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MICRONUCLEUS ASSAY OF RAINBOW TROUT

Y. Glushko, PhD, senior researcher
niko-yulia@ukr.net

Institute of Fisheries of NAAS, Kyiv, Ukraine

Genotoxic pollution of aquatic ecosystem describes the introduction of contaminants with mutagenic, tertogenic and carcinogenic potentials into its principal media and genome of the resident organisms. Biomarkers are biological responses to environmental chemicals at the individual level or below demonstrating departure from normal status. Fish are excellent subjects for the study of the mutagenic and carcinogenic potential of contaminants present in water because they can metabolize, concentrate, and store waterborne pollutants.

Micronucleus (MN) assay is an ideal monitoring system that uses aquatic organisms to assess the genotoxicity of water in the field and in the laboratory. Research reports maintained that it can be applicable to freshwater and marine fishes and that gill cells are more sensitive than the hematopoietic cells to micronucleus inducing agents. Recent research reports maintained that micronucleus formation in freshwater and marine fish is a function of water pollution caused primarily by heavy metals and polycyclic aromatic hydrocarbons. The micronucleus assay has been widely used to screen for chemicals that cause these types of damage. Rodriguez–Cea determined the sensitivity of micronucleus test in freshwater fish species for application in field surveys. The author studied three fish species namely: Brown trout (*Salmo trutta*), European eel (*Anguilla anguilla*) and European minnow (*Phoxinus phoxinus*) for their use as *in situ* pollution biomarker by measuring the micronucleus indices of their renal erythrocytes.

The purpose of our work is to determine the level of cytogenetic indicators of trout from the ecological condition of water. For cytogenetic analysis has been used micronucleus test and analysis of frequency of apoptosis of rainbow trout (*Oncorhynchus mykiss*) blood cells. We studied 15 individuals of rainbow trout have been grown in reinforced concrete pools of SLL "Ishkhan" of Chernivtsi region. Peripheral blood is obtained from the dorsal vessels of each individual by vertically puncturing of sterile syringe. The MN assay was performed as per the protocol of Davydov O.N. and Temnyhanov Y.D. but with own modifications. Blood smears were made onto grease-free pre-cleaned and marked slides by droping two drops of 0.6 % NaCl and one drop of blood. The slides were air-dried for 24h. After fixation in pure methanol for 30 min, the slides were allowed to air-dry and stained by the method of Romanowsky with standard Giemsa solution for 40 min. Slides were made for each fish and scored 3000 cells using oil-immersion under a light microscope (Primo Star Zeiss, 100×1.25). It has been counted the occurrence frequency of cytogenetic indicators (erythrocytes with micronuclei (EMN), lymphocytes with micronuclei (LMN), binuclear lymphocytes (BNL) and apoptosis). The diameter of the micronucleus (MN) should be less than one-third of the main nucleus. MN should be separated from or marginally overlap with main nucleus as long as there is clears identification of the nuclear boundary. Obtained results were expressed as ppm (‰). Statistical analysis was performed using the Student's t-distribution. Statistically significant differences were tested at 1 and 5 % levels.

Blood system of fish is very sensitive to changes of water environment, that's why the results micronucleus test in peripheral blood cells makes possible to evaluate the genotoxic effect onto fish genome. Results of our previous studies have been shown, that in the second year of individual development silver and bighead carps were characterized the lowest level of individual variability by the cytogenetic indicators comparatively with other age groups. Researchers from different countries report, that the level of cytogenetic mutations in peripheral blood cells of fish is directly depends not only from ecological condition of water bodies, but also from investigated species of fish.

As far as we observe increasing of environmental genotoxic effect onto the chromosomal apparatus of carps is very necessity to analyze the level of mutations. There isn't universal method for detection of all types of aberrations in fishes, but cytogenetic methods are most sensitive and reliable for detection of mutagenic effects of genotoxic agents *in vivo*. That is why we carried analysis of occurrence frequency of cytogenetic indicators (erythrocytes with micronuclei, lymphocytes with micronuclei, binuclear lymphocytes and apoptosis) in the peripheral blood cells of rainbow trout of SLL "Ishkhan" of Chernivtsi region.

The results of cytogenetic analysis have been demonstrated that group of rainbow trout was characterized comparatively not high level of erythrocytes with micronuclei (1.7±0.2 ‰). Also it has been observed that this fish were characterized lower level of lymphocytes with micronuclei (1.0±0.3 ‰) and binuclear lymphocytes (0.7±0.2 ‰), which dedicated about absence of negative factors pressure onto immune system of investigated fish. In turn, the increased level of apoptosis (3.1±0.2 ‰) in the group of rainbow trout may be a result of elimination of mutant lymphocyte and erythrocytes by this way. The results of our investigations have been demonstrated the stable cell homeostasis and suggested about favorable conditions for growing of fish.