Characteristics of protein, lipid, and carbohydrate metabolism of fish of the Kremenchuk Reservoir in the prespawning period

Natalia Rudyk-Leuska, Mykhailo Leuskyi, Nikolay Yevtushenko, Melaniia Khyzhniak, Igor Buzevich, Alina Makarenko, Ganna Kotovska, Iryna Kononenko

ABSTRACT
The paper presents the results of scientific research aimed at studying the peculiarities of metabolism in the body of seven species of mature fish in the Kremenchuk reservoir in the pre-spawning period under ecological conditions that differ from existing ones according to the Dnipro Reservoir Rules of Operation. Somewhat increased levels of total protein accumulation were found during this period in the muscles of zander, perch, and gibel carp. More statistically significant differences between the content of total protein in the liver and muscles were found in other fish species, in particular in roach it was 51.2%, in bream – 57.8%, in European flounder, and zope – 40.6%. Slightly elevated total lipids were found in the muscles of these fish. Thus, in the muscles of silver bream, it was 12.07 mg/g of raw weight, and in the muscles of gibel carp – 18.5 mg/g, while in the muscles of all other studied species of fish, this figure was in the range of 6.7 to 8.71 mg/g of raw weight. The glycogen content in the muscles of different objects of the Kremenchuk reservoir in the pre-spawning period was different. Its highest content was found in the gibel carp muscle, which reached 74 mg/g of raw weight. Significantly lower (2.7 times) was the level of glycogen accumulation in zander muscles and 3.2 times – in roach muscles. In the muscles of bream, European perch and silver bream found close, relatively low levels of glycogen, which was in the range of 10 – 13 mg/g of raw mass, and the lowest level of its accumulation was recorded in the muscles of the zope (only 4.9 mg/g). The glycogen content of the liver of all studied fish species significantly exceeded that recorded in their muscles.

Keywords: reservoir, fish, liver, white skeletal muscle, proteins, lipids, glycogen

INTRODUCTION
After wintering under certain temperature conditions and the presence of forage organisms for these fish species, the body's energy potential is gradually restored and prepared for one of the most important periods-spawning. This preparation is carried out in the so-called pre-spawning period, which is characterized by specific conditions of metabolism and the functional state of the organism. It is known that in this period, there is a differentiation and then trophoplasmic growth of oocytes and spermatogonia [1], [42].

In most fish species, the pre-spawning period is divided into two subperiods. The first subperiod is mainly associated with the differentiation of sexual products and is characterized as the protoplasmic growth of oocytes (period of low growth). The second subperiod (period of high growth) is mainly associated with the trophoplasmic growth of oocytes. According to these subperiods, adult fish's physiological and biochemical characteristics change [2], [41]. During the first subperiod, there is the most significant decrease in lipids in the body of fish, and during the second subperiod-intensification of protein growth [3], [43].

During the pre-spawning period, the fish liver activates the processes of protein synthesis associated with the differentiation and growth of generative tissue. Plastic material for forming sexual products of fish is food components, as well as reserves that are concentrated in the body due to feeding in the previous year [4], [5].

In fish that feed before spawning, partially restored energy resources ensure the maturation of sexual products and the spawning process itself [6].
However, it should be noted that the effectiveness of the pre-spawning period for the maturation of sexual products and preparation of the body for spawning is largely determined by the level and temperature of the reservoir.

It is known that the role of the Kremenchuk Reservoir is related to the need to maintain different water levels throughout the year. Thus, in particular, according to the Rules of Operation of the Dnipro Reservoirs, the capacity of this reservoir before floods is reduced by lowering the water level by 3.0 – 5.0 m. That is, only during this period, the maximum area of flooding and the useful volume of the reservoir is observed. However, the pre-spawning period of many fish species occurs somewhat earlier [7].

Meanwhile, the analysis of the decadal dynamics of the level regime of the Kremenchuk reservoir shows that in 2020 there was No. spring operation, as there were not enough flood waters, we recorded sufficient water filling in March-May, which did not reach the level of NSH only by 20 – 70 cm. A similar monthly water regime of the Kremenchuk Reservoir was also registered from 2017 to 2019 only with insignificant minimum and maximum values (±10 – 20 cm), which did not harm the ecological situation of the reservoir and the development of biota in it. The established level regime in the reservoir during this period was maintained at a sufficiently high level, recommended by the Interdepartmental Commission because of the low floods in the Dnipro due to global warming.

The established level and temperature regime of water can significantly impact the development of biota and, accordingly, the processes of preparation of the organism, especially mature fish, for spawning.

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 the pre-spawning period under the existing hydrological regime and complex effects of natural and anthropogenic factors.

With this in mind, our research aimed to assess the physiological status of mature fish of the Kremenchuk Reservoir with different types of nutrition in terms of metabolism in the pre-spawning period under existing environmental conditions recommended by the Interdepartmental Commission and under the influence of anthropogenic factors.

**Scientific Hypothesis**

We studied how changes in the content of glycogen, proteins, and lipids occur in the body of freshwater fish of the Kremenchuk Reservoir. The difference in indicators shows a change in the intensity and direction of their metabolic processes.

**MATERIAL AND METHODOLOGY**

Research fishing was carried out in the spring of 2021 in the pre-spawning period during monitoring studies of the middle part of the Kremenchuk Reservoir, one of the six largest reservoirs in the cascade on the Dnipro River in Poltava, Kirovohrad, and Cherkasy regions of Ukraine. Located between Kaniv and Kamyanka reservoirs and is formed by the dam of Kremenchuk HPP.

**Samples**

White skeletal muscles and liver of mature bream (*Abramis brama*), roach (*Rutilus rutilus*), white bream (*Blicca bjoerkna*), zander (*Sander lucioperca*), European perch (*Perca fluviatil*), zope (*Ballerus ballerus*) and gibel carp (*Carassius gibelio*) were selected as biological material for in-house processing. The collected material determined the total content of proteins, lipids, and glycogen (Figures 1, 2, 3, 4, 5, 6, 7).

**Figure 1** Bream (*Abramis brama*).

**Figure 2** White bream (*Blicca bjoerkna*).
Chemicals
Potassium hydroxide (KOH), anthrone (C₁₄H₁₀O), concentrated sulfuric acid (H₂SO₄), sodium hydroxide (NaOH), sodium carbonate (Na₂CO₃), potassium sodium tartaric acid (KNaC₄H₄O₆ × 4H₂O), copper sulfate (CuSO₄), Folin–Ciocalteu reagent (FC), vanillin reagent (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).

Animals and Biological Material
In total, 35 specimens were processed – mature bream (Abramis brama), roach (Rutilus rutilus), white bream (Blicca bjoerkna), zander (Sander lucioperca), European perch (Perca fluviatilis), zope (Ballerus ballerus) and gibel carp (Carassius gibelio) caught from the Kremenchuk reservoir.

Instruments
Set of grids with a mesh step from 30 to 100 mm (producer «CrayFish» Limited Liability Company, Finland). Electronic laboratory scales (TBE-0.15-0.001-a-2, producer «Inter-Synthesis» Limited Liability Company, Ukraine). Spectrophotometer Unico 280 UV/VIS (producer: ALTALAB Limited Liability Company, Ukraine).

Laboratory Methods
The content of total proteins in tissue samples was determined by the method of Lowry et al. (1951) [8], and the content of total lipids was determined using a phosphorovaniline reagent [9]. The anthrone method determined glycogen content in fish tissues [10].

Description of the Experiment
Sample preparation: When determining the chemical composition in the organs and tissues of fish from the reservoir were selected 5 specimens of fish of each species.
**RESULTS AND DISCUSSION**

Given that the metabolism of fish depends on environmental factors [2], in our opinion, it is appropriate to present information that characterizes the dynamics of the level and temperature regime of the Kremenchuk Reservoir in the spring of 2021 (pre-spawning period) [11], [12].

Based on the analysis of materials submitted by the Poltava Fish Protection Patrol, in the spring of 2021, in the pre-spawning and spawning periods, relatively high and stable indicators characterized the water level in the Kremenchuk Reservoir. Thus, in March, the water level fluctuated between 78.7 – 79.5 m, and in April - within was 79.5 – 80.8 m. Thus, in March, the water level in the reservoir was lower than NSH (81 m) by 2.3 – 1.5 m, and in April – 1.5 – 0.2 m.

The average daily water temperature of the Kremenchuk Reservoir in the pre-spawning and spawning periods fluctuated within: in March – within 3 – 5 ºC, in April – within 6 – 9 ºC, in May – 9 – 19 ºC.

One of the integral indicators that determine the physiological status of fish is metabolism, which is based on indicators of metabolic processes associated with the biosynthesis of proteins, lipids, carbohydrates, and other organic compounds that support the body in different seasons and its adaptation to changed ecological conditions of existence [13], [14], [15].

In addition, metabolic indicators are a kind of biomarkers that characterize not only the physiological status of fish but also the water quality and ecological status of water bodies they inhabit [16], [17], [18].

During the study period, the content of total proteins, lipids, and glycogen was determined in the liver – and organs characterized by its multifunctional activity, including protein-synthesizing, lipid-forming, glycogen-storing, and other functions, as well as white skeletal muscle [19], [20].

**Protein:** In the pre-spawning period, intensive processes of protein synthesis associated with the differentiation and growth of generative tissue occur. Reserve substances formed in the body in the previous period of feeding and components of food consumed by fish in the pre-spawning period are plastic materials for the formation of sexual products.

The main source of energy for protein synthesis in the pre-spawning period are fat reserves, which accumulate in significant quantities in the body, and food energy. During the period of energy mobilization, the absolute and
With increasing water temperature in the reservoir, there is an intensive development of natural fodder base, which contributes to increased food consumption and, accordingly, the use of its components in the biosynthesis of organic matter [30], [31].
In this respect, the results of studies are noteworthy, which found that during pre-spawning feeding, the content of total lipids in the white skeletal muscles of mature fish of the Kremenchuk reservoir was at about the same level, except for gibel carp and silver bream. Slightly elevated total lipids were found in the muscles of these fish (Table 3). Thus, in the muscles of silver bream, it was 12.07 mg/g of raw weight, and in the muscles of gibel carp – 18.5 mg/g, while in the muscles of all other studied species of fish, this figure was in the range of 6.7 to 8.71 mg/g of raw weight.

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

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Muscles</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zander</td>
<td>6.73 ±1.10</td>
<td>37.26 ±3.89</td>
</tr>
<tr>
<td>European perch</td>
<td>8.67 ±1.03</td>
<td>38.42 ±6.56</td>
</tr>
<tr>
<td>Gibel carp</td>
<td>18.50 ±2.18</td>
<td>40.46 ±3.95</td>
</tr>
<tr>
<td>Roach</td>
<td>8.71 ±1.05</td>
<td>50.41 ±7.01</td>
</tr>
<tr>
<td>Bream</td>
<td>8.41±1.91</td>
<td>40.00±3.84</td>
</tr>
<tr>
<td>Silver bream</td>
<td>12.07 ±2.16</td>
<td>50.11±10.53</td>
</tr>
<tr>
<td>Zope</td>
<td>6.89 ±1.03</td>
<td>38.16±2.70</td>
</tr>
</tbody>
</table>

Note: M is the simple arithmetic mean, and m is the error of the arithmetic mean.

Approximately the same content of total lipids in the muscles of the studied fish species of Kremenchuk Reservoir in the pre-spawning period may indicate the maximum accumulation of energy resources needed to ensure the functional activity of these tissues in the pre-spawning period and during spawning.

According to other researchers, the content of lipids in the meat of bighead carp of the Kremenchuk Reservoir averages from 4% to 6.6%, and in common carp meat from 3% to 4.6% (spring catch) [21].

It is noteworthy that the liver’s total lipids content in the pre-spawning period is higher than in the fish's muscles. Studies have shown that the total lipid content in the liver of gibel carp, European perch, bream, zope, and zander was about the same, relatively high level, which is 4 – 5 times higher than the values recorded in the muscles of these fish species.

The content of total lipids in the liver of roach and silver bream was slightly higher, which exceeded the average of other fish species by about 20 – 25%.

The significant level of accumulation of total lipids in the liver of fish in the pre-spawning period is primarily due to the high functional activity of the liver in the biosynthesis of lipids from digested food components and their deposition to energy protoplasmic growth of oocytes at the final stage of their development and spawning process.

According to the literature, a higher initial level of lipids (at the beginning of the pre-spawning period) provides a more intensive process of fish maturation and greater spawning. The author believes that the initial level of fat reserves in fish at the beginning of the pre-spawning period may be one of the important indicators of the readiness of fish for their maturation and spawning. The higher the fat content of fish, the faster it matures and therefore reaches a higher maturity.

In addition, fat reserves largely provide energy synthesis of generative tissue. In gamete formation, lipids partially pass to the gonads and are included in the yolk of oocytes as a nutrient, and after hatching and fertilization of eggs, they act as the main source of endogenous food during embryo development. Therefore, the level of fat reserves in the organs and tissues of fish and the intensity of their use in the maturation of the gonads largely depends on the effectiveness of the spawning process of fish. That is, indicators of the dynamics of lipid content in the organs and tissues of fish can be indicators of fish readiness for spawning.

Thus, in the body of the studied fish species, intensive fat accumulation in the pre-spawning period is established, as these accumulated reserve substances after wintering are spent on the energy supply of sexual production processes at the final stage of their development and spawning.

The results coincide with the data of other researchers, who proved that at the beginning of the pre-spawning period (III-IV stages of maturity of sexual products) in the organs and tissues of fish, there is an increase in protein and lipids [40].

In the pre-spawning period, when the process of gonadal formation takes place, the level of lipids in the muscles of the Kremenchuk Reservoir was quite high. Immediately before spawning (March), bream liver is maximally saturated with protein and the maximum amount of lipids.

At the end of the pre-spawning period, the total content of proteins and lipids decreases, which is due to the use of reserve substances in the process of trophoplasmic growth of oocytes and spermatoocytes.
Temperature conditions largely determine the rate of lipid formation in the fish liver. As the water temperature decreases, the lipid content in the liver decreases sharply, and it is replaced by glycogen, which in warm water reaches 18 – 20%, and at high temperatures decreases to 2 – 3%.

**Glycogen:** Glycogen plays an important role in the accumulation of energy in fish under anaerobic conditions due to its easy mobility, high degree of recovery, and ability to release energy. This is its specificity as a source of energy for fish. The main stores of glycogen are concentrated mainly in the muscles, but the most mobile "depot" of glycogen is in the liver of fish [32], [33], [34].

Muscle glycogen is mainly used as an energy source for fish movement, so its changes are related to seasonal physiological rhythms. Muscle glycogen stores are most abundant during fish wintering when food intake is sharply reduced. Used glycogen stores in muscles are constantly replenished not only due to their reserves in the liver but also as a result of its intensive biosynthesis from intermediate products of the breakdown of proteins and lipids, which are mobilized from muscle tissue and the liver itself as a result of processes gluconeogenesis [35], [36].

It should be noted that the use of lipids and glycogen in energy metabolism in fish is closely related. Thus, in fish glycogen can be synthesized from the products of fat metabolism, and lipids – from carbohydrate metabolism. The blood glucose level regulates the intensity of lipid mobilization from fat depots.

During the pre-spawning season, carbohydrate parameters usually increase by 1.5 – 2 times, due to the intensive nutrition of fish and an increase in the overall level of energy metabolism (Table 4).

**Table 4** Glycogen content in the organs and tissues of the studied fish of the Kremenchuk Reservoir in the pre-spawning period of the annual cycle of 2021 (M ±m, mg/g of raw tissue mass, n = 5).

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Muscles</td>
</tr>
<tr>
<td>Zander</td>
<td>27.34 ±8.43</td>
</tr>
<tr>
<td>European perch</td>
<td>13.35 ±2.85</td>
</tr>
<tr>
<td>Gibel carp</td>
<td>73.96 ±11.41</td>
</tr>
<tr>
<td>Roach</td>
<td>23.03 ±2.41</td>
</tr>
<tr>
<td>Bream</td>
<td>17.03 ±2.31</td>
</tr>
<tr>
<td>Silver bream</td>
<td>10.59 ±1.58</td>
</tr>
<tr>
<td>Zope</td>
<td>4.90 ±1.09</td>
</tr>
</tbody>
</table>

Note: M is the simple arithmetic mean, and m is the error of the arithmetic mean.

The glycogen content in the muscles of different objects of the Kremenchuk reservoir in the pre-spawning period was different. Its highest content was found in the gibel carp muscle, which reached 74 mg/g of raw weight. Significantly lower (2.7 times) was the level of glycogen accumulation in zander muscles (Table 2) and 3.2 times – in roach muscles. In the muscles of bream, European perch and silver bream found close, relatively low levels of glycogen, which was in the range of 10 – 13 mg/g of raw mass, and the lowest level of its accumulation was recorded in the muscles of the zope (only 4.9 mg/g).

The glycogen content of the liver of all studied fish species significantly exceeded that recorded in their muscles. The highest level of glycogen accumulation was found in the liver of gibel carp, which reached 270 mg/g of raw weight. The content of glycogen in the liver of zander and bream was significantly lower (1.8 – 2 times), respectively, compared to its maximum accumulation in the liver of gibel carp. Glycogen was three times lower in the liver of roach, European perch, silver bream, and zope, but they were significantly higher than those found in the muscles of these fish species.

Different glycogen content in the muscles and liver of different species of fish in the pre-spawning period, apparently, due to different needs of the body in its supply of energy during spawning, and may be related to the physiological processes occurring in fish in the pre-spawning period, and to some extent can be determined by environmental conditions that characterize the functional activity of the organism as a whole.

The dynamics of carbohydrate metabolism in fish are determined by the easy mobilization and recovery of carbohydrate reserves and the ability to release large amounts of energy in a short time.

There are two glycogen peaks in the muscles and liver of demersal fish. The first maximum is observed at the end of the feeding period when glycogen accumulates simultaneously with lipids. Then, during the winter, it is consumed, and its content increases again before spawning due to biosynthesis from the breakdown products of protein-lipid muscle complexes. In muscle, glycogen serves as a backup energy source for muscle function [36].

In the liver, glycogen is a carbohydrate reserve from which glucose is formed when certain enzymes are involved. Some carbohydrates in fish are converted into fats and stored in the liver and muscles. If necessary, glycogen is easily converted into glucose, which can participate in fish's energy supply of metabolic processes [37].
Glycogen levels in muscle, especially in the liver, are thought to be indicators of the body's physiological condition [38].

CONCLUSION
Analysis of the results of field and experimental studies shows that in the pre-spawning period under existing conditions in white skeletal muscle, the total protein content of all studied species of mature fish from the Kremenchuk Reservoir was relatively high (from 145 to 187 mg/g raw weight). Approximately the same and relatively high level of total protein in the muscles of fish with different types of food may indicate its low cost of accumulated reserves during the feeding period last year during the winter, on the one hand, as well as optimal environmental conditions and availability in the aquatic environment feed resources, which contributed to the high intensity of protein-synthesizing function of the fish liver in this period from another. Evidence of the liver's high activity in protein biosynthesis in the pre-spawning period is its higher total content, which is found in the liver compared to muscle. It should be noted that only a slight excess (11%) of total protein in zander liver and 19.6% in perch liver was found in the pre-spawning period, compared to its content in muscle. More statistically significant differences between the content of total protein in the liver and muscles were found in other fish species, in particular in roach it was 51.2%, in bream – 57.8%, in European flounder, and zope –40.6%. In the pre-spawning period, approximately the same content of total lipids was found in the muscles of most species of fish studied. Its content in gibel carp and silver bream muscles was slightly higher. Thus, in the muscles of silver bream, it was 12.07 mg/g of raw weight, and in the muscles of gibel carp – 18.5 mg/g, while in the muscles of all other studied species of fish, this figure was in the range of 6.7 to 8.71 mg/g of raw weight. The content of total lipids in the liver of all studied fish species was significantly higher than in the muscles, which may indicate high activity of lipid-forming function of the liver in the presence in the aquatic environment of sufficient components necessary for lipid biosynthesis. The glycogen content in the muscles of different objects of the Kremenchuk reservoir in the pre-spawning period was different. Its highest content was found in the gibel carp muscle, which reached 74 mg/g of raw weight. Significantly lower (2.7 times) was the level of glycogen accumulation in zander muscles and 3.2 times – in roach muscles. In the muscles of bream, European perch and silver bream found close, relatively low levels of glycogen, which was in the range of 10 – 13 mg/g of raw mass, and the lowest level of its accumulation was recorded in the muscles of the zope (only 4.9 mg/g ). The glycogen content of the liver of all fish species was higher than that of muscle. The highest glycogen content was recorded in the liver of gibel carp, zander, and bream, less – in other fish species. The high content of glycogen in the organs and tissues of fish indicates a high level of glycogen-storing function of the liver and its role in ensuring the energy supply of fish in spawning.

REFERENCES


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Conflict of Interest:
The authors declare No. conflict of interest.

Ethical Statement:
Following the protocols, No. 3/2021, No. 5/2021, and No. 7/2021 at the meeting of the Ethics Commission of the Faculty of Animal Husbandry and Aquatic Bioresources of the National University of Life and Environmental Sciences of Ukraine during the experimental catches signed Acts No. 1/3, 2/5 and 1/1 ie in the process of catching (all norms of the current legislation of Ukraine according to DSTU 2284:2010 are observed).

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