KAPITEL 5 / *CHAPTER 5* [5]

PHYSIOLOGICAL STATUS OF FISH OF KREMENCHUG RESERVOIR IN THE FEEDING PERIODS

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Introduction

One of the priority areas of research in the field of natural sciences today is to study the ecological status of water bodies of complex and fishery purposes and the biota that inhabits them, including ichthyofauna, under the complex influence of global warming and anthropogenic factors. This is due to the fact that there are already significant changes in the temperature of the aquatic environment of reservoirs of various types, resulting in significant violations of the functional activity of various physiological systems of aquatic organisms, reducing their productive characteristics and growth intensity, etc [13,20].

Over the last decade, there have been changes in the global climate, which have a significant impact on the climatic conditions of the formation of river runoff of Ukraine, causing changes in its surface water resources [1, 12, 13]. The Kremenchuk Reservoir did not avoid problems in this aspect. Based on the analysis of the dynamics of the hydrological regime in the period from 2015 to 2020, it is established that due to the low level of flood waters from the upper Dnipro and rivers flowing into the reservoir, the level regime in it does not meet the requirements of Dnipro reservoirs, and is at the highest level with slight fluctuations throughout the growing season, which is determined by the decision of the Interdepartmental Commission, due to the need to provide drinking water to the population of different regions located in the reservoir area and industrial enterprises, etc.

In addition, modern reservoirs for fishery purposes, including the Kremenchuk Reservoir, are characterized by significant contamination with toxicants of various chemical nature. The main sources of pollution of the Kremenchuk reservoir are industrial and domestic wastewater from Cherkasy. A significant amount of pollutants enters the Kremenchuk Reservoir with tributaries (Ros, Vilshanka, Supoy,

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Sula), which has a negative impact on the mouths of these rivers and adjacent bays.

According to the State Coinspection of Ukraine, the concentration of toxic substances in rivers that feed the reservoirs of the Dnipro cascade is 30-40 times higher than the maximum allowable levels.

Among the pollutants, the main priority toxic substances in the Kremenchuk Reservoir are heavy metals. Thus, the concentration of zinc, manganese, copper, nickel, cobalt and lead in water exceeded the maximum allowable norms [29]. In particular, in the cascade of Dnipro reservoirs in 1998, 192 cases of high pollution (over 10 MPC) were detected, of which 189 cases - heavy metals [15].

Changing environmental conditions caused by global warming for natural reproduction and rearing of fish requires in-depth monitoring observations aimed at establishing the peculiarities of metabolic processes occurring in the body of different species of mature fish, both seasonally and in different periods of the annual cycle (foraging, wintering, pre-spawning, spawning). The results of these studies can be a starting point for predicting the possible effects of the combined effects of global warming and anthropogenic factors of the aquatic environment on the ichthyofauna of water bodies of different types.

Despite the urgency of this problem, in the literature we have not found relevant information concerning the study of metabolic processes in the body of mature individuals of different species of fish of the Kremenchuk Reservoir in certain periods of the annual cycle under the existing hydrological regime and complex effects of natural and anthropogenic factors.

The literature presents only some information that reflects the indicators of metabolism in the body of certain species of fish of the Kremenchuk reservoir under environmental conditions provided by the Rules of operation of the Dnipro reservoirs [15]. Thus, in the work of O. Malyarevskaya and T.I. Birger (1978) data on the biochemical composition of breeds of bream and roach and its impact on the quality of their eggs and larvaeis is present [19]. Other studies found the peculiarities of the accumulation of total lipids in the body of some species of fish that inhabit different parts of the Kremenchuk reservoir [18]. A wider range of physiological and biochemical studies in the study of the physiological status of bream and roach of Sulynska Bay and the adjacent part of the Kremenchuk Reservoir were conducted in

spring and autumn 2004-2006, the results of which are presented in a monograph [21]. However, these and some other studies do not fully reflect the peculiarities of the course of metabolism in fish in the modern period with changes in environmental conditions.

With this in mind, the aim of our research was to assess the physiological status of mature fish of the Kremenchuk Reservoir with different types of nutrition in terms of metabolism in the foraging period of the annual cycle under existing environmental conditions recommended by the Interdepartmental Commission and under the anthropogenic factors.

Material and methods of research

Research fishing was carried out in the spring of 2021 in the pre-spawning period and in the autumn of 2021 in the feeding period during monitoring studies of the middle part of the Kremenchug reservoir. White skeletal muscles and liver of mature bream (*Abramis brama*), roach (*Rutilus rutilus*), white bream (*Blicca bjoerkna*), zander (*Sander lucioperca*), european perch (*Perca fluviatilus*), zope (*Ballerus ballerus*) and gibel carp (*Carassius gibelio*), which determined the total content of proteins, lipids and glycogen. The content of total proteins was determined by Lowry [32], the content of total lipids was determined using phosphorovaniline reagent [24]. The glycogen content in bream tissues was determined by the anthrone method [31].

The obtained digital material was subjected to statistical processing according to the program Statistica - 10.0.

The results of research

Shortly after spawning, the post-spawning period takes place, during which reserve substances are restored as a result of intensive post-spawning feeding of fish. During the feeding period, the metabolism in the body of mature fish is aimed at restoring the energy reserves used during spawning for somatic and linear growth, as well as the deposition of total lipids in the body cavity, liver and muscles, the maximum values of which are found in summer. After spawning of fish under optimal environmental conditions, there is a summer feeding period, during which

there is an intensive accumulation of protein in the organs and tissues and growth.

During this period, the main increase in plastic and energy substances occurs in the body of fish. The duration of the feeding period in fish of different ecology and origin is different. During this period there is a protein increase and accumulation of energy reserves, as well as a significant part of the growth of gonads. This period is characterized by the intensive accumulation in the organs and tissues of fish of a significant amount of reserve substances necessary for energy supply of the body's vital processes in winter.

Significant accumulation of muscle fat occurs during the colder periods of the feeding season and occurs in different years, either immediately after spawning or in the second half of the feeding period. However, a high level of fat accumulation in the organs and tissues of fish is observed under optimal environmental conditions that promote intensive nutrition, and the presence in the reservoir in sufficient quantities for this type of quality food. Thus, the most intensive biosynthesis of fat in European carp liver is observed in late July - early August [7].

Higher content of total lipids in the muscle tissue of fish is observed in autumn compared to the spring period, due to the temperature and intensity of fish nutrition [16].

Intensive fat accumulation at lower temperatures is caused by the preparation of the organism for winter conditions during the transition of the organism to the endogenous type of nutrition and ensuring all vital processes.

As the water temperature decreases during the period when the gonads are in the III stage of maturity, the amount of cavity fat continues to decrease, as well as the content of total lipids in the liver and muscles, which are spent on generative metabolism. Therefore, when the water temperature decreases at the end of the feeding period, there is a suspension of plastic metabolism (protein growth) and switching metabolism to intensive fat accumulation [6, 13].

Based on field and experimental research, it was found that the period of autumn feeding of fish was also characterized by a relatively high level of protein metabolism in the body of almost all studied fish species. The highest content of total protein in white skeletal muscle was found in zope and silver bream, in the muscles of which the protein content reached an average of 152 mg / g of raw tissue mass (table 1).

Table 1 - The content of total protein in the organs and tissues of the studied fish of the Kremenchuk reservoir in the feeding period of the annual cycle of 2021 (M ± m, mg / g of raw mass of tissue, n = 5)

Fish species	Protein	
	Muscles	Liver
Zander	108,37±3,26	80,4±3,94
European perch	143,65±9,85	138,13±7,88
Gibel carp	135,47±5,91	131,13±8,30
Roach	142,61±7,36	132,20±5,74
Bream	145,29±7,36	108,24±8,26
Silver bream	150,32±3,40	113,77±6,95
Zope	154,33±9,06	119,27±7,52

The content of total protein in the muscles of bream, European perch, silver bream and gilber carp was slightly lower and approximately the same, which ranged from 135-145 mg / g of raw weight. The lowest amount of total protein was recorded in zander muscles.

The established level of accumulation of total protein in the muscles of all studies of fish species of the Kremenchuk Reservoir in general corresponds to their species status of the chemical composition of white skeletal muscles in this period.

The obtained results may indicate a high level of protein-synthesizing function of the liver of the studied fish species in ensuring the processes of plastic metabolism and growth of fish, especially in summer. However, it is noteworthy that in all fish species studied, the content of total protein in the liver to varying degrees was slightly lower compared to muscle.

Thus, probably less total protein in the liver compared to muscle was found in zope (29.4%), silver bream (32.1%), bream (34.2%) and zander (34, 8%). Almost the same and high content of total protein in the liver and muscles of perch, gossip and crucian carp may be due to high functional activity of the liver during the summer feeding, which was aimed at ensuring plastic metabolism in the body of these fish and their growth.

It has been suggested that the lower content of total protein in the liver of zope, silver bream, bream and zander compared to muscle may be due to the use of a significant proportion of the hepatic protein precursor oovitelin in the maturation of

sexual products at different stages of trophoplasmic oocyte growth [17].

It is known that the shell of the egg yolk contains both proteins and lipids. It is proved that the proteins of oocyte membranes, as well as other components of membranes, are secreted by egg plasma. Proteins and lipids of the yolk, which are concentrated in its granules, are synthesized and supplied mainly by the liver [22]. Meanwhile, there is a view of the possible biosynthesis of some proteins by the oocytes themselves and the surrounding cells.

Thus, the spectrum of proteins and lipids and their amount in oocytes are largely determined by the course of protein and lipid metabolism in the body, ie its physiological state [3].

The metabolism of the oocyte and liver itself provides the biosynthesis of various spare substances, which are then used by the embryo for its development. Such substances include, above all, glycogen, proteins and lipids [2].

Therefore, during the feeding period in the liver of fish is the biosynthesis of components necessary for the formation of sexual products, as well as their transport to the gonads, where the processes of ovogenesis, ie maturation of germ cells in the female. This period is characterized by the accumulation in oocytes of nutrients necessary for further development of eggs, hatching pre-larvae, larvae growth, and ensuring their high viability. Such nutrients are proteins, lipids, various macro-and micronutrients, enzymes that are necessary for the conversion of egg yolk reserves into embryos. The degree of accumulation in the yolk of the necessary components is of great importance for the development of the embryo, because for the transition to external nutrition, the egg yolk is the only source of substrates and energy for embryo development [23].

In addition, during the period of vitelogenesis and maturation of the gonads, there are changes in the content of total serum protein, the ratio of protein fractions. Based on these and other studies, scientists have concluded that liver function is closely related to the processes of vitelogenesis [11]. On the other hand, it is known that the growth of oocyte vitelogenesis is closely related to liver function, as the bulk of the egg yolk is synthesized by the liver.

The period of trophoplasmic growth of oocytes, during which there is first a gradual and then rapid accumulation of nutrients in developing germ cells, requires

significant energy expenditure from the mother's body. To pass this period all the conditions that maintain the substances at the appropriate level are required, as well as the intensive process of vitelogenesis, in particular, nutrition, temperature and so on. The absence of these conditions leads to a delay in the development of germ cells [10].

In other fish species, it is possible that the processes of generative metabolism during the autumn feeding were in the final stages. It is known that in most fish widows the processes of trophoplasmic growth of oocytes at the stage of vitelogenesis begin in autumn (in October-November). The duration of vitelogenesis in oocytes is related to the spawning characteristics of fish. Thus, in fish with simultaneous spawning (bream, zander, roach, European perch), the processes of vitelogenesis are observed in autumn at lower water temperatures, which then intensify in the pre-spawning period at elevated water temperatures [26].

In fish with portioned spawning (silver bream, gilber carp), the process of vitelogenesis occurs in early spring and continues after the first portion of caviar is hatched.

Therefore, fish enter the period of trophoplasmic growth of oocytes depending on the timing of their spawning. Therefore, different levels of protein use by the liver in the processes of trophoplasmic growth of oocytes in the studied species of fish may be associated with different timing of their spawning, and, accordingly, the beginning of generative metabolism.

It should also be noted that the different content in the organs and tissues of fish total protein can be caused by different effects of environmental conditions on metabolic processes and the presence of food in the aquatic environment. It is assumed that in the autumn feeding period there is a decrease in the intensity of protein metabolism and switching metabolic processes to lipid metabolism [4].

Lipids. Lipids are known to play an extremely important role in fish. The physiological importance of lipids is due to the fact that they are quite concentrated, high in calories and the most convenient for storage, as well as a stable and economical source of energy in the body.

A special role belongs to lipids in the energy supply of generative metabolism, in particular during the formation of gametes, as well as in the processes of

embryonic and post-embryonic development of the organism and so on.

In many species of fish during the development of the gonads, the content of lipids in organs and tissues is reduced because the body's fat reserves are consumed during gametogenesis. The degree of reduction of lipids depends on the intensity of gonadal development and the environmental conditions in which the pre-spawning or feeding period occurs [29].

In addition to proteins, lipids, in particular the fraction of phospholipids, play a significant role in the processes of trophoplasmic growth of oocytes, as it is known that the bulk of cell membranes are phospholipids involved in the formation of intracellular membrane cells. Therefore, in the process of maturation of sexual products to the gonads comes not only synthesized by the liver protein oovitelin, but also certain fractions of lipids [17].

The content of lipids in the organs and tissues of fish also has a significant effect on the intensity of spermatogenesis, because the biosynthesis of nucleic acids in sperm is determined by the amount of fat in the body [30].

In the process of ovarian maturation, the liver plays a significant role in fish metabolism. In the early stages of oogenesis in the liver, in addition to proteins, there is an accumulation of lipids mainly in the form of fractions of triacylglycerols and phospholipids. Therefore, with the onset of vitelogenesis, the fat in liver decreases.

Lipids accumulated in organs and tissues, in particular, triacylglycerols are the main reserve energy source, which is involved in ensuring the body's energy expenditure, especially in cases where the body's energy needs are higher than exogenous energy intake [25].

The sequence of use of fat reserves in different species of fish is different. Thus, in fatty fish, liver lipids are primarily used to ensure the processes of generative metabolism. It is proved that the metabolic activity of lipids is the highest in this organ. Mesenteric fat, connective tissue fat, and muscle lipids are used later.

It should be noted that the accumulation of lipids occurs primarily and with the greatest intensity in those organs and tissues that consume it the fastest. It is also known that lipids play an extremely important role in the energy supply of the processes of plastic and generative metabolism and growth of fish, as well as various vital processes of the body, especially in winter.

In many species of fish, metabolism after spawning is accompanied by a restructuring of metabolism towards the accumulation of lipids. The intensity of feeding and the level of fat reserves, which reaches the fish at the end of the feeding period, is determined by the scale of energy expenditure of the body [29].

Studies conducted in autumn during the feeding period showed that the relative content of total lipids in the white skeletal muscles of almost all 7 species of mature fish of the Kremenchuk Reservoir was characterized by a lower level compared to their content in the liver (table 2).

Table 2 - The content of total lipids in the organs and tissues of the studied fish of the Kremenchuk reservoir in the feeding period of the annual cycle of 2021 (M ± m, mg / g of raw tissue mass, n = 5)

Fish species	Lipids	
	Muscles	Liver
Zander	65,25±8,71	105,63±6,44
European perch	12,65±1,15	57,62±6,64
Gibel carp	6,26±0,82	62,26±6,24
Roach	12,27±2,23	88,89±9,01
Bream	4,61±0,38	31,09±4,41
Silver bream	12,33±0,52	84,35±3,77
Zope	14,96±1,98	85,79±5,33

Among the studied fish species, the highest content of total lipids in muscles was found in zander, which reached 65 mg / g of raw tissue mass. In the muscles of other fish species, such as zope, roach, silver bream and European perch, the content of total lipids was about 4-5 times lower than its content in the muscles of zander. The lowest content of total lipids was recorded in the muscles of bream and gibel carp.

The content of total lipids in the liver of the studied fish species was much higher. Zander was also characterized by the highest level of accumulation of total lipids in the liver. The content of total lipids in the liver of roach, zope and silver bream was slightly smaller and almost the same (on average by 22%).

No significant differences were found between the content of total lipids in the liver of European perch and gibel carp. However, in the liver of these fish species, the

total lipid content was 75% lower compared to those recorded in the liver of zander. The lowest content of total lipids is found in bream liver.

It is noteworthy that the content of total lipids in the liver of zander, roach, zope, silver bream, gilber carp, European perch and bream exceeded the level of their accumulation in the muscles by 61.8%, while in the liver of roach, gilber carp, European perch and bream - almost 7, 6, 10, 5 and 7 times respectively.

The unequal content of total lipids in the muscles and liver of different species of fish may be due to the presence in the aquatic environment of sufficient food of appropriate quality and environmental conditions, as well as species specificity of metabolism in fish, which determine the level of metabolic processes with lipid-forming function of the liver.

Thus, the data of field and experimental studies indicate that during the autumn foraging period in fish there is an intensification of lipid metabolism, in particular a significant level of lipid-forming function of the liver, which also has a significant accumulation of synthesized lipids necessary for energy metabolism at the stage of trophoplasmic growth of oocytes and vital processes of the organism during the winter.

According to the literature, the most intensive lipid biosynthesis in fish liver (carp) occurs during July-August [8]. The maximum accumulation of lipids in the organs and tissues of fish is observed in late July, early August [19]. The total lipid content in the liver usually increases in parallel with the increase in fat content in the body cavities and in the muscles of the bream, ie during the summer period is mainly the deposition of energy resources of the body.

The intensity of fish feeding and accumulation of lipids in their organs and tissues largely depends on the availability of food. There is a close relationship between fat content and the amount of food in the pond. It is necessary to take into account the physiological state of the fish and the conditions of its consumption of food, its availability, water temperature, gas regime, water level fluctuations, etc. [28].

The intensity of feeding and the level of fat reserves of fish is determined by the scale of energy expenditure. It is well known that lipids, along with proteins, are the structural basis of cell organelles and membranes, as well as nerve fibers, etc., and

determine the direction and order of enzymatic reactions in cells.

The rate of lipid formation in fish liver is largely determined by temperature conditions. As the water temperature decreases, the lipid content in the liver decreases sharply and it is replaced by glycogen, the content of which in warm water reaches 18-20%, and at high temperatures decreases to 2-3% [14,16].

Intensive biosynthesis of fat in the liver in the summer is typical for fast-growing fish that eat wholesome food, and for fish that eat plant-based foods and are characterized by low growth rates. The rate of lipid biosynthesis in fish liver is largely determined by temperature conditions. As the water temperature decreases, the content of total fat in the liver decreases sharply and it is replaced by glycogen, the content of which in warm water in winter reaches 18-20%, and from high temperatures decreases to 2-3%. That is, in the autumn period with a decrease in water temperature there is a change in "fat metabolism" to "carbohydrate" [8, 14, 15]. This process is accompanied by a decrease in the content of total lipids in the liver, which causes an increase in its mass.

At the end of the feeding period, fish usually accumulate the maximum amount of fat and glycogen in the liver and muscles [27]. For example, in autumn, the glycogen content of carp liver was 10 times higher than that of muscle.

Glycogen. A significant role in the energy supply of the vital processes of the fish, especially during the winter, belongs to glycogen. During the autumn feeding period, the glycogen content in fish muscles usually increases slightly compared to the spawning period.

Studies have shown different levels of accumulation in the liver and white skeletal muscles of different fish species (Table 3).

Among the studied fish species, the highest content of glycogen was found in the muscles of gibel carp, 2.8 times less - in the muscles of roach, 4 times - in bream, 4.7 times - in zander, 6.7 times – European perch and zope, 10 times less - in silver bream.

The different levels of glycogen in the muscles of different species of fish are obviously largely due to the quantity and quality of fish feed, as well as the body's internal needs for using this energy component in ensuring the vital processes of fish, especially during winter.

Table 3 - Glycogen content in the organs and tissues of the studied fish of the Kremenchuk reservoir in the feeding period of the annual cycle of 2021 (M ± m, mg / g of raw tissue mass, n = 5)

Fish species	Glycogen	
	Muscles	Liver
Zander	16,50±1,27	102,47±3,41
European perch	11,52±0,78	80,89±7,73
Gibel carp	78,32±13,03	149,09±12,17
Roach	28,08±2,79	102,62±7,31
Bream	19,51±1,60	84,40±3,69
Silver bream	7,59±0,28	27,50±3,22
Zope	11,67±1,31	47,60±2,68

Significantly higher levels of glycogen accumulation were recorded in the liver compared to the muscles of all fish species studied.

The highest content of glycogen was also found in the liver of gilber carp. Glycogen content was found to be 46% lower in roach and zander liver, 76.5% and 84% less in bream and European perch hepatopancreas, respectively. The content of glycogen in the liver of zope was three times lower and 5.4 times lower in the liver of silver bream, compared with its content in the liver of gibel carp.

It is also noteworthy that the content of glycogen in the liver of all studied fish species significantly exceeded its values established in the muscles. Thus, in the liver of gibel carp glycogen content was almost twice its value in muscle, in roach 3.7 times, in zander - 6.2 times, in bream - 4.3 times, in European perch - 7 times , in zope - 4.7 times, in silver bream - 3.6 times.

The results of research indicate a high level of glycogen-storing function of the liver of various species of fish in the Kremenchuk Reservoir during the autumn feeding period, which is very important for energy supply of fish in the winter and spawning.

The high level of glycogen accumulation in the organs and tissues of fish is largely determined by the presence of food required for intensive biosynthesis in the liver of glycogen in the reservoir.

Thus, at the end of the feeding period in the organs and tissues of mature fish of Kremenchuk Reservoir the maximum level of accumulation of total protein, lipids

and glycogen is registered, which are necessary for energy support of generative synthesis and development of gonads and various vital processes and wintering period.

Conclusions

Studies have shown that under the current environmental conditions (relative to the constant level and water temperature) caused by global warming, in the autumn foraging period, the physiological status of adult fish with different types of food in the Kremenchuk Reservoir was characterized by relatively high metabolic rates. Evidence of this is the significant accumulation of total protein, lipids and glycogen in organs and tissues, including the liver and white skeletal muscles.

Most of the studied fish species (except gibel carp) during the autumn feeding period had a higher content of total protein in the white skeletal muscles compared to the liver. This is due to the fact that the main stage of protein accumulation in fish muscles occurs during the summer feeding period, and in the autumn feeding period there is some inhibition of plastic metabolism and switching to intensive fat accumulation [9].

In addition, the lower content of total protein in the liver of fish during this period can be explained by the fact that with the onset of a new stage of gametogenesis in most fish in the autumn, the precursor protein oovitelin is used. It is used to ensure the processes of generative metabolism to a large extent, in addition to food components. It also participates in the processes of trophoplasmic growth of oocytes and spermatocytes.

During the autumn feeding period, a high level of accumulation of total lipids in the liver of the studied fish species was found, which significantly exceeded the values recorded in the muscles. This is due to the high intensity of lipid-forming function of the liver, aimed at ensuring the processes of energy metabolism at the stage of trophoplasmic growth of oocytes and the vital processes of the organism during the winter.

With a decrease in water temperature in the autumn there is a change in "fat

metabolism" for carbohydrates. In addition to lipids, in the organs and tissues of different species of fish during the autumn feeding found intensive accumulation of glycogen, the high level of accumulation of which is largely determined by the presence in the reservoir of feed required for intensive biosynthesis of glycogen in the liver.

Thus, at the end of the feeding period in the organs and tissues of mature fish of Kremenchuk reservoir the maximum level of accumulation of total protein, lipids and glycogen, which are necessary for energy support of generative synthesis and development of gonads and various vital processes and wintering period had been recorded.

The unequal content in the organs and tissues of different species of fish of total protein, lipids and glycogen in the foraging period is largely due not only to the peculiarities of their metabolic processes, but also due to the presence in the aquatic environment of food and environmental conditions specific to each species of fish.

Thus, small fluctuations in water level and optimal temperature in the autumn feeding of fish with different types of food in the Kremenchuk reservoir were optimal ecological conditions for the development of natural forage and the body's processes of plastic, generative metabolism and energy supply of the body during wintering and spawning.