Intraspecific population admixture of a top piscivore correlates with anthropogenic alteration of freshwater ecosystems

Erik Eschbach (corresponding author), Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany – current adress: Thünen-Institute of Fisheries Ecology, Herwigstr. 31, Bremerhaven, Germany, +49 471 9446 0278, erik.eschbach@thuenen.de

Arne Wolfram Nolte, Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Biology, August-Thienemann-Str. 2, 24306 Plön, Germany – Department of Ecological Genomics, Institute for Biology and Environmental Sciences, Carl von Ossietzky University, 26111 Oldenburg, Germany, arne.nolte@uol.de

Klaus Kohlmann, Department of Ecophysiology and Aquaculture, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany, kohlmann@igb-berlin.de

Josep Alos, Instituto Mediterráneo de Estudios Avanzados, IMEDEA (CSIC-UIB). C/ Miquel Marqués 21, 07190, Esporles, Illes Balears, Spain, alos@imedea.uib-csic.es

Sandro Schöning, Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany, sandro.schoening@web.de

Robert Arlinghaus, Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany – Chair of Integrative Fisheries Management, Faculty of Life Sciences, Humboldt-Universität zu Berlin, Invalidenstrasse 42, 10115 Berlin, Germany, arlinghaus@igb-berlin.de

Abstract

Conservation of local genetic diversity is an important policy objective, but intraspecific genetic diversity can be transformed by natural ecological processes associated with anthropogenic changes in ecosystems. Environmental changes and a strong interconnection of drainage systems impact freshwater biodiversity from gene to population level. Populations can either become extinct or expand their range and accompanying secondary contacts can lead to genetic admixture. We investigated how the genetic population structure and the patterns of genetic admixture of *Esox lucius* L. (the northern pike) vary with the type of ecosystem and the integrity of the ecosystem assessed by measures under the European Water Framework Directive. The pike inhabits river, lake and brackish water ecosystems, where it is confronted with different ecological disturbances. We analysed 1,384 pike samples from the North, Baltic and Black Sea drainages and differentiated between metapopulations from each hydrogeographic region using genotypes from 15 microsatellites and mitochondrial *cyt b* sequences. Individual populations showed signs of genetic admixture ranging from almost zero to complete replacement by foreign genotypes. Hierarchical general linear modeling revealed a highly significant positive association of the degree of genetic admixture with decreasing ecological status. This may mean that populations in disturbed environments are more prone to influences by foreign genotypes or, alternatively, increased genetic admixture may indicate adaptation to rapid environmental changes. Regardless of the underlying mechanisms, our results suggest that anthropogenic alterations of natural freshwater ecosystems can influence genetic structures, which may lead to a large-scale reduction of intraspecific genetic diversity.

46

47 **Key words**

48 admixture - ancestry distribution – ecological modification – *Esox lucius* – stocking –

49 range expansion

Introduction

In the age of the Anthropocene, animal and plant populations have to cope with landscapes that are used and intensively altered by humans (Christie & Knowles, 2015; Ortego et al., 2015; Sexton et al., 2013), which is discussed as the most prominent factor leading to loss of genetic diversity and evolutionary potential, and which in turn can result in the irreversible loss of populations and species (Dulvy et al., 2003; Laurance & Useche, 2009; Smith & Bernatchez, 2008). However, before extinction occurs, genetic structure of species and populations is expected to undergo changes, which may reveal processes and functions that help organisms to adapt to changing environmental conditions (Arnold, 2016).

Studies investigating fish communities and populations demonstrated that aquatic ecosystems react particularly sensitive to ecological changes (e.g. Whitehead et al., 2017), like habitat fragmentation, increasing isolation of populations through migration barriers (Waples et al., 2017), artificial opening of new routes for migration and intentional transplantation of individuals through stocking and introduction (Laikre et al., 2010). Particularly, stocking as a widespread management practice mediates direct secondary contacts among populations leading to unpredictable outcomes as a plethora of studies have shown (Allendorf et al., 2001; Diana et al., 2017; Hansen, 2002; Marie et al., 2012; van Poorten et al., 2011). According to these investigations stocked fish either disappear without a trace or become established to varying degrees, which eventually leads to admixture of non-native with native populations or even complete replacement of native populations. For example, Englbrecht et al. (2002) showed that some native populations of arctic char (*Salvelinus umbla*) were not genetically affected despite massive stocking in natural lake systems, while other natural populations were replaced by stocked char of different origin after severe

eutrophication of an alpine lake. Therefore it is possible that environmental degradation through human-induced eutrophication was responsible for the natural population losing their buffering potential and rendering it vulnerable to invasion by non-native genotypes. As another example, Harbicht et al. (2014) identified a number of physico-chemical (oxygen, pH, temperature), morphometrical (surface area and depth of lakes) and topographical (elevation) factors that significantly influenced admixture in brook trout (*Salvelinus fontinalis*) as a result of stocking. Similarly, numerous studies have shown that hybridization is enhanced at the interspecific level in ecologically perturbed habitats, e.g. in African cichlids *Cichlidae* (Seehausen et al., 1997; 2008), sculpins *Cottidae* (Nolte et al., 2005), European whitefish *Corregonus 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 their exact nature and how they might affect intra- and interspecific hybridization. Although integrative measures of ecosystem status are readily available at national and international scales and can be indicative of the environmental challenges faced by a range of taxa in the wild, research linking ecosystem status and genetic structuring of freshwater vertebrates on large geographical scales is still scarce. In Europe, the Water Framework Directive was introduced as a comprehensive policy to monitor and improve freshwater ecosystem quality and its ecological status (European Commission, 2000). Accordingly, rivers and larger lakes are regularly assessed based on a range of biological indices, from phytoplankton to fish, to assess their ecological status and inform management actions. Genetic population structures within species, however, are currently not

100 considered in this context, although it is likely that the ecological status of water
101 bodies is systematically related to the meta-population structure of individual species.

102 *Esox lucius* L., the northern pike, may well be affected by contemporary
103 environmental change as e. g. by the loss of floodplains in rivers and nutrient inputs
104 in lakes (Craig, 1996, Skov & Nilsson, 2018). To fulfill their life-cycle pike strongly
105 depend on aquatic macrophytes providing shelter for early developmental stages and
106 camouflage to hunt for prey during the juvenile stage. After reaching sexual
107 maturation flood plains are essentially needed as spawning ground (Casselman &
108 Lewis, 1996; Craig, 1996; Raat, 1988). It is very likely that changes in these
109 ecological key-features affect infra- and intraspecific outcomes upon secondary
110 contacts caused by stocking (Cowx, 1994; Guillerault et al., 2018; Hühn et al., 2014)
111 or by active migration through canals connecting once separated water bodies
112 (Pauwels et al., 2013). For example, Gandolfi et al. (2017) studying the invasion
113 process of *Esox lucius* into closely related native Italian *Esox flaviae/cisalpinus*
114 (Lucentini et al., 2011; Bianco & Delmastro, 2011) populations, observed a mosaic-
115 type distribution of the two species and different degrees of genetic admixture,
116 possibly as a result of the different ecological status of the studied water bodies:
117 Lake Garda, which provides good ecological conditions for native
118 *E. flaviae/cisalpinus* to fulfill its natural life cycle, still seems to out-compete the
119 establishment of northern pike, while other Italian waters with a less good ecological
120 status were strongly prone to genetic introgression. Recently in Danish pike
121 populations at least two regional clusters were identified referring to the
122 hydrogeographic regions of the Baltic and the North Sea, which could be further sub-
123 divided at different river catchment scales. At the same time also deviations from
124 native signatures were observed on local scales, prompting the authors to speculate

125 human-assisted ecological changes affecting habitat quality as possible reasons
126 besides historical geological alterations (Bekkevold et al. 2015; Wennerström et al.,
127 2018).

128 The objective of the present study was to explore whether the ecological status of the
129 inland waterbodies in Germany has possibly influenced the genetic structure of
130 contemporary pike populations, particularly with respect to genetic admixture among
131 populations of *E. lucius*. Therefore we extended the approach by Bekkevold et al.
132 (2015) to a larger geographical scale across all principal drainage systems in
133 Germany. More specifically, we evaluated the presence of population structure that
134 permitted genetic assignment of individuals to their origins and used this information
135 to identify large-scale and local signs for intra-specific admixture. Finally we tested
136 whether the observed genetic patterns correlated with the ecological status of the
137 water bodies assessed according to the European Water Framework Directive.

138

139 **Material & Methods**

140 *Sampling and DNA extraction*

141 Sampling was performed in 2011 and 2012 applying a sampling scheme that covers
142 as many relevant water systems and types as possible over a wide geographical
143 area in Germany, accepting that not all sampling points could be sampled with the
144 same intensity. Nevertheless, we have established standards, i.e. a minimum of 10
145 individuals characterized by at least 14 microsatellites. The sample collection
146 comprised specimens from five river catchments draining into the North Sea, six
147 catchments draining into the Baltic Sea, and one catchment draining into the Black
148 Sea (Table 1). Three ecosystem types were covered including 26 lakes, 24 rivers

149 and three brackish coastal water areas (Table 1). Pike were sampled from water
150 bodies covering the complete range of ecological states, from very good (status 1) to
151 poor (status 5), as defined by the European Water Framework Directive (WFD,
152 2000/60/EC): two samples from status 1, seven from status 2, 22 from status 3, 10
153 from status 4 and nine samples from status 5 water bodies. 37 waters were classified
154 as natural and 13 were classified as heavily modified (see DRYAD deposited
155 material for details). For three small water bodies (Alte Würm, Kleiner Döllnsee, and
156 Schulzensee) no data according to the Water Framework Directive were available
157 because they are only generated for standing water bodies beyond 50 ha in size.

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

167

168 *Genetic marker analysis*

169 We employed nuclear as well as mitochondrial markers to infer population structure
170 and to compare our data with published data. Fifteen polymorphic microsatellites
171 (Table 2) for pike were selected according to Eschbach & Schöning (2013). These
172 were employed to analyze a subset of 1,384 samples of 53 populations with an

173 average sample size of 22.1 ± 9.8 (mean ± SD) individuals per population, and
174 96.4 ± 94.0 (mean ± SD) individuals per river catchment. Microsatellites were co-
175 amplified in multiplex PCR (Table 2) with a Thermocycler T Gradient machine
176 (Biometra, Goettingen, Germany) using the Qiagen® Multiplex PCR Kit (Qiagen,
177 Hilden, Germany). Forward primers were 5´-labeled with fluorescent dyes HEX, NED
178 or FAM (SMB Services in Molecular Biology GmbH, Berlin, Germany) (Table 2). PCR
179 started with 15 min at 95°C, followed by 35 cycles of 0.5 min at 94°C, 1.5 min at
180 58°C, 1.5 min at 72°C, and finishing with 10 min at 72°C. Fragments were sized with
181 an Applied Biosystems 3500xL Sequencer equipped with a 24-capillary array.
182 Chromatograms were evaluated with GeneMapper® Software v4.1 (Life
183 Technologies, Darmstadt, Germany).

184 Haplotype analysis of the mitochondrial cytochrome b gene (*cyt b*) was carried out to
185 link the present data set with the broad scale phylogeographic analysis by Skog et al.
186 (2014) using the primers described by Grande *et al.* (2004). DNA was extracted with
187 the ArchivePure DNA Cell/Tissue Kit (5 Prime GmbH, Hilden, Germany). The
188 Multiplex PCR Kit (Qiagen GmbH, Hilden, Germany) was used for PCR and
189 sequencing was performed with the BigDye Terminator v.3.1. Cycle sequencing Kit
190 by Applied Biosystems™, following the instructions of the manufacturers. Sequencing
191 was carried out on an Applied Biosystems 3100x Genetic Analyzer. A 1.2 kbp region
192 was amplified for a subset of 184 pike individuals belonging to 22 populations of 12
193 river catchments. The average sample size was 9.1 ± 2.1 (mean ± SD) individuals
194 per population and 16.7 ± 8.5 (mean ± SD) per river catchment. Individual forward
195 and reverse sequences were assembled using Seqman (DNA star package) and the
196 resulting contigs were checked by eye to correct sequencing errors. Sequences of
197 all main and sub haplotypes were deposited at the NCBI database (Acc. no.

198 KY399416 – KY399442).

199

200 *Genetic data analysis*

201 Microsatellite data were tested for the presence of null alleles with MICROCHECKER
202 2.2.3 (van Oosterhout et al., 2004) using 1,000 randomizations and applying a 95%
203 confidence interval. Total and mean numbers of alleles as well as heterozygosity (H_O
204 and Nei´s H_S) were calculated with FSTAT 2.9.3.2 (Goudet, 1995). GENEPOP 4.2
205 (Raymond & Rousset, 1995) was applied to test for Hardy-Weinberg deviations and
206 linkage disequilibrium setting the Markov chain parameters (MCMC) to 10,000
207 dememorizations, 20 batches and 5,000 iterations per batch.

208 STRUCTURE 2.3.2 (Falush et al., 2003) was used to infer the most likely population
209 structure based on microsatellite data of 53 pike populations. The calculation was
210 done with an admixture model without *a priori* population information, using a burn-in
211 period of 100,000 repeats, 100,000 subsequent MCMC repeats and 10 iterations for
212 each k value between one and 30. The most likely number of ancestral populations
213 was identified as the k value, where the change of likelihood dropped considerably
214 compared to subsequent values (Δk criterion). All individuals were assigned to each
215 of the ancestral gene pools as defined in the most likely STRUCTURE model. NA
216 describes the fraction of the genome inherited from the drainage basin-specific
217 lineage, as opposed to ancestry that most likely originated from a different river basin
218 according to the STRUCTURE model. To express all foreign genetic influences
219 (irrespective of their origins) in relation to NA, hybrid indices (HI) for each individual
220 were inferred from the individual NA values. Using the formula $HI = 1 - (2 \times |0.5 -$
221 $NA|)$ 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 ancestry contributed to an individual's genetic composition.

MSA 4.05 (Dieringer & Schlötterer, 2003) was employed to calculate genetic differences (Nei´s D_A (Nei, 1983), chord distances (Cavalli-Sforza & Edwards, 1967) and the proportion of proportiond 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).

Principal coordinate analysis as an alternative to identify genetic clusters was performed with GeneAlEx 6.5 (Peakall & Smouse, 2012) using the covariance matrix obtained from Fst values (Table S2).

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 that 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 were visualized 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, Suffolk, UK).

Environmental effects on genetic structure

585 pike individuals from 24 lakes and 392 pike individuals from 23 rivers were tested in two independent hierarchical general linear models (HGLM) to infer the effect of different ecological predictors on native ancestry (NA) and hybridization index (HI). These response variables (*y*) were composed of values within the standard unit interval $y_i \in [1,0]$, where *i* designated an individual fish. Special techniques were required for linear modelling with respect to binomial errors and beta distributed random effects to incorporate features such as heteroskedasticity or skewness commonly observed in this type of data (Cribari-Netom & Zeileis, 2010). The data comprised repeated measures within individual water bodies (*n* individuals from 50 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 waterbodies 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 (not modified or highly modified) and its ecological status as a numerical covariate from 1 (very good) to 5 (poor) according to the European Water Framework Directive. The raw data were retrieved from the Federal Institute of Hydrology (BfG, Koblenz, Germany: http://geoportal.bafg.de/mapapps/resources/ apps/had and http://geoportal.bafg.de/ mapnavigator) and were deposited in DRYAD. 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) considering a beta 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 minimal adequate model testing the main effects.

Results

Assessment of genetic markers

All of the 15 microsatellite loci proved to be highly polymorphic with a total number of 9 to 37 different alleles over all pike populations and a mean number of 3.5 to 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 ± SD) reflected in significant heterozygote deficiencies in 2.7 ± 1.6 loci (Fig. S1). Linkage disequilibrium was detected in 3.3% of all possible loci combinations after Bonferroni correction (Fig. S2). Because departures were distributed over many loci and populations, all loci were used for population genetic analysis.

For network analysis, a 1,174 bp region of the mitochondrial *cyt b* gene containing 48 variable positions was selected. With a total of 918 informative sites in 208 sequences analyzed (excluding sites with gaps and missing data; including reference sequences), the overall information content was relatively low (3.8%) as expected for pike.

Genetic structure of pike populations in Germany

Analysis of microsatellite data with STRUCTURE suggested a k value of either three or five as the most likely number of existing genetic clusters of pike (Fig. 1, showing the relevant k range only). However, five clusters were considered less likely based on the definition of the minimal Δk criterion. To further confirm the results obtained with STRUCTURE, we considered two additional genetic analyses based on microsatellite data. First, calculating genetic differences resulted in dendrograms (trees) with three main clusters. Although bootstrap values were mostly low (and therefore omitted from fig. 2), the overall topology of the consensus trees proved to be stable. Using Nei´s D_A yielded exactly the genetic structure of pike populations predicted by the assignments of STRUCTURE assuming three as the most likely number of k (Fig. 2). Moreover, the tree analysis showed that the severely admixed pike populations were grouped into the expected “new” genetic background, 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 (Fig. 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 (trees not shown). Second, principal coordinate analysis (PCoA) based on Fst values was employed (Fig. 3). Although variation was moderate (accumulated variance explained by axes 1 and 3 = 23.2% and 22.6% by axes 1 and 2) a clear clustering into three groups representing the drainage basins of the North, Baltic and Black Sea, respectively (Table 3), was obtained, 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 predicted by the STRUCTURE analysis, providing further evidence for k = 3.

Network analysis with mitochondrial *cyt b* sequences identified two of the three main haplotypes postulated by Skog et al. (2014). However, while mitochondrial haplotypes frequencies differed among drainage basins, there was not one to one correspondence of haplotypes with the main clusters identified here based on nuclear data. Of 184 sequences 159 (86.4%) grouped with five reference sequences defined as haplotype E, representing the northern clade of *E. lucius* (Fig. S3). 24 sequences (13.0%) grouped together with 16 haplotype B reference sequences, constituting the circumpolar clade, and one sequence grouped with three haplotype F reference sequences of the southern clade. The northern clade exhibited a star-like appearance consisting of the main haplotype, surrounded by 26 sub-haplotypes, deviating by one (N = 22) or two mutations (N = 4). Pike individuals from the North Sea hydrogeographic region were the dominant fraction (59.9%), while individuals from the Baltic and Black Sea regions contributed 30.2% and 10.7%, respectively. The circumpolar clade consisted mostly of pike from the Baltic Sea hydrogeographic region (87.5%) and only of a minority of pike from other regions (North Sea: 4.2%, Black Sea: 8.3%). It was separated from all but one (B14) haplotype B reference sequences by one mutation and appeared homogenous, except for two sub-haplotypes deviating by one mutation. The two clades (the main haplotypes E and B) differed by six mutations and were connected via a hypothetical ancestor. The closest representative of the southern clade, connected via the same ancestor, differed by four mutations from the northern and by six mutations from the circumpolar clade (Fig. S3).

Population structure among the major hydrogeographic basins

344 Admixture analysis based on microsatellites, revealed varying proportions of the
345 different genetic lineages in pike populations across hydrogeographic regions. The
346 Black Sea genetic cluster was most frequent in pike of the Danube and its tributary
347 rivers (65.3% in pie chart 1 of Fig. 4 – subsequently indicated as e.g. “65.3% in 1”) as
348 well as in pike of the big alpine lakes (77.5% in 1.1 and 91.5% in 1.2) (Table 3).
349 Elevated levels of the Black Sea lineage, however, were also found in the
350 geographically close Lake Constance (86.4% in 4.2) and the river Main (63.7% in
351 4.1), a big tributary of the river Rhine connected via a channel – the Rhein-Main-
352 Donau-Kanal – with the Danube. Interestingly, some water bodies in the very north of
353 Germany hosted a high proportion of Black Sea genetic imprint as well, such as pike
354 from the river Eider (50.1% in 7) and pike inhabiting Wittensee (58.8% in 2.2) and
355 Großer Plöner See (67.1% in 10).

356 The Baltic genetic cluster was most prevalent in pike populations of the coastal
357 waters of the Baltic Sea and water bodies draining into that basin, albeit with
358 considerable variation (49.4% to 89.7% in 3.1, 3.2, 8, 8.1, 9, 11 and 12). Among the
359 populations from Baltic tributaries, pike from the Oder main stream and from its
360 tributary river Neiße, showed signs of pronounced admixture with the Black Sea and
361 North Sea genetic lineages (in total 57.2% in 3).

362 The North Sea genetic cluster was dominant in populations of the river catchments of
363 Elbe (78.7% in 2 and 76.5% in 2.1, excluding Wittensee with only 31.7% in 2.2), Ems
364 (74.8% in 6) and Weser draining into the North Sea. Populations of the Weser
365 catchment were represented by pike of two big lakes, Steinhuder Meer and Edersee
366 (a reservoir), which differed in their admixture patterns. While populations of the
367 Steinhuder Meer were predominantly shaped by the North Sea genetic cluster
368 (84.3% in 5.1), this ancestry contributed relatively little to pike of the Edersee

population (34.5% in 5). Similarly, and unexpectedly, the river Rhine exhibited a higher proportion of the Baltic than of the North Sea genetic cluster (63.6% in 4).

Genetic admixture could also be read from the distance based consensus tree (Fig. 2) and the frequency based principal coordinate analysis (Fig. 3). E. g., the Rhine population (RHE2) appeared within the Baltic Sea cluster and pike from Großer Plöner See (GPS) were placed within the Black Sea cluster.

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 (Fig. 5). Based on ancestry coefficients (NA), the distributions of native *vs*. foreign genetic ancestries displayed a range from mostly pure native populations (green violin plots in Fig. 5 with mean NA ≥ 0.50) to hybrid swarms, with mostly admixed individuals (yellow violin plots in Fig. 5 with 0.25 < NA < 0.50). Moreover, some distributions were skewed towards foreign ancestry, with complete replacement of native ancestry in some populations (red violin plots in Fig. 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 (Fig. 5). This includes river populations of e.g. the Danube (DON, INN, NAB, ROT; see table 1 for explanation of IDs) and Oder catchments (ODE2, ODE7, NEI2) as well as lake populations of e.g. the Trave (GRA) and Elbe catchments (GKB). In other populations, such patterns were observed to a less extent, e.g. in lake populations of the Ucker (HAH) and Weser catchments (STM) and river populations of the Elbe catchment (HAV1, KAR).

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 Großer Plöner See (GPS) and of the rivers Neiße (NEI2) and Main (MAI), where a near complete replacement of native by foreign genetic identities was suggested by the most likely STRUCTURE model (Fig. 5) and confirmed by genetic distance trees (Fig. 2) and principal coordinate analysis (Fig. 3). Pike populations in Lake Constance, however, ought to be viewed differently in this regard due to their geological history (see discussion for further details). In the Baltic Sea hydrogeographic region, a coastal population (BAL2 in Fig. 5) and one freshwater population (WBS) exhibited pronounced native genetic signatures. Likewise, the North Sea hydrogeographic region harbored populations that appear to be rather typical and pure representatives of the respective genetic cluster (JAG, KRK, GST, MUR).

Correlation of hybridization levels with ecological quality

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 hybridisation index (HI) of pike populations. Specifically, the decline in the ecological status was highly significantly correlated with HI (Table 4). Each unit of decrease of the ecological status lead to an increase of HI by a factor (slope) of 0.25 (1.28 in raw scale) ± 0.08 with respect to the intercept (defined as the best ecological status). Accordingly, the HI increased by a value of 0.21 from the best (= 1 in Fig. 6) to the poorest (= 5) ecological status defined according to the European Water Framework

418 Directive. The estimate (in logit scale) of HI for lakes was -1.79 (0.17 in raw scale) ±
419 0.26, while it was -1.37 (0.26 in raw scale) ± 0.26 in rivers. These values indicated a
420 significantly stronger signal of past hybridization of pike populations in rivers as
421 compared to lakes (Table 4). While the hypothesis of a correlation between HI and
422 the type and ecological status of the water body was supported, relationships of HI
423 and the general level of modification of the water body (Table 4) and all two-level
424 interaction effects were not supported (data not shown).

425 In addition, the native ancestry (NA) exhibited a strong negative correlation with the
426 deterioration of the ecological status of the water bodies (Fig. 6), however this was
427 not statistically significant (Table 4). This held also true for the predictors “type” (i.e.
428 ecosystem type) and “modification” (i.e. general degree of modification) of the water
429 bodies (Table 4), as well as their two-level interactions (not shown), which were
430 therefore removed from the model with NA.

431

432 **Discussion**

433 *Differentiation of pike from different drainages*

434 Mitochondrial DNA markers have been previously used to reconstruct the
435 colonization patterns of pike in Europe after the last glaciations c. 15.000 years ago
436 (Nicod et al., 2004; Skog et al., 2014). Our own analysis of mitochondrial *cyt b*
437 sequences together with 15 polymorphic microsatellites (Eschbach & Schöning,
438 2013) allowed distinguishing lineages that seem typical for different drainage basins.
439 STRUCTURE analysis (Falush et al., 2003) of microsatellite data revealed a k value
440 of three as the most likely number of genetic lineages present in Germany (Fig. 1). A
441 second k value of five was deemed much less likely when judged against results

obtained with two other analysis performed with microsatellite data. Both the construction of genetic distance based consensus trees as well as a principal coordinate analysis based on allele frequencies argued in favor of only three genetic clusters. Each of them was regarded as representative of the hydrogeographic regions in the North Sea, Baltic Sea or Black Sea, suggesting that these were the most likely distribution areas of the ancestors after the retreat of the glaciers (Table 3). NETWORK analysis (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 (Fig. S3). 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.

Signatures of migration or stocking?

Recent secondary contacts and genetic admixture between divergent pike lineages have most likely increased as a result of anthropogenic activities. This included 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

467 populations naturally separated in space. Unfortunately, past stocking is generally
468 not well documented in Germany (Arlinghaus et al., 2015), but certainly has occurred
469 over decades in central Europe in pike and multiple other economically relevant
470 fishes (Cowx, 1994; Guillerault et al., 2018; Kottelat & Freyhof, 2007; Larsen & Berg,
471 2004). Given the poor locality-specific records, it is however impossible to take
472 stocking into account in a more detailed way than just to accept that it has happened
473 frequently across pike stocks (Arlinghaus et al., 2015). Nevertheless, the pike system
474 offers the opportunity to explore environmental factors that stabilize existing diversity
475 patterns or, conversely, promote admixture, without knowing which factor of
476 secondary contact was ultimately responsible. Pike of the Danube catchment and of
477 the brackish coastal areas of the Baltic Sea exhibited a clear dominance of native
478 ancestry as inferred from analysis of 15 polymorphic microsatellites (Fig. 4).
479 Likewise, relatively low admixture levels were observed in pike populations of the
480 Ems (pie-chart no. 6 in Fig. 4) and Weser catchments (pie-chart no. 5.1 in Fig. 4)
481 belonging to the North Sea hydrogeographic region. A possible explanation for the
482 persistence of autochthonous populations is either a low level of local stocking or
483 competitive exclusion of foreign genotypes by better-adapted native populations
484 (Englbrecht et al., 2002; Eschbach et al., 2014; Gandolfi et al., 2017). It has been
485 found before that brackish water populations are adapted to reproduction in low
486 salinity conditions, causing an increase in the mortality of stocked freshwater fish,
487 thereby preventing introgression despite decades of stocking (Jørgensen et al., 2010;
488 Larsen et al., 2005). Moreover, high density blocking can effectively counteract
489 establishment of immigrants from a distant population in an environment already
490 inhabited by locally adapted conspecifics as long as the local stock is naturally
491 reproducing at high levels (van Poorten et al., 2011; Waters, 2011). In line with this,

492 stocking experiments with pike showed that stocked individuals suffer from
493 substantially higher mortality than wild conspecifics, if the natural reproduction is
494 sufficient, reducing the potential for successful establishment (Arlinghaus et al., 2015;
495 Diana et al., 2017; Hühn et al., 2014).

496 In some water bodies investigated in this study, however, pike individuals showed
497 high foreign ancestries, e.g. in the rivers Neiße, Main, Rhine and Eider, as well as in
498 the lakes Wittensee and Edersee. This data suggested that much of the genetic
499 material from native populations may be replaced through the introduction of foreign
500 stocks, which in turn can maintain populations that are resilient to further invasion by
501 local genotypes. In this context, our analysis suggests a near complete replacement
502 of native pike in the lake Großer Plöner See, in the north of Germany that is presently
503 inhabited by pike characterized by microsatellite genotypes that are likely to originate
504 from the Danube catchment. Admittedly, the data set we used in this study is
505 heterogeneous and complex, which makes an analysis of the overall population
506 structure a difficult task. Still, sampling for this particular lake is above average
507 (N = 30 individuals), and the effect we observed according to the most likely
508 STRUCTURE model was unambiguous and supported by two other analytical
509 methods (genetic distance based consensus trees and allele frequency based
510 PCoA). Englbrecht et al. (2002) described a comparable case for the arctic char
511 (*Salvelinus umbla*) in Starnberger See (Bavaria, Germany), where resident fish have
512 been completely replaced by stocked fish. They argue that this was possible because
513 the lake became heavily eutrophied in the middle of the last century (Ruecker et al.,
514 1999), with resident char approaching near extinction, while non-resident char were
515 apparently adapted to deal with the novel environment. There is clear evidence that
516 such a complete replacement has occurred in a range of other stock-enhanced

populations of fishes (van Poorten et al., 2011). Thus, although no detailed ecological data was available for Großer Plöner See, it is possible that its native population might have undergone a similar fate and became invaded by stocked pike. This example shows that complete genetic swamping most likely by stocking is indeed a possible scenario even if the ecosystem appears healthy and of high integrity in present time. By contrast, the high proportion of Black Sea hydrogeographic region ancestry in pike of Lake Constance is likely a result of ancient natural connections with the Danube catchment and rather reflects native ancestry stemming from natural post-glacial dispersal, previously reported for perch (*Perca fluviatilis*) by Behrmann-Godel et al. (2004) for the same system. The minor proportions of other non-North Sea hydrogeographic region ancestries were likely due to human-assisted colonization via introduction and stocking, because southward gene flow from downstream areas of the river Rhine is not possible due to an insurmountable waterfall at Schaffhausen (Switzerland).

Pike populations in 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, 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

542 reflected natural catchment barriers and were likely caused by successful pike stock
543 enhancement activities in the past.

544 Pike of the lakes Großer Kossenblatter See, Drewitzer See and Ammersee exhibited
545 pronounced linkage disequilibria (Fig. S2), and all pike of lake Ammersee additionally
546 exhibited deviation from Hardy-Weinberg equilibrium as well as heterozygote
547 deficiencies (Fig. S1). In agreement with this, bimodal distributions of native and
548 foreign ancestries of individuals of some populations confirmed that they were not
549 genetically homogenous. This can be compared to bimodal hybrid zones, which are
550 often characterized by pronounced deviation from Hardy-Weinberg equilibrium due to
551 restrictions in panmixia (Allendorf et al., 2001; Redenbach & Taylor, 2003). Possible
552 scenarios to explain this pattern include that foreign pike genotypes are regularly
553 introduced at a large scale without much reproductive success. Alternatively, foreign
554 genotypes may be reproductively isolated to some extent so that they persist as a
555 distinct genetic population in parallel to the local population of pike. The latter
556 explanation is less likely because we know that stocked individuals that survive
557 readily hybridize with native pike (Arlinghaus et al., 2015), although there is evidence
558 of natal homing of pike in large standing water bodies, which can contribute to the
559 development of meta-populations within lake ecosystems (Miller et al., 2001).

560

561 *Impacts of ecosystem status on hybridization*

562 In other cases, we detected various degrees of foreign ancestry (Fig. 5),
563 documenting likely admixture between genetically distinct populations – an effect that
564 increased with the degradation of the ecological status of the recipient ecosystem.
565 We note that the quality of this inference depends on the sample sizes that were

566 available for each population as well as the degree of differentiation between the
567 presumed source populations. It might, therefore, be useful to revisit specific
568 populations with a more powerful study design and genome wide marker coverage.
569 Nonetheless, it was obvious that the three pike lineages readily hybridized upon
570 secondary contact. This result bears general questions on why hybridization
571 proceeded with different intensity in different pike populations and whether pike of
572 different origins are indeed isolated to some extent when they are brought into
573 secondary contact.

574 We found that the individual admixture levels in pike, expressed as a hybridization
575 index (HI), were not confined to a specific hydrogeographic region or any particular
576 river catchment therein. Instead it turned out that the HI increased significantly with
577 decreasing ecological quality of a water body. Albeit not statistically significant, we
578 observed a congruent decrease of native ancestry. Thus, environmental change
579 could have driven genetic changes in pike populations and individuals by affecting
580 the frequency of hybridization among populations brought into secondary contact.
581 The fact that the HI was only slightly lower in water bodies with low modifications as
582 compared to the HI of highly modified waters demonstrates that the admixture as
583 such occurs in all populations and is not restricted to highly modified habitats (Fig. 6).

584 Our analysis yielded a significantly higher HI in pike populations in rivers as
585 compared to lake-dwelling pike, which is likely due to fundamental ecological
586 differences between the two habitat types such as the increased natural connectivity
587 in rivers, resource availability, productivity, habitat structure, and community
588 composition (Irz et al., 2006; Hof et al., 2008). Most importantly, however, rivers and
589 lakes vary in stability and disturbance frequency, including exposure to catastrophic
590 floods, which occur more frequently in lotic than in lentic systems. Rivers of central

591 Europe also have been more strongly modified, e.g. by removal of connectivity to
592 floodplains and habitat simplification, which represent a central component of their
593 disturbance regime and at the same time constitute essential spawning habitat for
594 pike. In a recent meta-analysis comparing resistance of limnic, marine and terrestrial
595 ecosystems towards invasive species, Alofs & Jackson (2014) clearly demonstrated
596 that lentic systems displayed a higher biotic resistance than lotic systems, which is in
597 accordance with our findings of different susceptibilities towards hybridization in river
598 and lake pike populations.

599 Our observation that hybridization in pike appears to be favored in ecologically
600 perturbed water bodies raises important questions about the mechanisms. The effect
601 could first be caused due to an increase of foreign genotypes that managed to invade
602 a weakened native population (Englbrecht et al., 2002; Gandolfi et al., 2017).
603 Alternatively, genetically admixed fish could be more competitive in the face of
604 anthropogenic changes to the ecosystem. This would resemble the first step of a
605 hybrid speciation scenario, where intraspecific hybrids are expected to be most
606 successful when parental populations are not at their optimum (Abbott *et al.,* 2013;
607 Nolte & Tautz, 2010). Stelkens *et al.* (2014) showed that particularly the interactions
608 of genetic variants between distant *Saccharomyces* strains can lead to a better
609 survival in environments of decreasing quality. Thus, hybridization can create
610 biodiversity resulting in novel phenotypes and adaptive change in response to
611 environmental change (Arnold, 2016; Charlesworth & Willis, 2009; Edmands, 2007;
612 Sefc et al., 2017). Examples of these processes can be found among invaders
613 conquering new environments that were not occupied by populations of the
614 respective species before, as it was found for *Cottus* hybrids in the river Rhine (Nolte
615 et al., 2005; Stemshorn et al., 2011), but also for spiders (Krehenwinkel & Tautz,

2013) and some 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 worsening environmental conditions (Arnold 2016). 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.

Conclusions and implications

At the species level, large-scale hybridization, which extends over different hydrogeographic regions each with its own evolutionary history of genetic lines, is synonymous with genetic erosion (Epifanio & Philipp, 2001), i.e. it increases the fate of extinction due to the loss of evolutionary potential. Especially in a species such as pike, which is characterized by a low natural genetic variability compared to other freshwater fish, it may be important to maintain genetic diversity through different genetic lines. Our study clearly showed a novel relationship between ecosystem status, assessed under the European Water Framework Directive, and the genetic structure of northern pike. It supports the idea that habitat degradation can also have far-reaching consequences for genetic integrity within species and promotes efforts to further improve the ecological quality of lakes and rivers. In the case of pike, this would essentially mean reconnecting floodplains with rivers and reducing nutrient inputs into lakes, which would increase both population size and genetic biodiversity.

641

642 **Acknowledgements**

643 Particular thanks go to Yvonne Klaar, Asja Vogt, Jasmin Spamer and Elke Bustorf for
644 technical assistance in the lab, as well as Petra Kersten for invaluable advice on
645 microsatellite analysis. We are also indebted to Ute Mischke for kindly providing
646 additional ecological data of the different water bodies. We thank Jochem Kail for
647 creating the map in Fig. 4. We are very much obliged to the many fishers, anglers,
648 fisheries authorities and research organisations for providing tissue samples as well
649 as a lot of helpful information. We are grateful to the whole BESATZFISCH team and
650 Michael Monaghan and his POPGEN group for many fruitful discussions. Funding of
651 the current work was granted by the German Federal Ministry of Education and
652 Research within the project BESATZFISCH (www.besatz-fisch.de) in the Program for
653 Social-Ecological Research (Grant No. 01UU0907 to R. A.). A.N. was supported
654 through an ERC starting grant “EVOLMAPPING”.

655

656 **References**

657 Abbott, R., Albach, D., Ansell, S., Arntzen, J. W., Baird, S. J. E., Bierne, N. ... Zinner,
658 D. (2013). Hybridization and speciation. *Journal of Evolutionary Biology,* 28, 229-246.

659 Aguilar, A., Banks, J. D., Levine, K. F., & Wayne, R. K. (2005). Population genetics of
660 northern pike (*Esox lucius*) introduced into Lake Davis, California. *Canadian Journal*
661 *of Fisheries and Aquatic Sciences,* 62, 1589-1599.

662 Allendorf, F. W., Leary, R. F., Spruell, P., & Wenburg, J. K. (2001). The problems
663 with hybrids: setting conservation guidelines. *Trends in Ecology & Evolution 16*, 613-
664 622.

665 Alofs, K. M. & Jackson, D. A. (2014). Meta-analysis suggests biotic resistance in
666 freshwater environments is driven by consumption rather than competition. *Ecology,*
667 95*,* 3259-3270.

668 Arlinghaus, R., Cyrus, E. - M., Eschbach, E., Fujitani, M., Hühn, D., Johnston, F., *et*
669 *al*. (2015). *Hand in Hand für eine nachhaltige Angelfischerei: Ergebnisse und*
670 *Empfehlungen aus fünf Jahren praxisorientierter Forschung zu Fischbesatz und*
671 *seinen Alternativen*. Berichte des IGB, (Hand in hand for a sustainable angling
672 fishery: Results and recommendations from five years of practice-oriented research
673 on fish stocking and its alternatives. Reports of the IGB), Berlin, Germany.

674 Arnold, M. L. (2016). Anderson´s and Stebbins´ prophecy comes true: genetic
675 exchange in fluctuating environments. *Systematic Botany Monographs,* doi:
676 10.1600/036364416X690679.

677 Bandelt, H. - J., Forster, P., & Röhl, A. (1999). Median-joining networks for inferring
678 intraspecific phylogenies. *Molecular Biology and Evolution,* 16*,* 37-48.

679 Behrmann-Godel, J., Gerlach, G., & Eckmann, R. (2004). Postglacial colonization
680 shows evidence for sympatric population splitting in Eurasian perch (*Perca fluviatilis*
681 L.) in Lake Constance. *Molecular Ecology,* 2*,* 491-497.

682 Bekkevold, D., Jacobsen, L., Hemmer-Hansen. J., Berg, S., & Skov, C. (2015). From
683 regionally predictable to locally complex population structure in a freshwater top
684 predator: river systems are not always the unit of connectivity in northern pike (*Esox*
685 *lucius*). *Ecology of Freshwater Fish,* 24*,* 305-316.

686 Bianco, P. G. & Delmastro, G. B. (2011): Recenti novità tassonomiche riguardanti i
687 pesci d'acqua dolce autoctoni in Italia e descrizione di una nuova specie di luccio
688 (Recent taxonomic news about freshwater fish native to Italy and description of a

689 new species of pike). Researches on Wildlife Conservation (IGF publ.), 2 (suppl.): 1–
690 13.

691 Bittner, D., Excoffier, L., & Largiadèr, R. (2010). Patterns of morphological changes
692 and hybridization between sympatric whitefish morphs (*Coregonus spp*.) in a Swiss
693 lake: A role for eutropication? *Molecular Ecology,* 19*,* 2152-2585.

694 Bowcock, A. M., Ruíz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J. R., & Cavalli-
695 Sforza, L. L. (1994). High resolution human evolutionary trees with oligomorphic
696 microsatellites. *Nature,* 368, 455-457.

697 Casselmann, J. M. & Lewis, C. A. (1996). Habitat requirements of northern pike
698 (*Esox lucius*). *Canadian Journal of Fisheries and Aquatic Sciences,* 53*,* 161-174.

699 Cavalli-Sforza, L. L. & Edwards, A. W. F. (1967). Phylogenetic analysis: models and
700 estimation procedures. *American Journal of Human Genetics,* 19*,* 233-257.

701 Charlesworth, D., Willis, J. H. (2009). The genetics of inbreeding depression. *Nature*
702 *Reviews genetics*, 10, 783-796.

703 Christie, M. R. & Knowles, L. L. (2015). Habitat corridors facilitate genetic resilience
704 irrespective of species dispersal abilities or population sizes. *Evolutionary*
705 *Applications,* 8: 454-463.

706 Cowx, I. G. (1994). Stocking strategies. *Fisheries Management and Ecology,* 1, 15-
707 30.

708 Craig, J. (1996). Pike **-** Biology and exploitation**.** Fish and Fisheries Series, Springer
709 Netherlands.

710 Cribari-Netom, F. & Zeileis, A. C. (2010). Beta Regression in R. *Journal of Statistical*
711 *Software*, 34, 1-24.

712 Diana, M. J., Wagner, C. P., & Wahl, D. H. (2017). Differences in stocking success
713 among geographically distinct stocks of juvenile Muskellunge in Illinois lakes. *North*
714 *American Journal Of Fisheries Management*, 37, 633-643.

715 Dieringer, D. & Schlötterer, C. (2003). Microsatellite analyser (MSA): a platform
716 independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes,*
717 3,167-169.

718 Dulvy, N. K., Sadovy, Y., & Reynolds, J. D. (2003). Extinction vulnerability in marine
719 populations. *Fish and Fisheries,* 4, 25-64.

720 Edmands, S. (2007). Between a rock and a hard place: evaluating the relative risks
721 of inbreeding and outbreeding for conservation and management. *Molecular Ecology,*
722 16, 463-475.

723 Englbrecht, C. C., Schliewen, U., & Tautz, D. (2002). The impact of stocking on the
724 genetic integrity of Arctic charr (*Salvelinus*) populations from the Alpine region.
725 *Molecular Ecology,* 11, 1017-1027.

726 Epifanio, J. & Philipp, D. (2001). Simulating the extinction of parental lineages from
727 introgressive hybridization: the effects of fitness, initial proportions of parental taxa,
728 and mate choice. *Reviews in Fish Biology and Fisheries,* 109, 339-354.

729 Eschbach, E. (2012). Ascertaining optimal protocols for DNA extraction of different
730 qualities of pike (*Esox lucius*) tissue samples – a comparison of commonly used solid
731 phase extraction methods. *Environmental Biotechnology,* 8, 7-14.

732 Eschbach, E. & Schöning, S. (2013). Identification of high-resolution microsatellites
733 without *a priori* knowledge of genotypes using a simple scoring approach. *Methods in*
734 *Ecology and Evolution,* 4, 1076-1082.

735 Eschbach, E., Nolte, A., Kersten, P., Kohlmann, K., Kail, J., & Arlinghaus, R. (2014).
736 Population differentiation of zander (*Sander lucioperca*) across native and newly
737 colonized ranges suggests increasing admixture in the course of an invasion.
738 *Evolutionary Applications,* 7, 555-568.

739 European Commission 2000. Directive 2000/60/EC of the European Parliament and
740 of the Council of 23rd October 2000 establishing a framework for Community action
741 in the field of water policy. Official Journal of the European Communities, L327/1.
742 European Commission, Brussels.

743 Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure
744 using multilocus genotype data: linked loci and correlated allele frequencies.
745 *Genetics,* 164, 1567-1587.

746 Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum
747 likelihood approach. *Journal of Molecular Evolution,* 17, 368-376.

748 Fournier, D. A., Skaug, H. J., Ancheta, J., Ianelli, J., Magnusson, A., Maunder, M. N.
749 ... Sobert, J. (2012). AD Model Builder: using automatic differentiation for statistical
750 inference of highly parameterized complex nonlinear models. *Optimization Methods*
751 *and Software,* 27, 233-249.

752 Gandolfi, A., Ferrari, C., Crestanello, B., Girardi, M., Lucentini, L., & Meraner, A.
753 (2017). Population genetics of pike, genus *Esox* (*Actinopterygii, Esocidae*), in
754 Northern Italy: evidence for mosaic distribution of native, exotic and introgressed
755 populations. *Hydrobiologia,* 794, 73-92.

756 Goudet, J. (1995). FSTAT (ver. 1.2): a computer program to calculate F-statistics.
757 *Journal of Heredity,* 86, 485-486.

758 Grande, T., Laten, H., & Lopez, J. A. (2004). Phylogenetic relationships of extant

759 esocid species (*Teleostei*: *Salmoniformes*) based on morphological and molecular
760 characters. *Copeia,* 2004, 743-757.

761 Guillerault, N., Hühn, D., Cucherousset, J., Arlinghaus, R. & Skov, C. 2018. Stocking
762 for pike population enhancement. In: Biology and ecology of pike (C. Skov & P. A.
763 Nilsson, eds), pp. 215-247. CRC Press, Boca Raton, Florida.

764 Guillerault, N., Hühn, D., Cucherousset, J., Arlinghaus, R. & Skov, C. (2018). Biology
765 and ecology of pike. Boca Raton, FL: CRC Press.

766 Hansen, M. M. (2002). Estimating the long-term effects of stocking domesticated
767 trout into wild brown trout (*Salmo trutta*) populations: an approach using
768 microsatellite DNA analysis of historical and contemporary samples. *Molecular*
769 *Ecology,* 11, 1003-1015.

770 Harbicht, A. B., Alshamlih, M., Wilson, C. C., & Fraser, D. J. (2014). Anthropogenic
771 and habitat correlates of hybridization between hatchery and wild brook trout.
772 *Canadian Journal of Fisheries and Aquatic Sciences,* 71, 688-697.

773 Heath, D., Bettles, C. M., & Roff, D. (2010). Environmental factors associated with
774 reproductive barrier breakdown in sympatric trout populations on Vancouver Island.
775 *Evolutionary Applications,* 3, 77-90.

776 Hof, C., Brändle, M., & Brandl, R. (2008). Latitudinal variation of diversity in
777 European freshwater animals is not concordant across habitat types. *Global Ecology*
778 *and Biogeography,* 17, 539-546.

779 Hühn, D., Lübke, K., Skov, C., & Arlinghaus, R. (2014). Natural recruitment, density-
780 dependent juvenile survival, and the potential for additive effects of stock
781 enhancement: an experimental evaluation of stocking northern pike (*Esox lucius*) fry.
782 *Canadian Journal of Fisheries and Aquatic Sciences,* 71, 1508-1519.

783 Irz, P., Odion, M., Arguillier, C., & Pont, D. (2006). Comparison between the fish
784 communities of lakes, reservoirs and rivers: can natural systems help define the
785 ecological potential of reservoirs? *Aquatic Sciences,* 68, 109-116.

786 Jacobsen, B. H., Hansen, M. M., & Loeschcke, V. (2005). Microsatellite DNA analysis
787 of northern pike (*Esox lucius* L.) populations: insights into the genetic structure and
788 demographic history of a genetically depauperate species. *Biological Journal of the*
789 *Linnean Society,* 84, 91-101.

790 Jørgensen, A. T., Hansen, B. W., Vismann, B., Jacobsen, L., Skov, C., Berg, S.,
791 Bekkevold, D. (2010). High salinity tolerance in eggs and fry of a brackish *Esox*
792 *lucius* population. *Fisheries Managment and Ecology,* 17, 554-560.

793 Keller, S. R. & Taylor, D. R. (2010). Genomic admixture increases fitness in an
794 invasive weed. *Journal of Evolutionary Biology,* 23, 1720-1731.

795 Kottelat, M. & Freyhof, J. (2007). Handbook of European freshwater fishes. Berlin,
796 Germany: Kottelat, Cornol and Freyhof.

797 Krehenwinkel, H. & Tautz, D. (2013). Northern range expansion of European
798 populations of the wasp spider *Argiope bruennichi* is associated with global
799 warming-correlated genetic admixture and population-specific temperature
800 adaptations. *Molecular Ecology,* 22, 2232-2248.

801 Laikre, L., Schwartz, M. K., Waples, R. S., Ryman, N., & GeM Working Group (2010).
802 Compromising genetic diversity in the wild: unmonitored large-scale release of plants
803 and animals. *Trends in Ecology & Evolution,* 25, 520-529.

804 Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P.A.,
805 McWilliam, H., ... Higgins, D. G. (2007). Clustal W and Clustal X version 2.0.
806 *Bioinformatics,* 23, 2947-2948.

807 Larsen, L. K. & Berg, S. (2004). NOBANIS – Invasive alien species fact sheet –
808 *Stizostedion lucioperca.* https://www.nobanis.org.

809 Larsen, P. F., Hansen, M. M., Nielsen, E. E., & Loeschke, V. (2005). Stocking impact
810 and temporal stability of genetic composition in a brackish northern pike (*Esox lucius*
811 L.), population assessed using microsatellite DNA analysis of historical and
812 contemporary samples. *Heredity,* 95,136-143.

813 Laurance, W. F. & Useche, D. C. (2009). Environmental synergisms and extinctions
814 of tropical species. *Conservation Biology,* 23, 1427-1437.

815 Lucentini, L., Puletti, M. E., Ricciolini, C., Gigliarelli, L., Fontaneto, D., Lanfaloni, L.,
816 Bilò, F., Natali, M., & Panara, F. (2011): Molecular and Phenotypic Evidence of a
817 New Species of Genus Esox (Esocidae, Esociformes, Actinopterygii): The Southern
818 Pike, *Esox flaviae*. PLoS ONE, 6, 1–14, doi:10.1371/journal.pone.0025218

819 Marie, A. D., Bernatchez, L., & Garant, D. (2012). Environmental factors correlate
820 with hybridization in stocked brook charr (*Salvelinus fontinalis*). *Canadian Journal of*
821 *Fisheries and Aquatic Sciences,* 69, 884-893.

822 Miller, L. M., Kallemeyn, L., & Senanan, W. (2001). Spawning-site and natal-site
823 fidelity by northern pike in a large lake: mark–recapture and genetic evidence.
824 *Transactions of the American Fisheries Society,* 130, 307-316.

825 Miller, L. M. & Kapuscinski, A. R. (1996). Microsatellite DNA markers reveal new
826 levels of genetic variation in northern pike. *Transactions of the American Fisheries*
827 *Society,* 125, 971-977.

828 Miller, L. M. & Kapuscinski, A. R. (1997). Historical analysis of genetic variation
829 reveals low effective population size in a northern pike (*Esox lucius*) population.
830 *Genetics,* 147, 1249-1258.

831 Miller, L. M. & Senanan, W. (2003). A review of northern pike population genetics
832 research and its implications for management. *North American Journal Of Fisheries*
833 *Management,* 23, 297-306.

834 Moritz, C. (1994). Defining „Evolutionarily Significant Units“ for conservation. *Trends*
835 *in Ecology & Evolution,* 9, 373-375.

836 Nei, M., Tajima, F., & Tateno, Y. (1983). Accuracy of estimated phylogenetic trees
837 from molecular data. *Journal of Molecular Evolution,* 19,153-170.

838 Nicod, J. - C., Wang, Y. Z., Excoffier, L., & Largiadér, C. R. (2004). Low levels of
839 mitochondrial DNA variation among central and southern European *Esox lucius*
840 populations. *Journal of Fish Biology,* 64, 1442-1449.

841 Nolte, A. W., Freyhof, J., Stemshorn, K. C., & Tautz, D. (2005). An invasive lineage
842 of sculpins, *Cottus* sp. (*Pisces, Teleostei*) in the Rhine with new habitat adaptations
843 has originated from hybridization between old phylogeographic groups. *Proceedings*
844 *of the Royal Society B: Biological Sciences,* 22, 2379-2387.

845 Nolte, A. W. & Tautz, D. (2010). Understanding the onset of hybrid speciation.
846 *Trends in Genetics,* 26, 54-58.

847 Ortego, J., Aguirre, M. P., Noguerales, V., & Cordero, P. J. (2015). Consequences of
848 extensive habitat fragmentation in landscape-level patterns of genetic diversity and
849 structure in the Mediterranean esparto grasshopper. *Evolutionary Applications,* 8,
850 621-632.

851 Ouellet-Cauchon, G., Mingelbier, M., Lecomte, F., & Bernatchez, L. (2014).
852 Landscape variability explains spatial pattern of population structure of northern pike
853 (*Esox lucius*) in a large fluvial system. *Ecology and Evolution,* 4, 3723-3735.

854 Peakall, R. & Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel.
855 Population genetic software for teaching and research-an update. *Bioinformatics,* 28,
856 2537-2539.

857 Pedreschi, D., Kelly-Quinn, M., Caffrey, J., O'Grady, M., & Mariani, S. (2014).
858 Genetic structure of pike (*Esox lucius*) reveals a complex and previously
859 unrecognized colonization history of Ireland. *Journal of Biogeography,* 41, 548–560.

860 Powels I. S., Goethals, P. L. M., Coeck, J., & Mouton, A. M. (2013). Movement
861 patterns of adult pike (*Esox lucius* L.) in a Belgian lowland river. *Ecology of*
862 *Freshwater Fish,* 23, 373-382.

863 Raat, A. J. P. (1988). *Synopsis of biological data on the northern pike Esox lucius*
864 *Linnaeus, 1758.* Rome, Italy: FAO Fisheries Synopsis No. 30.

865 Raymond, M. & Rousset, F. (1995). GENEPOP (version 1.2): population genetics
866 software for exact tests and ecumenicism. *Journal of Heredity,* 86, 248-249.

867 Redenbach, Z. & Taylor, E. B. (2003). Evidence for bimodal hybrid zones between
868 two species of char (*Pisces: Salvelinus*), in northwestern North America. *Journal of*
869 *Evolutionary Biology,* 16, 1135-1348.

870 Ruecker, A., Grosser, S., & Melzer, A. (1999). History and causes of reed decline at
871 Lake Ammersee. *Limnologica,* 29, 11-20.

872 Seehausen, O., van Alphen, J. J. M. & Witte, F. (1997). Cichlid fish diversity
873 threatened by eutrophication that curbs sexual selection. *Science,* 277, 1808-1811.

874 Sefc, K. M., Mattersdorfer, K., Ziegelbäcker, A., Neuhüttler, N., Steiner, O., Gössler,
875 W*., &* Koblmüller, S. (2017). Shifting barriers and phenotypic diversification by
876 hybridization. *Ecology Letters,* 20, 651-662.

877 Sexton, J. P., Hangartner, S. B., & Hoffmann, A. A. (2013). Genetic isolation by
878 environment or distance: which pattern of gene flow is most common? *Evolution,* 68,
879 1-15.

880 Skog, A., Vøllestad, L. A., Stenseth, N. C., Kasumyan, A., & Jakobsen, K. S. (2014).
881 Circumpolar phylogeography of the northern pike (*Esox lucius*) and its relationship to
882 the Amur pike (*E. reichertii*). *Frontiers in Zoology,* doi:10.1186/s12983-014-0067-8.

883 Skov, C. & Nilsson, P. A. (2018). Biology and ecology of pike. Boca Raton, Florida:
884 CRC Press.

885 Sloss, B. L., Franckowiak, R. P., & Murphy, E. L. (2008). Development of new
886 microsatellite loci and multiplex reactions for muskellunge (*Esox masquinongy*).
887 *Molecular Ecology Resources,* 8, 811-813.

888 Smith, T. B. & Bernatchez, L. (2008). Evolutionary change in human-altered
889 environments. *Molecular Ecology,* 17, 1-8.

890 Stelkens, R. B., Brockhurst, M. A., Hurst, G. D. D., & Greig, D. (2014). Hybridization
891 facilitates evolutionary rescue. *Evolutionary Applications,* 7, 1209-1217.

892 Stemshorn, K. C., Reed, F. A., Nolte, A. W., & Tautz, D. (2011). Rapid formation of
893 distinct hybrid lineages after secondary contact of two fish species (*Cottus spec*.).
894 *Molecular Ecology,* 20,1475-1491.

895 Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011).
896 MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood,
897 Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and*
898 *Evolution,* 28, 2731-2739.

van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes,* 4, 535-538.

van Poorten, B. T., Arlinghaus, R., Daedlow, K., & Haertel-Borer, S. S. (2011). Social-ecological interactions, management panaceas, and the future of wild fish populations. *Proceedings of the National Academy of Sciences of the United States of America,* 108, 12554-12559.

Vonlanthen, P., Bitter, D., Hudson, A. G., Young, K. A., Müller, R., Lundsgaard-Hansen, B., ... Seehausen, O. (2012). Eutrophication causes speciation reversal in whitefish adaptive radiations. *Nature,* 482, 357-362.

Wang, J., Wang, C., Qian, L., Ma, Y., Yang, X., Jeney, Z., & Li S. (2011). Genetic characterization of 18 novel microsatellite loci in northern pike (*Esox lucius* L.). *Genetics and Molecular Biology,* 34, 169-172.

Waples, R. S., Elz, A., Arnsberg, B. D., Faulkner, J. R., Hard, J. J., Timmins-Schiffmann, E., & Park, L. K. (2017). Human-mediated evolution in a threatened species? Juvenile life-history changes in Snake River salmon. *Evolutionary Applications*, doi: 10.1111/eva.12468.

Waters, J. M. (2011). Competitive exclusion: phylogeography´s 'elephant in the room'? *Molecular Ecology,* 20, 4388-4394.

Wennerström, L., Bekkevold, D., & Laikre, L. (2018). Biology and ecology of pike. Boca Raton, Florida: CRC Press.

Wennerström, L., Olsson, J., Ryman, N., & Laikre, L. (2016). Temporally stable, weak genetic structuring in brackish water northern pike (*Esox lucius*) in the Baltic Sea

922 indicates a contrasting divergence pattern relative to freshwater populations.
923 *Canadian Journal of Fisheries and Aquatic Sciences,* 74, 562–571.

924 Whitehead, A., Clark, B. W., Reid, N. M., Hahn, M. E., & Nacci, D. (2017). When
925 evolution is the solution to pollution: key principles, and lessons from rapid repeated
926 adaptation of killifish (*Fundulus heteroclitus*) populations. *Evolutionary Applications,*
927 10, 762-783.

928

929 **Legends to figures**

930 **Fig. 1**: Admixture analysis revealed three or five genetic clusters as the most likely
931 numbers, as indicated by a decrease in Δk and an increase in variance of calculated
932 probabilities P(D). Only the relevant range of calculated k is shown here.

933 **Fig. 2**: Neighbor joining consensus tree based on 10,000 permutations for calculating
934 Nei´s genetic distance estimator D_A. Although bootstrap values were generally low,
935 the tree topology proved to be stable and was consistent with the most likely
936 STRUCTURE model predicting three main clades. Furthermore, admixed populations
937 (labeled with a diamond) grouped according to their predicted dominant genetic
938 background within the respective clades, e.g. the RHE2 population of the river Rhine
939 groups within the Baltic Sea clade (green branches) and the GPS population
940 sampled from Großer Plöner See, which is connected with the Baltic Sea is
941 positioned in the Black Sea clade (red branches). Branches of the North Sea clade
942 are drawn in blue.

943 **Fig. 3**: Principal coordinate analysis based on pairwise Fst values (see Table S2) of
944 all pike samples. Despite low levels of variation (23.2% accumulated variation of axis
945 1 and 3) the three main clusters predicted by the most likely model of STRUCTURE

946 were clearly resolved and admixed pike populations were positioned within the
947 correct genetic context.

948 **Fig. 4**: Map of Germany illustrating that genetic admixture on population level varied
949 strongly and was not confined to a particular hydro-geographic region or river
950 catchment therein. – Black, grey and white colors of the pie charts indicate genetic
951 ancestry proportion of Black Sea, North Sea and Baltic Sea hydrogeographic region,
952 respectively. Numbers indicate pooled populations as displayed in Table 3.

953 **Fig. 5**: Genetic admixture calculated per individual. Extension of a figure indicates
954 increased numbers of individuals with a certain proportin of native ancestry (column
955 2) or degree of hybridization (column 3). Mean values are indicated within each figure
956 as a dot. Column 1 indicates the number of individuals analyzed per sampling site.
957 Color code for native ancestry: green = NA ≥ 0.5, yellow = 0.25 < NA < 0.5,
958 red = NA ≤ 0.25, color code for hybridization index: green = HI ≤ 0.25,
959 yellow = 0.25 < HI < 0.5, red = HI ≥ 0.5.

960 **Fig. 6**: Correlation of native ancestries and hybridization indices with habitat type,
961 strength of modification and ecological quality (1 = very good to 5 = poor according to
962 EU water framework directive) as obtained with HGLM analysis (see Table 4 for
963 details). Baltic coastal waters (BAL2, BAL3 and BAL4) and freshwaters without
964 ecological information (AWU, SUS, KDO) were excluded from analysis. IDs of water
965 bodies are explained in Table 1.

966

967 **Legends to tables**

968 **Table 1**: Sampled water bodies, type of water body and geographic positions of
969 sampling sites within each of the three hydro-geographic regions. Sample
970 identification (ID) is given by a three letter code, which is used throughout the text.

971 **Table 2**: High-resolution microsatellites selected according to Eschbach & Schöning
972 (2013) for population genetic analysis of species with low genetic variability.

973 **Table 3**: Admixture analysis revealed three genetic clusters of pike populations
974 belonging to the hydro-geographic region of the North, Baltic and Black Sea,
975 respectively (shaded areas indicate highest proportion of ancestry). Some
976 populations exhibited high proportions of non-native ancestry (indicated in fat italic
977 writing). Sample IDs are explained in Table 1. Samples with the same number have
978 been pooled for a clearer presentation in Fig. 4.

979 **Table 4**: Results of hierarchical general linear mixed modelling (HGLM) to test the
980 effect of different linear predictors on the hybrid index (HI) and the native ancestry
981 (NA) controlling for the random variance attributed to the individuals sampled in
982 specific waterbodies nested within catchments (see Fig. 6). The table shows the
983 estimates (in logit scale) and their standard error (s.e.), the t-value statistics and their
984 p-value (Pr(>|t|). Two-level interactions were non-significant in all cases and removed
985 from the model. The estimates of the categorical variables were shown per one
986 category with respect to the other (intercept). Significance codes: 0 '***' 0.001 '**',
987 0.01 '*', 0.05 and '.' 0.1.

Legends to supplementary material

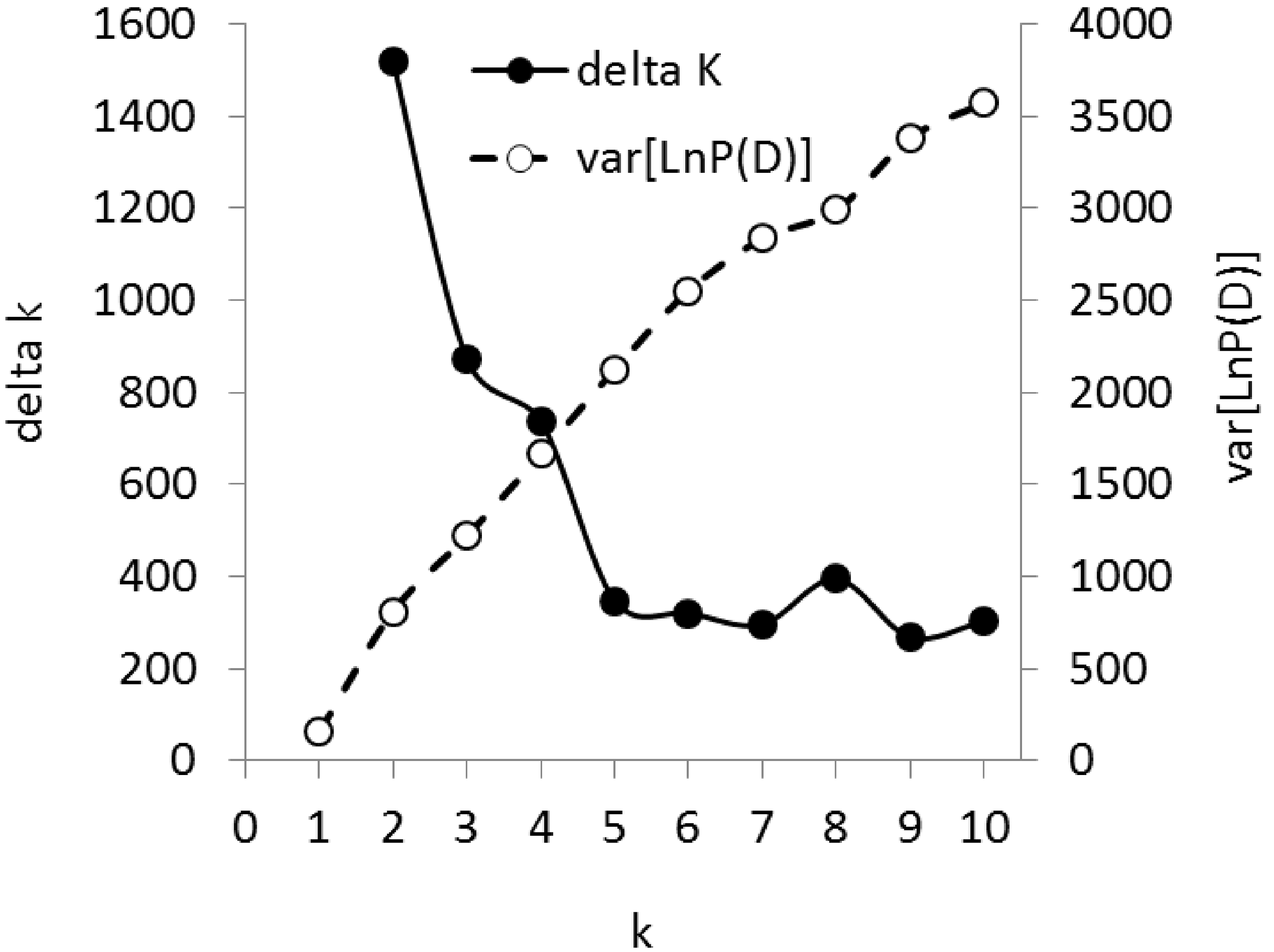
Fig. S1: Pike populations showing deviations from Hardy-Weiberg equilibrium (A) and heterozygote deficiency (B).

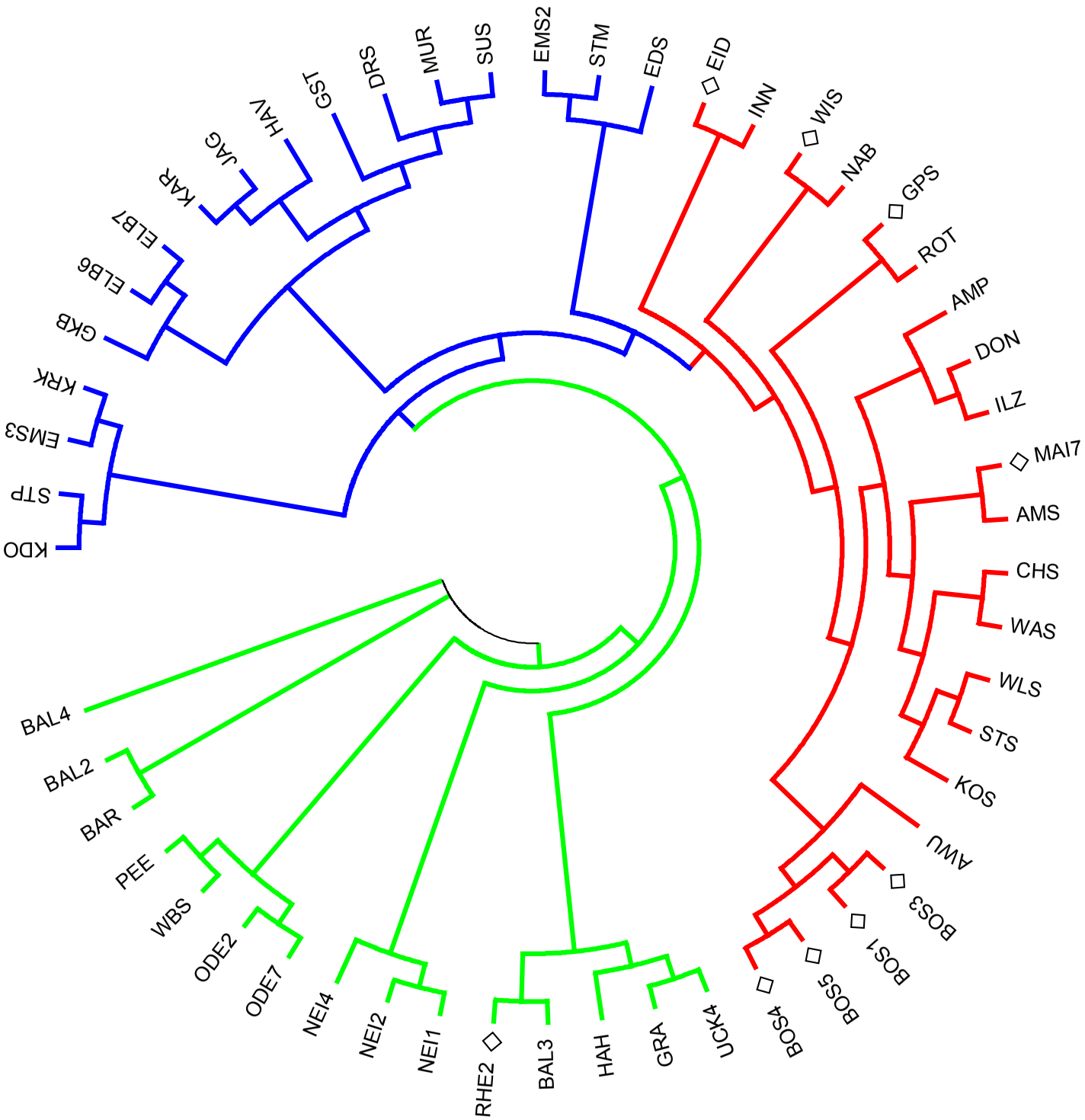
Fig. S2: Number of loci combinations exhibiting linkage disequilibria determined with and without Bonferroni correction.

Fig. S3: Network analysis based on *cyt* b sequences. B, E and F mark the circumpolar, northern and southern clades, respectively, of the northern pike according to Skog *et al.* (2014). – The size of the circles is proportional to the number of pike individuals with a certain haplotype; color code for samples: blue = North Sea, green = Baltic Sea, orange = Black Sea hydro-geographic region, gray = reference sequences, white = a mutation step, black = a hypothetical ancestor.

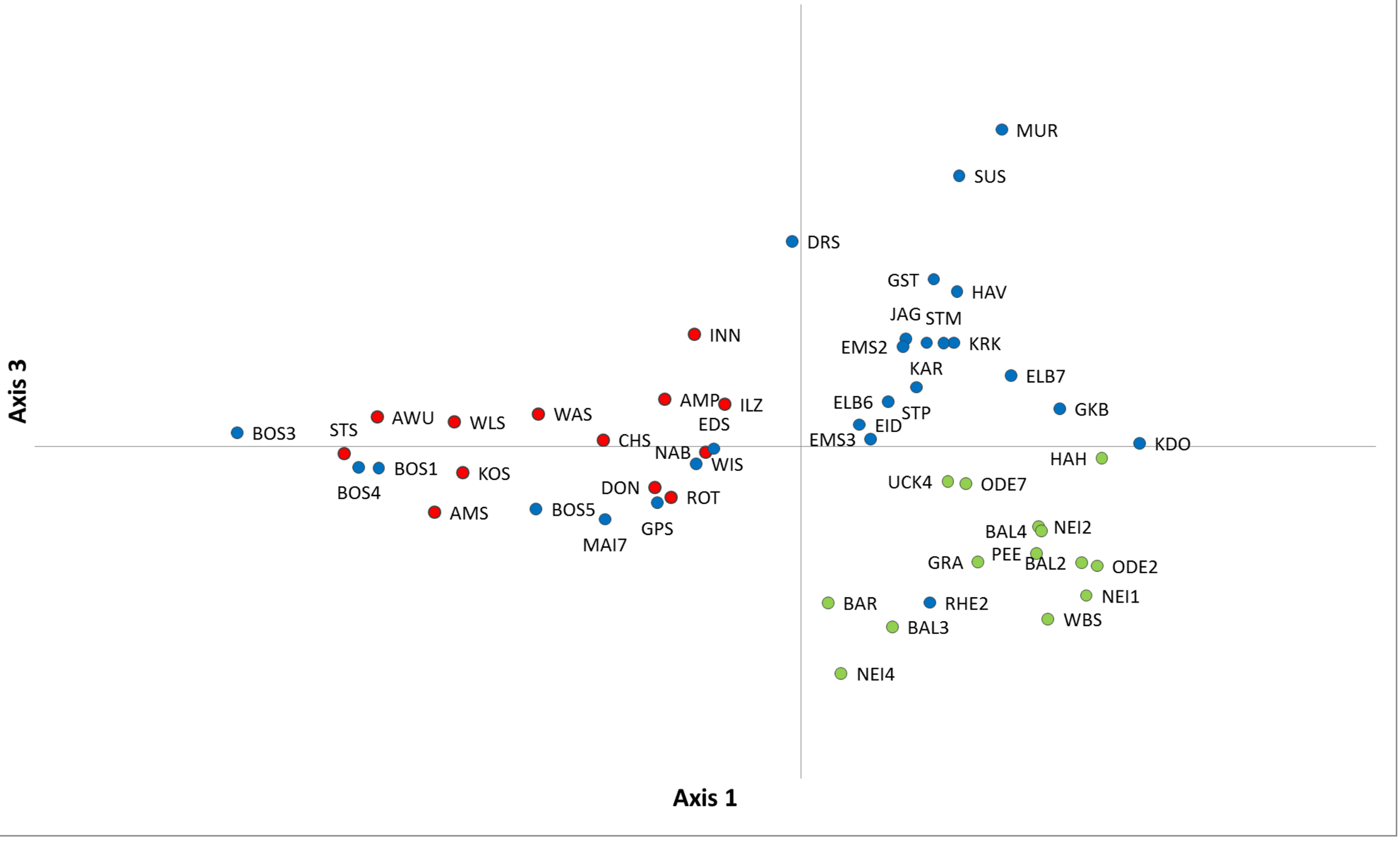
Table S1: Test for evidence of null alleles. **A**: number of alleles per locus and population (FSTAT 2.9.3.2). Total number of alleles = 6,969. **B**: Test for null alleles (MICROCHECKER 2.2.3). Total number of putative null alleles = 112, i.e. 1.6% of total number of alleles.

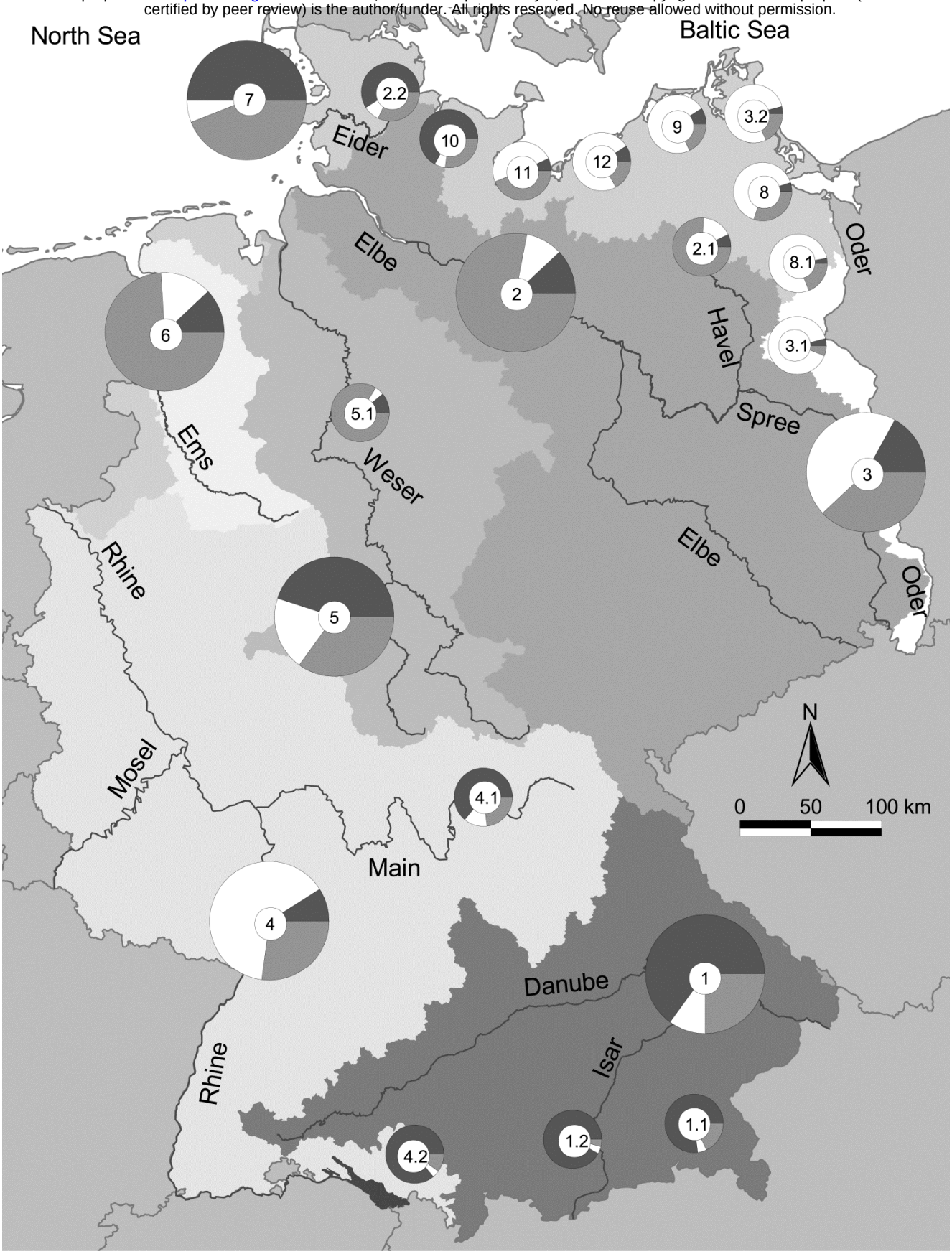
Table S2: Pairwise Fst (below diagonal) and p values (above diagonal) of 53 pike populations. Fst values were employed for principal coordinate analysis (Fig. 3).

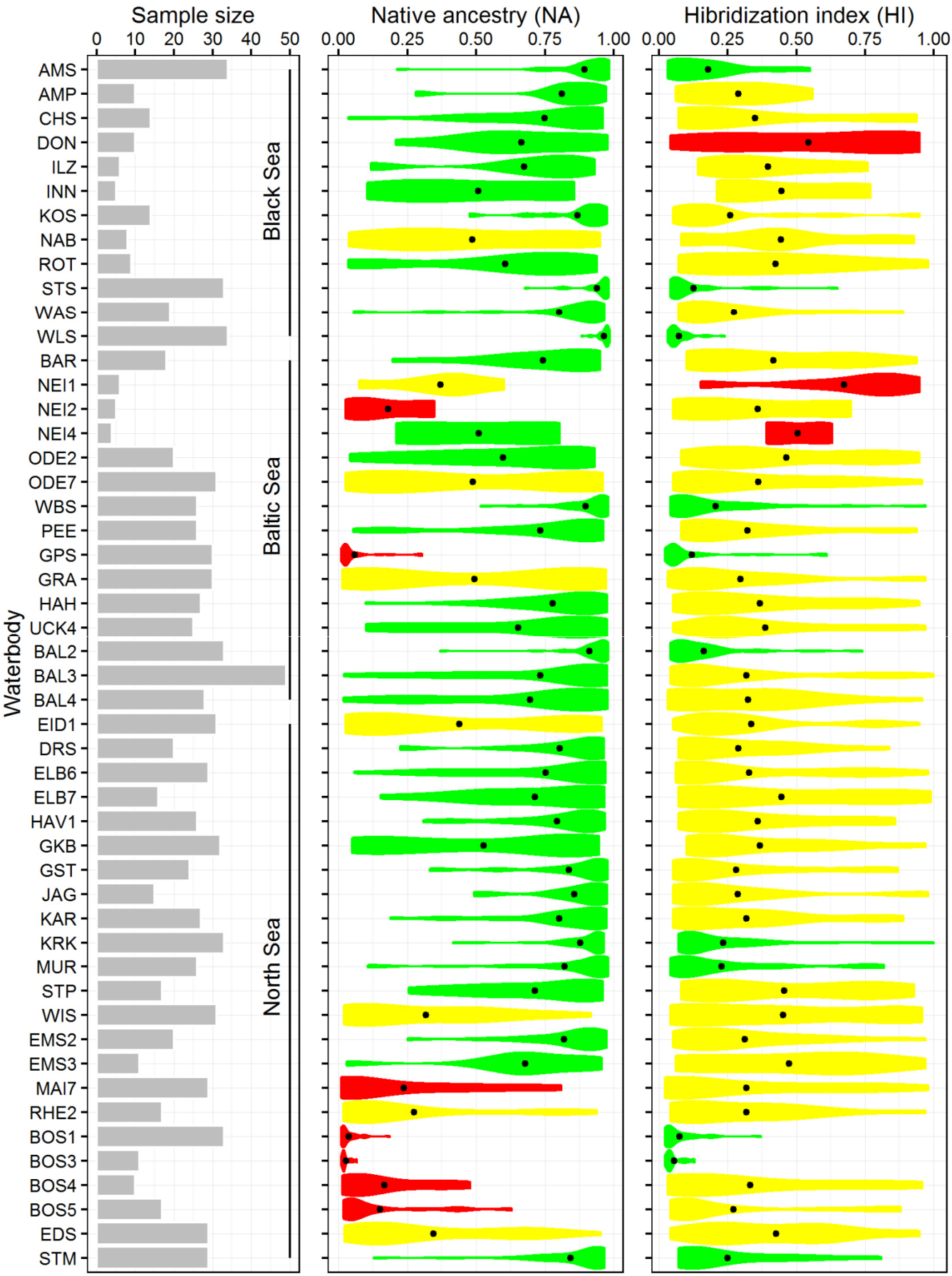

delta K
var[LnP(D)]
delta k
var[LnP(D)]
k
1600
1400
1200
1000
800
600
400
200
0
4000
3500
3000
2500
2000
1500
1000
500
0
0 1 2 3 4 5 6 7 8 9 10



Principal Coordinates (1 vs 3)







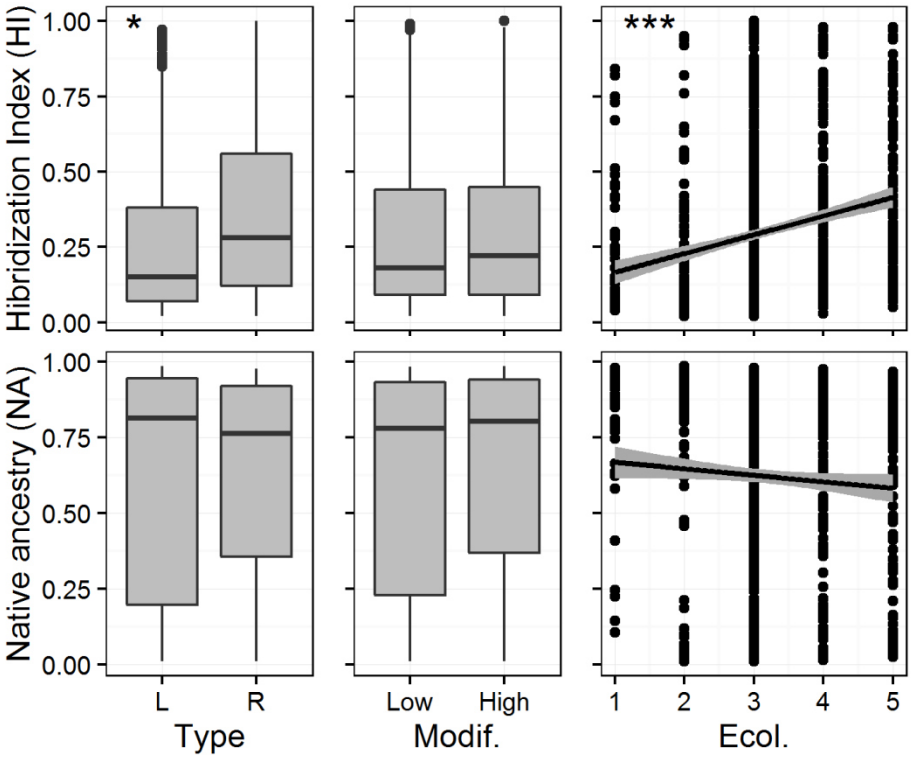


Table 1: Sampled water bodies, type of water body and geographic positions of sampling sites within each of the three hydro-geographic regions (HGR). Sample identification (ID) is given by a three letter code, which is used throughout the text (underlined codes indicate samples of which sub-samples have been taken to analyze the mitochondrial *cyt b* gene in addition to microsatellites).

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

Catchment	Waterbody	Type	ID	LAT	LONG	IC
	Großer Stechlinsee	l	GST	53°09´ N	13°01´ E	BB
	Jäglitz	r	JAG	52°52´ N	12°24´ E	BB
North Sea HGR (cont.):						
	Karthane	r	KAR	52°59´ N	11°47´ E	BB
	Kleiner Döllnsee	l	KDO	52°59´ N	13°34´ E	BB
	Krainke	r	KRK	53°13´ N	11°04´ E	NI
	Müritz	l	MUR	53°25´ N	12°41´ E	MV
	Schulzensee	l	SUS	53°09´ N	13°15´ E	BB
	Schwarze Elster	r	STP	51°28´ N	13°26´ E	BB
	Wittensee	l	WIS	54°23´ N	09°44´ E	SH
Ems	Ems	r	EMS3	52°58´ N	07°18´ E	NI
	Hieve	l	EMS2	53°24´ N	07°16´ E	NI
Rhine	Main	r	MAI7	50°01´ N	10°31´ E	BY
	Rhine	r	RHE2	49°09´ N	08°22´ E	BW
	Lake Constance	l	BOS1	47°41´ N	09°02´ E	BW
		l	BOS3	47°43´ N	09°13´ E	BW
		l	BOS4	47°33´ N	09°37´ E	BW
		l	BOS5	47°35´ N	09°31´ E	BW
Weser	Edersee	l	EDS	51°11´ N	09°03´ E	HE
	Steinhuder Meer	l	STM	52°28´ N	09°19´ E	NI

Type = type of waterbody: r = river, l = lake, c = coast, NN = no name; ID = sample identification code; geographic coordinates: LAT = latitude north (N), LONG = longitude east (E); IC = German federal state identification code: BB = Brandenburg, BW = Baden-Württemberg, BY = Bavaria, HE = Hesse, MV = Mecklenburg-Vorpommern, NI = Lower Saxony, SH = Schleswig-Holstein, SN = Saxony; HGR = hydro-geographic region; NN = name unknown.

Table 2: High-resolution microsatellites selected according to Eschbach & Schöning (2013) for population genetic analysis of species with low genetic variability.

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

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 3: Admixture analysis revealed three genetic clusters of pike populations belonging to the hydrogeographic regions of the North, Baltic and Black Sea, respectively (shaded areas indicate highest proportion of ancestry). Some populations exhibited high shares of non-native ancestry (indicated in fat italic writing).

		Proportion of ancestry:					Proportion of ancestry:		
ID*	no. in Fig. 4	North Sea	Baltic Sea	Black Sea	ID	no. in Fig. 4	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***					

* See Table 1 for definition of sample IDs. Samples with the same number have been pooled for a clearer presentation in Fig. 4

Table 4: Results of hierarchical general linear mixed modelling (HGLM) to test the effect of different linear predictors on the hybrid index (HI) and the native ancestry (NA) controlling for the random variance attributed to the individuals sampled in specific waterbodies nested within catchments (see Fig. 6). The table shows the estimates (in logit scale) and their standard error (s.e.), the t-value statistics and their p-value (Pr(>|t|). 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: 0 '***' 0.001 '**', 0.01 '*', 0.05 and '.' 0.1.

Hybrid index	Estimate	s.e	t-value	Pr(>|t|)	
(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	Estimate	s.e	t-value	Pr(>|t|)
(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