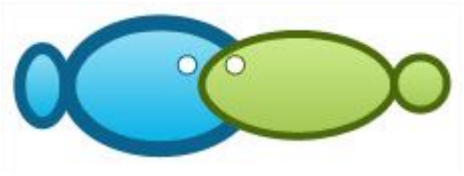

Comparative characteristics of indicators of protein, lipid and carbohydrate metabolism in fish with different types of nutrition and in different conditions of existence

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Abstract. Field research in the Kyiv and Kremenchug reservoirs of the Dnipro cascade has identified certain features in the content of proteins, lipids and carbohydrates in the liver and white skeletal muscle in benthoid (bream *Abramis brama*, roach *Rutilus rutilus*) and predatory (zander *Sander lucioperca*, perch *Perca fluviatilis*) species during the feeding period. The content of total protein in the liver and muscles of bream, roach and zander and in the muscles of perch from the Kyiv Reservoir slightly exceeded the value for these species of fish that inhabit the Kremenchug Reservoir. The content of total protein in the liver of zander during the feeding period slightly exceeded the value in it in bream and roach. Instead, the protein content in the muscles of bream and roach exceeded its value in the muscles of predatory fish from the Kyiv and Kremechuk reservoirs. The highest content of total lipids was found in the liver of roach, which inhabits both reservoirs. In contrast, the total lipid content in the liver of zander of the Kremenchug Reservoir was almost twice lower than its content in the liver of perch from the Kyiv Reservoir. The total content of lipids in the liver of bream and zander from the Kremenchug Reservoir was higher compared to the liver of these species of fish from the Kyiv Reservoir. The content of total lipids in the white skeletal muscles of all studied fish species from both Kyiv and Kremenchug reservoirs was found to be almost at the same level. The highest content of glycogen was registered in the liver of bream and roach of the Kremenchug Reservoir, which probably exceeded the value in the liver of these species of fish from the Kyiv Reservoir. In contrast, in the liver of predatory fish (zander, perch) from the Kyiv Reservoir, the glycogen content exceeded these values recorded by us in the liver of these fish from the Kremenchug Reservoir. The glycogen content in the muscles of bream and roach from both reservoirs was approximately the same. In the muscles of zander and perch from the Kyiv Reservoir, the glycogen content more than doubled the values recorded in the muscles of these species of fish from the Kremenchug Reservoir. Based on the obtained data, it can be concluded that roach and bream from the Kremenchug Reservoir are characterized by higher metabolic rates, which may be due to better environmental conditions and the availability of sufficient fodder base during the feeding period. The identified differences in the metabolic rates of different species of fish may also be related to the peculiarities of adaptation processes to the relevant environmental conditions and the specifics of feeding processes.

Key Words: bream, glycogen, Kremenchug Reservoir, Kyiv Reservoir, liver, perch, roach, total lipids, total protein, white skeletal muscle, zander.

Introduction. Global warming, which has been observed over the past few years and predicted by scientists in the coming years, has significantly affected the thermal and ice regime of the Dnipro reservoirs, and, consequently, the course of numerous intra-reservoir processes occurring in them (IPCC 2007; Vyshnevskyi 2020).

The increase in air temperature predicted by global warming scenarios leads to a significant increase in the maximum possible evaporation of water, especially in reservoirs, which can significantly affect various life processes of aquatic organisms, including fish, and the development and growth of forage organisms. As a result, global warming is having a significant destructive effect on most biological systems. All this can

have a negative impact on the bio- and fish productivity of water bodies in general (Lapina 1980; Adams & Peck 2008; Gleick 2000).

Particular attention in this aspect deserve issues related to the impact of changing environmental conditions on the physiological status of fish inhabiting the reservoirs of the Dnipro cascade. 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 the organs and tissues (Payuta & Florova 2019; Kofonov et al 2020).

The analysis of literature sources on this problem shows the lack of information on the current state of the ichthyofauna of the reservoirs of the Dnipro cascade under the combined effects of global warming and anthropogenic factors on the fish. In the available sources of information there are only data of physiological and biochemical studies aimed at studying the physiological status of certain species of fish inhabiting the reservoirs of the Dnipro cascade. Thus, in particular, there were earlier information on the content of total lipids in the muscles of some fish species of the Kremenchug Reservoir (Maliienko 1973), as well as on the content of total protein in the tissues of bream *Abramis brama*, roach *Rutilus rutilus* and pike *Esox lucius* of the same reservoir (Bilyk 1986). More detailed information on the physiological and biochemical characteristics of industrially valuable fish species of the Sulynska Bay of the Kremenchug Reservoir is presented in the monograph of Rudyk-Leuska & Yevtushenko (2010).

With this in mind, the aim of our research was to study the metabolic characteristics of some fish species of Kremenchug and Kyiv reservoirs by the level of accumulation of total proteins, lipids and carbohydrates (glycogen) in the liver and white skeletal muscles.

Material and Method. To study the physiological status of fish, fishing was carried out in the middle part of the Kremenchug Reservoir and the lower part of the Kyiv Reservoir in October 2020. A total of 10 fish catches were carried out in each reservoir. Fishing was carried out with nets with a mesh of 30, 36, 40, 45, 60 and 80 mm. A sample of fish of 25-30 specimens was selected. The average water temperature in the summer in these areas was: Kremenchug Reservoir - 19-26^{o}C, Kyiv Reservoir - 21-23^{o}C. The biological material was samples of liver and white skeletal muscle of mature bream *Abramis brama*, roach *Rutilus rutilus*, zander *Sander lucioperca* and perch *Perca fluviatilis*.

The content of total proteins in tissue samples was determined by the method of Lowry et al (1951). Briefly, 0.1 g of tissue was hydrolyzed for 1 hour in 10 mL of 10% NaOH at a temperature of 60°C. To 0.1 mL of the hydrolysate was added 10 mL of solution No. 3, and staining was carried out for 15 minutes. Then 1.0 mL of Folin's reagent diluted 1:1 with distilled water was added to the sample. The staining was carried out for 30 minutes. The extinction of the solution was determined on a spectrophotometer Unico 280 UV/VIS at 720 nm against control. The amount of protein was set according to the calibration schedule. Solution No. 3 was prepared from solutions No. 1 and No. 2 in a ratio of 9:1. Solution No. 1 was prepared on the basis of 0.1 n NaOH with the addition of 20 g Na_2CO_3 and 0.5 g of potassium, sodium tartaric acid. Solution No. 2 contained 1 g $CUSO_4$ per 1 liter of distilled water.

The content of total lipids was determined using phosphorovaniline reagent (Tits 2000). Briefly, 100 mg of tissue was hydrolyzed in 1.5 mL of concentrated sulfuric acid for 15 minutes. To about, 0.1 mL of the hydrolysate was added with 3 mL of vanillin reagent (10 mmol L^{-1} of vanillin and 11.5 mmol L^{-1} of phosphoric acid). The solution was stained for 40 min. The extinction of the solution was determined on a spectrophotometer Unico 280 UV/VIS at 530 nm against control. The amount of lipid was set according to the calibration schedule.

The content of glycogen was determined by the anthrone method (Severina & Solovyeva 1898). Briefly, 0.1 g of tissue was hydrolyzed for 1 hour in 3 mL of 30% KOH at a temperature of 100°C, then 0.9 mL of distilled water and 3 mL of 0.2% anthrone were added to 0.1 mL of the hydrolysate. Then the sample was boiled at 100°C for 10 minutes. The extinction of the solution was determined on a spectrophotometer Unico

280 UV/VIS at 620 nm against control. The amount of glycogen was established according to the calibration graph.

The caloric content of fish tissues was calculated by the formula:

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

where: P, G, L = the content of protein (P), glycogen (G) and lipids (L) in g per kg of raw weight; 4.184 = conversion factor from kcal to kJ.

The obtained digital material was subjected to statistical processing using the program Statistica-10.0. Significance between the study groups was assessed using Student's T-test at the level of probability ($p < 0.05$).

Results and Discussion. One of the indicators of the physiological status of fish and the peculiarities of the functioning and individual systems of the body in different periods of the annual cycle is the content of total protein in the organs and tissues. Our studies have shown that the content of total protein in the white skeletal muscles of bream, roach and zander in the autumn significantly exceeded the content of protein in the liver (Figure 1). Thus, the total protein content in the white skeletal muscles of bream from the Kyiv Reservoir exceeded the protein content in the liver by 63.8%, in the muscles of roach - by 147.1%, in the muscles of zander - by 16.1%, and in the muscles of perch - by 111.7%.

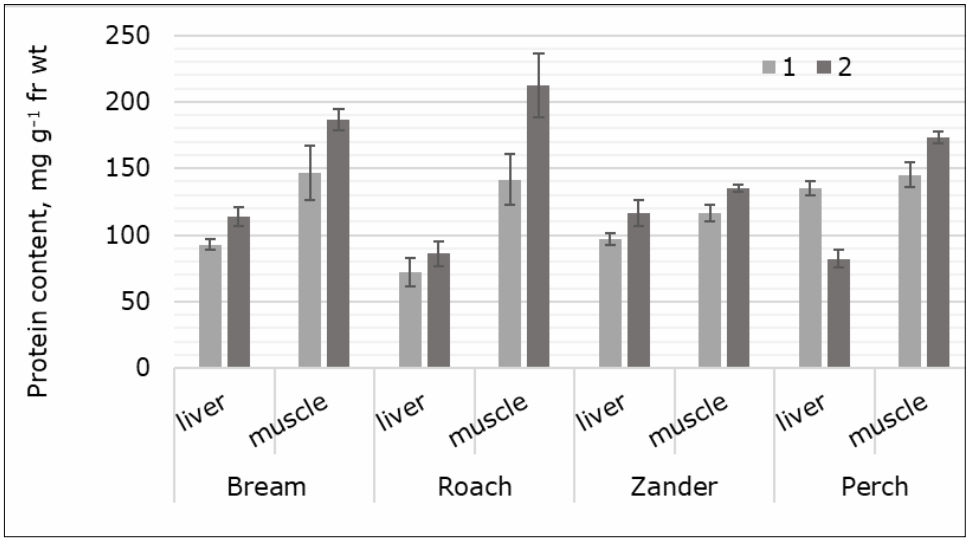

Figure 1. The content of total proteins in the liver and muscles of fish of Kremenchug and Kyiv reservoirs (mean ± standard error, mg g^{-1} of raw tissue, n = 5) (Note: 1 - Kremenchug Reservoir, 2 - Kyiv Reservoir).

When comparing the protein content in the liver of almost all species of fish, it can be noted that it contains less protein in fish from the Kremenchug Reservoir compared to Kyiv (Figure 1). First of all, this indicates that the conditions of this reservoir are favorable for the accumulation of lipids and glycogen in the liver. The warmer temperature of the reservoir in the summer allowed the fish to achieve a better physiological state in preparation for the next winter. Undoubtedly, the condition of the fodder base of the Kremenchug Reservoir plays a significant role, as its middle part is rather shallow compared to the lower part of the Kyiv Reservoir.

River perch stands apart from other studied fish species. This species is more cold-water and belongs to the boreal plain complex, other studied species - to the pontocaspian freshwater. The protein content in the liver of fish from the Kyiv Reservoir is lower, which is also associated with the accumulation of other energy-intensive compounds.

Muscle tissue has the same patterns of difference in protein content in different species of fish as in the liver. The total protein content in the white skeletal muscles of bream from the Kyiv Reservoir exceeded this figure in bream from the Kremenchug

Reservoir by 27.5%, in roach muscles - by 49.9%, in zander muscles - by 15.8%, and in the muscles of perch - by 19.6%. It is also characteristic that predatory fish species from the Kyiv Reservoir have a lower protein content compared to benthic fish species - bream and roach.

A slight difference in the content of total protein in the muscles and liver of perch from the Kremenchug Reservoir may be evidence of its active nutrition and high functional activity of the liver in the biosynthesis of protein from the components of the food it consumes. However, in fish inhabiting the Kyiv Reservoir, the difference in protein content in the liver and muscles is significant. Probably due to the earlier preparation of perch for wintering, the liver accumulates more other reserve substances, including lipids and glycogen, which is reflected in the protein in this organ.

A slightly higher level of total protein accumulation in the white skeletal muscles of bream and roach was found in studies conducted in 2004-2005 by N. Y. Rudyk-Leuska on fish inhabiting Sulynska bay and the open part of the Kremenchug Reservoir (Rudyk-Leuska & Yevtushenko 2010). It should also be noted that in the liver of these fish species at the end of the feeding period the total protein content was almost twice as high as in the liver of these fish species of Kremenchug Reservoir in 2020. Probably, this is due to the temperature regime of the reservoir: the water temperature in the summer during the research was lower by 1-3^{o}C than in 2020.

One of the important indicators that also characterize the physiological status of fish are lipids, which, along with proteins and carbohydrates, are involved in the construction of the cell membrane and are a mandatory component of protoplasm. In addition, lipids play a very important role in metabolic processes, in particular in the energy supply of the body's vital processes at different periods of the annual cycle, including the winter.

Analysis of the results of research presented in Figure 2 indicates the different content of total lipids in the liver and white skeletal muscles of bream, roach, zander and perch, which inhabit the Kremenchug and Kyiv reservoirs.

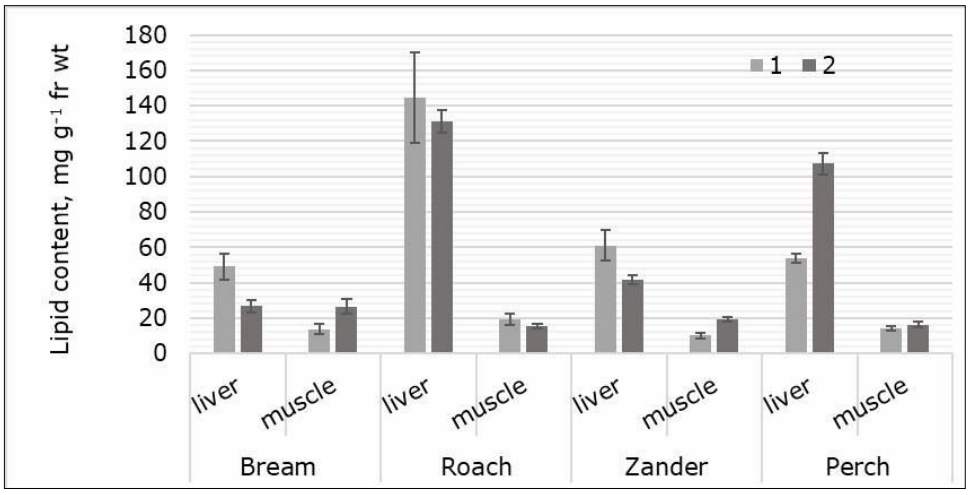

Figure 2. The content of total lipids in the liver and muscles of fish of Kremenchug and Kyiv reservoirs (mean ± standard error, mg g^{-1} of raw tissue, n = 5) (Note: 1 - Kremenchug Reservoir, 2 - Kyiv Reservoir).

The highest levels of total lipids are found in the liver and white skeletal muscle of roach. This is due to the peculiarity of the biology of this species. At the same time, the amount of total lipids in the liver of roach from the Kyiv Reservoir was lower by 10.2% than in fish from the Kremenchug Reservoir. At the same time there is a so-called fatty infiltration of a liver. This phenomenon is usually also observed under the influence of elevated temperatures on fish (Yosipova & Yevtushenko 2003). In our case, it is the temperature conditions that affected the degree of accumulation of total lipids in the organs and tissues of fish. Thus, fish from the Kyiv Reservoir tend to have less lipid

accumulation in the liver and muscles. This is consistent with the higher protein content in the roach from this pond.

The level of total lipids in the liver of zander was more than twice lower, which may also indicate its sufficiently high lipid-forming function during the feeding period. However, in the white skeletal muscle of this species, we recorded the lowest content of total lipids. In our opinion, this may be due to the greater locomotor activity of zander compared to other species. Due to this, the body consumes a significant amount of fat. In addition, the content of total lipids in the liver of zander from the Kremenchug Reservoir was 46.2% higher than that of fish from the Kyiv Reservoir.

The levels of total lipids in the liver and white muscles of bream and perch from the Kremenchug Reservoir were recorded at approximately the same level. However, this rate in the muscles of these species of fish was the same, but it was 3-4 times lower than in the liver. These indicators, in general, meet the generally accepted standards for these species of fish. The obtained results coincide with the data previously registered concerning the organs and tissues of bream of the middle part of the Kremenchug Reservoir (Yevtushenko et al 2007; Rudyk-Leuska & Yevtushenko 2010). In addition, our data are consistent with those of other researchers, who found that the total lipid content in bream muscle averaged 1.8-4.5% (Maliienko 1973; Dabrowski et al 1968; Zahorskikh & Kirsypuu 1990; Fedonenko 1995; Sidorov 1995; Kurhanskyi 1999).

When comparing the lipid content of roach, bream and zander, it is noticeable that at lower temperatures of the reservoir (Kyiv Reservoir) they accumulate less lipids in the liver, but more in the muscles.

Perch again differs from other studied species in the degree of lipid accumulation in both liver and muscles. In fish from the Kyiv Reservoir, the lipid content in the liver is 2.0 times higher, and in the muscles by 16.2% compared to the perch from the Kremenchug Reservoir.

It should be noted that according to data (Rudyk-Leuska & Yevtushenko 2010) in the open part of the Kremenchug Reservoir in 2004-2005 the total lipid content in the muscles of bream and roach was almost twice lower than in 2020, and the content of total lipids in the liver of these fish species was at about the same level. Which also indicates the influence of temperature conditions on the degree of accumulation of lipids in the tissues of these species of fish. In addition, under certain temperature conditions and the presence in the aquatic environment of a sufficiently high level of food organisms for fish, there is intensive consumption of food with a significant content of fat, which is deposited in the abdominal cavity of fish and liver.

An important role in the processes of energy metabolism in fish belongs to glycogen, which is the main energy component of white skeletal muscle. Its specificity is easy mobilization and fast recovery, as well as the ability to release large amounts of energy in a short period of time. It is also known that the main glycogen depots in fish are the liver and muscles. It has been found that in some species of fish the glycogen content in the liver can reach 10-15%, and in white skeletal muscle its content is normally maintained at 1.5% (Coban & Sen 2011).

According to the glycogen content, the studied fish species of different reservoirs were divided into two groups. The first is benthic (roach, bream), in which its amount in the liver is greater in fish from the Kremenchug Reservoir compared to Kyiv. The second group is predatory species that accumulate more glycogen in both the liver and muscles at slightly lower water temperatures during the growing season.

Among the studied fish species from the Kremenchug Reservoir, the highest glycogen content was found in the liver of bream and roach (9.5-12.5%) and in their white skeletal muscles (4.0-4.6%), and the lowest - in zander and perch liver (8.6%) and white skeletal muscles (2.3-2.8%) (Figure 3). The highest content of glycogen in the liver was in zander and perch (10.8-11.0%) and in the muscles (5.6-6.2%) of fish inhabiting the Kyiv Reservoir. Bream and roach from this reservoir contained 8.5-9.6% in the liver and 3.6-5.1% in the muscles.

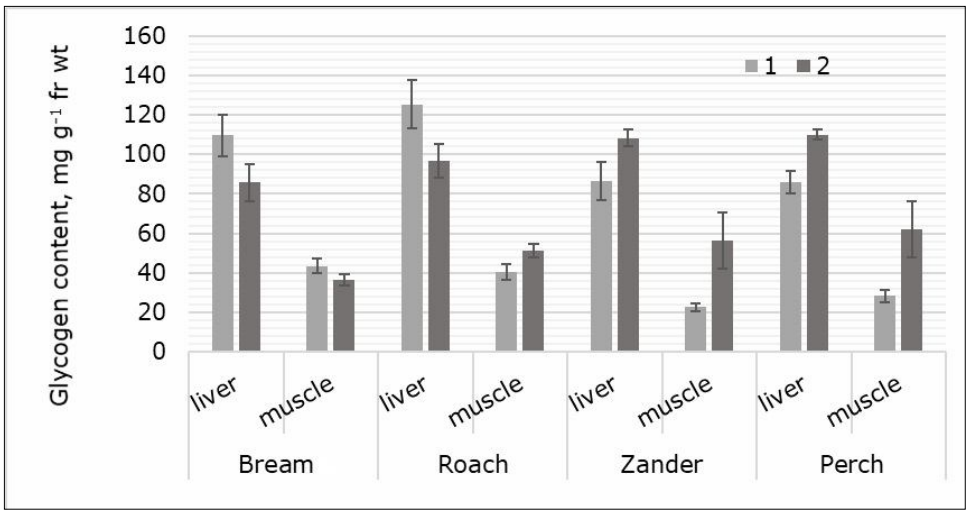

Figure 3. Glycogen content in the liver and muscles of fish of Kremenchug and Kyiv reservoirs (mean ± standard error, mg g^{-1} of raw tissue, n = 5) (Note: 1 - Kremenchug Reservoir, 2 - Kyiv Reservoir).

Undoubtedly, in addition to the temperature regime of the reservoir, the glycogen content in the tissues of benthoidal species of fish is influenced by the state of the forage base. But for predatory fish species, the latter factor is ineffective, because for them the feed base was sufficient in both Kremenchug and Kyiv reservoirs.

Roach and bream from the Kyiv Reservoir were less prepared for the next winter in terms of both lipid and glycogen accumulation compared to the Kremenchug Reservoir.

Particularly noticeable difference is between different species of fish in the biochemical response to rising water temperatures by the caloric content of their tissues. Representatives of the pontocaspian freshwater fauna (bream, roach and zander) are characterized by higher caloric content of fish liver from a warmer water body - Kremenchug (Figure 4). However, the white skeletal muscles of these fish species contain less energy at elevated temperatures. Separately, as mentioned above, behind the changes in this indicator is a boreal species - river perch, which has opposite patterns to other studied species. It adapts better to environmental conditions at fairly low water temperatures. Fish are distributed in the following order according to the caloric content - roach > perch > bream > zander.

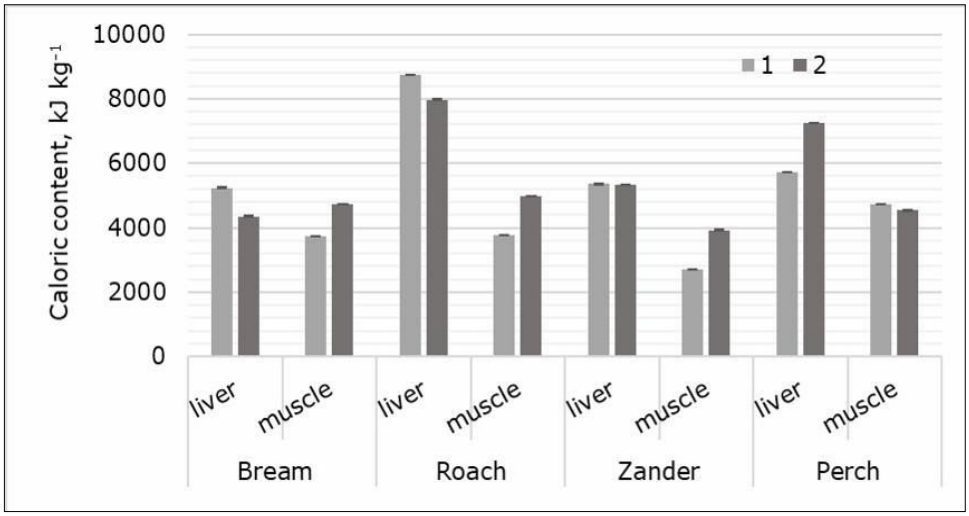

Figure 4. Total caloric content of fish tissues of Kremenchug and Kyiv reservoirs (Note: 1 - Kremenchug Reservoir, 2 - Kyiv Reservoir).

In 2020, no extreme increase in water temperature was observed in either the Kyiv or Kremenchug reservoirs. But some increase in water temperature within the optimum already has affected the physiological state of industrially valuable species of fish. There is no doubt that further research is needed in this direction, as it is known from model experiments that exceeding the temperature of 27^{o}C leads to negative phenomena and sometimes to the death of fish (Martseniuk et al 2018).

Conclusions. The temperature regime of the reservoir during the growing season significantly affects the physiological and biochemical processes, which are manifested in the degree of accumulation of spare substances in the liver and white skeletal muscles of major industrially valuable fish species, including roach, bream, zander and perch.

Analysis of scientific researches and their comparison with the materials of other researchers shows that the content of total protein, lipids and glycogen in the liver and muscles depends on the type of fish diet, their geographical distribution, water temperature and other environmental factors in the previous growing season. These indicators determine the degree of readiness of fish for the winter.

Roach, bream and zander synthesize a larger amount of glycogen lipids in the liver with higher caloric content with a lower protein content under the temperature and ecological conditions of the Kremenchug Reservoir, compared to the Kyiv Reservoir.

Perch is more adapted to cold-water existence, its biochemical parameters are the best in the Kyiv Reservoir.

Under the complex influence of natural and anthropogenic factors of the aquatic environment fish develop physiological and biochemical mechanisms aimed at adapting to ecological living conditions.

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