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River damming drives population fragmentation and habitat loss of the threatened Danube streber (*Zingel streber*): Implications for conservation

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Abstract

1. The Danube streber, *Zingel streber*, is a threatened and data-deficient percid fish endemic to the Danube catchment. The study provides the first data on distribution, life history, and genetic structure of the species at the upstream limit of its historic distribution (south-western Germany).
2. A 3-year survey effort with 143 fishing events identified several small, fragmentary populations covering only 7% of the historical range of the species. Census population sizes (N_c) of these subpopulations were estimated from mark–recapture data at <200 individuals. Effective population sizes (N_e), calculated from genetic data (microsatellite genotyping), were much smaller still, at <15 individuals, resulting in an N_c/N_e ratio of <0.25, strongly indicating that populations are seriously affected by genetic drift and inbreeding, and are thus facing a severe extinction risk.
3. Life-history parameters recorded during the study indicate a rapid life cycle, with both sexes probably attaining sexual maturity at the age of 1 year or older. Spawning commenced at the beginning of April and fecundity was low (~300–400 eggs per female).
4. Genetic analysis and mark–recapture data indicate that subpopulations of the streber live in effective isolation, separated by impassable weirs that significantly reduce genetic connectivity between subpopulations.
5. The species is rheophilic, and limited to sites with flow velocities of ~0.7 m s^{-1}. Hydropower infrastructure may thus also have diminished the availability of suitable habitat by reducing flow rates.
6. Only 32% of the historical range of the Danube streber is now estimated to be morphologically suitable for the species. Furthermore, relevant parts of this range are located upstream of dams and are therefore not accessible for natural recolonization.
7. The availability and accessibility of suitable habitats seem to be factors limiting the size of the remaining subpopulations.
8. Conservation actions should address the restoration of degraded river habitats and increase the connectivity between isolated subpopulations of the Danube streber.

KEYWORDS

endangered species, fish, hydromorphology, hydropower, renewable energy, stream

1 | INTRODUCTION

The impact of human activities has led to much more severe biodiversity declines in freshwater ecosystems than in terrestrial ecosystems, owing to the combined threats of overexploitation, pollution, flow modification, habitat degradation, and invasive non-native species (Dudgeon et al., 2006). In Europe, small-bodied rheophilic freshwater fish are especially prone to the risk of extinction (Reynolds, Webb, & Hawkins, 2005).

Zingel streber (Siebold, 1863), the Danube streber, is a rheophilic percid endemic to the catchment of the Danube. It is a bottom dweller with a preference for relatively deep water, and generally requires lotic river sections characterized by strong overflow and highly poriferous gravel or lithoidal substrata (Kováč, 2000; Zauner, 1996). As a consequence of the cryptic and nocturnal lifestyle of the species, however, studies are limited and further ecological information is scarce (Zauner, 1996).

Habitat degradation or loss, for instance through the regulation and fragmentation of rivers by weirs and dams, are considered a primary threat to the Danube streber (Freyhof, 2011; Kováč, 2000; Zauner, 1996). In addition to causing habitat loss, impassable weirs and dams also present barriers to formerly connected populations, causing genetic isolation and erosion of the gene pool (Knaepkens, Bervoets, Verheyen, & Eens, 2004; Wofford, Gresswell, & Banks, 2005). Impediment to the longitudinal and lateral movement of fish caused by manmade structures also limits the opportunities for species to avoid the adverse effects of stochastic environmental stressors, such as floods, droughts, or pollution events.

Although the global population of the Danube streber is currently rated as being of 'Least Concern' on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Freyhof, 2011), a much more complex and concerning picture emerges from local, regional, and national conservation assessments. Specifically, the species is regarded as 'Endangered' in Austria (Wolfram & Mikschi, 2007) and Slovenia (Povž, 1996), and as 'Critically Endangered' in the Czech Republic (Lusk, Hanel, & Lusková, 2004).

The limited information available from the federal state of Baden-Württemberg (Germany) indicates a critically endangered status for the Danube streber (Baer, Blank, Chucholl, Dußling, & Brinker, 2014), characterized by small, isolated occurrence and a negative population trend. The species is also listed as critically endangered in its Bavarian range (Bavarian Environmental Protection Agency, update of Red List of Fishes, July 2017). In line with these concerns, the conservation status of the species was recently assessed within the framework of the European Habitats Directive (Council of the European Communities, 1992) as 'unfavourable–inadequate', both in Baden-Württemberg and in Germany as a whole. As the Danube streber is generally not well represented by standard fish monitoring such as that carried out for the Water Framework Directive (WFD), population changes are likely to go unnoticed. The general paucity of information is reflected in the IUCN red list assessment, where the current population trend is listed as 'Unknown' (Freyhof, 2011).

Effective conservation strategies for the Danube streber require better knowledge of the present status of the species and the threats that it faces. To this end, the present study evaluated the status of the Danube streber population of Baden-Württemberg, in the south-western part of the Danube catchment, at the upstream limit of the historical distribution of the species (Figure 1; Klunzinger, 1881). The purpose of the study was to provide the first data on

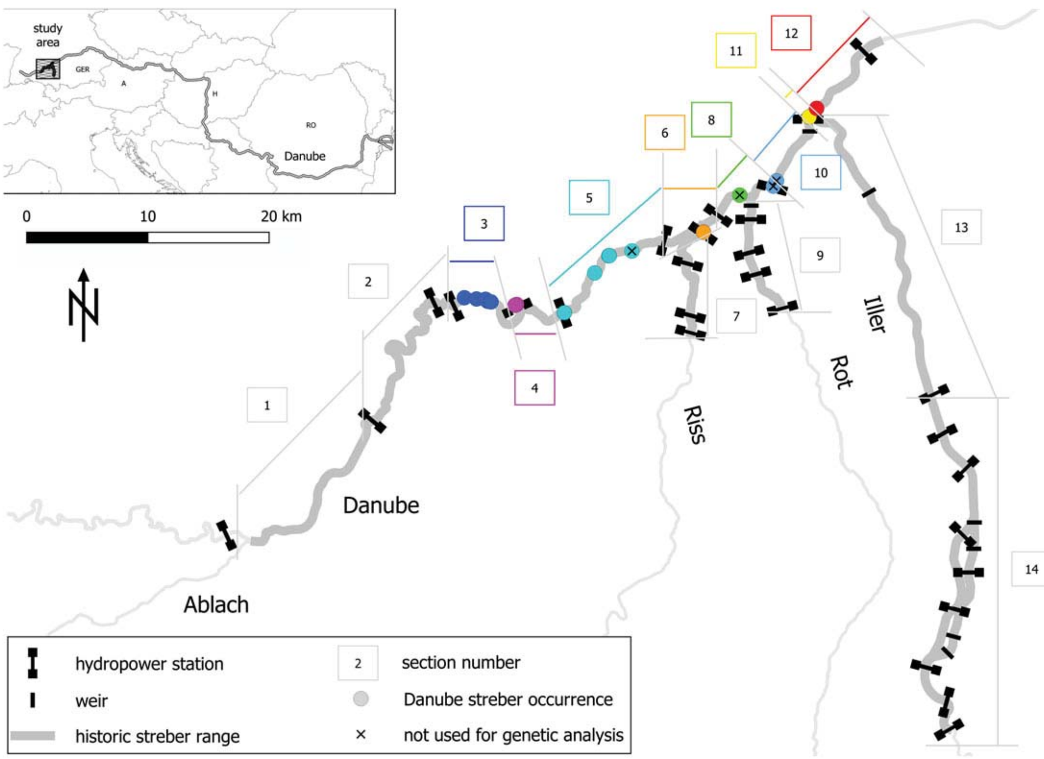

FIGURE 1 Study area, showing the locations of the hydropower stations and weirs. The historic range of the Danube streber was surveyed in 14 sections, demarcated by hydroelectric power stations. Sections where the presence of Danube streber was confirmed are highlighted in colour. The inset shows the location of the study area in relation to the whole Danube catchment area

population sizes, growth, and genetic structure in the context of habitat loss and fragmentation through river damming. The conservation implications of the findings are discussed, in order to provide guidance for stakeholders and regulatory agencies.

2 | METHODS

2.1 | Study area

The study area is a section of the River Danube between the confluence of the River Ablach and the city of Ulm, located in Baden-Württemberg, southern Germany (Figure 1). Historical literature suggests that this 90-km-long stretch was the principal habitat of a once-continuous population of Danube streber, and other historical records exist only for the lower reaches of the tributary streams – the Riss, the Rot, and the Iller (Dußling, 2016; Klunzinger, 1881; Königlich Statistisches Landesamt, 1895; Von Siebold, 1863; Wiedemann, 1885; Figure 1). These stretches collectively comprise around 181 km of river, and were surveyed for the presence of Danube streber as part of the present study.

The mean discharge of the Danube in the study stretch increases from less than 40 $m^3 s^{-1}$ at the upstream limit to 125 $m^3 s^{-1}$ at the border with Bavaria (near the city of Ulm). With a mean discharge of 75 $m^3 s^{-1}$, the Iller is the largest of the three tributary streams surveyed, with the similarly sized Rot and Riss being considerably smaller than the Iller (Table 1). Further hydromorphological features (catchment area, wetted width, and depth) of the rivers surveyed are summarized in Table 1.

The section of the Danube that runs through the study area historically featured runs, riffles, and fast-flowing deeper pools with coarse substrata (largely gravel and boulders), alternating with slow-flowing parts where the substrata were typically sandy or muddy, and featured submerged macrophytes. The construction of 12 hydropower stations on this stretch of river over the last 130 years (Figure 1) has led to considerable fragmentation, however, punctuating the physical river habitat with a string of artificial lakes, and altering water quality both upstream and downstream. The River Iller is also heavily used for the production of hydroelectricity (Figure 1 and Table S1).

Near-natural stretches are now very rare and in most reaches the Iller flows slowly, through the intake channels of nine power stations, leaving a very small volume of water passing along the original stream bed, especially in summer. Large parts of the River Iller flow through the State of Bavaria. The Rot and Riss are also used for the production of electricity, with a combined total of eight hydropower stations (Figure 1). Thus, although it is possible for the Danube streber to enter the lower reaches of all three tributary streams from the Danube River, movement further upstream is blocked after just 500 m on the Riss and 200 m on the Rot.

In total, there are 56 potential barriers to fish migration in the study area (Table S1). Of these, 37.5% (n = 21) have fish ladders or bypass systems designed to allow upstream migration, and 7.1% (n = 4) have structural concessions for downstream migration (Table S1). However, none of these mitigations meet the requirements for the Danube streber in terms of flow velocity, water depth, and connection to the river bed (Zauner 1996), and thus all 56 barriers must be considered effectively impassable for the species, at least in the upstream direction (Konrad pers. comm., Fisheries Administration Tübingen, responsible for the Danube).

The fish fauna of the study area is dominated by generalist species such as *Alburnoides bipunctatus* (Bloch, 1782) (spirlin), *Phoxinus phoxinus* (Linnaeus, 1758) (Eurasian minnow), and *Squalius cephalus* (Linnaeus, 1758) (chub). Formerly significant stocks of *Thymallus thymallus* (Linnaeus, 1758) (grayling) and *Chondrostoma nasus* (Linnaeus, 1758) (nase) are declining and endangered (Baer et al., 2014). The study area was also formerly a recognized fishing area for *Hucho hucho* (Linnaeus 1758) (huchen) (Klunzinger, 1881), which is now nearly extinct.

TABLE 1 Hydromorphological features and sampling effort (number of fishing events) for each of the 14 sampling sections

Section	River	Starting at hydropower station/river mouth	Section length (km)	Catchment area (km^2)	Mean discharge ($m^3 s^{-1}$)	Estimated mean wetted width (m)	Estimated average depth (m)	Fishing events (n)
1	Danube	River Ablach	16.2	2159	n.a.	10–15	0.7	16
2	Danube	HS Riedlingen	19.1	2916	n.a.	15–20	0.7	20
3	Danube	HS Alfredstal	8.4	3552	40	15–20	0.8	16
4	Danube	HS Munderkingen	5.2	3913	n.a.	15–20	0.8	7
5	Danube	HS Rottenacker	11.7	4021	n.a.	15–25	0.8	28
6	Danube	HS Öpfingen	5.6	4305	n.a.	15–25	0.9	11
7	Riss	HS Burgrieden	10.0	379	5	4–6	0.7	5
8	Danube	HS Ersingen	6.6	5015	n.a.	15–30	0.9	9
9	Rot	HS Obersulmentingen	10.0	296	4	4–8	0.8	4
10	Danube	HS Donaustetten	6.7	5366	54	20–30	1.1	11
11	Danube	HS Wiblingen	1.0	5408	n.a.	25–40	1.3	4
12	Danube	River Iller	8.2	8138	125	30–40	1.5	3
13	Iller	HS Untereichen	27.9	2730	54	n.a.	n.a.	1
14	Iller	HS Lautrach	44.5	n.a.	n.a.	n.a.	n.a.	8

The location of the sampling sections is shown in Figure 1; n.a., data not available.

2.2 | Sampling sections and fishing effort

The study area was divided into 14 sections of differing lengths (Figure 1 and Table 1), according to the locations of the hydropower facilities.

Fishing surveys were conducted between 2012 and 2014. A range of different habitats were fished in all sections, including slow-flowing parts with sandy substrata and deeper, fast-flowing parts with coarse substrata. Reaches with seasonally low or no water flow, and areas outside the state of Baden-Württemberg (including long stretches of the River Iller in sections 13 and 14), were not fished, resulting in small sample sizes in the River Iller. A total of 143 fishing events were conducted on stretches of 100–2200 m in length (Table 1). The eight stretches of water where Danube streber were detected were fished at least twice, amounting to 30 samplings in total.

Danube streber were captured by electrofishing (EFKO, straight DC, 300–600 V, 8 kW). On most occasions the fishing was conducted from a boat using an electrified dip net deployed downstream in deeper (>0.3 m) and faster-flowing (>0.25 m s^{-1}) sections of the river. To enable fishing in low visibility, an electrified benthic bottom trawl was modified according to the method described by Szalóky, György, Szekeres, Falka, & Csanyi (2012). This consisted of a rectangular steel frame (0.8-m wide, 0.4-m high) to which a 7-m-long drift net (mesh size 6 mm) was attached. A buoy attached to the codend of the net was used to indicate the position of the net while fishing. The frame was electrified with a copper anode cable, attached to the pulling rope ~2 m in front of the frame and connected to the electrofishing device on the boat through a 20-m-long cable. Results with the electrified dip net and the benthic bottom trawl indicated a comparable efficiency of fishing, and the catch per unit effort (CPUE) was thus defined as the number of Danube streber caught per 100 m of river stretch, independent of the method and river width. To calculate the actual range of the study species, the lengths of all river stretches (in km) in which Danube streber were detected were combined.

Each captured Danube streber was anaesthetized (clove oil, 0.1 mL L^{-1}), measured (total length, TL, to the nearest mm), and weighed (to the nearest g). Scales were taken for age determination. In addition, 246 individuals were each marked with a unique colour combination of visible-implant-elastomer (VIE) tags (Northwest Marine Technology, Seattle, WA, USA), fitted under the membrane posterior to the eye (Hale & Gray, 1998) and/or to the lower jaw (Figure 2). These supplemented the pattern of three dark caudal stripes and the 'tear dot' beneath the eye (Figure 2), which is unique to each individual. To check the reliability of the tagging, in spring 2012 10 Danube streber were held for 2 weeks in two plastic mesh cube enclosures with a volume of 1 m^3 (*cf*. Baer & Brinker, 2008), filled with autochthonous gravel from the Danube, and installed in the River Riss. Tissue was sampled from the caudal fin for genotyping (n = 187; Table 2). All fin clips were stored in 99.9% ethanol. After processing, all Danube streber were released back into the river section from which they were captured. All experiments were conducted according to the German Animal Welfare Act (TierSchG) and approved by the appropriate agency (Referat Tierschutz of Regierungspräsidium Tübingen: 1/12; AZ 35/9185.81-7 and 1/13, AZ 35/9185.82-2).

FIGURE 2 Marked (orange arrow) Danube streber, with its individually unique skin/scale pattern of stripes and 'tear dot' (grey arrow), in its natural habitat

TABLE 2 Number of Danube streber captured, catch per unit effort (CPUE as individuals caught per 100 m of river strech; mean, minimum, and maximum), and the sample size used for genetic analysis per sampling section

Section	Total no. caught	Mean CPUE ± SD	Min. CPUE	Max. CPUE	*n* for genetic analysis
1	0	0	0	0	0
2	0	0	0	0	0
3	16	0.37 ± 0.33	0.10	0.89	16
4	28	1.51 ± 1.13	0.13	3.00	22
5	91	0.72 ± 0.61	0.03	1.75	59
6	95	7.83 ± 10.87	0.00	27.00	43
7	0	0	0	0	0
8	3	0.08 ± 0.12	0.00	0.17	0
9	0	0	0	0	0
10	4	0.04 ± 0.06	0.00	0.08	0
11	25	4.17 ± 1.18	3.33	5.00	21
12	30	2.20 ± 0.92	1.20	3.00	26
13	0	0	0	0	0
14	0	0	0	0	0

2.3 | Estimation of contemporary habitat availability

To assess the contemporary habitat availability for Danube streber in the surveyed river sections, reach-scale data on river morphology were extracted from the 'waterway structural mapping of Baden-Württemberg' (https://www4.lubw.baden-wuerttemberg.de/servlet/is/48296/). Out of a total of 19 373 river segments extracted for the whole Danube catchment, 459 segments were retained for further analysis (mean length ± standard deviation (SD) of retained segments: 608 ± 294 m), covering the historical range of Danube streber in Baden-Württemberg (Dußling, 2016).

Morphological variables relating to the habitat preferences of Danube streber were selected based on the river segments in which the species was caught during the fishing survey. The selected variables were depth, width, gradient, substratum, and the influence of river damming (lentic conditions). Depth and width data previously recorded in non-dimensional, ordered categories (depth – very deep, deep, moderately deep, shallow, and very shallow; width – <1, 1–5, 5–10, 10–40, and >40 m) were transformed to a numeric scale and multiplied to calculate an approximation for river discharge using the approach of equal appearance intervals (Torgerson, 1958).

The river stretches in which Danube streber were found to be present all featured at least 85% adequate discharge, were non-lentic, with gradients of <2‰, and had substrata dominated by gravel, stones, or sand. Sections exhibiting these characteristics were thus defined as potentially suitable habitat for the species, and the actual length of river in the study area meeting each of these criteria was then calculated. The combination of all selected variables was used to determine the contemporary habitat availability for the Danube streber in relation to the historical range of the species. To assess the validity of this approach, the overlap between stretches with potential Danube streber habitat (model prediction) and the actual occurrence of the Danube streber (based on the fishing surveys) was calculated. To evaluate further the performance of the modelling approach, values were also calculated for false positives, false negatives, and Cohen's kappa (κ_n; Brennan & Prediger, 1981), which quantifies the possibility of correct predictions occurring by chance.

2.4 | Population size estimation

To assess the population size of streber and evaluate the effect of habitat fragmentation on relevant demographic parameters, the census population size N_c was estimated from mark–recapture data and an estimate of the effective population size N_e was calculated from genetic data (microsatellite data, see method below). Whereas N_c provides information about the number of adult individuals in the current population, N_e gives an indication of the genetic variance and likely losses in genetic variation. The N_e/N_c ratio points to factors that affect genetic variability in the population and may predict the extinction risk for the population (for a review, see Frankham 2007).

River sections 4, 5, and 6 were easily accessed, and recreational activities (canoeing, swimming, etc.) or predation pressures (cormorants) were low. These sections were therefore used for population size estimation.

Sections were fished three times during the summer and autumn of 2013. The fish present in these sections are isolated from each other by impassable hydropower infrastructure, and are thus considered distinct populations. For the purposes of analysis it was assumed that the population size remained constant between repeated electrofishing surveys, without recruitment or losses, that sampling was random, and that all individuals had an equal chance of capture in any given sampling event. The captured fish were marked on each sampling occasion, and N_c was estimated as a reciprocal according to PisciStat 1.2 (S. Blank, Langenargen, Germany), with the Schumacher and Eschmeyer method (Ricker, 1975), by applying the following formula:

$$\frac{1}{N_c} = \frac{\sum(M_t R_t)}{\left(C_t M_t^2\right)},$$

where M_t is the number of individuals previously marked (before time t), R_t is the number of recaptured fish (at time t), and C_t is the whole catch (at time t). According to Sokal & Rohlf (2003), the variance was calculated as follows:

$$\frac{1}{N_c} = \frac{\sum\left(\frac{R_t^2}{C_t}\right) - \left[\frac{\sum(R_t M_t)^2}{\sum C_t M_t^2}\right]}{s^{-2}},$$

where s is the number of samples included in the summations. The confidence interval was calculated according to the method described by Krebs (1989), as follows:

$$\frac{1}{N_c} \pm t_{(n-1,\infty)} s_{1/N_c}$$

The effective population size N_e was estimated for streber from the same river sections. Samples were available from two consecutive years in section 4 and from three consecutive years in sections 5 and 6, allowing for the use of the temporal method (Waples, 1989). As Danube streber exhibit overlapping generations, the default sampling strategy Plan I was chosen (Waples, 2005) and the *F*-statistic was calculated following the method described by Jorde and Ryman (2007), as implemented in NeEstimator (Do, et al. 2014). The census population size N_c calculated from mark–recapture data (described above) was used to calculate N_e, with an assumed generation time of 2 years. All microsatellite alleles were included for calculating the 95% confidence interval of N_e.

2.5 | Microsatellite genotyping

Population structure analyses were carried out for subgroups of Danube streber caught in different river sections. The populations from sections 8 and 10 were excluded because of small sample sizes (Table 2), leaving six local Danube streber subpopulations from sections 3, 4, 5, 6, 11, and 12. A further 34 Danube streber caught in the Hungarian Danube near the city of Isza (provided by Z. Szaloky, Hungarian Academy of Science, Centre for Ecological Research, DRI Department of Restoration and Animal Ecology) were incorporated into the population genetic analysis for the purposes of comparison.

Total genomic DNA was extracted following the standard salt extraction methodology described by Aljanabi and Martinez (1997). Danube streber were genotyped using the Type-it kit (Qiagen, http://www.quiagen.com), in which eight microsatellite loci were amplified in three batches. The first batch contained the markers *Za094*, *Za181*, and *Za107*, the second batch contained the markers *Za022*, *Za113*, and *Za165*, and the third batch contained the markers *SviL1* and *Za030* (Dubut et al., 2010; Wirth, Saint-Laurent, & Bernatchez, 1999). The polymerase chain reaction (PCR) cycle was 95°C for 5 min, then 27 rounds using a cycle of 95°C for 30 s, 50°C for 90 s, and 72°C for 30 s, finishing with 60°C for 30 min. All batches were fragment-analysed on an ABI 3130 genetic analyser (ThermoFisher Scientific, https://www.thermofisher.com). Allele calling was performed automatically using GeneMapper 4.0 (Applied Biosystems, now ThermoFisher Scientific).

2.6 | Testing for genetic variation and population structure

Genetic diversity within German Danube streber and Hungarian streber populations was estimated by calculating: expected (H_E) and observed (H_O) heterozygosity; the number of alleles per locus (A_N); allelic richness (A_R), using the sample-size independent rarefaction method; and the number of private alleles (A_P) per population using fstat 2.9.3.2 (Goudet, 2001).

Population structure was first calculated including all sampled individuals (n = 221) using STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000), under the admixture model with 50^5 burn-ins and 10^6 iterations in five replicate runs for a number of clusters (k = 1–7). The calculation was then repeated with the Hungarian samples (n = 187) excluded. STRUCTURE HARVESTER (Earl & VonHoldt, 2012) was used to implement the Evanno method (Evanno, Regnaut, & Goudet, 2005) to find the most probable number of genetic clusters, k, within the Danube streber population. To test microsatellite loci for genotyping errors, including stuttering, null alleles, and large allele drop-outs, the program MICROCHECKER 2.2.3 was used (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004), clustering samples population-wise. Indications of stuttering and null alleles were found for several loci (Table S3B), but no locus showed a consistent pattern of genotyping error in more than three populations. Therefore, all eight loci were used in all analyses.

Comparing the number of observed and expected heterozygotes, possible deviations from Hardy–Weinberg equilibrium (HWE) and tests for locus-by-locus linkage disequilibrium were calculated using ARLEQUIN 3.5 (Excoffier & Lischer, 2010). Pairwise F_{ST} values were calculated for the Hungarian Danube versus the German Danube populations, and between Danube populations, using ARLEQUIN 3.5. Excluding the Hungarian samples, multiple comparisons were corrected using the false discovery rate (FDR) of Benjamini and Hochberg (1995). Isolation by distance (Mantel test, 10 000 permutations) was examined by correlating genetic differentiation (F_{ST}) and distance in km along the stream between all German Danube populations. To test for partitioning of genetic variation, an analysis of molecular variance (AMOVA) was performed. Molecular variation was thus partitioned to within sampling years, among sampling years within Danube populations, and among Danube populations. Significance was determined by 10 000 permutations of the haplotypes.

To further test the effect on genetic connectivity of the hydropower installations, the streber population structure was examined in river sections fragmented by hydropower facilities and other sections where populations were fragmented only by stretches of unsuitable habitat. The effects of spatial distance on genetics were included as a covariate. Pairwise F_{ST} values were calculated (as implemented in ARLEQUIN 3.5) between all pairs of samples, which included four comparisons where the separation arose from natural variation in habitat type and six comparisons in which populations were separated by hydropower installations. The data were tested using the following generalized linear model (GLM):

$$Y_{ijk} = \mu + a_i + bj + (\mathrm{ab})_{ij} + \varepsilon_{ijk},$$

where Y_{ijk} is the genetic population differentiation (F_{ST}), μ is the overall mean, a_i is separation by hydropower (yes/no), b_j is the spatial distance between sample locations, $(ab)_{ij}$ is the interaction between separation and spatial distance, and ε_{ijk} is the random residual error. Post-hoc differences between sections with and without hydropower infrastructure were subjected to a Student's t-test.

3 | RESULTS

3.1 | Population range, growth, and size

In total, 293 Danube streber were caught during the study in stretches of the Danube between the cities of Untermarchtal and Ulm, and in the mouth of the River Riss (Figure 1 and Table 2). Most of those stretches were separated by hydropower weirs and dams (Figure 1). The combined length of all stretches of river with Danube streber totalled 12.8 km, approximately 7% of the historical range of the species (181.3 km) in the state of Baden-Württemberg (Table 3).

TABLE 3 Overview of the morphological variables selected to predict potential habitat suitability for the Danube streber, including data range/levels, the thresholds for the predicted presence of Danube streber, and the predicted suitable range in km and in relation to the historical range

Morphological variable	Variable range	Selection criteria (preference Danube streber)	Habitat length (km)§	Percentage in relation to the historical range§
Depth§width	52–1200	300–800	107.72	59%
Slope	0–51‰	<2‰	135.35	75%
Substratum	clay peat mud sand gravel stone boulder solid rock	sand (>50%) gravel (>50%) stone (>50%) not clay not peat	128.15	71%
Lentic conditions	0 0–10% 10–50% 50–100% 100%	not 100%	128.55	71%
Potentially suitable habitats for Danube streber (combination of all variables)			**58.03**	**32%**
Actual Danube streber occurrence in the historical range			12.82	7%
Actual Danube streber occurrence in the estimated suitable habitats			10.47	6%

§Outcomes for model variables are shown in grey, whereas the combination of all variables (final model) are shown in bold black; entries set in normal font juxtapose real catches with historical and modelled suitable habitat.

The upper limits of the Danube streber population range were marked by the power station at Alfredstal (the start of section 3; Figure 1) on the Danube and by Ersingen on the Riss (the end of section 7; Figure 1), with no captures outside this area despite extensive survey efforts (Figure 3 and Table 2).

Danube streber were found as single individuals or in small shoals of around three to six fish. The observed relative abundance was generally low. The CPUE was less than 1 in sections 3, 5, 8, and 10 (mean 0.04–0.75; Table 2), and greater than 1 in sections 4, 6, 11, and 12 (mean 1.51–4.17; Table 2). The lowest CPUE was recorded in sections 8 and 10, and the highest CPUE was recorded in section 6, which included the mouth of the River Riss (Table 2). No Danube streber were detected in slow-flowing or shallow stretches. All individuals were caught at depths between 0.4 and 1.8 m (mean 0.7 m, SD ±0.3 m) in flow velocities of 0.2–0.9 m s^{-1} (0.7 ± 0.1 m s^{-1}), and most were caught above coarse substrata (gravel, stones), sometimes near sandy areas.

Consideration of the observed length distribution, age determined by scale analysis, and recapture data suggests that the autumn Danube streber population of the area can be divided into four age classes: 0+ (young of the year), individuals between 54 and 71 mm, weighing 1.4 ± 0.5 g; 1+, individuals between 89 and 133 mm, weighing 10.3 ± 3.5 g; 2+, individuals between 140 and 162 mm, weighing 20.2 ± 5.0 g; and 3+, individuals >166 mm, weighing 33.6 ± 7.1 g. No older fish (4+ years or more) were detected. For each year, 60% of autumn-caught Danube streber belonged to the 1+ year class, whereas 0+ and 2+ classes accounted for 15 and 18%, respectively, and larger Danube streber (>160 mm) were comparably rare, at 4.1%. The longest Danube streber captured during the entire study had a total length of 184 mm.

Spawning of the Danube streber appears to commence in early spring, at the beginning of April. During that time, males exhibited small white tubercles along the head, and females showed larger, more rounded bellies. Natural spawning behaviour was observed in tagged individuals when single mature females were stocked with between three and five mature males in enclosures filled with gravel from their natural habitat. The males waited behind the female, sometimes nudging her tail or belly. During the spawning act the female released around 300–400 eggs, which were fertilized by the waiting males. The smallest sexually mature individuals recorded in the study were a 105-mm-long male and a 117-mm-long female.

No loss of tags was observed in any of the fish in the enclosures (*n* = 10). Of the streber marked and released back into the river (*n* = 246), 13 individuals (5.3%) were recaptured. Ten of these fish were recaptured within 2–10 months of marking, whereas only three individuals were retrieved after more than 1 year. All recaptures were made exclusively at their release site. The estimated adult census population sizes (N_c) ranged from 81 individuals in section 5 to 109 individuals in section 4, and 194 individuals in section 6 (see Table S2 for confidence intervals). Effective population sizes were much smaller than the corresponding census population sizes, and ranged from five in section 6 to 13 in section 4 and 19 in section 5 (Table 2). The corresponding N_e/N_c ratios were thus all <1, and ranged from 0.02 in section 6 to 0.12 in section 4 and 0.24 in section 5.

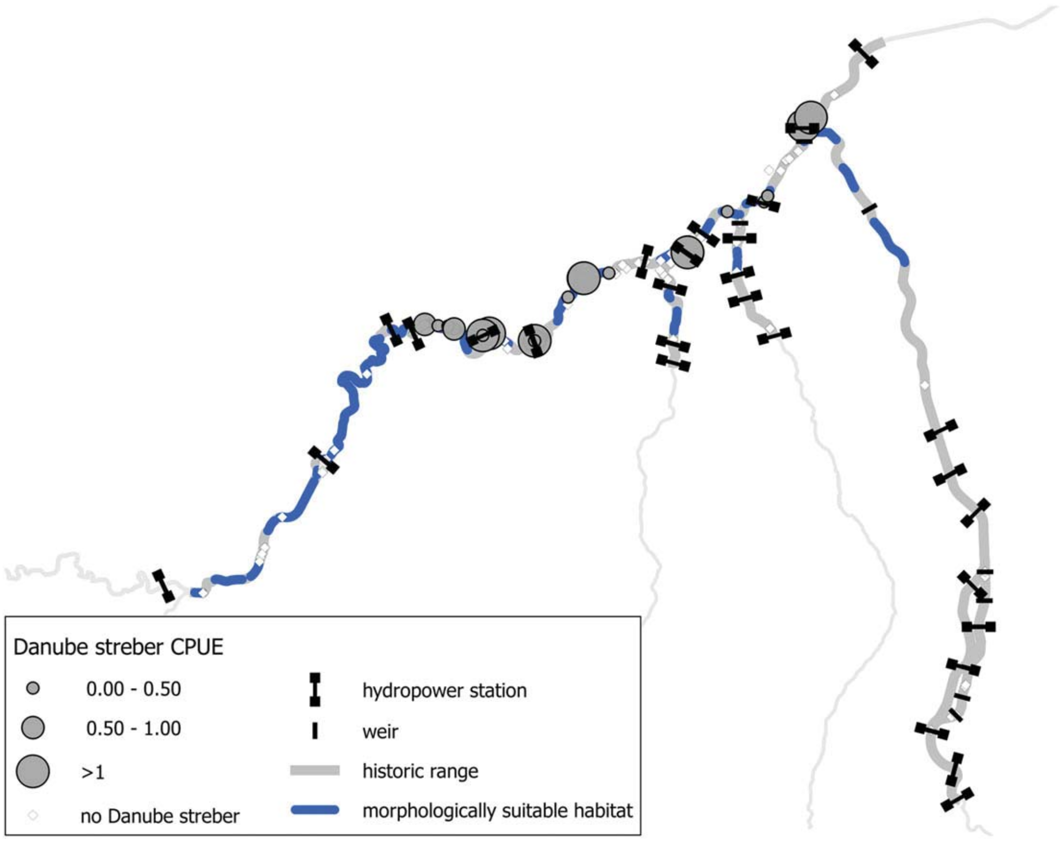

FIGURE 3 River stretches predicted as morphologically suitable for the Danube streber, in relation to the presence and absence of Danube streber, as assessed during the fishing surveys. CPUE: catch per unit effort

3.2 | Estimation of contemporary habitat availability

Only 32% (58 km) of the river within the historical range of the Danube streber in Baden-Württemberg (181.3 km) is now judged to provide morphologically suitable habitat for the species (Table 3). Suitable stretches are mostly found in the River Danube and in short isolated stretches of the rivers Riss, Rot, and Iller (Figure 3).

Of the 58.0 km of river within the study area identified as potentially suitable for Danube streber, only 10.5 km (18%) appears to be occupied by the species, which now appears to be absent from around 48 km of suitable habitat within its historical range. Around 9.1 km (71%) of the contemporary Danube streber range (12.8 km) was correctly predicted as suitable by the modelling approach (false-positive rate = 0.28). Of the 168.5 km where no Danube streber were detected, 120.9 km (72%) was correctly predicted as unsuitable (false-negative rate = 0.18). Cohen's κ_n was 0.45, which indicates that the model has a prediction ability that is fair to good (Fleiss, Levin, & Paik, 2013).

3.3 | Genetic diversity and evaluation of the population structure

For the complete data set, k = 2 clusters were determined as most probable by the Evanno method, separating Hungarian individuals from those sampled in the German Danube region (Figure 4 and Table S3A). Calculating the classical genetic diversity indices and pairwise F_{ST} comparisons revealed no deviation from HWE for the Hungarian samples (only two of eight microsatellite loci deviated from the HWE; Table S4A).

The pairwise F_{ST} value between Hungarian and German Danube populations was significant (F_{ST} = 0.051; P < 0.0001); however, in the German Danube sample, seven of the eight microsatellite loci investigated deviated from HWE, indicating further population substructuring within the German Danube streber populations (Table S4A).

Based on the second run of STRUCTURE using only populations from the German Danube, the Evanno method determined k = 2 clusters as most probable (Table S3B); however, an additional peak in the Δk distribution at k = 4 indicated further substructuring of the German Danube streber. Danube streber populations separated by hydropower infrastructure appeared to form genetically separated clusters on the structure bar plot (Figure 4). Furthermore, populations 11 and 12, which are not separated by a power station but by the mouth of the River Iller, also formed two clearly separated genetic clusters (Figure 4).

Based on these findings, the classical genetic diversity indices and pairwise F_{ST} values for the six populations of German Danube streber were calculated separately. Across all six investigated populations of German Danube streber, the mean observed and expected heterozygosities ranged from 0.71 to 0.78, and from 0.77 to 0.80, respectively. In the Hungarian population H_O and H_E were 0.77 and 0.87, respectively. The number of alleles (A_N) for the German populations ranged between 7.8 and 10.4, and the Hungarian streber population had a mean A_N of 17.4. Allelic richness (A_R) for the German populations ranged from 7.2 and 7.9, whereas A_N for the Hungarian streber was 13.3. The number of private alleles (A_P) ranged between 0 and 3, when comparing streber within the German Danube river sections, and 26, when including all German Danube streber compared against the Hungarian streber with 53 private alleles (Table 4). No deviation from the HWE was found in any of the populations. A maximum of two loci deviated from HWE in the populations of section 3, and one locus each for sections 11 and 12 (Table S4B). The apparent population substructuring of the German Danube streber was further evidenced by significant pairwise F_{ST} values between almost all populations. Only two pairwise comparisons were not significant (Table 5). There was no significant correlation between genetic distance (F_{ST}) and stream distance (km) between German Danube populations (correlation coefficient r = 0.152, P = 0.239; Figure 5). The analysis of molecular variance indicated that most genetic variation was found within sampling years (96%, F_{ST} = 0.038, d.f. = 347, P < 0.0001), with lesser but still highly significant variation among sampling years within populations (1.85%, F_{SC} = 0.019, d.f. = 9, P < 0.0001) and among populations (1.97%, F_{CT} = 0.020, d.f. = 5, P = 0.0001).

Hydropower dams had a significant effect on population structure in the two river sections tested (t = 2.45; d.f. = 8; P < 0.05; Figure 6).

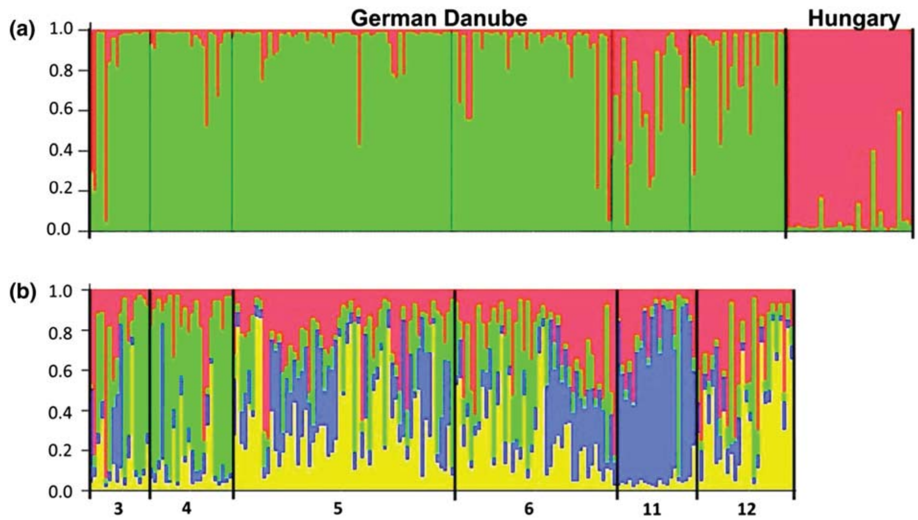

FIGURE 4 Structure bar plots showing the population structure of the Danube streber with the assignment probability (y-axis) per individual (x-axis). (a) Plot for k = 2, including streber from the German Danube and from Hungary. (b) Plot for k = 4, including only streber from the German Danube partitioned into six separate local populations (see Figure 1 for location and numbering of the populations and see text for details)

TABLE 4 Sample size and genetic diversity observed in populations of streber from six German Danube river sections and a location in Hungary

Population (sample size)	H_O (SD)	H_E (SD)	A_N (SD)	A_R (SD)	A_P
Danube 3 (16)	0.71 (0.18)	0.79 (0.10)	7.9 (3.3)	7.9 (3.3)	3*
Danube 4 (22)	0.74 (0.13)	0.77 (0.09)	7.8 (2.8)	7.2 (2.5)	0*
Danube 5 (59)	0.74 (0.10)	0.77 (0.08)	10.4 (3.7)	7.4 (2.5)	3*
Danube 6 (43)	0.71 (0.13)	0.78 (0.11)	10.0 (4.8)	7.8 (2.9)	2*
Danube 11 (21)	0.74 (0.18)	0.79 (0.08)	8.4 (3.7)	7.7 (3.2)	0*
Danube 12 (26)	0.78 (0.09)	0.80 (0.09)	9.0 (3.9)	7.9 (3.0)	2*
Germany (187)	0.74 (0.10)	0.80 (0.08)	8.9 (3.5)	7.6 (2.7)	26#
Hungary (34)	0.77 (0.14)	0.87 (0.14)	17.4 (3.5)	13.3 (5.0)	53#

H_O, mean observed heterozygosity; H_E, mean expected heterozygosity; A_N, mean allele numbers; A_R, allelic richness (rarefaction method); A_P, number of private alleles per population, either comparing streber between different German river sections (*) or comparing all German with Hungarian streber (#).

TABLE 5 F_{ST} values below, corresponding *P* values above diagonal, comparisons between all pairs of subpopulations of German Danube streber populations (Dan 3-12) and a Hungarian reference population (Hung)

	Dan 3	Dan 4	Dan 5	Dan 6	Dan 11	Dan 12	Hung
Dan 3	–	0.060	0.041	0.216	<0.001	0.004	<0.001
Dan 4	0.013	–	<0.001	0.001	<0.001	0.000	<0.001
Dan 5	**0.010**	**0.031**	–	0.003	<0.001	0.005	<0.001
Dan 6	0.006	**0.019**	**0.010**	–	<0.001	0.006	<0.001
Dan 11	**0.058**	**0.072**	**0.055**	**0.060**	–	<0.001	<0.001
Dan 12	**0.021**	**0.027**	**0.011**	**0.013**	**0.058**	–	<0.001
Hung	**0.060**	**0.064**	**0.064**	**0.057**	**0.055**	**0.049**	–

Significant values are set in bold after the calculation of the false discovery rate (FDR).

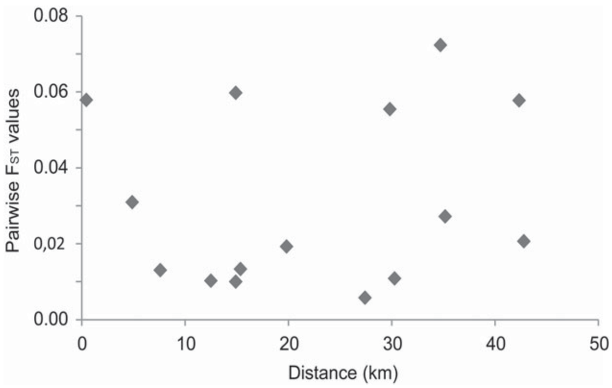

FIGURE 5 Scatterplot of genetic distance (F_{ST}) and river distance (km) between six streber subpopulations from the German Danube

Pairwise F_{ST} values (Table S6) between subsamples of streber collected within the same river section but separated by stretches of unsuitable habitat (n = 4) were significantly smaller than those between subpopulations separated by hydropower infrastructure (n = 6). The GLM (corrected r^2 = 0.82, P = 0.0039) revealed no effect of spatial distance or interaction of spatial distance and hydropower presence or absence (P > 0.05).

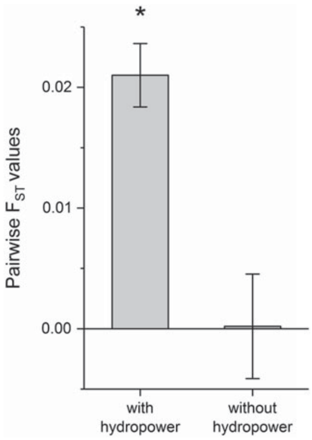

FIGURE 6 Bar graph of pairwise genetic distance (F_{ST}, mean ± SD) between subsamples of Danube streber collected within the same river section separated by unsuitable habitat ('without hydropower'; n = 4) or separated by dams ('with hydropower'; n = 6); hydropower dams had a significant effect on genetic distance (*)

4 | DISCUSSION

This study is the first to assess coherently the status of the critically endangered Danube streber at its upstream distribution limit in the Danube catchment. Historical evidence suggests that the population was once continuous (Klunzinger, 1881), but today it is fragmented into several small subpopulations (Figure 1) occupying just 7% of its historical range (Table 3). This declining range is an alarming example of the adverse effects of hydrosystem modification and fragmentation on rheophilic species. The expansion of hydropower schemes in this area over the last 130 years has reduced the naturally flowing portion of the Danube and has limited the area of habitat suitable for the Danube streber, with the result that the regional population is now artificially divided into several worryingly small subpopulations.

These results reinforce the critically endangered status of the Danube streber in the study area, and place significant doubt over the long-term viability of the remaining population. Of particular concern are the direct and indirect stressors caused by hydroelectric power stations, which effectively result in the fragmentation of populations (Gouskov, Reyes, Wirthner-Bitterlin, & Vorburger, 2016; Junge, Museth, Hindar, Kraabøl, & Vøllestad, 2014; Perkin, Gido, Costigan, Daniels, & Johnson, 2015), and the loss and degradation of the riverine habitat suitable for Danube streber (Fullerton et al., 2010).

While the genetic differences observed between populations of Danube streber in the current study were overall small, in line with findings for the closely related Rhône streber *Zingel asper* (Linnaeus, 1758) (Danancher, Izquierdo, & Garcia-Vazquez, 2008), the analysis confirms the genetic separation of subpopulations isolated by hydroelectric infrastructure, and of two further subgroups separated by functional habitat gaps. Comparably small genetic differences have also been found for other percid species: for example, for isolated yellow perch (*Perca flavescens* (Mitchill, 1814)) spawning populations in Lake Erie, and for European perch (*Perca fluviatilis* (Linnaeus, 1758)) subpopulations in Sweden and Germany (Bergek & Björklund, 2007, 2009; Gerlach, Schardt, Eckmann, & Meyer, 2001; Stepien, Behrmann-Godel, & Bernatchez, 2015). Stepien, Sepulveda-Villet, and Haponski (2015) have speculated that the relatively low genetic diversity may be characteristic of the percids, which might also relate to the streber. Laroche and Durand (2004) suggest that the genetic differentiation observed for *Z. asper* in the Rhône system may be principally a result of recent habitat fragmentation. Such isolation presents the potential for reduced evolutionary flexibility and the accumulation and fixation of mildly deleterious mutations (Knaepkens et al., 2004; Lynch & Lande, 1998). It was shown that for the Danube streber habitat fragmentation has reduced subpopulation sizes and increased the risk that genetic drift and/or inbreeding will reduce genetic variability and population fitness by fixation of deleterious alleles (Newman & Pilson, 1997). The small census population and even smaller effective population sizes recorded in the present study indicate that the fragmented streber subpopulations of the German Danube are severely affected by genetic drift and/or inbreeding, and are suffering from a significant loss of genetic variability. Such low effective population sizes and N_c/N_e ratios carry a significant risk of extinction (Newman & Pilson, 1997; Saccheri, Kuussaari, Kankare, & Vikman, 1998). It is interesting that the level of genetic variation is almost equally partitioned among sampling sites and among years (see AMOVA results), potentially indicating further as yet undescribed substructuring of populations within river sections. However, it could also be the result of age-specific reproduction rates, where one age class always dominates reproduction in a certain year (Luikart, Ryman, Tallmon, Schwartz, & Allendorf, 2010). Comparisons of allelic richness between the fragmented Danube streber populations in the Upper Danube and those of the Hungarian population found a decrease in richness affecting all but one of the loci investigated (Table 4, and Table S5 for single-locus estimates). In addition, the number of private alleles was much higher in the Hungarian population than in the fragmented German populations (Table 4), hinting further at the genetic depletion of these German populations. The most probable reason for the greater allelic richness observed in the Hungarian Danube streber population is the much larger population size (>500 individuals; Z. Szalóky, pers. comm.), probably within viable limits, compared with the fragmented and genetically poor population in Germany. However, it may also be an effect of locality, as allelic richness is often lower in upstream populations (like the German Danube streber) than in populations living downstream (like the Hungarian streber) (Wofford et al., 2005). The effective isolation of these population fragments is further highlighted by the finding that all recaptures of marked fish took place at the initial capture sites. Although the genetic separation of the Hungarian Danube streber from all individuals sampled in the German Danube region was an expected outcome, given the considerable geographic separation of the two populations (>800 km), it has implications for conservation management. Any restocking programmes should be carried out with autochthonous donor stocks originating within the German Danube to avoid any loss of local genetic adaptations and variability.

Today, the estimated population size of all the remaining Danube streber subpopulations is fewer than 200 individuals. Such small numbers pose a significant impediment to long-term population viability, and carry a high risk of extinction in the short term (Melbourne & Hastings, 2008; O'Grady, Reed, Brook, & Frankham, 2004; Soulé & Wilcox, 1980). According to Soulé & Wilcox (1980), the effective population size necessary to maintain adaptive genetic variation and thus long-term viability is 500 individuals. Lynch and Lande (1998) further suggested that the effective population size necessary to maintain long-term viability was 1000–5000. The small estimated population sizes recorded here were underlined by the fact that the CPUE of Danube streber was remarkably low, and that in some 100-m river stretches, captures were of single individuals. Despite the extremely low density and population size of the Danube streber, the sampled populations showed signs of successful reproduction and a normal age structure.

The recorded life-history parameters for the Danube streber indicate a rather rapid life cycle, with both sexes probably attaining sexual maturity at age 1+. This is in accordance with the population dynamics reported for the species in the Austrian Danube (Zauner, 1996), as well as for the Rhône streber (Labonne & Gaudin, 2005). Spawning of Danube streber in the present study was recorded at the beginning of April, corroborating the results of Zauner (1996), who gave mid-April as the spawning time in the Austrian Danube. The maximum recorded size in this study (TL 184 mm) is comparable with that recorded for the Austrian Danube (Zauner, 1996), but is slightly smaller than the overall largest known individual (230 mm; Zauner, 1996). Animals of the populations monitored in the Upper Danube apparently rarely exceed the age of 3+. This is shorter than the maximum 7+ reported by Zauner (1996) for Austrian streber; however, this was considered extraordinary and the mean lifespan in the Austrian population may actually be more in agreement with our results. The probable short average lifespan of 2–3 years of the Danube streber is underlined by the fact that very few marked Danube streber were recaptured after 1 year. Laroche and Durand (2004) estimated a short lifespan (2–3 years) and a high population turnover for the Danube streber, in line with the findings (low recapture rates) presented here, and relatively low adult annual survival rates (0.45–0.50) for the related Rhône streber. In addition, the fecundity of the Danube streber is low, at 300–400 eggs per female, compared with the 1117–1366 eggs per female estimated for the Rhône streber by Labonne and Gaudin (2006). Other European percids of comparable body length, such as *Perca fluviatilis*, release more than 6000 eggs per female (Dubois, Gillet, Bonnet, & Chevalier-Weber, 1996). Thus, overall, the results on population dynamics and growth complement the sparse data available from other Danube streber populations (Zauner, 1996), and reinforce the picture of a species with a rather rapid life cycle and a short mean lifespan.

A rapid life cycle may, in theory, translate to potentially rapid population recovery following environmental perturbations caused by stochastic events, such as droughts, flooding, or pollution. However, such recoveries depend on populations being able to perform longitudinal or lateral evasive movements, which is probably not the case in the severely fragmented Upper Danube. Moreover, the low fecundity of the Danube streber is likely to offset its rapid life cycle and may limit its potential for recovery (Kováč, 2000; Zauner, 1996). The results of the genetic analysis imply that downstream larval drift is unlikely. Thus, the potential for downstream colonization is questionable and rapid downstream colonization appears very unlikely. The results all point to a very limited capacity for natural re-colonization of the historical range, and a high risk of losing nearly the whole Danube streber population of the Upper Danube, for example in a single pollution event.

Danube streber exhibit highly specialized habitat requirements. The deeper, fast-flowing habitats featuring hard open substrata (coarse gravel) with which they are associated (Freyhof, 2011; Kováč, 2000; Zauner, 1996) were once characteristic of many reaches in the study area (Klunzinger, 1881); however, the creation of dams for hydropower stations has significantly altered many of these stretches, leading to a substantial loss of Danube streber habitat. In particular, it was estimated that only 32% of the historic habitat is still suitable for Danube streber, and only ~7% is still occupied by the species. The high overlap between estimated suitable habitat and actual occurrence of this species indicates the applicability of the estimation method. The results chime with those of Changeux and Pont (1995), who stated that numerous populations or subpopulations of *Z. asper* have probably disappeared from the Rhône system during the last 70 years owing to the construction of dams and weirs, and that this species today occupies only 17% of its historical range. A decisive factor in habitat loss is the close association of Danube and Rhône streber with high flow velocities. Zauner (1996) established a clear preference in Austrian Danube streber for water flowing at 0.35–0.65 m s^{-1}, with flows of ~0.6 m s^{-1} regarded as being optimum. Cavalli, Pech, and Chappaz (2003) only caught *Z. asper* in water flowing faster than 0.3 m s^{-1}. These findings match our data (the mean flow velocity at occupied sites was 0.7 m s^{-1}), and emphasize the strictly rheophilic habitat preferences of the Danube and Rhône streber. Consequently, the quasi-lentic conditions created by damming, characterized by slow-flowing water and soft-bottomed habitats, are consistently devoid of Danube streber. These essentially uninhabitable zones (Zauner, 1996) may themselves serve as barriers separating stretches of suitable habitat. In addition, large parts of the remaining suitable but unoccupied habitat are upstream of hydropower stations (Figure 2). Thus, both the availability and accessibility of suitable habitats may be limiting the size of the populations detected in the study area. Similar findings have been reported for Rhône streber (Labonne & Gaudin, 2005). To set ecological gain in context even at full capacity, the total energy production in the distribution area, which includes the rivers Riss, Rot and Danube, amounts to 59 Mio kWh a-1. This total represents less than 3% of hydropower generated in Baden Württemberg and meets only 0.74‰ of overall energy demand in the state (Table S1).

Every sixth species currently featured on the IUCN Red List of Threatened Species is classified as data deficient. Lack of knowledge and erroneous estimates of real species status hinder effective conservation measures (Bland et al., 2017), and the present example highlights the severe risk facing the Danube streber, which could easily be overlooked if careful monitoring with suitable methods is not carried out. Freshwater systems are home to a very large proportion of cryptic and inconspicuous species, which are often severely threatened by human shaping of surface waters, but whose plight often remains unnoticed. The present work demonstrates the urgent need to assess the status of these species and to set, direct, and prioritize global biodiversity targets (Convention on Biological Diversity Aichi targets; Trindade-Filho, de Carvalho, Brito, & Loyola, 2012).

As population size is a critical factor for long-term viability, and is directly related to the availability of suitable habitat, any further degradation or loss of Danube streber habitat should be avoided at all costs, especially as the monitored population fragments are already disturbingly small. Action to improve the conservation status of the Danube streber, as required by the EC Habitats Directive (Council of the European Communities, 1992), should focus on restoring degraded river habitats and increasing the connectivity between isolated population fragments. In particular, the creation of near-natural flowing conditions in heavily modified or quasi-lentic river stretches would benefit the Danube streber by providing more suitable habitat. Furthermore, passability hydropower plants should be provided by the construction of bypass channels designed to meet the rheophilic requirements of the Danube streber. One solution could be the creation of longer semi-natural bypass channels to allow Danube streber to avoid the extensive backwaters that are effective barriers in their own right. Such channels should be deep and fast flowing, with hard open substrata; these would also benefit other aquatic species, in particular the rheophilic fish that share habitat requirements with the Danube streber. A further, complementary conservation approach might include restocking with autochthonous donor stocks for population reinforcement (IUCN SSC, 2013); however, reinforcement stocking is rarely effective in situations where natural recruitment occurs (Baer & Rösch, 2008) – as evidenced by the present study for the Danube streber – and thus should only be considered when current threats (i.e. population fragmentation and degradation of suitable habitat) have been removed or sufficiently reduced (IUCN SSC, 2013). Finally, the remaining Danube streber populations should be carefully monitored to detect further population changes as they happen.

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