Comparative characteristics of the physiological state of fish under different climatic conditions on the example of Kremenchuk and Kakhovka reservoirs

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Abstract. Field studies at the Kremenchuk and Kakhovka reservoirs of the Dnipro Cascade established that the studied areas of the reservoirs did not differ significantly in terms of chemical composition of the water, biomass of phyto- and zooplankton and benthic organisms. The water temperature during the growing season has the most significant influence on the physiological state of fish. Thus, until July, the water temperature value in the Kremenchuk reservoir slightly exceeded its value in the Kakhovka reservoir, but during August-September, it was significantly lower in the areas of the Kremenchuk reservoir. Under these conditions, the content of proteins, lipids, and glycogen in the liver and white skeletal muscles of fish species, either demersal (bream, *Abramis brama*, roach, *Rutilus rutilus*, Gibel carp, *Carassius gibelio*, and white bream, *Blicca bjoerkna*) or predatory (zander, *Sander lucioperca*) were investigated in the autumn period. Research was conducted using generally accepted methods. It was established that the carp showed better adaptation to the temperature factor when a greater amount of reserve substances, in the form of lipids and glycogen, accumulated in its white muscles and liver, at elevated water temperature. The caloric content of carp tissues was the highest, compared to other fish species, in the conditions of the Kakhovka reservoir. Zanders revealed multidirectional processes of accumulation of spare substances. A higher amount of glycogen and proteins, with a higher caloric content, were observed in the white muscles of fish from the Kakhovka reservoir, while a lower content of glycogen, lipids and glycogen were found in the liver, compared to zanders from the Kremenchug reservoir. The roach, silver bream, and bream from the Kakhovka reservoir showed less readiness for the next winter in terms of the degree of accumulation of reserve substances in their tissues, due to the higher water temperature in September, while their energy resources were mostly spent on growth related needs.

Key Words: water temperature, biomass of phyto- and zooplankton, benthos.

Introduction. Temperature is one of the most important abiotic factors of both terrestrial and aquatic environments (Romanenko et al 1991). Its changes affect not only the speed of chemical reactions, but also determine the general physiological state of the body. For fish, even small changes in temperature can cause significant changes in metabolism (Jobling 1981). An increase in water temperature leads to a significant increase in water evaporation, especially in reservoirs, which can also significantly affect the various life processes of aquatic organisms, including fish, and the development and growth of food organisms. In connection with this, global warming of the climate has a significant destructive effect on most biological systems. All this can have a negative impact on the biological and fish productivity of water bodies as a whole. Climatic changes force living organisms to form compensatory mechanisms under the action of an adverse factor, through changes in the enzymatic activity of many biochemical reactions and in the content of accumulated reserve substances (Shulman & Finenko 2001).

Issues related to the influence of environmental conditions on the physiological status of fish habitats in the reservoirs of the Dnipro Cascade deserve special attention.

One of the integral indicators of the physiological state of fish is metabolism, which is determined by the content of proteins, lipids, carbohydrates, etc. in organs and tissues. Recently, the interest in conducting such research has gained great importance. This is explained by the fact that with the help of these indicators it is possible to obtain a information on the state of the organism in specific conditions of existence and also on the ecological status of a water body itself. Monitoring studies may indicate how to remediate the ecological damages caused by anthropogenic factors.

Based on the above, the aim of the study was to determine the influence of different temperature conditions, using the example of the Kremenchuk and Kakhovka reservoirs, on the content of glycogen, lipids and protein in the muscles and liver of 5 species of fish at the end of the growing season.

Material and Method

Description of the study sites. Experimental fish were caught in the middle of the Kremenchuk Reservoir (the village of Chervona Sloboda – the village of Lesky) and the river inlet to the Kakhovka Reservoir (the village of Bilenke), during the fall of 2021 (Figure 1).

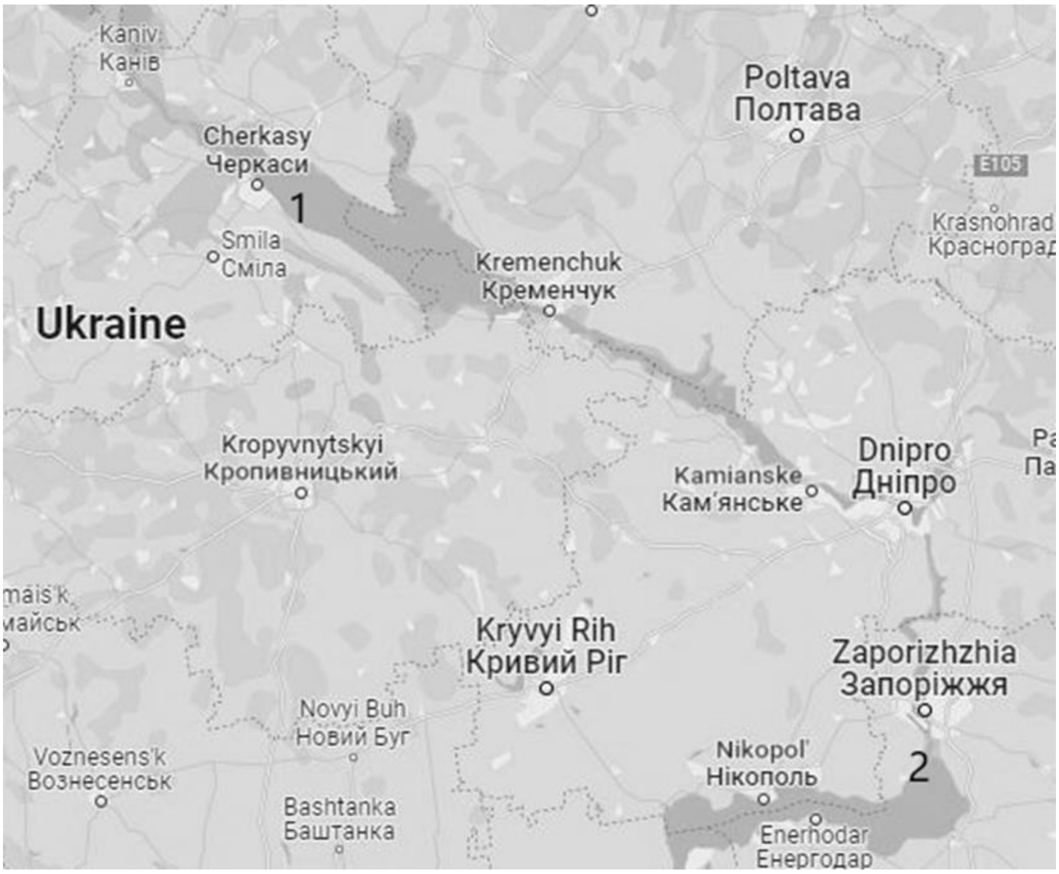

Figure 1. Areas of fish selection in the Kremenchuk and Kakhovka reservoirs. Note: 1 - v. Chervona Sloboda – v. Lesky (Kremenchuk reservoir), 2 – v. Bilenke (Kakhovka reservoir).

Determination of the chemical composition of water. Water samples were taken with a Rutner bathometer in the summer of 2021. Dissolved oxygen in water was determined by the iodometric method (ISO 5813: 1983, IDT, pH by the electrometric method (ISO 10523: 1994, MOD), iron – using 1,10-phenanthroline (ISO 6332), calcium by the ISO titrimetric method (ISO 6058), potassium by the atomic absorption spectrometry method (ISO 6059: 1984, IDT), total hardness by the titrimetric method using ethylenediaminetetraacetic acid (ISO 6059: 1984, IDT), permanganate oxidizability (index) by the titrimetric method (ISO 8467:2021), determination of chemical oxygen demand (bichromate oxidizability) by the titrimetric method (ISO 6060:1989 IDT). Ammonium ions were determined by the potentiometric method (ISO 6778: 1984, IDT), nitrites by the molecular absorption spectrometric method (ISO 6777:1984, IDT), nitrates by the spectrometric method using sulfosalicylic acid (ISO 7890-3:1998, MOD), compounds phosphorus by the spectrometric method (ISO 6878), hydrocarbons by the gravimetric method after solvent extraction (ISO 9377-1) (Arsan et al 2006).

Phytoplankton. The selection of phytoplankton samples was carried out in the summer of 2021, from the Kremenchuk Reservoir, at the sites of Karyer, Chervona Sloboda, Farvater, and from the Kakhovka reservoir inlet, at the sites of Bilenke, Bilenke-Malokaterinivka station, in the district Malokaterinivka. Phytoplankton was sampled with a Beckman bathometer, at different horizons. To preserve the samples, 40% formaldehyde was added at a ratio of 1:100. The sample was thickened using the sedimentation method. Phytoplankton samples were viewed in a special Najotta counting chamber of 0.01 cm^3, under an optical microscope, identified, and then all detected species of algae were counted per 1.0 dm^3 (Priymachenko 1981). Determination of the taxonomic composition of algae was carried out according to criteria from different authors (Korshykov 1938; Kondratieva 1968; Bukhtiyarova 1999). Phytoplankton biomass was quantified by the volumetric method (Topachevsky & Masyuk 1984; Shcherbak 2002; Arsan et al 2006).

Zooplankton. Zooplankton samples were taken from the surface layer of the reservoir using an Apstein plankton net (sieve No. 72) - by the method of water filtering through a net of 100 dm^3. After sampling, the sample was preserved with 4% formalin. Chamber processing of samples was carried out by the generally accepted hydrobiology counting-weighing method in a Bogorov chamber, under a MBS-9 stereoscopic microscope (Arsan et al 2006). Zooplankton organisms were identified to species using identifiers (Manuilova 1964; Mordukhay-Boltovskoy 1968; Mordukhay-Boltovskoy 1969; Kutykova 1977; Monchenko 1974; Kutykova & Starobogatova 1977). Determination of the individual mass of organisms was carried out according to the tables of individual masses (Mordukhay-Boltovskoy 1954; Mordukhay-Boltovskoy & Rivier 1987). The number and biomass were calculated per 1 m^3.

Benthos. Benthos samples were taken with an Ekman-Berge dredger. The soil lifted to the surface was washed and the organisms were fixed with a 10% formalin solution. Processing of the selected benthos samples was divided into two stages: (1) the delivered zoobenthos sample was washed from formalin and dirt residues; organisms were selected and divided into groups: insect larvae, molluscs, worms-leeches, oligochaetes, nematodes; the systematic position was determined under a binocular microscope or a hand magnifier, with the help of appropriate markers; organisms of each group were counted, weighed on torsion scales and recalculated per 1 m^2 of the reservoir; (2) the organisms of each of the selected groups were assigned species, measured, counted, mass determined and recalculated per 1 m^2 of the reservoir (Arsan et al 2006).

Methods of biochemical analysis. The biological material consisted of samples of the liver and white skeletal muscles of sexually mature individuals of bream, roach, zander, silver bream and crucian (silver) carp.

The content of total proteins in tissue samples was determined according to Lowry et al (1951). For this, 0.1 g of tissue was hydrolyzed for 1 hour in 10 mL of 10% NaOH, at a temperature of 60°C. 10 mL of solution No. 3 was added to 0.1 mL of the hydrolyzate and kept for 15 minutes. Then 1 mL of Folin's reagent, which was dissolved 1:1 with distilled water, was added to the sample. The solutions were kept for 30 minutes. Extinction of the solution was determined on a Unico 280 UV/VIS spectrophotometer at a wavelength of 720 nm against a control (0.1 mL of distilled water with the addition of all reagents). The amount of protein was determined using a calibration curve. Solution No. 3 was prepared from solutions No. 1 and No. 2 in a ratio of 1:9. Solution No. 1 was prepared on the basis of 0.1 N NaOH (1 L) with the addition of 20 g of Na_2CO_3 and 0.5 g of K, Na tartaric acid. Solution No. 2 contained 1 g of $CuSO_4$ per 1 L of distilled water.

The content of total lipids was determined by using the phosphor-vanillin reagent, with the standard set of reagents "Total lipids" (Filicit-Diagnostics) (Knight et al 1972).

The glycogen content was determined by the anthrone method (Severina & Solovieva 1986). 50-100 mg of tissue was added to the test sample in 3 mL of 30% KOH, and placed in a water bath (100°C) for 30 minutes. 0.1 mL was taken from the cooled hydrolyzate and 0.9 ml of distilled water was added. 3 mL of 0.2% anthrone reagent was added to the resulting mixture in an ice bath. The resulting reaction mixture was placed in a boiling water bath for 10 min. Then it was measured against a blank sample (1 mL of distilled water in 3 ml of 0.2% anthrone reagent) on a Photo-colorimeter KFK-2MP, at a wavelength of 620 nm (red light filter). The obtained results were expressed in mg g^{-1} of tissue.

The calorie content of fish tissues was calculated according to the formula (Savron et al 1967):

$$X\ (kJ\ g^{-1}) = (4.0 \times B + 4.0 \times G + 9.0 \times L) \times 4.184$$

Where:
B, G, L – the content of protein, glycogen and lipids, respectively, in g per kg of raw mass;
4.184 - the conversion factor of kcal in kJ.

Statistical analysis. The obtained digital material was subjected to statistical processing using the Statistica 10.0 program.

Results and Discussion. The water temperature during the fishing season, at the beginning of October, was of 8–10°C, but during the growing season it differed significantly between the two reservoirs. Thus, the average water temperature in the spring-summer period in the areas of the Kremenchuk Reservoir was: in May, of 14°C; in June, of 19.8°C; in July, of 24.6°C; in August, of 22.3°C; in September, of 15.9°C, while in the area of the Kakhovka reservoir it was of 14.8, 19.2, 24.6, 24.2, 17.0°C, respectively (Figure 2).

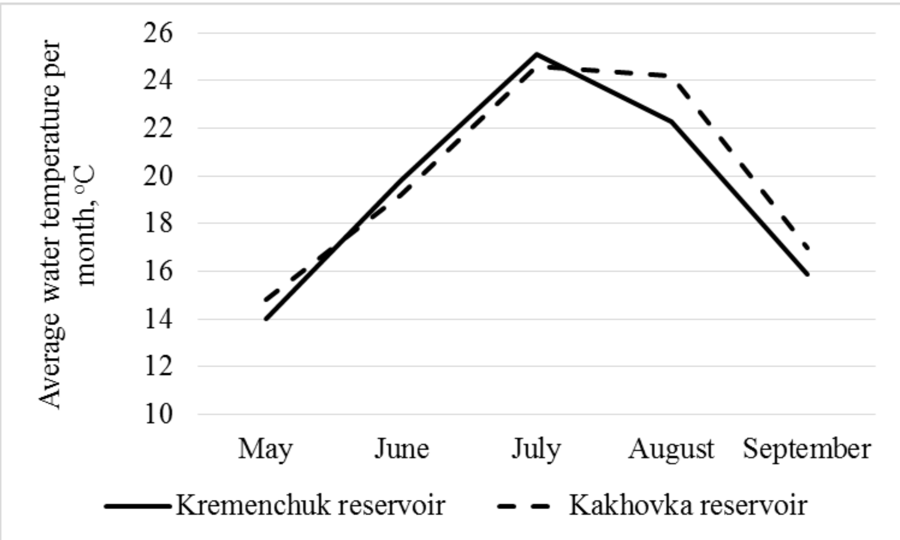

Figure 2. Average water temperature in the studied areas of the Kremenchuk and Kakhovka reservoirs during the growing season.

By July, the water temperature value in the Kremenchuk Reservoir slightly exceeded its value in the Kakhovka Reservoir, but during August-September, it was significantly lower in the areas of the Kremenchuk Reservoir.

The chemical composition of water in fishing areas is shown in Table 1. The Kremenchuk Reservoir has a slightly lower pH of the medium compared to the Kakhovka Reservoir. The concentration of water nitrogen and phosphorus compounds in these reservoirs does not differ significantly, under an almost similar anthropogenic influence. Concentration values of free ammonia, ammonium ions, nitrites, phosphorus and phosphates exceeding the maximum allowable concentrations were not observed. According to other water parameters, it can also be stated that the fish were not significantly affected by adverse factors, and their physiological state primarily depended on the water temperature during the growing season.

Table 1

Chemical indicators of water in the fishing grounds of the Kremenchuk and Kakhovka reservoirs, autumn 2021

Water quality indicators	*Kremenchuk reservoir*	*Kakhovka reservoir*	
	v. Chervona sloboda	*v. Bilenke*	*Station Bilenke-Malokaterinivka*
Hydrogen index, pH, pH units	7.2	8.8	8.6
Free ammonia, NH_3, mg N dm^{-3}	0.002	0.07	0.05
Permanganate oxidation, mg O dm^{-3}	15.1	12.6	11.5
Dichromate oxidation, mg O dm^{-3}	37.7	31.8	28.8
Ammonium nitrogen, NH_4^+, mg N dm^{-3}	0.66	0.72	0.78
Nitrites, NO_2^-, mg N dm^{-3}	0.03	0.07	0.07
Nitrates, NO_3^-, mg N dm^{-3}	0.36	0.20	0.28
Mineral phosphorus, PO_4^{3-}, mg P dm^{-3}	0.25	0.17	0.12
Total iron, Fe^{2+} + Fe^{3+}, mg Fe dm^{-3}	1.02	0.63	0.80
Calcium, Ca^{2+}, mg dm^{-3}	54.2	50.0	46.2
Magnesium, Mg^{2+}, mg dm^{-3}	7.3	30.1	19.4
Sodium + potassium, Na^+ + K^+, mg dm^{-3}	25.0	3.1	15.4
Hydrocarbons, HCO_3^-, mg dm^{-3}	207.5	231.7	195.6
Chlorides, Cl^-, mg dm^{-3}	20.8	34.5	34.3
Sulfates, SO_4^{2-}, mg dm^{-3}	14.8	16.3	17.1
Total hardness, mg-eq. dm^{-3}	3.3	4.8	3.8
Mineralization, mg dm^{-3}	329.6	366.6	328.4

According to the biomass abundance of phytoplankton, zooplankton, and benthos identified in the studied areas of the Kremenchuk and Kakhovka reservoirs can be considered medium feed (Ministry of Defense 2009; Kruzhilina & Kotovska 2013). The average biomass of food organisms in 2021 is: (1) in the Kremenchuk reservoir: from 0.035 to 1.231 mg dm^{-3} of phytoplankton, from 0.09 to 0.735 g m^{-3} of zooplankton, up to 2.44 g m^{-2} of soft macrozoobenthos and from 0.327 to 2.433 g m^{-2} of shellfish; (2) in the Kakhovka reservoir: from 0.009 to 7.947 mg m^{-3} of phytoplankton, from 0.001 to 0.444 mg m^{-3} of zooplankton and up to 10.0 g m^{-2} of soft macro-zoobenthos. Dominant species were blue-green and diatom algae, for the phytoplankton, and Cladocera, for the zooplankton. Environmental factors, in particular water temperature, significantly affect the metabolism of fish. Moreover, the temperature factor is of a decisive importance for the nutrition of fish, their growth and the degree of accumulation of reserve substances in organs and tissues. As the water temperature decreases, the plasticity slows down: fish that are in satisfactory conditions continue to grow, which consumes energy resources, while the storage of energy-intensive substances has not yet occurred in a significant manner.

Glycogen is a necessary material for the vital activity of fish; it mainly accumulates in the liver, which is the main source of glucose in the blood. The amount of glycogen depends on the physical, chemical and biological factors encountered by the fish (Koedprang et al 2002). The minimum amount of glycogen in the tissues is observed in the summer, and its maximum content in the months of September-October (Coban & Sen 2011). Unfavorable or stressful environmental factors cause a decrease in the glycogen reserves, first in the liver, and then in the muscles.

In connection with the different temperature conditions of the studied areas, the amount of glycogen accumulated in the muscles of roach and bream from the Kakhovka Reservoir was 2.3 times lower (Figure 3). The fish from this reservoir were in a state of growth, and glycogen was used for energy needs. The glycogen content in muscles did not differ in zander and crucian carps between reservoirs, while different contents were observed in silver breams.

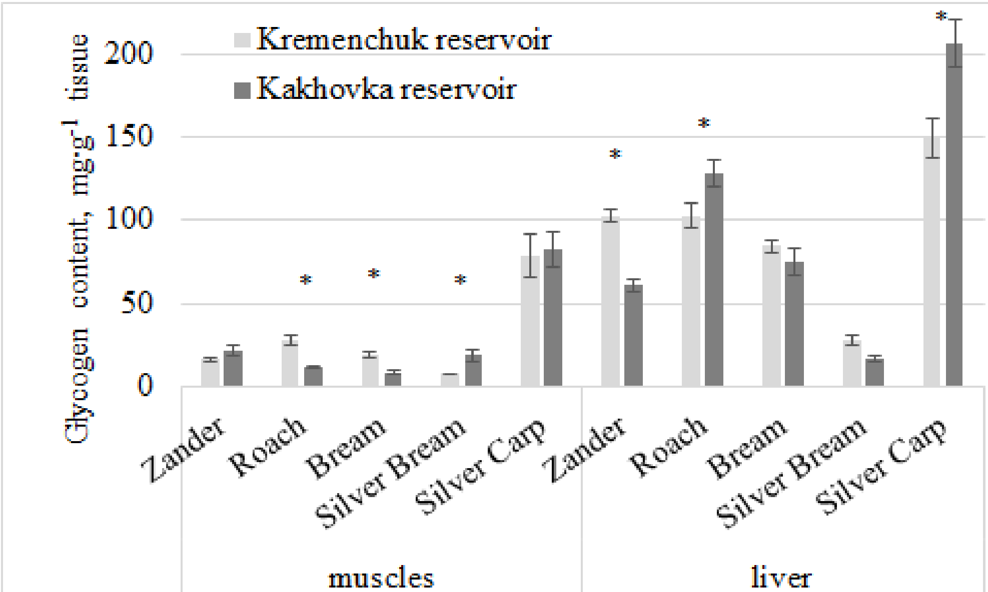

Figure 3. Glycogen content in white muscles and liver of fish from Kremenchuk and Kakhovka reservoirs, M±m, n=8 (* – significant difference (P<0.05), M – mean, m – standard errors of the mean, n – number of samples).

With the exception of crucian carp, in most species of fish, the content of glycogen in the liver of fish was either the same (bream, roach) in both reservoirs or lower in fish from the Kakhovka reservoir (zander, silver bream). Thus, the temperature regime of water bodies was reflected in the carbohydrate metabolism of fish. Lipids and their constituent fatty acids are the main organic components of fish and play an important role as a source of metabolic energy (Tocher 2003). Environmental factors such as season, temperature, and stage of reproduction affect the lipid content of tissues of different species (Love 1970).

As our studies have shown, the content of lipids in white muscles probably did not differ in all studied species of fish. An exception was the silver crucian carp, in which the content of lipids in the muscles of fish from the Kakhovka reservoir is 4.1 times higher (Figure 4). Also, the content of lipids was lower in the silver bream from the Kakhovka reservoir. However, the accumulation of lipids in the liver of all fish species of the Kakhovka Reservoir was probably lower. This once again indicates that, due to the higher water temperature in August and September in the area of the Kakhovka reservoir, the fish did not finish preparing for the next winter. Crucian carp, on the other hand, showed opposite patterns, probably due to its greater warmth at the end of the annual cycle.

One of the main factors affecting the intensity of metabolic processes of fish is the temperature of the environment (Brown 1993). During the vital activity of fish, proteins perform various functions, which also include the energy supply of the body. Using proteins and amino acids as an oxidizing substrate makes it possible to compensate for the inability to catabolize lipids (Ballantyne 2001). Thus, when 1 g of protein is broken down, approximately 16.7 kJ of energy is released (Metzler 1980). Obviously, the significant use of protein as a source of energy supply is caused by the growing energy needs of metabolic processes, which are accelerated due to an increase in the temperature of the external environment.

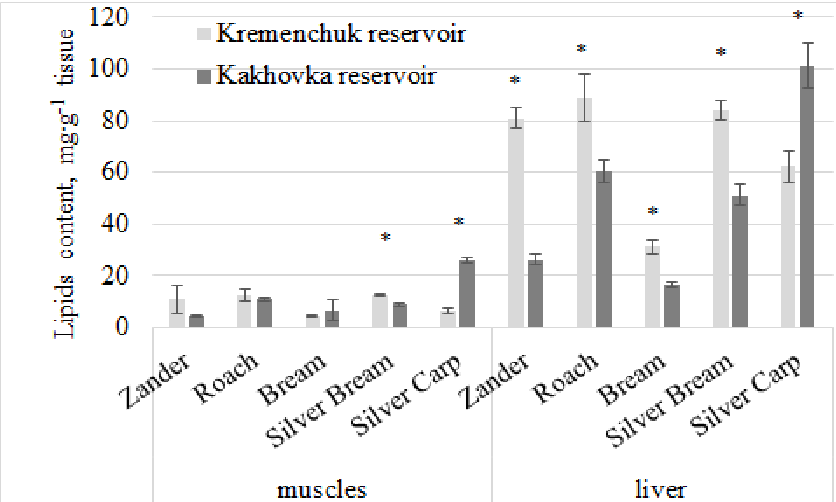

Figure 4. Lipid content in white muscles and liver of fish from Kremenchuk and Kakhovka reservoirs, M±m, n=8 (* – significant difference (P<0.05), M – mean, m – standard errors of the mean, n – number of samples).

According to our research, it was found that among the fish species studied, only the zander from the Kakhovka reservoir has a significant (1.9 times) excess of protein content in the white muscles, compared to fish from the Kremenchuk reservoir (Figure 5). The content of protein in muscles was higher in bream, roach, silver bream and crucian carp from the Kremenchuk reservoir. If, for the crucian carp from the Kakhovka reservoir, less accumulation of proteins in the muscles can be explained by a significant accumulation of other energy substrates – glycogen and lipids, this was not observed in other species.

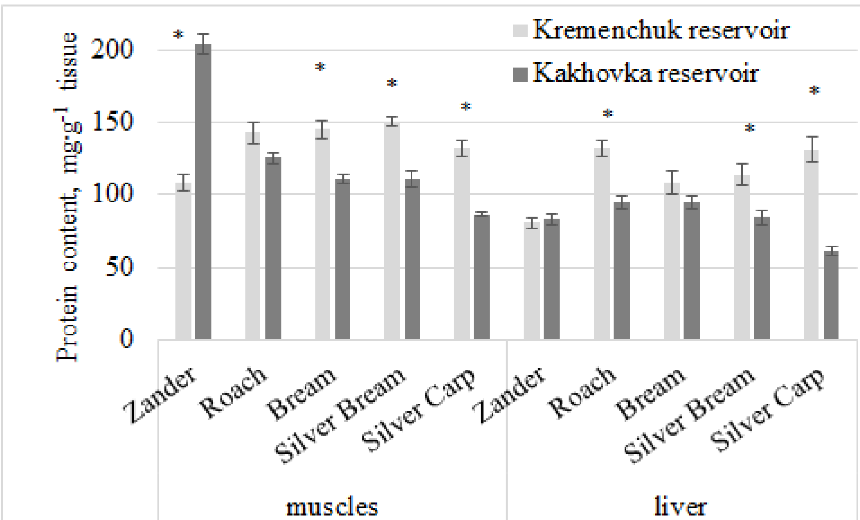

Figure 5. Protein content in white muscles and liver of fish from Kremenchuk and Kakhovka reservoirs, M±m, n=8 (* – significant difference (P<0.05), M – mean, m – standard errors of the mean, n – number of samples).

The liver protein content was either similar in fish from both reservoirs (zander, bream) or higher in fish from the Kremenchuk Reservoir (roach, silver bream, crucian carp). This once again emphasizes the influence of environmental factors, and primarily of the water temperature, during the growing season, on the degree of accumulation of reserve substances.

The characteristic of fish according to the caloric content of white muscles and liver turned out to be the most indicative under different environmental conditions. Thus, the value of this indicator in the muscles of zander from the Kakhovka reservoir exceeded its value in fish from the Kremenchuk reservoir by 1.6 times (Figure 6). However, the caloric content was 1.8 times lower in the liver of this species. This indicates that this predatory species continued to grow, and energy resources were spent on maintaining increased growth rates.

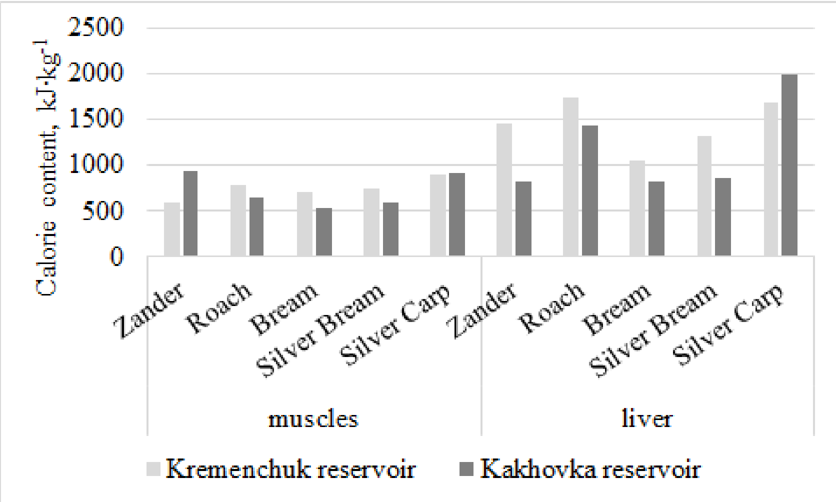

Figure 6. Average caloric content of white muscles and liver of fish from Kremenchuk and Kakhovka reservoirs, M±m, n=8 (* – significant difference (P<0.05), M – mean, m – standard errors of the mean, n – number of samples).

The crucian carp was characterized by other patterns. If the muscles of fish from both areas did not differ in caloric content, it was significantly higher (with 18%) in the liver of the crucian carp from the Kakhovsky Reservoir. It should be noted that the caloric content of gilbert carp liver was the highest among all fish species studied. This heat-loving species, despite a rather warm September, ends its annual cycle at the beginning of October and prepares for the upcoming winter.

Other types of fish (roach, bream, and silver bream) from the Kremenchuk reservoir were characterized by higher muscle and liver caloric values compared to individuals from the Kakhovka reservoir. A cooler September forced the fish to prepare for winter earlier, which was manifested in a greater accumulation of energy resources in the form of glycogen, lipids and proteins.

Conclusions. As showed by the current research, the selected sites in the Kremenchuk and Kakhovka reservoirs did not differ significantly in terms of hydrochemical and hydrobiological indicators. The main influencing factor on the physiological state of native fish species was water temperature. In the spring and until mid-summer, the water temperature in the Kremenchuk reservoir was higher. From the middle of summer to the beginning of autumn, the average monthly water temperature in the Kakhovka reservoir is with 1.1–1.9°C higher, compared to other reservoirs. Under these conditions, the crucian carp showed better adaptation to the temperature factor, with a greater amount of reserve substances in the form of lipids and glycogen accumulated in its white muscles and liver, at elevated water temperature. The caloric content of the crucian carp tissues was the maximum compared to other fish species and in the conditions of the Kakhovka reservoir. The zander, as the only studied predatory fish species, revealed multidirectional processes of accumulation of reserve substances. In the white muscles, a higher amount of glycogen, proteins and caloric content were observed in the specimens from the Kakhovka reservoir. In the liver, a lower content of glycogen, lipids and protein was found in the specimens from the Kakhovka, compared to those from the Kremenchuk reservoir. Roach, silver bream and bream showed less adaptability to the next wintering, in terms of the degree of accumulation of reserve substances in the tissues, in the Kakhovka reservoir due to the higher water temperature in September. Fish continued to grow and their energy resources were mostly spent on growth related needs.

Conflict of interest. The authors declare no conflict of interest.

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