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Microsatellite-Based Analysis of Genetic Diversity and Population Structure of Rainbow Trout (*Oncorhynchus mykiss*) Cultured in Ukraine

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Abstract

The genetic structure of rainbow trout farmed in Ukraine were characterized based on microsatellite loci. The selected set of SSR-markers (Simple-sequence repeats-markers) had a high degree of polymorphism that allowed determining the specificity of each local stock (average PIC value=0.785±0.034). The microsatellite analysis of rainbow trout from the studied stocks showed a high level of genetic diversity (uHe=0.825±0.030, PIC=0.785±0.034, I=1.836±0.127). The level of allelic diversity of the selected loci was high, where the average number of alleles per locus was 7.833, the effective number of alleles per locus was 5.687. The range of amplicon sizes of the studied loci and private alleles for each local group was determined. The cluster analysis showed the presence of three clusters. The range of genetic variability was presented in the graphical interpretation of the principal coordinates analysis (PCoA). Based on unbiased genetic distances, the Kharkiv and Transcarpathian local stocks were found to be the closest, while the Chernivtsi stock was the most distant from them.

Introduction

The rainbow trout (*Oncorhynchus mykiss*) is one of the most widespread cultured species in the world (Hrytsyniak, Hrynzhevskyi, Tretiak, Kiva, & Mruk, 2008; Stanković, Crivelli, & Snoj, 2015). Due to its biological plasticity, rapid weight gain, delicious taste and dietary properties, this fish is very attractive to consumers and manufacturers and its faming is commercially profitable (Hrytsyniak *et al.,* 2008). This species, native to North America was worldwide introduced since the end of the 19th century (Stanković *et al.,* 2015). Rainbow trout cultivation in Ukrainian aquaculture began in 1894 in Pushcha-Voditsa near Kyiv (Titarev, 1980; Hrynzhevskyi *et al.,* 2006) during the period of pan-European introduction (Hrytsyniak *et al.,* 2008). In recent years, about two thousand tons of trout have been produced annually at Ukrainian trout farms (Mruk, 2020).

Compared to other European countries, this is not a high amount; however, due to the creation of highly industrial trout farms, the production amounts of this valuable species in the country are increasing (Terterian, Terterian, & Kolos, 2011). Currently in Ukraine, there are about 50 large and small trout farms, 10 of which have an annual production of approximately 50 tons of fish and more (Mruk, 2020). Today, there are capacities to grow about 20,000 tons of rainbow trout and char using the existing production base in Ukraine. However, this is possible with the creation of national projects to support the owners of private trout farms. Several studies have been carried on by Ukrainian scientists devoted to the improvement of technologies for the cultivation of salmonids in the conditions of industrial farms, taking into account the specifics of natural conditions in Ukraine (Mruk, Terteryan, Didenko, & Khandozhivska, 2013; Haloyan, Mruk, Terteryan &

Kucheruk, 2017; Mendryhsora, Kurynenko, & Mruk, 2017). The development of new cultivation approaches with the use of modern methods of population genetic for the assessment of local stocks is aimed at improving the state of trout breeding, as well as at adopting practices of rational management of this type of aquaculture.

There are varieties of rainbow trout in different countries, which represent a wide range of variability in terms of fish-breeding, biological and genetic characteristics (Stanković *et al.,* 2015). Therefore, today an important task is to study the genetic structure and diversity of local stocks of a given species in order to establish the phylogenetic origin using modern tools of molecular genetic analysis.

The knowledge gained from the population genetic analysis of aquatic genetic resources (AqGR) is essential for the management and conservation of biological diversity (Kuciński *et al.*, 2015; Coates, Byrne, M., & Moritz*,* 2018) both in natural populations and in aquaculture (FAO, 2018). Modern methods of molecular biology allow obtaining a better characterization of aquatic genetic resources of aquaculture, which is of practical importance for maintaining its stable state. A prerequisite for determining the genetic potential of brood stocks is to study the peculiarities of the intraspecific organization of their genetic structures. This will allow monitoring changes in the gene pool of fish stocks and controlling the level of biodiversity.

FAO's 2019 State of the World's Genetic Resources report (FAO, 2019) indicates that among a number of modern molecular genetic markers along with SNP-markers, microsatellite loci (SSR) are one of the most proper and convenient for research. Variations in these codominant and selectively neutral DNA markers are used as an indicator of genetic variation (Silverstein, Rexroad, & King, 2004). Microsatellite loci are widely used to assess the genetic structure of rainbow trout in a number of researchers in different countries, allowing to provide a comparative analysis of the biodiversity of this species (Fishback, Danzmann, & Ferguson, 2000; Silverstein *et al.,* 2004; Johnson, Rexroad, Hallerman, Vallejo, & Palti, 2007; Yousefian *et al.,* 2012; Allen, Ferguson, & Danzmann*,* 2013; Ceyhun & Ciltas, 2013; Barat *et al.,* 2015; Abadía-Cardoso *et al.,* 2016; Artamonova, Yankovskaia, Holod, & Makhrov*,* 2016; Faccenda*,* Lunelli, Gandolfi, & Bozzi, 2018). Microsatellite analysis allows determining allelic diversity and heterozygosity, which are ones of the main primary indicators of polymorphism in the genetic structure of populations (Chistiakov, Hellemans, & Volckaert, 2006).

An important aspect in the formation of replacement broodstocks is the preservation of their genetic diversity in order to avoid inbreeding. In the practice of fish farming, there are several ways to renew the biodiversity of broodstocks like for example by using individuals from natural populations, where the level of biodiversity is higher, and by genetic control. Rainbow trout do not reproduce in natural conditions in Ukraine as in most countries in Western and central Europe (Stanković *et al.*, 2015; Lyach, 2020). To date, scientists have not reached a consensus to answer the question "why rainbow trout populations do not reproduce in the natural condition across much of Europe" (Stanković *et al.*, 2015). In Ukraine, natural reproduction was not recorded maybe because of human impact on natural and ecological complexes (over-fishing without complying with the basic rules of fish protection, hydraulic engineering, local water pollution) (Ustych, Scherbak, & Mruk, 2017). Therefore, its occurrence in natural watercourses in Ukraine is due exclusively to uncontrolled escapes from fish farms or to fish stocking for sport fishing. So, the main source for gene pool renewal in trout farms of Ukraine is the genetic material of already existing formed stock. In this regard, the knowledge on the features of the genetic structure of local stocks is an important and pressing task.

Therefore, the aim of our work was to analyze the microsatellite sequences of nuclear DNA of rainbow trout for assessing its genetic diversity in aquaculture of Ukraine.

Materials & Methods

Sampling

The materials for the study were local stocks from three trout farms of Ukraine. These trout farms are located in three regions of Ukraine located in three different temperate-climate ecotones: Forest-steppe, Kharkiv oblast (Novaya Vodolaga, n=35), Carpathian, Chernivtsi oblast (Berehomet, n=21), and Carpathian Mountains, Transcarpathian oblast (Turya Polyana, n=27) (Figure1).

These farms use a full-cycle system of fish rearing with on-site egg production that facilitates genetic control, while the majority of other trout farms in Ukraine import fertilized eggs from abroad. The studied local farms differ in culture technologies. Kharkiv and Chernivtsi local stocks are cultured in recirculation aquaculture systems (RAS), while the Transcarpathian stock is cultured using the classic technology with water supply from a natural source (river).

Blood of age-3 rainbow trout were used as biological samples for microsatellite analysis. Samples were collected in accordance with the provisions recommended by the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes (Strasbourg, 1986) ETS No.123. Blood from the caudal vein was taken using a sterile syringe with heparin (25 IU per 1 ml of blood). Samples were transported in refrigerators at 4°C. The blood was stored at -20°C until DNA extraction.

Total DNA was isolated according to the method recommended in the commercial DNA-Go kit (BioLabTech LTD). The concentration and purity of the obtained DNA were determined by a biophotometer
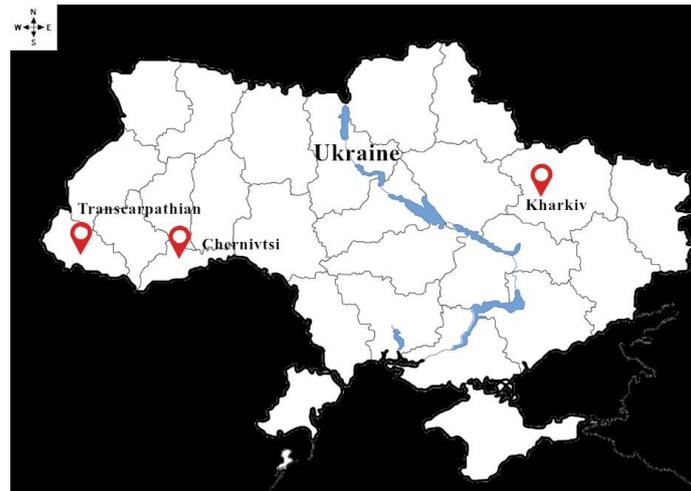
Figure 1. Geographical locations of three local stocks of rainbow trout used for the study.

(Eppendorf, Germany). The ratio of absorption at 260 and 280 nm (A 260 / 280) was used to assess the quality of the isolated DNA. Samples with A 260 / 280 values higher than 1.75 were used for further analysis.

Microsatellite Analysis

SSR primers shown in Table 1 were used for analysis. Polymerase chain reaction (PCR) was carried out in a Thermo scientific thermocycler (Arktik Thermal Cycler) using the Thermo Scientific DreamTaq Green PCR Master Mix (2X) under the following conditions: initial DNA denaturation at 94°C for 5-10 min (depending on the protocol for each type of primer) the following 30-35 cycles: DNA denaturation at 94°C - 30 sec, primer annealing at 58°C - 30 sec, chain synthesis at 72°C - 30 sec; final extension at 72°C for 5 - 10 min.

Amplicons were separated on a 3% agarose gel with ethidium bromide in 1 × TAE buffer. Foregrams were registered in UV light. The Thermo Scientific pUC19 DNA/MspI (HpaII) Marker was chosen as a fragment length marker.

Statistical Analysis

Amplicon analysis was performed in Totallab v.2.01 and Genalex 6.5 (Peakall & Smouse, 2006, 2012). The polymorphic information content (PIC) was calculated using the formulas generally accepted for codominant markers (Nagy *et al.,* 2012). Analysis of Molecular Variance (AMOVA) was performed using Genalex 6.5. Principal Coordinates Analysis (PCoA) (Jombart, Pontier, & Dufour, 2009) was performed using genetic distance data for 6 microsatellite markers in Genalex.

The STRUCTURE program (Pritchard, Stephens, & Donnelly, 2000; Falush, Stephens, M., & Pritchard, 2003) was used for cluster analysis by the Monte Carlo Markov Chain Scheme (MCMC) algorithm for Bayesian statistics. At the initial stage, the number of assumed clusters K ranged from 1 to 10 with Length of Burnin Period-10000, Number of MCMC Reps after Burnin - 50000 with 20 iterations for each cluster. STRUCTURE HARVESTER (Earl & von Holdt, 2011) was used to determine the most likely number of clusters by the Δ K method of Evanno, Regnaut, and Goudet (2005). After that, a secondary set

Table 1. Characterization of microsatellite loci selected for the study

Locus	Repeat motif	Genbank accession number	Marker sequence (5'→3')	PCR conditions			Cycles	Reference
				D	AT	*E*		
OMM 1032	$(AG)_{22}$	AF352737	F:GCGAGGAAGAGAAAGTAGTAG R:CCCATCTTCTCTCTGATTATG	94°C 30 sec	58°C 30 sec	72°C 30 sec	35	Rexroad *et al.* (2002)
OMM 1077	$(GATA)_9$	AF352748	F:GGCTGACCAGAGAAAGACTAGT TC R:TGTTACGGTGTCTGACATGC					
OMM 1088	$(GATA)_{12}$	AF352757	F:CTACAGGCCAACACTACAATC R:CTATAAAGGGAATAGGCACCT					
Str 15	$(GT)_{13}$	AB001058 (MST-15) *	F:TGCAGGCAGACGGATCAGGC R:AATCCTCTACGTAAGGGATTTGC				30	Estoup *et al.* (1993)
Str 60	$(CT)_{13}AC$ $CA(CT)_3$	AB001057 (MST-60) *	F:CGGTGTGCTTGTCAGGTTTC R:GTCAAGTCAGCAAGCCTCAC					
Str 73	$(GT)_{13}TTATC$ $T(GT)_3$	AB001056 (MST-73) *	F:CCTGGAGATCCTCCAGCAGGA R:CTATTCTGCTTGTAACTAGACCTA					

Notes: * , Sequences registered in GenBank according to the name used in the reference (Presa & Guyomard, 1996); F, forward primer's sequence; R, reverse primer's sequence; D, denaturation; AT, annealing temperature; E, extension.

was analyzed for the number of clusters from 2 to 4, Length of Burnin Period - 500000, Number of MCMC Reps after Burnin - 750000 with 20 iterations for each cluster. CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015) was used to average the iterations of each K value and to establish the probability of assigning each individual to the initial cluster (using a consensus membership probability, q-value). Graphical interpretation of the obtained results was performed in STRUCTURE PLOT (Ramasamy, Ramasamy, Bindroo, & Naik, 2014).

Unbiased genetic distances and identity were calculated according to Nei formulae (Nei, 1978), which are used in cases of a small number (up to 50) individuals from each studied population. The MEGA X package (Kumar, Stecher, Li, Knyaz& Tamura, 2018) was used to build an unrooted dendrogram using the method of unweighted paired average (UPGMA) (Nei, 1996).

Results

The microsatellite analysis of the genetic structure of local rainbow trout stocks from three Ukrainian fish farms showed that six of the selected SSR loci (OMM 1032, OMM1077, OMM 1088, Str 15, Str 60, Str 73) were polymorphic (Table 2). The number of alleles for all loci varied from 4 (Str 73) to 10 (Str 15), with an average value of 7.833. The average value of the effective number of alleles per locus was 5.687. A high Shannon index was recorded for all studied loci, while the average value was 1.836±0.127. These SSR loci showed a high level of polymorphism PIC=0.785±0.034. The probability of identity (PI) was in the range of 0.04-0.06 for the studied microsatellite loci, with the exception of the locus Str 73 (0.16). Str 73 was the least informative for the selected panel. Looking at the F_{ST} at marker level, the average genetic differentiation per locus was 0.137 (P=0.001). Among the studied loci, the largest differences of F_{ST} values were observed for the locus Str 73 (F_{ST}>0.25).

The established amplicon ranges for microsatellite loci are specific for the studied Ukrainian rainbow trout stocks (Figure 2). The locus OMM 1088 showed the smallest discrepancy in amplicon ranges among the three local stocks. The narrowest range of alleles was observed at the locus Str 73.

Each of the three studied Ukrainian rainbow trout groups had specific amplicon ranges and allele frequencies for six microsatellite loci (Table 3). Only the locus OMM 1088 had an allele that was found with a high frequency in each of the three groups (125 bp). At the same time, the frequencies of private alleles for each stock were determined and it was found that the Chernivtsi local group had the largest number of private alleles (9). A slightly lower number of private alleles (7) was recorded in rainbow trout of the Transcarpathian local stock. The smallest number of private alleles was found in the Kharkiv local rainbow trout group.
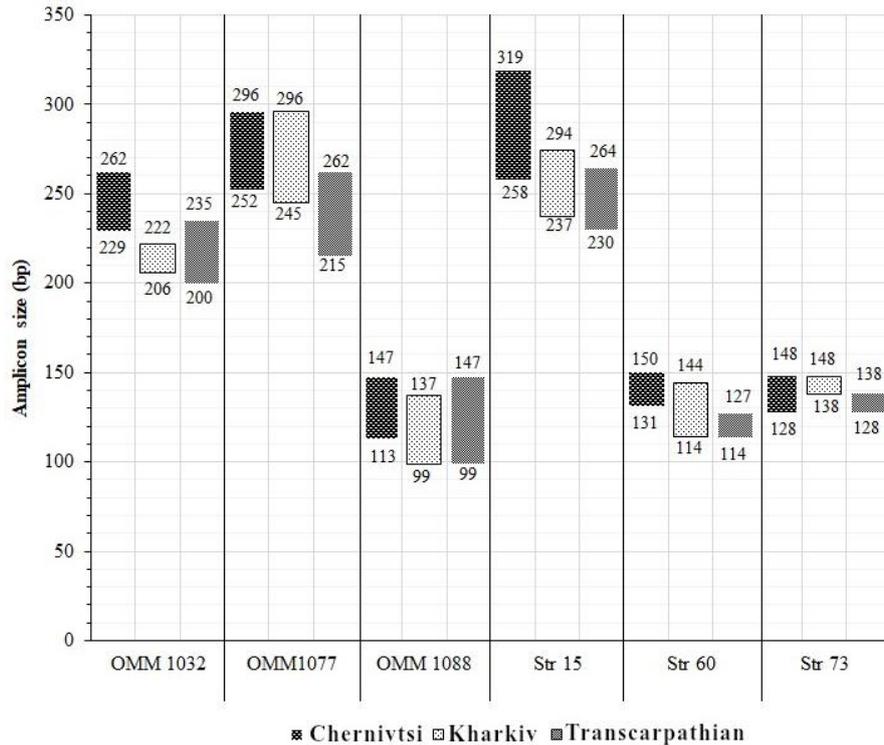
The average values of the number of alleles per locus (Na), the effective number of alleles per locus (Ne), and the Shannon index (I) in the Chernivtsi (Na=4.667±0.333; Ne=3.878±0.315; I=1.423±0.072), Kharkiv (Na=4.333±0.667; Ne=3.238±0.508; I=1.197±0.198) and Transcarpathian (Na=4.888±0.601; Ne=3.505±0.529; I=1.335±0.138) groups were approximately at the same level (Table 4). In each group, the PIC value was not lower than 0.5 indicating the informativeness of the marker set for studying the genetic diversity of rainbow trout populations.

The expected unbiased heterozygosity (uHe) for the three groups ranged from 0.658 (Kharkiv) to 0.786 (Chernivtsi) indicating an average level of heterogeneity. In general, for all loci, with the exception of OMM1077, a statistically significant deviation from the Hardy-Weinberg equation was observed (P<0.05) (Table 4). According to the results of the assessment of molecular variance (AMOVA) for the Ukrainian stocks of rainbow trout, 14% of the genetic variability was attributable to variability among populations, 86% - within populations, which confirmed the genetic similarity of the studied populations. According to the results of the PCoA analysis, the first and second coordinates described 11.82 and 10.42% of allelic variance, respectively (Figure 3).

Table 2. Main parameters of genetic diversity of SSR loci for Ukrainian local stocks of rainbow trout

Locus	Allele size (bp)	Na	Ne	I	uHe	PIC	PI	F_{ST} (p-level)
OMM 1032	200 – 262	9	6.719	2.014	0.867	0.834	0.04	0.146 (0.001)
OMM1077	215 -296	9	5.386	1.901	0.830	0.793	0.06	0.077 (0.008)
OMM 1088	99-147	7	5.360	1.795	0.829	0.789	0.06	-0.005 (0.520)
Str 15	230-319	10	7.042	2.095	0.875	0.842	0.04	0.088 (0.002)
Str 60	114-150	8	6.627	1.971	0.866	0.831	0.04	0.219 (0.001)
Str 73	128-148	4	2.988	1.238	0.681	0.618	0.16	0.288 (0.001)
Mean	-	7.833	5.687	1.836	0.825	0.785	-	-
SE	-	0.872	0.613	0.127	0.030	0.034	-	-

Notes: Na, Number of alleles per locus; Ne, Effective number of alleles per locus; I, Shannon information index; uHe, expected unbiased heterozygosity; PIC, polymorphic information content; PI, probability of identity; F_{ST}, fixation index (results of locus-by-locus AMOVA analysis); p-level, probability value P(rand>=data); SE, standard error of the mean.

Figure 2. Amplicon ranges by microsatellite loci for three studied rainbow trout groups.

Table 3. Frequency of alleles distribution at six microsatellite loci

Locus OMM 1032									
Allele	200	206	215	222	229	235	242	253	262
Chernivtsi	0.000	0.000	0.000	0.000	0.125	0.000	0.125	0.500	0.250
Kharkiv	0.000	0.300	0.300	0.400	0.000	0.000	0.000	0.000	0.000
Transcarpathian	0.278	0.111	0.222	0.222	0.111	0.056	0.000	0.000	0.000

Locus OMM1077									
Allele	215	225	245	252	262	270	276	285	296
Chernivtsi	0.000	0.000	0.000	0.063	0.188	0.188	0.250	0.063	0.250
Kharkiv	0.000	0.000	0.400	0.050	0.150	0.000	0.200	0.100	0.100
Transcarpathian	0.125	0.063	0.563	0.063	0.188	0.000	0.000	0.000	0.000

Locus OMM 1088							
Allele	99	105	113	118	125	137	147
Chernivtsi	0.000	0.000	0.125	0.125	0.250	0.250	0.250
Kharkiv	0.050	0.150	0.100	0.300	0.200	0.200	0.000
Transcarpathian	0.056	0.222	0.056	0.000	0.444	0.167	0.056

Locus Str 15										
Allele	230	237	242	250	258	264	274	292	305	319
Chernivtsi	0.000	0.000	0.000	0.000	0.143	0.000	0.000	0.286	0.143	0.429
Kharkiv	0.000	0.350	0.200	0.350	0.000	0.050	0.050	0.000	0.000	0.000
Transcarpathian	0.222	0.222	0.167	0.222	0.111	0.056	0.000	0.000	0.000	0.000

Locus Str 60								
Allele	114	122	127	131	135	140	144	150
Chernivtsi	0.000	0.000	0.000	0.125	0.125	0.375	0.250	0.125
Kharkiv	0.150	0.000	0.000	0.350	0.450	0.000	0.050	0.000
Transcarpathian	0.125	0.250	0.625	0.000	0.000	0.000	0.000	0.000

Locus Str 73				
Allele	128	134	138	148
Chernivtsi	0.333	0.167	0.167	0.333
Kharkiv	0.000	0.000	0.100	0.900
Transcarpathian	0.333	0.500	0.167	0.000

Notes: Frequencies of private alleles for each stock are underlined.

Table 4. Parameters of genetic diversity of rainbow trout (*O. mykiss*) groups

Locus	Na	Ne	I	PIC	PI	uHe	Prob	PHW
Chernivtsi								
OMM 1032	4	2.909	1.213	0.6050	0.17	0.700	0.001	***
OMM1077	6	4.923	1.667	0.7659	0.07	0.850	0.190	ns
OMM 1088	5	4.571	1.560	0.7456	0.08	0.833	0.000	***
Str 15	4	3.267	1.277	0.6414	0.15	0.747	0.002	**
Str 60	5	4.000	1.494	0.7119	0.10	0.800	0.000	***
Str 73	4	3.600	1.330	0.6713	0.13	0.788	0.006	**
Mean	4.667	3.878	1.423	0.690	-	0.786	-	-
SE	0.333	0.315	0.072	0.025	-	0.023	-	-
Kharkiv								
OMM 1032	3	2.941	1.089	0.627	0.19	0.695	0.000	***
OMM1077	6	4.082	1.583	0.768	0.09	0.795	0.133	ns
OMM 1088	6	4.878	1.670	0.793	0.07	0.837	0.000	***
Str 15	5	3.448	1.356	0.815	0.14	0.747	0.026	*
Str 60	4	2.857	1.161	0.740	0.19	0.684	0.034	*
Str 73	2	1.220	0.325	0.619	0.69	0.189	0.002	**
Mean	4.333	3.238	1.197	0.727	-	0.658	-	-
SE	0.667	0.508	0.198	0.034	-	0.097	-	-
Transcarpathian								
OMM 1032	6	4.909	1.673	0.7659	0.07	0.843	0.002	**
OMM1077	5	2.667	1.244	0.5860	0.18	0.667	0.097	ns
OMM 1088	6	3.522	1.475	0.6777	0.12	0.758	0.006	**
Str 15	6	5.226	1.706	0.7803	0.06	0.856	0.001	**
Str 60	3	2.133	0.900	0.4683	0.28	0.567	0.001	**
Str 73	3	2.571	1.011	0.5355	0.23	0.667	0.007	**
Mean	4.833	3.505	1.335	0.6356	-	0.726	-	-
SE	0.601	0.529	0.138	0.052	-	0.046	-	-

Notes: Prob –PHW, Hardy-Weinberg probability test: * $P<0.05$, ** $P<0.01$, *** $P<0.001$, n.s.=nonsignificant deviation.
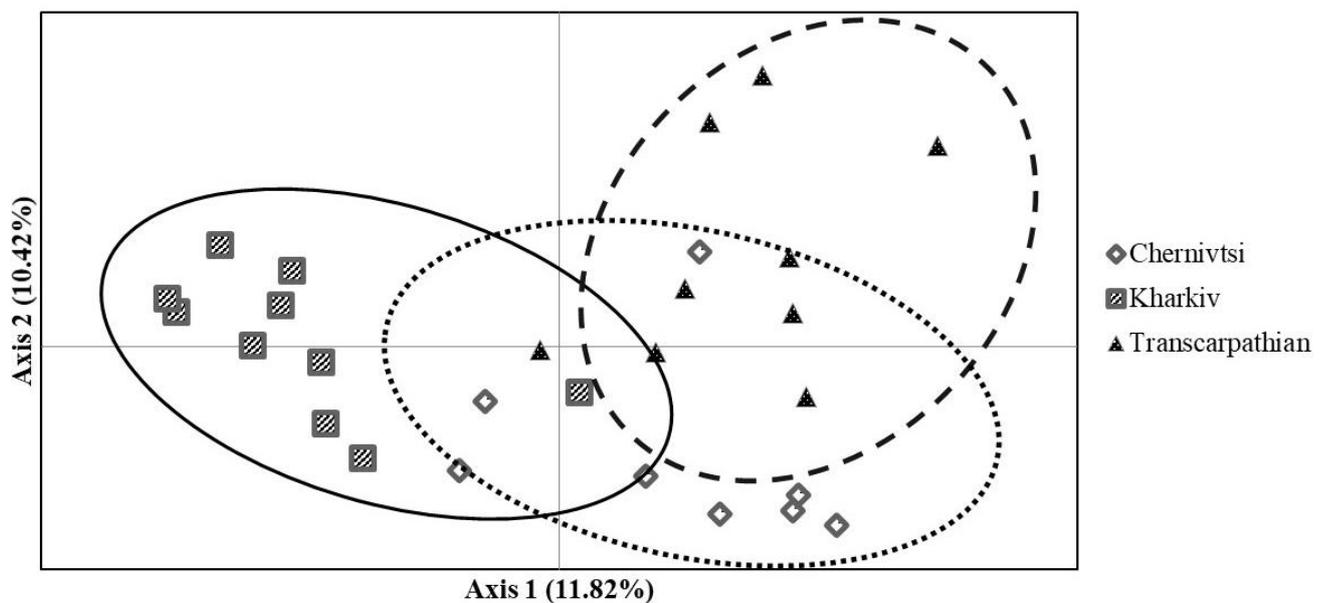

Figure 3. Principal component Analysis (PCoA) of the studies local stocks of rainbow trout.

According to ΔK, the most probable number of clusters was determined as K=3 (Figure 4). The cluster analysis results allowed building a barplot, which showed the genetic structure, boundaries and subdivisions of local rainbow trout stocks (Figure 5).

The largest unbiased genetic identity was recorded between the Kharkiv and Transcarpathian local stocks, while the lowest one was between the Transcarpathian and Chernivtsi groups of ranbow trout (Table 5). The phylogenetic relationships among the studied local rainbow trout stocks were presented in a UPGMA dendrogram (Figure 6) based on Nei's unbiased genetic distances (1978) (Table 5).

Discussion

Analysis of the genetic structure of local stocks of cultured rainbow trout was carried out using six SSR markers of different origins. Markers OMM 1032, 1077, 1088 (Rexroad, Coleman, Hershberger, & Killefer, 2002) were developed based on DNA of *O. mykiss* (Kamloop strain), while primers Str 15, 60, 73 (Estoup, Presa, Krieg, Vaiman, & Guyomard., 1993) were created from the DNA sequence of the brown trout *Salmo trutta*. Flanking regions of microsatellite loci were found to persist among closely related species (Presa & Guyomard, 1996; Artamonova *et al.,* 2016). These SSR loci occur in genomes of several salmonids and are polymorphic making them possible to be used for interspecific comparisons and phylogenetic reconstructions (Presa & Guyomard, 1996). For example, microsatellite loci OMM (1032, 1077, 1088) were used in the molecular genetic analysis of the Danube salmon (*Hucho hucho*) (Kuciński *et al.,* 2015) and taimen (*Hucho taimen*) (Kuang, Tong, Xu, Yin, & Sun, 2009), where these markers were highly polymorphic for the studied fish groups. The primers of

the Str group (15, 60 73) demonstrated high informativeness in studies on the genetic structure of rainbow trout populations (Estoup *et al.,* 1993; Presa & Guyomard, 1996) and Atlantic salmon (Estoup *et al.,* 1993; Presa & Guyomard, 1996). This suggests the advisability of the use of a selected set of microsatellite loci for other closely related fish species.

Quite often, studies carried out on rainbow trout (Fishback *et al.,* 2000; Guyomard *et al.,* 2006; Johnson *et al.,* 2007; Allen *et al.,* 2013; Faccenda *et al.,* 2018) use the OMM 1088 locus, which is in the linkage group RT-16. Such interest in this marker may be due to the significant level of association of the marker or linkage group with the spawning period (Fishback *et al.,* 2000; Allen *et al.,* 2013). Johnson *et al.* (2007) showed that the OMM 1088 locus had a high polymorphism index (PIC=0.79) in a study of a rainbow trout broodstock. In our studies, the value of the polymorphic information content was very similar and ranged from 0.6777 to 0.793. Our studies on three local stocks of rainbow trout demonstrated a high informativeness of Str loci (15, 60, 73) (PIC ranged from 0.618 to 0.842). The six microsatellite loci, which we have selected, were highly informative in the analysis of the genetic structure as evidenced by high numbers of alleles per locus, the value of the polymorphic information content (PIC>0.5) and the probability of identity.

Also, and importantly, amplification of this marker set can be carried out at the same primer annealing temperature. These facts allow reducing the costs and time of the analysis and forming multiplex systems later, and as a consequence, this will speed up the monitoring of populations of various fish species using molecular genetic markers.

Our studies demonstrated the features of the gene pool of local Ukrainian rainbow trout stocks. The
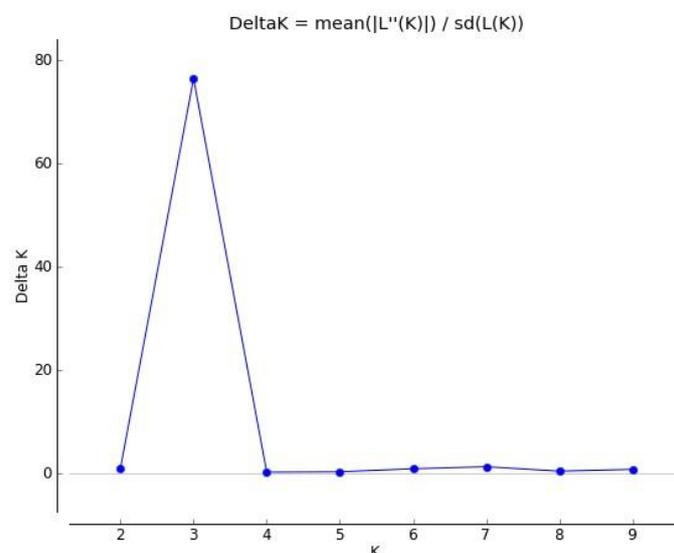

Figure 4. Graphical display of the determination of the most probable number of clusters (K) in the Structure Harvester program.
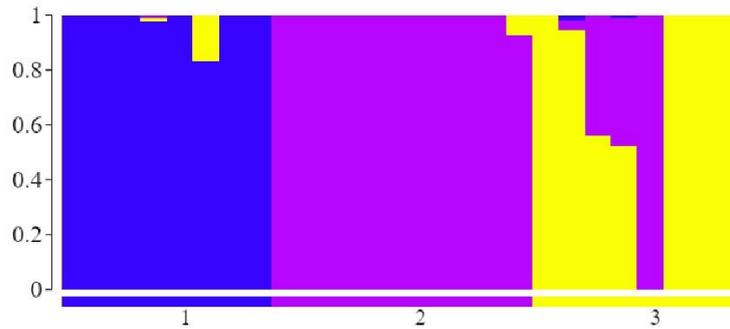
Figure 5. Results of the cluster analysis of local stocks of Ukrainian rainbow trout for six SSR loci using Structure 2.3.4 with K=3: vertically - the proportions of allele frequencies of the corresponding cluster (posterior probability), horizontally – local stock numbers: 1 - Chernivtsi; 2 - Kharkiv; 3- Transcarpathian.

Table 5. Pairwise Population Matrix of Nei Unbiased Genetic Distance (under the diagonal) i Nei Unbiased Genetic Identity (above the diagonal)

	Chernivtsi	Kharkiv	Transcarpathian
Chernivtsi	-	0.426	0.320
Kharkiv	0.852	-	0.455
Transcarpathian	1.139	0.787	-

Figure 6. UPGMA dendrogram of genetic relationships among 3 local stocks of rainbow trout using unbiased genetic distance based on 6 microsatellite loci.

number and frequency of alleles in different groups were determined, that is one of the measures to determine the diversity of the genetic structure. The allele range and frequency as well as the presence of private alleles in each local stock are often used to determine the differences among fish broodstocks (Yousefian *et al.,* 2012; Kucinski *et al.,* 2015; Artamonova *et al.,* 2016). Faccenda *et al.* (2018) used the OMM1088 marker in their studies on the rainbow trout stocks in Trentino (Italy). The number of alleles per locus was higher than in our studies and amounted to 12, while the F_{ST} index was 0.065 that correlated with our values (F_{ST} 0.06). Such a difference in the number of alleles per locus observed in the study of Faccenda *et al.* (2018) is probably due to a large number of the studied farms and, consequently, to the detection of a higher level of polymorphism at the investigated locus. The studies of Estoup *et al.* (1993), Presa & Guyomard (1996) on the rainbow trout determined that the number of alleles per locus was 8 for Str (MST) 15, 5 for Str (MST) 60, and 3 for Str (MST) 72. Among the loci Str 15, 60, 73, the smallest number of alleles per locus was observed for the locus Str 72. Studies on the brown trout (Ceyhun & Ciltas, 2013) also showed that the lowest average

number of alleles per locus was for the locus Str (MST)-73 (2.0) among the selected marker set.

When comparing the generalized parameters of genetic variability among Ukrainian stocks of rainbow trout with the literature, certain common features and differences can be identified. For the locus OMM 1032, Ukrainian local stocks have amplicons with heavier molecular weight in comparison with the data of Rexroad *et al.* (2002), since slow migration amplicons were 262 and 222 bp, respectively. However, the size of the "light" alleles (200 bp) and the number of alleles per locus were the same. For the locus OMM 1077 a larger number of alleles per locus and an extension of the molecular weight range were found. However, as for the locus OMM 1088, the Ukrainian stocks had only seven alleles per locus compared to twelve alleles detected in the study of Rexroad *et al.* (2002), and a shift in the molecular weight range: 99-147 bp and 113-170 bp, respectively.

A sufficiently high level of genetic diversity for six microsatellite loci was determined for the studied samples of rainbow trout, since the Shannon index was more than 1.5, which is considered high for SSR markers (Galinskaya, Shchepetov, & Lysenkov, 2019). The least

genetically diverse was the Kharkiv group (1.197±0.198), while the most diverse was the Chernivtsi group (1.423±0.072).

The recorded deviation from the Hardy-Weinberg equilibrium is expected for small isolated populations. In this case, it is recommended to measure the genetic differentiation for population genetic analysis (Evanno *et al.,* 2005; Hauser, Seamons, Dauer, Naish, & Quinn, 2006; Latch, Dharmarajan, Glaubitz, & Rhodes, 2006). To characterize the rainbow trout gene pool, we used the F_{ST} coefficient, which reflected the proportion of the total genetic diversity distributed among subpopulations (F_{ST}=0.137; P=0.001). However, low F_{ST} values according to Hedrick (2000) can be obtained with modern hypervariable markers that are characterized by many alleles.

The Principal Coordinates Analysis (PCoA), which was based on the analysis of genetic distances calculated using the AMOVA algorithm, reflected the variability range in local stocks of Ukrainian rainbow trout. However, the overlapping regions in the three groups show their genetic similarity.

Based on the values of Unbiased Genetic Distance and Unbiased Genetic Identity, the Kharkiv and Transcarpathian local stocks were shown to be the most genetically close by six studied microsatellite loci. This may indicate that the genetic material used for breeding at these trout farms was historically of the same origin. In our case, molecular methods are a tool that clarifies the issue of the origin and differentiation of stocks, because the rainbow trout has been imported repeatedly from Europe since the time of its pan-European introduction, and in most cases, this was done spontaneously. The stocks were formed by the method of mass selection (Hrynzhevskyi *et al.,* 2006).

Therefore, today most of the owners of trout farms are interested in cooperation with scientists in order to determine the state of the genetic structure of their local stocks, differentiate from other stocks and carry out their certification. The similarity of these two local stocks can also be seen in the barplot obtained from the cluster analysis (Figure 6). This may be due to the fact that more than 15 years ago the replacement broodstock at the Kharkiv farm was created using eggs from the Transcarpathian local stock. The most genetically distant seems to be the Chernivtsi stock.

Conclusion

The features of the genetic structure of rainbow trout in aquaculture of Ukraine were characterized using microsatellite loci. The selected set of SSR-loci had a high information content and a degree of polymorphism, which made it possible to determine the specificity of each local stock. This provides a basis for the use of these microsatellite loci for the analysis of biodiversity and differentiation of rainbow trout stocks in the conditions of industrial trout farms. The specific amplicon ranges were shown and the allelic diversity for the selected loci was characterized for each local stock. A cluster analysis of the studied local stocks showed the presence of three clusters. The phylogenetic analysis allowed determining the degree of genetic identity and differentiation. Specific overlap of allelic ranges for the analyzed loci, parameters of Nei's genetic identity (1972), and AMOVA test results identified 86% variability within populations indicating the genetic proximity of the studied local stocks. At the same time, the cluster analysis results, PCoA and the presence of private alleles of each local stock demonstrated the variability and specificity range of the studied groups of rainbow trout. Based on the results of the comparative analysis of the studied rainbow trout stocks, it can be concluded that, despite the similarity in their genetic structures, each group was characterized by specific features that were expressed in the amplicons sizes, frequencies of certain alleles, and the presence of private alleles.

The data on the genetic diversity based on microsatellite loci in rainbow trout reared in aquaculture conditions of Ukraine can be used as a methodological framework for monitoring the state of gene pools, which will provide recommendations for selective-breeding work with the aim of rational management of genetic resources of trout farms.

Ethical Statement

Samples were collected in accordance with the provisions recommended by the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes (Strasbourg, 1986) ETS No.123

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Author Contributions

Conceptualization: OB and ST, Data Curation: OB, ST, AM and OZ, Formal Analysis: OB, OZ and AD, Funding Acquisition: ST, Investigation: OB and OZ, Methodology: OB, ST and AM, Project Administration: OB and ST, Resources: OB, ST, AM and OZ, Software: OB and OZ, Supervision, visualization and writing - original draft: OB, ST, OZ and AD, Writing-review and editing: OB, OZ, AM and AD.

Conflict of Interest

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