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CYTOGENETIC ANALYSIS OF SILVER CARPS FROM SEPARATE REGIONS OF UKRAINE

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The study makes a comparative analysis of cytogenetic indicators (erythrocytes with micronuclei, lymphocytes with micronuclei, binuclear lymphocytes and apoptosis) in the peripheral blood cells of two-year-old silver carp from fish farms Khersonrybgosp, Lymanske and Galytskiy. It shows that a group of silver carp from fish farm "Galytskiy" was characterized by a higher level of EMN (2.9±0.3‰), LMN (1.6±0.3‰), BNL (1.3±0.2‰) and apoptosis (4.4±0.2‰) compared with the groups from fish farms Khersonrybgosp, Lymanske. Statistically significant intergroup differences were found in silver carp groups by the number of EMN (P <0.05) and apoptosis (P <0.01).

Keywords*: silver carp, micronucleus test, cytogenetic indicators, genomic mutations.*

Глушко Ю. М. Цитогенетичний аналіз білих товстолобиків окремих регіонів України

Проведено порівняльний аналіз рівня цитогенетичних показників (еритроцитів з мікроядрами, лімфоцитів з мікроядрами, двоядерних лімфоцитів та апоптозів) в клітинах периферійної крові дворічок білих товстолобиків рибних господарств «Херсонрибгосп», «Лиманське» та «Галицький». Встановлено, що група білих товстолобика РГ «Галицький» характеризується вищим значенням ЕМЯ (2,9±0,3‰), ЛМЯ (1,6±0,3‰), ДЯЛ (1,3±0,2‰) та апоптозів (4,4±0,2‰) порівняно з групами рибних господарств «Херсонрибгосп» та «Лиманське». Статистично достовірні міжгрупові відмінності виявлено в групах білих товстолобиків за частотою ЕМЯ (P<0,05) та апоптозів (P<0,01).

Ключові слова: *білий товстолобик, мікроядерний тест, цитогенетичні показники, геномні мутації.*

Глушко Ю. Н. Цитогенетический анализ белых толстолобиков отдельных регионов Украины

Проведен сравнительный анализ уровня цитогенетических показателей (эритроцитов с микроядрами, лимфоцитов с микроядрами, двуядерных лимфоцитов и апоптозов) в клетках периферической крови двух годовиков белых толстолобиков рыбных хозяйств «Херсонрыбхоз», «Лиманское», «Галицкий». Установлено, что группа белых толстолобиков РХ «Галицкий» характеризируется высшими значениями ЭМЯ (2,9±0,3‰), ЛМЯ (1,6±0,3‰), ДЯЛ (1,3±0,2‰) и апоптозов (4,4±0,2‰) сравнительно с группами рыбных хозяйств «Херсонрыбхоз» и «Лиманское». Статистически достоверные межгрупповые различия выявлены в группах белых толстолобиков по количеству ЭМЯ ($P<0,05$) и апоптозов ($P<0,01$).

Ключевые слова: *белый толстолобик, микроядерный тест, цитогенетические показатели, геномные мутации.*

Formulation of problem. The main direction of aquaculture in inland water bodies of Ukraine is pond fisheries as the main reserve for further raising of production amounts. Today, traditional objects of fisheries in aquaculture are carps of the Ukrainian framed and scaled breeds and phytophagous fish (silver carp, bighead carp and grass carp). Specimens of pond aquaculture are characterized by the different phenotypic and genotypic peculiarities, growth rate, fecundity, search ability, cold resistance, resistance to infectious diseases. Consequently, in pond fisheries are necessary complex knowledges of variability of populations genetic structure, level of somatic and generative mutagenesis, resistance to infectious diseases. At environmental conditions the complex of biotic and abiotic factors influence onto the homeostasis of fish. During the vegetative period in ponds are collected physical, chemical and biological mutagens which induce in fish the occurrence of different types of mutations (gene, chromosomal, genomic).

As far as increase the influence of environmental genotoxins onto the chromosomal apparatus of fish is very necessity to analyze the level of mutations for estimation of physiological state and make the forecast of viability, fruitfulness as a result of total action on their genotoxic agents. There is not 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*.

Study of problem. 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 [1]. 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. This is so because they can metabolize, concentrate, and store waterborne pollutants [2]. Since fish often respond to toxicants in a similar way to higher vertebrates with fast responses on low concentrations of direct acting toxicants, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans. The main application for model systems using fish is to determine the distribution and effects of chemical contaminants in the aquatic environment [3]. 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 [4]. 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. According to Hartwell and Fagr [1, 5], the incidence of micronuclei in fish and other aquatic life serves as an index of these types of damage and counting of micronuclei is much faster and less technically demanding than scoring of chromosomal aberrations. The micronucleus assay has been widely used to screen for chemicals that cause these types of damage [6, 7]. Rodriguez –Cea [8] 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 erythrocyte micronucleus test has been used with different fish species to monitor aquatic pollutants displaying mutagenic features in developed countries [9, 10, 11]. Kligerman demonstrated that fish inhabiting polluted waters have higher frequencies of micronuclei. The micronuclei frequencies may vary according to the season, the kind of pollution involved, and the species of fish. As to silver carp, unfortunately in Ukraine similar investigations did not perform at all.

Objectives and methods of investigation. That is why the purpose of our work is to determine depending the level of cytogenetic indicators of fish from the ecological condition of water and breeding conditions. For cytogenetic analysis has been used micronucleus test and analysis of frequency of apoptosis of blood cells of fish. Three groups of two years silver carps (10 individuals in each group) from fish farms: “Khersonrybgosp” Kherson region, “Limanske” Kharkiv region and “Galitski” Ivano-Frankivsk region has been sampling. Fish were collected from locations that represent different levels of contaminants. 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. [12] 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). There were 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; and

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.

Result of investigation. 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 of exogenic factors on the 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 [*13*]. 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 [14, 15].

Therefore, for objective assessment of the living conditions effect on the fish genome, we performed a comparative analysis of cytogenetic indicators of two years silver carps from different fish farms such as: "Khersonrybgosp", "Limanske" , "Galitski" (Fig.1).

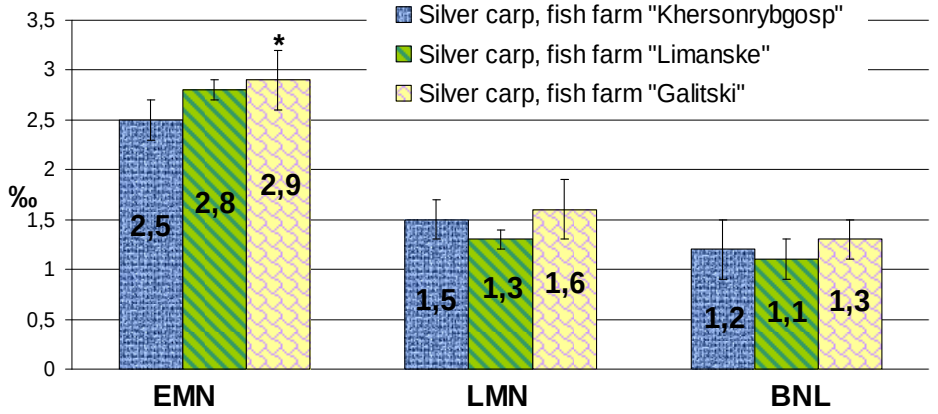

Fig.1. Level of cytogenetic mutations *in peripheral blood cells of two years silver carps from different fish farms*

It has been investigated, that group of silver carp from fish farm "Galitski" Ivano-Frankivsk region is characterized by the highest level of erythrocytes with micronuclei (2.9±0.3‰), lymphocytes with micronuclei (1.6±0.3‰), binuclear lymphocytes (1.3±0.2‰) compared with groups of fish from other investigated regions. It is suggested that water body of this fish farm has mutagens that cause occurrence of genetically defective erythrocytes and lymphocytes.

Statistically significant intergroups differences were find out by the frequency of EMN ($P < 0.05$). Carp fish erythrocytes are oval in shape with abundant smooth eosinophilic cytoplasm and a central, oval-shaped condensed nucleus (Fig. 2). It is make possible to differentiate normal cells and genetically defective which have micronuclei in the cytoplasm besides of the main nucleus. Micronuclei are cytoplasmic chromatin-containing bodies formed when acentric chromosome fragments or whole chromosomes lag during anaphase and fail to become incorporated into daughter cell nuclei during cell division. Because genetic damage that results in chromosome breaks or spindle abnormalities leads to micronucleus formation, the incidence of micronuclei serves as an index of these types of damage [1]. These structures are easy to visualize in erythrocytes (Fig.3.) and therefore can be used as a measure of chromosomal aberration.

As to the frequency of lymphocytes with micronuclei and binuclear lymphocytes (Fig.1) significant intergroup differences weren't observed.

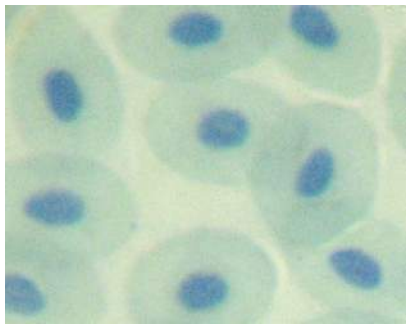

Fig. 2. Photomicrograph showing normalerythrocytes of silver carp

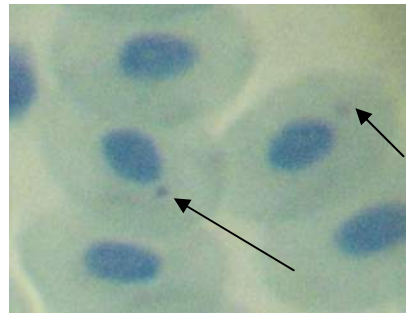

Fig. 3. Photomicrograph showing erythrocyte with micronucleus of silver carp

But if to analyze the total number of cytogenetic damages in lymphocytes, we can conclude that the immune system of silver carps from fish farm "Galician" has more pressure of genotoxic factors.

One more important indicator of cytodifferentiation of fish cells is apoptosis. Apoptosis is the process of high regulated programmed cell death with specific biochemical events lead to characteristic cell changes and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation. According to researchers [16, 17], apoptosis is the way of genetic defective cells death. Therefore, next step of our investigation was a comparative analysis of apoptosis frequency in peripheral blood cells of silver carps from different fish farms. These results are shown in table 1.

Table 1 - The level of apoptosis in peripheral blood cells of silver carp from different fish farms

Number of individuals	1	2	3	4	5	6	7	8	9	10	11	12	M±m, ‰
Fish farm “Khersonrybgosp”	4	3	4	5	4	4	4	4	4	3	3	4	3.8±0.2
Fish farm “Limanske”	2	3	3	3	3	3	3	3	3	4	4	3	3.1±0.2
Fish farm “Galitski”	5	5	6	4	4	4	4	4	4	4	5	4	4.4±0.2

It has been established that a group of silver carp from fish farm “Galitski” was characterized not only highest level by the results of micronucleus test and by the level of frequency of apoptosis (4.4±0.2 ‰). In fish organisms apoptosis has a very important role in the development and functioning of immune system and has a positive correlation with the concentration of carcinogens in the environment. Statistically significant intergroups differences were find out ($P<0.01$) between groups of fish from farms “Galitski” and “Limanske”.

Therefore, as to our opinion, the comparatively high level of apoptosis in a group of silver carp from fish farm “Galitski” was the result of eliminating of genetic defective (mutant) cells by this way and testimony of less favorable conditions for fish farming in this farm, compared with other investigated fish farms. The highest level of mutant cells as to results of micronucleus test in this group of silver carp also shows that the water body of this fish farm has relatively high concentration of genotoxins.

Conclusions. It is concluded from this study that micronucleus test and analysis of apoptosis frequency are the biomarkers of physiological state of fish and can be used for estimation and control the genotoxic effects of waterborne pollutants.

It has been established that the group of silver carp from fish farm "Galitski" is characterized higher level by the frequency of EMN, LMN, BNL and apoptosis compared with groups from fish farms "Khersonrybgosp", fish farm "Limanske". Statistically significant intergroups differences were find out by the frequency of EMN ($P<0.05$) between groups of silver carp from farms "Khersonrybgosp" and "Galitski" and by the frequency of apoptosis ($P<0.01$) between groups from farms "Galitski" and "Limanske".

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УДК 630*:582.475

ВПЛИВ ГОСПОДАРСЬКИХ ЗАХОДІВ НА ПРОДУКУТИВНІСТЬ ТА БУДОВУ СОСНОВИХ ДЕРЕВОСТАНІВ

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Установлено закономірності будови насаджень залежно від віку, способів створення, агротехніки вирощування. Досліджено вплив густоти і складу на ріст штучних і природних соснових насаджень та очищення дерев від гілок та сучків. Наведено результати вивчення залежності щільності деревини від густоти насаджень та умов місцезростання. Охарактеризовано розподіл об'єму стовбура на зону безсучкової деревини та зони зі зрощеними і незрощеними сучками зважаючи на загальну кількість стовбурів у чистих і змішаних насадженнях.

Ключові слова*: штучні насадження, природні деревостани, зруби, густота культур, запас, будова деревостанів, сучкуватість.*

Гриб В.М. Влияние хозяйственных мероприятий на продуктивность и строение сосновых древостоев

Установлены закономерности строения насаждений в зависимости от возраста, способов создания, агротехники выращивания. Исследовано влияние густоты и состава на рост искусственных и природных сосновых насаждений и очищение деревьев от сучков и ветвей. Приведены результаты исследования плотности древесины от густоты насаждений и условий местопроизрастания. Охарактеризировано распределение объема ствола на зону безсучковой древесины и зоны со сросшимися и не сросшимися сучками от общего количества стволов в чистых и смешанных насаждениях.

Ключевые слова*: искусственные насаждения, природные древостои, вырубки, густота культур, запас, строение насаждений, сучковатость.*

Gryb V.M.,The impact household activities on the productivity and constitution pine stands

Have revealed regularities the structure of plants depending on the age, way of creating, agricultural techniques of growing. Was investigated the influence of density and composition on