


Genetic population structure of a top predatory fish (northern pike, *Esox lucius*) covaries with anthropogenic alteration of freshwater ecosystems

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Abstract

1. *Esox lucius* L. (northern pike) inhabits river, lake, and brackish water ecosystems of the temperate region in North America and Eurasia. In many areas of its native range, the species is confronted with human-induced disturbances that can affect local and regional genetic diversity. Conservation of genetic diversity constitutes an important policy objective. Therefore, we investigated how the genetic population structure and patterns suggestive of hybridisation among subpopulations from different catchments vary with the type of ecosystem (river vs. lake) and the integrity of the ecosystem assessed using ecological status assessments of the European Water Framework Directive and indices of the wetland quality (in rivers) and trophic state (in lakes).
2. In total, 1,384 pike samples from the North, Baltic, and Black Sea drainages in Germany were analysed. We differentiated between metapopulations from each hydrogeographic region using genotypes from 15 microsatellites and mitochondrial *cyt b* sequences.
3. Individual populations showed signs of genetic admixture ranging from almost zero to complete replacement by foreign genotypes originating from different catchments. Hierarchical general linear modelling revealed a significantly positive association of the degree of genetic hybridisation with decreasing ecological status. An eroded population substructure indicative of hybridisation was also significantly elevated in rivers relative to lakes and in heavily modified water bodies. Results were consistent when using ecological status assessments by the Water Framework Directive and an alternative environmental quality index based on the health of wetlands in rivers and trophic state in lakes.
4. We speculate that macrophyte loss through loss of wetlands or in response to eutrophication fostered genetic mixing among different subpopulations, which were brought into secondary contact either due to stocking or due to natural dispersal from foreign catchments. Our findings suggest that anthropogenic alteration of

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freshwater ecosystems may influence the genetic structures present in a freshwater top piscivore at both local and regional scales.

KEYWORDS

admixture, ancestry distribution, ecological modification, secondary contact, stocking

1 | INTRODUCTION

Animal and plant populations are evolving in human-dominated landscapes (Christie & Knowles, 2015; Ortego et al., 2015; Sexton et al., 2013; Smith & Bernatchez, 2008). Studies investigating fish communities and populations have demonstrated that aquatic ecosystems are particularly sensitive to ecological changes (e.g. Whitehead et al., 2017), including habitat fragmentation, which increases isolation of populations through migration barriers (Waples et al., 2017). Alternatively, human activities are involved in connecting previously isolated aquatic ecosystems and fish populations, e.g. through the artificial opening of new routes for migration via the construction of artificial canals that connect previously disconnected catchments (Lin et al., 2020) or intentional transplantation of individuals through stocking and introductions (Laikre et al., 2010). In particular, stocking as a widespread fisheries-management and conservation practice mediates secondary contacts among fish populations, leading to multiple, difficult-to-predict outcomes, and possibly contributing to either the erosion or reinforcement of genetic differences through hybridisation (Allendorf et al., 2001; Diana et al., 2017; Hansen, 2002; Harbicht et al. 2014; Marie et al., 2012). Stocking and facilitated natural migration through human-made artificial connections among different river basins have substantially increased the speed of human-assisted secondary contact among subpopulations of fish relative to natural factors alone and thereby can shape contemporary genetic structures through rapid evolution (Lin et al., 2020; van Poorten et al., 2011).

Depending on local ecological conditions, particularly the degree of local natural recruitment, stocked fish either disappear without a trace or become established to varying degrees (Johnston et al., 2018), which eventually leads to admixture of non-native with native populations or even complete replacement of native populations (Englbrecht et al., 2002; van Poorten et al., 2011). Numerous studies have shown that hybridisation is enhanced at the interspecific level in ecologically perturbed habitats, e.g. in African cichlids *Cichlidae* (Seehausen et al., 1997), sculpins *Cottidae* (Nolte et al., 2005), European whitefish *Coregonus* spp. (Bittner et al., 2010; Vonlanthen et al., 2012), and trout *Oncorhynchus* spp. (Heath et al., 2010). All of these examples support the idea that the outcome of secondary contact between different populations or lineages is influenced by local ecological conditions, but as yet little is known about how local environmental conditions determine population structure of intensively exploited species at large geographical scales.

Esox lucius L., the northern pike, is a freshwater top predatory fish species. The species may well be affected by contemporary

environmental change caused by human activities, including loss of floodplains in rivers and elevated nutrient inputs in lakes that reduce the extent of submerged macrophytes (Craig, 1996; Skov & Nilsson, 2018). To fulfil their life cycle, pike strongly depend on aquatic macrophytes providing shelter for early developmental stages and camouflage to hunt for prey during the juvenile stage (Craig, 1996). After reaching sexual maturation, floodplains, and more generally submerged macrophytes, are equally essential, serving as spawning habitat for the phytophilic pike (Casselman & Lewis, 1996; Raat, 1988). Thus, changes in macrophyte extension through loss of floodplains or eutrophication of lakes affect the reproductive potential of pike, and may in turn also affect the stocking intensity of pike by motivating increased stocking efforts as natural recruitment declines (Cowx, 1994; Guillerault et al., 2018; Hühn et al., 2014). Gandolfi et al. (2017), studying the invasion process of *Esox lucius* into closely related native Italian *Esox flaviae/cisalpinus* (Bianco & Delmastro, 2011; Lucentini et al., 2011) populations, observed a mosaic-type distribution of the two species and different degrees of genetic admixture, possibly as a result of the different ecological status of the studied water bodies. Lake Garda provides good ecological conditions for the native *E. flaviae/cisalpinus* to fulfil its life cycle. Therefore, native pike seem to be able to out-compete the establishment of introduced northern pike. By contrast, other Italian waters with a poorer ecological status have been found to be strongly prone to genetic introgression (Gandolfi et al., 2017). Similarly, in Danish pike populations at least two regional clusters were identified referring to the hydrogeographic regions of the Baltic and the North Sea (see also Wennerström et al., 2018), which could be further sub-divided at different river catchment scales (Bekkevold et al., 2015). The authors speculated that local-scale deviations from native genetic signatures typical of catchments were the result of human-caused changes in local habitat quality (e.g. availability of submerged macrophytes) or could be due to historical geological alterations (Bekkevold et al., 2015). In addition, stocking may leave a strong legacy in the genetic structure of local pike populations (Möller et al., 2021), which will also be determined by the local environment (Larsen et al., 2005; Nordahl et al., 2019). The reviewed literature suggests that to fully understand the contemporary genetic structures in widely dispersed fish species, such as pike, the interaction of local ecological status in the context of the potential for human-assisted secondary contact is important to consider.

In Europe, in the 2000s the Water Framework Directive (WFD) was introduced as a policy to monitor and improve freshwater ecosystem quality and the ecological status of rivers and large lakes beyond 50 ha in dimension (European Commission, 2000). Therefore, all European rivers and large lakes are today regularly assessed based

on a range of biological indices, from phytoplankton to fish, to assess their ecological status and inform management actions (Hering et al., 2010). Although different taxa respond differently to anthropogenic drivers, the ecological assessment of lakes often tracks eutrophication signals, while hydromorphological changes are a dominant stressor in European rivers (Hering et al., 2013). Both drivers have negative effects on natural recruitment of pike (Craig, 1996; Raat, 1988; Skov & Nilsson, 2018), and hence indicators of ecological status of lakes may relate systematically to the population structure of pike. Genetic population structures within species, however, are currently not considered in the WFD. It is nevertheless likely that the ecological status of water bodies is systematically related to the meta-population structure of individual species. Other assessments of ecosystem status of freshwaters exist, e.g. the degree of wetland loss in rivers (BFN, 2009) or the degree of eutrophication in lakes (Hering et al., 2013), but similar to the WFD assessments, limited data exist to link these water body-specific indicators to genetic structure of individual fish species.

The objective of the present study was to explore whether the ecological status of the inland water bodies in Germany would be associated with the genetic structure of contemporary pike populations, particularly with respect to potential genetic admixture among populations of *E. lucius* of different hydrogeographic regions (i.e. catchments or basins). We evaluated the presence of population structure that permitted genetic assignment of individuals to their origins, and used this information to identify large-scale and local signs for intra-specific admixture. We then tested whether the observed genetic patterns correlated with the ecological status of the water bodies assessed according to the European WFD and, as a robustness check, according to an index of wetland status of rivers and the trophic state of lakes. We assumed that both indices would correlate with the quality of pike habitat and thus index the degree of natural recruitment of pike in a local ecosystem. We further assumed that the degree of local natural recruitment would affect how local populations would respond to the secondary contact with foreign genotypes (Hühn et al., 2014), and in turn how resilient the genetic structure of a local population would be against genetic invasion. We overall hypothesised that the ecological status of local ecosystems shapes the genetic structure of northern pike across Germany.

2 | METHODS

2.1 | Sampling and DNA extraction

Sampling was performed in 2011 and 2012 covering as many relevant water systems and types as possible over a wide geographical area in Germany. Our standard for inclusion in the study was a minimum of 10 individuals characterised by at least 14 microsatellites. The sample collection comprised specimens from five river catchments draining into the North Sea, six catchments draining into the Baltic Sea, and one catchment draining into the Black Sea (Table 1). Three ecosystem types were covered including 26 lakes,

24 rivers, and three brackish coastal water areas (Table 1). Pike were sampled from water bodies covering the complete range of ecological states according to the WFD, the quality of the wetlands (i.e. wetland loss) following a recent report by the German Agency for Nature Conservation (BFN, 2009) and publicly available information on trophic states in lakes. For three small water bodies <50 ha (Alte Würm, Kleiner Döllnsee, and Schulzensee), ecological status data following the European WFD were not available.

Fin and muscle tissue samples of pike were collected by commercial and recreational fishers, research organisations and state fishery authorities. Samples obtained as frozen tissues were thawed in absolute ethanol (Thomas Geyer) at room temperature and subsequently transferred to fresh ethanol following Eschbach (2012). Samples from research organisations were generally obtained preserved in ethanol, while samples from anglers were obtained air-dried. DNA of all types of samples was extracted with the nexttec™ DNA isolation kit (Biozym Scientific GmbH, Hess. Oldendorf, Germany) according to the manufacturer's instruction.

2.2 | Genetic marker analysis

We employed nuclear as well as mitochondrial markers to infer population structure and to compare our data with published data. Fifteen polymorphic microsatellites (Table 2, Table S1) for pike were selected for high information content according to Eschbach and Schöning (2013). These were employed to analyse a subset of 1,384 samples of 53 populations with an average sample size of 22.1 ± 9.8 (mean \pm SD) individuals per population, and 96.4 ± 94.0 (mean \pm SD) individuals per river catchment. Microsatellites were co-amplified in multiplex polymerase chain reaction (PCR; Table 2) with a Thermocycler T Gradient machine (Biometra) using the Qiagen® Multiplex PCR Kit (Qiagen). Forward primers were 5'-labelled with fluorescent dyes HEX, NED, or FAM (SMB Services in Molecular Biology GmbH; Table 2). Polymerase chain reaction started with 15 min at 95°C, followed by 35 cycles of 0.5 min at 94°C, 1.5 min at 58°C, 1.5 min at 72°C, and finishing with 10 min at 72°C. Fragments were sized with an Applied Biosystems 3500xL Sequencer equipped with a 24-capillary array. Chromatograms were evaluated with GeneMapper® Software v4.1 (Life Technologies).

Haplotype analysis of the mitochondrial cytochrome b gene (*cyt b*) was carried out to link the present data set with the broad scale phylogeographic analysis by Skog et al. (2014) using the primers described by Grande et al. (2004). DNA was extracted with the ArchivePure DNA Cell/Tissue Kit (5 Prime GmbH). The Multiplex PCR Kit (Qiagen GmbH) was used for PCR and sequencing was performed with the BigDye Terminator v.3.1. Cycle sequencing Kit by Applied Biosystems™, following the instructions of the manufacturers. Sequencing was carried out on an Applied Biosystems 3100x Genetic Analyzer.

A 1.2 kbp region was amplified for a subset of 184 pike individuals belonging to 22 populations of 12 river catchments. The mean \pm SD

TABLE 1 Sampled water bodies

Catchment	Waterbody	Type/ecological status	ID	LAT	LONG	IC
Black Sea HGR:						
Danube	Alte Würm	r/NA	AWU	48°13'N	11°27'E	BY
	Ammersee	l/2	AMS	48°00'N	11°07'E	BY
	Amper	r/3	AMP	48°27'N	11°49'E	BY
	Chiemsee	l/2	CHS	47°52'N	12°27'E	BY
	Danube	r/3	DON	48°44'N	11°09'E	BY
	Ilz	r/2	ILZ	48°38'N	13°26'E	BY
	Inn	r/3	INN	48°14'N	12°59'E	BY
	Kochelsee	l/2	KOS	47°39'N	11°21'E	BY
	Naab	r/3	NAB	49°05'N	11°56'E	BY
	Rott	r/4	ROT	48°23'N	12°45'E	BY
	Starnberger See	l/2	STS	47°53'N	11°18'E	BY
	Waginger See	l/4	WAS	47°56'N	12°46'E	BY
	Walchen See	l/2	WLS	47°35'N	11°20'E	BY
Baltic Sea HGR:						
Barthe	NN Lake (Barthe)	l/4	BAR	54°16'N	12°45'E	MV
Oder	Neiße	r/5	NEI1	51°54'N	14°41'E	BB
		r/5	NEI2	51°57'N	14°43'E	BB
		r/5	NEI4	52°03'N	14°45'E	BB
		r/5	ODE2	53°03'N	14°18'E	BB
	Oder	r/3	ODE7	52°11'N	14°41'E	BB
	Werbellinsee	l/3	WBS	52°54'N	13°41'E	BB
Peene	Peene	r/3	PEE	53°53'N	13°28'E	MV
Schwentine	Großer Plöner See	l/3	GPS	54°08'N	10°23'E	SH
Trave	Großer Ratzeburger See	l/4	GRA	53°43'N	10°45'E	SH
Ucker	Hardenbecker Haussee	l/4	HAH	53°14'N	13°31'E	BB
	Ucker	r/4	UCK4	53°31'N	13°59'E	MV
–	Schaproder Bodden	c/4	BAL2	54°30'N	13°07'E	D
–	Schlei	c/5	BAL3	54°30'N	09°40'E	D
–	Stettiner Haff	c/4	BAL4	53°48'N	14°04'E	D
North Sea HGR:						
Eider	Eider	r/3	EID1	54°19'N	09°09'E	SH
Elbe	Drewitzer See	l/1	DRS	53°32'N	12°21'E	MV
	Elbe	r/3	ELB6	53°12'N	10°57'E	NI
		r/3	ELB7	51°51'N	12°27'E	SN
	Gölper See (Havel)	l/5	HAV1	52°44'N	12°15'E	BB
	Großer Kossenblatter See	l/5	GKB	52°08'N	14°06'E	BB
	Großer Stechlinsee	l/3	GST	53°09'N	13°01'E	BB
	Jäglitz	r/4	JAG	52°52'N	12°24'E	BB
	Karthane	r/4	KAR	52°59'N	11°47'E	BB
	Kleiner Döllnsee	l/NA	KDO	52°59'N	13°34'E	BB
	Krainke	r/3	KRK	53°13'N	11°04'E	NI
	Müritzt	l/1	MUR	53°25'N	12°41'E	MV

(Continues)

TABLE 1 (Continued)

Catchment	Waterbody	Type/ecological status	ID	LAT	LONG	IC
Ems	Schulzensee	l/?	SUS	53°09'N	13°15'E	BB
	Schwarze Elster	r/4	STP	51°28'N	13°26'E	BB
	Wittensee	l/3	WIS	54°23'N	09°44'E	SH
	Ems	r/4	EMS3	52°58'N	07°18'E	NI
	Hieve	l/3	EMS2	53°24'N	07°16'E	NI
Rhine	Main	r/3	MAI7	50°01'N	10°31'E	BY
	Rhine	r/3	RHE2	49°09'N	08°22'E	BW
	Lake Constance	l/2	BOS1	47°41'N	09°02'E	BW
Weser		l/3	BOS3	47°43'N	09°13'E	BW
		l/3	BOS4	47°33'N	09°37'E	BW
		l/3	BOS5	47°35'N	09°31'E	BW
	Edersee	l/3	EDS	51°11'N	09°03'E	HE
	Steinhuder Meer	l/3	STM	52°28'N	09°19'E	NI

Note: Sampled water bodies, type of water body and geographic positions of sampling sites within each of the three hydro-geographic regions (HGR). Sample identification (ID) is given by a three-letter code, which is used throughout the text (italic codes indicate samples of which sub-samples have been taken to analyse the mitochondrial *cyt b* gene in addition to microsatellites). Ecological status follows the WFD (1 = very good to 5 = poor).

Abbreviations: BB, Brandenburg; BW, Baden-Württemberg; BY, Bavaria; c, coast; HE, Hesse; HGR, hydro-geographic region; IC, German federal state identification code; ID, sample identification code; geographic coordinates; l, lake; LAT, latitude north (N); LONG, longitude east (E); MV, Mecklenburg-Vorpommern; NA, not available; NI, Lower Saxony; NN, no name; r, river; SH, Schleswig-Holstein; SN, Saxony; Type, type of waterbody

Loci	References	5'-label	Multiplex No.	A _T	A _M	H _O	H _S
Elu87	1	NED	1	13	4.9	0.56	0.58
Eluc045	2	FAM		26	6.7	0.66	0.67
B451	3	HEX		37	13.3	0.79	0.89
PkB47	4	FAM	2	18	6.1	0.50	0.53
Elu19	5	NED		20	4.5	0.43	0.46
EL02	6	HEX		24	7.6	0.61	0.75
PkB16	4	NED		37	14.5	0.79	0.92
Elu76	5	FAM	3	23	7.3	0.61	0.71
EL27	6	NED		16	5.8	0.68	0.69
EmaD12a	7	HEX		34	12.9	0.86	0.90
EL01	6	FAM		25	6.6	0.61	0.64
EluB108	8	FAM	4	14	5.2	0.60	0.60
EluBe	8	NED		9	3.5	0.61	0.54
B24	3	HEX		28	10.9	0.86	0.88
Eluc033	2	NED		20	6.7	0.64	0.66

Note: High-resolution microsatellites selected according to Eschbach and Schöning (2013) for population genetic analysis of species with low genetic variability. References: 1 = Miller & Kapuscinski, 1996; 2 = Wang et al., 2011; 3 = Aguilar et al., 2005; 4 = Wildlife Forensics Laboratory, California, USA (internal report); 5 = Miller & Kapuscinski, 1997; 6 = Ouellet-Cauchon et al., 2014; 7 = Sloss et al., 2008; 8 = Launey et al., 2003. NED, FAM, and HEX are fluorescent dyes; A_T = total number of different alleles over all populations, A_M = mean number of different alleles per population, H_O = observed heterozygosity, H_S = heterozygosity according to Nei & Chesser (1983).

TABLE 2 Microsatellites used for genotyping

sample size was 9.1 ± 2.1 individuals per population and 16.7 ± 8.5 per river catchment. Individual forward and reverse sequences were assembled using Seqman (DNA star package) and the resulting contigs were checked by eye to correct sequencing errors. Sequences of all main and sub haplotypes were deposited at the NCBI database (Acc. no. KY399416–KY399442).

2.3 | Analysis of genetic data

Microsatellite genotypes were tested for the presence of null alleles with MICROCHECKER 2.2.3 (van Oosterhout et al., 2004) using 1,000 randomisations and applying a 95% confidence interval. Total and mean numbers of alleles as well as heterozygosity (H_O and Nei's H_S) were calculated with FSTAT 2.9.3.2 (Goudet, 1995). GENEPOP 4.2 (Raymond & Rousset, 1995) was applied to test for Hardy–Weinberg deviations and linkage disequilibrium using 10,000 Markov chain Monte Carlo dememorisations, with 20 batches and 5,000 iterations per batch.

Principal coordinate analysis (PCoA) with GeneAIEx 6.5 (Peakall & Smouse, 2012) based on the covariance matrix obtained from F_{st} values (Table S2) was used to visualise the most dominant axes of genetic divergence in a scatterplot. Next, MSA 4.05 (Dieringer & Schlötterer, 2003) was employed to calculate genetic differences (Nei's D_A , Nei et al., 1983), chord distances (CD, Cavalli-Sforza & Edwards, 1967) and the proportion of shared alleles (Bowcock et al., 1994) among all populations. To allow for bootstrapping, the permutation option was set to 10,000. Consensus trees were calculated subsequently with the NEIGHBOR and CONSENSUS packages of PHYLIP 3.695 (Felsenstein, 1981) and displayed with the software MEGA 5 (Tamura et al., 2011) (see supplement for all analysis for all three trees).

STRUCTURE 2.3.2 (Falush et al., 2003) was used to infer the most likely genetic population structure based on microsatellite data for pike from 53 sampling locations. The calculation was done with an admixture model without a priori sampling site information, using a burn-in period of 100,000 repeats, 100,000 subsequent Markov chain Monte Carlo repeats and 10 iterations for each k value between 1 and 30. The most likely number of ancestral populations was initially identified as the k value, where the change of likelihood dropped considerably compared to subsequent values (δk criterion) following Evanno et al. (2005). While the δk criterion implied a most likely genetic subdivision of only two clusters, our exploration of the results for a range of k values showed signs of additional genetic subdivision. Accordingly, based on the results from the PCoA, the genetic distance tree, the geographical settings and patterns of clustering in STRUCTURE, we based our subsequent analysis on a population model that assumed genetic subdivision according to three hydrogeographic regions ($k = 3$, North Sea, Baltic Sea, and Black Sea), in agreement with previous research in pike (Bekkevold et al., 2015; Wennerström et al., 2018). However, as a further test we also ran additional models for $k = 2$ to $k = 8$ to examine the robustness of our findings on the covariance of genetic structure and environmental status.

We used the STRUCTURE Q value that describes the fraction of the genome inherited from the drainage basin-specific lineage as native ancestry (NA). To express all foreign genetic influences in relation to NA, hybrid indices (HI) for each individual were inferred from the individual NA values. Using the formula $HI = 1 - (2 \times |0.5 - NA|)$ results in a value of 1.0, if the native and foreign ancestries contributed equally to an individual's genetic composition (maximal hybrid status, as found in a first-generation hybrid), and a value of 0, if only the native or only the foreign ancestry contributed to an individual's genetic composition. CLUSTAL X Version 2 (Larkin et al., 2007) was used to align all *cyt b* sequences along with 24 reference sequences of haplotypes described by Skog et al. (2014). The alignment was trimmed to a length of 1,174 bp, which contained the sites that were diagnostic for the groups of haplotypes described by Skog et al. (2014). This alignment was used to confirm the presence or absence of the respective haplotypes in the populations studied here. The relationship among all haplotypes was visualised using a median-joining network as described by Bandelt et al. (1999) that was constructed using the program NETWORK 4.6.1.3 (Fluxus Technology Ltd).

2.4 | Environmental effects on genetic structure

In total, 585 pike individuals from 24 lakes and 392 pike individuals from 23 rivers (excluding pike of brackish waters and of waterbodies without ecological information) were tested in two independent hierarchical general linear models (HGLM) to infer the effect of different ecological predictors (ecological status following the WFD and wetland/trophic state index as a second index of environmental quality) on NA and HI. The response variables (y) were composed of values within the standard unit interval $y_i \in [0,1]$, where i designated an individual fish. A link function (logit) was required for linear modelling with respect to binomial errors and β -distributed random effects to incorporate features such as heteroskedasticity or skewness commonly observed in environmental data (Cribari-Netom & Zeileis, 2010). The data comprised repeated measures within individual water bodies (n individuals from 47 different water bodies), and therefore, we considered the variance attributed to water bodies as a random effect. In addition, to account for a higher probability of natural exchange among individuals sampled in specific water bodies within a basin, water bodies were nested within catchments. We then modelled HI and NA on a set of predictors using a linear predictor with unknown coefficients and a link function (logit). The predictors considered were: the type of water body (lake or river); its level of modification following the classification of the European WFD (not modified or highly modified); and its ecological status as a numerical covariate from 1 (very good) to 5 (very poor) according to the European WFD or 1 (very good) to 4 (poor) of our index of wetland quality/trophic state (see below). The level of modification was derived from public data on the WFD, which distinguishes heavily modified water bodies from those that are not heavily modified as different policy goals apply to these two conditions. We included

degree of modification only as a control variable as we expected the most relevant information in our metric of ecological status and ecosystem type (river vs. lake).

The raw data for the ecological status following the WFD was retrieved from site-specific ecological assessment data supplied by the Federal Institute of Hydrology (Koblenz, Germany: https://www.bafg.de/DE/05_Wissen/01_InfoSys/WasserBLicK/WasserBLicK.html). To that end, we matched the GIS information on the pike sampling site with the nearest site for which a Water Framework Assessment metric was available. We used the ultimate ecological status assessment indicator provided by the Federal Institute of Hydrology for the latest reporting period after 2010, instead of taxa-specific values (phytoplankton, macrophytes, macrozoobenthos, or fish), because taxa-specific values would have increased the number of missing values for selected populations. Note that the assessment of ecosystem status in the European WFD follows a reference community-based approach for the major taxa groups and uses the poorest assessment results of any of the four taxa to identify the ecosystem status (Hering et al., 2010). Thus, although readily available across Europe, the metric may not perfectly represent the conditions relevant for pike.

As a second index we also used a combined status assessment of the wetland status for rivers (derived from the national wetland report, BFN, 2009) and of the trophic state of lakes. The trophic state of lakes was calculated as the trophic state index of the State Commission for Water (LAWA); it was transformed into an ordinal index: oligotrophic = 1, mesotrophic = 2, eutrophic = 3, and hypereutrophic = 4. The wetland index presented in BFN (2009) also ranged from 1 to 4, indicating the degree of wetland loss in a given site as: 1 = very good wetland status, 2 = good status, 3 = moderate status, 4 = bad status. The two indices were combined to form a wetland/trophic state index as a measure of ecosystem quality of rivers and lakes, which was assumed to directly index the ecosystem quality of pike habitat. We used this new index as a robustness check to see whether ecosystem quality of freshwater ecosystems explained pike population structure.

We used the glmmADMB of the R-package, built on the open source AD Model Builder nonlinear fitting engine, to fit two HGLM models (one for NA and another one for HI) to both indices of ecological status (status following WFD and wetland/trophic state index) considering a β response distribution type using the logit-link function (Fournier et al., 2012). The estimates of the fixed effects (in logit scale) as well as their standard error were estimated via the Laplace approximation. In the initial HGLMs, we included all two-level interactions among the predictors. Non-significant interactions were sequentially removed from the minimally adequate model testing the main effects.

The key model presented in the paper was fitted to the data following the three hydrogeographical basins ($k = 3$ in STRUCTURE). To account for the uncertainties associated with finding the correct number of genetic clusters in complex data and the possibility that finer-grained population structure was not sufficiently captured by our division into three hydrogeographic regions, we repeated

the modelling for results assuming a range of k values from 2 to 8. However, detailed parameter estimates are only reported for our preferred solution of $k = 3$ in the main text. Key parameter values for the impact of ecosystem status and ecosystem type of native ancestry and hybridisation for other k values are provided in Figures S4 and S5, serving as a robustness check.

3 | RESULTS

3.1 | Assessment of genetic markers

All of the 15 microsatellite loci proved to be highly polymorphic with a total number of 9–37 different alleles over all pike populations and a mean number of 3.5–14.5 different alleles per population (Table 2). The potential presence of null alleles was detected in 1.6% of alleles over all loci and populations (Table S1). Diversity measures for observed (H_O) and Nei's (H_S) heterozygosity ranged from 0.43 to 0.86 and 0.46 to 0.92, respectively, over all populations (Table 2). Populations showed deviation from Hardy–Weinberg equilibrium in 2.7 ± 1.9 loci (mean \pm SD) reflected in significant heterozygote deficiencies in 2.7 ± 1.6 loci (Figure S1). Linkage disequilibrium was detected in 3.3% of all possible loci combinations after Bonferroni correction (Figure S2). Because departures were distributed over many loci and populations, all loci were used for population genetic analysis.

A 1,174-bp region of the mitochondrial *cyt b* gene with 48 variable positions was selected for the network analysis (Figure S3); 208 sequences (including reference sequences) provided a total of 918 informative sites (excluding sites with gaps and missing data).

3.2 | Genetic structure of pike populations in Germany

Analysis of microsatellite genotypes with STRUCTURE suggested that a k value of 2 represented the most likely number of genetic clusters (Figure 1, showing the relevant k range only) when applying the δk criterion. Likelihoods for higher values of k continued to rise and did not visibly plateau, implying the presence of further, more subtle, population subdivision that could not be ignored. To assess the results obtained with STRUCTURE, we considered two alternative population genetic analyses based on microsatellite data, which supported a differentiation into three hydrogeographical regions was most plausible.

First, calculating genetic differences among populations as defined by sampling sites resulted in dendrograms (trees) with three main clusters that confirmed an overall grouping of samples according to hydrogeographic regions (Figure 2). Although bootstrap values were mostly low (and therefore omitted from Figure 2, see supplementary information for details), the overall topology of the consensus trees proved to be stable. The grouping based on Nei's D_A agreed with a genetic structure of pike populations as

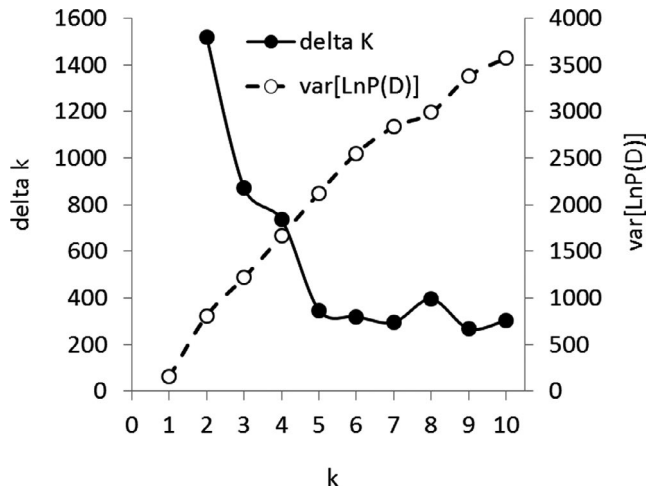


FIGURE 1 Admixture analysis revealed two genetic clusters as the most likely number, as indicated by a decrease in k and an increase in variance of calculated probabilities $P(D)$. This value probably underestimates the true population structure (see text), and thus this study explores the implications of a range of values of k . Only the relevant range of calculated k is shown here

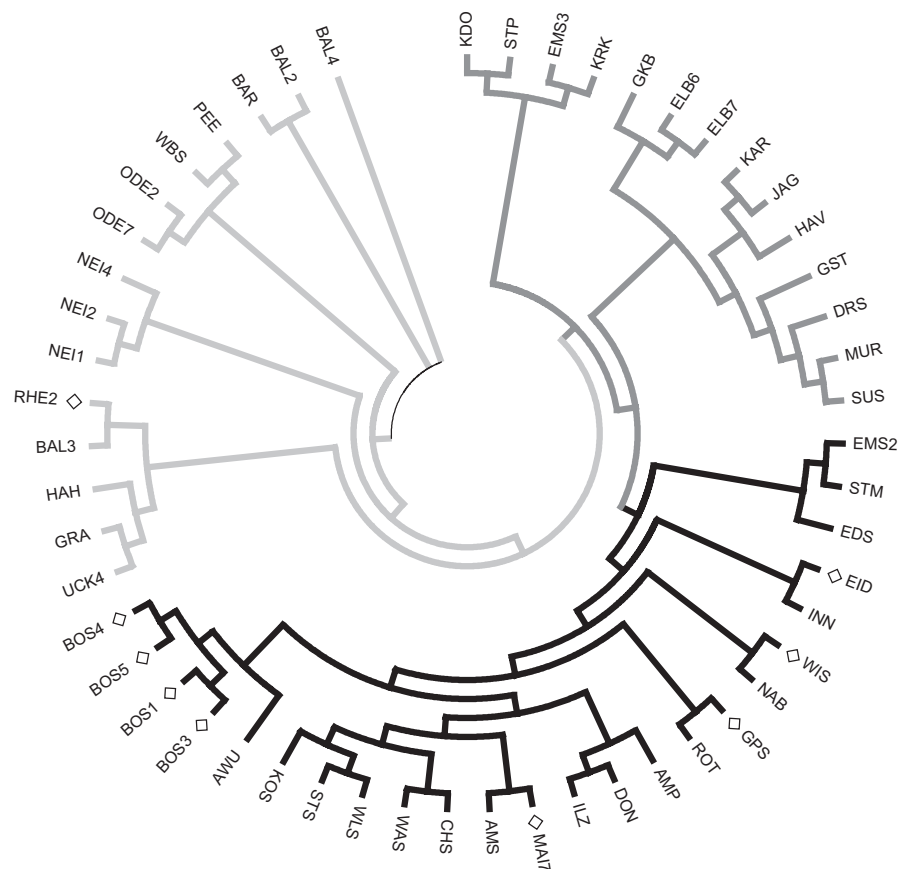
predicted by the assignments of STRUCTURE assuming three as the most likely number of $k = 3$ (Figure 2). Moreover, the two analyses also agreed in that the tree analysis showed that the severely admixed pike populations were grouped into the expected new alternative genetic backgrounds, e.g. the Rhine population (RHE2)

in the Baltic Sea hydrogeographic group or pike of Großer Plöner See (GPS) in the Black Sea hydrogeographic group (Figure 2). Trees based on the two other distance measures - chord distances and proportion of shared alleles—yielded the same basic structure of trees, but showed one and three deviation/s compared to the predictions of STRUCTURE, respectively (see supplement for details).

Second, PCoA based on F_{st} values was employed (Figure 3). Although variation was moderate (accumulated variance explained 23.2% by axes 1 and 2) the clear clustering into three groups reflected differences among the drainage basins of the North, Baltic, and Black seas, respectively (Table 3), which supported a k value of three predicted by the STRUCTURE analysis. Using the PCoA, severely admixed populations were as well positioned in the genetic background as predicted by the STRUCTURE analysis, providing further evidence for $k = 3$. Accordingly, we focused the following analysis on a model assuming genetic subdivision into three hydrogeographic regions as this model appeared best supported by the overall signal of the genetic data.

Network analysis with mitochondrial *cyt b* sequences identified two of the three main haplotypes postulated by Skog et al. (2014). However, while mitochondrial haplotype frequencies differed among drainage basins, there was no one-to-one correspondence of haplotypes with the main clusters identified here based on nuclear data. Accordingly, the haplotypes cannot be used to assign individuals to populations with certainty or to

FIGURE 2 Neighbour-joining consensus tree based on 10,000 permutations for calculating Nei's genetic distance estimator D_A . Although bootstrap values (see supplementary materials) were generally low, the tree topology proved to be stable and was consistent with the most likely STRUCTURE model predicting three main clades (Figure S6). Furthermore, populations with strong signals of foreign admixture according to the structure analysis (labelled with a diamond) grouped according to their predicted dominant genetic background within the respective clades, e.g. the RHE2 population of the river Rhine groups within the Baltic Sea clade (light grey coloured branches) and the GPS population sampled from Großer Plöner See, which is connected with the Baltic Sea is positioned in the Black Sea clade (black coloured branches). Branches of the North Sea clade are drawn in dark grey



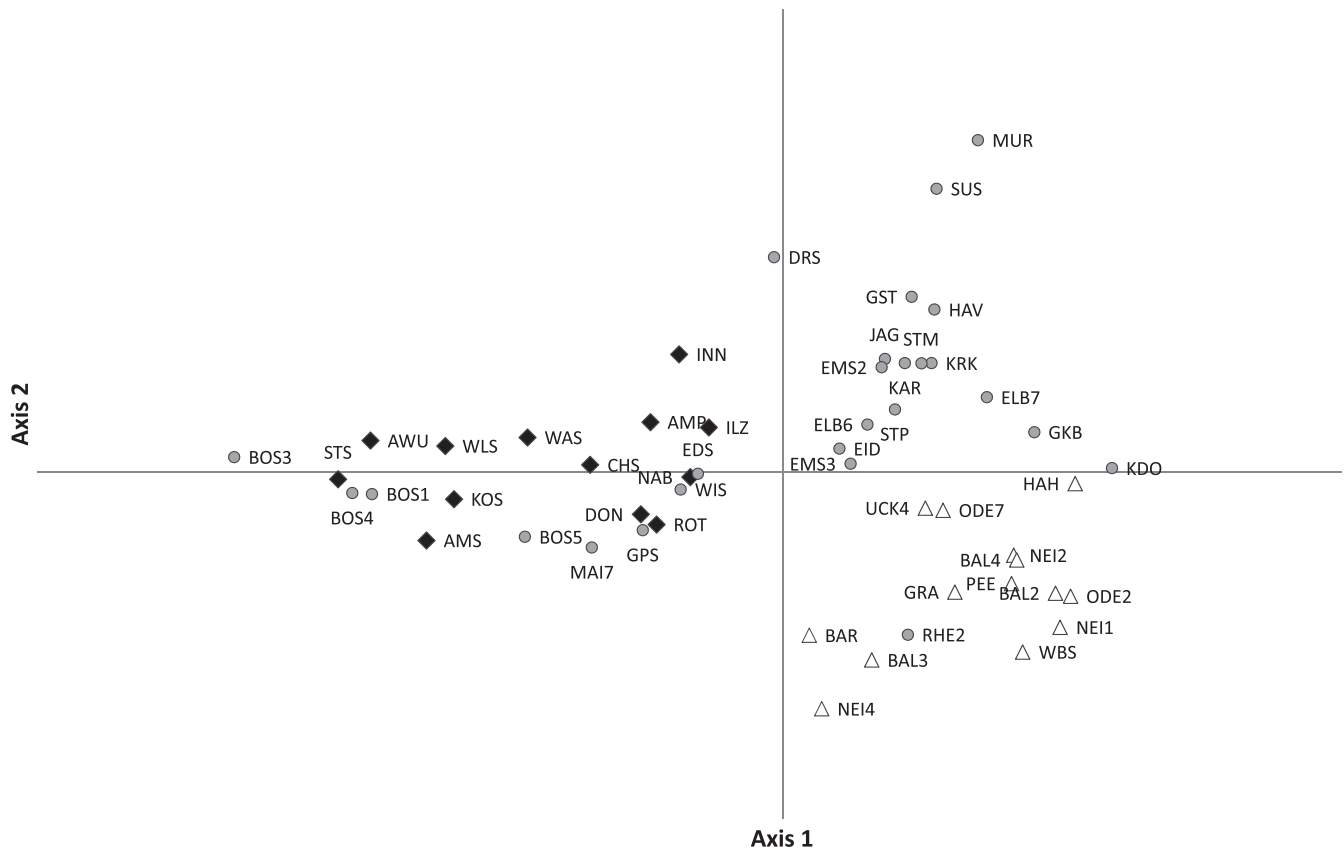


FIGURE 3 Principal coordinate analysis based on pairwise F_{st} values (see Table S2) of all pike samples. Despite low levels of variation (23.2% accumulated variation of axis 1 and 2) the three main clusters predicted by the most likely model of STRUCTURE (Figure S6) were clearly resolved and admixed pike populations were positioned within the correct genetic context

reconstruct an individual's ancestry (see supplement for a more detailed analysis).

3.3 | Population structure among the major hydrogeographic basins

Admixture analysis based on microsatellites revealed varying proportions of the different genetic lineages in pike populations across hydrogeographic regions. The Black Sea genetic cluster was most frequent in pike of the Danube and its tributary rivers (65.3% in pie chart 1 of Figure 4—subsequently indicated as e.g. 65.3% in 1) as well as in pike of the big alpine lakes (77.5% in 1.1 and 91.5% in 1.2; Table 3). Elevated levels of the Black Sea lineage, however, were also found in the geographically close Lake Constance (86.4% in 4.2) and the river Main (63.7% in 4.1), a large tributary of the river Rhine connected via a channel—the Rhein–Main–Donau Kanal—with the Danube.

The Baltic genetic cluster was as expected most prevalent in pike populations of the coastal waters of the Baltic Sea and water bodies draining into that basin, albeit with considerable variation (49.4–89.7% in 3.1, 3.2, 8, 8.1, 9, 11 and 12). Among the populations from Baltic tributaries, pike from the river Oder main stream and from its tributary river Neiße, showed signs of pronounced admixture

with the Black Sea and North Sea genetic lineages (in total 57.2% in three). By contrast, populations of brackish pike around the Island of Rügen in north-eastern Germany showed relatively high levels of the Baltic genetic cluster. However, for these populations the distance-based consensus tree (Figure 2) did not necessarily group the subpopulations sampled within the brackish water and in the tributaries draining into the Baltic Sea neatly by their geographic distance, suggesting the potential for saline-adapted subpopulations. While the genetic distance aligned with short geographic distance for the fish of lake within the catchment of the river Barthe (BAR) and the fish sampled from the nearby brackish lagoon Schaproder Bodden (BAL 2), fish from the river Peene (PEE)—a tributary draining into the estuary of the River Oder close to the mouth of the Baltic grouped closer to populations from river Oder (ODE 2 and ODE 7) and an inland lake (Werbellinsee, WBS) than to the pike sampled from the nearby brackish water of Stettiner Haff (BAL 4)—the estuary of the river Oder.

The North Sea genetic cluster was dominant in populations of the river catchments of Elbe (78.7% in 2 and 76.5% in 2.1, excluding Wittensee with only 31.7% in 2.2), Ems (74.8% in 6) and Weser draining into the North Sea. Unexpectedly, the river Rhine exhibited a higher proportion of the Baltic than of the North Sea genetic cluster (63.6% in 4), which was also mirrored in the distance-based consensus tree (Figure 2) and the frequency-based principal coordinate

TABLE 3 Admixture analysis of pike populations

ID	No. in Figure 4	Proportion of ancestry:			ID	No. in Figure 4	Proportion of ancestry:		
		North Sea	Baltic Sea	Black Sea			North Sea	Baltic Sea	Black Sea
AMP	1	0.140	0.048	0.812	ODE2	3	0.306	0.598	0.096
AWU	1	0.138	0.040	0.821	ODE7	3	0.416	0.487	0.097
DON	1	0.225	0.111	0.664	NEI1	3	0.450	0.370	0.181
ILZ	1	0.218	0.108	0.674	NEI2	3	0.629	0.180	0.191
INN	1	0.278	0.215	0.507	NEI4	3	0.176	0.509	0.316
NAB	1	0.407	0.106	0.487	WBS	3.1	0.068	0.897	0.035
ROT	1	0.312	0.084	0.605	BAL2	3.2	0.066	0.911	0.022
CHS	1.1	0.188	0.063	0.750	BAL3	3.2	0.218	0.733	0.050
WAS	1.1	0.169	0.031	0.801	BAL4	3.2	0.263	0.695	0.042
AMS	1.2	0.037	0.070	0.894	RHE2	4	0.275	0.636	0.089
KOS	1.2	0.093	0.039	0.867	MAI7	4.1	0.236	0.127	0.637
STS	1.2	0.036	0.026	0.937	BOS1	4.2	0.037	0.059	0.904
WLS	1.2	0.021	0.015	0.963	BOS3	4.2	0.027	0.019	0.954
ELB6	2	0.752	0.043	0.205	BOS4	4.2	0.166	0.043	0.791
ELB7	2	0.714	0.155	0.131	BOS5	4.2	0.150	0.044	0.806
HAV1	2	0.793	0.097	0.110	EDS	5	0.345	0.204	0.451
KAR	2	0.801	0.104	0.095	STM	5.1	0.843	0.051	0.107
JAG	2	0.855	0.062	0.083	EMS3	6	0.677	0.195	0.128
KRK	2	0.878	0.078	0.044	EMS2	6	0.819	0.076	0.105
STP	2	0.713	0.142	0.144	EID1	7	0.439	0.060	0.501
MUR	2.1	0.821	0.157	0.023	UCK4	8	0.299	0.653	0.048
SUS	2.1	0.952	0.026	0.021	HAH	8.1	0.188	0.778	0.034
GST	2.1	0.837	0.054	0.108	PEE	9	0.183	0.732	0.085
GKB	2.1	0.527	0.375	0.097	GPS	10	0.270	0.059	0.671
KDO	2.1	0.649	0.308	0.043	GRA	11	0.439	0.494	0.068
DRS	2.1	0.802	0.078	0.120	BAR	12	0.169	0.743	0.088
WIS	2.2	0.317	0.095	0.588					

Note: Three genetic clusters of pike populations were identified belonging to the hydrogeographic regions of the North, Baltic, and Black seas, respectively (shaded areas indicate highest proportion of ancestry). Some populations exhibited high shares of non-native ancestry (indicated in italic writing). See Table 1 for definition of sample IDs. Samples with the same number have been pooled for a clearer presentation in Figure 4.

analysis (Figure 3). Accordingly, the Rhine population (RHE2) appeared within the Baltic Sea cluster, and pike from GPS were placed within the Black Sea cluster.

3.4 | Admixture at the individual level

Genetic admixture was examined at the level of individuals within pike populations to assess the homogeneity of ancestries and investigate for possible signs of population substructure (Figure 5). Based on ancestry coefficients (NA), the distributions of native versus foreign genetic ancestries displayed a range from mostly pure native populations (populations in Figure 5 with mean NA ≥ 0.50) to sampling sites with mostly admixed individuals (populations in Figure 5 with $0.25 < \text{NA} < 0.50$). Moreover, some distributions were skewed

towards foreign ancestry, with complete replacement of native ancestry in some other populations (populations in Figure 5 with mean NA ≤ 0.25). The frequency distribution of ancestry coefficients in some populations showed signs of bimodality, that is, individuals may fall into different groups that differ in their ancestry coefficients (Figure 5).

Ancestry distributions within pike populations of the Black Sea hydrogeographic region generally exhibited higher proportions of native ancestries (particularly in pike of the alpine lakes), whereas the two other hydrogeographic regions were comprised of populations in which individual genotypes suggested high proportions of foreign genetic material. The most extreme examples included pike of GPS and of the rivers Neiße (NEI2) and Main (MAI), where a near-complete replacement of native by foreign genetic identities was found (Figure 5) and confirmed by genetic distance trees (Figure 2)

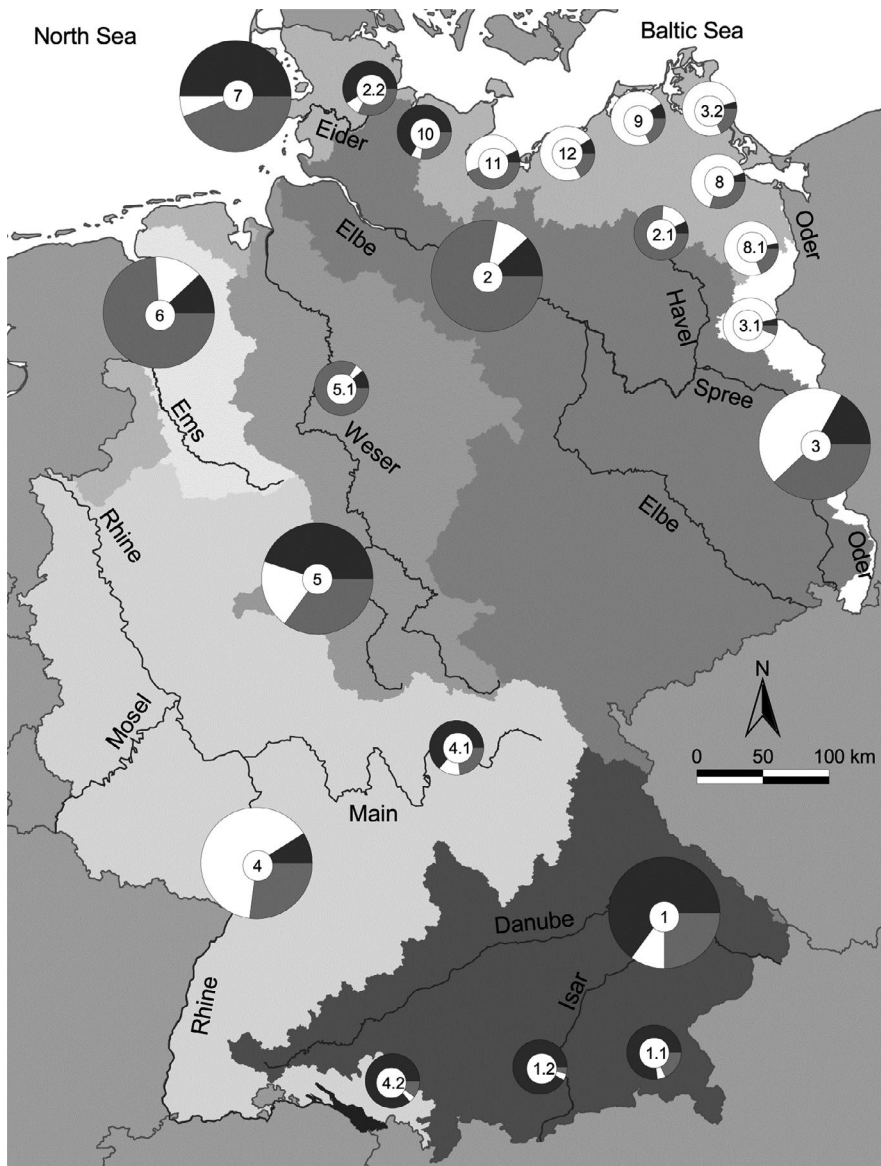


FIGURE 4 Map of Germany illustrating that genetic admixture on population level varied strongly and was not confined to a particular hydro-geographic region or river catchment therein. Black, grey, and white in the pie charts indicate genetic ancestry proportion of Black Sea, North Sea, and Baltic Sea hydrogeographic region, respectively. Different catchments are indicated by the shaded areas. Numbers indicate pooled populations as displayed in Table 3. Large pies indicate river populations, small pies lake populations

and principal coordinate analysis (Figure 3). Pike populations in Lake Constance (e.g. BOS1 and 3), however, should be viewed differently due to their geological history (see discussion for further details). In the Baltic Sea hydrogeographic region, a coastal population (BAL2, Schaproder Bodden in Figure 5) and one freshwater population (WBS, Werbellinsee) exhibited pronounced native genetic signatures. Likewise, the North Sea hydrogeographic region harboured populations that appear to be rather typical and pure representatives of the respective genetic cluster (JAG, KRK, GST, MUR).

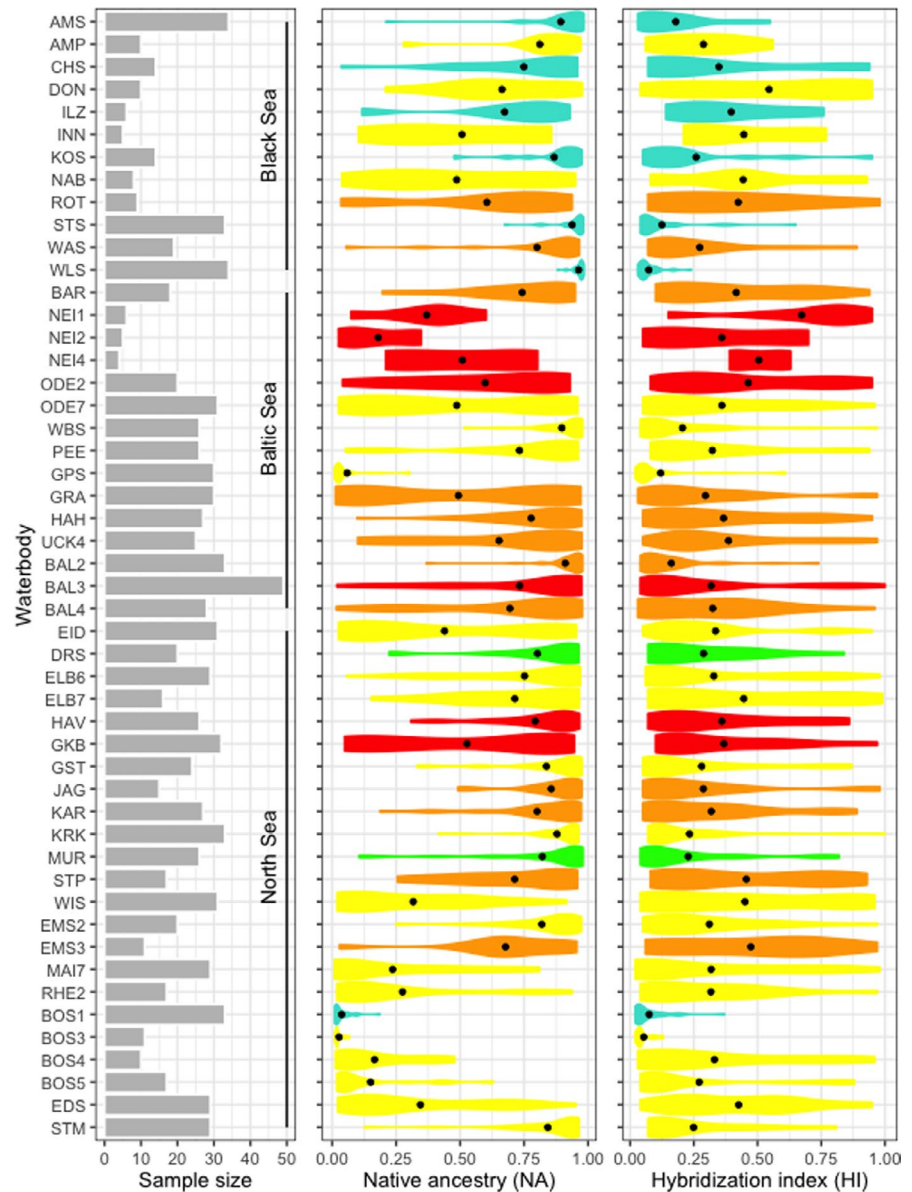
3.5 | Correlation of hybridisation levels with ecological status and ecosystem type

Employing hierarchical general linear models revealed that the ecological status of the water body as well as the type of ecosystem had a significant effect on the HI of pike populations. Specifically, the decline in the ecological status highly significantly covaried with

HI (Table 4). Each unit of decrease of the ecological status led to an increase of HI by a factor (slope) of 0.19 (1.21 in raw scale) \pm 0.05 with respect to the intercept (defined as the best ecological status). Results were consistent when using the ecological status defined according to the European WFD (Table 4). The estimate (in logit scale) of HI for lakes was -1.40 (0.24 in raw scale) \pm 0.18, while it was -1.02 (0.36 in raw scale) \pm 0.12 in rivers. These values indicated a significantly stronger signal of past hybridisation of pike populations in rivers as compared to lakes (Table 4). While the hypothesis of a covariance between HI and the type and ecological status of the water body was supported, relationships of HI and the general level of modification of the water body (Table 4) and all two-level interaction effects were not supported (data not shown).

The NA exhibited a similar association with the deterioration of the ecological status of the water bodies (Figure 6); however, the relationships were not statistically significant (Table 4). This held also true for the predictor *type* (i.e. lake vs. river) and *modification* (i.e. general degree of water body modification) of the water bodies

FIGURE 5 Genetic admixture calculated per individual. Extension of a figure indicates increased numbers of individuals with a certain proportion of native ancestry (column 2) or degree of hybridisation (column 3). Mean values are indicated within each figure as a dot. Column 1 indicates the number of individuals analysed per sampling site. Colour code for native ancestry and hybridisation index refer the ecological status according to the European WFD: 1 (very good—green), 2 (good—turquoise), 3 (average—yellow), 4 (poor—orange), and 5 (very poor—red) [Colour figure can be viewed at wileyonlinelibrary.com]



(Table 4), as well as their two-level interactions (not shown), which were therefore removed from the model with NA.

The results mentioned above for the relationship with the ecological status following the WFD was mirrored by the covariance of the hybridisation index with the indicator for wetland quality/trophic state (Table 4). As before, increasing losses of the wetlands and increasing eutrophication significantly increased the degree of hybridisation among subpopulations of pike (Table 4, Figure 6). Signs for hybridisation were also more pronounced in rivers relative to lakes (Table 4, Figure 6).

The environmental regression model was also fitted to ancestry coefficients as derived from STRUCTURE analyses assuming a range of k values from 2 to 8 to examine the robustness of the findings. While the same trends for covariation were detected across most values of k , the results were not significant for a $k = 2$ (Figures S4 and S5). By contrast, the outcomes of the covariation of ancestry measures with ecological status as detected for a

model assuming $k = 3$ genetic clusters were significant for k values of up to six (Figures S4 and S5), in all cases showing that the degree of hybridisation increased with declining environmental quality (independent of the index) and was always larger in rivers relative to lakes.

4 | DISCUSSION

Our analysis of the population genetic structure of pike from 53 sampling sites covered lakes and major rivers from all three hydrogeographic regions in Germany. Admittedly, we were unable to assign the pike genotypes unambiguously to a certain number of genetic clusters, which we attribute to a shallow and complex population structure previously reported for pike populations (Bekkevold et al., 2015; Jacobsen et al., 2005; Pedreschi et al., 2014; Wennerström et al., 2018). A shallow genetic population structure

TABLE 4 Results of hierarchical general linear mixed modelling

Model 1	Estimate	SE	t-value	Pr(> t)
Hybridisation index				
(Intercept)	-1.401	0.176	-7.98	<0.001***
Type (river)	0.376	0.119	3.16	<0.01**
Modification (high)	-0.128	0.125	-1.03	0.3
Ecological status	0.186	0.053	3.49	<0.001***
Native ancestry				
(Intercept)	0.731	0.44	1.66	0.096
Type (river)	-0.247	0.239	-1.030	0.301
Modification (high)	0.389	0.266	1.460	0.143
Ecological status	-0.16	0.104	-1.550	0.122
Model 2	Estimate	SE	t-value	Pr(> t)
Hybridisation index				
(Intercept)	-1.81	0.3	-6.32	<0.001***
Type (river)	0.57	0.17	3.34	<0.001***
Modification (high)	-0.35	0.19	-1.84	0.07
Ecological status—WTS	0.32	0.11	2.89	<0.01**
Native ancestry				
(Intercept)	-1.49	0.84	-1.78	0.07
Type (river)	0.23	0.47	0.48	0.63
Modification (high)	-0.5	0.54	-0.94	0.35
Ecological status—WTS	0.34	0.31	1.1	0.27

Note: The effect of different linear predictors on the hybridisation index and the native ancestry was tested controlling for the random variance attributed to the individuals sampled in specific waterbodies nested within catchments (see Figure 6). The table shows the estimates (in logit scale) and their standard error (SE), the t-value statistics and their p-value (Pr(>|t|)) for the model fitted with ecological quality according to EU Water Framework Directive (model 1) and the wetland and trophic status—WTS (model 2). Two-level interactions were non-significant in all cases and removed from the model. The estimates of the categorical variables were shown per one category with respect to the other (intercept). Significance codes: * Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; and *** Significant at the 0.001 probability level.

of German pike populations was also supported by our analysis of mitochondrial haplotypes. Pike populations are not known to contain deeply divergent lineages in central Europe (Nicod et al., 2004; Skog et al., 2014). A haplotype NETWORK (Bandelt et al., 1999) of *cyt b* haplotypes assigned about 90% of pike originating from the hydrogeographic region of the Baltic Sea to the circumpolar clade and almost 60% of pike originating from the North Sea region to the northern clade as described by Skog et al. (2014). In our data, the southern clade, identified as a third mitochondrial haplotype by Skog et al. (2014), was represented by only a single individual among 21 pike from the Danube catchment. Thus, although our analysis of mitochondrial haplotypes agrees with the general findings from Skog et al. (2014), our data showed that lineage sorting of mitochondrial

haplotypes has not proceeded to a point where haplotypes alone are sufficient to distinguish the lineages of pike studied here. Hence, the strongest support for the existence of three evolutionarily significant units (Moritz, 1994) of pike with different distribution areas was supported by multilocus microsatellite analyses.

4.1 | Genetic structure of pike in Germany

Using microsatellite markers, clear signs for genetic substructure were found and manifest as groups of individual sampling sites that containing mostly one genetic ancestry. Genetic clustering also revealed that pike from some sampling sites had genetic ancestry in multiple populations, which could be a sign of genetic admixture or be caused by a lack of resolution of the available genetic data (Lawson et al., 2018). A striking pattern in the data was that this inability to clearly assign a single genetic ancestry appeared to be particularly prevalent in rivers and was also correlated with ecological status. This suggests that a lack of genetic distinctness may not only be caused by the connectivity of ecosystems (which is likely to be more pronounced in rivers compared to isolated lakes, in particular when river catchments are connected via canals and other migration routes) but may also be related to an erosion of population structure that is associated with human activity.

Secondary contacts between divergent pike lineages are very likely to have increased in the last centuries as a result of anthropogenic activities. Humans have facilitated natural migration through human-made artificial connections among different river basins as well as stocking of economically important fish species. The latter represents an important factor that increases the potential for gene flow between populations naturally separated in space (Arlinghaus et al., 2015) and is known to shape the genetic diversity of the congeneric muskellunge (*Esox masquinongy*) in North America (Roegemont et al., 2019). Both processes are expected to lead to admixed genotypes and may lead to an erosion of population-specific genetic variance. Unfortunately, past stocking is generally not well documented in Germany (Arlinghaus et al., 2015), but certainly has occurred over decades in central Europe in pike and multiple other economically relevant fishes (Cowx, 1994; Guillerault et al., 2018; Kottelat & Freyhof, 2007; Larsen & Berg, 2004).

Our work relies on correlative evidence and thus cannot be conclusive. It is likely that the generally low level of genetic differentiation in pike populations (Wennerström et al., 2018) and the variation in sample size in the different populations did not recover the exact genetic structure present across Germany. Nevertheless, some of our findings are consistent with expectations and allow the suggestive identification of environmental factors that may stabilise existing diversity patterns or, conversely, promote admixture. A few examples follow:

Pike of the Danube catchment and of the brackish coastal areas of the Baltic Sea, for example, exhibited a clear dominance of native ancestry as inferred from analysis of 15 polymorphic microsatellites. Similar results were recently reported by Möller et al. (2021) from pike populations around Rügen. Likewise, relatively low admixture

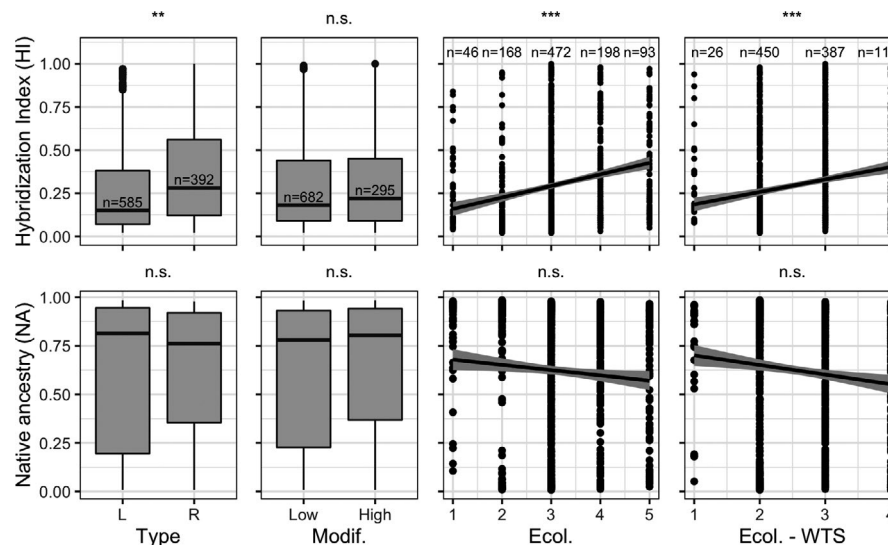


FIGURE 6 Relationships of native ancestries and hybridisation indices with habitat type, strength of modification, ecological quality (1 = very good to 5 = poor according to EU Water Framework Directive and the combined wetland/trophic status for pike ecology—WTS—from 1 = very good to 5 = very poor) as obtained with HGLM analysis (see Table 4 for details). Baltic coastal waters (BAL2, BAL3 and BAL4) and freshwaters without ecological information (AWU, SUS, KDO) were excluded from analysis. IDs of water bodies are explained in Table 1. Significance codes: n.s. non-significant, * Significant at the 0.05 probability level, ** Significant at the 0.01 probability level and *** Significant at the 0.001 probability level

levels were observed in pike populations of the Ems and Weser catchments belonging to the North Sea hydrogeographic region. A possible explanation for the persistence of autochthonous populations is either a low level of local stocking or competitive exclusion of foreign genotypes by better-adapted native populations (Englbrecht et al., 2002; Eschbach et al., 2014; Gandolfi et al., 2017; Larsen et al., 2005; Möller et al., 2021; Roegemont et al., 2019).

In other water bodies pike individuals showed high foreign ancestries, e.g. in the rivers Neiße, Main, Rhine, and Eider, as well as in the lakes Wittensee, GPS, and Edersee. These data suggested that much of the genetic material from native populations may be replaced through the introduction of foreign stocks. Note that the high proportion of Black Sea hydrogeographic region ancestry in pike of Lake Constance could be a result of ancient natural connections with the Danube catchment and may represent native ancestry stemming from natural post-glacial dispersal, previously reported for perch (*Perca fluviatilis*) (Behrmann-Godel et al., 2004).

Pike populations in several other water bodies exhibited high levels of genetic admixture. When source populations are adjacent, admixture can be explained by natural immigration through man-made connections such as the Main–Donau Kanal linking the Danube with the river Main (Powels et al., 2013). However, we also detected signatures of admixture between rather distant source populations, e.g. between pike of the rivers Oder in the east and Rhine in the west or between the rivers Danube in the south and Eider in the very north of Germany. Stocking, rather than migration, is a more likely explanation here (Monk et al., 2020), because migration would probably have created a more coherent geographical pattern. Our data are in line with genetic structures of pike populations in Denmark at the intra-specific level

(Bekkevold et al., 2015) and Italy at the inter-specific level (Gandolfi et al., 2017), both of which not always reflected natural catchment barriers and were probably caused by successful pike stock enhancement activities in the past. Similar results exist for muskellunge in North America (Roegemont et al., 2019).

4.2 | Impacts of ecosystem status on hybridisation

We detected various degrees of mixed ancestries in a range of sampling sites, either documenting admixture between genetically distinct populations or a lack of clear population differentiation. This effect was significantly associated with rivers and increased with the degradation of the ecological status of ecosystems. While the ecological status indicator of the WFD does not directly indicate which ecological factor was involved, our wetland/trophic state index is strongly suggestive that the loss of key pike habitat, particularly loss of access to submerged macrophytes, correlated with the increasing admixture rates. The quality of this inference depends on the sample sizes that were available for each population as well as the degree of differentiation between the presumed source populations. Given the low resolution of microsatellites, it is certainly useful to revisit specific populations with a more powerful study design and genome wide marker coverage. Before such research becomes available, the current analysis suggests that the pike lineages hybridised upon secondary contact. This result bears general questions on why hybridisation proceeded with different intensity in different pike populations and whether pike of different origins are indeed isolated to some extent when they are brought into secondary contact. Note again that the secondary contact might have been caused by

stocking or any other factors, e.g. increased connectivity of water bodies among catchments.

We found that the individual admixture levels in pike, expressed as an HI, were not confined to a specific hydrogeographic region or any particular river catchment therein. Instead, it turned out that the HI increased significantly with decreasing ecological quality of a water body, and this result was robust for k values from three to six and for two different indices of ecological status. Albeit not statistically significant, we observed a congruent decrease of native ancestry with habitat modification. Thus, our data suggest environmental change drove genetic changes in pike populations and individuals by affecting the frequency of hybridisation among populations brought into secondary contact. The fact that the HI was only slightly lower in water bodies with low modifications as compared to the HI of highly modified waters implies that the admixture as such occurs in all populations and is not restricted to highly modified habitats.

Our analysis yielded a significantly higher HI in pike populations in rivers as compared to lake-dwelling pike, which is probably due to fundamental ecological differences between the two habitat types such as the increased natural connectivity in rivers, resource availability, productivity, habitat structure, and community composition (Hof et al., 2008; Irz et al., 2006). Most importantly, however, rivers and lakes vary in stability and disturbance frequency, including exposure to floods or minimum water flows, which occur more frequently in lotic than in lentic systems. Rivers of central Europe also have been more strongly modified, e.g. by removal of connectivity to floodplains and habitat simplification (BFN, 2009), which represent a central component of their disturbance regime and at the same time constitute essential spawning habitat for pike. In a meta-analysis comparing resistance of limnic, marine and terrestrial ecosystems towards invasive species, Alofs and Jackson (2014) demonstrated that lentic systems displayed a higher biotic resistance than lotic systems, which is in accordance with our findings of different susceptibilities towards hybridisation in river and lake pike populations.

Our observation that a lack of population genetic differentiation and signs for admixture in pike appears to be favoured in ecologically perturbed water bodies raises important questions about the mechanisms. The effect could first be caused by an increase of foreign genotypes that managed to invade a weakened native population (Englbrecht et al., 2002; Gandolfi et al., 2017) and it could be affected by demographic processes (Mathieu-Bégné et al., 2019). Alternatively, genetically admixed fish could be more competitive in the face of anthropogenic changes to the ecosystem. This would resemble the first step of a hybrid speciation scenario, where intraspecific hybrids are expected to be most successful when parental populations are not at their optimum (Abbott et al., 2013; Nolte & Tautz, 2010). Stelkens et al. (2014) showed that particularly the interactions of genetic variants between distant *Saccharomyces* strains can lead to a better survival in environments of decreasing quality. Thus, hybridisation can create biodiversity, resulting in novel phenotypes and adaptive change in response to environmental change (Charlesworth & Willis, 2009;

Edmands, 2007; Sefc et al., 2017). Examples of these processes can be found among invaders conquering new environments that were not occupied by populations of the respective species before, as it was found for *Cottus* hybrids in the river Rhine (Nolte et al., 2005; Stemshorn et al., 2011), but also for spiders (Krehenwinkel & Tautz, 2013) and plants (Keller & Taylor, 2010). Likewise, in a previous study we observed increased intraspecific genetic diversity of zander (*Sander lucioperca*) in water bodies, where this fish species had been introduced in the late 19th century, a pattern that would be in line with an advantage of admixed individuals in the course of an invasion (Eschbach et al., 2014). Thus, new combinations of genes from different evolutionary backgrounds might enable fast adaptation, and thus increase the chance to survive under declining environmental conditions. However, careful future studies are needed to distinguish the adaptive scenario outlined here from neutral explanations that are related to abrupt changes in propagule pressure in fluctuating environments.

4.3 | Conclusions and implications

Our study revealed a novel relationship between ecosystem status, assessed under the European WFD and via a wetland/trophic state index, and the genetic structure of northern pike across Germany. Due to the limitations in sample size, our wide geographical scope and the lack of time series data, our findings of the relationship of genetic structure and environmental status in pike necessitates more careful analysis using genetic markers of higher resolution. However, our work supports the novel hypothesis that habitat degradation can affect the genetic integrity within pike. Efforts to improve the ecological quality of lakes and rivers could therefore promote the maintenance of genetic structure. In the case of pike, this would mean reconnecting floodplains with rivers, or wetlands to lakes or brackish lagoons (Nordahl et al., 2019), and reducing nutrient inputs into lakes to increase macrophyte abundance. These actions would probably increase the pike population size and help to maintain local genetic biodiversity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

cyt *b* haplotypes of pike are available from the NCBI database: KY399416 to KY399442. Microsatellite genotypes of pike are available from Dryad Digital Repository: <https://doi.org/10.5061/dryad.tdz08kpmz>.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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