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The role of extreme environmental factors in mass fish mortality: a case study of reservoirs in Belarus

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ABSTRACT. The article examines the cases of mass death of fish in the reservoirs of Belarus, occurring without an obvious reason. Possible abiotic and biotic factors are analyzed, including oxygen deficiency, the "blooming" of water by blue-green algae, parasitic and infectious diseases, as well as the hydrochemical characteristics of the aquatic environment. The need for comprehensive and long-term monitoring is emphasized in order to identify the true causes of death and develop effective measures to reduce the negative impact on the ichthyofauna. Based on the collected data, an approach is proposed for assessing and minimizing environmental risks for fish populations.

Keywords: Fishing, hydrochemistry, phytoplankton, parasitology, hematology

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1. Introduction.

In recent years, cases of "unmotivated" (i.e., without an explicit factor) mass deaths of fish or individual fish species have become more frequent in the reservoirs of Belarus. Such cases attract the attention of not only ichthyologists, but also the general public, as they are an alarming signal of violations in the functioning of aquatic ecosystems (Ramachandra et al., 2016; Svobodova et al., 1993; Davie and Pera, 2021). The death of fish was observed, as a rule, either during a period of consistently high air and water temperatures, or during their abrupt change. Such seasonal timing indicates a possible relationship between the physico-chemical parameters of the aquatic environment and the physiological state of aquatic organisms, but does not allow us to identify a specific cause-and-effect mechanism. Seasonal fish mortality has been noted in many studies and had various causes (Oskarsson et al., 2018; Godinho et al., 2019; Landsberg et al., 2020; San Diego-McGlone et al., 2024).

However, neither an increase in temperature, nor its sudden changes, nor fluctuations in other hydrochemical parameters (pH, dissolved oxygen, organic and mineral substances), nor the presence of blue-green algae and opportunistic bacteria in the water, nor pollution – none of these factors individually explains fish mortality or serves as its direct cause. This indicates the multifactorial nature and complexity of the phenomenon under consideration. Studies conducted in various regions with similar environmental situations also confirm that all indicators are closely related to each other (Sukhovilo et al., 2024; Kirvel et al., 2023; Vezhnavets, 2024).

To determine the true cause, it is necessary to consider all these factors in combination and to monitor them for a sufficiently long time. Only a systematic approach based on comprehensive hydrobiological, hydrochemical, ichthyological and ichthyopathological observations can provide an adequate understanding of the changes taking place and their consequences for the aquatic biota.

Despite the obvious importance of this problem, no clear causes and mechanisms of the lethal effects of environmental conditions on the ichthyofauna of reservoirs have been established so far during the massive "freezes" during the periods mentioned above. This indicates a lack of knowledge of both local and global environmental processes affecting aquatic communities.

A review of the probable causes of fish death suggests that many abiotic and biotic factors may be involved. It is necessary to take into account not only direct effects, but also indirect ones, such as stress factors that reduce the body's immune defenses and increase its sensitivity to pathogens. Since the death of fish in reservoirs in most cases was not a one-time

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event, it covered a certain period and was recorded for 6 species of native and non-native fish, as well as one species of commercial invertebrates (crayfish), it can be assumed that one common or complex of similar negative factors affected. Such selectivity and temporal extent indicate a systemic disturbance of the ecological balance in water bodies.

Among the most likely causes of noted fish death, the following should be considered: deficiency of dissolved oxygen (Burkholder et al., 1999; Abdel-Tawwab et al., 2019), an active reaction of the medium (Landsberg et al., 2020; Brown et al., 1983), salt and biogenic composition of water (Demeke and Tassew, 2016); "blooming" of water with the presence of cyanotoxins (Jewel et al., 2003; Sevrin-Reyssac and Pletikosic, 1990); parasitic and infectious diseases (Faruk, 2018; Overstreet, 2021); bacterial contamination of the water mass (Popovic et al., 2019; Dar et al., 2020). It is important to note that each of these factors can have both individual and synergistic effects on aquatic organisms, which makes it difficult to determine the dominant cause.

Without a clear understanding of the factors and mechanisms behind the pathological process, efforts to prevent the lethal effects of extreme conditions on the ichthyofauna of Belarusian reservoirs will remain short-term and symptomatic. This will not allow effective development and implementation of preventive measures aimed at preserving biodiversity and sustainability of aquatic ecosystems. The collected, processed and analyzed material on the hydroecological state of water bodies, ichthyological and ichthyopathological studies, together allow us to draw conclusions about the possible causes of fish death and develop a methodology to minimize the impact of established negative factors on the fish population of reservoirs and watercourses.

2. Materials and methods.

Reservoirs and watercourses where fish deaths were recorded were selected as observation sites during the research period. The observations covered 2 reservoirs in the Mogilev region of Belarus: the Chigirinsky reservoir – the Drut River system; the Osipovichskoye reservoir – the Svisloch River system. The location of these reservoirs is shown in Figure 1.

The collection and processing of samples to characterize the hydrochemical regime of the water bodies were carried out in accordance with the methods described by Plotnikov (2017) and Kozhina (1977). Samples were taken from both surface and bottom layers, resulting in an integrated indicator. To characterize the microalgae community during the open water period, samples were taken and the collected phytoplankton material was processed in accordance with the methods of hydrobiological studies (Oksiuk, 1993; GOST 17.1.1.04-77, 1977).

For molecular genetic analysis, phytoplankton samples were fixed in 96% ethanol. A set of Genomic DNA purification reagents (Thermo Scientific, Lithuania) was used to isolate DNA from samples, in accordance with the manufacturer's attached instructions. The following primers were used for amplification: HEPF 5'-TTTGGGGTTAACTTTTTT-GGGCATAGTC and HEPR 5'-AATTCTTGAGGCTGTAAATCGGGTTT (Jungblu and Neilan, 2006). The amplification program was as follows: 2 min at 92 °C, followed by 35 cycles of 92 °C for 20 s, 52 °C for 30 s, and 72 °C for 1 min. In the final cycle, the elongation step was extended to 12 min. The resulting PCR products were analyzed by horizontal electrophoresis in 1.5% agarose gel using 0.5% TAE (Tris-acetate) buffer (20 mmol/L Tris-OH, 0.5 mmol/L EDTA, 7.8 mmol/L CH_3COOH, pH 7.6). The gel was stained with ethidium bromide. A VDS-CL gel scanner (Amersham Biosciences, USA) was used to display the electrophoresis results.

Samples for water contamination were taken directly at the reservoir in Eppendorf tubes (1.5 ml) by immersion in the water column. To determine the total microbial contamination of the water, a series of dilutions was prepared in such a way that from 30 to 300 colonies of microorganisms grew on Petri dishes. The dilution was prepared as follows: 1 ml of test water was taken from each reservoir with a sterile pipette and

Fig.1. Location of research objects on the map of Belarus.

9 ml of distilled water was added (dilution 1:10), thoroughly mixed. Next, 1 ml was taken from tubes with a dilution of 1:10 with a sterile pipette and added to tubes with 9 ml of distilled water, obtaining a dilution of 1:100. 1 ml of probiotic was added to a sterile Petri dish at a dilution of 1:100 and filled with warm (450) meat peptone agar (MPA). It was left at room temperature until it solidified, then placed in a thermostat at 35 °C for 24 hours. After a day, the growth of microbial colonies on the surface and in the MPA thickness was evaluated based on 1 ml of undiluted samples of the studied water (Musselius, 1983) was used as a comparison parameter.

Microbiological examination of fish, sampling for seeding and the method of seeding microbiological material were carried out in accordance with the methods of bacteriological research (Musselius, 1983; Golovina, 2003; Methodological guidelines, 1999). Crops from internal organs, blood, exudate (if any) fish were produced according to the standard procedure on a solid nutrient medium (micopeptone agar - MPA). After incubating the crops in a thermostat at 20-280 ° C for 24 to 48 hours, their morphology was studied. The bacterial species was determined using the Oxi – test and Api 20E test systems. Hematological and biochemical parameters were studied for the fish caught during the control catches: total serum protein, amount of hemoglobin, ESR, content of erythrocytes and leukocytes. Hematological studies were performed according to the methods (Ivanova, 1982; Golovina, 1989). The evaluation of the results was carried out based on the normative blood parameters of carp fingerlings and data from literary sources (Golovina, 1989).

3. Results and discussions.

Analyses of the quality of the water mass during the subglacial period were carried out in the reservoirs of Chigirinskoye (Drut river system) and Osipovichskoye (Svisloch river system), where cases of mass fish death were noted. The results of the main indicators are presented in Table 1. Hydrochemical indicators of water quality are shown in Figure 2.

The temperature regime in the analyzed reservoirs corresponded to the seasonal distribution and was unlikely to have negative consequences for fish. In reservoirs, reverse stratification of the water mass was observed, with a slight increase in water temperature from the surface of the ice to the bottom.

The gas regime in the Chigirinskoye and Osipovichskoye reservoirs was marked by a slight decrease in the dissolved oxygen content relative to the optimum, but the values did not exceed the critical values for fish. The active reaction of the medium was alkaline (pH = 8.0-8.1), which is due to the predominance of reducing reactions.

According to the salt composition, the water of the Chigirinskoye and Osipovichskoye reservoirs belongs to the bicarbonate-calcium type with an average (4.5-4.7) hardness. Concentrations of biogenic elements, whose participation in redox reactions is reflected in the gas regime, may be of limiting importance for fish during the subglacial period. Increased concentrations of ammonium nitrogen and mineral phosphorus were observed in both reservoirs, and nitrite nitrogen in the Osipovichskoye reservoir. The latter is classified by pollution indicators as "heavily polluted" and "very dirty" (Oksiuk, 1993). This is due to the fact that the reservoir serves as a receiver of the waters of the Svisloch River and the discharge of wastewater treatment plants in Minsk (Fig. 3). Ammonium nitrogen at elevated pH values (more than 7.5) is capable of converting into toxic free ammonia and causing acute toxicosis of fish. The background indicator of the safe content of ammonium nitrogen for surface reservoirs for fisheries purposes is up to 0.39 mg/l, whereas during the ice age, concentrations of ionized ammonium reached 0.86 mgN/l in the Chigirinskoye reservoir, and 1.89 mgN/l in the Osipovichskoye reservoir. Under the prevailing temperature background of the water, such an ammonium ion content is able to maintain a concentration of non-ionized ammonia of about 0.01 mg/l, which does not exceed the permissible values for fish ponds. Consequently, acid toxicosis at the observed temperature background was not a threat to the life of the fish.

In general, according to the indicators of water transparency, its salt composition and oxidizability, the analyzed reservoirs should be attributed to the eutrophic type of reservoirs (α- and β-mesozoic).

Table 1. Main indicators of water quality during the ice age.

Indicators	Units of measurement	Reservoir	
		Chigirinskoye	Osipovichskoye
Transparency	m	1.0	0.7
Temperature	°C	1.6	1.9
pH	units	8.0	8.1

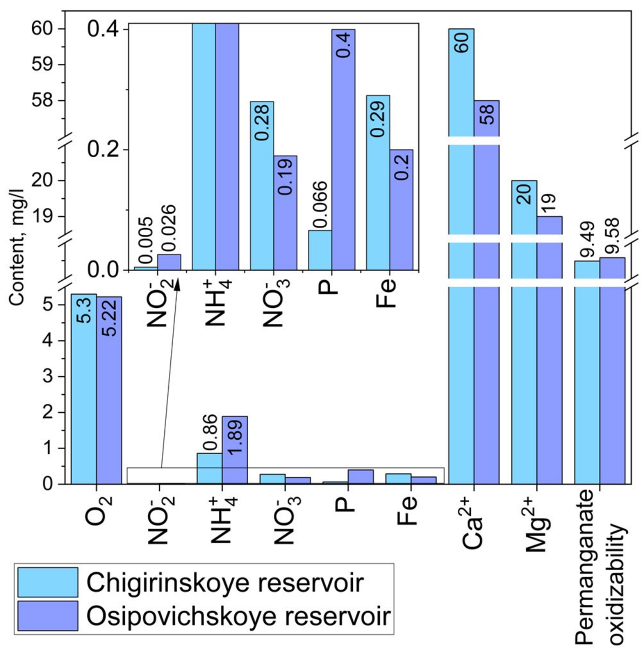

Fig.2. Hydrochemical indicators of water quality during the ice age.

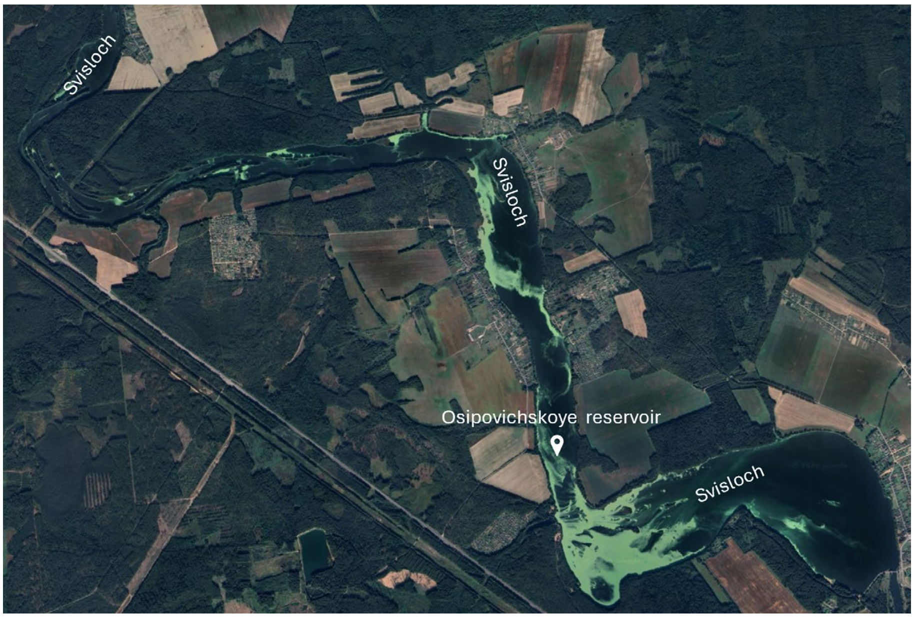

Fig.3. Osipovichskoye reservoir.

Thus, in the subglacial period, the main limiting factor is, first of all, the oxygen regime of reservoirs, other environmental factors are only indirectly important through their effect on the gas regime of reservoirs.

During the ice-free period (the period of open water), seasonal observations (spring, summer-autumn) and analyses of the quality of the water mass were carried out.

In the early spring period, the hydrochemical regime is largely determined by the recent flood and the level of dissolved biogens removed from the catchment area. Due to low photosynthetic activity during this period, concentrations of biogens (nitrogen and phosphorus compounds) can reach higher values than in other months of the open season. The temperature regime in the analyzed reservoirs corresponded to the seasonal distribution and was unlikely to have negative consequences for fish (Table 2). Homothermy of the water mass was observed in the waters, with a slight increase in water temperature at the surface.

The gas regime in reservoirs under conditions of melting ice cover and low water temperatures was quite favorable (Fig. 4). The active reaction of the medium is alkaline (pH = 7.8-8.5), which is due to the predominance of reducing reactions and the seasonal development of cold-loving microalgae.

Thus, analyzing the hydrochemical indicators for the year of observations, the following conclusions can be drawn. The level of nitrate nitrogen turned out to be only slightly increased, and the remaining parameters of the aquatic environment corresponded to sanitary and environmental standards. This suggests that the fixation of the mass death of fish could not be caused solely by the current chemical parameters of the water.

A similar question can be observed in the work of Soler et al. (2021), where it was noted that even with the restoration of water quality (in particular, the reduction of ammonia to standard values), the biological condition of fish continued to remain disturbed. This indicates delayed or chronic exposure to pollutants, or their combination with other stressful factors. A number of other studies have described cases where fish deaths were caused by significantly higher concentrations of substances. Values from 1 to 8 mg/l were critical for ammonia, at which behavioral disorders, oxidative stress, and immunosuppression were observed in Barbus meridionalis (Soler et al., 2021).

Table 2. Main indicators of water quality in the early spring period.

Indicators	Units of measure-ment	Reservoir	
		Chigirinskoye	Osipovichskoye
Transparency	m	0.6	0.5
Temperature	°C	7.1	7.8
pH	units	8.5	8.5

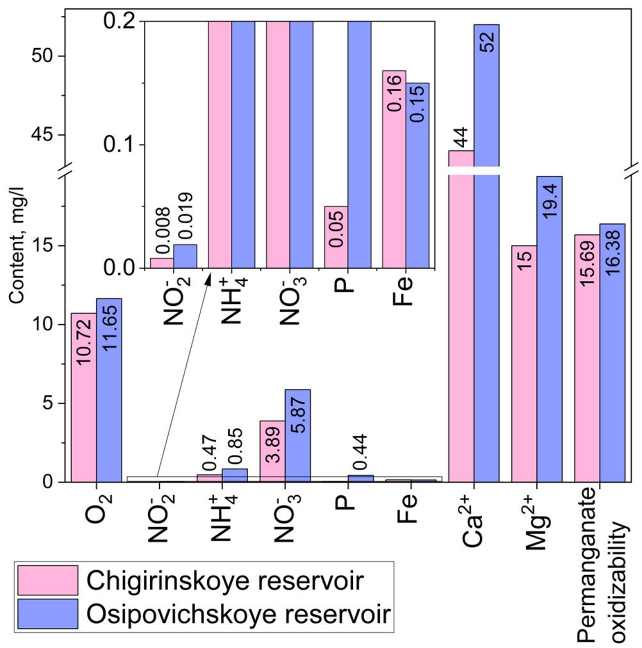

Fig.4. – Hydrochemical indicators of water quality in the early spring period.

Literature data indicate that significantly higher concentrations of pollutants are required for the occurrence of a toxic effect on aquatic organisms. Thus, in a study using Daphnia magna, it was found that concentrations of ammonium nitrogen above 40 mg/l and nitrites above 6 mg/l lead to a decrease in vital activity and an increase in mortality, while nitrates and phosphates did not have a noticeable toxic effect at the studied levels (Serra et al., 2019). Analysis of the effects of nitrates on freshwater organisms showed a significant decrease in survival (about 62%) and growth (about 29%) at concentrations significantly higher than natural background levels (Isaza et al., 2020).

In your case, the absence of extreme values for the main pollutants makes the version of acute chemical poisoning unlikely. Based on a comparison of our own data with the results of scientific research, we can conclude that the indicators of aquatic chemical parameters observed in our case, including a slightly elevated level of nitrate nitrogen, could not be the only cause of fish death.

According to the salt composition, the reservoir water belongs to the bicarbonate-calcium type with an average hardness of 3.4 to 4.7. A slight decrease in total hardness is due to a seasonal factor (the influx of less mineralized waters from the winter flood), which affects the overall ratio of alkaline earth metal ions, usually supplied with groundwater. The reaction of the environment (pH) and concentrations of some biogenic elements, whose participation in redox reactions affects the gas regime, may have a limiting effect for fish in the spring. The increased concentrations of ammonium nitrogen and mineral phosphorus noted during the subglacial period have decreased slightly as a result of chemical and bacterial destruction, but remained quite high. The content of toxic non-ionized ammonia is observed at the trace level and in this form is not capable of having a negative effect on fish. At the same time, the content of the final product of nitrification, nitrate nitrogen, has increased in the Chigirinsky and Osipovich reservoirs, which highlights the degree of anthropogenic pollution in both reservoirs. The exact combination of forms of mineral nitrogen in water against the background of a weakly alkaline or alkaline reaction of the medium can affect the processes of excretion of metabolic products (in the form of ammonia) from fish organisms, causing damage to the gill apparatus in some of them (for example, carp, silver carp), followed by contamination by microflora and partial death of fish.

Water quality indicators in the summer period are determined by the peculiarities of nutrition and the nature of the watershed of reservoirs, the level of intra-reservoir production processes and the degree of development of primary producers.

The temperature regime (Table 3) in the analyzed reservoirs corresponded to the seasonal distribution and could was unlikely to have negative consequences for fish. Homothermy of the water mass was observed in the waters, with a slight increase in water temperature at the surface.

The characteristics of the chemical composition of the water masses of the surveyed water bodies are shown in Figure 5. The gas regime in reservoirs in conditions of open water and high photosynthesis intensity of primary producers was quite favorable and did not limit the vital activity of the majority of fish species. The active reaction of the medium is alkaline (pH = 8.2-8.8), which is due to the predominance of reducing reactions and the seasonal development of microalgae.

According to the salt composition, the reservoir water belongs to the bicarbonate-calcium type with an average hardness of 3.4 to 4.7. A slight decrease in total hardness compared to the winter-spring periods is due to a seasonal factor (the influx of less mineralized waters with snow flooding), which affects the overall ratio of alkaline earth metal ions, usually coming from groundwater. Concentrations of biogenic elements, whose participation in redox reactions affects the gas regime, may be of limiting importance for fish. The increased concentrations of ammonium nitrogen and mineral phosphorus observed during the subglacial period decreased as a result of chemical and bacterial degradation, remaining high only in the Osipovichskoye reservoir, which is subject to constant pollution from catchment and municipal wastewater. The content of toxic non-ionized ammonia is noted at the trace level and in this form is not capable of having a negative effect on fish. The final product of nitrification is the nitrate nitrogen content significantly decreased in the

Table 3. Main indicators of water quality in the summer and autumn period.

Indicators	Units of measurement	Reservoir	
		Chigirinskoye	Osipovichskoye
Transparency	m	0.8	0.9
Temperature	˚C	20.3	18.8
pH	units	8.2	8.8

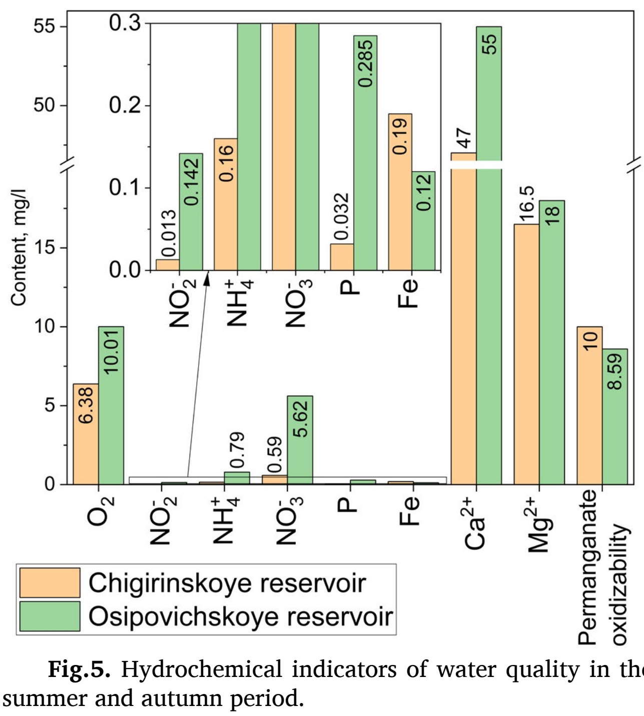

Fig.5. Hydrochemical indicators of water quality in the summer and autumn period.

Chigirinsky reservoir. In the Osipovichsky reservoir, the nitrate nitrogen content also decreased slightly, but still remained at a high level, which underlines the degree of anthropogenic pollution. This can affect the intensity of the processes of excretion of large products of protein metabolism through the gill apparatus and contribute to the occurrence of gill diseases (Oksiuk, 1993; Golovina, 2003).

Based on the results of a comprehensive analysis of pollution and assessment of the levels of trophism of water bodies, a characteristic of the ecological state of reservoirs at the time of the survey is given (Table 4). The correspondence of saprobicity classes to trophic levels is represented as follows: β-mesosaprobic correspond to the class of ev trophy (weakly eutrophic); α-β-mesosaprobic correspond to the class of eutrophic; α-meso-saprobic and polysaprobic correspond to the class of hypertrophy (highly eutrophic).

The analysis of phytoplankton development and detection of cyanotoxins was carried out. The community of planktonic algae in the analyzed reservoirs was represented by taxa of the main systematic groups that determine the intensity of "blooming" of reservoirs. The quantitative development of an algal community during the period of open water is determined by the course of warming up of water masses and the rate of change of dominant taxa and divisions. At the time of the survey, the species (qualitative) composition of communities by water bodies was not very diverse (Table 5).

The maximum indicators of population and biomass development were recorded for the Chigirinskoye reservoir – 2819.34 thousand specimens/l and 47.24 mg/l (Fig. 6). The peaks of phytoplankton development in the spring period were noted. In the Osipovichskoye reservoir, this was due to the development of relatively cold-resistant diatoms. With the warming of the water and the change of dominant groups, the quantitative indicators of development decreased. The early peak of phytoplankton development in the Osipovichi reservoir may be explained by the large volume of conditionally treated waters flowing through the Svisloch River, whereas for the Chigirinskoye reservoir, diffuse sources of pollution in the catchment area or discharges of insufficiently treated waters from higher-lying communal facilities could be the reason.

In general, representatives of the β-mesosaprobic zone predominated among the phytoplankton species in the Chigirinskoye reservoir, while representatives of the α–β-mesosaprobic zone predominated in the Osipovichskoye reservoir, which gives reason to characterize the waters as «moderately polluted». A comparative analysis of the cases of mass death of fish and the level of quantitative development of planktonic algae does not provide grounds for their direct relationship. Fish deaths were observed both at the peak of phytoplankton development ("bloom") and at moderate or relatively small levels. It is obvious that the degree of algae development can directly affect the gas regime and indirectly affect the bacterial background of the reservoir during the destruction of dying cells.

To identify the presence of cyanotoxin genes in the analyzed reservoirs, condensed recorded samples of phytoplankton were selected. Based on the PCR analysis, the genes responsible for the synthesis of cyanotoxins such as microcystins were not detected in phytoplankton samples from the Osipovichskoye and Chigirinskoye reservoirs.

The results obtained during the study are consistent with the data of other authors. A number of studies have emphasized that although phytoplankton can affect the ecosystem of a reservoir, the mass death of fish is most often caused not by the very fact of blooming, but by accompanying environmental changes, in particular hypoxia caused by the decomposition of biomass.

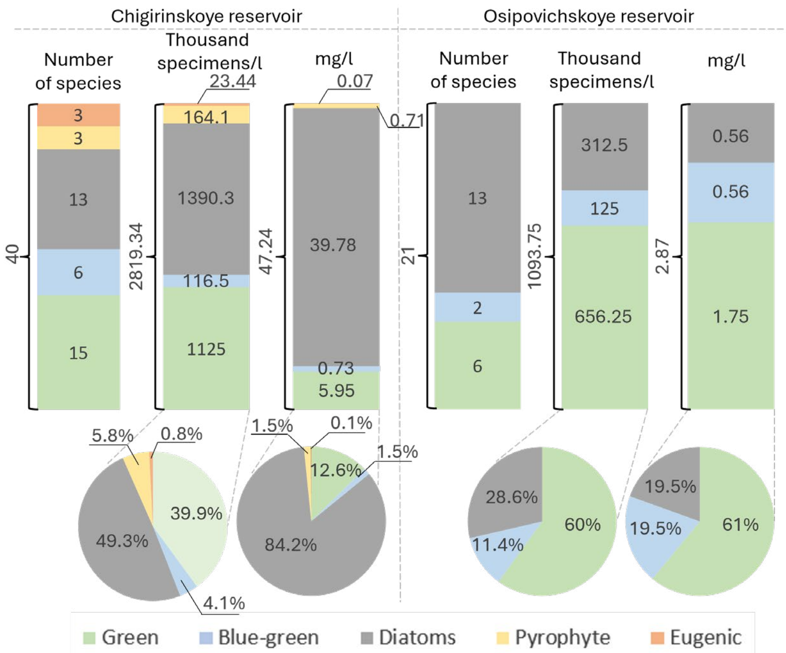

Fig.6. Quantitative indicators of phytoplankton development.

Table 4. Assessment of the water quality of the analyzed reservoirs

Indicators	Reservoir	
	Osipovichskoye	Chigirinskoye
Total microbial count, CFU/ml	381-430	1180-1400
Saprobity index	2.73	2.11
Assessment of the level of saprobity	α-mesosaprobic	β-mesosaprobic
Assessment by trophic level	β-eutrophic	α-eutrophic

For example, blooms of Prymnesium parvum in northern reservoirs led to the death of hundreds of tons of fish, while toxins such as prymnesin were of decisive importance, rather than the quantitative biomass of algae (Oh et al., 2023). Similarly, in the case of Karenia mikimotoi and Karenia brevis, both hypoxic conditions and the release of highly active toxic substances that affect the gill tissue of fish and cause mass mortality have been recorded (Oh et al., 2023).

However, even in the absence of pronounced toxicity, decomposition of dense algae biomass can cause oxygen deficiency. Such cases have been described in studies concerning eutrophic lakes and reservoirs, where peak bio-mass values lead to nocturnal hypoxia and death of ichthyofauna (Chislock et al., 2013; Wallace and Gobler, 2021).

It is worth noting that, according to a number of models for predicting mass fish deaths, the most significant predictors are dissolved oxygen concentration, water temperature, salinity, and chlorophyll content, while the fact of flowering does not always lead to negative consequences (Yniguez and Ottong, 2020; Ting and Chen, 2024).

Table 5. Qualitative composition of phytoplankton by surveyed water bodies

Type and department of algae	Chigirinskoye	Osipovichskoye	Type and department of algae	Chigirinskoye	Osipovichskoye
Green			**Diatoms**		
Scenedesmus quadricauda	+	+	*Navicula sp.*	+	+
Scenedesmus quadricauda setus	+	-	*Tabellaria flocculosa*	-	+
Sc. acuminatus	+	-	*Tabellaria fenestrata*	+	
Coelastrum sphaericum	-	+	*Gomphonema sp.*	-	+
Coelastrum microporum	+	-	*Nitzshia sp.*	+	+
Oocystis borgei	+	+	*Stephanodiscus hantzschii*	+	+
Didimocystis sp.	+	-	*Gyrosigma acuminatum*	+	+
Tetrastrum glabrum	+	+	*Cyclotella comta*	+	+
Tetraedron caudatum	+	-	*Cyclotella meneghiniana*	-	+
Micractinium pusillum	+	-	*Pinnularia sp.*	+	-
Kirchneriella lunaris		+	*Amphora ovalis*	+	-
Ankistrodesmus agustus	+	-	*Asterionella formosa*	+	-
Ankistrodesmus pseudomirabilis	+	+	*Melosira granulata*	+	+
Korschikoviella sp.	+	-	*Synedra ulna*	+	-
Oocystis lacustris	+	-	*Cocconeis pediculus*	+	+
Micractinium pusillum	+	-	*Cymbella sp.*	+	+
Golenkinia radiate	+	-	*Fragillaria sp.*	-	+
Blue-green			*Stephanodiscus hantzschii*	-	+
Microcystis aeruginosa	+	+	**Pyrophyte**		
Microcystis sp.	+	-	Peridinium sp.	+	-
Aphanizomenon flos – aquae	+	-	Cryptomonas marssonii	+	-
Oscilatoria limnetica	+	-	*Rhodomonas pusilla*	+	-
Oscillatoria amoena	+	-	**Eugenic**		
Oscillatoria redeckei	+	-	*Trachelomonas volvocina*	+	-
Spirulina sp.	-	+	*Euglena viridis*	+	-
			Phacus sp.	+	-
Total, taxa	Chigirinskoye – 40; Osipovichskoye – 21				

Thus, the data obtained in this study on the observed death of fish at both high and moderate levels of phytoplankton development, as well as the absence of microsystin genes in the analyzed samples, are confirmed in the literature. This suggests that the key mechanisms of death could be changes in the gas regime and possible bacterial contamination during the destruction of algae, rather than the direct toxic effect of phytoplankton.

The following indicators were determined. The intensity (massiveness) of invasion (II) is the minimum and maximum number of parasites in one infected individual fish; the infestation or extent of invasion (EI) is the number of infected fish specimens to the number of studied specimens, expressed as a per-centage.

Osipovichskoye reservoir

Carp – 2 specimens, silver crucian carp – 4 specimens, bream – 6 specimens, rudd – 1 specimen were examined. Blood samples were taken from bream, bream and silver crucian carp for hematological analysis. A complete parasitological analysis showed the presence of the following parasites. 1 specimen of the monogenetic fluke Diplozoon paradoxum was found in scraping from carp gills. The carriage of metacercariae of trematodes *Diplostomum sp.* (16 and 24 pairs/fish) was noted in the eye lenses of both carp. Rudd has 1 cyst of the *Rhipidocotyle illense* trematode in its gills, and cysts of the *Paracoenogonimus ovatus* trematode (1-9 pairs in the field of vision), as well as trematodes of an indeterminate species, are abundant in muscle tissue. *Posthodiplostomum cuticola* cysts were found on the bream body surface (EI – 100%, II – 8-85 pairs/fish), a large number of monogenetic *Dactylogyrus sp.* cells were found in gill scrapings (EI - 100%, II – 1-3 pairs in the visual field), and metacercariae *Diplostomum sp.* (EI – 100%, II – 13-27 pairs/fish). Metacercariae of the trematode *Paracoenogonimus ovatus* were found in the muscle tissue of 2 bream specimens (EI - 33%, II – 1-2 pairs in the visual field), and 1 specimen of the cestode *Khawia sinensis* was found in the intestine. Single *Dactylogyrus sp*. (EI – 25%, II – 1-2 in the visual field) were found on the gills of the silver crucian carp, and 1 metacercarium *Diplostomum sp*. was found in the lens of the eye of 1 specimen.

Chigirinskoye reservoir

10 silver crucian carps and 5 breams specimens were examined. Clinical examination revealed severe hyperemia of the abdomen and the bases of the pectoral and ventral fins in 5 specimens of silver crucian carps and 2 specimens of bream. Splitting of the fin rays was noted in 8 specimens of the silver crucian carps. In 1 specimen of the silver crucian carp, an ulcer was found on the surface of the body in a pocket of scales. Upon autopsy, the features and pathological condition of the internal organs were not revealed.

Bream, 5 specimens. Metacercariae of *p. Diplostomum* trematodes were found in the eyes (EI – 100%, II – 4-40 pairs/fish). Monogenetic flukes of *Dactylogyrus sp.* were found on the gills of 4 specimens (EI – 80%, II – 1-9 pairs/fish).

Silver crucian carp, 10 specimens. Metacercarium Diplostomum sp. was found in the eye lenses of 1 specimen (EI – 10%, II – 1 pair/fish).

The research results have shown that the degree of infection of fish with ecto- and endoparasites is different. Basically, it is at the carrier level. The parasitic factors themselves could hardly have caused the mass death of fish.

In addition, a bacteriological study of fish was conducted.

Osipovichskoye reservoir

Carp – 2 specimens, silver crucian carp – 4 specimens, bream – 6 specimens, rudd – 1 specimen were examined. Primary bacteriological crops produced very weak growth. For further work, only 2 strains were isolated: from the blood of 1 specimen of carp and from the blood of 1 specimen of silver crucian carp – oxy-positive, gram-negative rods. Using the API 20E test system, they were identified as *Aeromonas hydrophila*.

Chigirinskoye reservoir

Microbiological cultures of material from parenchymal organs, blood and contents of silver crucian carp ulcer (4 specimens) were performed. Oxidase-negative gram-negative cocci have been isolated from the liver. Oxidase-negative gram-negative rods, which are defined *as Stenotrophomonas maltophilia*, and oxidase-negative gram-positive cocci, which are defined as *Micrococcus sp*, were isolated from the kidneys. Oxidase-positive gram-negative rods have been isolated from the spleen, which are identified as *Aeromonas hydrophila* and *Proteus mirabilis*. Oxidase-positive gram-negative rods have been isolated from the blood, which are identified as *Vibrio sp*. and *Pasteurella pneumatropica*, as well as oxidase-negative gram-positive rods. A mixture of microorganisms from oxidase-negative gram-positive and gram-positive cocci and gram-negative rods was isolated from the contents of the ulcer.

The greatest real threat to fish is, first of all, the bacteria *Aeromonas hydrophila*, and other forms that can cause diseases, such as representatives of *Vibrio* and *Proteus*, pose a potential threat. Conditionally pathogenic bacteria (genera *Aeromonas, Vibrio, Proteus*, etc.) are natural components of the aquatic ecosystem, but under certain conditions (stress situations or a violation of the immune status of fish) they can cause an acute course of diseases, accompanied by significant death of diseased individuals. The remaining representatives of the bacterioflora isolated from fish during the reporting period belong to the saprophytic microflora. They have been found singly in certain fish species and can be characterized as pathogenic symbionts. However, with excessive development, even pathogenic forms can pose a certain threat of losses. In principle, saprophytes themselves are not capable of causing an acute infectious process in fish, however, penetrating into the body of fish and developing in large quantities in internal organs and blood, they can cause significant harm due to their inactivity and the release of metabolic products into the fish tissue. They can be especially dangerous for fish with reduced immunity for any reason (exhaustion, stress factors, exceeding the temperature threshold, etc.).

The following indicators of fish blood were also studied: total serum protein, amount of hemoglobin, ESR, content of erythrocytes and leukocytes. The results are presented in Table 6.

Table 6. Blood counts of fish in the surveyed reservoirs

	Total whey protein, g/l	Amount of hemoglobin, g/l	ESR, mm/h	Red blood cell count, 10^{12}/l	White blood cell count, 10^{9}/l
Osipovichskoye reservoir					
Silver crucian carp, average	**39.9**	**83.0**	**3.8**	**1.77**	**26.0**
The reference value	**51.0**	**88**	**-**	**1.60**	**51.0**
Bream, average	47.7	101.0	1.8	1.62	43.6
Physiological control	47.0	96	-	1.70	49.0
Carp, average	24.9	104.5	2.0	1.40	23.8
The reference value	**30 – 45**	**85.1 ± 2.3**	**up to 4**	**1.5 ± 0.04**	**24.5 ± 4.3**
Chigirinskoye reservoir					
Silver crucian carp, average	37.5	129.8	3.8	2.1	27.5
The reference value	**51.0**	**88**	**-**	**1.60**	**51.0**

Analyzing the results of a general blood test, including the level of total protein and ESR (Table 6), it can be concluded that the silver crucian carp from the Chigirinskoye reservoir was exposed to the greatest external effects of negative factors. The total protein level in its blood was 37.5 g/l, which is lower than the reference value (51 g/l), and the hemoglobin level significantly exceeded the reference value (88 g/l) and amounted to 129.8 g/l. The erythrocyte level was higher than the reference value (1.6×10^{12}/l) and amounted to 2.1×10^{12}/l. The content of leukocytes and ESR was close to the reference value. A low value of total protein indicates a decrease in the body’s resistance, and a high level of hemoglobin indicates the possible ingestion of toxic components into the fish’s body. An increase in the level of red blood cells indicates a low oxygen level in the blood of the fish. In the silver crucian carp from the Osipovichskoye reservoir, blood counts did not significantly differ from the reference values. It was noted that the blood and serum values of silver crucian carp, carp and bream from the Osipovichskoye reservoir did not significantly differ from the reference values for each species.

The level of cortisol in blood serum is shown in Table 7. Cortisol levels in fish depend on a number of factors. In this case, we can talk about their high level in both bream (622 ± 18.4 nmol/l) and silver crucian carp (815 ± 40.4 nmol/l and 768 ± 58.8 nmol/l, respectively) both in the Osipovichskoye reservoir and in the Chigirinskoye reservoir. Since the indicators of the total blood count, total protein and ESR did not differ significantly from the reference values in these fish, it can be concluded that an increase in the level of cortisol is not associated with pathological processes in the body. Perhaps this increase is due to the activation of physiological processes, for example, related to the maturation of gonads and spawning.

The results of the studies showed that the fish studied in the two reservoirs did not have significant differences in the level of cortisol in their blood serum. Thus, it can be concluded that the conditions in which the examined fish were located have the same effect on their hormonal status.

4. Conclusion

The surveyed reservoirs are classified as eutrophic (groups α- and β-eutrophic). According to the saprobicity index, all reservoirs are classified as mesosaprobic (groups β- mesosaprobic and α- mesosaprobic), with satisfactory gas and temperature conditions. According to the indicators of some water components, the actual values exceed accepTable limits (high levels of biogenic pollution, primarily from compounds of mineral nitrogen and phosphorus), but generally do not reach critical values for fish life.

Table 7. Cortisol level in the blood of the examined fish

Osipovichskoye reservoir		Chigirinskoye reservoir
Bream	Silver crucian carp	Silver crucian carp
600	600	1040
600	600	600
540	1020	535
620	1040	1040
750	-	600
-	-	1086
-	-	475
average 622 ± 18.4	average 815 ± 40.4	average 768 ± 58.8

The composition of planktonic communities is represented by a number of taxa of algae, which determine the level of development and intensity of water «blooming». The change of dominants in the phytoplankton structure occurs in the diatom-green–blue–green direction and is determined by hydrological conditions and the rate of warming of water masses. The taxonomic composition and quantitative development of algae emphasize the eutrophic nature of water bodies.

The bacterial background was within the normal range. In the autumn period, the contamination of waters with saprophytic microflora decreases to minimal values.

No direct connection was found between the mass death of fish and the magnitude of the development of saprophytic microflora. Once in the body of fish along with the detritus consumed, saprophytic microflora can contaminate internal organs and tissues, causing an infectious process against the background of a decrease in the natural immune status.

Ichthyopathological studies have shown the degree of infection of fish with ecto- and endoparasites at the carrier level, the parasitic factors themselves could hardly be the cause of the mass death of fish.

A number of bacteria have been isolated from the blood and parenchymal tissues of fish, some of which are identified as conditionally pathogenic representatives of *p. Aeromonas, Vibrio* and *Proteus*, capable of developing an infectious process.

Hematological studies have shown a decrease in the level of total protein in silver crucian carp against the background of a significant increase in hemoglobin levels. There was no excess of ESR and leukocyte count, which indicates the absence of pressure from pathogenic and (or) conditionally pathogenic microflora. Violations of blood and serum parameters could be caused by toxic effects or, as a result, oxygen deficiency, as evidenced by a recorded increase in cortisol levels in silver crucian carp (815±40.4-768±58.8 nmol/l), probably associated with the activation of physiological processes (maturation of the body during partial spawning).

The main reason for the noted deaths of silver crucian carp is the eutrophication of reservoirs, which causes the intensive development of phyto- and bacterioplankton with subsequent contamination by the microflora of the internal organs of fish against the background of a decrease in the resistance of the fish body as a result of probable intoxication and extended partial spawning.

Conflict of interest

The authors declare no conflicts of interest.

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