Genetic structure of rainbow trout *Oncorhynchus mykiss* (Salmoniformes, Salmonidae) from aquaculture by DNA-markers

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The rational use of valuable fish species from aquaculture is difficult to implement without knowledge of the state of the genetic structure of local stocks. Different types of DNA markers can be used to achieve the goals of selection and breeding work. The genetic structure of a local stock of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) (Salmoniformes, Salmonidae) farmed in Ukraine was studied using DNA-markers: microsatellite (SSR-markers – single-sequence repeats-markers) and intermicrosatellite (ISSR – inter-simple sequence repeat). Five fragments of trinucleotide: microsatellite motifs with a single anchor nucleotide at the 3'-end were used as a primer for analysis by the ISSR-PCR method. Totally, 85 amplicons were obtained across the five loci, of which 92.9% were polymorphic. The total number of alleles ranged from 10 (marker (ACC)<sub>G</sub>) to 23 (marker (AGC)<sub>G</sub>). The following monomorphic amplicons were determined for the studied local stock of rainbow trout: according to marker (CTC)<sub>C</sub> – 770 and 520 bp bands, for the marker (GAG)<sub>C</sub> – 345, 295 and 260 bp, and for the marker (AGC)<sub>C</sub> – 350 bp. The average number of polymorphic bands per locus was 15.8. The selected ISSR primers had a level of polymorphic information content above the average. The most effective markers for molecular-genetic analysis of rainbow trout were (AGC)<sub>G</sub> and (AGC)<sub>C</sub> according to the percentage of polymorphic bands, marker index, effective multiplex ratio and resolving power. The selected ISSR loci allow the genetic structure of the studied local stock to be characterized using the total and the effective number of alleles per locus (Na and Ne were 1.9 and 1.4, respectively), the Shannon index (average value I was 0.4) and the unbiased expected heterozygosity (mean uHe = 0.3). Microsatellite-based analysis showed features of the genetic structure of the local stock of rainbow trout at six microsatellite loci (OMM 1032, OMM 1077, OMM 1088, Str 15, Str 60, Str 73). Allelic diversity was established and alleles with the highest frequency and most typical for the given stock were identified. The Shannon index and unbiased expected heterozygosity were determined using SSR-markers and were 1.42 and 0.79, respectively. This depicts the complexity of the population structure, a high level of genetic diversity and indicates a high level of heterozygosity of local stock. The “gene pool profile” established as a result of ISSR-PCR in the future will help to differentiate local stocks of rainbow trout in aquaculture of Ukraine. Microsatellite markers provide the ability to determine individual features of genetic variation of local populations and to conduct the management of genetic resources on fish farms.

**Keywords** fish population genetics; ISSR-PCR; microsatellite marker; genetic polymorphism; population structure.

**Introduction**

Modern methods to control the genetic diversity of local stocks play an important role in increasing efficiency and accelerating breeding work in aquaculture (Chiu et al., 2012). Breeding programs in combination with molecular biology techniques can optimize the use of aquatic genetic resources in aquaculture (Saad et al., 2012). Molecular genetic markers are effective population genetic tools to resolve such issues as mechanisms of adaptation to the environmental condition of fish species, protection of biodiversity, assessment of inbreeding effects and stock identification (Olaganju, 2019). The knowledge of the specifics of the formation of genetic structure will create a platform for obtaining local groups of individuals with the desired economic and valuable characteristics.

The primary task for the development of such programs is the study of polymorphism at the intraspecific level. ISSR-PCR (inter-simple sequence repeats) are one of the most convenient and cheapest tools of molecular genetic analysis for solving this problem. These dominant ISSR-markers allow polyclonal genotyping of individuals to be carried out using a single microsatellite locus (Egorova et al., 2018). Multilocus intermicrosatellite analysis (ISSR-PCR) makes it possible to study genetic biodiversity and identify species-specific features, which can be used to create a “gene pool standard” of a breed based on ISSR-fingerprint (Stolpovski et al., 2010; Labastida et al., 2015; Komarova et al., 2018). Genetic certification becomes an integral part of modern breeding standards and undoubtedly facilitates combating falsifications. ISSR markers are widely used to study various fish species: rainbow trout (Melnikova et al., 2010; Perfileyeva et al., 2018), sterlet (Kornavova et al., 2018), tilapia (Saad et al., 2012), cyprinids (Mariutsa et al., 2016). A number of works are devoted to the study of the genetic profile of marine fish (Yusufzai et al., 2016) and, to a greater extent, exotic fish species, for example, Family Osphronemidae (Abu-Aiwaity et al., 2017), Pangasius species (Ly & Yen, 2019), parrotfish (Saad et al., 2013) in Ukraine. A population genetic analysis using intermicrosatellite loci has already been performed on sturgeon (Dubin, 2012) and cyprinids (nahorniuk et al., 2013; Hrytsyniak et al., 2015; Mariutisa et al., 2016). However, the rainbow trout cultured in Ukraine has not been studied yet using ISSR markers.

On the other hand, investigations of genetic variability are conducted using microsatellite markers (SSR – single sequence repeats). They are characterized by wide distribution in the genome, have large allelic polymorphism among individuals and are closely connected with genes of known function. SSR-markers were proven to be essential in studies of the genetic structure of populations for providing management of fish stocks (Olaganju, 2019). Analysis of literature sources in recent years shows that microsatellites were predominantly used in genetic studies of rainbow trout in different countries (Ban et al., 2015; Abadia-Cardoso et al., 2016; Faccenda et al., 2018) to assess the genetic variability of stocks and evaluation of their relationships, reconstruction of the admixture history. Abadila-Cardoso et al. (2016) showed the possibility of identifying the differ-
ences in natural populations of *Oncorhynchus mykiss*Walbaum, 1792 from individuals of farmed stocks and assessing the impact on rainbow trout from natural water sources during stocking. Moreover, currently, microsatellites along with SNP-markers (single nucleotide polymorphisms markers) are actively used for the analysis of quantitative trait loci (QTL). It allows determination of the breeding value of individuals, prediction of their productivity at an early age, determination of the efficiency of selection and response to the intensity of selection (Olagunju, 2019).

Therefore, the purpose of our research was to study the genetic profile of rainbow trout in aquaculture of Ukraine using SSR- and ISSR-markers.

### Material and methods

The selection of fish for the study was carried out taking into account the provisions recommended by the European Convention for the Protection of Vertebrate Animals used for Research and other Scientific Purposes (Strasburg, 1986). The rainbow trout of the Chernivtsi local stock (Berehomet, Chernivtsi region) (Fig. 1) was selected as an object for the study of the genetic structure by ISSR- and SSR-markers. Fin clips were collected from the age-3+ group (n = 21) and stored in 96% ethanol at a temperature of 4 °C until DNA isolation. DNA was isolated using a DNA-Go commercial kit (BioLabTech LTD). A biophotometer (Eppendorf, Germany) was used to assess the quantity and quality of the isolated DNA.

ISSR genotyping of rainbow trout was performed using five fragments of trinucleotide microsatellite loci (Table 1), which have already been actively studied in other fish species (Dubin, 2012; Nahornik et al., 2013; Hrytsyniak et al., 2015; Mariutsa et al., 2016). The following SSR-markers were used (Table 1): OMM 1032, OMM 1077; OMM 1088 (Strasbourg, 1986). The rainbow trout of the Chernivtsi local stock was used to assess the quantity and quality of the isolated DNA.

**Table 1**

| Type of marker | Symbol | Repeat Motif | Sequence (5'-3') | iD | Cycles | D | AT | E | fE |
|---------------|--------|--------------|-----------------|----|--------|---|----|---|----|
| **ISSR**      |        |              |                 |    |        |   |    |   |    |
| A             | (CIC)C | 5’-CTC/CTC/CTC/CTC/CTC/CTC-3’ | 95°C 2 min 32 94°C 30 s 60°C 30 s 72°C 2 min 72°C 10 min | 94°C 10 min 35 94°C 30 s 58°C 30 s 72°C 30 s 72°C 5 min |
| B             | (GAG)C | 5’-GAG/GAG/GAG/GAG/GAG/GAG-3’ | “” | “” | “” | “” | “” | “” |
| C             | (AGC)G | 5’-ACC/AGC/AGC/AGC/AGC/AGC-3’ | “” | “” | “” | “” | “” | “” |
| D             | (ACC)G | 5’-ACC-ACC-ACC-ACC-ACC-ACC-3’ | “” | “” | “” | “” | “” | “” |
| E             | (AGC)C | 5’-ACC/AGC/AGC/AGC/AGC/AGC-3’ | “” | “” | “” | “” | “” | “” |

**SSR**

| OMM 1032 (AG)2 | 5’-GAG/GAG/GAG/GAG/GAG/GAG-3’ | 94°C 10 min 35 94°C 30 s 58°C 30 s 72°C 30 s 72°C 5 min |
| OMM 1077 (GATA)2 | 5’-GAC/GAG/GAG/GAG/GAG/GAG-3’ | “” | “” | “” | “” | “” | “” |
| OMM 1088 (GATA)2 | 5’-CCT/AGG/AGC/AGC/AGC/AGC-3’ | “” | “” | “” | “” | “” | “” |
| Str 15 (MST-15)* (GT)3 | 5’-GTC/AGG/AGC/AGC/AGC/AGC-3’ | 94°C 5 min 30 “” | “” | “” | “” | “” |
| Str 60 (MST-60)* (CT)3 | 5’-CTC/AGG/AGC/AGC/AGC/AGC-3’ | “” | “” | “” | “” | “” | “” |
| Str 73 (MST-73)* (GT)3 | 5’-TATC/TATC/TATC/TATC/TATC/TATC-3’ | “” | “” | “” | “” | “” | “” |

Notes: * – sequences registered in GenBank according to the name used in the reference (Pres & Guyomard, 1996); F – forward primer’s sequence; R – reverse primer’s sequence; ID – initial DNA denaturation; D – denaturation; AT – annealing temperature; E – extension; fE – final elongation; “” – conditions are similar to those indicated in this column above.

The Polymorphism Information Content (PIC) was assessed using methods (Nagy et al., 2012) generally accepted for codominant markers and the GENeOm for ISSR-markers (Abuzayed et al., 2016). The following parameters were used to determine the information content of ISSR primers: effective multiplex ratio (EMR), marker index (MI), resolving power (Rp), which were calculated using methods (Prevost & Wilkinson, 1999).

### Results

A total of 85 amplicons was obtained by genotyping rainbow trout with the use of five ISSR markers and 92.9% of the amplicons were polymorphic (Table 2). The molecular weight of the amplified fragments ranged from 170 to 1900 bp (Fig. 2). Amplicon size and their frequencies show the “gene pool profile” of rainbow trout cultivated in aquaculture of Ukraine by ISSR-markers. The total number of amplicons per locus (NTB) ranged from 10 (marker D and B) to 23 (marker C). For three of the five studied loci, 6 conservative bands, or so-called monomorphic bands were identified, the number of which ranged from 1 to 3 per locus. For marker A, these were amplicons with a molecular weight of 770 and 520 bp, for marker B – 345, 295 and 260 bp, and for marker E – 350 bp.

The mean value of alleles per locus and the effective number of alleles per locus (Ne) Shannon’s information index (I), unbiased expected heterozygosity (uHe), and the percentage of polymorphic loci (PPL).

For further statistical processing, the presence of the ISSR-PCR amplicon was noted as “1”, and the absence – as “0”. POPGENE version 1.32 (Yeh & Boyle, 1997) and GenAlEx v 6.5 (Peakall & Smouse, 2006, 2012) were used to determine the number of alleles per locus (Na), effective number of alleles per locus (Ne), Shannon’s information index (I), unbiased expected heterozygosity (uHe), and the percentage of polymorphic loci (PPL).

![Fig. 1. The phenotype of the rainbow trout cultivated in the local Chernivtsi stock](image)

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(marker C). The marker index, which serves as a general measure of the usefulness of the marker system, increased in this order B < D < A < E < C. The resolution power (Rp) of the selected ISSR loci grew in the sequence D < B < A < C < E.

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Perfilyeva et al. (2018) found a species-specific allele (400 bp) for a Kazakhstan population of rainbow trout using markers (CAG)$_{T}$-T and (CAG)$_{7}$. Several studies (Melnikova et al., 2010; Komarova et al., 2018; Perfilyeva et al., 2018), which were carried out on rainbow trout using intermicrosatellite loci, are indicative of the fact that ISSR-markers are promising for intraspecific differentiation of populations. As noted by Sulimova et al. (2011), the information content and convenience of the analysis using ISSR-PCR method has increased due to the development of software for statistical processing of results, such as, for example, Structure. Stolpovskii et al. (2010) used ISSR loci to analyze the genetic structure and determine the so-called “gene pool profile” and “gene pool standard.” It’s possible to determine the correspondence of the genetic structure of individuals to the gene pool standard according to the major bands and amplification frequencies, which occurred with a high frequency (more than 40.0%), as well as further cluster analysis. Studies in this field, as shown in some works (Sulimova et al., 2011; Komarova et al., 2018), allows ISSR-markers to be used to create genetic passports of breeds or intrarbreed types. Therefore, at the present stage, intermicrosatellite analysis is highly informative, universal and indicative for the study of biodiversity and identification of differences in animal populations.

At the same time, Faccenda et al. (2017) showed that the results of microsatellite analysis were essential to understanding the state of genetic resources of each individual stock. Since, in addition to significant genetic variation within populations, there is also significant subdivision reflected at the inter-population level. Faccenda et al. (2017) concluded that it was possible to give recommendations on the rational management of local stocks for breeding pure bred broodstocks based on the data of the SSR-analysis. Faccenda et al. (2017) showed that OMM markers (including the OMM 1088 marker used in our work) were promising for effective work with local stocks for their differentiation.

We concluded that ISSR markers are convenient for interspecies identification and creation of genetic passports since they are universal in this aspect and are applicable for different animal species. Microsatellite markers are more convenient for identifying intraspecific polymorphism of rainbow trout and can be used as routine tools for solving the goals of fish farming at the current level of the development of molecular genetic methods.

**Conclusion**

The information content of ISSR primers for studying the gene pool of rainbow trout and monitoring its state was determined. The genetic profile of rainbow trout by ISSR- and SSR-markers has been obtained, which will allow intra- and interspecific identification to be carried out, and gene pool standards of breed to be introduced in the future that will be developed and approved in Ukraine. The obtained results indicate that the DNA markers used may be useful for monitoring the genetic diversity and inbreeding rate of local stocks of rainbow trout in aquaculture.

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