ACTA ICHTHYOLOGICA ET PISCATORIA (2020) 50 (2): 161–170 DOI: 10.3750/AIEP/02494

GENETIC CHARACTERIZATION OF SELECTED POPULATIONS OF EUROPEAN PERCH, *PERCA FLUVIATILIS* (ACTINOPTERYGII: PERCIFORMES: PERCIDAE), IN THE WATERS OF NORTH-WESTERN POLAND WITH RECOMMENDATIONS FOR FISHING AND STOCKING POLICIES

Wawrzyniec WAWRZYNIAK[1], Jolanta KIEŁPIŃSKA*[2], Przemysław CZERNIEJEWSKI[1], and Anna BUGAJ[1]

[1] *Department of Commodity, Quality Assessment, Process Engineering and Human Nutrition, West Pomeranian University of Technology in Szczecin, Poland*

[2] *Department of Aquatic Bioengineering and Aquaculture, West Pomeranian University of Technology in Szczecin, Poland*

Wawrzyniak W., Kiełpińska J., Czerniejewski P., Bugaj A. 2020. Genetic characterization of selected populations of European perch, *Perca fluviatilis* (Actinopterygii: Perciformes: Percidae), in the waters of north-western Poland with recommendations for fishing and stocking policies. Acta Ichthyol. Piscat. 50 (2): 161–170.

Background. European perch, *Perca fluviatilis* Linnaeus, 1758, is a predatory freshwater fish present also in the coastal waters of the Baltic Sea. The available catch data indicate that the population profile of this species is disrupted, and the condition of the population is not stable. The aim of this study was to determine the diversity and genetic structure of and assess phylogenetic links and gene flow between selected perch populations originating from various types of reservoirs in northern Poland.

Materials and methods. A total of 392 perch were collected from selected bodies of water in north-western Poland and the fin samples were preserved in 96% ethanol or stored for short periods at –20°C. Genomic DNA was isolated, while for further mitochondrial DNA analysis, a 1081-bp fragment of the control region was amplified. Amplification products were sequenced and subjected to restriction analysis (PCR-RFLP) in order to determine the genetic diversity of the control region of mtDNA. The obtained sequences were analyzed using bioinformatics tools, and phylogenetic trees were generated to determine the degree of diversity between individuals in selected perch stocks.

Results. Seven haplotypes were identified. Ten polymorphic sites were defined, and the degree of genetic diversity between selected haplotypes was determined. Based on the distribution of perch haplotypes, stocks from Pomeranian Bay (haplotypes F, D), the Szczecin Lagoon (haplotypes D, F, H), and the more homogeneous stocks from Pomeranian Bay (haplotypes F, D) and the Kamieński Lagoon (D, F) can be distinguished. Analysis of phylogenetic relations allowed identifying stocks with the most conserved genome, similar to the standard perch genome, and stocks that have become more genetically distant, probably due to the diversity of the inhabited ecosystems or natural hybridization observed in this species.

Conclusions. European perch inhabiting waters of north-western Poland is a species with high plasticity, without isolated stocks or populations that can be clearly distinguished on a genetic basis. It does, however, have a tendency for mitochondrial genomic modifications, which is likely due to the broad spectrum of ecosystems inhabited by the species. Due to the homogeneity of the perch populations from the Szczecin Lagoon, the Kamieński Lagoon, and Lake Myśliborskie, they should be used for the restoration of populations in water bodies in which their genetic structure is severely disrupted. For the same reason, the policy of perch catches should be modified. The authors suggest increasing the protected size to 20 cm and limiting the catches of the species as by-catch by modifying fishing tools, including the introduction of a seasonal prohibition of tools that might eliminate individuals of 15–16 cm in length from natural waters in their spawning period.

Keywords: Percidae, mtDNA, genetic diversity, haplotypes

* Correspondence: Jolanta Kiełpińska, Katedra Bioinżynierii Środowiska i Akwakultury, Zachodniopomorski Uniwersytet Technologiczny w Szczecinie, ul. Kazimierza Królewicza 4, 71-550 Szczecin, Poland, phone: +48914496663, e-mail: (JK) *jkielpinska@zut.edu.pl*, (WW) *wwawrzyniak@zut.edu.pl*, (PC) *pczerniejewski@zut.edu.pl*, (AB) *abugaj@zut.edu.pl*, ORCID: (JK) 0000-0002-9627 0368, (PC) 0000-0001-8553-9109.

INTRODUCTION

European perch, *Perca fluviatilis* Linnaeus, 1758, is one of the most common fish species in the inland waters of Europe (Kottelat and Freyhof 2007). It is currently heavily exploited due to its abundant occurrence, a high value of the meat, and export potential (Gillet et al. 2013). Also, in the Polish waters, a dynamic increase in the catches of this species has been observed in recent decades (Wysokiński 1998, Czerniejewski et al. 2006). The intense exploitation of perch can lead to a decline of its population. Stocking is a common method of supporting populations at risk. It involves the supplementation of individuals obtained by pre-rearing under controlled conditions into wild populations. Unfortunately, these activities have not taken into account the presumed genetic uniqueness of each population, and the only criterion for selection of the material is the economic criterion, i.e., lower price of the juveniles or lower transport costs (Jurczyk and Brzuzan 2004). In view of the above, it seems necessary to estimate the existing resources of the genetic diversity and their relations in natural waters. Moreover, in addition to its widespread occurrence and economic importance, as well as high adaptability to changing environmental conditions (Craig 1987), perch is one of the best model species for the analysis of the postglacial history of this freshwater fish (Nesbø et al. 1999). Perch tolerates a wide range of temperatures, with the optimum between 8 and 27°C and it was among the first colonizing species during the Pleistocene interglacial periods (Jerzmańska and Raczyński 1991, Ŝerbuha 1993). Because of its high fecundity and lack of specific requirements for spawning, it easily colonizes new habitats such as lakes, dam reservoirs, or canals (Thorpe 1977). The literature regarding the genetic diversity of perch is relatively scarce and provides data only on some European populations, mainly Scandinavian ones (Refseth et al. 2011, Nesbø et al. 1998b, 1999). However, several groups of perch differing in mtDNA have been distinguished in Europe. To date, researchers have focused on the impact of isolation and fragmentation of the environment on the structure of the population of the species (Gerlach et al. 2001, Behrmann-Godel 2004, 2006), as well as analysis of the phylogenetic links within the family Percidae (Nesbø et al. 1998a, Song et al. 1998). Bergek and Olsson (2009) confirmed, that populations of perch appear to cluster in different schools in the lake that harbor genetically differentiated groups of fish.

The majority of studies have also aimed to analyze the genetic diversity and phylogeographic links between the populations of European perch as a reflection of the past glacial refugia and postglacial colonization by this species (Nesbø et al. 1999). Most studies involving assessment of the diversity of the sequences of the mtDNA control region in European perch have taken into account the fragment adjacent to trnT, while the fragment adjacent to the trnF gene is poorly known. Moreover, no studies of the genetic diversity of perch have been conducted in Poland, and no genotyped perch populations have been described.

The aim of this study was to determine the diversity and genetic structure of selected perch populations originating from various types of reservoirs in northern Poland and to assess phylogenetic links and gene flow between them. The authors also indicated the possible usefulness of the studied bodies of water as sources of pre-rearing material to be used in the stocking of natural waters.

MATERIAL AND METHODS

Sample collection. The material for genetic analysis was collected within 2005–2009 from the Baltic Sea (Pomeranian Bay), estuary (Szczecin Lagoon, Kamieński Lagoon, and Lake Dąbie), river (Oder River), and inland lake (Lake Myśliborskie). The perch were acquired from commercial fishers using fyke nets. For genetic analysis, the cartilage and bone tissues from the caudal fin were used. Tissue fragments from a total of 392 European perch were collected, with a detailed summary presented in Table 1. Before analysis, the biological samples were preserved in 96% ethanol or stored for a short period at –20°C.

DNA isolation. Isolation of genomic DNA was conducted using the Genomic Mini AX Tissue kit (A&A Biotechnology) in accordance with the provided protocol,

Table 1

The number of individuals of perch, *Perca fluviatilis*, from which isolated tissues for genetic analysis were obtained in 2005–2009

Sample origin	Coordinates	*N*	*n*	
			Sequenced	Digested
Pomeranian Bay (Świnoujście)	54°07′37″N, 014°16′51″E	55	12	40
Szczecin Lagoon 1	53°44′82″N, 014°22′62″E	52	7	41
Szczecin Lagoon 2	53°47′78″N, 014°23′58″E	40	6	32
Szczecin Lagoon 3	53°42′48″N, 014°30′52″E	41	7	33
Kamieński Lagoon	53°56′19″N, 014°42′36″E	40	9	31
Lake Dąbie	53°31′49″N, 014°39′20″E	56	8	45
Oder (Dziewoklicz)	53°22′43″N, 014°32′3,5″E	51	8	41
Lake Myśliborskie	52°55′21″N, 014°51′66″E	57	6	45
Total		392	63	308

N = Total number of samples collected, *n* = number of samples used; for statistical analysis, only a part of the material for which amplification products had been obtained was used.

and using the phenol–chloroform extraction method (Green and Sambrook 2012). Based on spectrophotometric measurements using Nanodrop 2000 (Thermo Scientific) DNA concentration of 70–80 μg · mL^{-1} were estimated. The high molecular weight of the isolated DNA was confirmed by electrophoresis on 1% agarose gel with ethidium bromide in 1 × TBE buffer.

DNA amplification. For the analysis of the polymorphism of the mtDNA control region, two pairs of primers were used: HV2 and CSB-3. The quality of the obtained products was checked by electrophoresis on 1.5% agarose gel (Basic Proto AgaroseTM) with ethidium bromide in 1 × TBE buffer. To determine the size of the obtained fragments, a molecular weight marker (MassRuler, Low Range from Fermentas) was used.

Amplification of the control region. For the analysis of mitochondrial DNA, a fragment of the control region controlling of one of the most variable fragments of 1018 base pairs was selected. Amplification of this fragment was conducted using the primers pairs described by Nesbø et al. (1998a)—HV2: TTCCCCGGTCTTGTAAACC, and CSB-3: TATTCCTGTTTCCGGGG using a Mastercycler Gradient (Eppendorf, Germany). The reaction mixture in a final volume of 20 μL contained 1 μL of DNA template, 5 μL of 1 × PCR buffer, 0.5 μL of dNTP (10 pmol), 2.5 mM $MgCl_2$, 0.2 mM of each primer, 0.5 U Taq polymerase (Novazym) and deionized nuclease-free water. The conditions of PCR were as follows: initial denaturation at 96°C for 5 min, proper denaturation at 96°C for 1 min, primer annealing at 51°C for 1 min, synthesis at 72°C for 2 min, final extension at 72°C for 10 min. Steps described in points 2 to 4 were repeated 29 times. Amplification products were purified using GeneMATRIX PCR/DNA Clean-Up Purification Kit (EURx).

Polymorphism of the amplified mtDNA control region

DNA sequencing. Bidirectional Sanger sequencing of the selected PCR products (n = 63) of the 1081 bp control region was ordered from Genomed, Warsaw, Poland. Using the BioEdit software (Hall 1999), alignment of the obtained mtDNA sequences was performed. Use of the BLAST service allowed checking the consistency of the obtained sequences with the Y14724 (Nesbø at al. 1998a) sequence submitted to NCBI and checking any previous submissions of the determined haplotypes in GenBank.

PCR-RFLP. Due to the large size of the analyzed control region, Primer3 software was used to design two pairs of primers (Table 2) covering the analyzed fragment. Primer sequences specific for selected fragments of the mtDNA control region were used in a PCR amplification. The reaction mixture in a final volume of 20 μL contained 1 μL of DNA template, 5 μL of 1 × PCR buffer, 0.5 μL of dNTP (10 pmol), 2.5 mM MgCl2, 0.2 mM of each primer, 0.5 U Taq polymerase (Novazym) and deionized nuclease-free water. The amplification was conducted using a Mastercycler Gradient (Eppendorf, Germany). The conditions of the PCR reaction were as follows: initial denaturation at 95°C for 5 min, denaturation at 96°C for 55 s, primer annealing at 59°C for 55 s, synthesis at 72°C for 55 s, and a final extension at 72°C for 5 min (stages from points 2–4 were repeated 32 times). Using the Webcutter V2.0 software, an in silico analysis of the sequences of the mtDNA control region (1081 bp) was conducted, and restriction enzymes recognizing the analyzed polymorphic sites were identified. As a result, nine restriction enzymes (Fermentas) were selected (Table 3).

PCR products for the investigated fragments of the analyzed sequence were restriction-analyzed by adding 4 units of a restriction enzyme with a buffer to 10 μL of the amplification product. The mixture was incubated overnight at a temperature recommended by the manufacturer. The obtained restriction fragments were separated on agarose gel (Reducta Proto AgaroseTM) with ethidium bromide in 1 × TBE buffer. Their percentage concentration was dependent on the size of the separated DNA fragments. In order to determine the size of the products, a molecular weight marker was used. The separated products were visualized under UV light.

ACRS-P+CR. As it was impossible to analyze a transition located in position 299 of the control region sequence using the PCR-RFLP technique, a modified method was used. ACRS-PCR (Amplification-Created Restriction Sites-Polymerase Chain Reaction) (Haliassos et al. 2001) involved the introduction of an artificial restriction site in the PCR product by changing the third base from the 3′ end in the "front" primer sequence (Table 4).

Amplification of the selected fragment of the control region (236 bp) was conducted using a Mastercycler Gradient (Eppendorf, Germany). The reaction mixture in a final volume of 20 μL contained 1 μL of DNA template, 5 μL of 1 × PCR buffer, 0.5 μL of dNTP (10 pmol), 2.5 mM $MgCl_2$, 0.2 mM of each primer, 0.5 U Taq polymerase (Novazym), and deionized nuclease-free water. The conditions of PCR were as follows: initial denaturation at 95°C for 5 min, proper denaturation at 96°C for 55 s, primer annealing at 59°C for

Table 2

Sequences of primers pairs used in the PCR amplification of the mtDNA control region for RFLP analysis

Primer	Primer sequence (5′→3′)	Annealing temperature [ºC]	Primer length [bp]	Product size [bp]	Location
CR1F	CCCCTAACGCTTCAAAGAAA	59	20	183–193	21–40
CR1R	TGTCCTTGAATGCCCCTTAT		20		184–203
CR3F	CCTGGCATTTGGTTCCTACT	59	20	218–226	490–509
CR3R	GCAGAGGAAATGTTCAACCTTATT		24		693–716

Location = location in the Y14724 sequence submitted to NCBI.

55 s, synthesis at 72°C for 55 s, final extension at 72°C for 5 min (stages from points 2–4 were repeated 32 times). The obtained product was digested with 4 units of restriction enzyme (Fermentas, Table 5) according to the manufacturer's manual. The mixture was incubated overnight at a temperature recommended by the manufacturer. Digestion products were separated on a 2% agarose gel with ethidium bromide and visualized under UV light.

Statistical analyses of the genetic diversity of the control region. Using Mega 4.1 software (Kumar et al. 2008), phylogenetic trees of haplotypes were generated using neighbor-joining (NJ) and maximum parsimony (MP) methods. Unrooted phylogenetic trees were generated to present phylogenetic relations between the haplotypes detected in the investigated area. Sequences published by the authors were arranged and aligned using Mega 4.1 software. A fragment common to all sequences was selected and using the MP method, a phylogenetic tree showing relations between the haplotypes detected in this study and the haplotypes detected by other researchers, was generated. The MP and NJ methods were used in generating dendrograms as described by Zdziennicki and Misiewicz (2014). Subsequently, using TCS software (Clement et al. 2000), relations between the haplotypes were determined by generating a network of phylogenetic relations between haplotypes, known as the minimum spanning network. Nesting was conducted in accordance with the indications given by Templeton et al. (1992), and Templeton and Sing (1993). The most likely structure of the population was determined using Samova software (Dupanloup et al. 2002). For sub-populations determined by the software, haplotype and nucleotide diversity and the frequencies of each haplotype were calculated using Arlequin 3.11 software (Excoffier et al. 2005).

Table 3

Restriction enzymes used to analyze the genetic diversity of the mtDNA control region by PCR-RFLP

Polymorphism	Enzyme	Recognized sequence	Location in relation to the Y14724 sequence [bp]	Digestion temperature [ºC]
103CR1/*Nhe*I	***Nhe*I**	G↓CTAGC CGATC↑G	103	37
129CR1/*Hin6*I	***Hin6*I**	G↓CGC CGC↑G	129	37
130CR1/*Hin6*I	***Hin6*I**	G↓CGC CGC↑G	130	37
135CR1/*Hin6*I	***Hin6*I**	G↓CGC CGC↑G	135	37
553CR3/*Taq*I	***Taq*I**	T↓CGA AGC↑T	553	37
559CR3/*Cac8*I	***Cac8*I**	GCN↓NGC CGN↑NCG	559	37
655CR3/*Alu*I	***Alu*I**	AG↓CT TC↑GA	665	37
669CR3/*Aci*I	***Aci*I**	C↓GCC GCG↑G	669–673	37

Table 4

Primer sequence used in the amplification of the mtDNA control region by ACRS-PCR, with a marked change in the "front" primer sequence

Primer	Primer sequence (5′→3′)	Annealing temperature [ºC]	Primer length [bp]	Product size [bp]	Location in the Y14724 sequence submitted to NCBI
CR2F	AGCATATAGACCTTTATCTAACAT**G**TA		27	236	272–298
CR2R	GTAGGAACCAAATGCCAGGA		20		489–508

Table 5

The restrictive enzyme used in the analysis of the genetic diversity of the mtDNA control region by ACRS-PCR

Polymorphism	Enzyme	Recognized sequence	Region	Digestion temperature
299CR2/*Ras*I	***Ras*I**	GT↓AC CA↑TG	299	37ºC

RESULTS

Detection of D-loop mtDNA polymorphism. The results of the sequencing of the control region of 1081 bp in 63 randomly selected individuals originating from 8 areas (Table 1) compared with the GenBank Y14724 sequence showed 10 polymorphic sites (Fig. 1).

Analysis of the polymorphism of the control region for the other 329 individuals was conducted using the RFLP and ACRS methods. To this end, specific primers were designed to cover the previously detected mutations. Further analysis was conducted with only those samples whose PCR products were pure and specific, as assessed by electrophoresis of 5 µL of the product on 1.5% agarose gel with ethidium bromide (Fig. 2).

Analysis of diversity of mtDNA haplotypes. In the investigated area, based on the analysis of 1081-bp fragments of the mtDNA control region, seven haplotypes were identified (Table 6). Ten polymorphic sites, which accounted for 0.98% of the analyzed sequences, were found. Among the ten polymorphic sites observed in the sequence, there were two deletions (a 3-nucleotide and a 5-nucleotide), one insertion (10-nucleotide), two transversions and five transitions. As shown in Table 7, based on the distribution of perch haplotypes, stocks from Pomeranian Bay (haplotypes F, D), the Szczecin Lagoon (haplotypes D, F, H), and the more homogeneous stocks from Pomeranian Bay (haplotypes F, D) and the Kamieński Lagoon (D, F) can be distinguished. An interesting aspect of the results is the presence of similar genetic stocks of perch in the Kamieński Lagoon and Lake Myśliborskie (D, F), although they are not connected and there is no migration of perch between them. Fish from Lake Dąbie the Oder River constitute one genetic stock with three haplotypes (F, F1, H).

Figure 3. presents the generated phylogenetic tree of perch from the investigated areas of north-western Poland. The maximum parsimony method (A) presents the relations between the detected perch haplotypes, taking into account the smallest necessary number of nucleotide changes that occurred over the course of evolution and substantiate the obtained result. This allows including the rate of mutation in the interpretation of the result but does not provide any guidance regarding the time of divergence. As it appears from the MP analysis, the genetically closest haplotypes are D and F that prevail in north-western Poland. In turn, haplotypes F1 and H are genetically remote and were probably formed by the isolation of a stock of perch from the Oder River catchment area, as

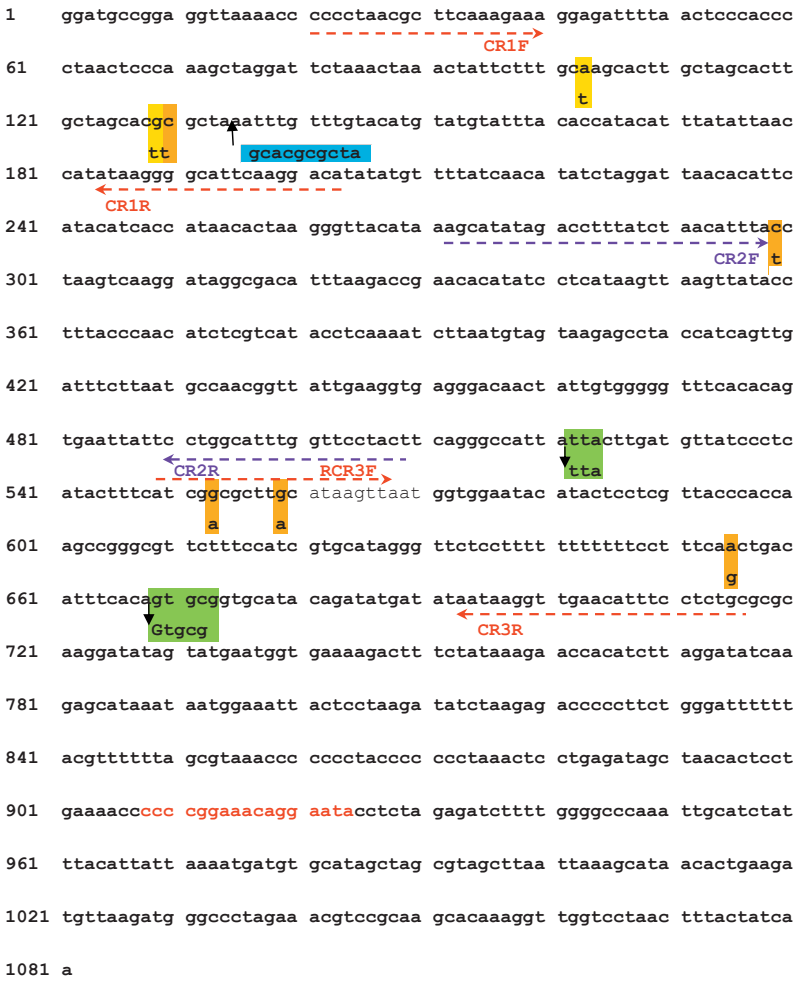

Fig. 1. The sequence of the control region of perch, *Perca fluviatilis*, mtDNA (GenBank Y14724) with marked mutations detected in the study: transversion, transition, and insertion, deletion

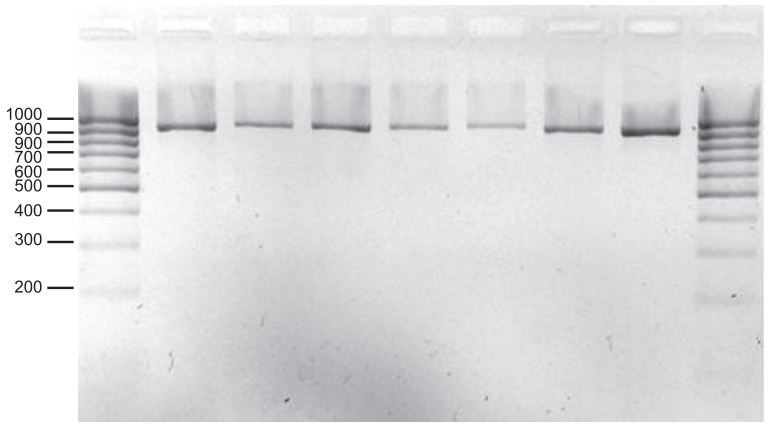

Fig. 2. Electrophoretic separation of amplification products of perch, *Perca fluviatilis*, using primers sequences; M = molecular weight DNA: pUC19 x Mspl (Serva Electrophoresis); Lanes: 1–3 CR1, 4–6 CR2

Table 6

Haplotypes of perch, *Perca fluviatilis*, detected in north-western Poland

Haplotypes 1–430 bp	Haplotypes 1–891 bp	Positions of mutations										
		103	129	130	135	299	522	553	559	655	669	*n*
		A	G	C	INS	C	DelT	G	G	A	DelG	
D	DA	T					–	A	A		–	90
F	FA	T				T	–				–	120
F	FB	T				T	–	A	A		–	86
F1	F1A	T	T	T		T	–	A	A		–	31
D	DB	T					–	A	A	G	–	12
F	FC	T				T	–	A	A	G	–	9
H	HA	T	T	T	+	T	–	A	A		–	23
											Total	371

n = number of fish individuals; INS = insertion GCACGCGCTA, DelT = deletion TTA, DelG = deletion GTGCG.

Table 7

Distribution of haplotypes of perch, *Perca fluviatilis*, in north-western Poland

Location	Haplotypes within 1–430 bp	Haplotypes within 1–891 bp
Pomeranian Bay	F, D	FA, FC, DB
Szczecin Lagoon 1	D, F, H	FA, DA, FB, HA
Szczecin Lagoon 2	D, F, H	FA, DA, FB, HA
Szczecin Lagoon 3	D, F, H	FA, DA, FB, HA
Kamieński Lagoon	D, F	DA, FB
Lake Dąbie	F, F1, H	FA, F1A, HA
Oder–Dziewoklicz	F, F1, H	FA, F1A, HA
Lake Myśliborskie	D, F	DA, FB

haplotype H is also occasionally present in the Szczecin Lagoon. This is probably due to dilution of the gene pool of the species or a previously undescribed phenomenon of natural hybridization. It can be noted that in the NJ model (B), whose principle is to find two objects with the smallest distance between them, haplotypes D and F were also the most genetically homogeneous. In this case, it seems likely that haplotype F (FA) is the most distant from the reference (GenBank acc. No. Y14724) perch genotype. Therefore, it is not only indicated as stocking material but also as a material with the highest diversity of the conserved mtDNA region.

DISCUSSION

The Szczecin Lagoon, due to its location, is of great importance as an ecological corridor for many fish species. Fish migrations in the Szczecin Lagoon can be divided into passive and active (Wiktor 1960, Dudko 2008). The former refers mainly to fish larvae that float freely, moved by water currents. In the Szczecin Lagoon, fish larvae

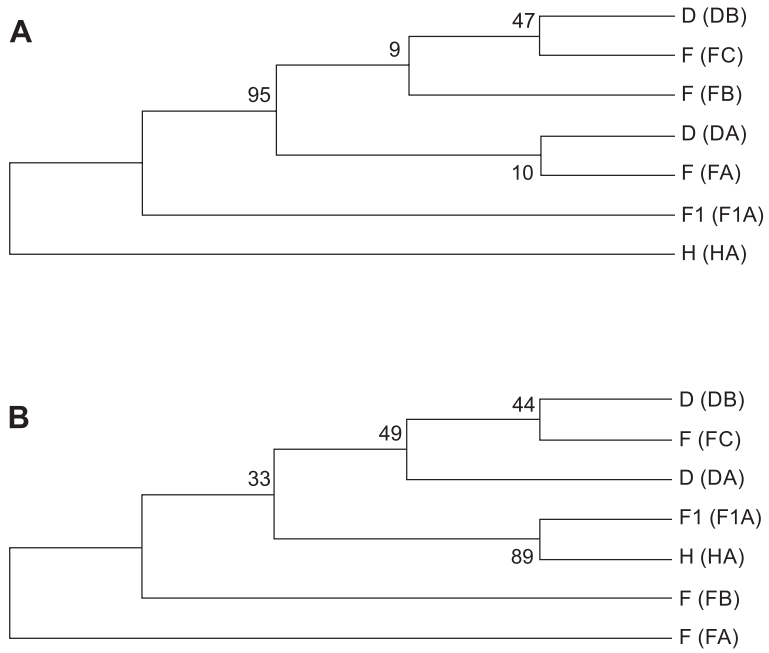

Fig. 3. Phylogenetic tree generated using: (**A**) Maximum Parsimony method (bootstrap = 1000 iterations), (**B**) Neighbor-Joining method (Kimura's 2-parametric model, bootstrap = 1000 iterations), showing the relations between the haplotypes of perch, *Perca fluviatilis*, occurring in the investigated area

in the form of ichthyoplankton are transported through the Piast Canal and the Świna Strait to Pomeranian Bay. However, there are no results of direct studies of the migration of larvae and juvenile fish, and of the scale of this phenomenon (Wolnomiejski and Witek 2013). Migrations of adult fish, i.e., active roaming in the Szczecin Lagoon and between the different parts of the Oder River estuary, are an important factor supporting the stocks of freshwater fish in Pomeranian Bay, but also in other parts of the Oder River estuary, such as Lake Dąbie and the Oder itself. The period and destinations of migrations for both reproductive and grazing purposes depend on the species of fish. A commercially important group of fish in the Szczecin Lagoon are species of the family Percidae, i.e., perch and pikeperch, *Sander lucioperca* (Linnaeus, 1758). According to Szypuła and Rybczyk (2001), the whole Oder estuary is inhabited by one population of perch that migrates between different parts of these waters. These are typical periodic migrations for grazing and spawning. Migrations of perch from Pomeranian Bay to the Szczecin Lagoon are also confirmed by analyses conducted by Garbacik-Wesołowska and Boberski (2000). Perch inhabiting the coastal zone of the Baltic Sea probably pass the longest distances among the known perch stocks—their range is up to 80 km (Król 2008). Perch spawning period in the Szczecin Lagoon occurs in late April and early May, depending on the thermal conditions of these waters, and therefore April is the time in which the highest biomass is obtained in fish catches besides the autumn period (Czerniejewski et al. 2006). After spawning, a part of the perch population migrates from the Szczecin Lagoon to Pomeranian Bay (Czerniejewski et al. 2006), where the fish form concentrated grazing stocks (Król 2008). After the incubation of eggs, the larvae of these fish at the spawning sites are observed at concentrations exceeding 2000 larvae · 100 m^{-3} of water, mainly in the eastern part of the lagoon (unpublished data by MIR PIB Gdynia). The majority of juvenile individuals (age groups 0+ and 1+) do not migrate to the waters of Pomeranian Bay, but remain in the waters of the lagoon, although there have been observations of grazing corridors of these fish leading also to the estuaries of the Ina and Gowienica rivers (Keszka and Tański unpublished[*]). Perch of the older age groups migrate all over the Szczecin Lagoon and are dispersed at all depths (Wysokiński 1998), and subsequently move to their grazing sites in Pomeranian Bay. Biological and environmental analysis of perch in the waters of north-western Poland indicates inconsistencies in the data regarding the formation of taxa below the rank of species, such as group, population or form. As early as in the 1970s, it was proven, based on the analysis of the species composition of parasitic fauna, that stocks of a species that have no contact with each other may form even in the same reservoir (Wierzbicki 1970). According to Dąbrowski (2005), the analysis of countable and measurable characteristics of perch from one reservoir did not reveal essential differences in body proportions compared to perch occurring at comparable aquatic habitats. However, they present different morphological forms depending on the abundance of food in the reservoir they inhabit. They can be defined as forms with a highly arched back in reservoirs rich in food, and elongated forms with a

[*] Keszka S., Tański A. 2008. Bonitacja zlewni Iny oraz dopływów dolnej odry i jej estuarium, będących w użytkowaniu rybackim przez Okręg PZW w Szczecinie. III etap Bonitacja rzek Gowienicy i Wołczenicy wraz z dopływami. [Assessment of fish resources in the catchment area of the Ina river and its estuary, as used for fishing by the Szczecin unit of PZW. Stage III: assessment of the Gowienica and Wołczenica rivers and their tributaries] Report for the Polish Angling Association, Szczecin. [In Polish.]

poorly arched back in reservoirs poor in food. A study by Fokina et al. (2015) revealed a possibility of genetic diversity of two perch populations whose geographical area of occurrence was limited to the catchment area of one river in Latvia. The distance between the two lakes from which the fish were sampled was approx. 60 km. The authors determined a genetic marker allowing molecular identification of these two populations. This means that gene transfer is not only affected by an environmental barrier but probably is a product of many factors, such as the optimal temperature for spawning or significant changes in the quality of the environment. The importance of these factors in shaping gene transfer in perch is also suggested by Bergek et al. (2010). The impact of the environment on gene transfer is very difficult to describe, as there are multiple barriers to the free exchange of genes in the aquatic environment, even if the reservoirs are physically connected. As suggested by Olsson et al. (2011), stretches of very deep water can constitute a barrier to gene transfer. Studies conducted in the coastline waters of Sweden allowed the authors to separate the populations of perch formed by genetically homogeneous stocks in the central part of the Baltic Sea, and those inhabiting the region of the Gulf of Bothnia. The authors mentioned that perch is a species useful in the local management of ichthyofauna resources in Sweden. Different conclusions were reached by Sepulveda-Villet et al. (2009) who analyzed genetic markers of mtDNA for all five Great Lakes located on the USA–Canada border. They demonstrated the presence of 21 haplotypes of American yellow perch, *Perca flavescens* (Mitchill, 1814), and at the same time established that the genetic distance between them is relatively low compared to the intraspecific genetic diversity of other fish species in that region. Gene transfer between the populations of perch also poses a risk of introducing genes from other species into the "wild-type" perch genotype. A case of natural hybridization of pikeperch and perch was described by Kahilainen et al. (2011). It involved hybridization occurring in one river in Finland, confirmed by morphological, anatomical and genetic analysis of the hybrids. Between 1964 and 2018 neither Odra River nor Dąbie Lake has been stocked (Anonymous unpublished[*]). Information on stocking perch in Pomeranian Bay is missing and this issue was confirmed also by the fishermen during the survey. According to aforementioned it might be concluded that the observed three haplotypes of perch identified in the Odra River and Dąbie Lake, taking into account the lack of stocking activities, may be regarded as a local population. In Pomeranian Bay, the presence of the two perch haplotypes, including haplotype F, identified for each sampling site, confirms that this population has high plasticity which does not require any further support. It is possible that the perch population from Pomeranian Bay will not reach the age balance, repeating the situation from the Szczecin Lagoon where *P. fluviatilis* constitutes as much as 32% but is represented mostly by juveniles under 20 cm. Hence it might be concluded that perch stock in the future will require stocking activities, especially when young individuals after 2–3 years will reach reproduction age and as shown by data (Wawrzyniak et al. unpublished[**]) their number and similarly gene pool is reduced. This supports the genetic regression process and in consequence decreases the condition of the population.

According to our study and the cases of intraspecific diversity of perch described in the literature, the plasticity of this species can be considered as high. However, it does not lead to the appearance of different separate taxonomic units (species or subspecies), probably due to the tendency for distant migrations for grazing and spawning, as well as the occurrence in very diverse environments. Such geographical conditions do not give enough time for the preservation of evolutionary changes at a genotype level. In recognition of the economic and ecological importance of the species as an element of the Polish ichthyofauna, analyses of the genomic purity of the stocking material need to be introduced. There is a substantiated risk of the formation of natural hybrids and contamination of the perch gene pool with material from other, phylogenetically similar species. In the waters of north-western Poland, we managed to confirm the presence of a pure genetic pool of perch inhabiting the Szczecin Lagoon, Kamieński Lagoon, and Lake Myśliborskie, which is an important reason for the introduction of regulations aimed to improve the management of the gene pool of this species. These bodies of water should be the only permitted catch sites in north-western Poland for obtaining material intended for pre-rearing and stocking.

ACKNOWLEDGMENTS

The presently reported study has been financed by the West Pomeranian University of Technology in Szczecin, Poland in the frames of an internal doctoral grant to AB.

REFERENCES

Behrmann-Godel J., Gerlach G., Eckmann R. 2004. Postglacial colonization shows evidence for sympatric population splitting of Eurasian perch (*Perca fluviatilis* L.) in Lake Constance. Molecular Ecology **13** (2): 491–497. DOI: 10.1046/j.1365-294X.2003.02082.x

Behrmann-Godel J., Gerlach G., Eckmann R. 2006. Kin and population recognition in sympatric Lake Constance perch (*Perca fluviatilis* L.): Can assortative shoaling drive population divergence. Behavioral Ecology and Sociobiology **59** (4): 461–468. DOI: 10.1007/s00265-005-0070-3

Bergek S., Sundblad G., Bjorklund M. 2010. Population differentiation in perch *Perca fluviatilis*: Environmental

[*] Anonymous [Different years.] Operaty rybackie. [Fisheries reports.] Repozytorium Operatów Rybackich Wydziału Nauk o Żywności i Rybactwa, Zachodniopomorski Uniwersytet Technologiczny w Szczecinie, Szczecin, Poland.

[**] Wawrzyniak W., Czerniejewski P., Neja Z., Raczyński M., Król S., Kiełpiński M., Szulc M., Tomaszkiewicz A. 2017. Program badań na Zalewie Szczecińskim i Jeziorze Dąbie w roku 2017 polegający na ocenie stanu zasobów ryb, ze szczególnym uwzględnieniem populacji sandacza, okonia, płoci i leszcza. [Program of studies conducted in the Szczecin Lagoon and Lake Dąbie in 2017, focused on assessing fish resources, particularly zander, perch, common roach and common bream.] Szczecin. [In Polish.]

effects on gene flow? Journal of Fish Biology **76** (5): 1159–1172. DOI: 10.1111/j.1095-8649.2010.02565.x

Bergek S., Olsson J. 2009. Spatiotemporal analysis shows stable genetic differentiation and barriers dispersal in the Eurasian perch (*Perca fluviatilis* L.). Evolutionary Ecology Research **11** (5): 827–840.

Clement M., Posada D., Crandall K. 2000. TCS: A computer program to estimate gene genealogies. Molecular Ecology **9** (10): 1657–1659. DOI: 10.1046/j.1365-294x.2000.01020.x

Craig J. 1987. The biology of perch and related fish. Timber Press, Portland, OR, USA.

Czerniejewski P., Robakowski P., Raczyński M., Wawrzyniak W., Bugaj A. 2006. Struktura gatunkowa i wielkość połowów ryb w Zalewie Szczecińskim na początku XXI wieku. [Species structure and the size of catches of fish in the Szczecin Lagoon in the early 2000s.] Pp. 182–194. *In*: Furmańczyk K. (ed.) Brzeg morski zrównoważony. Zintegrowane Zarządzanie Obszarami Przybrzeżnymi w Polsce – stan obecny i perspektywy. [Sustainable sea shore. Integrated Coastal Zone Management in Poland—Current status and future prospects.] Print-Group, Szczecin. [In Polish.]

Dąbrowski J. 2005. Cechy przeliczalne i mierzalne okonia (*Perca fluviatilis* L.) z jeziora Gopło. [Countable and measurable traits of perch (*Perca fluviatilis L.*) from Lake Gopło.] Zeszyty Naukowe Akademii Techniczno-Rolniczej w Bydgoszczy, Zootechnika **35** (245): 117–124. [In Polish.]

Dudko S. 2008. Wędrówki sandaczy pomiędzy Zatoką Pomorską a Zalewem Szczecińskim. [Zander migrations between the Pomeranian Bay and the Szczecin Lagoon.] Pp. 173–180. *In*: Wawrzyniak W., Formicki K., Bartel R., Dunin-Kwinta I. (eds.) Rybackie perspektywy pobrzeża południowego Bałtyku III. [Prospects and Perspectives of Fisheries in the Costal Zone of the Southern Baltic III.] Foka, Szczecin. [In Polish.]

Dupanloup I., Schneider S., Excoffier L. 2002. A simulated annealing approach to define the genetic structure of populations. Molecular Ecology **11** (12): 2571–2581. DOI: 10.1046/j.1365-294X.2002.01650.x

Fokina O., Grauda D., Rashal I. 2015. Genetic diversity of two perch *Perca fluviatilis* populations of the Latgale region. Pp. 96–98. DOI: 10.17770/etr2015vol2.280 *In*: Environment, Technology, Resources. Proceedings of the 10th International Scientific and Practical Conference, Vol. II, Rezekne, Latvia.

Garbacik-Wesołowska A., Boberski E. 2000. Stan zasobów ryb Zalewu Szczecińskiego oraz strefy przybrzeżnej Wybrzeża Zachodniego i warunki ich eksploatacji. [Current status and exploitation of fish resources of the Szczecin Lagoon and the western Baltic coastal zone in Poland.] Studia i Materiały Morskiego Instytutu Rybackiego **72** (B): 77–104. [In Polish.]

Gerlach G., Schardt U., Eckmann R., Meyer A. 2001. Kin-structured subpopulations in Eurasian perch (*Perca fluviatilis* L.). Heredity **86** (2): 213–221. DOI: 10.1046/j.1365-2540.2001.00825.x

Gillet C., Lang C., Dubois J.P. 2013. Fluctuations of perch populations in Lake Geneva from 1984 to 2011 estimated from the number and size of egg strands collected in two locations exposed to different fishing practices. Fisheries Management and Ecology **20** (6): 484–493. DOI: 10.1111/fme.12037

Green M.R., Sambrook J. 2012. Molecular cloning: A laboratory manual. 4th edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

Haliassos A., Chomel J.C., Tesson L., Baudis M., Kruh J., Kaplan J.C., Kitzis A. 1989. Modification of enzymatically DNA for the detection of point mutations. Nucleic Acids Research **17** (9): 3606. DOI: 10.1093/nar/17.9.3606

Hall T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series **41**: 95–98.

Jerzmańska A., Raczyński P. 1991. Middle Pleistocene freshwater fishes from interglacial deposits near Bełchatow, Poland. Folia Quaternaria **61** (62): 109–123.

Jurczyk Ł., Brzuzan P. 2004. Charakterystyka genetyczna lipieni południowo-zachodniej Polski na podstawie badań mitochondrialnego DNA. [Genetic characteristics of grayling in south-western Poland based on mitochondrial DNA studies.] Archives of Polish Fisheries **12** (Suppl. 2): 247–251. [In Polish.]

Kahilainen K.K., Teacher A.G.F., Kähkonen K., Vinni M., Lehtonen H., Merilä J. 2011. First record of natural hybridization and introgression between pikeperch (*Sander lucioperca*) and perch (*Perca fluviatilis*). Annales Zoologici Fennici **48** (1): 39–44. DOI: 10.5735/086.048.0104

Kottelat M., Freyhof J. 2007. Handbook of European freshwater fishes. Publications Kottelat, Cornol and Freyhof, Berlin.

Król S. 2008. Wędrówki okoni pomiędzy Zalewem Szczecińskim a Zatoką Pomorską. [Perch migrations between the Szczecin Lagoon and the Pomeranian Bay.] Pp. 181–190. *In*: Wawrzyniak W., Dunin Kwinta I., Bartel R. (eds.) Ryby wędrowne w Polskiej gospodarce wobec nowej polityki wspólnotowej. III Rybackie perspektywy pobrzeża południowego Bałtyku. [Migratory fish in the Polish economy in respect to the new EU policy. III The fishing prospects for the southern Baltic coastal zone.] Foka, Szczecin. [In Polish.]

Kumar S., Nei M., Dudley J., Tamura K. 2008. MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. Briefings in Bioinformatics **9** (4): 299–306. DOI: 10.1093/bib/bbn017

Nesbø C.L., Arab M.O., Jakobsen K.S. 1998a. Heteroplasmy, length and sequence variation in the mtDNA control regions of three percid fish species (*Perca fluviatilis*, *Acerina cernua*, *Stizostedion lucioperca*). Genetics **148** (4): 1907–1919.

Nesbø C.L., Fossheim T., Vollestad L.A., Jakobsen K.S. 1999. Genetic divergence and phylogeographic relationships among European perch (*Perca fluviatilis*) populations reflect glacial refugia and postglacial colonization. Molecular Ecology **8** (9): 1387–1404. DOI: 10.1046/j.1365-294x.1999.00699.x

Nesbø C.L., Magnhagen C., Jakobsen K.S. 1998b. Genetic differentiation among stationary and anadromous perch (*Perca fluviatilis*) in the Baltic sea. Hereditas **129** (3): 241–249. DOI: 10.1111/j.1601-5223.1998.00241.x

Olsson J., Mo K., Florin A.B., Aho T., Ryman N. 2011. Genetic population structure of perch *Perca fluviatilis* along the Swedish coast of the Baltic Sea. Journal of Fish Biology **79** (1): 122–137. DOI: 10.1111/j.1095-8649.2011.02998.x

Refseth U.H., Nesbø C.L., Stacy J.E., Vøllestad L.A., Flejd, Jakobsen K.S. 2002. Genetic evidence for different migration routes of freshwater fish into Norway revealed by analysis of current perch (*Perca fluviatilis*) populations in Scandinavia. Molecular Ecology **7** (8): 1015–1027. DOI: 10.1046/j.1365-294x.1998.00423.x

Sepulveda-Villet O.J., Ford A.M., Williams J.D., Stepien C.A. 2009. Population genetic diversity and phylogeographic divergence patterns of the yellow perch (*Perca flavescens*). Journal of Great Lakes Research **35** (1): 107–119. DOI: 10.1016/j.jglr.2008.11.009

Ŝerbuha [Shcherbukha] A.Â. 1993. O rodstve iskopaemyh i sovremennyh taksonov roda *Perca* (Osteichthies Percidae). [On the relations between fossil and recent taxa in the genus *Perca* (Osteichthies Percidae).] Vestnik Zoologii **1993** (4): 39–40. [In Russian.]

Song C.B., Near T.J., Page L.M. 1998. Phylogenetic relations among percid fishes as inferred from mitochondrial cytochrome *b* DNA sequence data. Molecular Phylogenetics and Evolution **10** (3): 343–353. DOI: 10.1006/mpev.1998.0542

Szypuła J., Rybczyk A. 2001. Age, growth and condition of perch in the Odra Estuary (1991–1999). Folia Pomeranae Universitatis Technologiae Stetinensis Piscaria **218** (28): 151–164.

Templeton A.R., Crandall K.A., Sing C.F. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics **132** (2): 619–633.

Templeton A.R., Sing C.F. 1993. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. Genetics **134** (2): 659–669.

Thorpe J.E. 1977. Morphology, physiology, behavior, and ecology of *Perca fluviatilis* L. and *P. flavescens* Mitchill. Journal of the Fisheries Research Board of Canada **34** (10): 1504–1514. DOI: 10.1139/f77-215

Wierzbicki K. 1970. The parasite fauna of the perch, *Perca fluviatilis* L. of Lake Dargin. Acta Parasitologica Polonica **18** (1): 45–55.

Wiktor J. 1960. Zarys warunków biologicznych Zalewu Szczecińskiego. [Summary of the biological conditions in the Szczecin Lagoon.] Polish Archives of Hydrobiology **7** (20): 7–27. [In Polish.]

Wolnomiejski N., Witek Z. 2013. The Szczecin Lagoon ecosystem: The biotic community of the Great Lagoon and its food web model. Versita, London, UK. DOI: 10.2478/9788376560502

Wysokiński A. 1998. Fishery management in the Szczecin Lagoon. Bulletin of the Sea Fisheries Institute **3** (145): 65–81.

Zdziennicki F., Misiewicz A. 2014. Algorytmy konstruowania dendrogramów stosowanych przy analizie filogenetycznej mikroorganizmów. [Dendrogram constructing algorithms applied in phylogenetic analysis of microorganisms.] Postępy Nauki i Technologii Przemysłu Rolno-Spożywczego **69** (1): 68–80.

Received: 5 June 2018
Accepted: 8 April 2020
Published electronically: 1 June 2020