KAPITEL 6 / *CHAPTER 6* [6]
THE IMPACT OF ANTHROPOGENIC XENOBIOTICS ON AQUATIC LIFE

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Introduction.

The structure of aquatic ecosystems, in addition to natural factors, depends on a significant number of external factors that affect the state of populations, interspecific relationships, composition and properties of the aquatic environment, physiological and biochemical processes in aquatic organisms, species composition and number of fish fauna [1].

However, despite a number of environmental protection measures, anthropogenic pressure on aquatic ecosystems is increasing due to the increased discharge of significant volumes of process wastewater from industrial enterprises and energy facilities, and recently also from large livestock production complexes, food processing plants, and veterinary medicine facilities into natural and artificial water bodies [2].

Waste from these enterprises contains a significant amount of water pollutants and can disrupt the functioning of natural ecosystems not only in the area of their production activities but also beyond, entering the soil, groundwater and surface water with wastewater [3].

At the same time, the problem of the impact of livestock facilities on aquatic ecosystems requires in-depth and comprehensive research on the components of pollution in wastewater, their impact on the hydrochemical composition of water, embryonic development processes, morphological characteristics, morphophysiological parameters, and the state of plastic metabolism in fish tissues, as the main representatives of aquatic ecosystems, in order to find effective molecular markers of aquatic organisms to characterise and assess the ecological status of water bodies.

6.1. Features of ontogenetic development of freshwater fish under the influence of xenobiotics.

Pollution of water bodies by wastewater from industrial enterprises and, in recent years, livestock farms containing a significant amount of xenobiotics of organic and

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mineral origin leads to deterioration of aquatic ecosystems, including changes in ichthyocenoses. The latter are important bioindicators of environmental monitoring of anthropogenic impact on aquatic ecosystems. Such bioindicators include different species of fish, the content of toxic compounds in water and tissues of aquatic organisms, the activity of a number of enzymes in tissues, the morphological composition of blood, morphometric features, and indicators of plastic metabolism in tissues [4].

Studies have shown that the most sensitive to the effects of unfavourable factors of the aquatic environment in fish are the embryonic, larval and fry periods of development, as opposed to juvenile and adult age. It has been established that the maximum changes in development, including embryo death under the influence of toxicants of various nature, occur during the embryonic period at the stage of crushing and gastrulation, and then feeding of pre-larvae, especially after their transition to mixed feeding [5].

The ionic composition of water, temperature, oxygen content, pH and water illumination are considered to be mandatory abiotic factors, and the content of carbon dioxide, hydrogen sulfide, and ammonia in water is considered to be optional abiotic factors. The effect of the above factors on fish is significantly enhanced by anthropogenic load, which causes significant changes in the content of these components in water due to the ingress of xenobiotics of various chemical natures into water bodies. The organism of different fish species, depending on the type of component, adapts to changes in its content within certain limits, which is determined by the reaction rate and ensures homeostasis. The reaction of fish to the action of exogenous (abiotic) factors in early ontogeny can be manifested by changes in a number of morphophysiological parameters, namely survival rate, rate of embryonic and postembryonic development, growth of embryos, larvae and fry, such meristic traits as the number of myomers, vertebrae, scales, etc., intensity of respiration and metabolic processes, occurrence of structural and functional developmental disorders in embryos, larvae and partially in fry. The effect of endogenous factors on fish in early ontogeny, in addition to affecting a number of morphophysiological parameters, changes the rate of growth and development, linear indicators of fish in certain periods of development, plastic and meristic traits [6].

The impact of toxic substances on fish occurs at all stages of their development, from fertilisation of eggs to mature individuals. However, the most vulnerable link in the ontogenetic development of fish is the embryonic and early postembryonic period.

Therefore, the reaction of fish to chemical water pollution due to anthropogenic impact is usually determined by the effect of a toxic factor in the early stages of ontogeny. In addition, the developmental stages of fish that are most exposed to toxicants are used to justify the MPCs for water pollutants. The same stages of fish development are also taken into account when developing methods for biotesting natural water bodies in case of water pollution with xenobiotics of various origins [7].

It is known that the reaction of fish to various types of pollutants depends on their type, concentration in water, mechanism of action, depositing capacity, rate of detoxification and excretion, as well as other factors. The impact of various water pollutants on fish is associated with disruption of the central nervous system, which causes dysfunction of various organs and body systems [8]. The negative effect of organic water pollutants on fish is manifested at the population, genetic, morphophysiological and molecular levels. This is confirmed by the impact of various pesticides and detergents on fish, which is manifested in an increase in the number of chromosomal aberrations in germ cells, changes in the number of vertebrae, and the occurrence of axial skeletal abnormalities in roach [7]. Some organic water pollutants have a positive effect on the development of carp eggs at low concentrations, while at high concentrations they have the opposite effect. Among the water pollutants in fish farming ponds, nitrogen-containing compounds such as nitrates, nitrites and ammonia attract special attention of researchers. In fish, exposure to water nitrates, which penetrate into the blood and tissues through the gills, causes significant changes in metabolic processes in the body associated with methemoglobinemia, hypoxia, and anaemia [8].

Fish are more affected than nitrates by ammonium nitrogen, which can cause toxicosis, activate the mechanisms of its binding and transformation into less toxic compounds [9].

As noted earlier, various types of pollutants have the most negative impact on fish in the early stages of ontogeny. The effect of toxicants on fish during this period depends on the concentration in water, the rate of penetration through the egg shell into the prevertebral fluid, and the cumulative ability of xenobiotics. As evidenced by the data obtained, it is likely that the proteins of the egg shell form stable complexes with water pollutant molecules that prevent their penetration into the cell. This may be one of the defence mechanisms against the negative impact of toxicants on the embryonic development of fish [10].

Indicators of embryonic development of fish were examined visually on newly

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collected female carp eggs, which were artificially fertilised, sown in Petri dishes and the number of dead eggs, the number of live embryos and larvae that were excluded was monitored. The acute toxicity of xenobiotics was determined on fish eggs and embryos in accordance with the requirements of ISO 7346-1, ISO 7346-2 and ISO 7346-3 [275, 405-409]. Embryonic development of fish was characterised by the stages of egg development and larval hatching. Body abnormalities in carp larvae were determined by their appearance at certain stages of their development. In embryos, the underdevelopment of certain body parts, abnormal shape and size of the yolk sac, saber-shaped body, hydrocele, and heart failure were monitored.

6.2. The impact of wastewater on the embryonic development and survival of carp larvae.

Ammonia is present in the water of most of the studied water bodies as a product of the breakdown of proteins and amino acids of plants and animals, microorganisms and freshwater fish excrement. The source of ammonia in pond water is also liquid manure and manure effluents from livestock facilities, as mentioned above.

It is known that fish resistance to ammonia is species-specific, but these differences are insignificant and are already evident even with short-term exposure. Therefore, the maximum permissible levels of ammonia in water for different fish species differ significantly. These factors were taken into account in further studies. Control over the number of dead carp embryos, which was carried out during critical periods of egg development, showed that during the period of blastodisc fragmentation and morula formation at a concentration of NH4+ of 0.05 mg/dm^3, the number of dead eggs was 23.2%, at a concentration of 0.5 mg/dm^3 - 84%, at a concentration of 5 mg/l - 89.1%, while in the control this figure was 33.9%.

After gastrulation, the death of carp embryos in the experimental variants under the influence of ammonia, determined in three sequences of studies, was approximately at the same level and amounted to 61.7 %, 68.7 and 54.4 %, respectively. The total number of live eggs in the control was 28.6 %, while in water with a concentration of NH4+ of 0.5 mg/dm^3, more than 80 % of embryos died at the segmentation stage and before the separation of the tail section. Before hatching, the mortality rate of eggs in this group reached 86.3 %. At a concentration of NH4+ in water of 0.5 mg/dm^3, the number of live eggs was 20.3 %. In the third series of studies with a water NH4+

concentration of 5 mg/dm^3, embryos died during the formation of eye vesicles. Earlier hatching of some embryos was observed at a concentration of NH4+ in water of 0.5 mg/dm^3. However, 3.5 days after fertilisation, the number of embryos released from the shell at NH4+ concentrations of 0.05 and 0.5 mg/dm^3 was the same. The highest egg loss was observed in all experimental variants during the first two days. The remaining live eggs became resistant to ammonium ions and practically did not die in the following days. During hatching, the egg yield in all experimental groups increased, averaging 7-8%. The surviving individuals continued to exist until the end of the yolk sac suction.

Over time, at the initial stages of splitting, eggs developed with an ammonia concentration of 5 mg/dm3 were ahead of embryos from other experimental variants in this respect. At a certain survival rate of eggs, the water environment with an ammonia concentration of 0.05 and 0.5 mg/dm^3 was both stimulating and protective up to a certain point in embryo development.

The obtained results confirm the conclusion about the toxic effect of ammonia and accelerated hatching of pre-larvae followed by the onset of death.

In some cases, the process of embryo development under the influence of ammonia is disrupted, which manifests itself in morphological deformities, late eye pigmentation, dehydration of embryos and leads to their death.

Studies on the effect of different doses of wastewater on the development of carp larvae were conducted on three-day-old individuals obtained from artificially fertilised fish eggs. The carp larvae were placed in water to which wastewater with a concentration of NH4+ of 0.05 (first), 0.5 (second) and 5 mg/dm^3 (third experimental group) was previously added. The characteristics of carp larvae development indicators under the influence of pig farm wastewater are given in Table 1.

The analysis of the results showed that at the initial stage of the experiment, namely 2, 4, 6, 12 and 24 hours after its start, the impact of pig farm wastewater on the larval survival was insignificant. All specimens in both the experimental and control groups remained alive, and their safety was 100 % (see Table 1).

There was no significant effect of wastewater even in the concentration of ammonia content of 0.05 (first), 0.5 (second) and 5.0 mg/dm^3 (third experimental group) on carp larvae on the second day of the experiment. The survival rate of carp larvae during this period in the experimental groups did not differ from the control and was 100 %. Only on the fourth day of the experiment in the third experimental group,

Table 1 -The impact of pig farm wastewater on the survival of carp larvae, pcs, %, n=20

Research period through:	Groups			
	control	experimental (ammonia concentration, mg/dm^3)		
		0,05	0,5	5,0
2 hours	20/100	20/100	20/100	20/100
4 hours	20/100	20/100	20/100	20/100
6 hours	20/100	20/100	20/100	20/100
12 hours	20/100	20/100	20/100	20/100
24 hours	20/100	20/100	20/100	20/100
2 days	20/100	20/100	20/100	20/100
4 days	20/100	20/100	20/100	25/25
5 days	20/100	17/85	12/60	1/5
6 days	20/100	3/15	1/5	died
7 days	17/85	died	died	-

Note: In Table 3.11, the number of live carp larvae is given in the numerator; the percentage of survival is given in the denominator.

where the ammonia content of the pig farm wastewater was 5.0 mg/dm^3, a significant larval mortality was observed, which amounted to 75 % of the initial number. A significant decrease in the survival rate of carp larvae was observed in all experimental groups on the fifth day of the experiment. Thus, in the first experimental group, the mortality rate of carp larvae was 25 %, in the second experimental group - 40 % and in the third - 95 %, while in the control group the number of live larvae was 100 % (see Table 1).

Further studies with carp larvae showed that when exposed to an ammonia concentration of 5.0 mg/dm^3, the mortality rate was 100 % on the sixth day. In other variants of the experiment, at NH4+ concentrations of 0.05 and 0.5 mg/dm^3, 100 % larval mortality was observed on the seventh day of the experiment. At the same time, on the sixth day, the survival rate of carp larvae in the first experimental group was 15 %, and in the second only 5 %, while in the control group it was 100 %.

It should also be noted that in water with a concentration of NH4+ of 5 mg/dm^3, in the first minutes of exposure to the toxicant, the larvae froze, did not respond to water movement and touch. However, after 20 minutes, active chaotic movement of the larvae was observed, indicating a negative effect of ammonia on them. After one hour of exposure, the larvae made random movements in the horizontal and vertical directions, rose to the water surface and sank to the bottom again. After 2-4 hours from the beginning of the experiment, the larvae lost their activity and became sedentary. Similar processes in the activity of the larvae of this group were observed over the next five days. The larval behavior in water periodically changed from active to passive.

In all the studied larvae, a gradual discoloration of the body covers was observed due to a decrease in the size of pigment spots. On the sixth day of the experiment, all larvae in this group died. In most of the dead larvae, the body was bent at right angles in the swim bladder area, the eyes acquired a dark silver hue and were enlarged in size.

Conclusion.

The results obtained from the experiment on the impact of pig farm wastewater on the development of carp larvae clearly indicate their toxic effects on carp fish, especially during early postnatal development. Since the oxygen content in the water, pH and water temperature remained virtually unchanged during the cultivation of carp larvae, which excludes the influence of these factors on the development of larvae during sensitive periods, it is possible to assert the direct rather than indirect toxic effect of ammonia and other wastewater pollutants on fish. The behavior of the larvae at the initial stages of the experiment, i.e. on the first day, indicates a slight toxic effect of wastewater on the larvae. As the time spent by the larvae in this environment increased, their condition remained stable over the next day, which may indicate the effect of their adaptation to these pollutants. Thus, the ability of ammonia to accumulate in the body of fish was confirmed, which enhances its ability to adversely affect embryogenesis, causing early death of larvae in variants with the highest concentration of the toxicant. It is also possible that fish may be affected by other toxic substances in wastewater, and control of dead fish larvae may be an important criterion for assessing the ecological state of ponds when polluted by various wastewater from pig farms.