ASSESSMENT OF THE GENETIC STATUS OF ICHTHYOFAUNA IN KAZAKH PART OF THE CASPIAN SEA REGION USING MICRONUCLEUS TEST

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Abstract

The analysis of micronucleus frequency in fish caught in the Kazakhstan part of the Caspian region in the territories of Mangistau and Atyrau regions was carried out. We determined the component composition of sediment samples in the field of fish catch. Fish from carp family was subjected by micronucleus analysis. The nature of violations indicates that in some places, anthropogenic pressure has chemical and radiological component. It noted the correspondence between the cytological and cytogenetic abnormalities in fish erythrocytes and the results of content of man-made pollutants in samples of sediments taken in the trapping field of test animals.

Key words: *micronucleus test, fish, genetic status, Caspian Sea, Kazakhstan*

1. INTRODUCTION

The complex interaction of mutagenic environmental factors differ in multilevel (environment, body, tissue, cell) and multi-directional characteristics. Since the experimental study of all possible options for assessing the potential mutagenicity of complex mixtures and combined mutagenic effects is not real, it is necessary to estimate the total mutagenicity in the habitat of living organisms. One approach to solving the complex problems of the organization and carrying out of genetic monitoring of environmental pollution is to conduct research in the field of environmentally contaminated regions.

One of the modern and the most promising environmental assessment of the quality of the environment is bio indication methods. The advantages of using bio-indicators for the integrated assessment of different levels of complexity of biological systems is that they respond not only to the individual pollutants, but also to the whole complex of influencing substances with certain reactions of the organism as a whole.

One indicator of the state of organism is a cytogenetic homeostasis, manifested in maintaining the karyotype (Il'inskikh, Ilinskikh & Nekrasov 1983). Genetic homeostasis can be characterize by using micronucleus test, the essence of which consists in counting the frequency of cells with micronuclei (Schmid 1975). They are formed mainly from chromosomal material with centromeres devoiced during formation of chromosomal aberrations and fallen behind at a stage of anaphase from total divergent chromosomes.

For all its uniqueness as a water reservoir and habitat the Caspian Sea and its coast have long been in critical condition from the point of view of ecology. To date, Atyrau and Mangistau oblasts of Kazakhstan were involved in a number of projects that involve both industrial and agricultural sectors of the economy. The conditions of production and processing of oil and gas, unsustainable exploitation of land and water resources to a large extent affect the unique natural complex of the Republic of Kazakhstan.

The aim of this work is the evaluation of the genetic status of fish fauna in monitoring zones of the Kazakhstan part of the Caspian (Atyrau and Mangistau oblasts) with the micronucleus test. One of the most sensitive areas of Atyrau region to environmental stresses are river basin Zhaiyk (Ural) and Zhem (Emba), where are carried out a significant part of economic activity, in Mangistau region Caspian Sea directly.

2. MATERIALS AND METHODS

The object of study was a natural fish populations of carp family in the Caspian region: common rudd (Scardinuserythrophtalmus), white bream (Bliccabjoerkna), common bream (Abramisbrama), asp (Leuciscus (Aspius) aspius) and the North-Caspian roach (Rutilusrutiluscaspicus). Catching fish fauna was carried out in 2 areas of the Kazakhstan part of the Caspian Sea region - Atyrau (Atyrau, Kulsary, conservation Inderborg) and Mangystau (Aktau, Cape Sagyndyk, Fort-Shevchenko).

For micronucleus analysis, we conducted selection, fixation and staining of the collected peripheral blood samples of biomarker animals and performed microscopic analysis of samples. From Atyrau region in 2015 examined - 52 animals. In Mangistau region in 2016 examined 22 animals.

To perform the hematology laboratory studies (preparation and analysis of cytogenetic preparations) peripheral blood samples were taken. Blood was collected from the caudal vessels by cutting fish tail immediately after the catch. In some cases the blood for the study of the fish was taken from the heart, stump tail or gill veins. Selection of the blood collection method depended on the size of the fish and the blood volume required for analysis. Peripheral blood smear preparations were prepared in conventional manner in the field. Cameral processing of products was carried out in laboratory conditions. Peripheral blood smears were fixed in 96% ethanol for 30 minutes, dried and stained with Romanovski-Giemsa for 5 minutes. During the microscopic analysis of each specimen we examined by 10,000 red blood cells (Ilyinskikh, Novitsky, Vanchugova & Ilinskikh 1992).

Chemical analysis of sediment samples was conducted in the laboratory of physical and chemical methods of analysis and ecology of "Institute of Chemical Sciences named after A.B. Bekturov" (Almaty, Kazakhstan). Quantitative analysis of objects of environment samples for heavy metal content was carried out with the sample preparation of samples of sediments (silt). Determination of PAHs was carried out by high performance liquid chromatography using a fluorimetric UV detector" (Borisova & Salnikov 1991).

In the analysis of the data we used the standard methods of statistical analysis (Rokitsky 1978).

3. RESULTS AND DISCUSSION

3.1. Determination of component composition and the presence of environmental contaminants in sediment samples

According to an analysis of published data on priority contaminants in the Caspian region, the determination of component composition of taken for analysis sediment samples was limited to certain hydrocarbons, petroleum products and heavy metals.

Table 1. Contents of chemicals in sediment samples taken in the field of biomaterials gathering in the Caspian region

Selection Point	Cd, mg/kg	Pb, mg/kg	Ni, mg/kg	Co, mg/kg	Cr, mg/kg	Hydrocarbons	Petroleum products
MPC, soil	0,5	6,0	4,0	5,0	6,0	-	5,0
Kulsary	0,80	11,6	26,1	3,22	14,3	5,8370	12,973
Inderborg	0,64	2,94	17,5	2,76	15,3	6,4950	15,651
Atyrau	1,10	16,8	53,7	9,80	39,4	3,9440	6,830
Port of Bautino	1,65	30,12	5,37	4,35	5,68	0,3727	11,9
Cape Sagyndyk	1,49	12,49	5,12	4,43	5,54	0,1775	3,89
Aktau	1,27	15,67	7,60	3,56	5,27	0,9723	21,6

Results of the content of hydrocarbons and oil products in bottom sediments of Atyrau region, taken within the boundaries of Kulsary (Southern coast of undrained Lake Kamyskol in which runs one of the branches Zhem (Emba)) and from the mainstream Bagyrlay river, 11 km north - west from village Inderborsky are practically at the same level. The sample of sludge from the Ural main riverbed (the right-bank part of the river, 30 km from Atyrau upstream) is the most clean on these indicators.

In the Mangistau oblast in the Caspian region the most polluted in terms of hydrocarbons and petroleum products is the bottom sediments taken from the shore of the Caspian Sea in the city Aktau, where amateur fishing is allowed. The content of hydrocarbons in the bottom sediments of Bautino is two times lower than in Aktau, and the content of oil products is three times lower. The most clean of the surveyed places is Cape Sagyndyk.

If the content of hydrocarbon sludge sample from the main river bed of Ural (near the city of Aktau) was the cleanest, then it is the most polluted by the content of heavy metals. Bottom sediments in the vicinity of Kulsary and Inderborg in the heavy metal content, as well as in the analysis of the content of hydrocarbons and petroleum products, are at the same level of pollution.

Results of heavy metals in sediment samples taken in the field of biomaterials collection show that in all the investigated locations, except Atyrau maximum permissible concentrations is normal only in cobalt. The highest values of the maximum permissible concentration were found from the content of cadmium and lead at all points except for the Inderborg. At all points of the Atyrau region, significant exceedances of MPC for nickel and chromium were revealed.

The accumulation of heavy metals in the bottom sediments of the Caspian Sea is characterized by a number of specific features. Lead in the bottom mud is inactive, but it is well extracted from the deposits by lamellar and gastropod mollusks. Perhaps that is why, against the background of the general "purity" of the chemicals studied at Sagyndyk Point, a twofold increase in lead content is observed, since the bottom sediments at this point are represented by small pebbles and shells of mollusks. The weak solubility of lead causes it to flow with river runoff in a suspended state, causing the mosaic distribution of the element in the bottom mud. Zones with a reduced lead content gravitate to the Volga coast and the Ural furrow. The higher contents of the element are found in shallow muddy plots. The maximum amounts of elements in the silty mass of the bottom coincide with the area of development of fine-silt sediments. Significant amounts of metals participate in migration along trophic chains, accumulating in shells and soft tissues of mollusks, and then in fish.

The average content of elements in the dry mass of the soil, obtained (Barkeliev 2002) for the vast territory of the northeastern part of the sea, was: cadmium <0.02-0.34 (average 0.073); lead <2.0-8.0 (average 3.0); chrome 4.0-27.0 (average 10.0); nickel <4.0-27.0 (average 10.0) mg / kg.

Comparison of the data on the content of chemical elements in the Caspian bottom sediments for the current period shows a significant increase in the values of cadmium and lead, the content of chromium and nickel remains at the same level as for marine deposits in the northeastern part of the sea.

3.2. Conducting a micronuclear test in fish of the Kazakhstan part of the Caspian region

The ichthyofauna of the Caspian basin, according to various estimates, has from 100 to 126 species and subspecies of fish. A distinctive feature of the Caspian ichthyofauna is the high endemism observed from the genus category to the subspecies level. At the subspecies level the ichthyofauna of the Caspian Sea is endemic by 100%, species - 43.6%, genera - 8.2%. The number of species is dominated by carp, bovine and herring fish. There are no less than 76 species and 47 subspecies of 17 families inhabiting the sea and deltas of the rivers, but only a part of them occurs in the Kazakhstan waters (Naseka & Bogutskaya 2009; Kazancheeva 1981). In connection with this micronuclear analysis, the fish of one of the most common families, the carp family, were exposed.

The most important freshwater reservoirs and fishery facilities in the Atyrau oblast are the Ural (Zhayik) and Zhem (Emba) rivers, and the assessment of their ecological well-being is of primary importance for the ecological balance of the region.

During the field work, material was collected that fit the characteristics of the current state of widely distributed species according to observation seasons. The animals under threat of extinction and included in the IUCN and the Red Book of Kazakhstan list were not used in the work.

In the 2015 season, four species of fish are caught in the outskirts of Kulsary in the Atyrau Oblast in the places of the Zhem (Emba) and Kamyskol Lake (Common rudd-*Scardinus erythrophtalmu*, Pike - *Esox Lucius,* Percum - *Percafluviatilis*, Roach - *Rutilusrutilus*). In the Inderborskiy Atyrau oblast in the Bagrylay river, two species of fish are caught (Common carp - *Cyprinus carpio;* Perch - *Percafluviatilis)*. In the vicinity of Atyrau from the sections of the river Chernaya Rechka, r. Ural were caught by fish of 7 species (Common carp - *Cyprinus carpio*, Common rudd - *Scardinus erythrophtalmus*, Perch - *Percafluviatilis,* Roach - *Rutilusrutilus,*Aspius - *Leuciscus (Aspius) aspius*, Silver crucian - *Carassius gibelio*, Gustera - *Blicca bjoerkna*;).

From the data presented, it follows that in the three monitoring sites of the Atyrau Oblast surveyed in 2015, 8 species of fish from 3 orders are shown to live. To compare the current biological diversity of the ichthyofauna with previously established facts, a comparison was made between data on the systematic representation of water bodies in the Ural and Emba (Zhem) river basins for 2013 and 2015 (Figure 1).

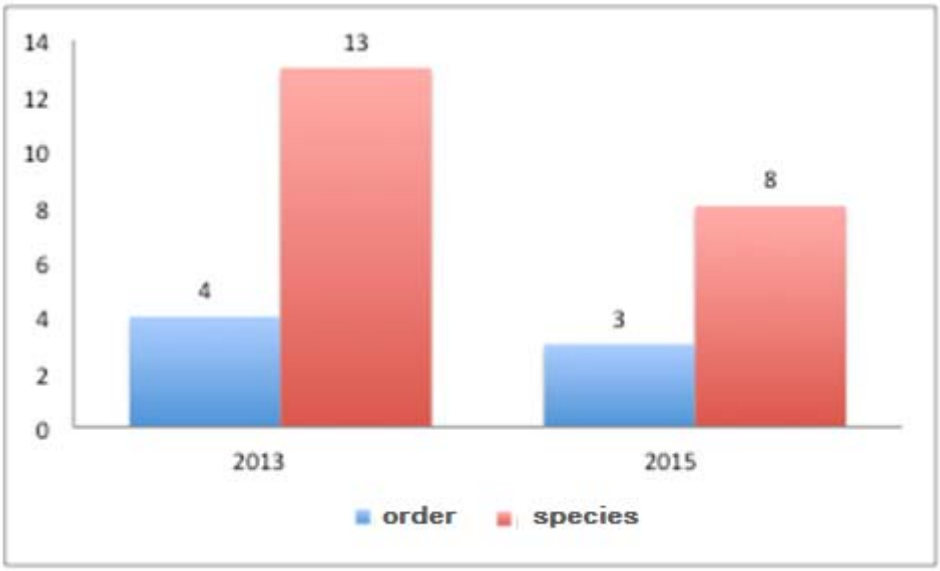


Figure 1. Comparative data on the systematic presentation of ichthyofauna

From the comparative data of the diagram it follows that the biodiversity of fish in the season of 2015, taking into account the catch season, the specifics of the collection of material and the timing of the work, can be considered within the norm at the present stage.

Since there is no permanent river network in the Mangystau Oblast, in the 2016 season the catch of the ichthyofaunawas carried out directly in the Caspian Sea within the Mangistau region. In the vicinity of the city of Aktau, four species of fish are caught (Aspius - *Aspiusaspius*, *Gobiusmacrophthalmus*, *Rutilusrutiluscaspicus, Clupeonellacultriventris*). At the monitoring point of Fort-Shevchenko, fish were caught in the Bay of Bautino. At this point, three species of fish are caught (*Rutilusrutiluscaspicus*, Bream - *Abramisbrama*, *Gobiusmacrophthalmus*). Two species of fish were caught at Cape Sagindyk (*Rutilusrutiluscaspicus* and *Gobiusmacrophthalmus*). Thus, in three

monitoring sites of the Mangistau region, surveyed in 2016, five species of fish from 3 orders are shown to live.

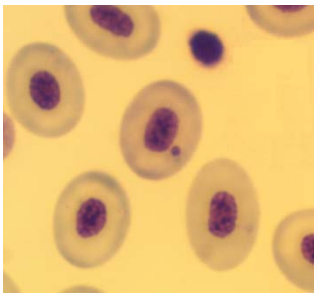
Based on zoological data, the largest number of fish caught in the Atyrau region belonged to two species of the Cyprinidae family (*Cyprinidae*) - *Scardinuserythrophtalmus* and *Bliccabjoerkna*. In the Mangistau region, the largest number of caught fish belonged to three species of the cyprinid family (*Cyprinidae*) - *Rutiluscaspicus*, *Abramisbrama* and *Leuciscus (Aspius) aspius*. Therefore, for the cytogenetic analysis, preparations of these species of fish of the Cyprinidae families were used. For hematological studies from 74 fish specimens, blood was taken for the preparation of cytological preparations. As further cytogenetic analysis showed, there is no difference between these species of fish of the same family, neither in the morphology of the erythrocytes, nor in the frequency and spectrum of the abnormalities, therefore, they were taken into account in the statistical processing together. Similarly, according to the data of a number of authors, an analysis of 7 fish species of the Taimyr Peninsula showed that the observed species differences are not significant and do not have statistically significant differences (Kryukov & Kochkarev 2013).

During the cytogenetic examination, all disturbances in the structure of erythrocytes, which differ from the normal morphology of erythrocytes, characteristic for this species, were recorded. Micronuclei in a microscope are visible as round, oval, different sizes of densely colored bodies with a clear contour. Different types of micronuclei probably correspond to the types of chromosome disorders that have arisen. By the size of micronuclei, one can judge the changes that have occurred in the chromosomal set of cells. Thus, the appearance of cells with large micronuclei is mainly associated with violations of the fission spindle, or the lag of entire translocated or dicentric chromosomes, and the appearance of cells with small micronuclei is mainly due to structural aberrations of chromosomes (straggling acentric fragments) (Kovaleva 2008). However, the correlation analysis of the frequency of chromosomal abnormalities and the frequency of micronuclei carried out by many cytogenetics in the study of objects at different levels of organization, including humans, did not reveal a connection between these indicators, which indicates a different nature, or at least the existence of additional mechanisms for the appearance of micronuclei (Kolyubaeva, Raketskaya, Borisova & Komar 1995). This is confirmed by the fact that micronuclei can be detected in the absence of cell division as a result of previous division. The core first forms a blade, which then separates and forms a micronuclei - this process is well illustrated by micronuclei with uneven edges pressed against the core. It has also been suggested (Kuzina 2013) that the non mitotic formation of micronuclei is the pathway for the release of genetically defective chromatin (Ilyinskikh, Novitsky, Vanchugova & Ilinskikh 1992).

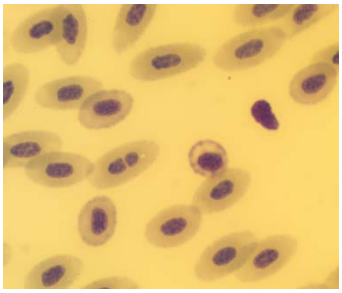
Additional information on cytological processes that occur in response to the effects of stress factors of the environment can be obtained by analyzing other signs of nuclear disturbances. Thus, amitosis of erythrocytes indicates the development of degenerative processes in the body of fish, caused by various causes, including chemical genotoxicants. Cell division by amitosis is accompanied by a direct division of the nucleus, when it, tugging, assumes a dumbbell shape. It is characteristic that various forms of amitosis arise in different physiological states of the body, which, as observed in marine and other fish, have their specious specific features. The division of the nucleus can also occur without constriction of the cytoplasm. Erythrocytes become binuclear, while a chromatid bridge can be observed between separating parts of the nucleus (Yarzhombek, Limanskii & Shcherbina 1986).

During the cytogenetic analysis of peripheral blood of fish, the following disturbances in the structure of erythrocytes were recorded: erythrocytes containing one or two micronuclei; binuclear erythrocytes; erythrocytes containing two nuclei connected by one or more strands - amitosis (bridge); erythrocytes having various disorders of the cytoplasm (with a "tail", a change in shape, absence of cytoplasm, vacuolation of the cytoplasm); protrusion or invagination of the nuclear envelope of the erythrocyte (Figure 2). It is known that in many cases these changes are accompanied by compensatory processes occurring in tissues, for example, with functional overload, fasting, after poisoning or denervation (Kuzina 2013). In addition, there are published data that nuclear and cytoplasmic anomalies of this nature can result from gamma radiation inducing geno- and cytotoxicity (Anbumani & Mohankumar 2015).

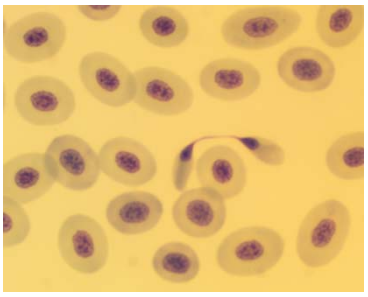
The results of cytogenetic analysis of fish caught in the monitoring points of the Caspian region of Atyrau and Mangistau oblasts are presented in Table 2.



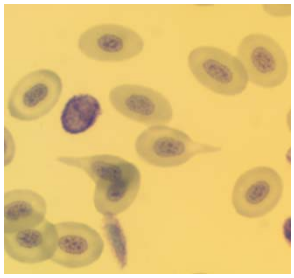
Erythrocyte with micronucleus



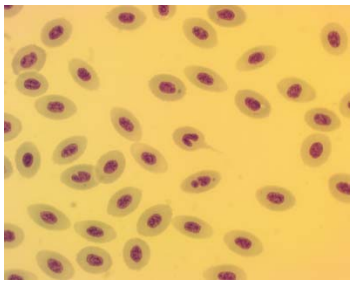
Binuclear erythrocytes



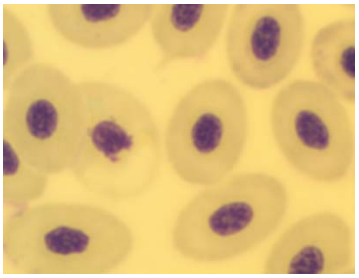
Amitosis



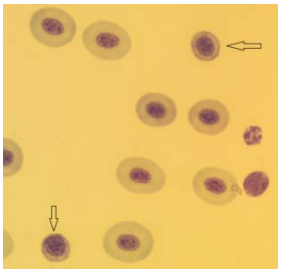
Violation of the cytoplasm in the form of a "tail"



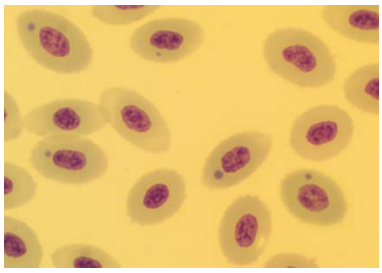
Invagination of the nuclear envelope



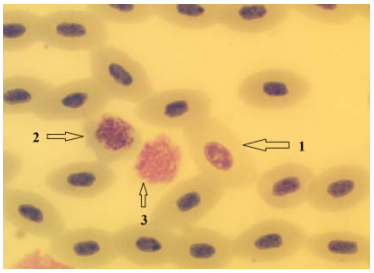
Protrusion of the nuclear envelope



Absence of cytoplasm



Vacuolation of the cytoplasm



Different stages of erythrocyte destruction

Figure 2. Erythrocytes of peripheral blood of fish with cytogenetic disorders

Table 2. Results of cytogenetic analysis of fish erythrocytes caught in the Kazakhstan part of the Caspian region

Catching location	Number of cells	One micro-nucleus	Two micro-nucleus	Amito-sis	Tail	Anomalies of nuclear envelope	Binuclear erythrocytes
Atyrau	210000	0,036± 0,004	0,0057± 0,001	0,0124±0,002	0,0005±0,0005	0,017± 0,003	0,007± 0,002
Kulsary	160000	0,026± 0,004		0,008± 0,002		0,009± 0,002	
Inderbor	150000	0,031± 0,004		0,009± 0,002		0,007± 0,002	
Aktau	70000	0,076± 0,010		0,012± 0,004	0,013± 0,004	0,018± 0,004	0,012± 0,003
Bay of Bautino	100000	0,071± 0,008		0,021± 0,005	0,025± 0,005	0,067± 0,008	0,002± 0,001
Cape Sagyndyk	50000	0,048± 0,010		0,014± 0,005	0,010± 0,005	0,023± 0,005	0,008± 0,004

The highest percentage of peripheral blood red blood cell abnormalities and the micronucleus frequency in them was recorded in the surveyed fish of the Cyprinidae families caught in the Caspian Sea within the Mangistau region. At the same time, they revealed practically the entire spectrum of the cytological abnormalities described above. Fish caught in the vicinity of Aktau were registered with erythrocytes with two micronuclei and the highest frequency of binuclear erythrocytes. Probably, these results can be explained by the influence of radiation factors on the examined ichthyofauna caused by the close location (5 km from Aktau) of the drainage lake-settler Koshkar-Ata, which is the "tailing pond" of the radiation waste.

In erythrocytes of carp fish caught in Bautino Bay, the average percentage of micronuclei was 0.071 ± 0.008%, which does not differ significantly from the data obtained in Aktau (0.076 ± 0.010%). However, some representatives of the carp family caught in the Bay of Bautino noted significant changes in the cytological character. Two fish (*Rutiluscaspicus)* showed changes in the morphology of erythrocytes - in one erythrocyte with a more rounded shape and light nucleus and cytoplasm, in the other, on the contrary, with more elongated cells and dark cytoplasm (method, staining time and dye concentration are identical). With what these differences are related at this stage, it is very difficult to express, perhaps it is caused by a physiological condition or some kind of disease. Also, one fish had a vacuolization of the cytoplasm of about 3% of erythrocytes and an invagination of the nuclei to 1%. Another representative of the carp family recorded up to 20% of red blood cells at different stages of destruction. Most of the fish analyzed did not exceed 0.5-1%. The presence of these disorders in the composition of peripheral blood with a certain statistical frequency can be considered as evidence of the manifestation of chronic toxicosis of the body of fish. On the frequency of occurrence of degenerative disorders in the analysis of erythrocytes of peripheral blood of fish from the Bay of Bautino, exceeds similar indicators from the city of Aktau.

In the erythrocytes of fish caught at Cape Sagyndyk, the smallest percentage of micronuclei in erythrocytes of the Cyprinidae family (0.048 ± 0.010%) is observed, which is probably due to the lack of anthropogenic activity of humans in this area and corresponds to the definition of the lowest chemical contamination of bottom sediments, although there is a twofold increase MPC for lead. Nevertheless, the entire spectrum of observed cytological disturbances is also recorded here.

In comparison with the fish of the Cyprinidae family caught in the Caspian Sea, the surveyed river fishes of this family caught in the Caspian region within the Atyrau region recorded less frequent

disturbances in the structure of red blood cells of peripheral blood. The highest frequency and spectrum of cytological disturbances was detected in fish caught in the vicinity of Atyrau (0,0781 ± 0,006%).

The frequency of micronuclei and cytological abnormalities in fish erythrocytes caught in the vicinity of Kulsary and in the Inderborg does not differ significantly. The examined individuals from this region did not have binuclear erythrocytes, erythrocytes with 2 micronuclei and amytic anomalies.

A comparative analysis of the results of micronuclear analysis from the three surveyed items shows that differences are observed only in the spectrum of detected violations in fish caught in the vicinity of the city of Atyrau. At the same time, the frequencies of the micronuclei themselves are at the same level, and there are no reliable differences between them. The absence of reliable differences in micronuclei frequencies in the study of fish from the city of Atyrau and Kulsary and Inderborg village indicates the general pollution of the Caspian water resources of the Atyrau region.

Various species of fish of the carp family have been studied by many researchers. At the same time, the frequency of micronuclei in different literary sources is sometimes different. The share of cells containing micronucleus in the sample of *Cyprinus carpio* found in the river Sulak was on average 1,2±0,21‰, in erythrocytes of fish contained in artificial conditions, an average of 0.3 ± 0.05 ‰ micronuclei (Abdullaeva & Gafurova 2016). Also, many researchers have shown that the background level of micronuclei in fish is 0.5-1 ‰ (Il'inskikh, Ilinskikh & Nekrasov 1983; Kazancheeva 1981). These data roughly correspond to the results obtained by us. At the same time, other authors demonstrate the micronucleation rate at 0.25 ± 0.03% in Ukrainian scaly carp (Gritsiak, Glushko & Tarasyuk 2013), 0,47±0,001% in *Cyprinus carpio* (Kryukov, Klimov & Krasova 2016) or even 2.91 ± 0.15% in bream from the Volga-Caspian canal (Kuzina 2013).

Thus, the conducted cytogenetic studies make it possible to draw the following conclusions:

- Cytogenetic disorders (micronuclei) in erythrocytes of peripheral blood of fish indicate the presence of mutagenic factors in the composition of water pollutants at monitoring points of animal selection and increased anthropogenic load.
- Cytological abnormalities in the peripheral blood erythrocytes of the animals under study indicate the development of degenerative processes in the body, caused by various causes, including ecological ones.
- The results of the cytogenetic examination of fish of the carp family are observed to correspond to the level of chemical contamination (hydrocarbons, oil products, heavy metals, nitrates) at all points of the study.
- The absence of reliable cytogenetic differences in the frequency of micronuclei and cytological abnormalities in fish in Atyrau and Mangistau provinces testifies to the general ecological pollution and anthropogenic impact in the major cities-ports of the Caspian territories of Kazakhstan.

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