Assessment of the Impact of Integrated Pollution on the Monitoring of Biodiversity in Fisheries

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Abstract. Results are presented of the comparative analysis of frequency of erythroid cells with micronuclei and "tailed" nuclei and of the interphase nuclei indices of red blood cells in the peripheral blood of bester, a sturgeon hybrid. The differences are found in the frequency of occurrence of abnormal red blood cells between the two groups of fish kept in cages in natural water (river) and in ponds with water supplied through a sedimentation basin. Despite these differences, the number of erythrocytes with chromosomal abnormalities in the bester of both groups is close to normal. However, the number of erythroid cells with micronuclei and "tailed nuclei" prevailed in the fish held in river cages. It was suggested that comparative cytogenetic monitoring of fish can be used not only to characterize the environment of water bodies, but also promote the rational organization of artificial reproduction of aquatic organisms that would contribute to the conservation of biodiversity of aquatic biological resources.

1. Introduction

The problem of biodiversity conservation of organisms living in water, including fish, becomes especially acute in connection with the growing economic activity of people, for instance, hydro-construction and navigation, development of hydrocarbon reserves, intensification of agriculture and industrial production. Human activities cause contamination of aquatic biota, which significantly affects the state of fish populations and their biodiversity.

The system of controlling the influence of pollution on aquatic biota, which exists at the present time, is based on the detection by analytical methods of maximum permissible concentrations of certain substances and sanitary and hygienic indicators, which does not provide objectivity in assessing the ecological well-being of water bodies [1]. High levels of chromosomal aberrations in erythrocytes of peripheral blood of fish are noted only in the zone of intense contamination when drilling wells.

This necessitates integral methods of monitoring the state of hydrobionts, which will allow us to assess the prospects of fisheries and the development of aquaculture. One such method is a micronuclear test, a highly sensitive indicator of toxic effects on living organisms. It characterizes chromosomal instability, the index of which determines the frequency of occurrence of micronuclei and "tailed" nuclei of cells [2, 3, 4].

Pollutants are biologically active substances that accumulate in aqueous organisms, disrupt the work of enzyme systems, lipid structure of cells, processes of maturation of sexual products, fertilization, development and other functions of the body. Therefore, an important task for the
conservation of biodiversity of water bodies is to conduct research and introduce into practice environmental tests designed to determine the influence of external factors on the functional state of aquatic organisms.

2. Material and methods of research

Bester specimens for research were sampled from the broodstock held in cages installed in the river and from the fish in the pond, water supply to which was carried out through a settling pond.

Blood samples were obtained from the dorsal artery. Blood specimens were prepared by the conventional method [5]. The smears obtained were air-dried and fixed with 96% ethyl alcohol. Blood smears of fish were stained according to Romanovsky – Giemsa method. Additionally, staining with the fluorochrome DAPI was applied, the specimens stained were put under a defatted cover glass for 20 minutes. After rinsing with distilled water, PBS was used. The ready specimens were covered with a defatted cover glass and sealed with rubber glue. The specimens were kept in the dark and cool place.

Detection of nuclei abnormalities in erythrocytes. Nuclei abnormalities in blood erythrocytes, namely, micronuclei and "tailed" nuclei, were evaluated on hematological specimens using an Olympus microscope (Japan) equipped with an oil-immersion objective (100x magnification). Specimens stained with DAPI fluorochrome were examined with a green filter, and the micronuclei that were conspicuous in the field of view were detected. The frequencies of occurrence of cells with micronuclei and with "tailed" nuclei were determined in samples of 10 000 erythrocytes and expressed in ppm (‰).

Detection of fibrillar centers in interphase cells. Specimens intended for the detection of fibrillar centers were prepared according to N.N. Mamayev et al. [6]. The blood smear was fixed with a mixture of glacial acetic acid and ethyl alcohol in a ratio of 1:3 for 15 minutes. Then the specimen was rinsed with distilled water and after drying the silver staining method was applied. With this technique, proteins were removed from the specimen, creating a peculiar background later. Calculation of the index of fibrillar centers was carried out according to the following formula:

\[ I_f = \frac{\sum FC}{n} \]

where

- \( I_f \) - index of fibrillar centers;
- \( \sum FC \) - the sum of the fibrillar centers found in the cells;
- \( n \) is the number of cells analyzed.

3. Results

As a result of the study of hematological preparations, the Howell-Jolly bodies (Figure 1) and the "tailed" erythrocytes were identified (Figure 2).

**Figure 1.** Micronucleus in the erythrocyte. Coloring with a fluorescent dye. O6.100x; ŧk.10x.

**Figure 2.** "Tailed" nucleus in the erythrocyte. Coloring with a fluorescent dye. 100x magnification 10x lens.
Micronuclei are the remains of the nuclear (chromatin) substance formed as a result of degradation of erythrocytes nuclei. They were rounded, chromatin-containing formations, 1.5-2 microns in size, mostly one in a cell.

Abnormalities of nuclei of the "tail" type looked like thin outgrowths - "tails" branching off the nucleus, no more than one per nucleus. The length of a "tail" was 1.5-2.0 μm, 0.1 μm thick. In some cases, the "tail" ended by the terminal expansion of the body in the form of a teardrop.

Types of the revealed nuclear abnormalities corresponded to the accepted morphological criteria of micronuclei [7] and "tailed nuclei" in erythrocytes [8].

The degree of genome stability is determined by the number of interphase nucleoli and fibrillar centers of ribosomal cystrons in erythrocytes (Figure 3).

In the process of pre-ribosome synthesis, nucleolus formation occurs only in active nucleolus-forming regions of chromosomes. According to H.G. Schwarzacher and R.B Everson [9], a dense fibrillar component is the central structure of the nucleolus, reflecting the r-DNA transcription and r-RNA processing.

Comparative micronucleus analysis of spontaneous chromosomal instability has revealed some significant differences in the frequencies of occurrence of micronuclei and "tailed" nuclei in the better from different habitats (Table 1).

Table 1. Frequency of occurrence of erythrocyte nuclear abnormalities in the blood of better, %.

| Place of sampling | Frequency of occurrence of micronuclei in erythrocytes | Frequency of erythrocytes with the "tailed" nuclei |
|-------------------|------------------------------------------------------|--------------------------------------------------|
|                   | X ± m      | Cv    | X ± m    | Cv     |
| Ponds             | 0.93±0.10 | 47.3  | 0.22±0.03 | 56.2   |
| Cages             | 1.54±0.23 | 52.2  | 0.32±0.08 | 64.7   |

According to the results of our studies presented in Table 1, the frequency of micronuclei occurrence in the red blood cells of the better raised in ponds is 35% lower than in the fish from cages in the river, and the frequency of occurrence of erythrocytes with "tailed" nuclei is by 30% lower in the fish from ponds.

Results of comparative analysis of interphase nucleoli of ribosomal cystrons in better blood are presented in Table 2.

Table 2. Index of interphase nucleoli in red blood cells of better.

| Place of sampling | IINEa | Range       | Cv  |
|-------------------|-------|-------------|-----|
| Ponds             | 7.02±0.16 | 5.62-8.53 | 12.8|
| Cages             | 6.40±0.25 | 5.42-7.08 | 9.8 |

a Index of interphase nucleoli in erythrocytes
The index value of fibrillar centers in the bester from cages exceeded this parameter in fish from ponds by 8.8%, but without significant differences.

4. Discussion
The presence of abnormal erythrocytes indicates the onset of the development of pathological processes in the blood of the bester. Perhaps this is a reaction of the body to the complex influence of abiotic and biotic factors of the habitat. At the same time, the presence of a small number of micronuclei (0.93-1.54%) and "tailed" nuclei (0.22-0.32%) may be a manifestation of the compensatory-adaptive response of an organism to unfavorable stress factors [21, 22]. Moreover, our studies have shown differences in the degree of manifestation of pathological disorder in the bester blood, depending on the maintenance conditions.

Water supply of ponds through the system of water supply channels and a settling pond favorably influences the chemical composition of water due to more intensive self-cleaning processes. Improvement of habitat conditions contributed to a decrease in cytotoxic effects, in particular, to a smaller number of anomalies of the nuclei of erythrocytes. This was manifested in a significantly lower incidence of micronuclei and "tailed" nuclei of erythrocytes by 30% or more in pond fish. The influence of external factors, especially pollutants, on the level of chromosome mutations in fish erythrocytes is confirmed in the scientific literature [23-25].

According to G.M. Palatnikov et al. [26] there were 6.50 ± 0.5 micronuclei per 1,000 red blood cells in the peripheral blood of breeders of the stellate sturgeon Acipenser stellatus caught in the zone of intensive drilling of wells, while in that of the Russian sturgeon Acipenser gueldenstaedtii there were 8.25 ± 0.75 micronuclei. Among the breeders caught in the Lencoran conditionally clean zone, these parameters were the following: 1.75 ± 0.25 for Acipenser stellatus, 2.25 ± 0.5 micronuclei for Acipenser gueldenstaedtii. The authors believe that parameters of the Lenkoran breeders do not exceed the background mutation rates and are admissible in genotoxic relation. Being guided by these data, it is necessary to recognize that judging by the indices of frequency of chromosomal abnormal erythrocytes the fish of both groups studied were up to the norm.

As provided by the indices of interphase nucleoli in erythrocytes, the bester is characterized by a rather high transcriptional and proliferative activity. Probably, this is due to their ancient origin and, accordingly, high adaptive plasticity, which determines their stability and ability to rapid physiological changes in the body under the influence of constantly changing external factors.

Interphase nucleoli are formed by special sections of chromosomal DNA called nucleolus organizer regions or nucleoli of ribosomal cistrons in blood. These sites encode and synthesize ribosomal RNA [27]. The frequency of occurrence of morphological anomalies in the nuclei of interphase cells reflects the degree of occurrence of chromosomal mutations. The value of indices of interphase nucleoli of erythrocytes ranged over 5.42-8.53 testifies to sufficient stability of the genome in the bester under conditions in question.

5. Commentary
1. The micronucleus test is a highly sensitive index of genotoxic effects for a wide variety of abiotic environmental factors, even with minor changes in it.
2. Pathomorphological changes in erythrocytes caused by unfavorable conditions in the habitat are common to many aquatic animals, which is proved by literature data on applying micronuclear tests.
3. Numerous studies emphasize the informativeness and important prognostic value of micronuclear test data.
4. The informativeness and simplicity of micronuclear test methods make it possible to use them effectively for the rapid processing of large fish samples as quick tests for the fish monitoring of water bodies and to obtain an integral assessment of the toxic load.
5. According to literature data, since aberrations of chromosomes occur in all cells under the influence of this or that factor, a micronuclear test with germ cells can be used to predict the quality of fish progeny.
Comparative cytogenetic monitoring of fish can characterize not only the ecological situation of water bodies, but also contribute to the rational organization of artificial reproduction of hydrobionts and the development of measures aimed at improving water bodies. This will help to preserve the biodiversity of aquatic biological resources.

6. References

[1] Patin S A 1997 *Ecological problems of development of oil and gas resources of the sea shelf* (Moscow: VNIRO)

[2] Miroshnikov A B, Ogrel L Yu and Balyatinskaya L N 2000 *Ecology and Industry of Russia* Biotests p 36

[3] Bochkov N P, Demin Yu S and Luchnik N V 1972 Classification and methods of accounting for chromosome aberrations in somatic cells *Genetics* 8 (5) 133-41

[4] Proshin S N, Kravtsov V Yu, Olnev M G, Yakovlev A F and Vakhtin Yu B 1998 Chromosomal bridges and "tailed" nuclei in populations of malignant cells *Genetics* 34 (1) 61

[5] Arabadzhi A A, Kryukov V I 2006 *Proc. Conf. of Young Scientists and Specialists of Department of Biotechnology and Veterinary Medicine May 11-12, 2006 (Orel)* pp 6-10

[6] Grishanin A K, Stepunov V A, Pavlov D F and Chuiko G M 1993 Study of the mutagenic effect of cadmium dichloride and naphthalene on erythrocytes of Orechromis mossambicus P. by micronucleation method *Genetics* 29 (7) pp 1213-1317

[7] Grishanin A K 1996 *Biology of Inland Waterbodies* (Newsletter No 1) pp 94-96

[8] Yakovlev A F, Tyukachev M V, Kravtsov V Yu, Donskaya P V and Popova A A 1996 The frequency of erythrocytes with micronuclei in the peripheral blood of fish of the sturgeon family *40th Scientific and Technical Conf. of University Teachers (Astrakhan)* pp 60-61

[9] Hooftman R N, de Raat W K. 1982 Induction of nuclear anomalies (micronuclei) in the peripheral blood erythrocytes of the eastern mudminnow Unbra pygmaea by ethyl methanosulphonate *Mutat. Res.* 104 pp 147-53

[10] Weeks B A, Anderson D P, DuFour A P, Fairbrother A, Goven A J, Lahvis G P and Peters G 1989 Immunological biomarkers to assess environmental stress *Biomarkers. Keystone* 3 pp 521-25

[11] Vandermeulen J H 1990 Time and dose response of aryl hydrocarbon hydroxylase in fingerling trout *Salvelinus fontinalis* *Biochem. Physiol* 95C 169-175

[12] Petukhova, G A 2000 Ecological and genetic characteristics of the effect of oil pollution on plant and animal test objects *Proc. Int. Symp on Ecology June 14-16 2000 (Petersburg)* vol 2 pp 334

[13] Kryuchkov V N 2004 *Ekologo-morphological features of pathology and adaptation of organs and tissues of fish under the influence of toxicants* (Thesis for a Doctor’s degree Makhachkala) 46 pp

[14] Mamedov Ch A, Palatnikov G M, Mekhtiev A A, Kasimov R Yu 2008 *Proc. Conf. on Comprehensive Approach to the Problem of Conservation and Restoration of the Bioresources of the Caspian Basin (Astrakhan)* pp 371-375

[15] Akhmetova V V, Basina S B 2015 Evaluation of the morphological and biochemical picture of the blood of carp fishes raised in the Ulyanovsk region *Bulletin of the Ulyanovsk State Agricultural Academy* 3 (31) pp 55

[16] Ivanova N T 1983 *Atlas of blood cells of fish* (Moscow) 184 pp

[17] Mamaev N N, Kovalyeva O V, Amineva K K, Gudkova A Y, Maier Y B, Polykarpov I S, Schneider Y A, Proshin S N and Lebedev L V 1998 AgNORs in cardiomycocytes from surgical patients with coronary heart disease *Mol. Pathol.* 51 (4) pp 218-221

[18] Muller W U, Streffer C 1995 *Advances in mutagenesis research* ed G Obe / (Springer-Verlag) pp 1-134

[19] Prokofov-Belgovskaya A A 1961 Radiation damage to chromosomes in the early stages of salmon development *Tsitologiya* 3 (4) pp 437-45
[20] Schreinemachers D M, Everson R B 1991 Effect of residual splenic function and folate levels on the frequency of micronucleated red blood cells in splenectomized humans Mutat. Res 263 pp 63-67
[21] Zhiteneva L D, Poltavtseva T G and Rudnitskaya O A 1989 Atlas of normal and pathologically altered fish blood cells (Rostov-on-Don) 112 pp
[22] Kuzina T V 2011 Changes in the structure of the nucleus of erythrocytes of peripheral blood of commercial fish of the Volga-Caspian canal, Bulletin of Moscow State Regional University. Ser. Natural. Science 2 pp 50-57
[23] Zakhidov S T, Chebotareva Yu V, Savvaitova K A and Maksimov V A 1996 Cytogenetic study of haematopoietic fish cells from the Norilo-Pyasina water bodies (Taimyr) Izvestiya RAN. Biological series 2 pp 10-15
[24] Yakovlev A F, Tyukachev MV, Kravtsov V Yu, Donskaya P V and Popova A A 1996 Frequency of erythrocytes with micronuclei in the peripheral blood of sturgeon fishes 40th Scientific and Technical Conf. of University Teachers (Astrakhan) pp 60-61
[25] Abdullaeva N M, Gafurova S S 2016 Analysis of erythroid cells of cyprinids (Cyprinus carpio L.) on the basis of micronuclear testing Izvestiya (Samara Scientific Center, RAS) 18 (2) pp 263-266
[26] Palatnikov G M, Abbasova A R, Mamedov Ch A and Kasimov R Yu 2004 Assessment of the quality of sturgeon breeders by using micronuclear test Aquaculture of sturgeons: achievements and prospects 3rd Int. Conf. March 2004 (Astrakhan) pp 200-202
[27] Perry R P 1967 The nucleolus and the synthesis of ribosomes Prog. Nucleic Acid Res. Mol. Biol.. 6 pp 219-257