at juvenile stage. Finally, our study did not address effects on diversity in mitochondrial DNA and maternal epigenetic effects (transgenerational epigenetics). Those factors may negatively affect offspring fitness under a low effective number of female breeders despite high levels of multiple paternity (Wolf and Wade, 2009; Matos, 2012).

6. Conclusions

Captive breeding is considered an important addition to habitat restoration in the management of endangered freshwater mussels. Our results highlight the importance of genetic monitoring in captive breeding and how insights into mating patterns may help to improve breeding protocols. If conditions are suitable, fertilisation in nature can be a highly efficient approach in breeding freshwater pearl mussels. If fertilisation takes place in the hatchery, our results show the importance of allowing for multiple paternity and of genetic monitoring, adjusting the relative contribution of brood mussels if required.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gecco.2019.e00564.

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