IOP Conference Series: Earth and Environmental Science

PAPER • **OPEN ACCESS**

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To cite this article: E D Khetsuriani *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **272** 022227

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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

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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).

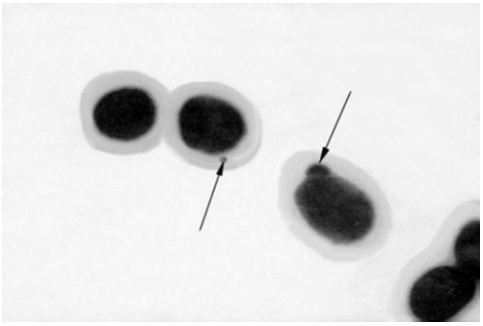


Figure 1. Micronucleus in the erythrocyte. Coloring with a fluorescent dye. Об.100х; ок.10х.

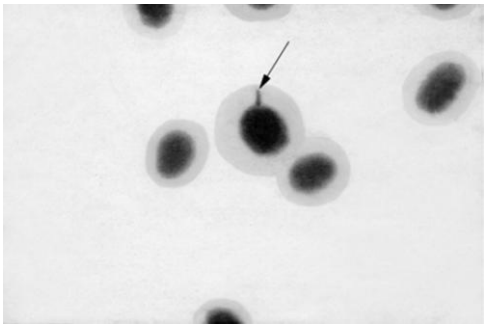


Figure 2. "Tailed" nucleus in the erythrocyte. Coloring with a fluorescent dye. 100x magnification 10x lens.

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).

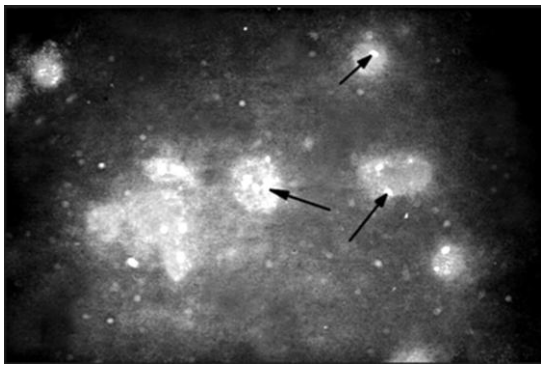


Figure 3. Fibrillar centers of ribosomal cistrons in fish red blood cells. Silver staining. 100x magnification 10x lens.

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 bester from different habitats (table 1).

Table 1. Frequency of occurrence of erythrocyte nuclear abnormalities in the blood of bester, ‰.

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 bester 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 bester blood are presented in table 2.

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

Place of sampling	IINE[a]	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.

6 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.

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