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A conservation genetics perspective on supportive breeding: A case study of the common nase (*Chondrostoma nasus*)

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Abstract

1. Supportive breeding programmes are becoming increasingly crucial for the conservation of many declining freshwater fishes such as the European common nase, *Chondrostoma nasus*. However, small relict populations are genetically highly vulnerable, and supportive breeding can have a detrimental impact on the genetic composition of the cultured offspring (e.g. as a result of inbreeding, genetic drift, and adaptation to captivity).
2. This study monitored the genetic effects of a continuing supportive breeding programme of common nase by comparing the genetic diversity of two wild spawning populations with the respective wild offspring and the progeny from captive breeding originating from spawners of the two wild populations, considering genetic variability, genetic differentiation, and inbreeding effects using nine microsatellite markers.
3. Despite low genetic differentiation, the offspring from captive breeding and from one of the natural populations (River Sims) were remarkably different genetically, as indicated by pairwise analyses of genetic divergence (F_{ST} from 0.028 to 0.070; Jost's D_{EST} from 0.080 to 0.205) and the discriminant analysis of principal components. The mean number of alleles and mean allelic richness in the captive-bred offspring and also in the wild offspring of the River Sims were lower than for wild populations of spawners and natural offspring of the River Mangfall, and signs of inbreeding effects were detected ($F = 0.106$ for captive bred and 0.048 for natural offspring).
4. The observed effects can probably be attributed to the limited number of spawners (two females and three males) used for captive breeding. In addition, the results support previous evidence on recruitment problems of the Sims population, such as a reduced hatching success.
5. Collecting fertilized eggs from the wild and rearing them in captivity (repatriation approach) could be an alternative to stripping a limited number of spawners and thereby to improve the conservation of genetic diversity of natural populations.

Bernhard C. Stoeckle, Melanie Mueller, Christoffer Nagel and Juergen Geist, equal contribution.

KEYWORDS
captive breeding, fish population genetics, freshwater fish conservation, genetic monitoring, rheophilic cyprinids, hatchery effect

1 | INTRODUCTION

As a result of the strong decline of many freshwater fish and mussel populations, ex-situ conservation programmes, including captive breeding and restocking, are gaining increasing importance in their conservation (Targońska, Żarski & Kucharczyk, 2008; Roques et al., 2018; Lamothe & Drake, 2019; Lepič, Blecha & Kozák, 2019; Strayer et al., 2019; Manubens et al., 2020; Wetjen et al., 2020; Geist et al., 2021). A successful implementation of supportive breeding measures is particularly crucial if populations are declining very quickly or where species are not able to survive in natural habitats despite extensive in-situ habitat restoration efforts (Mameri et al., 2018; Manubens et al., 2020). One example is the common nase, *Chondrostoma nasus* L. (subsequently referred to as nase), a key species of European river systems that was once very widespread. Nase has a unique role in the food web of central and eastern European rivers, both in the top-down direction by grazing benthic algae, which can significantly increase hyporheic oxygen supply (Hübner et al., 2020), and in the bottom-up direction by providing an important food source for the highly endangered Danube salmon (*Hucho hucho*, L.) (Šubjak, 2013). Nase is considered a medium-age species, which usually reaches an age of 8–15 years (Blahak & Lusk, 1995; Lusk, Jurajda & Peňáz, 1995). Sexual maturity is attained in 4–7 years (Lusk, Jurajda & Peňáz, 1995) with a fecundity of 15,000 to 20,000 eggs per kilogram body weight (Harsanyi & Aschenbrenner, 1995). Populations of this species began to decrease in the 20th century (Lepič, Blecha & Kozák, 2019) and have now declined to highly endangered relict populations in many places (Peňáz, 1996; Targońska, Żarski & Kucharczyk, 2008; Wetjen et al., 2020). This reflects the high sensitivity of rheophilic cyprinids to structural habitat degradation and pollution (Targońska, Żarski & Kucharczyk, 2008). Restoration measures to improve habitat quality and connectivity (Pander et al., 2017; Meulenbroek et al., 2018; Ramler & Keckeis, 2019; Nagel et al., 2020) are currently supplemented by several short-term ex-situ methods to support declining populations and to repopulate rivers after local extinction. These methods comprise the translocation of adults to establish new populations (conservation translocation) (Ovidio et al., 2016; Præbel et al., 2021), and restocking with captive-bred larvae and juveniles (Targońska, Żarski & Kucharczyk, 2008; Lepič, Blecha & Kozák, 2019; Wetjen et al., 2020).

Supportive breeding measures in nase and other fish species are usually based on stripping wild spawning adult fish, which are caught by electrofishing, and the release of juvenile progeny later after they have reached a certain size in the hatchery (Targońska, Żarski & Kucharczyk, 2008; Thorstensen et al., 2019; Wetjen et al., 2020). Spawning adult nase are easily accessible as they congregate to spawn. Nase larvae can be reared in ponds with a high rate of success, which makes supportive breeding and restocking a potentially valuable conservation tool for this species (Mameri et al., 2018; Lepič, Blecha & Kozák, 2019). However, supportive breeding can have a detrimental impact on the genetic diversity (i.e. loss of genetic diversity) of the offspring generation owing to genetic drift and selection effects (i.e. adaptation to captivity) (Roques et al., 2018; Thorstensen et al., 2019). Selection can occur in different phases of the breeding programme. Capture of potential parents usually takes place only for a limited period (Klupp & Geist, 2018); moreover, only animals that are ready to spawn can be used, and these may not be representative of the population. Fertilization (artificial mixing of eggs and sperm) and rearing conditions (e.g. temperature and water chemistry) during incubation may have selective effects in the offspring (Klupp & Geist, 2018). Individuals that are best adapted to the rearing conditions have an advantage, possibly resulting in higher survival rates. Feeding can also be selective, especially when spawners from wild populations are used. In this case, often only a small proportion of juvenile fish accepts the food (Klupp & Geist, 2018). The genetic effects of maladaptation can cause significant physiological and morphological changes (Latorre et al., 2020), eventually resulting in lowered lifetime success compared with individuals hatched in the wild and reduced adaptive potential to changing environmental conditions (Fraser et al., 2019). The use of only a limited number of broodstock individuals not fully representing the genetic diversity of the original population can enhance genetic drift and inbreeding in the offspring (Franklin, 1980; Brown et al., 2000). This has already been demonstrated in captive breeding programmes of other aquatic species using neutral markers (Geist et al., 2021; Rojas et al., 2021). The intentional translocation of populations for conservation purposes can also result in adverse genetic consequences, such as founder effects, genetic drift, and inbreeding depression (IUCN, 2013). It is surprising that only a few studies (primarily focused on salmonids: Rytwinski et al., 2021) have attempted to assess the consequences of this management practice on population genetics (Præbel et al., 2021), yet the controversy about the advantages and disadvantages of supportive breeding and stocking is increasing.

For many freshwater fish species, the effects of artificial reproduction on genetic diversity are well known, and genetically informed management strategies exist (Wetjen et al., 2020), particularly for economically important species such as salmonids. For vanishing species such as nase, which have a limited commercial value or interest to recreational anglers, data are deficient despite their high importance in the food web of European rivers. The genetic structure of nase populations has received limited interest in fisheries management, and data are known only from a few studies in the Rhine catchment in Switzerland (Hudson, Vonlanthen &

Seehausen, 2014) and Germany, the Rhône in France (Gollmann et al., 1997; Devaux et al., 2015), and the upper Danube in Germany (Gollmann et al., 1997), all revealing low to moderate levels of genetic differentiation among populations and reduced heterzygosity in several populations (Gennotte et al., 2014).

In this study, a continuing captive breeding programme of nase focused on monitoring genetic effects was assessed. Captive breeding has been carried out by local fishery clubs for several years in two tributaries of the River Inn in Germany (the Mangfall and the Sims), where two of the major remaining spawning sites within the River Inn system are located (Nagel et al., 2020). Owing to the limited number of ripe females at any given time, the breeding programme typically relies on small numbers of parents, making it necessary to look for genetic effects in the offspring, as observed previously for other species (e.g. Danube salmon; Geist et al., 2009). The nase populations in the Mangfall and Sims have been reported by the local anglers to be morphologically different and to exhibit a distinct homing behaviour. Previous investigations of the effectiveness of restoring spawning areas have shown that spawning sites differ in habitat quality and that these populations differ in demographic structure as well as larval survival rates (Duerregger et al., 2018; Nagel et al., 2020).

The objectives of genetic monitoring were to (i) assess whether there is genetic differentiation between the two populations, (ii) evaluate the genetic diversity of populations and their suitability for reintroduction, and (iii) test for genetic differences between progeny from captive breeding and wild offspring. These results were intended to be used for suggesting ways of improving future conservation management of the nase populations, and for populations of other rheophilic cyprinids where similar propagation programmes are in place.

2 | MATERIAL AND METHODS

2.1 | Tissue sampling and DNA extraction

All the work conducted in this study passed an ethical review and was approved by the government authorities (fish sampling permit no. 31–7562; fin clipping permit: ROB-55.2-2532.Vet_03–20-1).

This study was designed to monitor the impact of the common breeding practice (Harsanyi & Aschenbrenner, 1995) on patterns of genetic variation in nase as described in local rearing guidelines and carried out by the local angling clubs in the River Inn catchment. Spawning nase are caught in close proximity to the spawning sites in two tributaries (Mangfall and Sims) and gametes are stripped directly in the field. Subsequently, fertilized eggs are brought to the hatchery and reared to a size of ~10 cm before restocking.

Electrofishing was conducted by wading in the River Mangfall (12°6′23.52″E; 47°50′46.66″N; 1 April, 2020) and in the River Sims (12°9′1.02″E; 47°51′4.20″N; 2 April, 2020) using a portable generator (1.5 kW; Grassl, Schoenau, Germany). Prior to further handling, all fish were anaesthetized with MS-222 (tricaine methanesulfonate; concentration according to Adam, Schürmann & Schwevers, 2013). Subsequently, sex was determined and total length (TL) measured to the nearest millimetre. A tissue sample (~0.25 cm^2) was taken from each fish by clipping the pelvic fin and immediately preserving it in 96% ethanol. Scales were used to identify the age of each specimen. This procedure was conducted with 30 fish each from the Mangfall and Sims populations (Table 1). In both rivers, the female-to-male ratio was ~1:3, matching the range of sex distribution described in previous studies (Lusk, Jurajda & Peňáz, 1995). Mean fish length and age were greater in the Sims population (Table 1).

In both populations, eggs of two females each were stripped and immediately fertilized by the milt of three males using the 'dry method' (i.e. mixing eggs and milt before adding water after about 1 min). This cross-breeding ratio resembles the artificial reproduction procedure most commonly described in published literature (Keckeis et al., 2000). The total number of females that can be stripped is restricted by the readiness of the females to spawn, which is limited to a few hours only each spawning season (Harsanyi & Aschenbrenner, 1995). Consequently, several females need to be caught in order to have a few that release eggs. To minimize disturbance of the already overaged and threatened relict population of the nase, the local angling clubs only use the described low number of spawners. As this study was intended to monitor their common practice of artificial breeding, the total number of spawners was not increased for genetic monitoring.

After stripping, egg stickiness was removed by the addition of fresh milk, as described in Targońska, Żarski & Kucharczyk (2008). Fertilized eggs were then brought to the hatchery and incubated at a water temperature of 10–12 °C. After hatching, larvae were reared in nature-like ponds until the following spring (see Lepič, Blecha & Kozák, 2019). To test for the effect of selective breeding on genetic diversity, 51 individuals were sampled from the breeding ponds in

TABLE 1 Overview of all treatment groups, including spawning adults from the Mangfall (AM) and the Sims (AS) populations, natural offspring (LM and LS) from these populations, and offspring from the breeding station (BMS). Total length (TL) and age are given as mean values plus/minus *SD*.

Code	River	Stage	*N*	Females/males	TL (mm)	Age (years)
AM	Mangfall	Adult	30	12/18	459 ± 31	8.8 ± 1.4
AS	Sims	Adult	30	9/21	493 ± 29	9.8 ± 1.6
LM	Mangfall	Larval	48	NA	10 ± 1	>0
LS	Sims	Larval	48	NA	10 ± 1	>0
BMS	Mangfall/Sims	Juvenile	51	NA	102 ± 5	>1

Note: Sex could not be determined in larval and juvenile stages.
Abbreviation: NA, not available.

spring 2021 with fin clips being used for further genetic analysis (Table 1).

To assess genetic diversity of the natural offspring in the year 2020, two drift nets were placed downstream of each spawning site in the River Mangfall and River Sims 2 weeks after spawning — see Nagel et al. (2020) for technical details. Drift nets were deployed for 5 days and 2 h each day to catch nase larvae emerging from the spawning sites. Drift nets were also placed upstream of the spawning sites to account for potential bias from larvae drifting from upstream stretches. No larvae were caught in these upstream nets, suggesting that all captured larvae originated from the observed spawning grounds. From all nase larvae caught, 48 were selected from each river for further genetic analysis. To gain representative subsamples of the natural offspring, the larvae selected comprised a subsample from each day of larval emergence, resulting in five subsamples from the River Mangfall and five from the River Sims (Table 1, Supporting Information Table S1). As larval emergence was distinctly higher in the River Mangfall, subsamples represented 10–15% of the total larvae caught each day in this river and ~30% of the total larvae caught each day in the River Sims (Supporting Information Table S1). Genomic DNA was extracted from fin clips and larvae, applying the standard phenol–chloroform method (Sambrook, Fritsch & Maniatis, 1989); DNA samples were then stored at −20°C for subsequent analyses.

2.2 | Polymerase chain reaction and genotyping

Nine microsatellite loci were analysed; these have been used in earlier population genetic studies on freshwater cyprinids (Mesquita et al., 2003; Muenzel et al., 2007; Vyskočilová, Šimková & Martin, 2007) and *C. nasus* populations (Hudson, Vonlanthen & Seehausen, 2014). Owing to overlapping allele size ranges of markers, two loci sets were generated for polymerase chain reaction (PCR) multiplexing (Set1: SARN7G5, LSOU08, SARN2F11B, LC290, SARN7K4, LSOU21; Set 2: LSOU05, SARN7F8, LC27) according to Hudson, Vonlanthen & Seehausen (2014), and forward primers were labelled with three different fluorescent dyes: TAMRA, HEX, and 6FAM.

Multiplex PCR reactions were performed using a Qiagen Multiplex PCR kit (Qiagen, Düren, Germany) in a total volume of 15 µl with the following components: 7.5 µl of the Qiagen-Mix, 3.9 µl (Set 1) and 5.1 µl (Set 2) high-performance liquid chromtography water, 0.2 µl of each primer (0.2 pmol μl^{-1}), and 1.2 µl DNA (20–25 ng μl^{-1}). PCR products were separated on an ABI PRISM 377 Sequencer (Applied Biosystems, Foster City, CA, USA). GeneMapper Software v. 4.0 (Applied Biosystems) was used to score the genotypes.

2.3 | Data analysis

Microsatellite allele frequencies, the mean number of alleles per locus *A*, allelic richness A_R as a standardized measure of the number of alleles corrected for sample size, expected and observed heterzygosities, H_E and H_O respectively, and inbreeding coefficient F_{IS} were calculated using Fstat v. 2.9.3 (Goudet, 2001). Genepop v. 4.7.3 (Rousset, 2008) was used to test genotypic distributions for conformance with Hardy–Weinberg expectations using the probability test (Haldane, 1954), and to estimate the significance of genotypic differentiation between these population pairs. All probability tests were based on the Markov chain method (Guo & Thompson, 1992; Raymond & Rousset, 1995) using 10,000 dememorization steps, 100 batches, and 5,000 iterations per batch. The number of distinct multilocus genotypes was determined using the R-package POPPR v. 2.8.348 (Kamvar, Tabima & Grünwald, 2014). Pairwise analyses of genetic divergence (F_{ST} and Jost's D_{est}; Wright, 1965; Jost, 2008) among populations and offspring were made using GENALEX v. 6.5 (Peakall & Smouse, 2012). The R-package Adegenet v. 2.1.1 (Jombart, 2008) in R v. 3.6.2 (R Core Team, 2019) was used to determine mean individual inbreeding coefficients F_{ind} for each source population and the offspring by calculating for each individual the probability of being homozygous at a locus $p(h) = F + (1-F)\sum_i p_i^2$ and summing up log-likelihoods over all microsatellite loci to account for multilocus genotypes, where *F* refers to the probability that an individual inherited two identical alleles from a single ancestor and p_i refers to the frequency of allele *i* in a population. In addition, relatedness between individuals within populations based on the *F*-value of the 2mod program (Ciofi et al., 1999) was estimated, quantifying the probability that two alleles share a common ancestor within a population. This measure is correlated with effective population size. The effective population size of the parental generation using the linkage disequilibrium method (Waples & Do, 2010) was estimated with Neestimator v. 2 (Waples & Do, 2008; Do et al., 2014).

The genetic structure of samples was visualized using discriminant analysis of principal components (DAPC; Jombart, Devillard & Balloux, 2010) as implemented in the software package Adegenet v. 2.1.1 (Jombart, 2008). This method was selected because it is less sensitive to uneven sampling (Puechmaille, 2016). DAPC first transforms the data using principal component analysis and then performs a discriminant analysis on the retained principal components. Results of the DAPC were visualized by the RGB transformation of the three discriminants. Similar generated colours thus correspond to the similar genetic composition of respective individuals or populations (Jombart, Devillard & Balloux, 2010). The data were assessed for potential genotyping errors, such as null alleles, short allele dominance (large allele dropout), or scoring errors, by using the computer program MICRO-CHECKER 2.2.3 (van Oosterhout et al., 2004).

3 | RESULTS

3.1 | Genetic integrity and differentiation

The overall fixation index F_{ST} was 0.0313 and mean Jost's D_{EST} was 0.0922. Pairwise F_{ST} values ranged from F_{ST} = 0.0061 (adults from

TABLE 2 Pairwise estimates of Jost's D_{EST} value (Jost, 2008) between populations from the rivers Mangfall (AM), Sims (AS), natural offspring (LM and LS), and offspring from breeding station (BMS) above diagonal, with F_{ST} (Weir & Cockerham, 1984) below the diagonal

Code	AM	AS	LM	LS	BMS
AM	–	0.0180	0.0190	0.0800	0.1340
AS	0.0061	–	0.0430	0.1150	0.0811
LM	0.0064*	0.0139*	–	0.0780	0.1492
LS	0.0275***	0.0366***	0.0261***	–	0.2051
BMS	0.0477***	0.0276***	0.0511***	0.0700***	–

Significance levels: *, $P < 0.05$; ***, $P < 0.001$.

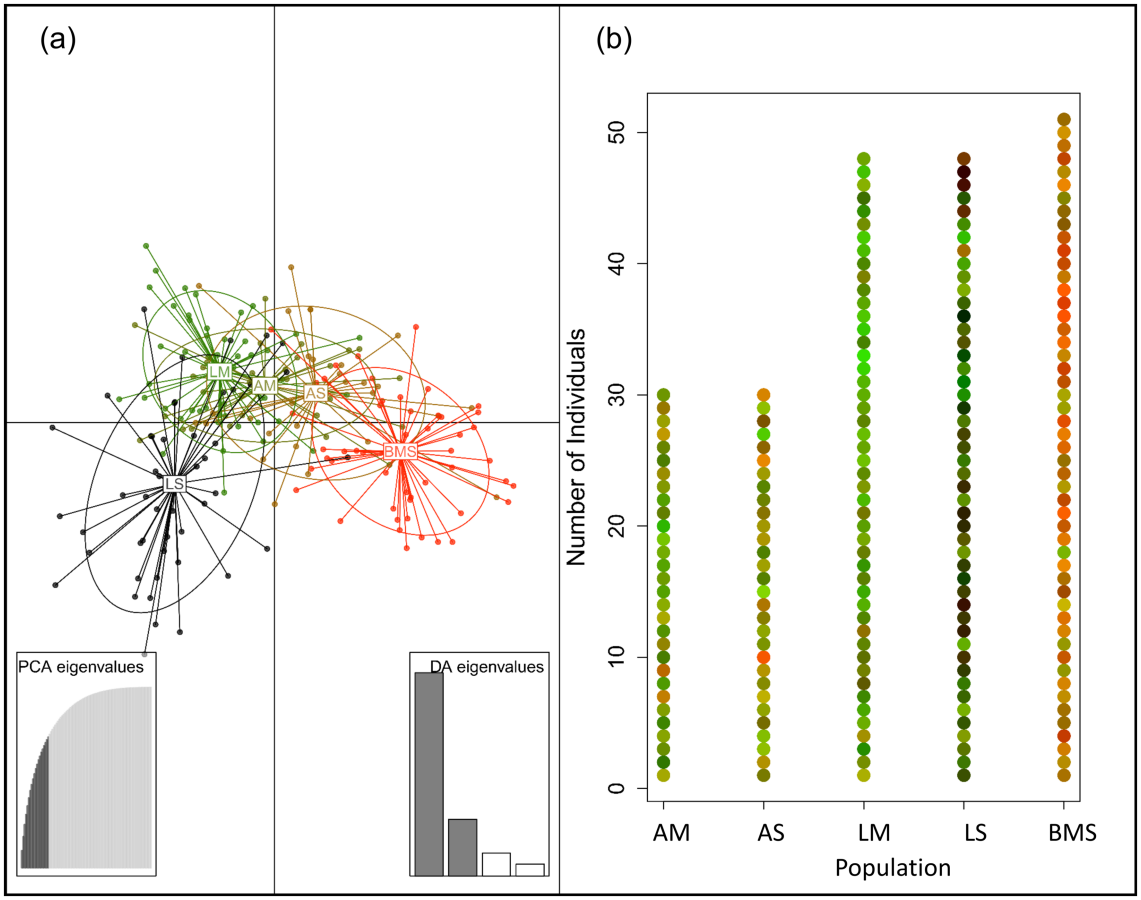

FIGURE 1 (a) Clustering of Mangfall (AM) and Sims (AS) populations, natural offspring from Mangfall and Sims (LM and LS), and offspring from the breeding station (BMS) based on discriminant analysis of principal components (DAPC) using the first 20 principal components and two discriminant functions explaining 87% of the total variation of the data (axis 1: 67%; axis 2: 20%). Individuals are depicted as individual dots; populations are represented by inertia ellipses and mean population colour based on the DAPC. DA: discriminant analysis; PC: principal component analysis. (b) Individual genetic composition of adult individuals from the rivers Mangfall (AM) and Sims (AS), natural offspring (LM and LS), and offspring from the breeding station (BMS) based on DAPC using the first 20 principal components and three discriminant functions. The colour of the dots corresponds to the results of the DAPC, with similar colours indicating similar genetic composition.

Mangfall [AM]–adults from Sims [AS]) to $F_{ST} = 0.0700$ (natural offspring from Sims [LS]–offspring from breeding station [BMS]). D_{EST} values were generally low, ranging from 0.0180 (AM to AS) to 0.2051 (LS to BMS), with higher levels of differentiation occurring between BMS, LS, and all other populations, as well as natural offspring from Mangfall (LM) (Table 2). All other populations were closely related with maximum $F_{ST} = 0.0139$, as also indicated by the similar colours in the DAPC (Figure 1). The effective population size of the parental generation for LM was 198.8 (parametric 95% confidence interval [CI]: 83.4 to infinite), for LS 19.1 (parametric 95% CI: 14.5 to 25.6), and for BMS 4.3 (parametric 95% CI: 3.5 to 7). The result of discriminant analysis of principal components (20 principal components explaining 87% of the total variation; axis 1: 67%; axis 2: 20%) is consistent with computed F_{ST} and D_{EST} values and graphically

illustrates the genetic differentiation between source populations from Mangfall (AM), Sims (AS), natural offspring (LM and LS), and offspring from the breeding station (BMS) (Figure 1).

3.2 | Genetic variability

MICRO-CHECKER did not detect any genotyping errors or signs of possible null alleles among the dataset. Deviations from Hardy–Weinberg equilibrium were observed both in LS and in offspring from the breeding station (BMS). LS and BMS exhibited a deficit and excess of heterozygotes respectively. Values of observed heterozygosity H_O in *C. nasus* populations and their offspring ranged from $H_O = 0.652$ (LS) to $H_O = 0.769$ (BMS). Allelic richness A_R ranged from 6.1 (BMS) to 9.0 (AS). The highest individual inbreeding coefficient was detected for LS ($F_{ind} = 0.241$), and the highest *F*-values were found in LS ($F = 0.048$) and BMS ($F = 0.106$) (Table 3). These latter two samples were the ones that significantly deviated from Hardy–Weinberg equilibrium.

3.3 | Comparison of captive-bred and natural offspring

Both A_R and *A* were lower in the natural offspring of the Sims (LS) and in the captive-bred juveniles (BMS) relative to their source populations AM and AS (Table 3). Adults and juveniles from Mangfall (AM and LM) and adults from Sims (AS) formed a closely related genetic cluster (Figure 1). High levels of genetic differentiation were observed between LS as well as BMS and all other groups (AM, LM, and AS) (Table 2, Figure 1).

4 | DISCUSSION

This study shows that first-generation, captive-bred offspring of wild nase exhibit decreased genetic diversity and potential inbreeding effects. This underlines the importance of using genetic tools in conservation and management programmes, especially those that incorporate supportive breeding (i.e. propagation and release). These results indicate that captive breeding should be a management technique of last resort and not the only long-term strategy for the conservation of threatened fish species. This is also confirmed by the results of a long-term captive breeding programme in the upper Lahn River in Germany, which has been reported to be unsuccessful (Schwevers & Adam, 1997; Wetjen et al., 2020). Most endangered fish species, including nase, exhibit small and declining relict populations, making them especially vulnerable to inbreeding depression and decreased adaptive potential (Pavlova et al., 2017; Roques et al., 2018; Fraser et al., 2019). Under such conditions, genetic monitoring allows the identification of optimal source populations, suitable parental individuals for breeding, and genetic assessment of offspring. In order to ensure the genetic integrity of reintroduced populations, both adult spawners, which represent the gene pool of the original population, and the stocked offspring should be examined (Wetjen et al., 2020).

In the dataset presented here, captive-bred juveniles (BMS) showed a significantly lower mean number of alleles and mean allelic richness than wild populations and a pronounced increase in their inbreeding coefficient ($F = 0.106$). Moreover, there was evidence of deviations from Hardy–Weinberg equilibrium associated with an excess of heterozygotes, most likely due to the fact that only a few spawners were used (Balloux, 2004). However, no change in heterozygosity could be detected in BMS when compared with wild populations and wild offspring. In nase, reproduction is characterized by polyandric and polygynic spawning behaviour (Peňáz, 1996), theoretically resulting in a high level of genetic exchange in intact river systems. The lower genetic diversity in BMS probably results from the limited number of spawners used in the supportive breeding programme (only two females and three males), resulting in a loss of alleles compared with wild populations. Præbel et al. (2021) showed similar genetic effects for translocated populations of *Coregonus lavaretus* L.

Compared with wild offspring from the River Mangfall, wild offspring from the River Sims had reduced genetic variability, expressed by a lower mean number of alleles, lower observed heterozygosity, increased inbreeding coefficient ($F = 0.48$), and a deficit of heterozygotes. Results from Neestimator showed that the parental generation of the Sims is smaller (Ne = 19.1) than that of the Mangfall (Ne = 198.8). Previous studies found that the River Sims

TABLE 3 Population genetics summary statistics for *Chondrostoma nasus* populations and their offspring and offspring from breeding station

Code	*N*	MLG	*A*	A_R	H_E	H_O	F_{IS}	F_{ind}	*F*	HW
AM	30	30	9.1	8.9	0.734	0.741	−0.009	0.185	0.003	
AS	30	30	9.3	9.0	0.762	0.759	0.004	0.181	0.004	
LM	48	48	9.1	8.3	0.740	0.741	0.007	0.190	0.015	
LS	48	47	8.3	7.8	0.740	0.652	0.120	0.241	0.048	***
BMS	51	51	6.7	6.2	0.725	0.769	−0.062	0.175	0.106	***

Note: Significance levels of all tests after sequential Bonferroni correction (Rice, 1989); ***, $P < 0.001$. Abbreviations: *N*, number genotyped; MLG, number of distinct multilocus genotypes; *A*, mean number of alleles; A_R, mean rarefied allelic richness; H_E, expected heterozygosity; H_O, observed heterozygosity; F_{IS}, inbreeding coefficient; F_{ind}, individual inbreeding coefficient; *F*, *F*-value based on the 2mod program; HW, results of Hardy–Weinberg probability tests for deviation from expected Hardy–Weinberg proportions.

population exhibited a significantly altered egg surface structure compared with the River Mangfall population (Nagel et al., 2021), as well as reduced hatching success under controlled incubation conditions (Duerregger et al., 2018), resulting in a significantly reduced recruitment under natural spawning conditions (Nagel et al., 2020). These outcomes may have resulted from genetic factors associated with inbreeding or demographic aspects such as the older age of spawners affecting fecundity (Keckeis et al., 2000); however, the levels of genetic variation detected here suggest that genetic factors are important in explaining the difference between these populations. In this study, local fisheries managers mixed offspring from both rivers in the hatchery. This is a commonly used approach because of space constraints, which is well in line with existing guidelines (Harsanyi & Aschenbrenner, 1995). Supportive breeding programmes in nase are only rarely informed by genetic monitoring (Lutz et al., 2021), as these efforts often include private activities of angling clubs, without knowledge transfer between scientists and managers (Lundmark et al., 2019). Using individuals from multiple populations within one river system may increase genetic diversity and the ability to adapt to changing environments, as well as decrease the risk of inbreeding depression, particularly for populations with reduced gene flow (Lutz et al., 2021; *Macquaria australasica*, Cuvier 1830, in Australia; IUCN, 2013). Outcrossing can have positive effects — such as superior fitness of progeny (heterosis) (Whitlock, Ingvarsson & Hatfield, 2000) — if the populations included evolved in similar environments and are not locally adapted (Lehnert et al., 2014), and the degree of genetic divergence among individuals from all the populations is not too high (Præbel et al., 2021). These requirements should be carefully considered before applying an outbreeding approach, as it may otherwise have unintended effects, such as reduced adaptation ability of the offspring (IUCN, 2013). However, differences in local adaptations are highly unlikely for the Sims and Mangfall populations. The two river systems are both sub-catchments of the River Inn, and the spawning sites are only ~5 km apart (Nagel et al., 2020). It is highly likely, therefore, that the artificial breeding programme in this study area would benefit from including adult breeders from both the Mangfall and Sims populations, while avoiding inclusion of juveniles from the Sims catchment because of their reduced effective population size and associated effects. Cost-effective genetic monitoring is now feasible for evaluating the effectiveness of different management strategies and can inform the decision-making process (Lutz et al., 2021).

There are various ways in which the existing supportive breeding programme can be improved. If the current approach of collecting parents from the wild is maintained, then an increase in the number of parents is critical. This can be realized by increasing the number of spawners in each collection event, especially including a larger number of ripe males, or by carrying out multiple collection events within the spawning period. A general rule-of-thumb for conservation-oriented breeding programmes is to use 50 male and 50 female spawners (Klupp & Geist, 2018) to minimize the risk of selection and drift effects. These theoretical guidelines conflict with practical recommendations for nase (Harsanyi & Aschenbrenner, 1995) and existing supportive breeding programmes in this species owing to constraints of hatchery space and the availability of broodstock spawners. Consequently, by way of mitigation, rearing activities should be maintained over several years, as this minimizes the risk of genetic bottlenecks, genetic drift, and selection effects, as previously discussed in the captive breeding of endangered freshwater pearl mussel (Geist et al., 2021).

Alternatively, a repatriation approach, where fertilized eggs or hatchlings are collected from the wild and reared in captivity until they have reached sufficient age or size (Thorstensen et al., 2019), may be a more suitable approach for conservation management than using a limited number of adult spawners. This approach maintains natural breeding behaviour, avoids early selection pressures, and reduces domestication selection (Osborne et al., 2020). It also includes more parental lines, increasing the likelihood of representing the genetic diversity found in wild populations, as observed in a study on captive-bred white sturgeon (Thorstensen et al., 2019). Repatriation of early life stages (i.e. eggs or larvae) following a hatchery-based breeding phase has also resulted in lower relatedness among offspring and in a higher number of reproducing adults in lake sturgeon (*Acipenser fulvescens*; Crossman et al., 2011). It was also found to transmit variation successfully from adults through the larvae and into the repatriated population of the razorback sucker (*Xyrauchen texanus*; Dowling et al., 2005). Such management strategies, however, should be applied very carefully, and only with suitable populations. In a first step, validation is needed to test whether the sampling of early life stages of natural offspring truly represents more genetic diversity than captive breeding does.

The effective long-term conservation management of nase and other rheophilic cyprinids should include demographic and genetic monitoring of populations. However, supportive breeding cannot replace in-situ measures to restore natural reproductive capacity (Manubens et al., 2020). It should only be applied, therefore, in addition to instream and catchment restoration measures (Geist & Hawkins, 2016; Knott et al., 2019), as even small-scale habitat restoration, such as cleaning spawning gravel, have proved their effectiveness to increase reproductive success in the wild quickly and effectively (Nagel et al., 2020). Careful captive breeding of nase populations may be highly beneficial in streams where populations have declined to a few individuals or have become extinct.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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