

ANNOTATION

dissertation for the degree of Doctor of Philosophy (PhD) in the specialty "8D05101 - Biology"

Study of the morphogenesis of the grass carp *Ctenopharyngodon Idella* Valenciennes under the conditions of pollution of a fish-feeding reservoirs

General characteristics of the work. The dissertation is devoted to the study of the morphogenesis of grass carp (*Ctenopharyngodon idella*) from various fishery reservoirs (the Ile River and the Kapchagay Reservoir).

Relevance of the dissertation topic. Currently, increasing anthropogenic pressure on the environment leads to the destabilization of natural ecosystems, a decrease in biological diversity and an increase in morbidity among the population [1]. Of great concern in the world is the pollution of water bodies as sources of clean drinking water and habitats for aquatic organisms, including commercial fish [2]. Thus, it can be said that the biodiversity of aquatic organisms reflects the ecological state of the reservoir. Today in Kazakhstan it is almost impossible to find clean rivers, lakes, reservoirs and other habitats for aquatic organisms [3]. Pollution of water bodies occurs as a result of uncontrolled development of agriculture and industry [4]. At the same time, not only water, but also bottom sediments and soils of coastal zones are polluted with various xenobiotics. Many domestic researchers note a decrease in the number of native and introduced fish species, as well as other aquatic organisms in the Ile River and the Kapshagay Reservoir [5-6].

The Ile River is one of the main waterways of the southeastern region of Kazakhstan. It provides up to 80% of the total water inflow into Lake Balkhash, flowing into its western part. In the 60-70s, a dam and the Kapshagay State District Power Plant were built to regulate the river flow, resulting in the formation of an artificial reservoir with a volume of 28.14 km³. Currently, the Kapshagay Reservoir is the second largest reservoir in Kazakhstan, which is not only a source of hydraulic energy, but also a reservoir for the needs of irrigation, fish farming and a recreational and resort area for residents of the metropolis of Almaty and the Almaty region [7]. In this regard, the Ile River and the Kapshagay Reservoir are subject to anthropogenic load and may contain a variety of pollutants with toxic and genotoxic activity. Many pollutants can cause sterility, metabolic disorders and various functions of aquatic organisms [8]. As is known, fish and other aquatic organisms grow and develop in the aquatic environment. Disruption of their embryogenesis as a result of exposure to unfavorable conditions leads to the appearance of defects and deformities that reduce the viability and survival of the species, resulting in the death of entire populations and depletion of bioresources [9].

All of the above indicates the importance and necessity of conducting a comprehensive study of water, development, and the state of adult forms of valuable commercial fish from various reservoirs of Kazakhstan.

Objective: To study the morphogenesis of grass carp (*Ctenopharyngodon idella*) from various fishery reservoirs (the Ile River and the Kapchagay Reservoir,

pond farming).

Research objectives:

1. To determine the chemical composition of surface waters from the Ile River, Kapshagay Reservoir and pond farms in the Almaty region.
2. To study the effect of heavy metals on the development of grass carp in an experiment.
3. To determine the expression level of the Wnt- β catenin genes of the signaling pathway regulating the embryonic development of grass carp under normal conditions and under the influence of heavy metals.
4. To study the role of oxidative stress and metallothionein in the development of grass carp under normal conditions and under the influence of heavy metals.
5. Determine the content of heavy metals (zinc, copper, lead) in the visceral organs of grass carp from the Ile River, Kapshagay Reservoir and pond farm
6. Determine the content of metallothionein, superoxide dismutase and catalase activity in the liver of adult grass carp from the Ile River, Kapshagay Reservoir and pond farm
7. Conduct a genotoxic analysis of blood cells of grass carp from the Ile River, Kapshagay Reservoir and pond farm
8. Study the histostructure of visceral organs of grass carp from the Ile River, Kapshagay Reservoir and pond farm

Object of the study: eggs, embryos, larvae, adults, blood and visceral organs of grass carp from different reservoirs of Kazakhstan.

Subject of the study: chemical composition of water from different sections of the Ile River and the Kapchagay Reservoir, pond farming, embryogenesis of white amur, cyto- and genotoxicity of heavy metals detected in the surface waters of the studied reservoirs.

Research methods. Chemical, embryological, biochemical, molecular biological, genetic, histological and statistical methods were used in the dissertation.

Scientific novelty of the study:

1. For the first time, using physicochemical methods, the content of heavy metals such as Zn, Cu and Pb in the surface waters of the Ile River and the Kapshagay Reservoir during the study period was established to exceed the MAC for fishery water bodies.
2. For the first time, based on embryo mortality rates and identified deviations from the normal development of grass carp larvae, it was established that the following TMs have the greatest embryotoxic and teratogenic effect: Pb > Cu > Zn.
3. For the first time, it was shown that heavy metals: Zn, Cu and Pb suppress the expression of the Wnt - β catenin genes of the signaling pathway regulating the normal development of grass carp, which leads to embryotoxic and teratogenic effects (lethality and deformities incompatible with the survival of larvae under these conditions).
4. For the first time, a dose-time dependence of the metallothionein (MT) protein content, superoxide dismutase (SOD) and catalase (CAT) activity on the effective concentration of the studied TM in grass carp larvae during

development was established, indicating the activation of oxidative stress in them.

5. For the first time, accumulation of Zn, Cu and Pb was established in the liver, gills and intestines of grass carp caught in different parts of the Ile River and the Kapchagay Reservoir (№ 1-4), except for fish from sections №5 and № 6.
6. For the first time, structural abnormalities and damage to the DNA of erythrocytes in the peripheral blood of grass carp from the Ile River and the Kapchagay Reservoir were detected, indicating the effect of mutagenic factors on fish.
7. For the first time, a decrease in the MT content and SOD and CAT activity in the liver of adult grass carp from different parts of the Ile River was revealed. In this case, the greatest development of oxidative stress was observed in fish in section №1 (upper reaches of the Ile River), which is probably associated with the pollution of water with heavy metals in this section and their accumulation in visceral organs.
8. For the first time, using histological and morphometric research methods, it was established that the highest incidence of fish with histopathologies of the gills, liver and intestines was observed in sections along the upper reaches of the Ile River and in the Kapchagay Reservoir near the dam and the city of Konayev (№1-4), to a lesser extent in the northern part of the Kapchagay Reservoir near the pumping stations (№5).

Thus, in this complex work, using highly informative methods of physicochemical analysis, toxicological, embryological, molecular genetic, biochemical, histological and morphometric methods, the surface waters of the Ile River, the Kapshagay Reservoir and the pond farm of the Almaty region, the morphogenesis of larvae and the state of adult forms of one of the valuable commercial fish – grass carp, caught in different parts of these reservoirs of Kazakhstan were studied. As a result of the studies, pollution of the surface waters of the studied reservoirs with heavy metals was revealed, especially Zn, Cu and Pb, the content of which exceeded the MAC for fishery reservoirs. In experiments on eggs and larvae of white amur, the processes of morphogenesis were studied under the influence of the identified pollutants (heavy metals) and their adverse effects were established, leading to high mortality of embryos and anomalies in the development of larvae. The basis of the embryotoxic and teratogenic action of these pollutants is the suppression of the expression of the Wnt/ β -catenin signaling pathway genes and the activation of oxidative stress. The experimental data were confirmed by the detection of heavy metals in the visceral organs of adult white amur from different parts of the Ile River and the Kapshagay Reservoir, as well as anomalies in the morphology and DNA damage of blood cells, histostructural pathologies of the visceral organs. The pathogenetic mechanism of such changes in adult fish, as well as in larvae, was the activation of oxidative stress, an indicator of which was the suppression of the work of antioxidant enzymes (SOD and CAT) and a decrease in the content of metallothionein protein, which binds TM and protects against oxidative stress. The identified biochemical, structural and mutagenic disorders in white amur raise certain concerns about the state of not only aquatic organisms, but also the biota

in the Balkhash region as a whole, as well as the health of the population consuming fish products and water from the studied reservoirs.

Based on the obtained results, the following conclusions can be made:

1. Physicochemical analysis of surface waters from different sections of the upper reaches of the Ile River and the Kapchagay Reservoir revealed the presence of the following heavy metals: Pb, Co, Mn, Cd, Cu, Zn, Fe, Ni, Cr, however, the content of only three of them: Zn, Cu and Pb exceeded the MAC for fishery reservoirs. The greatest excess of the MAC for Zn, Cu and Pb was found in the surface waters of the upper reaches of the Ile River, the Kapchagay Reservoir (near the dam of the State District Power Plant and the city of Konayev), and the smallest - in its northern part (near the pumping stations). At the same time, the pH of the water, the content of dissolved oxygen, nitrates and nitrites in the water were at the MAC level.
2. Biotesting of the effect of Zn, Cu and Pb on the development of grass carp, one of the valuable commercial species, revealed their dose-dependent embryotoxicity and teratogenicity: embryo lethality, various morphological anomalies in surviving larvae, and growth retardation. The highest percentage of developmental disorders was 51.6% at a lead concentration of 100SHRK compared to 7.2% in the control group. Moreover, the embryotoxic and teratogenic effect among the studied metals was manifested in the following order: lead > copper > zinc.
3. The effect of Zn, Cu and Pb on grass carp larvae significantly suppressed the expression of the *Wnt1*, *Gsk3 β* , *β -catenin*, *Lef1*, *Axin2*, *Myca* and *Cd1* genes of the Wnt/ β -catenin signaling pathway. In particular, the expression of target genes was significantly reduced at 100 MPC of zinc, while copper and lead were reduced at 10 and 100 MPC.
4. A dose-time dependence of the MT content, SOD and CAT activity in grass carp larvae during development was established when exposed to different concentrations of Zn, Cu, Pb. Exposure to these TM at concentrations of 10 and 100 MPC caused a statistically significant increase ($p \leq 0.05$) in the MT content, SOD and CAT activity in fish larvae in the period from 24 hours to 144 hours of development.
5. The content of Zn, Cu and Pb in the visceral organs of grass carp from different parts of the Ile River, Kapchagay Reservoir and pond farm was determined, which exceeded the MAC standards in almost all study points, except for № 5 (northern part of the Kapchagay Reservoir) and № 6 (pond farm). At the same time, the content of Zn, Cu and Pb exceeded the MAC in the organs of grass carp in the following sequence: liver > gills > intestines.
6. The following morphological abnormalities of blood cells were revealed: micronuclei, amitosis, invagination of nuclei, apoptosis, vacuolization of cells. The total percentage of morphological abnormalities of erythrocytes compared to the control group was: the highest in the upper reaches of the Ile River by 3.9 times, and the lowest in the area of the northern pumping station of the Kapchagay Reservoir (№ 5) by 1.4 times. The level of DNA damage at point №1 was 55.7%; №2 - 51%; №3 - 41%; №4 - 29.5%; №5 - 35%, while in the

control group only 17.5% of damage was recorded.

7. The activity of antioxidant enzymes (SOD and CAT) and the content of metallothionein in the liver of grass carp taken for the study from the Ili River and the Kapchagay Reservoir decreased. This, in turn, causes oxidative stress. The most pronounced change in the oxidative stress index was observed at study point №1 (the upper reaches of the Ili River): the amount of MT increased by 1.5 times; SOD activity by 1.7 times; CAT activity decreased by 2.0 times.
8. Histological and morphometric studies have shown that the highest incidence of fish with histopathologies of the gills, liver and intestines is observed in the Ile River (upper reaches) and in the Kapchagay Reservoir near the dam and the town of Konayev (№1- 4), and to a lesser extent in the northern part of the Kapchagay Reservoir near the pumping stations (№ 5). Fish from the pond farm (№ 6) were practically healthy. The main histopathological changes in grass carp from the above-mentioned sections of the Ile River and Kapchagay were: in the gills - degenerative changes in the second-order gill filaments (lamellae), hyperplasia and necrosis of gill epithelial cells; in the liver - vascular congestion, expansion of the Disse spaces, fatty degeneration and necrosis of hepatocytes; in the intestines - edema of the submucosal layer, inflammatory infiltrates, sloughing of epithelial cells at the tips of intestinal villi. Many of these destructive processes are incompatible with the life of fish, which leads to a decrease in biodiversity and bioresources of water bodies.

Scientific and practical significance of the dissertation: The obtained results complement and expand the available scientific information on the embryogenesis of lower vertebrates, in particular, fish in the conditions of anthropogenic impact on their habitat. Thus, based on biotesting of the surface waters of the Ile River and the Kapshagay Reservoir, conducting a comprehensive biochemical, genetic, morphological and morphometric study of the development of white amur larvae, an important conclusion was made on the pathogenesis of the negative impact of heavy metals on aquatic organisms, leading to their death as a result of the activation of oxidative stress and the initiation of mutation processes. The results of the dissertation can be used in the development of sanitary and hygienic standards for fishery reservoirs, for the organization of safe fish farming and catching commercial fish, will contribute to sustainable development in this sector of the economy, improving the environment and public health. The results of the conducted research have been introduced into the educational process at the Department of Biodiversity and Bioresources of the Kazakh National University in the course “Cell Biology and Histology”, 2nd year of bachelor’s degree in doctoral studies in the specialty “5B095102 – Biology” (Appendix A).

The main provisions submitted for defense:

1. In the surface waters of the Ile River and the Kapshagay Reservoir, the content of Zn, Cu and Pb exceeded the MAC for fishery water bodies.
2. According to the indicators of embryo mortality and the identified deviations from the normal development of grass carp larvae, the greatest embryotoxic and teratogenic effect is possessed by: lead > copper > zinc.

3. The level of expression of the Wnt - β catenin genes of the signaling pathway regulating the development of grass carp is suppressed when exposed to Zn, Cu and Pb.
4. The content of metallothionein (MT) and the activity of antioxidant enzymes (SOD and CAT) in grass carp larvae under the influence of heavy metals significantly increased. On the contrary, the content of MT and the activity of SOD and CAT in the liver of adult fish from different parts of the Ile River and the Kapshagay Reservoir decreased.
5. The content of heavy metals (Zn, Cu and Pb) exceeded the MAC level in the visceral organs of grass carp from different parts of the Ile River and the Kapchagay Reservoir, which caused the development of pathological processes in the fish organism.
6. Surface waters from different parts of the Ile River and the Kapchagay Reservoir showed high genotoxic activity, inducing DNA damage to grass carp blood cells with a statistically significant frequency.
7. The highest incidence of fish with histopathologies of the gills, liver and intestines was observed in the Ile River (upper reaches) and in the Kapchagay Reservoir (near the dam and Konayev town), but to a lesser extent - in the northern part of the reservoir. Fish from the pond farm of the Almaty region were practically healthy.

The applicant's personal contribution consists of direct participation in collecting material for the dissertation, analyzing the results obtained, and statistically processing quantitative data. The author independently analyzed, summarized, and presented the results obtained, and formulated conclusions. The text of the dissertation is written according to a plan agreed upon with the scientific supervisors. The share of the author's personal participation in joint publications is proportional to the number of co-authors.

Compliance of the work with the scientific research program. The dissertation was completed within the framework of the project of the Ministry of Education and Science of the Republic of Kazakhstan, grant №AP14869740 "Biotesting of water and bottom sediments of the Ili River and the Kapchagay Reservoir and forecasting environmental risks for the biodiversity of the studied ecosystems" (2022-2024).

Verification of the results of the dissertation. The results of the dissertation research were presented and discussed at the following international scientific conferences:

- International scientific conference of students and young scientists "World of Farabi", Kapchagay, Republic of Kapchagay, April 6-9, 2020;
- At the 5th International Scientific Conference «Reviews of Modern Science» (February 1-2, 2024) Zürich, Switzerland;

Publications. The main results of the dissertation are published in 8 scientific papers, including 2 articles with quartile indicators Q2 (SJR - 0.659) and Q3 (SJR - 0.505). in high-level scientific journals included in the Web of Science and Scopus databases; 4 articles in domestic periodicals recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and

Higher Education of the Republic of Kazakhstan; 2 abstracts were published in the proceedings of an international conference.

Structure and volume of the dissertation. The dissertation is written on 94 pages of text. It includes normative references, designations and abbreviations, introduction, literature review, research materials and methods, research results and their discussion, conclusion and a list of 227 references. The dissertation contains 26 figures and 10 tables.