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RESEARCH ARTICLE

Genetic characteristics of Atlantic salmon (*Salmo salar*) stocking material released into Polish rivers

Dariusz Kaczmarczyk, Rafał Bernaś, Anna Nitkiewicz, Martyna Gadomska, Dorota Fopp-Bayat

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Abstract. In Poland, stocking rivers with Atlantic salmon (*Salmo salar* L.) began in the nineteenth century, and become intensive in the 1990s. Currently, it is performed by the Polish Angling Association, and institutions involved in the program "Stocking Polish Marine Areas." The aim of this study was to evaluate the genetic variation of the salmon stocked in recent years in Poland. For this purpose, salmon stocks from three broodstocks and two river populations (Słupia and Parsęta) were investigated. Eleven microsatellite loci were amplified. The total number of alleles detected across all loci ranged from 46 to 81 in the stocks. The average observed heterozygosity across all investigated loci was 0.59; and the expected heterozygosity was 0.58. The populations remained in Hardy–Weinberg equilibrium. The average Garza-Williamson *M* index value for all populations was low suggesting a reduction in genetic variation because of the founder effect. Genetic distance among populations was high between the Lithuanian stock (Rutki and Parsęta) and the group of Latvian origin (Aquamar–Żelkówko–Słupia). Several admixture traces were recorded in all stocks with the smallest evidence of this in the Rutki stock. Inbreeding values per generation were typical of those observed in breeding stocks (0–3%). The effective population size values were low or very low for all the stocks (*Ne 15-37)*.

Keywords: Atlantic salmon, stocking, endangered species, genetic variation, inbreeding, kinship analysis

D. Kaczmarczyk [✉], A. Nitkiewicz
National Inland Fisheries Research Institute, Department of Pond Fishery, Oczapowskiego 10, 10-719 Olsztyn, Poland
E-mail: d.kaczmarczyk@infish.com.pl

R. Bernaś
National Inland Fisheries Research Institute, Department of Migratory Fish, Rutki 49, 83-330 Żukowo, Poland

M. Gadomska
University of Gdańsk, Faculty of Biology, Wita Stwosza 59, 80-308 Gdansk, Poland

D. Fopp-Bayat
University of Warmia and Mazury in Olsztyn, Department of Ichtiology and Aquaculture, Oczapowskiego 5, Olsztyn 10-719, Poland

Introduction

The European occurrence of Atlantic salmon (*Salmo salar* L.) extends from northern Portugal to northwestern Russia, including Iceland, the British Isles, and the Baltic Sea (Parrish et al. 1998). Unfortunately, as a result of mainly anthropogenic factors, such as hydrotechnical development of rivers, water pollution, or excessive exploitation, over the last one hundred years many natural populations in the Baltic Sea basin area have been extirpated, and the condition of the remaining ones has deteriorated significantly (HELCOM 2011). Of the 90 wild historical Baltic salmon

populations, only about 30 survive and maintain native salmon populations (Koljonen 2001, ICES 2023).

Native Atlantic salmon in Poland became extinct in the mid twentieth century (Bartel 2001). Salmon first disappeared from the upper Vistula River in the 1950s, from its lower course after the opening of the dam in Włocławek in the 1960s, and during the same period from most rivers in Pomerania. By the late 1980s, salmon had also disappeared from the Drawa and Odra river basins (Bernaś et al. 2016). In response to the loss of native salmon populations, Poland launched a restoration program for this species. The Latvian population from the Daugava River was selected as the source population since it was the closest available population geographically at that time (Bartel 2001). After importing eggs several times, the Aquamar broodstock was established in Miastko. This is the source of material used in systematic stocking of fish that began in and has been carried out since the late 1990s. The tributaries of the upper Vistula were stocked mainly with hatchlings and fry, while the lower Vistula and its tributaries were stocked with smolts. In the Oder basin, stocking was carried out mainly in the Drawa River, but the main Pomeranian rivers were also stocked (Bartel 1997). After a decade, salmon from the Lithuanian Neman River was imported to Poland several times. This happened because it was possible to obtain fish from a population that was closer geographically and genetically to our extinct lines (Bernaś et al. 2016). Currently, small stocks of Lithuanian salmon are kept at the NIFRI Department of Salmonid Research in Rutki and in the Żelkówko hatchery. Stocking of these fish began in 2021 (Drawa River). Occasionally, if necessary, males from this stock were used for spawning if it was not possible to obtain wild males during artificial spawning performed by the Polish Angling Association (Słupia, Parsęta).

Taking into account the above, the salmon restitution program in Poland, which continues to this day, can only be considered moderately successful. Currently, small natural populations, the spawning efficiency of which is monitored annually, occur only in the Słupia, Parsęta, Łupawa, and Drawa rivers (Bernaś et al. 2009, ICES 2023).

Release of captive-bred individuals of marine and anadromous salmonid species into rivers is a method used frequently to prevent population declines and to increase harvest opportunities (Kitada 2018). While the release of hatchery produced juveniles is often crucial for keeping endangered populations alive, it is known that this method of supporting wild populations may also cause numerous negative effects on their genetic characteristics. One of them is a consequence of using non-local broodstocks for the production of stocking material. This can result in changes of the genetic characteristics of the population that is being maintained (Kohout et al. 2012, Valiquette et al. 2014). Moreover, inbreeding and domestication effects can decrease survivability of hatchery produced individuals in the wild environment (Christie et al. 2012a). Its known that potential problems that are closely related with supporting wild populations with hatchery produced juveniles include the risk of reducing genetic variation (Machado-Schiaffino et al. 2007), and, consequently, reducing the effective population size. This is despite its increased population size measured in the number of individuals, and this is known as the Ryman–Laikre effect (Ryman and Laikre 1991, Christie et al. 2012b, Waples et al. 2016). Hatcheries also induce selection pressure in maintained stocks which can result in different, sometimes opposite selection processes that can occur in breeding stocks and wild populations (Bernaś et al. 2020). The aim of this study was to assess the genetic variability of breeding stocks used for salmon stocking in Poland and to compare them with populations from the Słupia and Parsęta rivers.

Material and methods

Fish samples

Material for the research was collected in 2019 from fish intended for stocking from the Aquamar and Żelkówko hatcheries (Latvian/Daugava strain) and from the salmon stock from Rutki (Lithuanian/Neman

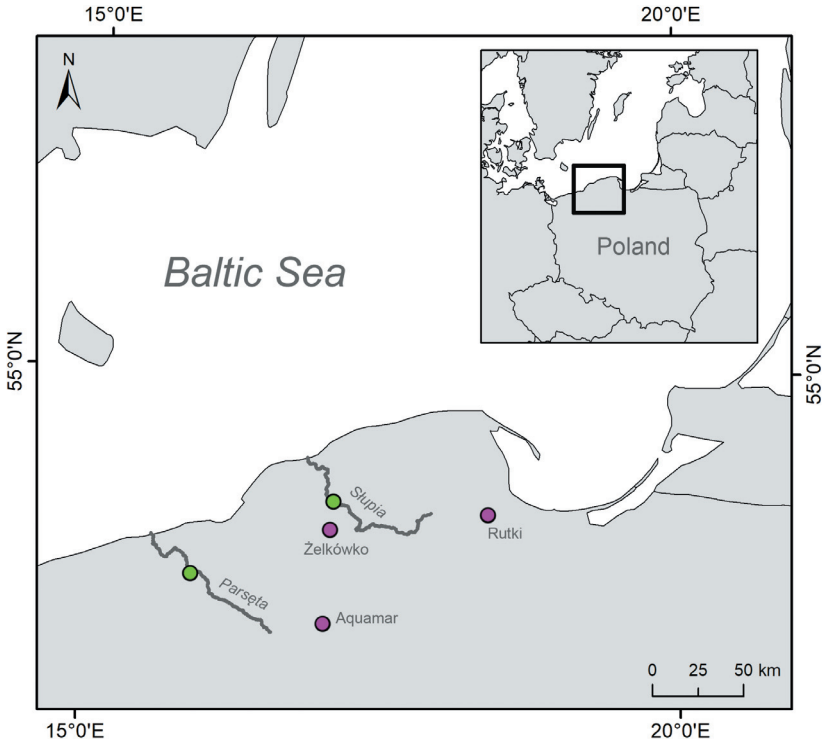

Figure 1. Sampling locations of the fish investigated in this study.

strain). However, since 2021, the Żelkówko hatchery has had only Lithuanian salmon. The analysis also included samples of salmon caught in the Słupia and Parsęta rivers during electrofishing between 2016 and 2022. These were fish of various ages, mainly ascending spawners but also 0+ juveniles. In total, 250 individuals, 50 from each location (Fig. 1) comprised the sample. Each fin fragment was placed in a separate 1.5 ml Eppendorf tube and conserved in 96% ethanol.

DNA extraction

Genomic DNA was extracted and purified from fin tissues using a Sherlock AX DNA Extraction and Purification Kit or a DNA and Genomic Mini AX Tissue SPIN DNA Extraction and Purification Kit. The extraction procedure was performed following the manufacturer's recommendations (A&A Biotechnology). DNA samples were stored at a temperature of -20°C. The integrity of the DNA samples was inspected visually following electrophoresis in a 1.5% agarose gel stained with Midori Green Advance DNA Stain (NIPPON Genetics, Japan). All agarose gels were photographed using a gel imaging system and the images were recorded digitally. Samples of the DNA yields were quantified by spectrophotometric analysis; only samples containing more than 30 ng/μl^{-1} of double-stranded DNA qualified for the PCR stage.

PCR amplification

The assessment of genetic variation was based on twelve polymorphic microsatellites. The set of markers used in these studies included the following microsatellite loci*: Ssa202*, *SSaD486*, *Ssa197*, *SSsp1605*, *SSspG7*, *SSa289*, *SSsp2213*, *SSsp2215*, *Ssa171*, *SSsp2210*, *SSsp2216*, and *Ssa14*. The primer sequences used for amplification of loci *Ssa171*, *Ssa197*, and *Ssa202* were taken from O'Reilly et al. (1996*),* loci *SSsp1605*, *SSsp2210*,

Table 1
Primer sequences and repeat motifs of the microsatellite fragments investigated (McConnell et al. 1995, O'Reilly et al. 1996, Paterson et al. 2004, King et al. 2005) and the set numbers used in amplification with the multiplex PCR technique

Locus	Repeat motif	[5' label] Primer sequence	Multiplex set nr.
Ssa14	TC, AC, CA	F: [VIC]ACATCCACACCGTCTGTCAA R: CACGTCATCAAGCAGAGGAA	IV
Ssa171	TG, TGTA	F: [6-FAM]GGTGACCTCATGGAAGCATT R: AGCTACTGAAACCCTTTGGC	amp separately*
Ssa197	GT, TG, GTGA	F: [NED]AGGTTTCAGAGCCCTCATCA R: CATGTGAACTCTGAAGCCCA	I
Ssa202	CA, CTCA	F: [PET]GGACAGTGAGGGACGCAGAC R: CCGTAAAATTTGGGGGCTAGA	I
Ssa289	GT	F: [6-FAM]CGGTATCGGTGCATCCCTAAA R: AACAGCGCGAGCGTCATTC	amp separately*
SsaD486	TAGA	F: [6-FAM]GTGAAGCATGGCATAGCACA R: CAGGAAAGTGCCAGCATACAC	I
SSsp1605	GATA	F: [6-FAM]CGCAATGGAAGTCAGTGGACTGG R: CTGATTTAGCTTTTTAGTGCCCAATGC	II
SSsp2210	GTTA	F: [6-FAM]AAGTATTCATGCACACACATTCACTGC R: AAGTATTCATGCACACACATTCACTGC	IV
SSsp2213	GTTA	F: [PET]ATGTGGAGGTCAACTAACCAGCGTG R: ATCAATCACAGAGTGAGGCACTCG	III
SSsp2215	GTTA	F: [NED]ACTAGCCAGGTGTCCTGCCGGTC R: AGGGTCAGTCAGTCACACCATGCAC	III
SSsp2216	GTTA	F: [NED]GGCCCAGACAGATAAACAAACACGC R: GCCAACAGCAGCATCTACACCCAG	IV
SSspG7	GTTA	F: [NED]CTTGGTCCCGTTCTTACGACAACC R: TGCACGCTGCTTGGTCCTTG	II

*amp separately – locus amplified in singleplex PCR mode

SSsp2213, *SSsp2215*, *SSsp2216*, and *SSspG7* from Paterson et al. (2004), locus *SSa289* from McConnell et al. (1995) and locus *SSaD486* from King et al. (2005). The primer sequences, repeat motifs, and their accession numbers are given in Table 1. PCR amplification was performed in a 30 µl reaction. The forward primer of each primer pair was 5' end labelled with fluorescent dyes (6FAM, VIC, NED, and PET). Ten of twelve of the markers investigated were amplified using multiplex PCR reactions in the following sets: multiplex I; multiplex II; multiplex III; multiplex IV. Microsatellites *Ssa171* and *SSa289* were amplified separately (Table 1).

The initial PCR conditions for amplifying microsatellites in multiplex sets I–IV were taken from Grandjean et al. (2009). The PCR reaction was started with the denaturation of DNA at a temperature of 95°C for 15 minutes. Next, 35 cycles were performed: each cycle consisted of denaturation (30s at 95°C), annealing (30s at 58°C), and elongation (90s at 72°C). After the last cycle, the final elongation was performed for 60 minutes at 60°C. Because amplification of markers *Ssa171* and *Ssa289* was not successful at the annealing temperature proposed by Grandjean et al. (2009), we amplified these DNA fragments separately at annealing temperatures of 48°C (*Ssa171*) and 54°C (*Ssa289*). The other temperatures and thermal profiles used in the amplification of these two microsatellites were the same as given above. PCR reactions were performed in a Mastercycler X50a thermocycler (Eppendorf, Germany) in Hot-Start PCR mode. The PCR product was verified by electrophoresis in a 2% agarose gel and stained using Midori Green Advance DNA Stain (NIPPON Genetics, Japan).

Genotyping

Genotyping was performed in four sets that included the microsatellites in Table 1. The genotyping of

microsatellite *Ssa171* was performed together with markers amplified in multiplex set III and *Ssa289* with multiplex set II. The lengths of the amplified DNA fragments were determined using an Applied Biosystems 3130 Genetic Analyzer with GS500LIZ (Applied Biosystems) size standards. Fragment size and allele determination were performed using GeneMapper 3.0 software (Applied Biosystems) following the manufacturer's recommendations.

Statistical analyses

Genetic variability was measured using observed heterozygosity (H_o), expected heterozygosity (H_e) (Nei 1987), and allelic frequency. The number of alleles per locus, allele frequency, allelic range, allelic diversity (*AD*), and the total number of alleles (*N*) were computed with MSA software (Dieringer and Schlötterer 2003).

An exact test for Hardy–Weinberg proportions (Nei 1987) was used to test for deviations from H–W equilibrium. The test was performed separately for each locus in each population as well for all loci in given populations. This test was performed with Arlequin 3.5.2 software (Excoffier and Lischer 2010). The number of steps in the Markov chain was 1,000,000, and the number of dememorization steps was 100,000. The deviations were considered significant if $p \leq 0.05$.

The occurrence of bottleneck or founder effects and its influence on within-population genetic variability was based on the Garza-Williamson *M* index (the number of alleles divided by the allelic range). This index (Garza and Williamson 2001), including Excoffier's adjustment, was calculated with Arlequin 3.5.2 software (Excoffier and Lischer 2010). The index is based on the observation that in bottlenecked populations the number of alleles observed in an allelic range is less prone to reduction than the allelic range itself. This is because the allelic range is the difference in the number of nucleotides of the longest and of the shortest alleles of a microsatellite fragment divided by length of the microsatellite's repeat motif, so unless the longest or shortest allele is changed, the allelic range will not be changed. The average value of the *M* index calculated across all microsatellite markers ranged from 0.8 to 1, which is typical of populations in which genetic variation has not been reduced because of a bottleneck. *M* values from 0.68 to 0.8 indicate the possibility of founder and/or bottleneck effects, and values lower than 0.68 indicate strong founder and/or bottleneck effects.

Genetic divergence among stocks was analyzed using two different methods: the fixation index (F_{ST}) (Wright 1951) and the variation in average allelic size ($\delta\mu^2$) (Goldstein et al. 1995). F_{ST} values and their statistical significance were calculated with Arlequin 3.5.2 software (Excoffier and Lischer 2010). The size of the genetic distance based on F_{ST} values and their ranges were interpreted according to Wright (1978) and Balloux et al. (2002). Higher values of this coefficient (closer 1.0) and statistical significance indicate larger genetic differences between pairs of populations, whereas lower values (closer to 0.0) and a lack of statistical significance indicate genetic similarity. Genetic divergence was also estimated using the sample size independent $\delta\mu^2$ method (Goldstein et al. 1995) calculated with MSA software (Dieringer and Schlötterer 2003). With this method, higher values indicate larger genetic differences among populations and smaller values indicate minor differences.

The contribution of the specific components of genetic variance to the total variance observed among all five stocks investigated was estimated with hierarchical analysis of molecular variance (AMOVA) (Excoffier and Slatkin 1995, Michalalakis and Excoffier 1996). These calculations were performed using Arlequin 3.5.2 software (Excoffier and Lischer 2010) with 1,000 permutations. The threshold for significance was set at $p = 0.05$. The evaluation of inbreeding was based on Wright's F_{IS} inbreeding coefficient (Wright 1951) and the comparison of observed heterozygosity and expected heterozygosity. The positive values of this coefficient indicate inbreeding and are negative for outbreeding. This coefficient was calculated with Arlequin 3.5.2 software (Excoffier and Lischer 2010) as a part of AMOVA locus by locus analysis and averaged across the loci.

The threshold for significance for results of F_{IS} analysis was set at p = 0.05.

STRUCTURE 2.3.4 was used to detect genetic structure and gene flow (Pritchard et al. 2000). The Evanno method (ΔK) was used (Evanno et al. 2005) to infer the true number of clusters (K) based on the rate of change in log probability among consecutive K values, which ranged from K = 1 to K = 6. Five iterations of each K were performed with 200,000 burn-ins and 200,000 Markov Chain Monte Carlo (MCMC) repetitions. To this end, the Clumpak program was employed to identify the optimal alignment of inferred clusters across different values of K (Kopelman et al. 2015).

In order to describe family structure, parentage analysis was assessed for every single broodstock/population using Colony 2.0.6.6. (Jones and Wang 2010). We applied non-default COLONY job settings including typing error rate 0.001, mating system I with male and female polygamy, mating system II with inbreeding, medium run length, and analysis method FL. The rest of the settings were default. The main goal was full-sib and half-sib dyad detection, determining the number of families, and estimating effective population size *Ne*.

Results

Genetic diversity in stocks

Allelic diversity

Of the twelve microsatellites included in this study, eleven were successfully amplified in all the stocks investigated. Microsatellite *Ssa171* was amplified in fish from the Rutki stock where the marker was polymorphic (alleles 241 and 247), while that from the Parsęta stock was monomorphic (allele 247). This microsatellite failed to amplify in any other stocks from the Aquamar–Żelkówko–Słupia group probably because of a mutation in the flanking regions of this microsatellite DNA. Consequently, this marker was omitted from calculations. Across the eleven microsatellites amplified in fish from all locations, 97 alleles were detected. Except locus *SsaD486*, all the microsatellites investigated were polymorphic, but the polymorphisms differed among the stocks. Some of them were highly polymorphic with more than ten alleles (Table 2). Within the stocks investigated, the degree of polymorphism across all loci assessed was

Table 2
Genetic properties of the stocks investigated. Number of alleles detected at investigated loci, total number of alleles (*N*), allelic diversity (*AD*), observed (H_o) and expected (H_e) heterozygosity calculated across all loci in the stocks investigated

Locus	Rutki	Parsęta	Aquamar	Żelkówko	Słupia	Across stocks
Ssa14	2	2	5	4	6	9
Ssa197	5	8	8	8	11	13
Ssa202	4	3	6	4	7	8
Ssa289	2	2	2	2	2	2
SsaD486	1	1	1	1	1	1
SSsp1605	2	4	5	5	6	6
SSsp2210	4	3	5	4	8	8
SSsp2213	6	6	6	11	8	12
SSsp2215	7	12	10	14	14	16
SSsp2216	9	9	10	11	11	14
SSspG7	4	4	5	5	7	8
N	46	54	63	69	81	97
AD	4.18	4.91	5.73	6.27	7.36	8.82
H_o	0.59	0.50	0.67	0.55	0.65	0.59
H_e	0.56	0.45	0.63	0.59	0.67	0.58

moderate or high (from 46 alleles in Rutki to 81 alleles in Słupia). Except for loci *SsaD486* and *Ssa289*, all the stocks differed in the number of alleles detected at a given locus and also in the overall number of alleles identified across all loci investigated. The allelic richness was moderate or high and ranged from 4.18 alleles per locus in the Rutki stock to 7.36 in the Słupia stock (Table 2).

Heterozygosity, Hardy–Weinberg equilibrium, and inbreeding coefficient

To evaluate genetic variation in the stocks, observed (H_o) and expected (H_e) heterozygosity were calculated (Table 2). In general, the genetic variation described with this indicator was moderate, with average values for observed and expected heterozygosity of 0.59 and 0.58, respectively. The values of H_o and H_e for individual stocks varied considerably. In the Rutki, Parsęta, and Aquamar stocks the number of observed heterozygotes exceeded the number of expected heterozygotes ($H_o > H_e$), but in the Żelkówko and Słupia stocks the frequency of homozygote genotypes was lower than expected ($H_o < H_e$) (Table 2). In all the stocks, the mean H_o value was close to the average percentage of heterozygotes (H_e) expected at H–W equilibrium. When calculated across all markers, departures from this equilibrium were not significant ($p > 0.05$). Departures were only found at the level of individual loci (Table 3), and departures at more than two loci were observed only in the Parsęta and Żelkówko stocks.

Positive values of F_{IS} indicating inbreeding were found in the Żelkówko and Słupia stocks. In the Żelkówko stock the value of this indicator was much higher (and significant at $p = 0.01$) than in the Słupia stock where it was close to a neutral 0.00 value and not significant ($p > 0.05$). Negative values of F_{IS} indicating outbreeding were found in the Rutki, Parsęta, and Aquamar stocks and were similar across them (Table 3) and not significant ($p > 0.05$).

Bottleneck and founder effects

The average Garza-Williamson *M* index value across the stocks investigated was 0.58. This value was lower than 0.68, which indicated that founder and/or bottleneck effects had a significant impact on genetic variations in these stocks (Table 3). The *M* value was the highest in the Aquamar stock (0.61), which was

Table 3
Results of the test for deviation from the Hardy–Weinberg equilibrium, inbreeding coefficient (F_{IS}), value of the Garza-Williamson index (*M*)

	Stock				
Locus	Rutki	Parsęta	Aquamar	Żelkówko	Słupia
SSa14	-	-	-	-	-
SSa197	-	-	*	-	-
Ssa202	-	*	-	-	*
SSa289	*	-	-	*	-
SsaD486	mn	mn	mn	mn	mn
Sssp1605	-	-	-	-	-
SSsp2210	-	*	-	-	-
SSsp2213	-	-	*	*	-
SSsp2215	-	*	-	*	-
SSsp2216	-	*	-	*	-
SSaspG7	*	-	-	-	-
F_{IS}	-0.07	-0.08	-0.07	0.08*	0.04
M	0.52	0.58	0.61	0.58	0.59

(mn) – monomorphic locus
(-) – no deviation or no significant deviation
(*) – significant values, $p \leq 0.05$

Table 4
Genetic differences among stocks based on $\delta\mu^2$ and F_{ST} values

		FST				
		Rutki	Parseta	Aquamar	Żelkówko	Słupia
$\delta\mu^2$	Rutki	x	0.28	0.16	0.20	0.16
	Parsęta	2.60	x	0.17	0.15	0.15
	Aquamar	3.46	4.97	x	0.03	0.02
	Żelkówko	4.39	5.69	0.61	x	0.03
	Słupia	2.31	2.52	0.93	1.10	x

the only stock where the *M* index was greater than 0.60. The lowest *M* value was in the Rutki stock (0.52), which suggested a slightly larger reduction in the genetic variation of this stock (Table 3).

Genetic divergence among stocks

Based on F_{ST} values, the genetic distances among most of the stocks were large (F_{ST} range of 0.15–0.25) (Table 4). The largest genetic distance (F_{ST} of 0.28) was found between the Rutki and Parsęta stocks. A large genetic distance was observed between the Rutki and Parsęta groups of stocks and all the other stocks (Aquamar–Żelkówko–Słupia). In the group of stocks that included Aquamar–Słupia–Żelkówko, the genetic distances were similar among each stock and were low (F_{ST} from 0.02 to 0.03). All the genetic differences among the stocks were significant at $p < 0.05$.

The magnitudes of the genetic distances among stocks were also estimated using the $\delta\mu^2$ method (Table 4). This method confirmed that the genetic distances among the Rutki, Parsęta, and the Aquamar–Słupia–Żelkówko group of stocks were large, although some of the specific results differed from those reported above. The greatest genetic distance estimated with the $\delta\mu^2$ method was that be-

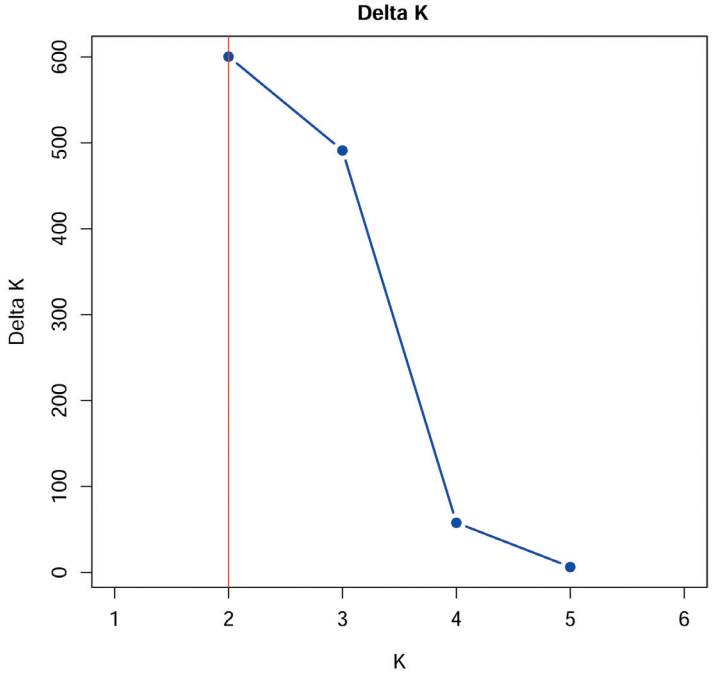

Figure 2. STRUCTURE ΔK plot for finding the best K fit for the data using the Evanno et al. (2005) method.

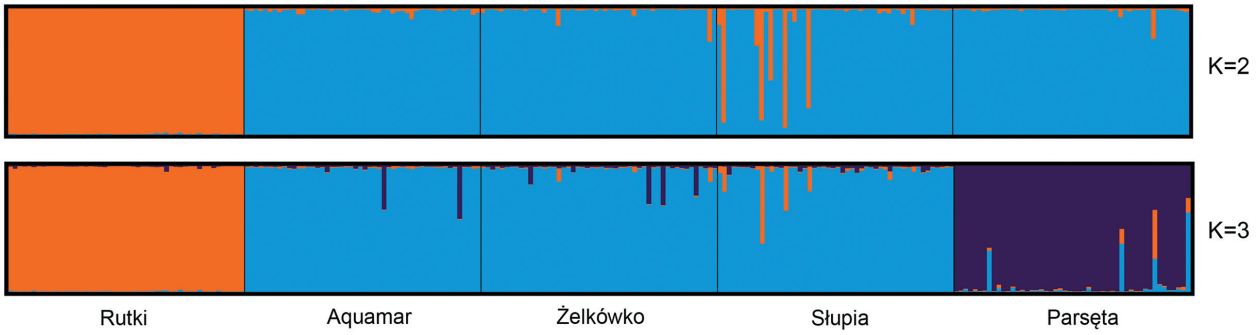

Figure 3. Clustering of salmon from the stocks/populations analyzed with putative K = 2 (upper bar) and K=3 (lower bar). Each individual is represented by a column divided into K shades with each shade representing a cluster.

tween the Parsęta and Żelkówko (5.69) and between the Rutki and Żelkówko (4.39) stocks. This method also uncovered genetic similarity within the Aquamar–Żelkówko–Słupia group of stocks where $\delta\mu^2$ among them ranged from 0.61 to 1.10.

The genetic distance among stocks was confirmed with Bayesian analysis performed with STRUCTURE 2.3.4 software. The Bayesian estimation of genetic structure and individual membership indicated that the maximum value of ΔK was for K = 2 (ΔK = 600.4) (Figure 2) and next for K=3 (ΔK 491.2). In the K=2 scenario, salmon from the Aquamar, Żelkówko, Słupia, and Parsęta stocks had its own cluster (blue cluster). The Rutki stock had another separate cluster (orange). The scenario for K=3 was similar, but in this approach, Parsęta salmon had its own cluster (violet) (Figure 3). Several admixture traces were recorded in all the locations studied with the least evidence in Rutki.

Hierarchical Analysis of Molecular Variance (AMOVA) revealed that the variation of all samples was 3.720 and the sum of squares was 1,801.5. The most important component of this variation was the variation within individuals (3.257 and the sum of squares 851.5). This class of variation was responsible for 87.5% of total variance among all samples. Other components were variation among populations and among individuals within populations, but their contribution to total variance was much lower (0.516, sum of squares 218.6 and 13.9%) and (-0.053, sum of squares 770.6 and -1.4%), respectively.

The analysis of familial structure and effective population size calculated in COLONY showed that proportions of unrelated individuals within samples were high and ranged from 88.64% in Parsęta to 95.24% in Żelkówko. Full-sib proportions varied from only 0.12% (Słupia) to 2.12% (Rutki), and half-sib proportions ranged from 4.16% to 10.40% (Table 5 and Fig. 4). The effective population size estimates were low and ranged from 15 to 37 (Table 5). Major differences were observed between the Rutki

Table 5

Results from the sibship assignment method employed by COLONY. Full-sib and half-sib dyads are displayed both as absolute values and relative frequencies (brackets). Full-sib families concern all detections, including single detections. The last column is the effective population size

Stock	Fullsib families	Unrelated dyads	Fullsib dyads	Halfsib dyads	Ne (CI95 L-U)
Rutki	26	2229	53 (2.12%)	218 (8.72%)	15 (8-30)
Aquamar	46	2335	7 (0.28%)	158 (6.32%)	28 (17-49)
Żelkówko	39	2381	15 (0.60%)	104 (4.16%)	37 (23-65)
Słupia	47	2367	3 (0.12%)	130 (5.20%)	36 (22-61)
Parsęta	35	2216	24 (0.96%)	260 (10.40%)	16 (9-34)

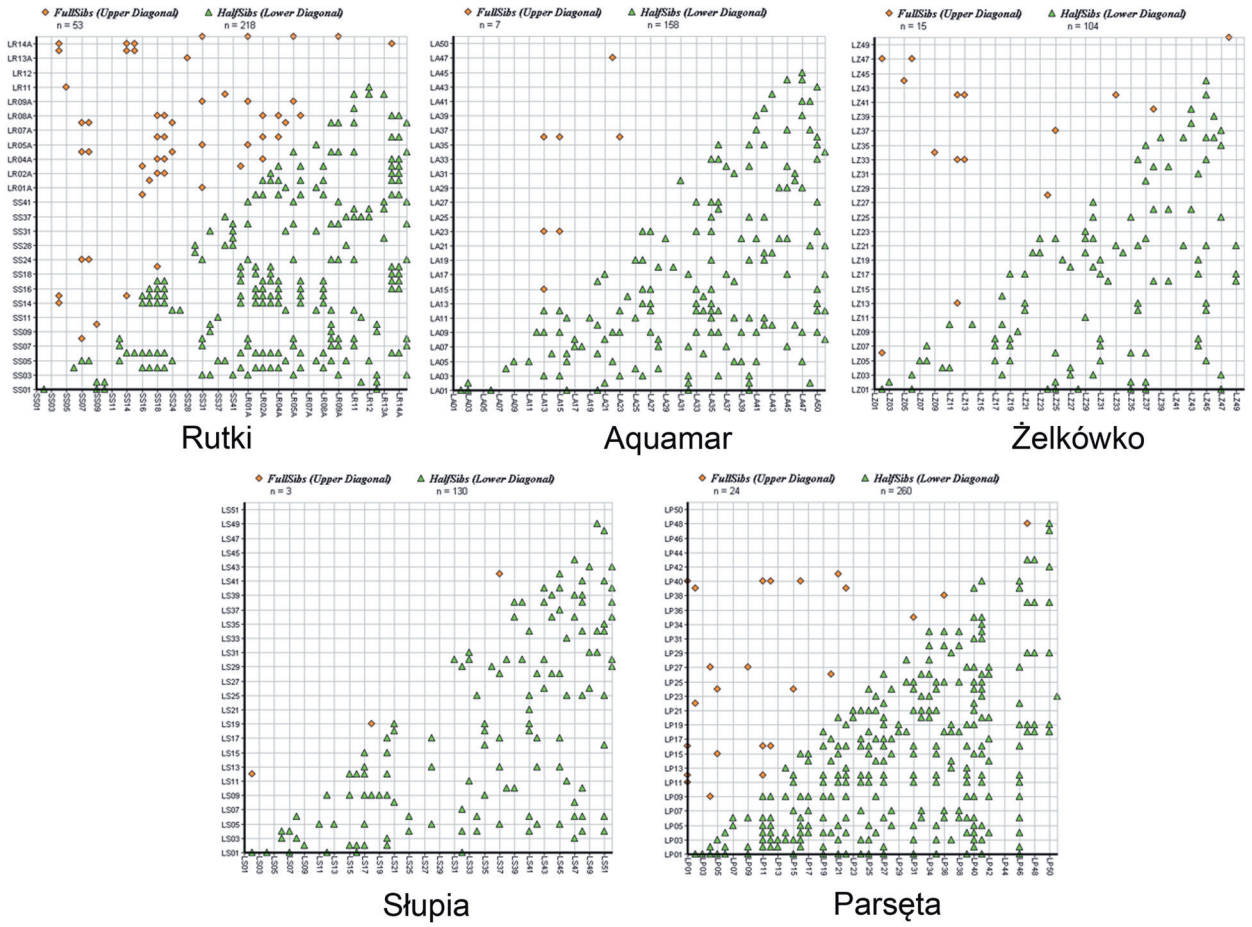

Figure 4. Relatedness within the salmon stocks/populations analyzed showing the estimated number of full-sib and half-sib detections.

and Parsęta group of stocks and the remaining stocks.

Discussion

Genetic variation in stocks

The comparison of microsatellite DNA variation revealed that the genetic diversity in the five salmon stocks/populations (97 alleles at eleven microsatellite loci, average H_o = 0.59) was slightly lower than that usually found in studies on microsatellite variation in Baltic Sea salmon populations (205 alleles at nine loci, average H_o = 0.72) (Säisä et al. 2005) and 29 North American and European salmon populations (266 alleles at 12 loci, average H_o in Europe 0.73) (King et al. 2001). However, these or even lower values of observed heterozygosity were also found in various Baltic rivers and not only in small ones (e.g., Tonteri et al. 2005, 2010, Vasemägi et al. 2005, Koljonen 2006, Ryynänen et al. 2007) but even in the largest salmon population in the Baltic Sea from the Torniojoki River (Tonteri et al. 2007). It is likely that genetic isolation and the low effective population size were key factors in determining the low genetic variation in the fish from these five Polish stocks. However, it should be noted that the population of salmon from the Neman, represented here by the broodstock from Rutki, is

itself characterized by low genetic variability resulting from population decline in the second half of the twentieth century (Leliűna and Virbickas 2006, Poćwierz-Kotus et al. 2015, Bernaś et al. 2016).

It is difficult to find an answer to the question how stable over the years are of those relative low levels of genetic variation in material released to Polish rivers. The level of genetic variation and genetics characteristics of fish released may depend on the number of fish used in artificial spawning, their individual characteristics, and the quality of gametes produced by each spawner. Consequently, both the number of alleles as well as observed heterozygosity might change over the years. A good, apparently effective method is to refresh the Latvian salmon stock by importing new material from Daugava every few years, as Aquamar does. The Rutki and Żelkówko stocks, which now have salmon from the Lithuanian line, are more difficult to renew because of difficulties obtaining roe from Lithuania. Consequently, it is necessary to ensure the frequent exchange of individuals between these two stocks in order to increase the effective population size and overall variability.

Founder and bottleneck effects are known to be important factors that determine genetic variation in broodstocks and genetic characteristics of hatchery-supported populations (Exadactylos et al. 2007). The results of a Garza-Williamson *M* index lower than the critical value of 0.68 (Garza and Williamson 2001) suggested that none of the five stocks studied avoided a reduction in genetic variation. However, there are some differences in the scale of and reasons for this reduction among the stocks. The lowest values observed in the Rutki stock were related to the demographic history of Neman salmon and the limited number of individuals with which the broodstock was created. In the remaining stocks analyzed, the *M* index values were similar, which resulted from their origin (Latvian line), although the distinctiveness of the Parsęta population could be explained by the founder effect. The Parsęta salmon issue requires further analysis, and this will be resolved in the near future. As future work, we plan to compare the Parsęta salmon with neighbouring Baltic populations and extinct Polish lineages to better understand its genetic position and the processes that are occurring within the stock.

Progressive inbreeding in broodstocks is a well-known problem in the conservation of human-dependent fish species. Conservation biologists routinely face the dilemma of keeping small, population specific broodstock, wherein inbreeding depression may ensue, or mixing one stock with other, which may exacerbate population declines via outbreeding depression (Houde et al. 2011). It is worth noting that the actual level of inbreeding or outbreeding in Polish stocks is not high enough to be a problem for stocking material production, but monitoring inbreeding and other indicators of genetic variation is recommended.

Genetic distance and structure

Salmonid biology and spawning behavior result in increased genetic differences among populations. Under natural conditions each salmon population is an independently evolving unit (Fraser et al. 2010) usually with limited gene flow among neighboring populations.

Some Baltic Sea salmon populations, especially those located in the Gulf of Bothnia, are reported to be highly homogenized (F_{ST} in range 0.031–0.076), and this process continues to progress (Östergren et al. 2021). However, some other salmon populations from the Baltic Sea show high differences, especially when comparing populations from distinct evolutionary lineages ($F_{ST} > 0.2$) (e.g., Poćwierz-Kotus et al. 2015, Bernaś et al. 2016). In the stocks investigated in this study, there is only a large genetic difference between the Rutki and Parsęta stocks and a slightly a smaller distance among the Aqamar, Żelkówko, and Słupia stocks. Moreover, the successful amplification of microsatellite *Ssa171* from DNA samples taken from the Rutki and Parsęta stocks and the unsuccessful amplification from the three other stocks is yet another result that indicates the genetic differences between the Rutki and Parsęta stocks and the group of the other three stocks.

Bayesian analysis also indicated that, despite the significant genetic distance, the population from

Parsęta clusters in the case of K = 2 with the populations of Latvian origin. However, in the case of the K = 3 approach, Parsęta creates its own cluster (violet), which was also present in the Aquamar salmon. This supports the theory that this situation is probably due to genetic drift that has occurred in the Parsęta salmon. Therefore, the Rutki and Parsęta stocks can be assessed as different from the Aqamar–Żelkówko–Słupia group. The genetic differences among the latter three salmon stocks were the smallest and close to those reported earlier in a study based on single nucleotide polymorphism (SNP) by Poćwierz-Kotus et al. (2015) and Östergren et al. (2021). The similarity of these three populations can be explained by the same ancestral population based on the Daugava strain. In turn, the fact that the Rutki salmon differed so clearly from the others is because it is from the Lithuanian strain that originated from different evolutionary units; this is also demonstrated in SNP analysis (Poćwierz-Kotus et al. 2015).

The presence of several signals of admixture in the Słupia population with Lithuanian genotypes can be explained by the fact that this concerns individuals that are the offspring of females from Słupia fertilized with Lithuanian males (from Żelkówko) during artificial spawning that were sampled as parr during electrofishing in 2022 (NIFRI data). Similar signals of mixing concern several salmon from Parsęta, with the difference that they were caught and sampled as adult ascending fish (2021–2022).

Familial structure and effective population size

The inbreeding values per generation were in the range most frequently observed in breeding stocks (1–3%) (Ryman 1994, Tave 1999) but only if we consider full-sibling detection, which is more robust and important. Half-sib estimation showed a higher share of related individuals in all the stocks studied, but especially in the Rutki and Parsęta salmon. These values resulted indirectly from the fact that part of the samples analyzed were from juveniles that were potentially related and the tendency of the COLONY algorithm to overestimate the number of half-sibs (Ackerman et al. 2017). General the *Ne* values were low or even very low for all the locations studied. Taking into account the size of the parental stocks and the population sizes, this was expected. The results were decidedly lower than those recommended for breeding stocks (Tave 1999) and for wild populations (Frankham et al. 2014) They were also lower than those found in breeding stocks of sea trout from Aquamar and Dąbie (Wąs-Barcz and Bernaś 2023).

Summary

The results of this work provide information on the genetic diversity of salmon stocks used to stock Polish rivers and on the genetic composition of two populations from the Słupia and Parsęta rivers. They also provide valuable information that can be used for the active protection of this species in Poland and in the Baltic Sea.

Moreover, the list of the genetic profiles of the fish investigated during this study will be used to construct a database of genetic profiles of salmon that represent Polish populations and stocks. This database will be used in future work on assessing the results of the management program for this species.

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ORCID iD

Dariusz Kaczmarczyk https://orcid.org/0000-0002-0109-9407
Rafał Bernaś https://orcid.org/0000-0002-4295-6436
Anna Nitkiewicz https://orcid.org/0000-0003-2610-1814
Dorota Fopp-Bayat https://orcid.org/0000-0002-9537-1108

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