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Genetic structure of Amur carp selected in «Karpatskyi vodohrai» Ltd.

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The purpose. To study genetic structure of breed herd of Amur carp selected in «Karpatskyi vodohrai» Ltd. (Lviv oblast) with the use of different types of markers and to determine the level of somatic mutagenesis by the microkernel test. **Methods.** Laboratory researches, computer statistical analysis. **Results.** Genetic structure of Amur carp on separate types of molecular-genetic markers (DNA-markers, genetic-biochemical systems) is studied. Species-specific features of genetic structure on probed loci are determined. The level of actual and expected heterozygosity is calculated. Increased allele and genotypic variety of genetic structure can be caused by a little heightened intensity of the carried out selection work. The detected excess of heterozygotes on separate loci testifies to presence of stabilization processes of genetic structure. Research of the level of somatic mutagenesis is also carried out at use of micronucleus test. **Conclusions.** At use of different types of molecular-genetic markers the information on genetic structure of a carp and its variety on genome level is gained. These results testify to the following: genome preserved resistant gene complexes, despite of significant selection effort. The applied genetically-statistical approaches can be used for monitoring genetic structure of groups of a carp of Ukraine, determination of the level of consolidation and phylogenetic links between them with their subsequent genetic certification. Results of researches of the level of somatic mutagenesis at use of micronucleus test shown that the probed group of fishes was characterized by average level of erythrocytes with micronucleus MNE (4,7±0,3‰), low level of lymphocytes with micronucleus and binucleate lymphocytes which total has made 3,5±0,3‰ that in its turn testifies to satisfactory conditions of selection.

Key words: *Amur carp, genetic structure, DNA-marker, genetic-biochemical marker, polymorphy, micronucleus test.*

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INTRODUCTION. The history of the introduction of the Amur wild carp in Ukraine began in 1954. The interest to Amur wild carp is associated with its higher cold-resistance, disease resistance, rapid growth rate and overall survival compared to the bark, which causes a general increase in fish productivity of ponds by 18-20% when cultivating carp - wild carp hybrids, which has a significant heterosis effect. Effective management of fish breeding in the territory of Ukraine involves the use of a number of breeding and breeding methods to improve the productivity of grown fish. An important component of the breeding process and the provision of fish farming to pure-breeding highly productive breeding material is a continuous genetic monitoring of reproductive potential of the population, which allows it to control its condition and make adjustments. Identification of genotypes by individual genes provides opportunities for studying the dynamic changes in the frequencies of alleles in the breeding process, which allows for the control of breeding work [1].

Despite the large number of methods for marking the variability of genetic material, genetic-biochemical systems are the most widely used for solving genetic selection problems in carnivores, and polymorphism analysis of various DNA regions and nucleotide sequences of DNA (DNA markers) [2].

The study of the genetic structure of the Amur wild carp populations on the territory of Ukraine is also important from the standpoint of population genetics, since they provide an opportunity to investigate the

mechanisms and level of changes at the genetic level of the Amur wild carp in the process of acclimatization.

The purpose of the research - the purpose of our work was to study the genetic structure of the Amur wild carp of “Karpatskiy vodograi” Ltd. using different types of markers and to study the level of somatic mutagenesis using the micronucleus test.

Materials and methods. As a material for research, blood samples from the caudal vein of the Amur wild carp (*Cyprinus carpio haematopterus*), “Karpatskiy vodograi” Ltd (n = 31 individuals) were used. The total DNA was isolated according to the standard method for using Gene JET Whole Blood Genomik DNA Purification Mini Kit (USA). The DNA concentration was determined on an Eppendorf Bio Photometr. Three microsatellite markers were used to study the genetic structure of Amur wild carp: MFW 06, MFW 15, MFW 23 [3].

Table 1 Nucleotide sequences of primers

Microsatellite locus	**Primers 5'-3'**	**The temperature of the annealing of the primers (°C)**
MFW 15	F: CTCCTGTTTTGTTTTGTGAAA R: GTTCACAAGGTCATTTCCAGC	54
MFW 23	F:GTATAATTGGGAGTTTTAGGG R:CAGGTTTATCTCCCTTCTAG	55
MFW06	F: ACCTGATCAATCCCTGGCTC R:TTGGGACTTTTAAATCACGTTG	55

PCR was performed on the "Termo scientific" amplifier (Arktik Termal Cycler, USA) in the following temperature mode: 5 minutes at 94 C; 33 cycles: 1 min for 94 C, 30 seconds for 53-55 C (depending on the locus), 30 seconds for 72 C; 5 min for 72 C. A 25 μl reaction mixture contained: 67 mM Tris-HCl (pH 8.8), 17 mM (NH4) 2SO4, 0.01% Tween-20, 0.2 mmol dNTP, 1 unit Tag-polymerase, 50 ng DNA, 1.7 mmol $MgCl_2$ and 0.2 μm primers. Electrophoretic separation of amplification products was carried out in 4% agarose gel using 1 × TAE buffer. Treatment and analysis of gels were performed using the Totallab v2.01 program. Statistical processing of the results was performed in the GelStat program.

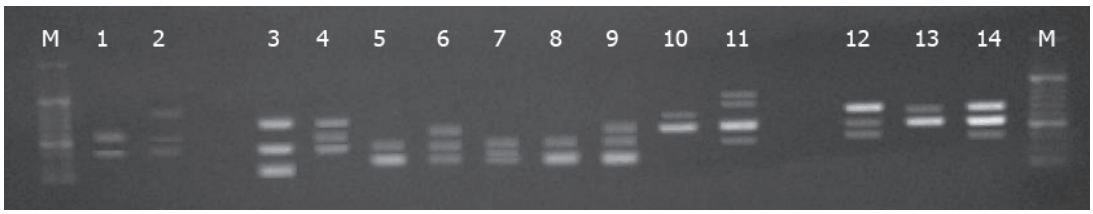
It has been analyzed the studied populations by the genetic-biochemical markers using the distribution of allelic and genotypic frequencies by transferin (Tf), esterase (Est) and albumin (Alb), malate dehydrogenase (MDH), malic-enzyme (ME), carboxyangidase CA), Electrophoretic distribution of proteins was carried out in polyacrylamide 9% gel followed by histochemical staining [4]. Mathematical processing of data was performed using the computer program "BIOSYS" [5]. The deviation of the actual frequencies from the theoretically expected from the Hardy-Weinberg ratio was calculated using Pearson's criterion [6]. The critical value of $\chi 2$ was taken for a 5 % significance level.

In this work it has been investigated the level of cytogenetic indicators using the micronucleus test of peripheral blood cells. In blood smears, the frequency of erythrocytes with micronuclei (EMN), binuclear lymphocytes (BNL), lymphocytes with micronuclei (LMN), apoptosis were calculated. Obtained results were expressed as ppm (‰). Slides were analyzed using the binocular light microscope (Primo Star Zeiss, 100×1.25). The cells were photographed with a Canon (PowerShot G6) camera. The statistical significance of differences in the frequencies of cytogenetic anomalies between animal groups was assessed according to the Student's criterion (tS) [7].

Results. *DNA markers*. The genotypes of the Amur wild carp specimens were analyzed using three microsatellite DNA loci: MFW 06, MFW 15, MFW 23. In the course of the work, the optimal conditions for SSR-PCR analysis were selected. The studies allowed to determine the factors that have the greatest impact on the amplification of SSR alleles of the Amur wild carp namely, the concentration of the DNA preparation, the concentration of the primer in the reaction mixture and the number of amplification

cycles. To obtain clear and reproducible alleles for each locus, the optimal conditions for performing the PCR were individually selected. Examples of the received SSR spectra are shown below (Fig. 1)

A



B

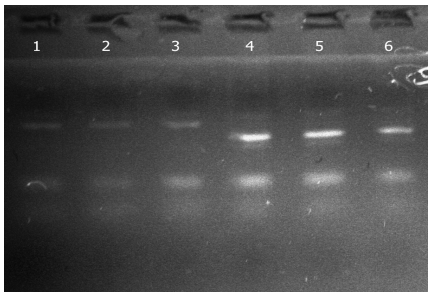


Fig. 1. Electrophoretic distribution of SSR-PCR amplification products for loci MFW 15 (A 10-14), MFW 06 (A 1-9), MFW 23 (B 1-6), M - molecular weight marker.

In the studied group of genotypes for 3 microsatellite loci, only 16 alleles with a molecular weight of 90 p.n. were detected. - 280 p.n. The number of alleles per locus ranged from 5 to 6. The most polymorphous was the MFW 15 marker (6 allelic variants were detected), and 5 alleles were detected for the use of markers MFW 23 and MFW 06 (Table 2).

Table 2 Observed allele frequencies in Amur wild carp species of “Karpatskiy vodograi” Ltd.

Locus		**Frequency, %**
MFW 15	230	21,87
	245	12,49
	255	6,265
	260	31,25
	272	15,63
	280	12,49
MFW 23	90	17,24
	110	24,14
	122	10,35
	140	13,79
	145	34,48
MFW 06	120	20,00
	140	20,00
	150	33,33
	165	10,00
	180	16,66

The allelic variants with a length of 260 pairs of nucleotides (pn) were found at the MFW 15 with the highest frequency of 31, 25% (found in 83% of individuals), and with the lowest frequency of 6.27%, allelic variants of 255 p.n. , which was detected in 17% of the examined individuals. The use of the MFW marker 23 revealed that the allele length of 145 p.n. met with the highest frequency - 34.48% (found in

83% of individuals), 110 p.n with a frequency of 24.14%, 90 pp - 17.24%, 140 pp - 13.79% and allelic version with length of 122 p.n. found with the lowest frequency - 10.35% (determined in 25% of individuals). As a result of research on the use of a microsatellite loop MFW 6, 5 allelic variants with a molecular weight of 120-180 p.n. With the highest frequency (33.3%), the allele was 150 p.n. (found in 80% of individuals), and with the lowest (10%) - allelic variant of 165 p.n. (determined in 25% of the studied individuals). According to the calculations of allele frequencies, the main indicators of genetic variability were determined (Table 3).

Таблиця 3 Characteristics of microsatellite DNA loci

Locus	Alelles rang size	n_a	n_e	Ho	He	F
MFW 15	230-280	6	4,566	0,706	0,781	0,096
MFW 23	90-145	5	4,237	0,773	0,764	-0,011
MFW 06	120-180	5	4,545	0,704	0,780	0,097
Середнє	-	5,33	4,45	0,728	0,775	0,061

n_a – number of alleles, n_e – the effective number of alleles;

Ho – observed heterozygosity, He – expected heterozygosity; F - average inbreeding rate.

Indicators of expected heterozygosity were used to calculate the inbreeding rate, which expresses the degree of inbreeding in the population. The highest level of available heterozygosity was recorded for the MFW 23 locus (77.3%), the lowest for the MFW 06 locus (70.4%). Estimated values of the expected (He) heterozygosity were generally higher than the observed value (Ho). The greatest discrepancy between the observed heterozygosity (Ho) and the expected (He) heterozygosity is evidenced by its deficiency, which was characteristic of the investigated population of Amur wild carp for the use of the microsatellite locus MFW 06. The actual increase in homozygosity in this locus may be due to many factors, including the selection of individuals for specific productive qualities, as well as the possibility of the appearance of zero alleles, but this assumption requires a series of more extensive research.

The effective number of alleles (an indicator that characterizes the locus by frequency of occurrence of alleles) in the studied sample of genotypes ranged from 4.23 (MFW 23) to 4.57 (MFW 15). The average effective number of alleles per locus was 4.45. The calculation of the index of inbreeding F of individuals relative to the population showed a slight excess of heterozygotes by the MFW 23 locus (F = -0.011). The average inbreeding rate (F) was 0.061, indicating no inbreeding in the investigated population (Table 3).

Genetic-biochemical systems. As a result of research on the genetic structure of Amur wild carp, genetic and biochemical systems revealed five allelic forms according to the transferrin locus: TfA, TfB, TfC1, TfC2, TfD (Table 4). The most common genotypes are those that include the TfC1, TfC2 alleles. A comparison of the actual and theoretical frequencies of genotypes revealed the presence of a small excess of heterozygotes. In pedigree tribe, the frequency of the Tf A allele was the highest and was 0.417. The lowest frequency was the allele Tf V (0.050). The analysis of the correspondence of the actual distribution with respect to the Hardy-Weinberg Law on the transferrin locus has shown that the actual heterozygosity in the population (He = 0,900) is higher than expected (H0 = 0,733).

Some researchers note the presence of high level of the Tf D allele in the Far Eastern Sasan (q = 0.64), whereas in European populations of carp and carp, this allele is found at a low frequency [8, 9, 10]. In the group of zealous farms of the “Karpatskiy vodograi” Ltd., we also detected this allele of transferrin that encountered a frequency of 0.150 and the frequency of occurrence is characteristic of European populations of Amur wild carp, where as for the Far Eastern population there is a predominance of the allele D. The revealed allelic variants have created 13 Combinations of genotypes out of 15 possible. The genotypes of C2C2, C2D have not been revealed at all, and in the vast majority there are genotypes AC1C2 and AD. The available homozygosity for the transferrin locus was 25%, and the available heterozygosity, respectively, was 75%.

European populations of Amur wild carp are characterized by a high (64%) frequency of allele A [10]. The frequency of the slow allele D varied depending on the population under study in the range of 10-15.5%. The proportion of the allele B in the populations we investigated was in the range of 10-20.7%, which is characteristic of European populations of safflowers [11].

Table 4 **Frequencies of alleles of polimorphic loci of these genotypes in the population of the Amur wild carp of "Karpatskiy vodograi" Ltd.**

Locus	Allele	Frequency	Genotype	Number of genotypes		χ 2	P
				observed	expected		
TF	A	0,417	AA	3	5.085	7	74.9
			AB	2	1.271		
			AC_1	8	6.356		
			AC_2	4	3.390		
			AD	5	3.814		
	B	0,050	BB	0	0.051		
			BC_1	1	0.763		
			BC_2	0	0.407		
			BD	0	0.458		
	C_1	0,250	C_1C_1	3	2.034		
			C_1C_2	3	2.288		
			C_1D	0	0.475		
	C_2	0,133	C_2C_2	0	0.470		
			C_2D	0	0.385		
	D	0,150	DD	1	1.220		
				H_0 = 0,733	**He** = 0,900		
EST	F	0,450	FF	5	5.949	49	48.4
	S	0,550	FS	17	15.102		
			SS	8	8.949		
				H_0 = 0,503	**He** = 0,567		
ALB	A	0,550	AA	6	8.949	5	3.0
			AB	21	15.102		
	B	0,450	BB	3	5.949		
				H_0 = 0,503	**He** = 0,700		
MDH	F	0,683	FF	12	14	6	12,8
			FS	17	13		
	S	0,317	SS	1	3		
				H_0 = 0,690	**He** = 0,495		
ME	F	0,533	FF	6	8,5	6	21,3
			FS	20	15		
	S	0,467	SS	4	6,5		
				H_0 = 0,862	**He** = 0,500		
CA	F	0,666	FF	11	13,3	6	8,14
			FS	18	13,3		
	S	0,334	SS	1	3,3		
				H_0 = 0,552	**He** = 0,437		

Ho – observed heterozygosity, He – expected heterozygosity

In population-genetic studies, the Est-1 locus is polymorphic and is represented by three genotypes - FF, FS, and SS. Two zones of esterase were found: F is fast and S is slow. Frequency of occurrence of allelic variant F - 0,450, S - 0,550. The observed statistically significant excess of heterozygotes (17; 15) by the EST locus (χ 2 = 0.491, p = 0.448) is observed. By the Est loci, the EstS frequency was prevalent. An unbalanced state of the esterase locus was observed in the population, since there is a statistically significant surplus of heterozygotes according to the Hardy-Weinberg law.

According to the locus of albumin, in the carp, like in most other types of fish [8,10,11], two alleles A and B. The prevalence of the allele variant A was 0.550. The most in equilibrium state are the allele frequencies behind the albumin loci. According to this locus, there was an excess of heterozygote AB (21; 15).

The results of MDH locus studies revealed two electrophoretic variants of this malate dehydrogenase enzyme: F - and S - forms. The MDH-F frequency (Table 4) was prevalent in the studied herd and a balanced state was observed for this locus, as there are no statistically significant differences in the actual distribution of genotypes from the expected Hardy-Weinberg distribution. Although there is a tendency towards the excess of actual heterozygous genotypes above the theoretically expected ones in the Amur carp in this locus.

We detected two allelic forms of malignant-enzyme: ME-S and ME-F. In the distribution of allele frequencies (Table 4), the rapid population (0.533) prevailed in the population under study. Distribution of genotypes by locus of ME revealed a slight tendency to excess heterozygotes, the value of actual heterozygosity was 0.862.

Locus carbonic anhydrase in the study group Amur carp was polymorphic and contained the gene of "fast" (F) and "slow" (S) allelic variants of the enzyme (Table 4). Since the distribution of actual genotypes was not statistically different from the theoretical one, it can be argued that the eagle population is balanced by this locus.

In assessing the dynamics of the genetic status of the population, an important parameter is heterozygosity (H). The mutation process, the different types of selection, the drift of genes and other factors of population dynamics often affect the heterozygosity of the population, especially in a limited flow of genes, and therefore its evaluation is a prerequisite for population research. By the magnitude of the growth of heterozygosity in the high polymorphic loci, one can make an assessment of the effective size of the parent population, or the optimal quantitative ratio of females and males in the population and its magnitude [9,12]. Of the six studied loci, the largest difference between the observed heterozygosity and the expected value was found in the Alb, ME, TF locus (Table 5).

Table 5 **The average heterozygoticity for the delivery of genetic-biochemical systems**

Locus /H	**TF**	**EST**	**MDH**	**ME**	**ALB**	**CA**	**For all loci**
H_e	0,900	0,567	0,495	0,500	0,700	0,437	0,599±0,033
H_o	0,733	0,503	0,690	0,862	0,503	0,552	0,640±0,016

Ho – observed heterozygosity, He – expected heterozygosity

The differences between the observed and the expected heterozygosity indicate an incident in the growth of the frequencies of the individual alleles in the ancestors, due to the inadequate level of exchange of genes between the subpopulations of the Amur wild carp due to the effect of isolation on them. The analysis of the correspondence of the actual distribution with respect to the Hardy-Weinberg distribution of the albumin locus revealed that the expected heterozygosity in the population (He = 0,700) is higher than the observed (H0 = 0,503).

According to some authors, the increase in the frequency of the occurrence of certain alleles and the decrease in the frequency of others is possible in the course of artificial selection on any fishing grounds and depends on the conditions of fish retention [12, 13].

Cytogenetic analysis. Many studies have been shown that the hematopoietic system of fish is a very sensitive to changes in the state of the aquatic environment. Therefore the observing the morphologically changing of blood cells allows as to give a quick answer about the sensitivity of farmed fish to the action of genotoxins. The level of chromosomal aberrations and genomic mutations in fish peripheral blood cells directly depends not only on the ecological conductions of the reservoir, but also on the species of investigated aquaculture representatives [14, 15, 16]. In their studies, Fergh and El Shahew for the use of the micronucleus test on four fish species showed different values of chromosomal aberrations [17].

Smears of blood in the nucleus of erythrocyte are small in compact nuclei In blood smears nuclear erythrocytes had small and compact ovals nuclei with a with a good visualized cytoplasm and characterized by a longitudinal diameter of 12.4 to 17.8 μm, and transverse from 7.1 to 10.2 μm. This feature made it possible to easily differentiate them and to calculate micronuclei separately for each cell group. Also, binuclear leukocytes BNL, which are somewhat elevated in peripheral blood cells, reflect the disturbances in the final stage of mitotic division - cytokinesis, apoptotic cells, and "amyotrophic" red blood cells, that is, erythrocytes in the state of division.

Monitoring studies of the level of somatic mutagenesis among representatives of Amur wild carp for micronucleus test were performed. The results of cytogenetic analysis showed that the study group was characterized by an average level of erythrocytes with micronuclei EMN (4.7 ± 0.3 ‰). In addition, we observed that these fishes are characterized by a relatively low level of lymphocytes micronuclei (Fig. 1) and dual-core lymphocytes, the total number of which was (3.5 ± 0.3 ‰), which indicates satisfactory conditions of breeding.

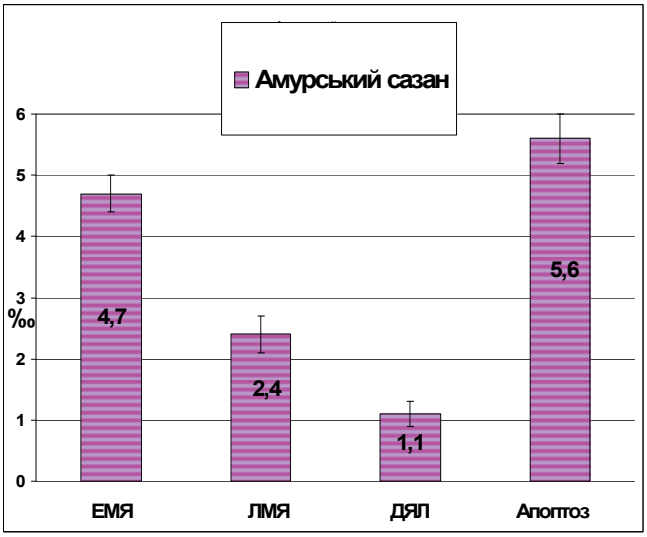


Figure 1. The level of cytogenetic indices in the peripheral blood cells of the Amur wild carp of “Karpatskiy vodograi” Ltd

The increased level of apoptosis (5,6 ± 0,4‰) in the study of Amur wild carp in spring may be the result of the elimination of mutant lymphocytes and erythrocytes.

Conclusions.

Thus, using various types of molecular genetic markers (DNA markers, genetic and biochemical systems), information was obtained on the genetic structure of the carp and its diversity at the genomic level. These results indicate that in her genome persistent gene complexes, despite in-vitro hybridization, have been preserved.

The study of the level of somatic mutagenesis for using the micronucleus test was conducted. The study group was characterized by an average level of erythrocytes with micronuclei EMN (4.7 ± 0.3 ‰), low levels of lymphocytes with micronuclei and dual-core lymphocytes, the total number of which was (3.5 ± 0.3 ‰), indicating satisfactory conditions for breeding.

Bibliography

1. *Hrytsyniak I.I.* (2010). Naukove zabezpechennia rozvytku akvakultury ta pidvyshchennia efektyvnosti vykorystannia vodnykh bioresursiv vnutrishnikh vodoim Ukrainy. [Scientific support for the development of aquaculture and enhancement of the use of water resources of the internal waters of Ukraine]. *Rybohospodarska nauka Ukrainy.* 1:4–13. [in Ukrainian].

2. *Tarasiuk S.I., Hrytsyniak I.I.* (2013). Molekuliarno-henetychni metody v rybnytstvi: monohrafiia. [Molecular genetic methods in fish culture: monograph]. Kyiv: Ahrarna nauka. 312. [in Ukrainian].

3. *Crooijmans R., Bierbooms V., Komen J.* et al. (1997). Mikrosatellite markers in common carp *(Cyprinus carpio* L.). *Animal Genetics.* 28:129–134.

4. *Glik B., Pasternak Dzh.* (2002). Molekulyarnaya biotehnologiya: principy i primenenie. [Molecular Biotechnology: Principles and Applications]. Moskva: Mir. 589. [in Russian].

5. *Swofford D.L., Selander R.B.* (1981). BIOSYS-1: a Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematic. *J. Heredity*. 72: 281–283.

6. *Zhivotovskij L.A.* (1991). Populyacionnaya biometriya. [Population biometrics]. Moskva: Nauka. 271. [in Russian].

7. *Plohinskij N.A.* (1970). Biometriya. [Biometrics]. Moskva: Izd-vo MGU, 367. [in Russian].

8. *Grytsiniak I., Nagornyuk T., Mariutsa A., Tarasjuk S.* (2013). Analysis of genetic structure of Ukrainian scaled and framed carps of the Antoninsky-Zozulenets. *Czasopism naukowych informujemy, że wydawane przez Instytutu Zootechniki czasopismo «Roczniki Naukowe Zootechniki»* — Balice. Poland. 40(2):145–153.

9. *Xiao T., Lu C., Xu Y.* et al. (2015). Screening of SSR markers associated with scale cover pattern and mapped to a genetic linkage map of common carp (*Cyprinus carpio* L.). *J. Appl Genet*.. May. 56(2): 269. doi: 10.1007/s13353-014-0250-9. Epub 2014 Oct 23.

10. *Kras S.I., Horodna O.V., Tarasiuk S.I.* (2010). Henetychna dyferentsiatsiia populiatsii amurskoho sazana VAT «Sumyrybhosp» ta VAT «Donrybkombinat». [Genetic differentiation of populations of Amur salmon of OJSC "Sumiribhosp" and OJSC "Donbribcombinat"]. *Naukovyi visnyk Lvivskoho natsionalnoho universytetu veterynar-noi medytsyny i biotekhnolohii imeni S. Z. Hzhytskoho*. No 12. 3(45). Part. 3. 59–65. [in Ukrainian].

11. *Kras S.I., Stovbinskyi V.I., Bodnar H.I., Tarasiuk S.I.* (2011). Osoblyvosti henetychnoi struktury amurskoho sazana. [Features of the genetic structure of Amur carp]. *Rybohospodarska nauka Ukrainy.* 1:62–67. [in Ukrainian].

12. *Fallahbagheri F., Pourkazemi M., Dorafshan S.* (2013). Genetic analysis of wild common carp, *Cyprinus carpio* L. in the Anzali wetland, the Caspian Sea. *Iranian J. of Fisheries Sciences.* 12(1):1–11.

13. *Kras S.I., Tarasiuk S.I., Mariutsa A.E.* (2011). Henetychna kharakterystyka stada amurskoho sazana (*Cyprinus carpio haematopterus*) rybohospodarstva «Lisnevychi». [Genetic characteristic of the Amur carp (*Cyprinus carpio haematopterus*) fish farm "Lisnevichi"]. *Naukovyi visnyk LNUVMBT imeni S.Z. Gzhytskoho.* № 4 (50). T. 13. Part. 3. 155–160. [in Ukrainian].

14. *Torres de Lemos C., Milan Rödel P., Regina Terra N.* et al. (2007). River water genotoxicity evaluation using micronucleus assay in fish erythrocytes. *Ecotoxicology and Environmental Safety*. 66(3):391–401.

15. *Kamel Ahmad, Jaber Salehl.* (2010). Clastogenic studies on Tandaha Dam water in Asser. *Mediterranean Environment*. 16(1):33–42.

16. *Grytsiniak I., Glushko Y., Tarasjuk S*. (2013). Cyto-genetic profile of the Ukrainian carp. *Czasopism naukowych informujemy, że wydawane przez Instytutu Zootechniki czasopismo «Roczniki Naukowe Zootechniki»* — Balice. Poland, 40(1):45–53.

17. *Galickij V.A.* (2008). Vozniknovenie eukarioticheskih kletok i proishozhdenie apoptoza. Citologiya. [The origin of eukaryotic cells and the origin of apoptosis. Cytology]. 47(2):103–120. [in Russian].