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Comparison of Genetic Diversity Indicators in Samples of Artificial and Natural Populations of Russian Sturgeon and Beluga at the Mouth of the Ural River

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Abstract

Background: Despite its ecological adaptability, sturgeon exhibits a low nuclear DNA evolution rate, enabling the use of the same primer sets for analyzing different Acipenseriformes species. The purpose of the paper was to analyze the indicators of genetic polymorphism of sturgeon populations in closed water supply systems compared with natural populations for conservation and restoration.

Methods: 147 sturgeon specimens, including *Acipenser gueldenstaedtii* and *Huso huso*, were selected from natural populations in the Ural River delta and artificial populations in aquaculture farms. DNA was extracted from fin tissues, and genetic diversity was assessed using seven short tandem repeat markers.

Result: The study showed significant genetic diversity in natural and artificial populations. Natural *A. gueldenstaedtii* populations demonstrated a higher level of genetic diversity (He=0.871, Ne=8.691) compared with artificial *A. gueldenstaedtii* populations (He=0.829, Ne=5.980). Similarly, artificial *H. huso* populations showed lower genetic diversity (He=0.554, Ne=2.704) than natural populations (He=0.663, Ne=3.238).

Conclusion: The analysis showed a deficiency of heterozygotes at many loci due to inbreeding, which highlights the importance of genetic management in aquaculture practice. The results highlight the need to implement genetic management strategies in sturgeon aquaculture to preserve genetic diversity and prevent inbreeding. Regular genetic monitoring and strategic breeding programs are recommended to ensure the sustainability and persistence of cultivated and natural sturgeon populations.

Introduction

Industrial aquaculture using recirculating aquaculture systems (RAS) is a developing industry globally due to the increased demand for commercial sturgeons and black caviar. Kazakhstan has a problem associated with low fish consumption per capita up to 4.3 kg/year, with a recommended rate of 14 kg/year. Therefore, the relevance of the development of the fish farming industry has increased significantly. As in many other countries, in Kazakhstan, traditional fishing is gradually giving way to artificial breeding and rearing of fish as a result of the anthropogenic impact of human activities on rivers and seas [1,2]. This method of fish farming is an effective tool for replenishing the natural sturgeon populations on the verge of extinction. The cultivation of sturgeon species in RAS allows for preserving pure populations (clean lines) without obtaining hybrids due to the control of fertilization processes, whereas in natural conditions, the number of pure populations of sturgeon decreases. In the natural environment, most sturgeon species have a low reproductive purity of the population due to changing environmental conditions (temperature, current, spawning sites, etc.) [3,4]. Despite the advantages of growing sturgeon in RAS, one of the main constraining problems is the limited choice of pairs for the reproduction of sturgeon, which determines the need to identify brood fish and breeding stock of fish to exclude closely related crosses, which will allow preserving the existing genetic structure and avoiding depletion of the gene pool [5,6].

When selecting brood fish pairs in fish farms, the risk of closely related crossing increases, inevitably leading to an increase in homozygosity in offspring and inbred depression, i.e., a decrease in vitality and fertility and an increase in harmful recessive mutations in young fish [7-10]. Yu.P. Altukhov, G.D. Ryabova, and E.I. Shishanova note that under artificial conditions, genetic diversity is depleted, and the reproduced population is increasingly less adapted to the environment and does not possess the full genetic diversity of the natural population [11-13]. Therefore, when carrying out measures aimed to maintain the number of sturgeons by releasing young fish into their natural habitat, it is necessary to apply a competent genetic approach allowing an assessment of the genetic diversity of the breeding stock and the degree of kinship of brood fish to exclude genetic degeneration of the population due to inbreeding.

In response to the transition to new conditions of existence (changes in the chemical composition of water and temperature [14], the appearance of toxic agents [15,16]), fish react with adaptations. When breeding fish in RAS, new factors (thermal waters, artificial feeds, high planting density, and industrial cultivation methods) create stressful conditions for fish [17].

In the conditions of constantly acting altered factors of existence, the role of stable genotypes ensuring selective success increases [18]. Therefore, there is a need to study the processes that form the genetic structure of rearing and breeding stock in these conditions. The formation of valuable rearing and breeding stock as an alternative to the removal of fish from natural populations for reproduction requires consideration of the impact of artificial reproduction conditions on changes in their genetic diversity.

A modern approach to studying the genetic diversity of fish populations and artificial fish stock and interspecific and species identification of specimens associated with the effective selection of brood fish pairs and the establishment of their geographical origin is the use of molecular markers [19]. Depending on the task, various approaches are used to study the genetic characteristics of sturgeons. Thus, studies have been conducted to evaluate the polymorphism of isoenzyme markers by electrophoresis of blood proteins in sturgeon [12,20] and the genetic variability of domesticated and natural sturgeon stock [21]. On the other hand, the identification of sturgeons, interspecific hybrids, and commercial products is based on the study of nucleotide sequences using such methods for detecting DNA polymorphism as RAPD (Randomly Amplified Polymorphic DNA). These markers were used in the work of K.V. Rozhkovan et al. [22] for the identification of four interspecific hybrids from the crossing of *Acipenser schrenckii* × *Acipenser baerii* × *Acipenser ruthenus*. Another of the main methods of nuclear DNA research is the analysis of the allelic composition of microsatellite loci. These DNA sections, which are not subjected to selective pressure and evolve at their own pace, gradually accumulate inherited differentiating features, the analysis of which allows for interspecific, intraspecific, and individual identification of sturgeons [23]. By analyzing the polymorphism of microsatellite loci, we established the species affiliation of sturgeon (*Acipenseridae*) and identified specimens of hybrid origin [23]. We established the geographical affiliation of specimens from fish farms [24] and assessed the genetic variability of aquacultural brood fish of Siberian sturgeon of various origins from 13 farms in the Russian Federation [25]. We studied the genetic diversity of natural populations of sterlet *A. ruthenus*, *Acipenseridae*, and two rearing and breeding stocks in the Perm Territory [26] and comparatively assessed the genetic structure of the sterlet population in the Irtysh River and the rearing and breeding stock in Tyumen [27] and indicators of genetic diversity and originality of three natural populations and three rearing and breeding

stocks of sterlet (*A. ruthenus*, *Acipenseridae*) in the Volga Federal District [28]. We studied the geographical genetic structure of the lake sturgeon (*Acipenser fulvescens*) in its Canadian range [29] and the genetic structure of Atlantic sturgeon populations from 13 spawning rivers [30]. Based on the 18S rDNA sequencing data, we analyzed the phylogenetic relationships of the Amur sturgeon *A. schrenckii* [31]. We developed population genetic markers based on the variability of inter-satellite DNA [32]. Based on the literature data, the study of the genetic diversity of sturgeon is relevant to this day, which stimulates the continuation of scientific research to solve existing problems.

Despite its high ecological adaptability, sturgeon is characterized by a low rate of nuclear DNA evolution [33], which allows for using the same sets of primers for the analysis of different *Acipenseriformes* species [34].

Thus, the study aimed to analyze and compare the genetic polymorphism of sturgeon populations grown in closed water supply systems (CWSS) compared with natural populations.

Methods

Sampling

In total, 147 specimens belonging to the sturgeon family were analyzed in our study (104 *Acipenser gueldenstaedtii* representatives, 43 *Huso huso* representatives). All specimens, details of sampling and on the origins, population are indicated in Table 1.

Samples were taken from 40 *A. gueldenstaedtii* specimens and 26 *H. huso* specimens. Samples were also taken from the natural population (64 *A. gueldenstaedtii* specimens and 17 *H. huso* specimens) caught in the delta of the Ural River and contained in RAS at the Ural-Atyrau Sturgeon Fish Hatchery RSE, which is a complex of engineering structures designed to grow young sturgeon species to replenish the stocks in the basin of the Caspian Sea. The Laboratory of Ichthyology and Aquaculture of Zhangir Khan West Kazakhstan Agriculture and Technical University is a modern laboratory aimed at conducting scientific research at aquaculture facilities, including on sturgeon grown in RAS. UNKOPPA is the largest aquaculture complex in Central Asia, where about 35 tons of sturgeon are grown (breeding stock, rearing and breeding stock, commercial fish, and young fish) and which serves as a technical and research basis for the introduction of new developments in fish farming and industrial aquaculture.

DNA extraction

DNA extraction, genotyping, and processing of the results were performed in the Laboratory of Biotechnology and Diagnostics of Infectious Diseases of the Testing Center of the Zhangir Khan West Kazakhstan Agriculture and Technical University.

Fragments of the pectoral fin were selected as material for DNA extraction and short tandem repeat (STR) analysis. Each selected sample was assigned an identification laboratory number. DNA was extracted from fin tissues using a commercial DNA-Extran-2 kit (SYNTOL, Russia). The quality and quantity of the isolated DNA material was determined using a spectrophotometer.

Genetic analysis

For genotyping, we used a GeneXpert-Sturgeon reagent kit (SYNTOL, Russia) designed for the genetic typing of sturgeon species. Genotyping was performed at seven STR loci. Characteristics of all seven loci are indicated in Table 2, including primer sequence. This kit allows the typing of most specimens of sturgeon species. The genotyping results according to these loci can be reproduced in other laboratories; all these loci are amplified in multiplex polymerase chain reaction (PCR).

The amplification reaction was carried out according to the manufacturer's instructions to the genotyping kit on the ProFlex PCR system thermal cycler (Applied Computer Systems, USA).

The separation of the obtained DNA fragments was carried out by capillary electrophoresis in a polymer gel using the AB 3500 genetic analyzer (Applied Microsystems, USA). The interpretation of the analysis results was carried out using the GeneMapper 6.0 software (Applied Biosystems).

Data analysis

Genetic diversity was analyzed using a specialized add-in for Microsoft Excel, GenAlEx 6.51 [39]. We evaluated the following indicators: allele frequency, number of alleles per locus, effective number of alleles (Na), observed (Ho) and expected (He) heterozygosity, fixation index (Fis), and Nei genetic distance index [40]. Cluster analysis was performed using the Structure 2.3.4 software based on the Markov Chain Monte Carlo (MCMC) method [41]. The number of clusters (C) ranged from 1 to 10. To analyze the results of Structure and identify the most optimal C value, a portable version of the Structure Harvester V. 0.6 software was used, which uses Evanno methods to calculate the Delta K index and determine the optimal number of clusters by sequentially iterating through them [42]. The results of the Structure launches were summarized using the CLUMPP software [43]. The resulting Q-matrices were presented in the form of histograms.

Results

Phenotypic traits

As a result of the study of DNA polymorphism at seven STR loci, 104 specimens from four studied *A. gueldenstaedtii* samples showed 16 alleles at the AoxD161 locus, 28 alleles at the Afug41 locus, 13 alleles at the LS19 and Afug135 loci, 23 at the Afug37 locus, 20 at the Spl173 locus, and 17 at the AoxD165 locus. Concerning the 43 specimens in three studied *H. huso* samples, 5 alleles were found at the AoxD161 locus, 12 at the Afug41 locus, seven alleles each at the LS19, Afug135, Afug37, and Spl173 loci, and eight alleles at the AoxD165 locus . Some of the identified alleles were detected with a low frequency (less than 0.1), which may indicate the risk of loss of these alleles in the future in the studied populations.

Among the belugas, allele 100 at the AoxD161 locus (more than 0.6 in each population), allele 198 at the Afug135 locus (frequency from 0.4 to 0.7 depending on the population), and allele 190 (AoxD165) with a frequency from 0.6 to 0.92 in the artificial population of UNKOPPA (HHa1) were distinguished with high frequency. As for the samples of Russian sturgeon, the maximum frequency of alleles did not exceed 0.4, and a decrease in the number of alleles and, accordingly, an increase in frequencies was more typical for the artificial population of *A. gueldenstaedtii* (AGa).

The I index characterizes genetic diversity; the higher this indicator, the higher the level of diversity in the sample. In our case, the values of I are high in the samples of Russian sturgeon from natural populations (more than 2) and slightly lower in the sample from an artificial population, decreasing up to 1.8 at the Afug135 locus. Samples from beluga populations are characterized by lower I index values from 0.345 to 1.691, while the lowest index values were observed in the HHa1 sample.

The Ho level in almost all loci in the Russian sturgeon samples was below the He level, which indicates a deficiency of heterozygotes, probably as a result of inbred crossing in the population. This is also indicated by the positive value of the F index. The situation differs among the three samples from the beluga populations. In the HHn and HHa1 samples, Ho is higher than He in most loci (the F index is negative), while in the HHa2 sample, only one locus (LS19) out of seven is characterized by a higher level of Ho (0.786) over He (0.673). This fact suggests that the HHn and HHa1 samples have a higher level of heterozygosity compared to the HHa2 sample.

There are not many works in scientific literature on the genetic structure of sturgeons, and even fewer used STR markers for genotyping; there is a wide variety of microsatellite loci suitable for study. Therefore, there is not much work to compare the data we obtained with those available in world databases.

In the samples of the Russian sturgeon, compared with the samples of the beluga, a significantly higher number of alleles is observed for each locus, which is associated with the tetraploidy of these sturgeon species. However, in this image, a decrease in the number of alleles in the AGa sample can be traced for almost every locus except AoxD161, where the number of alleles is comparable to samples from natural populations. The reverse situation is typical for samples from beluga populations: the number of alleles in the sample from the artificial HHa2 group differs favorably in almost all loci from the other two samples, while the situation is similar in the HHn and HHa1 samples. This fact indicates a lack of brood fish in the natural environment and RAS at UNKOPPA and a better selection of parent pairs in Laboratory of Ichthyology and Aquaculture of Zhangir Khan West Kazakhstan Agriculture and Technical University compared with the second RAS studied by us.

The He value was established for the study of genetic diversity. The He values are high, especially for the Russian sturgeon, as a polyploid species, while for the beluga, which is a diploid, it was significantly lower and ranged from 0.554 to 0.663. For the total sample of sturgeon, the He was 0.750. For the samples of Russian sturgeon, this indicator is the highest in the sample AgN3 (He=0.871), and the lowest in the sample AGa (He=0.829). Among the beluga, the highest He value was characterized by the HHa2 sample (He=0.663), and the lowest value was found in the HHa1 sample (He=0.554).

In all groups of Russian sturgeon, the possibility of inbreeding can be considered since they had a lack of heterozygotes for all the studied loci, the only exception was the Afug41 locus in the *A. gueldenstaedtii* AGa group. In the studied beluga groups in HHa2, six of the seven studied loci had a deficiency of heterozygotes, which indicates the possibility of inbreeding in this group.

The Russian sturgeon has the highest Na and Ne values in the AGn1 sample (Na=14,571; Ne=8,691), and the lowest in the AGa sample (Na=10,429; Ne=5,980). In beluga, the highest Na and Ne values were observed in the sample of artificial origin HHa2 (5,571 and 3,238, respectively), and the lowest in the sample of natural origin HHn (3,714 and 2,822, respectively). The low indicators of genetic diversity of the natural beluga sample can be explained by the fact that, first, the number of brood fish of this species is limited in natural conditions and, second, a small number of specimens were studied.

Results demonstrated that Na is the average number of alleles per locus, Na freq. ⩾5% is the average observed number of alleles with a frequency of ⩾5% per locus, Ne is the effective number of alleles, No. of

Code	Sturgeon species	N	Sampling date	Population	Sampling place
AGn1	*A. gueldenstaedtii*	19	23.11.2022	natural	Ural-Atyrau Sturgeon Fish Hatchery
AGn2	*A. gueldenstaedtii*	20	10.04.2023	natural	Ural-Atyrau Sturgeon Fish Hatchery
AGn3	*A. gueldenstaedtii*	25	27.06.2023	natural	Ural-Atyrau Sturgeon Fish Hatchery
AGa	*A. gueldenstaedtii*	40	20.09.2022	artificial	Laboratory of Ichthyology and Aquaculture of Zhangir Khan West Kazakhstan Agriculture and Technical University
HHn	*H. huso*	17	23.11.2022	natural	Ural-Atyrau Sturgeon Fish Hatchery
HHa1	*H. huso*	12	16.05.2023	artificial	Educational and Scientific Complex of Experimental Industrial Production of Aquaculture (UNKOPPA)
HHa2	*H. huso*	14	07.09.2023	artificial	Laboratory of Ichthyology and Aquaculture of Zhangir Khan West Kazakhstan Agriculture and Technical University

Table 1: Organoleptic characteristics of probiotic strains isolated from fish intestines.

Locus	Primer sequence (5'-3')	Length of the amplicons, base pairs (bps)	Repeat structure	Link to the source
AoxD161	F: GTTTGAAATGATTGAGAAAATGC R: TGAGACAGACACTCTAGTTAAACAGC	90-153	ATCT	[35]
Afug41	F: TGACGCACAGTAGTATTATTTATG R: TGATGTTTGCTGAGGCTTTTC	182-274	ATCT	[36]
LS19	F: CATCTTAGCCGTCTGGGTAC R: CAGGTCCCTAATACAATGGC	124-166	GTT	[37]
Afug135	F: GCCAATTCCTGAAATATACCAG R: CGAAACCGCTTCAGACCTT	184-276	ATCT	[36]
Afug37	F: CAGGGAATCATGAGCACACG R: TGGCGCAGGATTTTGACAC	140-222	ATCT	[36]
Spl173	F: GGCTTTTGTCTGAAACGTCC R: TGGTGTGTGATTTTGAAGGC	226-324	ATCT	[38]
AoxD165	F: TTTGACAGCTCCTAAGTGATACC R: AAGCCCTACAACAAATGTCAC	158-244	ATCT	[35]

Table 2: Characteristics of seven microsatellite loci isolated from bacterial strains.

Code	AGn1	AGn2	AGn3	AGa	HHn	HHa1	HHa2
AGn1	0.000	-	-	-	-	-	-
AGn2	0.010	0.000	-	-	-		-
AGn3	0.004	0.009	0.000	-	-	-	-
AGa	0.023	0.023	0.021	0.000	-	-	-
HHn	0.106	0.108	0.097	0.117	0.000	-	-
HHa1	0.123	0.118	0.117	0.139	0.098	0.000	-
HHa2	0.076	0.075	0.076	0.096	0.090	0.065	0.000

Table 3: Nei Genetic Distance between isolated bacterial strains.

private alleles is the average number of unique alleles in a population, and He is the expected heterozygosity. Thus, the average number of alleles with a frequency of ⩾5% was ≈50% for samples from natural populations of *A. gueldenstaedtii* (AGn1, AGn2, AgN3) and more than 60% for the AGa sample. For samples from *H. huso* populations, the number Na freq. ⩾5% was a significant 70% in the HHa1 sample, 79% in the HHa2 sample, and 88% in the natural HHn population sample. The effective number of alleles was more than 50% of the average number of alleles for each sample, while unique alleles were found in each group of samples (No. of private alleles) except a sample of *H. huso* from UNKOPPA (HHa1).

Analysis of genetic distances

The average intra-group distances of the Russian sturgeon and beluga for each of the species were approximately the same, and the distances between natural and artificial groups were greater, as expected, as Nei Genetic Distance indicated in Table 3.

There is a clear division of natural and artificial samples in both *A. gueldenstaedtii* and *H. huso*. There is a high similarity of two samples caught from natural conditions at different times, namely, AGn1 selected on 23.11.2022 and AgN3 selected on 27.06.2023. Here, repeated sampling from the same specimen is excluded, since newly caught specimens were scanned for the presence of a chip and chipped in case of its absence; all obtained genotypes were checked for duplication.

Cluster analysis

When conducting a cluster analysis of the genotyping results in the Structure program, the C value was set from 1 to 10. However, in all cases, starting from C =4, the clustering process became uninformative. The determination of Delta C values by the Evanno method showed that the most optimal number of clusters in the analysis of all samples (n= 147) of both fish species was three, with Delta C=727.544. Clustering results of the sturgeon fish at C=3 can be seen in Figure 1.

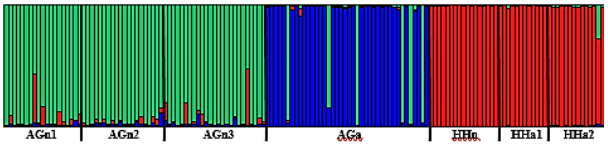

Figure 1: Clustering analysis of sturgeon fish samples showing genetic relationships at three distinct groupings (C=3).

As can be seen from Figure 1, a cluster formed from 3 samples of *A. gueldenstaedtii* (green) of the natural population of the Ural-Atyrau Sturgeon Fish Hatchery is distinguished. A sample from the artificial population of Russian sturgeon of the Laboratory of Ichthyology and Aquaculture of Zhangir Khan West Kazakhstan Agriculture and Technical University is distinguished in a separate cluster (blue). A group of one natural and two artificial populations of beluga is formed in a separate cluster (red). As can be seen in the figure, even though all populations are kept in different conditions, there are cross-values from other species. This indicates the presence of common alleles, since genetic markers are preserved due to common origin. Nevertheless, with a separate analysis of the data for each species of studied sturgeon in the samples of belugas, there is also a division into two clusters (C=2 with Delta C=155.679), a sample of belugas from the natural population forms a separate cluster. In the case of Russian sturgeon samples, no changes are observed in a separate analysis, and two clusters are clearly distinguished separating samples from natural and artificial populations. Results of clustering of Russian sturgeon at C=2 are indicated in Figure 2.

As a result of the analysis of the structure of the studied groups of Russian sturgeon using modeling in STRUCTURE v.2.3.4 [25] based on the results of genotyping for seven STR loci, we showed two distinct clusters (C=2). In Figure 2, 2 clusters are distinguished. Formed from 3 samples of *A. gueldenstaedtii* (red) of the natural population of the Ural-Atyrau Sturgeon Fish Hatchery and a cluster of the artificial population of Russian sturgeon of the Laboratory of Ichthyology and Aquaculture of Zhangir Khan West Kazakhstan Agriculture and Technical University (blue).

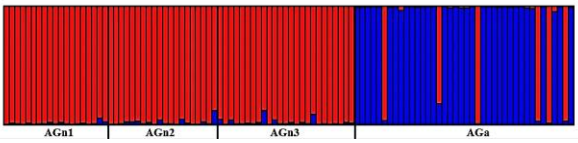

Figure 2: Clustering analysis of Russian sturgeon samples showing genetic groupings at two clusters (C=2).

Based on the data on the genetic polymorphism of the studied samples of Russian sturgeon and beluga, the following recommendations are given:

1. To preserve the genetic resources of *A. gueldenstaedtii*, it is recommended to use a sample from the confluence of the Ural River with the Caspian Sea and contained in RAS at the Ural-Atyrau Sturgeon Fish Hatchery (AGn1 and AgN3) with the highest indicators of genetic diversity (He=0.867; Ne=8.691 and He=0.871; Ne=8.268);
2. To compensate for the damage caused to reservoirs by fishing and human economic activity, it is recommended to release sturgeons from the rearing and breeding stock of the Ural-Atyrau Sturgeon Fish Hatchery, the Laboratory of Ichthyology and Aquaculture of Zhangir Khan West Kazakhstan Agriculture and Technical University, and UNKOPPA into the Ural River.

One of the main goals of genotyping and studying the genetic diversity of rare species of fish and sturgeons is to preserve the gene pool and prevent its depletion. The selection of parental pairs and the development of sturgeon crossing schemes becomes an important task. The data allows us to determine the degree of kinship of specimens based on microsatellite analysis data. The general sample of sturgeons is characterized by high He (He=0.750) and Ne values (Ne=5.676).

The indicators of genetic diversity in Russian sturgeon are higher in the groups AGn1 (He=0.867; Ne=8.691) and Agn3 (He=0.871; Ne=8.268), *A. gueldenstaedtii* from the Laboratory of Ichthyology and Aquaculture of Zhangir Khan West Kazakhstan Agriculture and Technical University caught at the confluence of the Ural River with the Caspian Sea, and lower values are observed in the sample from the Laboratory of Ichthyology and Aquaculture of Zhangir Khan West Kazakhstan Agriculture and Technical University AGa (He=0.829; Ne=5.980).

In beluga, the indicators of genetic diversity are higher in the group from the Laboratory of Ichthyology and Aquaculture of Zhangir Khan West Kazakhstan Agriculture and Technical University (He=0.663; Ne=3.238), and lower in the group from UNKOPPA (He=0.554; Ne=2.704).

In our case, with a maximum degree of kinship equal to 1, 100% of alleles in both specimens will be repeated and, conversely, if both specimens do not have the same alleles, the degree of kinship will be 0. Thus, when selecting parental pairs in RAS, it is recommended to identify (chip), as well as genotyping each specimen, and select pairs with the lowest total number of alleles. The maximum permissible value of the degree of kinship can be set at 0.25. However, it is necessary to separately consider the situation with the state of the gene pool and the number and type of genetic markers studied in each population and strive for lower values of the degree of kinship when developing crossing schemes.

Discussion

The study of genetic polymorphism was carried out using microsatellite markers, the effectiveness of which for sturgeon has been shown in many works [23,44-47].

Our findings unveiled notable differences in the genetic diversity between natural and artificial populations of sturgeon species (*Acipenser gueldenstaedtii* and *Huso huso*). The data we have obtained are consistent with the opinion of the authors [52], the presence of hexaploid individuals in *A. gueldenstaedtii*, low I index value for natural beluga populations, lower *Ho* values in Russian sturgeon compared to Beluga specimens which showed higher *Ho* values. Our data displayed that *AoxD161* locus, *Afug135*, and *AoxD165* from the artificial population of *Hh* displayed a higher frequency, which is in direct contrast to the data from these authors [52]. As in our previous work, a hexasomal distribution of alleles was found at the LS19 locus in four *A. gueldenstaedtii* specimens caught from the natural environment [48].

The data we obtained were slightly consistent with the opinion of the authors [53], who studied the six microsatellite loci of which *AoxD16*, *AoxD165*, and *AfuG41* aligned with ours and revealed that belugas showed higher *He* values at *AoxD161* and *AfuG41* loci than those observed. In our research, *He* values in *AfuG41* loci were consistently higher in every *Hh* population but *AoxD161* was significantly low.

The highest genetic distance was recorded in *Hhn*, which is consistent with the results of the authors [52]; the low genetic distance among *AG* specimens aligns with these authors [54], and our findings on the clustering of the conclusion that values of C or K > 3 were uninformative was confirmed by [52]. Inbreeding within artificial populations may occur due to the absence of heterozygotes in several loci, which agrees with the work reported by Roques et al. (4) on *Acipenser sturio*. Thus, annual monitoring and broadening the sturgeon species range are necessary for preventing the formation of such related pairs of parents. These actions are consistent with proposals made by previous studies [55].

A. Dudu et al. [49] conducted a study of 33 samples of belugas caught in the Danube River in different years (2001-2007). The analysis was carried out on seven microsatellite loci, of which only one coincides with the loci used by us (LS19). The number of alleles in this locus was nine, whereas in ours, in each of the three samples, it did not exceed five for each sample and seven for the total number of belugas studied. R. Matache [50] focuses on the genotyping of 29 beluga whales caught in the Caspian Sea, where 10 alleles were found at the LS19 locus.

N.A. Nebesikhina et al. [51] analyzed 439 brood fish of Russian sturgeon from the repair and breeding stock of the Azdonrybvod and 167 specimens caught during the spawning period in the Sea of Azov. The study was conducted on five STR markers (An20, Afug41, Afug51, AoxD161, AoxD165), the number of alleles at matching loci was not much lower than the values we obtained, except the AGa sample; the Ho and He indicators were significantly higher than ours and approached 1. According to Nebesikhina et al., the indicators of genetic diversity of samples from the natural environment were lower than the corresponding values for samples from artificial populations, although the opposite was observed in our work.

From our study, we obtained data that suggest species with high mean Ne and He and low genetic distance (*AGn1, AGn3*) should be implemented in RAS to increase genetic diversity, although, It is believed that RAS systems might mitigate inbreeding; the data received from our study suggest otherwise, and this may result from inadequate inbreeding or mismanagement. This finding calls for further research, a reassessment of the current RAS system, and more stringent monitoring

The obtained data on the genetic polymorphism of natural groups and those grown in RAS can be used to preserve the gene pools of Russian sturgeon and beluga typical for a region. The Ho and F values indicate a deficiency of heterozygotes in most of the studied samples as a result of closely related interbreeding within populations, which indicates a decrease in genetic diversity and a shortage of brood fish. This work should be carried out annually, covering a larger number of specimens and species of sturgeon, to monitor the state of indicators of genetic diversity, develop crossing schemes, and prevent the formation of parental pairs with a high level of genetic kinship.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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