

Genetic diversity management of sculpin (*Cottus* spp.) and brown trout (*Salmo trutta*) in the Palatinate Forest-North Vosges Biosphere Reserve

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Abstract – Protected areas can make an important contribution to the conservation of genetic diversity in the current biodiversity crisis. We have examined two representative freshwater fish taxa, *Cottus* spp. and *Salmo trutta*, in 15 midmountain headwaters of the Franco-German Palatinate Forest-North Vosges Biosphere Reserve in Central Europe to facilitate freshwater genetic diversity protection. Population genetic analyses of microsatellites and mtDNA showed lower genetic diversity, but distinctly differentiated genetic structure in *Cottus* spp., and higher diversity, but less differentiated structure in *Salmo trutta*. Phylogenetic analyses of mtDNA designated most sculpin to *Cottus gobio*, but also identified the first known population of *Cottus rhenanus* in the region. In addition to species-specific recommendations, we derived stream-specific guidance in an attempt to make optimal use of the combined genetic information on both taxa for habitat-oriented management prioritization and improved conservation of freshwater genetic diversity.

Keywords: Biodiversity loss / salmonids / cottids / River Rhine / Alsace

1 Introduction

The present biodiversity crisis (Barnosky *et al.*, 2011) includes global loss of genetic variation in wild populations (Leigh *et al.*, 2019). Intraspecific genetic variation is a foundational component of biodiversity, promotes ecosystem structure and functioning, and supports nature's contributions to people (e.g., Des Roches *et al.*, 2021; Hoban *et al.*, 2022). Nevertheless, information on genetic variation is still not sufficiently considered in current conservation policy and decision making but improved standardization of collecting, harmonizing, and interpreting genetic data can help to better operationalize interspecific variation for conservation and management (Hoban *et al.*, 2022 and citations therein). Regional assessments can not only improve the useability of their findings for regional managers and authorities, but also contribute to enable large-scale analyses by using standardized fundamental metrics (Genetic Composition EBVs; Hoban *et al.*, 2022).

Freshwater habitats are biodiversity hotspots (Strayer and Dudgeon, 2010), but their current loss of species is disproportionately higher than in marine or terrestrial ecosystems (Tickner

et al., 2020) and many European freshwater fish species show negative population trends (e.g. Deinet *et al.*, 2020). Protection and restoration of critical freshwater habitats has been suggested as a priority action to “bend the curve of freshwater biodiversity loss” (Tickner *et al.*, 2020). Yet, international efforts to conserve and facilitate the sustainable use of biodiversity currently require more actions and goals specific to the conservation of freshwater ecosystems and their species, genetic and functional diversity (Darwall *et al.*, 2018).

Biosphere reserves and the UNESCO Man and Biodiversity (MAB) program aim to enable the protection and, at the same time, the sustainable use of biodiversity, and many reserves include valuable freshwater habitats. Conservation of biodiversity is one main function of biosphere reserves and this explicitly includes genetic variation particularly for their core zones. Activities in surrounding buffer zones should support scientific research and monitoring. To support the implementation of these goals, we have studied the genetic diversity and population structure of characteristic fish species in headwaters of a Central European biosphere reserve and propose a habitat-centered approach to aid in the development of habitat management measures to conserve genetic diversity.

The Palatinate Forest-North Vosges Biosphere Reserve at the French-German border extends broadly over a densely forested low mountain range. The landscape is characterized

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by numerous fine substrate dominated brooks and streams. The freshwater sculpin bullhead (*Cottus gobio*) and brown trout (*Salmo trutta*) are, as in many other western and central European low mountain areas, typical fish species of the region and both are listed as index species of the biosphere reserve (Simon, 2003).

Sculpin and brown trout often occur syntopically, but differ in many ecological traits relevant for appropriate conservation and management measures. For instance, brown trout have better swimming capacities than the benthic, swim bladder-less sculpin (e.g., Taugbøl *et al.*, 2019; Egger *et al.*, 2021). Thus, their dispersal abilities differ and gene flow between sculpin populations may be affected more strongly by small river barriers (e.g., culverts, ramps) than between brown trout populations (e.g. Junker *et al.*, 2012; cp. Hale *et al.*, 2016). Further, in contrast to sculpin, brown trout are highly appreciated for recreational fisheries and hence often intensively managed. Finally, in western and central Europe these species are often the only ray finned fish species in small headwaters. Therefore, the combined study of these two species in particular with high resolution microsatellite markers is a promising approach to help managers make decisions about conserving genetic diversity from a habitat-based perspective, rather than just from the perspective of a single species.

In Europe there are currently 15 species of freshwater sculpin in the genus *Cottus* that are recognized (Freyhof *et al.*, 2005). Three of these species occur in the broader study area in the Upper Rhine region, namely *C. gobio*, *C. perifretum* and *C. rhenanus* (Freyhof and Kottelat, 2008a, 2008b; Freyhof, 2011). *Cottus perifretum* and *C. rhenanus* were first described in the taxonomic revision of European *Cottus* species by Freyhof *et al.* (2005). To our knowledge, no detailed distribution mapping of the three species has taken place in the Upper Rhine tributaries since then. Thus, basic data for appropriate management, even at the species level, is lacking. Furthermore, morphological characters of *C. gobio* and *C. rhenanus* are highly variable (Freyhof *et al.*, 2005), so that species identification in the field may be very difficult or even impossible, especially for practitioners. This problem can further contribute to the lack of species distribution data. However, all three relevant species correspond well to genetic clades or haplogroups found in earlier studies (Freyhof *et al.*, 2005) by Englbrecht *et al.* (2000) and Volckaert *et al.* (2002). Thus, analyses of mitochondrial DNA (mtDNA) can enable species identification and help in overcoming knowledge gaps regarding the current distribution of sculpin species in the Palatinate Forest-North Vosges Biosphere Reserve.

To assist in improving the conservation and management of the genetic diversity in significant freshwater habitats in the Palatinate Forest-North Vosges Biosphere Reserve and to contribute to current global efforts to better integrate genetic diversity in conservation policies, we examined sculpin and brown trout by means of high-resolution microsatellite markers and mtDNA control region sequencing. For these purposes, we provide (1) a comprehensive population genetic characterization of these representative freshwater fish from 15 streams and (2) discuss possible implications of our findings for habitat-centered management and conservation in the reserve. Furthermore, mtDNA analyses revealed the presence of two different *Cottus* species in the reserve, which is

additional and valuable information for conservation and management.

2 Materials and methods

2.1 Study sites

The Franco-German transboundary “Palatinate Forest-North Vosges” UNESCO Biosphere Reserve is located between the departments Bas Rhin and Moselle in north-east France and the federal state Rhineland-Palatinate in south-west Germany. The reserve covers an area of 3018 km². The landscape is characterized by densely forested low mountain ranges of Triassic Buntsandstein formations and the numerous small headwaters and rivers are predominantly fine substrate-rich, siliceous, and oligotrophic. In the streams there are numerous barriers, especially small ones, such as culverts and steps for bed stabilization, but also impoundments and weirs. Typically, slopes are between one and eight percent. We investigated 15 stream sites in five sub-catchments east of the main watershed in the reserve between rivers Moselle and Rhine, i.e., direct tributaries of the Rhine. All sites were in or at the border of buffer areas of the biosphere reserve, except one site (*Que*) in a transition area and one site (*Bab*) just outside the reserve (Fig. 1, Tab. 1).

Sites were selected to minimize the potential influence of fisheries management, i.e., all surveyed streams were so small that they are unlikely to be used for fishing, and no evidence of recent stocking was found for any of the waters before sampling. However, the full stocking history of the region and the selected sites ultimately remains unclear because we could not a priori rule out or assume undocumented stocking in the study areas or immigration of stocked fish. The population *Bab* outside of the reserve was included because until several years ago it served as a donor population for brown trout rearing for stocking efforts in nearby regions (e.g. Black Forest).

2.2 Sampling

In June 2019, and March 2021 (sites *Sub*, *Deb*, *Que*) backpack electrofishing was performed with partners (see acknowledgements) to acquire tissue samples. Fishes were anaesthetized, their species, or genus, and total length were documented, and fin clips were taken. Tissue samples were preserved in 96% ethanol on site and stored at −20 °C in the lab.

2.3 Genetic analysis

Tissue was incubated with 5% Chelex 100 (Bio-Rad) and 0.06 mg Proteinase K (Qiagen) at 56 °C for 2 h and 100 °C for 30 min for DNA extraction. Nuclear DNA (*n* = 721) was then examined at ten microsatellite loci for sculpin (*n* = 255), and twelve loci for trout (*n* = 466) respectively, in six (multi load) multiplex-PCRs. Detailed locus and primer information (Tab. S1-1), PCR protocols (Tab. S1-2), and cycling parameters (Tab. S1-3) are provided in Supplemental Information S-1. At locus Cgo1016 we found one allele (“136”) in one sculpin population (*Bab*) that appeared to fall out of the regular repeat length and was highly influential in

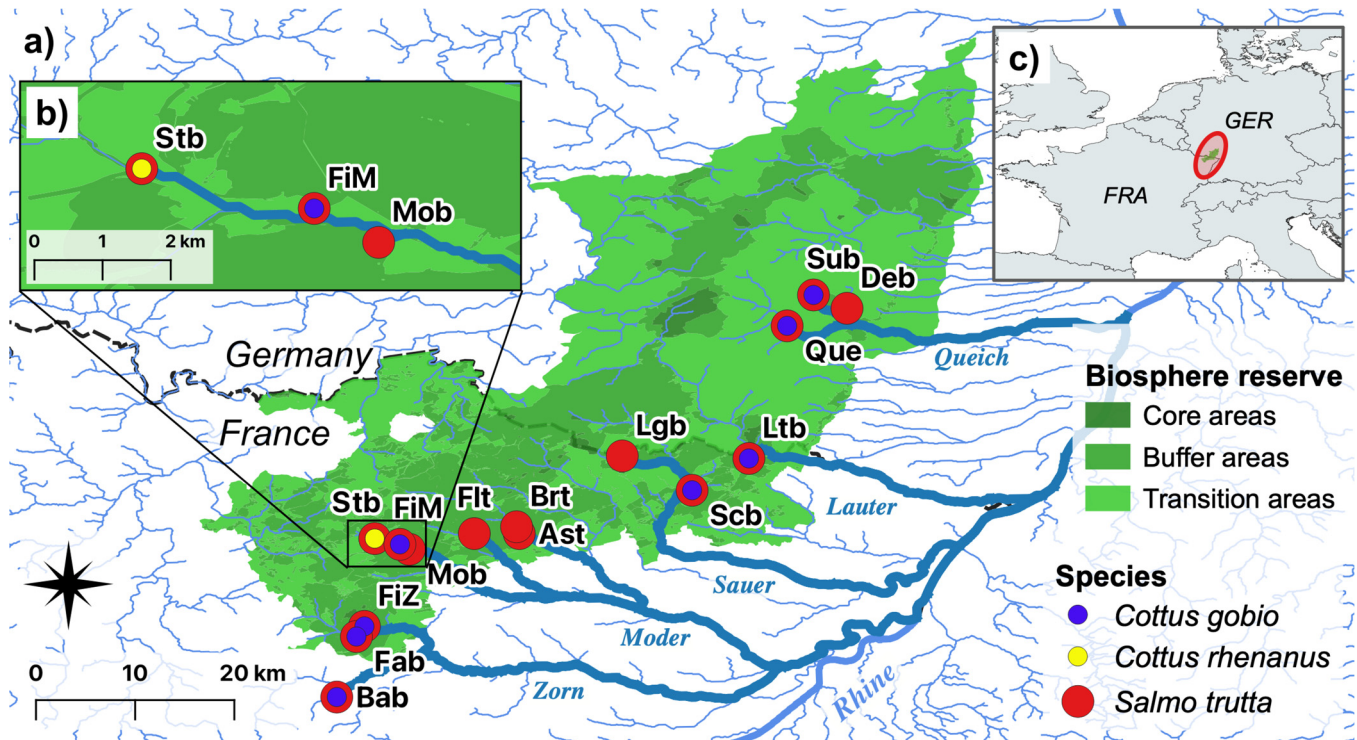


Fig. 1. Location of the study waters in the Palatinate Forest-North Vosges Biosphere Reserve at the French-German border (a). Green areas indicate the different zones of the reserve. Circles mark the studied streams and their colors show the taxa found. Population codes are given in Table 1. The shortest distances between all study sites are highlighted in the river network by thicker, dark blue lines. Catchments are given by the respective stream names (cp. Tab. 1). River Rhine is indicated by a thicker line. The dashed line marks the border between France and Germany. For better visibility and scale, the area with sites *Stb*, *FiM* and *Mob* in the Moder catchment is enlarged in b). The location of the biosphere reserve in the border area between north-west France and south-west Germany in central Europe is shown in c).

data analyses. Thus, we bidirectionally sequenced 3 homozygous samples with allele “136” and 4 homozygous samples with allele “137”, to rule out that this allele is an artefact (e.g., scoring error), as detailed in Tables S1-4 and S1-5.

The mitochondrial control region of 99 sculpin and 100 brown trout was amplified with primers PST (5'-CCCAAAGCTAAAATTCTAAAT-3') and FST (5'-GCTTTAGTTAAGCTACGC-3') (Cortey and Garcia-Marin, 2002) and bidirectionally sequenced (Tabs. S1-6 and S1-7). Sequencing was primarily done to identify sculpin species and we analyzed 10–12 specimens per site (except *Ltb*: 5). To allow further comparisons between sculpin and brown trout and complement microsatellite analyses, we additionally sequenced 10–12 brown trout from those 9 sites where both taxa were present (Tab. 1). Sequences (mtDNA and microsatellite Cgo1016) were edited and aligned using the software geneious prime (Biomatters Ltd.) and the Megablast implementation was used with mtDNA haplotypes to identify identical sequences deposited in GenBank and all haplotypes found in this study were submitted to GenBank (accession numbers OP186296–OP186308).

We determined the size range of all microsatellite alleles [base pairs (bp)] and the total number of alleles per locus and consequently rejected one locus (Cgo91) for sculpin and one locus (OMM1323) for brown trout because they were

monomorphic (Tab. S1-1). To inspect data quality, occurrence of null alleles was estimated after Brookfield (1996) with the null.all function of the PopGenReport package v. 3.0.4 (Adamack and Gruber, 2014) using first the whole dataset per species and then the single populations per species in R v. 4.1.2 (R Core Team, 2021). Deviations from Hardy-Weinberg equilibrium (HWE) were tested per locus and population using the exact test of the hw.test function of the pegas v. 1.1 R package (Paradis, 2010) with 10,000 Monte-Carlo iterations.

2.4 Genetic diversity and inbreeding

To characterize genetic diversity, we calculated the means over microsatellite loci of the number of alleles (N_A), the allelic richness (A_R) standardized for sample size, and the expected and observed heterozygosity (H_{exp} and H_{obs}) with standard errors of means (SEM) per population and per catchment for both taxa using the R packages adegenet v. 2.1.5 (Jombart, 2008; Jombart and Ahmed, 2011) and PopGenReport (function *alleleRich*). Furthermore, haplotype diversity h was calculated as $h = n/(n-1) (1 - \sum x_i^2)$, with sample size n and frequency of haplotype x_i (Nei and Tajima, 1981) per population to describe mitochondrial genetic diversity. Inbreeding was estimated by using the fixation index $F_{IS} = (H_{exp} - H_{obs})/H_{exp}$.

Table 1. Population codes, sampling locations, and number of analyzed specimens per marker type (N_{CR} : control region sequences, N_{MS} : microsatellites) and taxon. Catchments are ordered from south to north, which corresponds to the downstream direction of the receiving river Rhine. Populations within catchments are ordered in downstream direction, which also approximately reflects proximity.

Catchment Population	Body of water	Coordinates (UTM 32M)	N_{CR}		N_{MS}	
			<i>Cottus</i> spp.	<i>Salmo trutta</i>	<i>Cottus</i> spp.	<i>Salmo trutta</i>
<i>Zorn</i>			36	32	102	107
Bab	Baerenbach	375447.15680; 5396564.6897	12	10	37	33
Fab	Fallbaechel	377627.40796; 5405730.6837	12	11	27	39
FiZ	Fischbaechel (Zorn)	378462.24592; 5407265.3805	12	11	38	35
<i>Moder</i>			22	21	68	173
Stb	Schuesselthalbaechel	379786.63228; 5420614.7842	12	11	34	23
FiM	Fischbaechel (Moder)	382295.75465; 5419675.6300	10	10	34	35
Mob	Moosbaechel	383224.97281; 5418900.0363				33
Flt	Fliessthal	389786.09058; 5421159.3078				31
Brt	Bronnthal	394045.02555; 5422122.1583				15
Ast	Aspenthall	394295.21662; 5421075.5775				36
<i>Sauer</i>			12	11	34	70
Lgb	Langenbach	404821.47026; 5432709.9444				35
Scb	Schmelzbach	411725.57522; 5427366.2698	12	11	34	35
<i>Lauter</i>			5	12	12	33
Ltb	Lauterbaechel	417484.30630; 5432125.8136	5	12	12	33
<i>Queich</i>			24	24	39	83
Que	Queich	421594.89412; 5452213.1976	12	12	18	22
Sub	Sulzbach	424288.94587; 5456877.2369	12	12	21	35
Deb	Dernbach	427598.86447; 5454803.2605				26
		Σ	99	100	255	466

2.5 Genetic diversity standardization between taxa

To make the genetic diversity information from both species in a habitat-centered approach more accessible to regional managers who are rather inexperienced in using genetic data, a standardization was performed for the two diversity parameters H_{exp} and A_R . The basic ideas here were that (1) in those streams where the genetic diversity values for both species are highest, the management priority should be strongly oriented towards conserving the current status, while for those waters where diversity values for both species are lowest, the focus should be on improvement measures and that (2) the minima and maxima of observed values [$\pm$ standard error of means (SEM)] reflect the actual framework of action of regional managers. The standardization was thus calculated as “scaled expected heterozygosity” $_{sc}H_{exp} = ((H_{exp} - \min(H_{exp} - SEM)) / (\max(H_{exp} + SEM) - \min(H_{exp} - SEM))) * 10$ and likewise as “scaled allelic richness” $_{sc}A_R$, so that per species the highest unscaled value corresponds to 10 and the lowest unscaled value to 0. Bringing these values for both species on a uniform scale then allows a graphical representation that (1) focuses on the specific habitats, (2) presents both taxa equally weighted, and (3) equates the two reference frames for management. The essential purpose of the suggested standardization is to align the actual regional framework of management action between taxa. Additionally, correlations of the scaled values between the species were tested using Pearson’s product moment correlation coefficient (PPMCC; R function `cor.test`), including only sites without both taxa present, to elucidate whether the studied habitats as such have an influence on the genetic diversity.

2.6 Genetic differentiation and structure

Differentiation of populations was measured by the number and frequency of private microsatellite alleles and their occurrence at the levels of loci, individuals and realized alleles using the `private_alleles` function of the `poppr` v. 2.9.3 (Kamvar *et al.*, 2014, 2015) R package. Also, the number of private alleles was standardized for sampling size by using a resampling approach: we used the smallest sampling size (sculpin: 12, trout: 15) to draw random individuals from each population and then determined the number of private alleles per population 100,000 times. The means of these reiterations per population provide an estimate of the number of private alleles that would have been expected if only the smallest sample size had been used in all populations, and thus allow direct comparisons between populations (cp. Kalinowski, 2004). Furthermore, we calculated pairwise Jost’s D (Jost, 2008) between all populations with the `pairwise_D` function of the `mmod` v. 1.1.3 (Winter, 2012) R package from microsatellite data. An analysis of molecular variance (AMOVA) was performed with the `poppr.amova` function (`poppr` package) to estimate the contribution to the differentiation of the variance within individuals, between individuals within populations, between populations within catchments, and between catchments using microsatellite and mtDNA data. Sculpin population *Ltb* was excluded from the AMOVA after it was identified as a different species of *Cottus* than the other sculpin (see below). The `randtest.amova` function (`ade4` package v 1.7.18; Dray and Dufour, 2007) was used with 1,000 permutations to test statistical significance. Finally, we used the function `find.clusters` (`ade4` package) to determine

Table 2. Genetic diversity values of sculpin (*Cottus* spp.) and brown trout (*S. trutta*) from nine and eleven microsatellite loci respectively, per population and catchment (italic). Shown are mean number of alleles (N_A), mean allelic richness (A_R), mean observed (H_{obs}) and expected (H_{exp}) heterozygosity, and mean fixation index F_{IS} . Numbers in brackets are standard errors of means.

Catchment population	N_A		A_R		H_{exp}		H_{obs}		F_{IS}	
	C. spp.	<i>S. trutta</i>	C. spp.	<i>S. trutta</i>	C. spp.	<i>S. trutta</i>	C. spp.	<i>S. trutta</i>	C. spp.	<i>S. trutta</i>
<i>Zorn</i>	6.11 (1.09)	10.82 (2.42)	4.61 (0.70)	8.81 (1.89)	0.61 (0.09)	0.68 (0.06)	0.44 (0.08)	0.61 (0.06)	-0.02 (0.04)	0.10 (0.04)
Bab	3.67 (0.69)	7.55 (1.49)	3.04 (0.51)	5.86 (1.01)	0.46 (0.07)	0.65 (0.05)	0.41 (0.09)	0.64 (0.06)	0.11 (0.13)	0.03 (0.03)
Fab	2.89 (0.39)	8.00 (1.69)	2.67 (0.33)	6.29 (1.19)	0.44 (0.07)	0.65 (0.06)	0.51 (0.10)	0.60 (0.07)	-0.13 (0.09)	0.09 (0.04)
FiZ	3.56 (0.58)	7.73 (1.50)	3.05 (0.44)	6.16 (1.08)	0.43 (0.08)	0.66 (0.05)	0.44 (0.10)	0.60 (0.07)	0.03 (0.07)	0.08 (0.08)
<i>Moder</i>	3.33 (0.41)	12.09 (2.95)	2.86 (0.20)	9.32 (1.92)	0.54 (0.04)	0.74 (0.05)	0.17 (0.03)	0.66 (0.04)	0.03 (0.07)	0.11 (0.03)
Stb	2.56 (0.29)	7.27 (1.29)	2.09 (0.22)	6.12 (0.93)	0.23 (0.06)	0.70 (0.04)	0.18 (0.06)	0.65 (0.05)	0.23 (0.10)	0.08 (0.05)
FiM	2.00 (0.17)	8.91 (1.80)	1.60 (0.18)	7.21 (1.26)	0.15 (0.06)	0.72 (0.05)	0.16 (0.07)	0.73 (0.05)	-0.02 (0.04)	-0.01 (0.05)
Mob		8.18 (1.55)		6.43 (1.02)		0.71 (0.05)		0.72 (0.06)		-0.01 (0.07)
Flt		4.45 (0.67)		3.71 (0.53)		0.53 (0.07)		0.55 (0.07)		-0.07 (0.06)
Brt		3.64 (0.41)		3.55 (0.38)		0.57 (0.04)		0.67 (0.05)		-0.16 (0.06)
Ast		6.36 (1.17)		5.46 (0.87)		0.66 (0.05)		0.63 (0.06)		0.07 (0.06)
<i>Sauer</i>	2.44 (0.44)	7.73 (1.80)	2.28 (0.35)	6.81 (1.37)	0.39 (0.08)	0.70 (0.05)	0.34 (0.07)	0.63 (0.06)	0.23 (0.10)	0.10 (0.07)
Lgb		5.64 (0.94)		5.06 (0.75)		0.65 (0.06)		0.63 (0.06)		0.03 (0.06)
Scb	2.44 (0.44)	5.73 (1.13)	2.28 (0.35)	4.83 (0.79)	0.39 (0.08)	0.67 (0.04)	0.34 (0.07)	0.64 (0.06)	0.12 (0.06)	0.05 (0.07)
<i>Lauter</i>	2.22 (0.55)	8.55 (1.84)	2.05 (0.44)	7.97 (1.65)	0.29 (0.09)	0.74 (0.04)	0.29 (0.10)	0.75 (0.05)	0.11 (0.13)	-0.02 (0.05)
Ltb	2.22 (0.55)	8.55 (1.84)	2.05 (0.44)	6.73 (1.23)	0.29 (0.09)	0.74 (0.04)	0.29 (0.10)	0.75 (0.05)	0.01 (0.14)	-0.02 (0.05)
<i>Queich</i>	3.44 (0.47)	10.27 (2.36)	2.94 (0.33)	8.78 (1.86)	0.47 (0.07)	0.74 (0.04)	0.31 (0.06)	0.70 (0.04)	-0.13 (0.09)	0.04 (0.04)
Que	2.67 (0.33)	7.73 (1.50)	2.43 (0.26)	6.53 (1.11)	0.34 (0.06)	0.71 (0.04)	0.32 (0.07)	0.71 (0.05)	0.04 (0.10)	-0.01 (0.05)
Sub	2.67 (0.44)	7.00 (1.51)	2.40 (0.33)	5.86 (1.09)	0.36 (0.06)	0.69 (0.05)	0.30 (0.08)	0.71 (0.05)	0.13 (0.14)	-0.02 (0.02)
Deb		7.36 (1.18)		6.22 (0.88)		0.71 (0.04)		0.69 (0.05)		0.02 (0.07)

genetic clusters based on the Bayesian Information Criterion (BIC), which we then applied in a Discriminant Analyses of Principle Components (DAPC; Jombart, 2008; Jombart and Ahmed, 2011) to visualize population structure and differentiation. Finally, we constructed haplotype networks with the haploNet function (package pegas) to visualize mitochondrial population structure.

2.7 Genetic species identification

To allow species identification of sculpin based on mitochondrial haplotypes we further added the following reference sequences to the haplotype network:

- All 59 haplotypes from Volckaert *et al.* (2002), which provide the relevant haplogroups, later designated to species.
- Haplotype Cot65 from Šlechtová *et al.* (2004), as we retrieved sequences identical to their haplotype Cot66, which was found in the same location.
- Sequence AB188166 from Yokoyama and Goto (2005), found in *C. poecilopus*, as an outgroup.

3 Results

Sculpin were only present at nine out of 15 sites, while brown trout were found at all sites. Thus, the distribution of sculpin appeared fragmented in the region. Overall, the population genetic results also indicate a much stronger structuring of sculpin compared to brown trout. Genetic diversity in general was higher in brown trout than in sculpin.

The occurrence of null alleles was indicated for all nine microsatellite loci in sculpin, *i.e.*, zero was not included within the 95% confidence interval of the bootstrap estimate, but when estimated per population null alleles were indicated for only two loci in four, and in one out of nine populations. In brown trout null alleles were indicated at ten out of eleven loci and the frequency was generally lower than in sculpin. The estimates per population showed only a few indications for the presence of null alleles at three loci, which appeared to be randomly distributed across populations (Tab. S2-1). Deviations from HWE were found in sculpin populations at all loci except one. Significant deviations ($p < 0.05$) for six loci were found in only one population each. For the other two loci deviations were found in two or four populations. The highest number of loci with deviations from HWE per population was three (*Stb*). In brown trout two loci showed significant deviations from HWE in more than half of the populations (eleven and eight, respectively). The number of populations with deviations ranged between one and five for the other loci. No deviation from HWE was found in one population (*Flt*). In the other populations the number of loci with significant deviations ranged between one (*Brt*, *Sub*) and six (*Deb*) loci (Tab. S2-2). Overall, these two indicators of the microsatellite data quality appeared to be randomly distributed across loci and populations, and therefore we believe they did not substantially affect our results.

3.1 Genetic diversity

In general, genetic diversity of sculpin was lower, compared to brown trout. The lowest values of mean allelic richness, and observed and expected heterozygosity found in brown trout populations were higher than the highest respective values found in sculpin populations (Tab. 2). The A_R per population was lowest in *FiM* and highest in *Brt*, and lowest in Lauter and highest in the Zorn catchment in sculpin. In brown trout populations, the lowest A_R was in the *Brt* population and the Sauer catchment and highest in *FiM* population and Moder catchment. The lowest H_{exp} was in sculpin population *FiM* and brown trout population *Flt*, and the highest H_{exp} was 0.46 in sculpin population *Bab* and in brown trout population *Ltb*. Fixation index F_{IS} differed by > 0.1 from 0 in five of the nine sculpin populations, but only in one brown trout population, indicating possible inbreeding in several sculpin populations. Further details and additional genetic diversity values are given in Table 2.

Sequencing of the mitochondrial control region revealed seven haplotypes in sculpin and six haplotypes in brown trout. We found five previously undescribed haplotypes in sculpin and two in brown trout (Tab. S3-1). Haplotype diversity h was considerably higher in brown trout [0.00 (*Bab*) to 0.78 (*Stb*)] than in sculpin (Tab. S3-2). Only in two sculpin populations, *FiZ* and *Que*, was h larger than zero (0.41 and 0.30). The only brown trout population in which $h=0$ was *Bab*, and the only haplotype found here was by far the most frequent haplotype, HTSal01.

Scaled genetic diversity values $_{sc}A_R$ and $_{sc}H_{exp}$ ranged from 0.84 (*FiM*) to 7.66 (*FiZ*), and 1.45 (*FiM*) to 8.36 (*Bab*) respectively, in sculpin populations. In brown trout populations these ranges were from 0.72 (*Brt*) to 7.62 (*FiM*), and 2.24 (*Flt*) to 8.70 (*Ltb*) respectively (Fig. 2). We found only a very weak (PPMCC: -0.41), statistically non-significant ($p=0.280$) correlation for $_{sc}A_R$, but a closer (PPMCC: -0.83) and significant ($p=0.006$) correlation for $_{sc}H_{exp}$ between sculpin and brown trout populations.

3.2 Genetic differentiation and structure

Private alleles were found more frequently in sculpin than in brown trout (Tab. 3). In sculpin populations the range was between zero (*Fab*) and eleven (*Bab*). Standardized for sampling size, the corresponding estimated numbers ranged between 0.32 and 9.80. In four populations (*Stb*, *Sub*, *Scb*, *Bab*) all individuals carried at least one private allele. Except population *Fab* (no private alleles) the lowest number (4) of individuals with private alleles was in population *FiM*. In brown trout no private alleles were present in five (*Ast*, *Brt*, *Que*, *Scb*, *FiZ*) out of 15 populations and ranged between one and five in the other populations. Standardized private alleles ranged between 0.03 and 3.46. The number of individuals carrying private alleles in these populations was between one (*Lgb*) and ten (*Deb*, *Sub*).

The pairwise Jost's D distances between sculpin populations were generally larger than between brown trout populations with nuclear and mitochondrial markers (Fig. 3, Tab. S2-4). The largest distance based on microsatellite data between sculpin populations was 0.78 (*FiM-Stb*, *FiM-Ltb*).

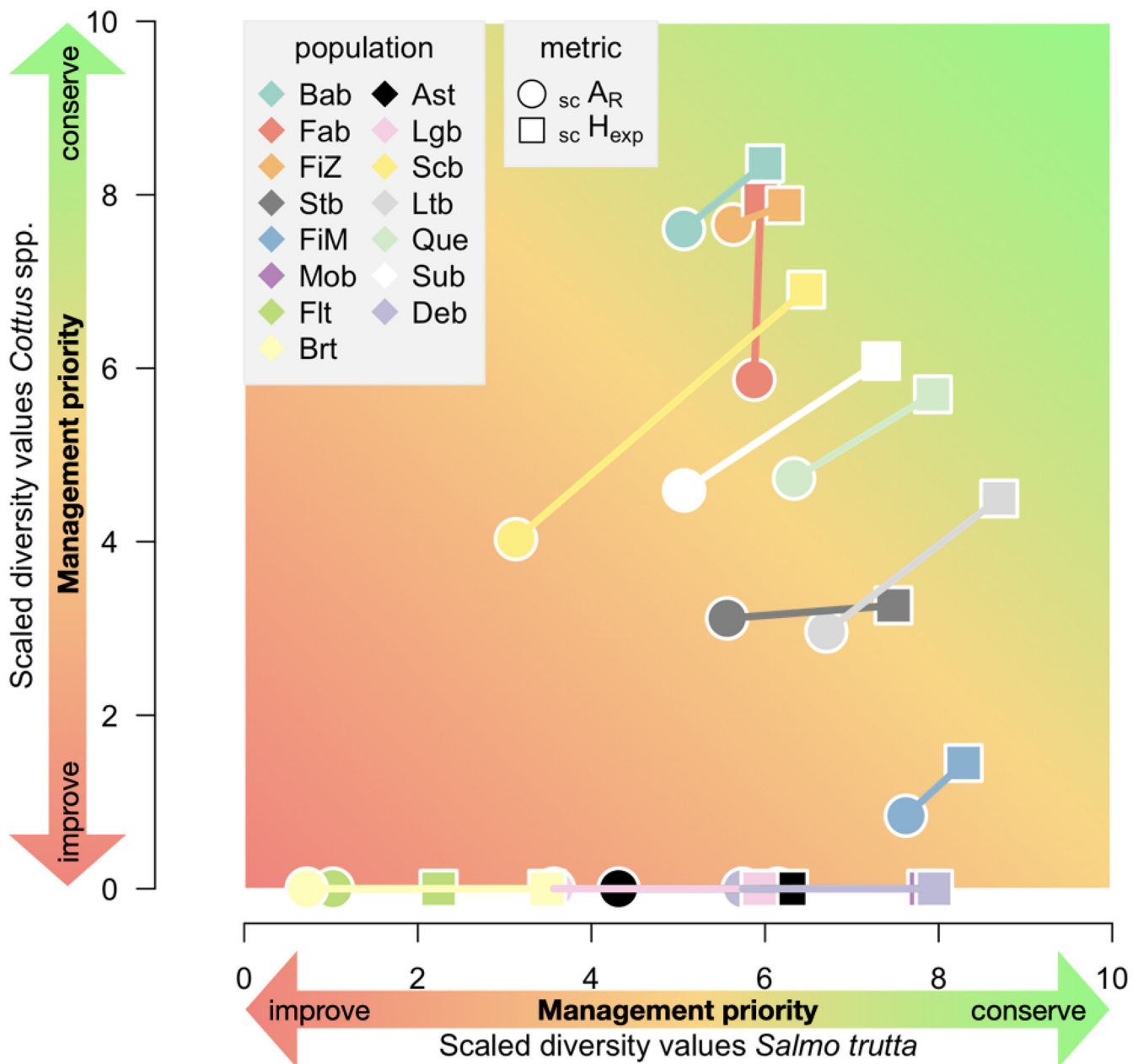


Fig. 2. Scaled allelic richness $_{scAR}$ and scaled expected heterozygosity $_{scH_{exp}}$ of sculpin (*Cottus* spp.; y-axis) and brown trout (*Salmo trutta*; x-axis) populations. The metric ($_{scAR}$ or $_{scH_{exp}}$) is indicated by symbols and colors show populations. The scaling is explained in the main text. The color gradient in the background of the graph and the arrows along the axes visualize possible management priorities from “improve” where genetic diversity is lowest, to “conserve”, where genetic diversity is highest.

The smallest distance of 0.26 was between *FiZ* and *Fab* in the Zorn catchment. Overall, sculpin populations *FiM* and *Stb* consistently had the largest genetic distances from other populations. In brown trout populations *Flt* and *Brt* appeared more genetically distant from the other populations. The highest distance was 0.27 (*Flt*-*Brt*). This was the only distance between brown trout populations, that was higher than the lowest distance between sculpin populations. All pairwise distances were highly significant ($p < 0.001$), except between brown trout populations *FiZ* and *Fab* in the Zorn catchment ($p = 0.015$), which was also the smallest distance between

brown trout populations. Based on mtDNA data the largest distances between populations were 0.74 in sculpin (*Scb*-*Stb*) and 0.12 in brown trout (*Scb*-*Sub*). Distances of 0.00 were found between two sculpin and between six brown trout populations. Distances between sculpin populations were mostly statistically significant ($p < 0.05$), but between brown trout populations less than half of the distances were significant (Tab. S2-4).

The analysis of molecular variance revealed that in bullhead (*C. gobio*) the differentiation within individuals contributed the most (47%) to the total variation followed by

Table 3. The number of different private alleles (N_{PA}), the number of different private alleles standardized for sampling size ($stdN_{PA}$), number of loci with private alleles (N_{PAloc}), number of individuals with private alleles (N_{PAind}), and number of realized private alleles ($N_{PAallel}$) per population of sculpin (*Cottus* spp.) and brown trout (*S. trutta*). Numbers in brackets are percentages of the respective units, and standard error of means for $stdN_{PA}$.

Catchment population	N_{PA}		$stdN_{PA}$		N_{PAloc}		N_{PAind}		$N_{PAallel}$	
	<i>C. spp.</i>	<i>S. trutta</i>	<i>C. spp.</i>	<i>S. trutta</i>	<i>C. spp.</i>	<i>S. trutta</i>	<i>C. spp.</i>	<i>S. trutta</i>	<i>C. spp.</i>	<i>S. trutta</i>
<i>Zorn</i>										
Bab	11 (33.33)	3 (3.61)	9.80 (0.003)	2.57 (0.004)	7 (77.78)	2 (18.18)	37 (100.00)	4 (12.12)	221 (33.18)	4 (0.56)
Fab	0	1 (1.14)	0.32 (0.002)	1.34 (0.002)	0	1 (9.09)	0	5 (12.82)	0	5 (0.59)
FiZ	7 (21.88)	0	7.85 (0.003)	1.36 (0.003)	5 (55.56)	0	31 (81.58)	0	62 (9.09)	0
<i>Moder</i>										
Stb	8 (34.78)	3 (3.75)	8.19 (0.003)	3.08 (0.003)	6 (66.67)	3 (27.27)	34 (100.00)	7 (30.43)	326 (54.15)	7 (1.39)
FiM	1 (5.56)	5 (5.10)	1.41 (0.003)	3.46 (0.004)	1 (11.11)	4 (36.36)	4 (11.76)	9 (25.71)	4 (0.65)	11 (1.44)
Mob		2 (2.22)		1.70 (0.003)		1 (9.09)		4 (12.12)		5 (0.69)
Flt		1 (2.04)		1.46 (0.002)		1 (9.09)		5 (16.13)		5 (0.74)
Brt		0		0.36 (0.002)		0		0		0
Ast		0		0.17 (0.001)		0		0		0
<i>Sauer</i>										
Lgb		1 (1.61)		0.45 (0.002)		1 (9.09)		1 (2.86)		1 (0.13)
Sch	8 (36.36)	0	7.20 (0.002)	0.03 (0.001)	5 (55.56)	0	34 (100.00)	0	198 (32.46)	0
<i>Lauter</i>										
Ltb	3 (15.00)	5 (5.32)	3.00 (0.000)	2.65 (0.003)	3 (33.33)	5 (45.45)	11 (91.67)	8 (24.24)	16 (7.48)	9 (1.24)
<i>Queich</i>										
Que	3 (12.50)	0	2.98 (0.004)	0.79 (0.003)	3 (33.33)	0	5 (27.78)	0	8 (2.52)	0
Sub	3 (12.50)	3 (3.90)	3.97 (0.003)	2.18 (0.002)	2 (22.22)	3 (27.27)	21 (100.00)	10 (28.57)	39 (10.32)	10 (1.30)
Deb		2 (2.47)		3.21 (0.003)		2 (18.18)		10 (38.46)		11 (1.92)

the between-populations-within-catchments variation (32%). Between-catchments variation also contributed notably (18%). The contribution of the within-individual variation was very dominant (88%) in brown trout, while the other hierarchical sources of variation played only a minor role (Tab. 4).

For bullhead the AMOVA of mtDNA data showed that differentiation between catchments contributed by far the most to the genetic variation (90%), while differentiation between populations within catchments and within populations is negligible (9% and 1%; Tab. 4). In contrast, differentiation between catchments is absent (<0%) in brown trout and within population differentiation accounts for most of the total variation (77%), but was not statistically significant ($p = 0.751$; Tab. 4).

Cluster analysis identified nine genetic clusters in sculpin and ten clusters in brown trout (Fig. 4). The DAPC showed considerable differentiation between genetic clusters in sculpin, but the clusters of brown trout formed a barely differentiated cloud. Only brown trout cluster 7 appeared somewhat separated. Cluster 7 consisted almost exclusively of trout from population *Flt*. With this exception, there were no clear relationships between brown trout genetic clusters and populations or catchments (Fig. 4, Fig. S4-1). In contrast, genetic clusters of sculpins corresponded very well with single populations and also catchments. Especially clusters 1 and 6, but also 2 and 9, appeared very distinct (Fig. 4, Fig. S4-2). A remarkable exception were clusters 1 and 7. These two clusters divided the population *Bab*, and cluster 1 was the most separated one. It was noticeable that all sculpin in cluster 1 were homozygous at locus *Cgo1016* with an irregular (*i.e.*, out

of the repeat motif) allele (“136”) and this allele caused the population to separate into two genetic clusters. Sequencing of the locus showed a deletion in a series of eleven (allele “137”) thymine nucleobases adjacent to the (GT)_n repeat motif (Fig. S4-3, base position 77), and the allele was thus handled as a normal allele.

The distribution of mtDNA haplotypes over populations appeared more differentiated in sculpin compared to trout. Three sculpin haplotypes (HTCot01, HTCot04, HTCot06) were found in more than one population, while the other four haplotypes were exclusive to a single population each (Fig. 5). Brown trout haplotypes appeared in at least two (HTSal05, HTSal06), and in up to eight (HTSal01) out of nine populations (Fig. S3-1).

3.3 Species identification and distribution

The comparison with the GenBank database and the haplotype network identified haplotype HTCot07 as *C. rhenanus* (Fig. 5). The 732 base pairs sequence was identical with haplotype HV18 from Volckaert *et al.* (2002) and with sequence MF326941 from Fast *et al.* (2017). The other six haplotypes were assigned to *C. gobio*. Haplotype HTCot07 was only found in population *Stb* in the Moder catchment and all twelve specimens examined in this population carried haplotype HTCot07 (cp. Fig. 4). Flow line distance between *Stb* and *FiM* is only approximately 3.1 km (Fig. 1b), and in population *FiM* we found the most common *C. gobio* haplotype HTCot06 exclusively.

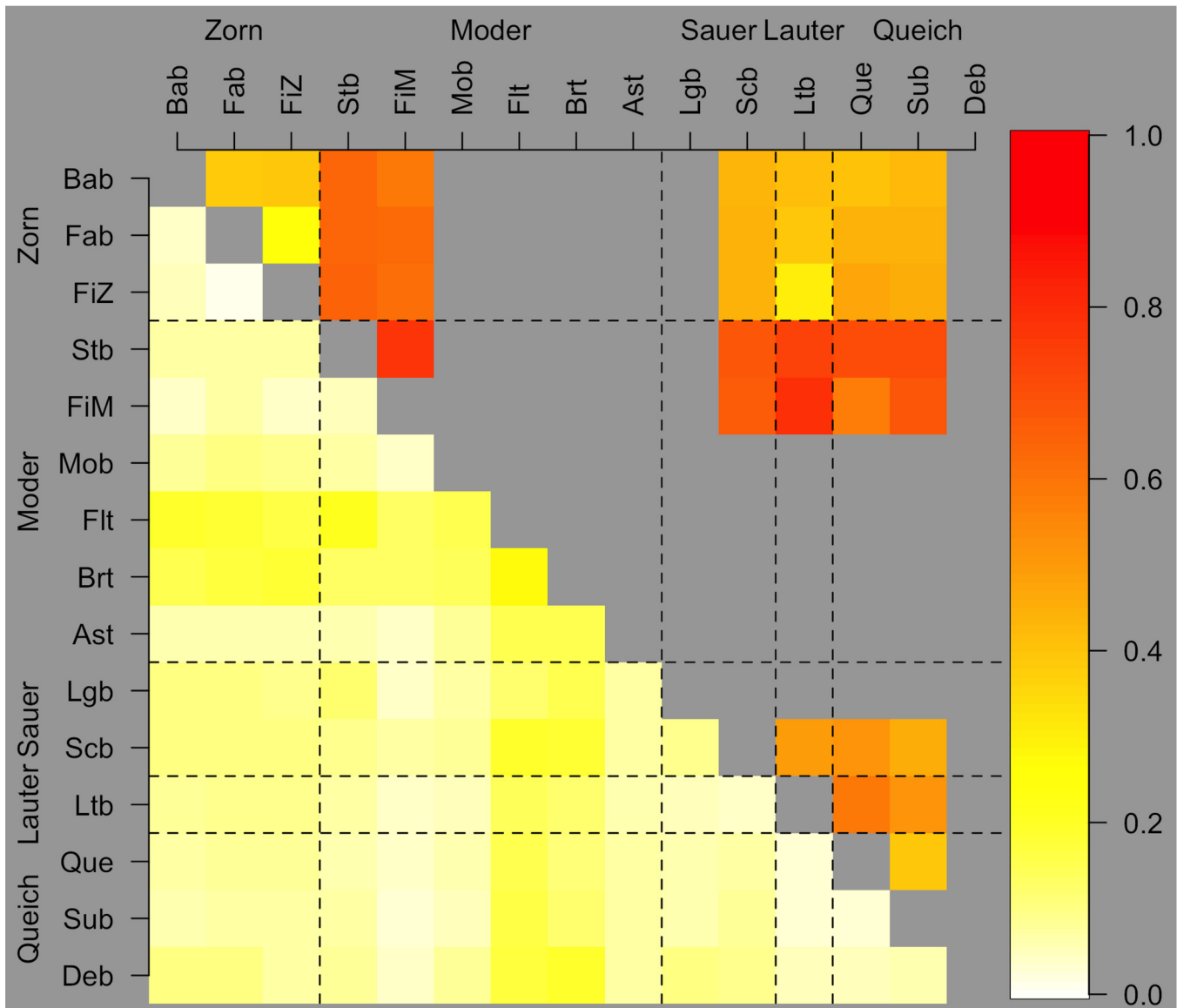


Fig. 3. Pairwise Jost's D genetic distances between brown trout (*Salmo trutta*) populations below the diagonal and sculpin (*Cottus* spp.) populations above the diagonal, based on eleven, and nine microsatellite loci respectively. The dashed lines indicate five catchments. All distances were statistically significant (cp. Tab. S2-3).

4 Discussion

Our study revealed significant differences for conservation and management between sculpin and brown trout. First, brown trout were found in all streams, but sculpin (*C. gobio* and *C. rhenanus*) in only nine out of 15 streams. Apparently, sculpin populations are fragmented, while brown trout populations could be more closely connected. Therefore, we assume that gene flow between sculpin populations is interrupted, while it might be maintained, even over larger distances, between brown trout populations. Furthermore, we assume that both taxa must have been present in all suitable streams during the original colonization of the area. The present fragmented distribution of the sculpin probably results from local extinction events due to environmental stochasticity or anthropogenic damage events. The presence of brown trout

in all streams may be explained by their better dispersal ability (including successful passage of minor barriers) and/or because they were restocked in affected areas (*cf.* below). Accordingly, special attention should be paid to the connectivity between sculpin populations in future stream management. However, it is essential to also consider the genetic integrity of the populations, or species.

4.1 Genetic diversity and inbreeding

Overall, microsatellite and mtDNA data showed that the genetic diversity of brown trout was higher compared to sculpin. Lowered genetic diversities may indicate small population sizes and/or inbreeding. Thus, the relatively low genetic diversity of sculpin populations was in accordance with the observed fragmented distribution (*cf.* Knaepkens *et al.*, 2004;

Table 4. Results of the analysis of molecular variance based on nine and eleven microsatellite loci for bullhead (*C. gobio*), and brown trout (*S. trutta*) respectively, and based on sequencing of the mitochondrial control region, with degrees of freedom (df), sum of squares (SS), expected mean squares (EMS), variance components (VC), percentage variation (%) and *p* values derived from a randomization test.

Source of variation	df		SS		EMS		VC		%		<i>p</i>	
	<i>C. gobio</i>	<i>S. trutta</i>	<i>C. gobio</i>	<i>S. trutta</i>	<i>C. gobio</i>	<i>S. trutta</i>	<i>C. gobio</i>	<i>S. trutta</i>	<i>C. gobio</i>	<i>S. trutta</i>	<i>C. gobio</i>	<i>S. trutta</i>
Microsatellites												
Between catchments	4	4	861.35	270.72	215.34	67.68	1.24	0.09	17.92	1.07	>0.001	0.088
Between populations within catchments	3	10	396.16	487.56	132.05	48.76	2.22	0.68	32.21	8.11	0.008	>0.001
Between individuals within populations	213	451	775.40	3540.32	3.64	7.85	0.20	0.28	2.87	3.39	>0.001	>0.001
Within individuals	221	466	719.05	3395.17	3.24	7.29	3.24	7.29	47.01	87.44	0.014	>0.001
MtDNA												
Between catchments	4	4	406.56	9.93	101.64	2.48	6.14	−0.07	89.99	−7.82	>0.001	>0.001
Between populations within catchments	3	4	21.33	14.96	7.11	3.74	0.58	0.28	8.57	31.18	>0.001	>0.001
Between individuals within populations	79	91	7.83	62.42	0.10	0.69	0.10	0.69	1.45	76.65	0.002	0.751

Junker *et al.*, 2012). It is likely that most of the sculpin populations are isolated and small. Stronger deviations of F_{IS} from zero may be interpreted as indicative for inbreeding ($F_{IS} > 0.1$) or outbreeding ($F_{IS} < -0.1$). Sculpin population *Sib*, the only *C. rhenanus* population (see below), had a low genetic diversity and F_{IS} was 0.23, so inbreeding could be relevant in this population. Hybridization with *C. perifretum* could possibly provide an alternative explanation, but the known hybrid lineage seems to be restricted to larger rivers, while small headwaters harbor pure populations of the parental species (Nolte *et al.*, 2005; Stemshorn *et al.*, 2011). Furthermore, we could not confirm the presence of *C. perifretum* in the region (cp. below). Other populations with $|F_{IS}| > 0.1$ include *Bab*, which had the highest genetic diversity amongst sculpin and *Fab*, where F_{IS} was negative (−0.13). Overall, we think inbreeding is currently not a major concern for sculpin populations in the biosphere reserve. However, increasing the presumably small population sizes may be helpful and an appropriate measure to prevent inbreeding in the long term. Depending on the specific situation, this could be achieved, for example, by limiting input of sandy sediments and facilitating in-stream erosion to prevent or remove clogging and thus provide more suitable nesting sites with unclogged stones and gravel. Another management option is the removal of barriers to adjacent suitable and unoccupied habitat (cf. Knaepkens *et al.*, 2004). Both measures potentially increase the effective population size and thus counteract possible inbreeding. In brown trout population *FIS* generally deviated lesser from zero, with the exception of *Brt* where F_{IS} was −0.16. We believe that in- or outbreeding is not currently of major concern for brown trout populations in the biosphere reserve either.

Our analyses of the mitochondrial control region discovered five previously unknown sculpin haplotypes and two new brown trout haplotypes. Since our study was the first study using this method in the region, this is not surprising, although a relatively large amount of comparable data is already available for both taxa in Europe [e.g., Volckaert *et al.*, 2002;

Šlechtová *et al.*, 2004; Stemshorn *et al.*, 2011 (sculpin), and Cortey *et al.*, 2009; Kohout *et al.*, 2012; Lerceteau-Kohler *et al.*, 2013 (brown trout)]. In any case, these results highlight the important role that biosphere reserves can play in protecting genetic diversity.

The only brown trout population with $h=0$ was the former donor population *Bab*. The only haplotype found here was the by far most frequent haplotype HTS101 (Tab. S3-2, Fig. S3-1). Ultimately, it remained unclear whether the use of population *Bab* as a donor for a brood stock contributed to the further spread of this haplotype in the region. Even though, in contrast, genetic diversity values of *Bab* based on microsatellites were not noticeably low, the brood stock itself should be evaluated if it is to continue to be used to produce fish for stocking.

4.2 Scaled genetic diversity

The genetic diversity of populations of different species, derived from different sets of microsatellite loci, cannot be compared directly. Even within a single species it can be problematic to compare absolute diversity values between regions or even studies (e.g. Schmidt *et al.*, 2017). Consequently, it is difficult to evaluate such values, as it is necessary for management decisions. However, to arrive at optimal management at the local/regional level, it seems appropriate and purposeful to use all available values from the region as an “absolute” evaluation standard. Thus, we attempted to overcome these difficulties by bringing diversity values for both species on a uniform scale. At the same time, this approach should make it easier to rank streams, with equal consideration given to the genetic situation of both taxa, along a continuous axis of management priorities from “improve” to “conserve.”

No stream harboring both taxa could be assigned unambiguously to the “improve” range or the “conserve” range of the management spectrum (Fig. 2). This was also

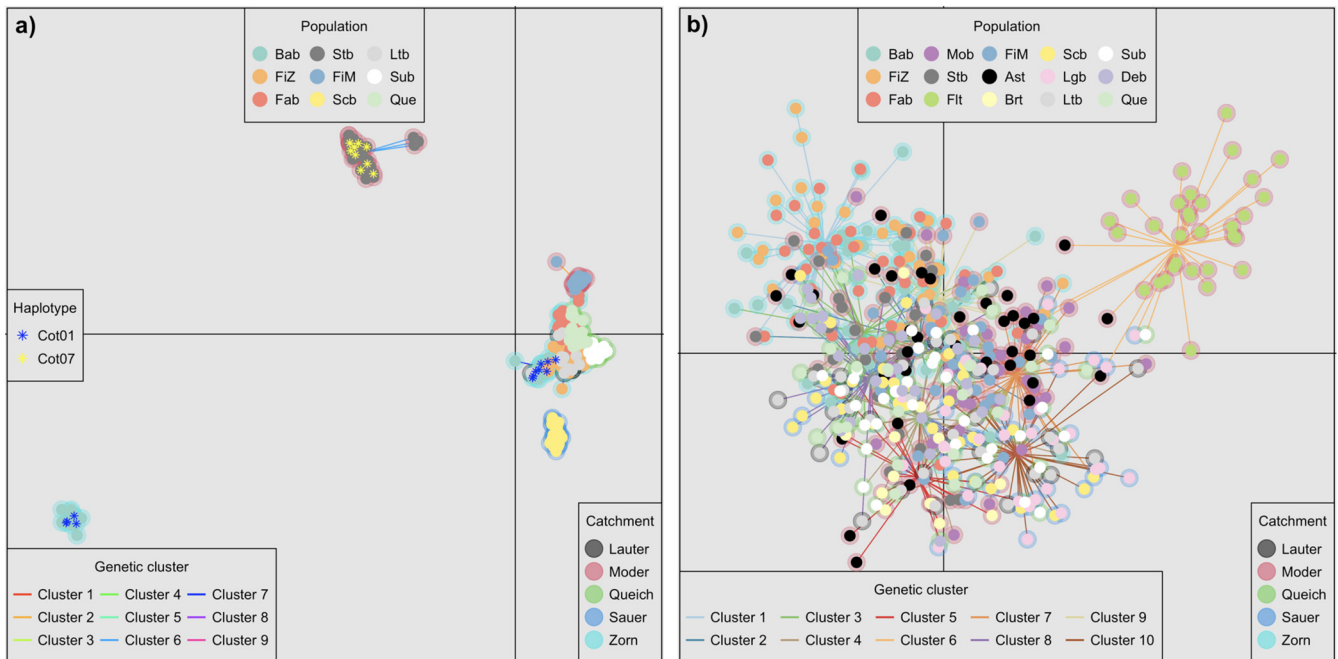


Fig. 4. Discriminant Analyses of Principal Components (x-axis: PC 1, y-axis PC 2) of sculpin (*Cottus* spp., a) and brown trout (*Salmo trutta*, b). Lines between individuals and “group centers” show the genetic clusters. Larger/outer circles indicate the catchment and smaller/inner dots the population where the individual fish were sampled. Individual membership probabilities per cluster are shown in Fig. S4-1 and S4-2. For sculpin populations *Bab* and *Stb* additional asterisks show the mtDNA control region haplotypes. Haplotype HTCot01 was designated to *C. gobio* and HTCot07 to *C. rhenanus* (cp. Tab. S3-1).

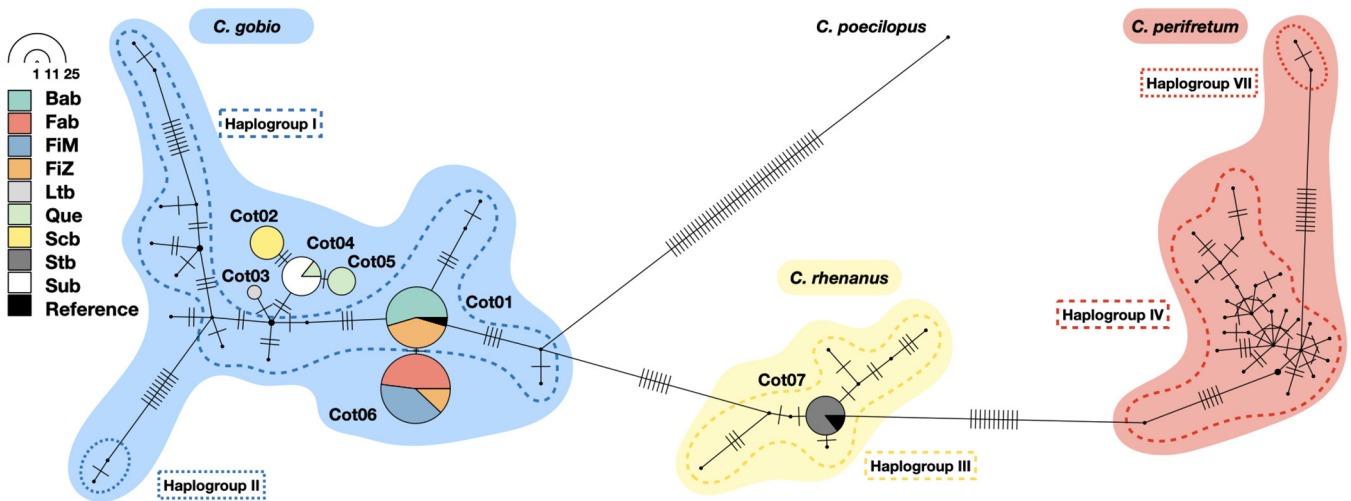


Fig. 5. Network of sculpin (*Cottus* spp.) haplotypes. Pie charts represent haplotypes and their size shows the haplotype frequency. The colors of chart slices indicate the populations (cp. Tab. 1). The number of mutational steps between haplotypes is shown by the number of tick marks on connecting branches. Colored dashed and dotted lines mark the haplogroups I, II, III, IV and VII after Volckaert *et al.* (2002). Haplotype HTCot01, or Cot66 (Šlechtová *et al.*, 2004), was not included in Volckaert *et al.* (2002), but was positioned in between group I haplotypes. Haplotype HTCot07 equals haplotype HV18 in haplogroup III. Colored background areas indicate species designations. Haplogroups I and II plus the haplotypes HTCot01 to HTCot06 from this study were considered as *C. gobio*. Haplogroup III, including haplotype HTCot07/HV18 was considered as *C. rhenanus*. Under *C. perifretum* we included haplogroups IV and VII.

reflected by the absent ($_{sc}A_R$) or weak ($_{sc}H_{exp}$) correlation between the scaled diversity values of the two taxa. Most closely, *Bab* and *FiZ* in the Zorn catchment could be assigned towards the “conserve” priority, albeit genetic diversities of the brown trout populations in these streams are more in the middle range of the spectrum of this species. The relation of the two scaled metrics to each other and within streams and between taxa is necessarily somewhat different, which resulted in further ambiguities. For example, $FiZ_{sc}A_R$ and $_{sc}H_{exp}$ differ only slightly, for *Fab* both values are nearly equal for brown trout, but differ for sculpin, and for *Scb* both values differ for both taxa (Fig. 2). However, these results showed that improvement is an adequate management option in all streams, as in all cases at least one taxon would benefit. But it also became apparent in which streams special care is needed to not worsen the situation of populations already in a genetically good condition, most obvious in population *FiM*. Interestingly, the scaled diversity values were lowest for brown trout populations *Flt* and *Brt*, and somewhat less pronounced in *Ast* and *Lgb*, where no sculpin were found. We suggest that (habitat) improvement should be the management priority for these streams.

Taken together, mitochondrial genetic diversity (h) of both taxa was low (zero) in *Bab* and high in *FiZ*. Analogous to the microsatellite evaluations, these two populations may be used to guide the path along an axis of management priorities from “improve” to “conserve”.

4.3 Genetic differentiation and structure

Altogether, genetic differentiation was more pronounced between sculpin populations and their genetic population structure followed, in contrast to brown trout, and to some extent the geographical stream structure. The number of private alleles, *i.e.*, alleles found exclusively in a single population, indicates differentiation between populations, but also points, in a broader sense, to the genetic uniqueness of populations. Sculpin populations *Bab*, *FiZ*, *Stb*, and *Scb* stand out by a particularly high number of private alleles (N_{PA} , $_{std}N_{PA}$, N_{PAloc}). In populations *Bab*, *Stb*, *Scb*, and *Sub* every sampled sculpin carried at least one private allele, and in population *Ltb* over 90% (N_{PAind} , Tab. 3). Therefore, at least for these populations, the value of genetically unique population characteristics should be carefully considered, *e.g.*, when planning measures to improve habitat connectivity. The generally lower number of private alleles in brown trout populations may be partially explained by the larger number of populations studied. Higher numbers of private alleles were found in populations *Stb*, *FiM*, *Ltb*, *Sub*, and *Deb*. However, the differences compared to the further populations were clearly less pronounced than with sculpin. Since we assume that the brown trout populations are much better connected (migratory behavior, less restricted by small in-stream-obstacles, less patchy distribution) than the sculpin populations, genetic uniqueness is rather of secondary importance here.

Pairwise Jost’s D genetic distances were also generally higher between sculpin populations than between brown trout populations. Especially sculpin populations *Stb* and *FiM* (both Moder catchment), but also *Ltb*, differed strongly from all

other populations. In brown trout populations *Flt* and *Brt* stood out a bit. Again, these results underscore that genetic uniqueness should be weighed when (re-) connecting sculpin populations. For instance, populations *Stb* and *FiM* should not be connected to increase their genetic diversity, although they are geographically close to each other if there is an impassable barrier between them. A better alternative would be to consider whether opportunities can be created for these populations to reach unoccupied habitats to increase effective population size. In the *Mob*, also nearby, no sculpin was found. In case there are other sculpin nearby, these should also be genetically investigated before measures are taken.

The AMOVA also showed differences between taxa relevant to management (Tab. 4). The within-individuals component (heterozygosity; microsatellites) alone accounted for nearly 90% of the genetic variance of brown trout. The contributions of the higher levels, *i.e.*, between individuals, between populations, and between catchments, were accordingly low. The AMOVA based on mtDNA also showed the lowest level, *i.e.*, the within populations component, contributed most (77%). To keep intraspecific genetic diversity high, one of the common recommendations is not to mix populations across catchment boundaries (*e.g.* Laikre, 1999; Baer *et al.*, 2007). We expect such (sub-) catchment-based management strategies to be less effective in the case of the biosphere reserves brown trout. Genetic diversity conservation should focus more on individual populations to maintain the high diversity within individuals. In bullhead mtDNA variation between catchments contributed by far the most (90%) to the total variation but based on microsatellites variation between populations (32%) contribute considerably more to the total variation than variation between catchments (18%). Therefore, a strictly catchment-based management is a requirement, but would likely be insufficient to conserve the total genetic variation and a population-based strategy seems more purposeful.

In brown trout the DAPC revealed a more pronounced differentiation of population *Flt* in the Moder catchment than shown by the previous analyses. All trout from this stream were members of the same genetic cluster (cluster 6) with a probability of 100%, and only very few trout from other streams were assigned to this cluster (Fig. S4-1). All other clusters were composed of fish from several, sometimes all (except *Flt*), streams. Thus, there was almost no apparent relation between genetic population structures and the stream system, and therefore catchment-based, geographic conservation units could hardly be established here, as it was possible for Mediterranean brown trout in major catchments in the Latium region in Central Italy (Rossi *et al.*, 2022), for example.

Stocking can cause genetic homogenization of populations (*e.g.* Klütsch *et al.*, 2019; Fitzpatrick *et al.*, 2020), and thus can explain the low differentiation between the brown trout populations. Our sampling sites were selected to minimize potential effects of stocking, but stocking, also in connected streams, cannot be completely ruled out, as demonstrated by an unexpected observation of a single “tiger trout” (*Salmo trutta x Salvelinus fontinalis*; total length 132 mm) during our sampling in *FiZ*. On the other hand, stocking can also be without genetic consequences in a given stream, if stocked fish migrate out, are caught, or die before being reproductive (*e.g.* Ferguson, 2006).

In sculpin, the DAPC further highlighted the genetic population structures shown by the previous analyses. Populations *Stb* and *Sch* formed exclusive clusters and population *FiM* an almost exclusive cluster (Fig. S4-2). Interestingly, the two most widely separated clusters (clusters 1 and 7) could be attributed to different causes, which imply different consequences for the management. Cluster 1 was formed exclusively from sculpin with an irregular allele at one locus (all specimen homozygous). This allele could be related to a single deletion in the flanking region of the microsatellites' repeat motive. Therefore, we believe that it shows a rather recent, unique mutation event and was inherited only partially within population *Bab* since then. Thus, the cluster probably indicates a family structure. Accordingly, the implications for management are not as serious as they might initially appear based on the very strong separation of cluster 1 alone. We think it is both necessary and sufficient to ensure the conservation status of the *Bab* population in the long term, in order to enable its possible independent evolutionary development. The cluster 7 was exclusive to population *Stb*. Analyses of mtDNA concluded that this population is a *C. rhenanus* population, whereas all other sculpin populations were bullhead, *i.e.*, *C. gobio*. Accordingly, the population *Stb* is the only known occurrence of the species *C. rhenanus* in the study area to date (see below), and it is imperative that this species status be adequately addressed in future management of the biosphere reserve. It is remarkable that a single point mutation on a single microsatellite locus led to the formation of a cluster (cluster 1, *C. gobio*) that appeared more differentiated from conspecific clusters than a cluster of a second species (cluster 7, *C. rhenanus*). This demonstrates the sensitivity of the cluster analyses, but urges caution in deriving management implications, which differed for the two clusters in our case.

4.4 Sculpin taxonomy and distribution

The discovery of *C. rhenanus* in the headwater *Stb* in the Moder catchment was rather unexpected, because to the best of our knowledge this species was unknown in the Palatinate Forest-North Vosges Biosphere Reserve before and does not occur in any of the five studied catchments according to IUCN distribution data (Freyhof and Kottelat, 2008a). The closest catchments with occurrences are the Speyerbach catchment, which directly borders the Queich catchment to the north, and the western drainages of the Northern Vosges and Palatinate Forest (Freyhof and Kottelat, 2008a). In the study area, on the other hand, occurrences of *C. perifretum* could have been expected in addition to *C. gobio* based on IUCN distribution data (Freyhof and Kottelat, 2008b; Freyhof, 2011). Compared to the large number of small streams in the study area, our sample size of 15 stream sections was relatively small. Considering that the shortest distance between *C. rhenanus* and *C. gobio* populations was only about 3 km, it seems all the more likely that extended fine scale surveys, including the western drainages, would find further occurrences of *C. rhenanus*. Occurrences of *C. perifretum* in the region can furthermore not be ruled out. Our study focused on headwaters and this species may be found further downstream (*cf.* Nolte *et al.*, 2005; Stemshorn *et al.*, 2011). *Cottus rhenanus* has only

been given species rank after the last amendment of EU Habitats Directive (Council Directive 92/43/EEC) Annex II. It was split from *C. gobio*, which is listed under the Annex II, and thus should inherit the same protective status.

Interestingly, our newly found *C. gobio*-haplotypes HTCot02, HTCot03, HTCot04, and HTCot05 grouped somewhat together, and apart from the two most common haplotype HTCot1 and HTCot06 in the network, and these haplotypes occurred exclusively in single streams or catchments (Fig. 5, Tab. S3-2). It is possible that these haplotypes are exclusive to the Palatinate Forest-North Vosges region and are therefore advised to be specially protected. Probably, the taxonomy of European freshwater sculpin, especially the “*C. gobio*-complex” (or haplogroups I/II), is not conclusively resolved (Freyhof *et al.*, 2005, *cf.* Stemshorn *et al.*, 2011). Our findings suggest that further investigating the sculpin in the wider Upper Rhine region may be an important contribution to overcome taxonomic uncertainties and thereby may help to further improve the freshwater biodiversity management with regard to this fish genus.

4.5 Perspective

Our study provides fundamental information on intraspecific genetic variation of two characteristic freshwater fish in headwaters in a protected area. We found pronounced differences between the taxa relevant for management and conservation, and suggested exemplified approaches to adequately consider genetic diversity and structure in the conservation management of freshwater habitats at a regional scale. Our attempt to combine genetic information of taxa indicated possible ways to ensure and improve the protection of genetic diversity in a protected area in a habitat-oriented manner in the future. We derived stream-specific and species-specific recommendations for the Palatinate Forest-North Vosges Biosphere Reserve, but the examples and approaches could be useful and further developed in other areas and/or with other taxa to facilitate management decisions.

We used standard metrics to describe the genetic diversity (richness and evenness), genetic differentiation (number of units and differentiation between units), and inbreeding (*cp.* Hoban *et al.*, 2022). Therefore, our genetic composition data may not only be of use regionally, but may also be considered in larger contexts, as needed to improve biodiversity conservation policies. Furthermore, standardized monitoring of genetic diversity, in contrast to other biodiversity components, is currently carried out by very few countries, although such monitoring could contribute significantly to global biodiversity conservation (Hoban *et al.*, 2022). This study provides a possible contribution to monitor genetic diversity in the biosphere reserve or in large-scale analyses.

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Supplementary Material

Supplemental Information S1. Locus and primer information (Tab. S1-1), PCR protocols (Tab. S1-2) and cycling parameters (Tab. S1-3) of six multiplex PCRs for microsatellite analyses. PCR protocols (Tab. S1-4) and cycling parameters (Tab. S1-5) for the sequencing of microsatellite locus Cgo1016. PCR protocols (Tab. S1-6) and cycling parameters (Tab. S1-7) for mtDNA control region amplification.

Supplemental Information S2. Null allele estimates (Tab. S2-1), Hardy-Weinberg equilibrium tests (Tab S2-2) and pairwise Jost's D distances based on microsatellite (Tab. S2-3) and mtDNA sequencing (Tab S2-4) data.

Supplemental Information S3. *Cottus* spp. and *Salmo trutta* haplotypes (Tab. S3-1), haplotype diversity *h* (Tab. S3-2), and network of *Salmo trutta* haplotypes (Fig. S3-1).

Supplemental Information S4. Discriminant Analysis of Principal Components membership probabilities to genetic clusters (Fig. S4-1, Fig. S4-2) and alignment of sequenced microsatellite locus Cgo1016 (Fig. S4-3).

The Supplementary Material is available at <https://www.kmae-journal.org/10.1051/kmae/2023005/olm>.

References

- Adamack AT, Gruber B. 2014. PopGenReport: simplifying basic population genetic analyses in R. *Methods Ecol Evol* 5: 384–387.
- Baer J, George V, Hanfland S, Lemcke R, Meyer L, Zahn S. 2007. Gute fachliche Praxis fischereilicher Besitzmaßnahmen. Schriftenreihe des Verbandes Deutscher Fischereiverwaltungsbeamter und Fischereiwissenschaftler e.V.: 14.
- Barnosky AD, Matzke N, Tomiya S, Wogan GOU, Swartz B, Quental TB, Marshall C, McGuire JL, Lindsey EL, Maguire KC, Mersey B, Ferrer EA. 2011. Has the Earth's sixth mass extinction already arrived? *Nature* 471: 51–57.
- Brookfield JFY. 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. *Mol Ecol* 5: 453–455.
- Cortey M, Garcia-Marin J-L. 2002. Evidence for phylogeographically informative sequence variation in the mitochondrial control region of Atlantic brown trout. *J Fish Biol* 60: 1058–1063.
- Cortey M, Vera M, Pla C, Garcia-Marin J-L. 2009. Northern and Southern expansions of Atlantic brown trout (*Salmo trutta*) populations during the Pleistocene. *Biol J Linn Soc* 97: 904–917.
- Darwall W, Bremerich V, De Wever A, Dell AI, Freyhof J, Gessner MO, Grossart H-P, Harrison I, Irvine K, Jähnig SC, Jeschke JM, Lee JJ, Lu C, Lewandowska AM, Monaghan MT, Nejstgaard JC, Patricio H, Schmidt-Kloiber A, Stuart SN, Thieme M, Tockner K, Turak E, Weyl O. 2018. The Alliance for Freshwater Life: A global call to unite efforts for freshwater biodiversity science and conservation. *Aquat Conserv Mar Freshw Ecosyst* 28: 1015–1022.
- Deinet AS, Scott-Gatty K, Rotton H, Twardek WM, Marconi V, McRae L, Baumgartner LJ, Brink K, Claussen JE, Cooke SJ, Darwall W, Eriksson BK, Garcia de Leaniz C, Hogan Z, Royte J, Silva LGM, Thieme ML, Tickner D, Waldman J, Wanningen H, Weyl OLF, Berkhuysen. 2020. Living Planet Index (LPI) for migratory freshwater fish. World Fish Migration Foundation, The Netherlands.
- Des Roches S, Pendleton LH, Shapiro B, Palkovacs EP. 2021. Conserving intraspecific variation for nature's contributions to people. *Nat Ecol Evol* 5: 574–582.
- Dray S, Dufour A-B. 2007. The ade4 package: Implementing the duality diagram for ecologists. *J Stat Softw* 22: 1–20.
- Egger B, Wiegler J, Seidel F, Burkhardt-Holm P, Emanuel Hirsch P. 2021. Comparative swimming performance and behaviour of three benthic fish species: The invasive round goby (*Neogobius melanostomus*), the native bullhead (*Cottus gobio*), and the native gudgeon (*Gobio gobio*). *Ecol Freshw Fish* 30: 391–405.
- Englbrecht CC, Freyhof J, Nolte A, Rassmann K, Schliewen U, Tautz D. 2000. Phylogeography of the bullhead *Cottus gobio* (Pisces: Teleostei: Cottidae) suggests a pre-Pleistocene origin of the major central European populations. *Mol Ecol* 9: 709–722.
- Fast K, Aguilar A, Nolte AW, Sandel MW. 2017. Complete mitochondrial genomes for *Cottus asper*, *Cottus perifretum*, and *Cottus rhenanus* (Perciformes, Cottidae). *Mitochondrial DNA Part B* 2: 666–668.
- Ferguson A. 2006. Genetic impacts of stocking on indigenous brown trout populations. Science Report: SC040071/SR. Environment Agency, Rio House, Waterside Drive, Aztec West, Almondsbury, Bristol, BS32 4UD.
- Fitzpatrick SW, Bradburd GS, Kremer CT, Salerno PE, Angeloni LM, Funk WC. 2020. Genomic and Fitness Consequences of Genetic Rescue in Wild Populations. *Curr Biol* 30: 517–522.e5.
- Freyhof J. 2011. *Cottus gobio*. The IUCN Red List of Threatened Species 2011: e.T5445 A97802083. <http://dx.doi.org/10.2305/IUCN.UK2008.RLTS.T5445A11147263.en>.
- Freyhof J, Kottelat M. 2008a. *Cottus rhenanus*. The IUCN Red List of Threatend Species 2008: e.T135710 A4188537. <http://dx.doi.org/10.2305/IUCN.UK2008.RLTS.T135710A4188537.en>.
- Freyhof J, Kottelat M. 2008b. *Cottus perifretum*. The IUCN Red List of Threatend Species 2008: e.T135511 A4135554. <http://dx.doi.org/10.2305/IUCN.UK2008.RLTS.T135511A4135554.en>.
- Freyhof J, Kottelat M, Nolte AW. 2005. Taxonomic diversity of European *Cottus* with description of eight new species (Teleostei: Cottidae). *Ichthyol Explor Freshw* 16: 107–172.
- Hale R, Morrongiello JR, Swearer SE. 2016. Evolutionary traps and range shifts in a rapidly changing world. *Biol Lett* 12: 20160003.
- Hoban S, Archer FI, Bertola LD, Bragg JG, Breed MF, Bruford MW, Coleman MA, Ekblom R, Funk WC, Grueber CE, Hand BK, Jaffé R, Jensen E, Johnson JS, Kershaw F, Liggins L, MacDonald AJ, Mergeay J, Miller JM, Muller-Karger F, O'Brien D, Paz-Vinas I, Potter KM, Razgour O, Vernesi C, Hunter ME. 2022. Global genetic diversity status and trends: towards a suite of Essential Biodiversity Variables (EBVs) for genetic composition. *Biol Rev* 97: 1511–1538.
- Jombart T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24: 1403–1405.
- Jombart T, Ahmed I. 2011. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* 27: 3070–3071.
- Jost L. 2008. GST and its relatives do not measure differentiation. *Mol Ecol* 17: 4015–4026.

- Junker J, Peter A, Wagner CE, Mwaiko S, Germann B, Seehausen O, Keller I. 2012. River fragmentation increases localized population genetic structure and enhances asymmetry of dispersal in bullhead (*Cottus gobio*). *Conserv Genet* 13: 545–556.
- Kalinowski ST. 2004. Counting Alleles with Rarefaction: Private Alleles and Hierarchical Sampling Designs. *Conserv Genet* 5: 539–543.
- Kamvar ZN, Tabima JF, Grünwald NJ, Souza V. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2: e 281.
- Kamvar ZN, Brooks JC, Grünwald NJ. 2015. Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. *Front Genet* 6: 208.
- Klütsch CFC, Maduna SN, Polikarpova N, Forfang K, Aspholm PE, Nyman T, Eiken HG, Amundsen P-A., Hagen SB. 2019. Genetic changes caused by restocking and hydroelectric dams in demographically bottlenecked brown trout in a transnational subarctic riverine system. *Ecol Evol* 9: 6068–6081.
- Knaepkens G, Bervoets L, Verheyen E, Eens M. 2004. Relationship between population size and genetic diversity in endangered populations of the European bullhead (*Cottus gobio*): implications for conservation. *Biol Conserv* 115: 403–410.
- Kohout J, Jaskova I, Papousek I, Sediva A, Slechta V. 2012. Effects of stocking on the genetic structure of brown trout, *Salmo trutta*, in Central Europe inferred from mitochondrial and nuclear DNA markers. *Fish Manag Ecol* 19: 252–263.
- Laikre L. 1999. Conservation Genetic Management of Brown Trout (*Salmo trutta*) in Europe. Concreted action on identification, management and exploitation of genetic resources in the brown trout (*Salmo trutta*) (TROUTCONCERT; EU FAIR CT97-3882).
- Leigh DM, Hendry AP, Vázquez-Domínguez E, Friesen VL. 2019. Estimated six per cent loss of genetic variation in wild populations since the industrial revolution. *Evol Appl* 12: 1505–1512.
- Lerceteau-Kohler E, Schliewen U, Kopun T, Weiss S. 2013. Genetic variation in brown trout *Salmo trutta* across the Danube, Rhine, and Elbe headwaters: a failure of the phylogeographic paradigm? *BMC Evol Biol* 13: 176.
- Nei M, Tajima F. 1981. DNA polymorphism detectable by restriction endonucleases. *Genetics* 97: 145–163.
- Nolte A, Freyhof J, Stemshorn K, Tautz D. 2005. An invasive lineage of sculpins, *Cottus* sp. (Pisces, Teleostei) in the Rhine with new habitat adaptations has originated from hybridization between old phylogeographic groups. *Proc R Soc B-Biol Sci* 272: 2379–2387.
- Paradis E. 2010. pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics* 26: 419–420.
- R Core Team. 2021. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.
- Rossi AR, Talarico L, Petrosino G, Crescenzo S, Tancioni L. 2022. Conservation genetics of mediterranean brown trout in central Italy (Latium): A multi-marker approach. *Water* 14: 937.
- Schmidt T, Zagars M, Roze A, Schulz R. 2017. Genetic diversity of a Daugava basin brown trout (*Salmo trutta*) brood stock. *Knowl Manag Aquat Ecosyst* 418: 55.
- Simon L. 2003. Leitarten im Biosphärenreservat Pfälzerwald-Nordvogesen.
- Šlechtová V, Bohlen J, Freyhof J, Persat H, Delmastro GB. 2004. The Alps as barrier to dispersal in cold-adapted freshwater fishes? Phylogeographic history and taxonomic status of the bullhead in the Adriatic freshwater drainage. *Mol Phylogenet Evol* 33: 225–239.
- Stemshorn KC, Reed FA, Nolte AW, Tautz D. 2011. Rapid formation of distinct hybrid lineages after secondary contact of two fish species (*Cottus* sp.). *Mol Ecol* 20: 1475–1491.
- Strayer DL, Dudgeon D. 2010. Freshwater biodiversity conservation: recent progress and future challenges. *J North Am Benthol Soc* 29: 344–358.
- Taugbøl A, Olstad K, Bærum KM, Museth J. 2019. Swimming performance of brown trout and grayling show species-specific responses to changes in temperature. *Ecol Freshw Fish* 28: 241–246.
- Tickner D, Opperman JJ, Abell R, Acreman M, Arthington AH, Bunn SE, Cooke SJ, Dalton J, Darwall W, Edwards G, Harrison I, Hughes K, Jones T, Leclère D, Lynch AJ, Leonard P, McClain ME, Murruven D, Olden JD, Ormerod SJ, Robinson J, Tharme RE, Thieme M, Tockner K, Wright M, Young L. 2020. Bending the Curve of Global Freshwater Biodiversity Loss: An Emergency Recovery Plan. *BioScience* 70: 330–342.
- Volckaert FAM, Hänfling B, Hellemans B, Carvalho GR. 2002. Timing of the population dynamics of bullhead *Cottus gobio* (Teleostei: Cottidae) during the Pleistocene. *J Evol Biol* 15: 930–944.
- Winter DJ. 2012. mmod: an R library for the calculation of population differentiation statistics. *Mol Ecol Resour* 12: 1158–1160.
- Yokoyama R, Goto A. 2005. Evolutionary history of freshwater sculpins, genus *Cottus* (Teleostei; Cottidae) and related taxa, as inferred from mitochondrial DNA phylogeny. *Mol Phylogenet Evol* 36: 654–668.

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