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Investigation of internal organs and additive tissue of hybrid hypophthalmichthys (*Hypophthalmichthys spp.)* as a promising raw material for the production of dietary nutritional products

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ABSTRACT

Preservation of the nutritional value of fish and the useful qualities of its rich composition is extremely important. The urgent task of the food industry is to develop and create quality food products that meet modern production trends and compete in domestic and foreign markets. This scientific paper describes studies aimed at assessing the specific weight (%) of essential nutrients (glycogen, proteins, and lipids) in particular organs and tissues of different size and mass groups of the hybrid of silver and bighead carp experimental ponds and reservoirs of Ukraine. To achieve the goal in the research process, fish farming, biochemical (study of total protein, lipids, and carbohydrates), and statistical (mathematical processing of research results) research methods were used. In all size and mass groups of the hybrid of silver and bighead carp from ponds and reservoirs in 2017, 2018, and 2019, mostly satisfactory values of general metabolism indicators were found - glycogen, proteins, and lipids in the liver, gills, and muscles of fish. In annual fish of winter ponds, total protein and glycogen content in all organs and tissues was slightly reduced. The organisms of biennial fish from feeding ponds were characterized by fluctuations in the content of glycogen in the liver (it was the highest in fish, 3.28 – 3.33%). Significant fluctuations in the total protein content of muscle, liver, and gills and a slight excess of glycogen in the liver and lipids in the gills of three-year-olds were observed in the reservoirs. The difference found in the availability of essential nutrients in the body of the studied fish indicates a change in the intensity and direction of their metabolic processes. However, their physiological condition at the time of the study was within normal limits.

Keywords: pond, reservoir, proteins, lipids, glycogen.

INTRODUCTION

The accumulation of glycogen, proteins, and lipids in fish is influenced by many biotic and abiotic factors **[1]**. The above factors can be significantly influenced by a number of factors, hydrochemical state of water, temperature, gas regime of the reservoir, seasons, and meteorological factors **[2]**. Significant dependencies can be made by the species, age, and sex of fish, stocking density and the presence of invasions, and the level of contamination of the reservoir with various nutrients or toxic substances (heavy metals, petroleum products, and their derivatives) **[3]**, **[4]**. Oil and petroleum products are the most common toxicants in the aquatic environment **[5]**. Their characteristic feature is that these substances are slowly destroyed under natural conditions, sometimes change their chemical form, and gradually accumulate in various ecosystem components **[6]**. Oil harms fish's metabolism, inhibiting the activity of various localized enzymes that carry out the hydrolysis of protein and carbohydrate components of food, which leads to an increase in total protein. Accumulating heavy metals in fish can cause significant disturbances in cellular metabolism **[7]**, causing oxidative damage to proteins and nucleic acids. Biogenic elements, which include nitrogen compounds, enter the body through the gill apparatus and intestines of fish, damaging their epithelial layer. In addition, they can adversely affect other internal organs, including the liver, kidneys, and spleen of fish. In the future, this can lead to metabolic disorders, which can be reflected in the neurohumoral regulation of metabolism and enzymatic activity, changing the content of energy-intensive compounds. This can affect fish survival, the quality and quantity of gametes, and hence the species diversity of ichthyofauna **[8]**. The toxic effect of phenolic compounds on fish is manifested in the disruption of protein biosynthesis, barrier functions of membranes, etc., which affects the processes of balance, respiration, and motor activity. In fish it causes anxiety and, subsequently, seizures and death. That is why phenols are classified as toxic paralytics **[9]**. Chromium has mutagenic and teratogenic properties, as its effects on fish can cause significant changes in physiological, histological, biochemical, and genetic parameters **[10]**. One of the main components in the body of fish is protein, and its content depends on the rate of linear growth. In addition, in fish, proteins can be used as alternative energy sources, which, if necessary (with the participation of aminotransferases) play a role in the processes of adaptation to the negative factors of the environment **[11]**. Studies of internal organs, particularly the liver, provide objective information that can be used to determine the general physiological state of the body **[12]**. It is known that the liver is an important organ of metabolism **[13]**. Among its main functions are the processes of digestion and the processes of protein metabolism (synthesis and decomposition). The cleavage of proteins into amino acids is subsequently broken down to form ammonia or urea or is involved in protein-synthetic processes. The level of lipid accumulation is directly dependent on the fatness of fish, and the direction of lipid metabolism varies depending on the stage of ontogenesis, sex, and phase of the reproductive cycle. For example, the lipid content in the liver of males and females differs and depends on the stage of maturity of the gonads **[14]**. Lipids are the basis for all intracellular membranes and play a significant role in cell metabolism **[15]**. Violation of homeostasis of the body always indicates the presence of pathology or stress. The total lipid content indicates the activity of anabolic and catabolic processes and the mobilization of lipids as a source of energy **[16]**. After all, lipids can be used in adaptive rearrangements of structural components of cells, tissues, and organs **[17]**. For aquatic organisms, the accumulation of many lipids provides vital support. It determines the survival of individuals when changing environmental factors in their combination, taking into account the peculiarities of the annual cycle of the organism **[18]**. Glycogen is a polysaccharide that stores carbohydrates, especially in liver and muscle tissue. In addition, it is a labile, readily available energy-intensive compound that can be converted to glucose when urgently needed (e.g., toxic substances entering fish tissues). Glycogen is also a source of chemical energy and a regulator of blood pressure. The content of glycogen in the tissues of fish's liver may decrease under the influence of pollution or deficiency of water-soluble oxygen caused by significant energy expenditure to overcome stress **[19]**. Under adverse conditions, detoxification and antioxidant protection systems change in fish tissues. Stimulation of detoxification mechanisms requires additional energy expenditure **[20]**, usually accompanied by suppression of energy metabolism. However, in the end, the effective work of regulatory and coordinating mechanisms ensures the organism's adaptation to changing conditions **[21]**.

Scientific Hypothesis

The difference in the availability of essential nutrients (glycogen, proteins, and lipids) in the body of the silver and bighead carp hybrid may indicate a change in the intensity and direction of their metabolic processes. At the same time, among the relevant studies, this area remains insufficiently studied. However, previously extensive work has been carried out, including collecting ichthyological material with a subsequent study of the chemical composition of their organs and tissues.

MATERIAL AND METHODOLOGY

The research was conducted in the spring, summer, and autumn periods from 2017 to 2019 in ponds based on the training, research, and production laboratory of fish farming of the National University of Life and Environmental Sciences of Ukraine (TRPLF NULES of Ukraine) of Ukraine, village Nemishayevo, Kyiv region (Polissya zone); State Enterprise "Experimental Farm" Nyvka " of the Institute of Fisheries of the National Academy of Agrarian Sciences (SEEF "Nyvka" IF NAAS) of Ukraine, Kyiv; (ponds are on the border of the zones (it is along the Nivka River that the Forest-Steppe is divided to the south and Polissya to the north)); Bila Tserkva Experimental Hydrobiological Station of the Institute of Hydrobiology of the National Academy of Sciences (BEHS IHB NAS) of Ukraine, Bila Tserkva (Forest-steppe zone), Kosiv (Kyiv region) and Velykoburlutsky (Kharkiv region) reservoirs (Forest-steppe zone).

Samples

Collection of ichthyological material was carried out during stocking and catching fish in ponds and reservoirs. The number of stocks in the ponds was 6, and the number of catches was 18; Velykoburlutsky Reservoir had 1 stocking, catch – 2, Kosiv Reservoir – 2 stocks, and 2 catches. The material for the study was a youth of the year, annuals, biennials, and triennials of the hybrid of silver and bighead carp (Figure 1, Figure 2, and Figure 3).

Figure 1 Biennial of silver and bighead carp hybrid caught in the autumn from a feeding pond No. 1 of TRPLF NULES of Ukraine.

Figure 2 Catch of silver and bighead carp hybrid biennials in the wintering pond No. 119 of SEEF "Nyvka" IF NAAS.

Figure 3 Collection of ichthyological materials (biennials of the hybrid of silver and bighead carp) in the spring during the capture of the winter pond No. 119 of SEEF "Nyvka" IF NAAS.

Chemicals

Potassium hydroxide (KOH), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).

Anthrone ($C_{14}H_{10}O$), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).

Concentrated sulfuric acid (H_2SO_4), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Sodium hydroxide (NaOH), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Sodium carbonate (Na_2CO_3), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Potassium sodium tartaric acid ($KNaC_4H_4O_6 \times {}_4H_2O$), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Folin–Ciocalteu reagent (FC), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Vanillin reagent (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).

Animals and Biological Material

45 specimens of the hybrid of silver and bighead carp of different size and mass groups (youth of the year, annuals, biennials, triennials) caught from ponds, and 35 specimens from reservoirs were processed.

Instruments

Net 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).
Technical electronic scales (BTHE-6-H1K-1, producer "Inter-Synthesis" Limited Liability Company, Ukraine).
Counting chamber of Najotta (producer "Laboratory equipment" Limited Liability Company, Ukraine).
Binocular microscope (XSP-139B LED Ulab, producer "Laboratory equipment" Limited Liability Company, Ukraine).
Apstein's grid (producer "ADS-Lab" Limited Liability Company, Ukraine).
Bogorov counting chamber (producer "ADS-Lab" Limited Liability Company, Ukraine).
Stereoscopic microscope (MBS-9, producer "Laboratory equipment" Limited Liability Company, Ukraine).

Laboratory Methods

The content of total protein in organs and tissues was determined by Lowry et al. (1951) **[22]**, lipids using the standard commercial kit "Total Lipids" (Philisit-Diagnostics, Ukraine), and glycogen - using anthrone reagent.

Description of the Experiment

Sample preparation: When determining the chemical composition in the organs and tissues of fish from reservoirs in different periods, selected 5 specimens of fish differing in weight and age.

Number of samples analyzed: 135 samples of tissues and organs (liver, white muscles, and gill petals) were taken from the fish caught in the ponds, and 105 samples were taken from the reservoirs to determine the number of proteins, fats, and carbohydrates.

Number of repeated analyses: In the experimental ponds and the Kosiv Reservoir, the repetitions of the experiments were twofold, and in the Velykoburlutsky Reservoir - one-time.

Number of experiment replication: The number of repetitions of each experiment to determine one value was 5 times.

Design of the experiment: The content of total proteins in tissue samples was determined by the method of Lowry et al. (1951) **[22]**. Briefly, 0.1 g of tissue and organ 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, the sample added 1.0 mL of Folin's reagent diluted 1:1 with distilled water. 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 based on 0.1 n NaOH with the addition of 20 g Na_2CO_3 and 0.5 g of potassium, and 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 a phosphorovaniline reagent. Briefly, 100 mg of tissue was hydrolyzed in 1.5 mL of concentrated sulfuric acid for 15 minutes. 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. 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.

Statistical Analysis

The statistical evaluation of the results was carried out by standard methods using statistical software Statgraphics Centurion XVII (StatPoint, USA) – multifactor analysis of variance (MANOVA), LSD test.

Statistical processing was performed in Microsoft Excel 2016 in combination with XLSTAT. Values were estimated using mean and standard deviations. We calculated the arithmetic mean (unweighted) value (M), and the arithmetic mean error (±m), which made it possible to estimate with a certain probability the deviation of the arithmetic mean deviation Fulton fatness rate. The statistical reliability of the results of the research was provided by analyzing samples with the number of fish from 10 to 25 specimens.

RESULTS AND DISCUSSION

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 **[23]**.

The general indicators of metabolism of annual hybrids of white with variegated silver carp, which were caught from winter ponds in the spring of 2017 – 2018, are shown in Table 1.

Table 1 General indicators of metabolism of annual hybrids of white with variegated silver carp caught from winter ponds, M ±m, n = 5.

Cloth	Glycogen, %		Albumen, %		Lipids, %	
	Spring, 2017 ye.	Spring, 2018 ye.	Spring, 2017 ye.	Spring, 2018 ye.	Spring, 2017 ye.	Spring, 2018 ye.
Pond No 101 SE DG "Nivka" IRG NAAS						
Muscles	4.3 ±0.02	0.45 ±0.01	13.69 ±0.04	13.62 ±0.03	0.38 ±0.01	0.35 ±0.01
Cv	8.33	6.58	0.68	0.47	8.32	6.48
Liver	2.11 ±0.02	2.07 ±0.02	12.78 ±0.02	12.87 ±0.01	3.56 ±0.03	3.59 ±0.02
Cv	2.28	2.23	0.41	0.18	1.98	1.10
Branchia	0.46 ±0.01	0.40 ±0.02	12.29 ±0.02	12.32 ±0.03	0.42 ±0.01	0.40 ±0.01
Cv	6.97	10.32	0.36	0.47	9.12	4.14
Rate No 2. NNVLR NULES of Ukraine						
Muscles	0.37 ±0.01	0.33 ±0.01	13.30 ±0.02	13.35 ±0.01	0.28 ±0.01	0.30 ±0.01
Cv	7.21	9.25	0.28	0.17	5.26	4.98
Liver	1.92 ±0.01	1.89 ±0.02	12.29 ±0.02	12.35 ±0.01	2.87 ±0.02	2.90 ±0.02
Cv	1.72	1.92	0.34	0.18	1.88	1.64
Branchia	0.38 ±0.01	0.34 ±0.01	12.12 ±0.09	12.11 ±0.09	0.32 ±0.01	0.35 ±0.01
Cv	5.26	9.55	1.59	1.69	9.88	6.48
Rate No. 5 BEGS IGB NASU						
Muscles	0.28 ±0.01	0.30 ±0.01	11.19 ±0.04	12.60 ±0.31	0.26 ±0.01	0.28 ±0.01
Cv	10.75	11.10	0.80	6.01	6.34	5.05
Liver	1.77 ±0.01	1.83 ±0.02	10.92 ±0.08	12.19 ±0.07	2.78 ±0.01	2.83 ±0.02
Cv	1.29	1.99	1.61	1.29	1.03	1.77
Branchia	0.31 ±0.01	0.26 ±0.01	10.76 ±0.06	12.05 ±0.11	0.29 ±0.01	0.34 ±0.01
Cv	8.60	9.12	1.21	2.12	6.13	7.58

Note: Cv – In this table and in the following – the coefficient of variation.

The average values of the share of glycogen in most organs and tissues of annuals of the hybrid of silver and bighead carp in 2017, and 2018 were at the level of 0.5% and below (Figure 4). Exceptions were the indicators of the specific gravity of glycogen in the liver of bighead carp: in the winter pond No. 101 – above 2%; in winter ponds No. 2 and No. 5 – less than 2% in both years of research. Thus, a higher level of glycogen in the liver of annual fish is evident compared to its presence in the muscles and gills of the youth of the year of silver and bighead carp hybrids.

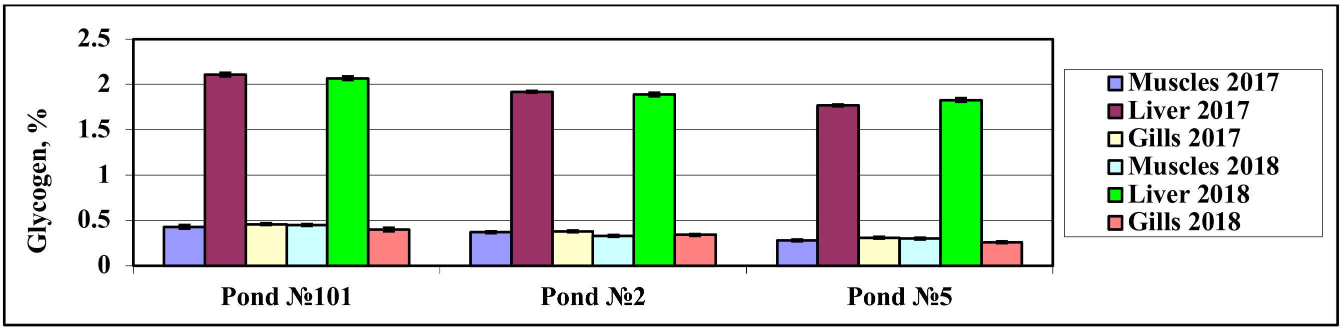

Figure 4 The average values of glycogen (%) in the organs and tissues of annuals of the hybrid of silver and bighead carp from winter ponds in 2017 and 2018.

The average values of the specific weight of protein in most organs and tissues of annuals of the hybrid of silver and bighead carp in 2017 and 2018 were almost evenly distributed in them, at the level of 10 – 14% (Figure 5). Exceptions were the indicators of the proportion of protein content in the winter pond No. 5 – they were lower (about 10%) and fluctuated over the years.

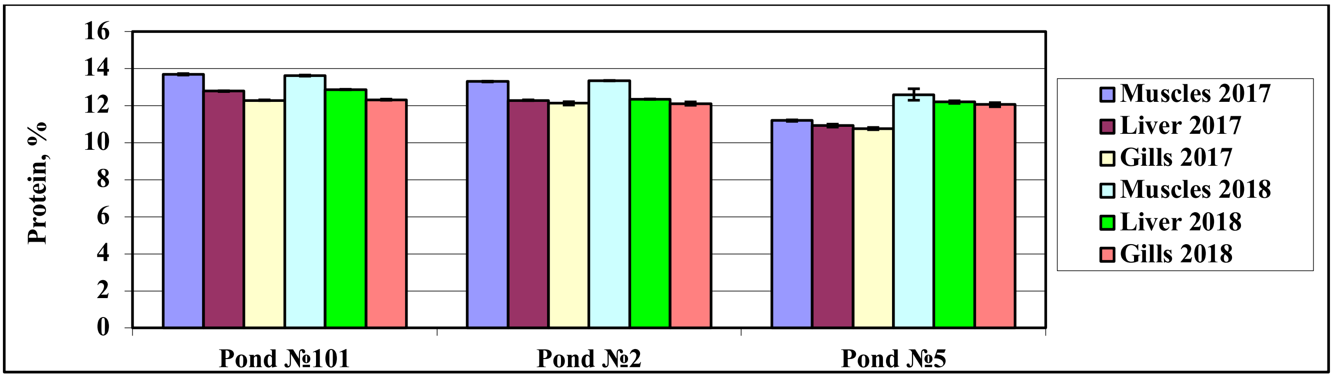

Figure 5 Average values of total protein (%) in the organs and tissues of annuals of silver and bighead carp hybrid from winter ponds in 2017 and 2018.

The average values of the specific weight of lipids in most organs and tissues of annuals of the hybrid of silver and bighead carp in 2017 and 2018 were, like glycogen, at 0.5% and below (Figure 6). Exceptions were the indicators of the proportion of lipids in the liver of bighead carp: in the winter pond No. 101 – above 3.5%; in winter ponds No. 2 and No. 5 – about 3% in both years of research. Thus, a higher level of lipids (as well as glycogen) is present in the liver of annual fish compared to its content in the muscles and gills of the youth of the year of the hybrids of silver and bighead carp.

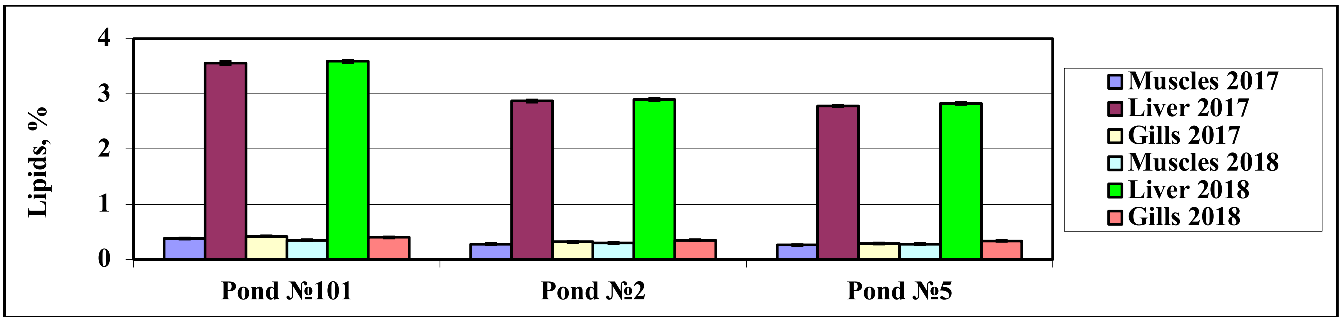

Figure 6 The average values of lipids in the organs and tissues of annuals of the hybrid of silver and bighead carp from wintering ponds in 2017 and 2018.

Thus, according to the results of studies conducted in 2017 and 2018, it was found that the concentration of glycogen, total protein, and lipids in fish organs and tissues of fish from SEEF "Nyvka" IF NAAS and TRPLF NULES of Ukraine was satisfactory. In annual fish from BEHS IHB NAS in 2017, the total protein and glycogen content in organs and tissues was slightly reduced, and the number of lipids was within normal limits.

The results indicate that the annuals of the hybrid of silver and bighead carp in the winter significantly reduce or stop the trophic activity and switch partially or completely to endogenous nutrition.

Due to the use of energy compounds by fish, there is a gradual decrease in their content during the winter. During this period, the most vulnerable are fish, especially those that did not have enough spare nutrients before winter. The data obtained probably indicates that the fish were not fully prepared for winter because they did not have enough energy compounds.

Winter is one of the most difficult periods in fish life, especially for the young, in the first year of life **[24]**. With the lack of feeding, the effective consumption of natural foods by annual herbivorous fish is almost stopped in late August or the first decade of September. Almost from the third decade of September, the youth of the year, in ponds without food, to ensure viability begin to use endogenous nutrients from their own "depot" **[25], [26]**. Adverse hydrological factors (low temperature, lack of oxygen, etc.) could also affect wintering conditions. The temperature largely regulates the intensity of metabolism and fish development rate **[27]**, **[28]**.

At low temperatures, lipids during critical physiological stress become the body's main energy source. Their synthesis provides the need for fish for lipids in the body due to lipids, which are part of the natural and artificial feed base.

Lipids of "peripheral" organs (muscles, gills, intestines) are mainly used, and lipids of a brain and a liver remain for maintenance of regulatory functions **[29]**, **[30]**, **[31]**. To stabilize the water temperature and create the desired wintering conditions, an important factor is the ice cover of the ponds, playing a significant role in heat exchange between the water column and atmospheric air. Direct ice cover ensures the stability of water temperature throughout the winter, which allows youth of the year to effectively use the accumulated nutrients without increased energy costs **[32]**, **[33]**, **[34]**. The specifics of the south of Ukraine against the background of a general warming of the atmosphere were the most sensitive to wintering of annuals of carp, which significantly affects the specific conditions of the zone, the water temperature under the action of periodic warming during wintering. Each such warming leads to increased mobility of youth the year in the winter. In the practical absence of food and the impossibility of their effective assimilation, mobility leads to deterioration of the general physiological state, reduced body resistance, overweight, and, as a result, low annual yield, which has been proven, quite reliably by previous studies **[35]**, **[36]**, **[37]**.

The resistance of fish to wintering conditions increases with age, and accordingly, fewer nutrients are used. Its condition after wintering is better compared to younger age groups of fish (Table 2).

Table 2 General indicators of metabolism of a two-year-old white hybrid with variegated silver carp caught from feeding ponds, M ±m, n = 5.

	Glycogen, %		**Albumen, %**		**Lipids, %**	
	Autumn, 2017 ye.	**Autumn, 2018 ye.**	**Autumn, 2017 ye.**	**Autumn, 2018 ye.**	**Autumn, 2017 ye.**	**Autumn, 2018 ye.**
Pond No. 101 SE DG "Nivka" IRG NAAS						
Muscles	0.68 ±0.01	0.66 ±0.01	14.53 ±0.03	14.57 ±0.03	0.57 ±0.02	0.55 ±0.02
Cv	2.64	4.05	0.39	0.53	7.30	6.58
Liver	3.33 ±0.03	3.28 ±0.03	13.97 ±0.04	13.93 ±0.02	5.65 ±0.04	5.69 ±0.02
Cv	1.77	1.22	0.63	0.39	1.41	0.80
Branchia	0.76 ±0.01	0.74 ±0.01	12.92 ±0.02	12.96 ±0.01	0.82 ±0.02	0.74 ±0.02
Cv	3.22	3.31	0.34	0.25	5.26	6.96
Rate No. 2 NNVLR NULES of Ukraine						
Muscles	0.55 ±0.03	0.57 ±0.02	14.30 ±0.09	14.21 ±0.07	0.46 ±0.01	0.45 ±0.01
Cv	11.64	7.55	1.33	1.07	6.57	4.97
Liver	2.87 ±0.06	2.84 ±0.05	12.85 ±0.03	12.83 ±0.02	4.68 ±0.01	4.64 ±0.01
Cv	4.61	3.80	0.56	0.32	0.68	0.71
Branchia	0.62 ±0.02	0.65 ±0.02	12.45 ±0.04	12.43 ±0.04	0.71 ±0.02	0.69 ±0.02
Cv	6.17	6.40	1.24	0.73	5.82	6.96
Rate No. 5 BEGS IGB NASU						
Muscles	0.43 ±0.01	0.42 ±0.01	14.03 ±0.14	14.22 ±0.22	0.39 ±0.01	0.41 ±0.02
Cv	5.20	4.02	2.19	3.41	5.88	8.46
Liver	2.51 ±0.06	2.61 ±0.02	12.77 ±0.01	12.81 ±0.03	4.61 ±0.14	4.66 ±0.06
Cv	5.02	1.63	0.18	0.51	6.92	3.07
Branchia	0.52 ±0.01	0.49 ±0.02	12.32 ±0.06	12.29 ±0.06	0.63 ±0.01	0.65 ±0.01
Cv	3.85	7.38	1.06	1.05	4.74	3.52

After wintering in annual local carp, obtained by crossing Nyvka plant line of small-scale intra-breed type of Ukrainian frame breed and Nyvka intra-breed type of Ukrainian scaly breed, it was found that fat consumption was 21.7 and 27.6%, respectively, for control and research group, protein consumption 11.1 and 13.3% **[30]**.

The average values of biochemical parameters of biennials of the hybrid of silver and bighead carp caught from feeding ponds in 2017 and 2018 were 0.5% and above (Figure 7).

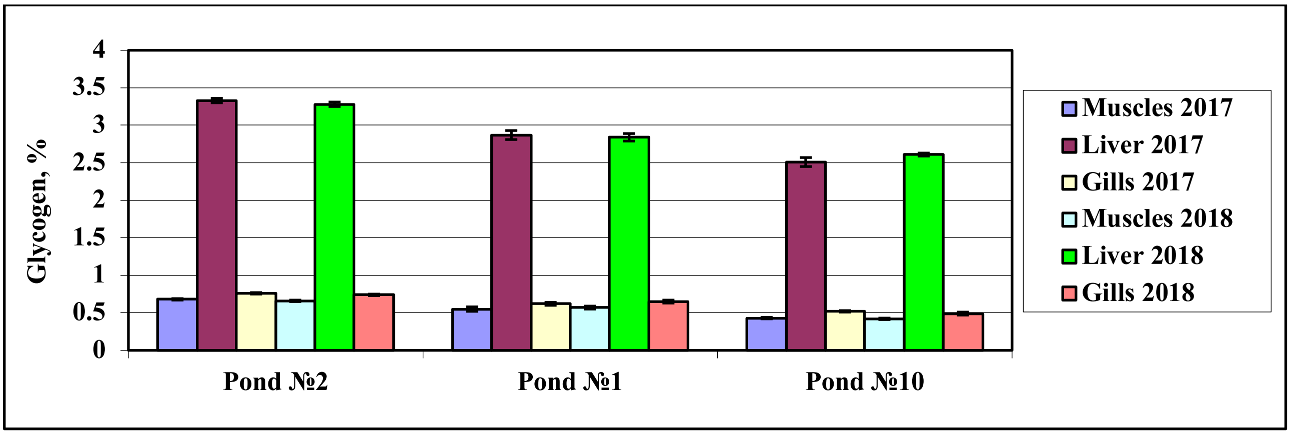

Figure 7 The average glycogen (%) values in the organs and tissues of biennials hybrid of silver and bighead carp from feeding ponds in 2017 and 2018.

Exceptions were indicators of glycogen content in fish's liver from feeding ponds: in pond No. 2, 3 – 3.5%; in pond No. 1, 2.5 – 3% and pond No. 10, the lowest about 2.5% in both years of research. Thus, in the liver of biennials, a higher level of glycogen is evident than its presence in the muscles and gills of young hybrids of silver and bighead carp.

The average values of the proportion of protein in most organs and tissues of biennials of the hybrid of silver and bighead carp in 2017 and 2018 fluctuated markedly by year and in particular organs and tissues: in muscle, the figures were at 14 – 14.5%; in the liver at the level of 12.5 – 14% (the highest pond fish No. 2); in gills – at least at the level of less than 12% (Figure 8).

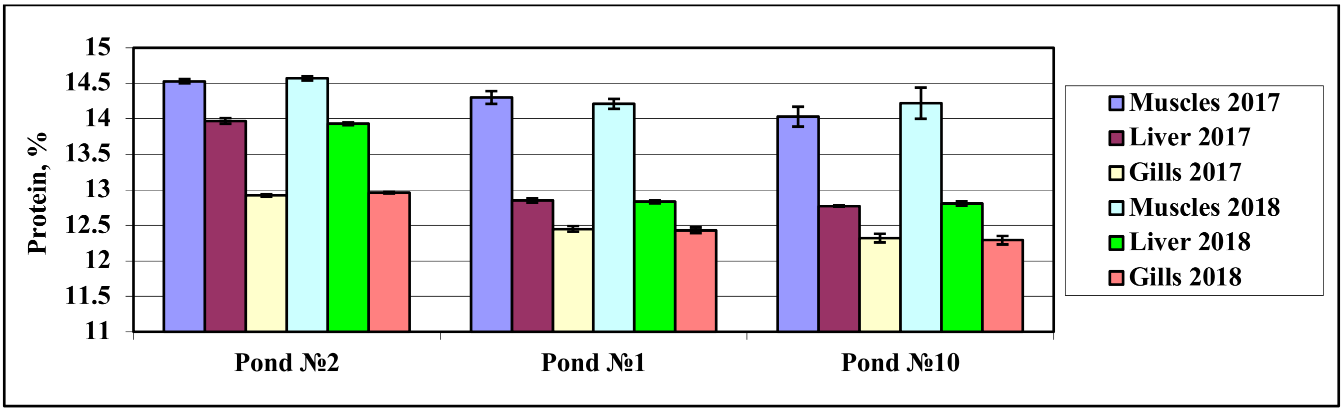

Figure 8 The average protein (%) values in the organs and tissues of biennials of the hybrid of silver and bighead carp from feeding ponds in 2017 and 2018.

The average lipids values in most organs and tissues of biennials of the hybrid of silver and bighead carp from feeding ponds for 2017 and 2018 were at 1% and below (Figure 9). Exceptions were indicators of lipid content in the liver of feeding ponds: pond No. 2 above 5 – 6%; ponds No. 1 and No. 10 4 – 5% in both years of research. Thus, a higher level of lipids is present in the liver of biennials and annuals compared to its content in the muscles and gills of the youth of the year hybrid of silver and bighead carp.

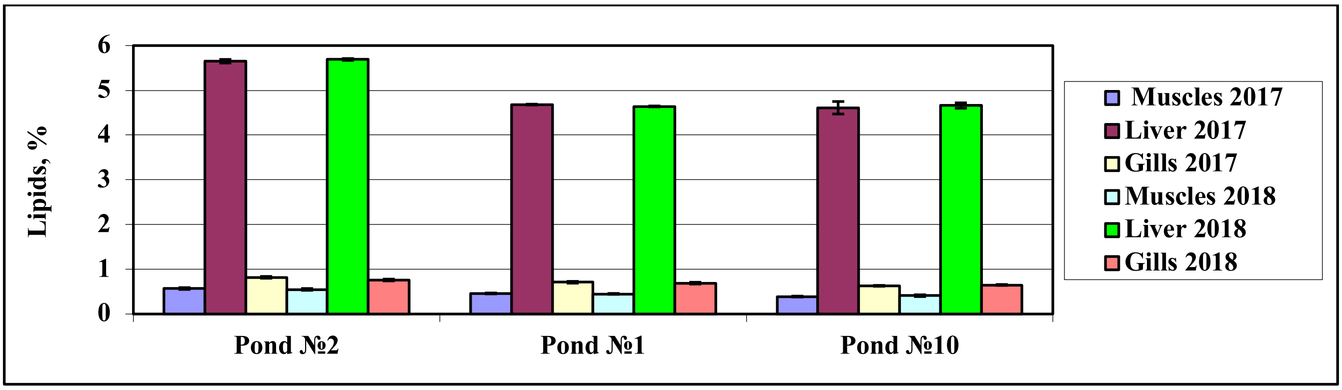

Figure 9 The average lipids (%) values in the organs and tissues of biennials hybrid of silver and bighead carp from feeding ponds in 2017 and 2018.

A study of biennials of Galician carp conducted by scientists at the „Korop" farm showed that skeletal muscle protein was 16.2% and lipid was 8.3%. This indicates a satisfactory physiological condition before landing on winter ponds **[38]**.

The results of studies of the level of energy compounds in the body of biennials of the hybrid of silver and bighead carp in research farms during 2017 and 2018 did not reveal significant physiological and biochemical status violations. It was recorded that the content of glycogen in the liver of biennials from SEEF "Nyvka" IF NAAS during 2017 and 2018 was the highest at 3.28 – 3.33%, compared with fish from other studied farms. All fish were characterized by fluctuations in the liver's glycogen content, which can be explained by the significant rate of mobilization of this substance to meet the energy problems of fish in a particular period and the short recovery time.

Table 3 shows the general metabolism indicators of biennials of white hybrids with variegated silver carp caught from winter ponds in the spring of 2018 – 2019.

Table 3 General indicators of metabolism of biennials of white hybrid with variegated silver carp caught from winter ponds, M ±m, n = 5.

Cloth	Glycogen, %		Albumen, %		Lipids, %	
	Spring, 2018 ye.	**Spring,** 2019 ye.	**Spring,** 2018 ye.	**Spring,** 2019 ye.	**Spring,** 2018 ye.	**Spring,** 2019 ye.
Pond No. 101 SE DG "Nivka" IRG NAAS						
Muscles	0.60 ±0.01	0.59 ±0.01	14.33 ±0.07	14.39 ±0.05	0.50 ±0.02	0.49 ±0.03
Cv	3.33	3.88	1.07	0.75	9.59	13.77
Liver	2.98 ±0.04	2.91 ±0.01	13.93 ±0.24	13.89 ±0.30	5.42 ±0.06	5.39 ±0.03
Cv	3.33	1.04	3.93	4.85	2.59	1.37
Branchia	0.66 ±0.04	0.60 ±0.03	12.87 ±0.28	12.95 ±0.12	0.75 ±0.02	0.70 ±0.02
Cv	12.08	12.87	4.91	2.10	4.84	5.24
Rate No. 2 NNVLR NULES of Ukraine						
Muscles	0.48 ±0.03	0.52 ±0.01	13.99 ±0.15	13.89 ±0.04	0.38 ±0.03	0.36 ±0.02
Cv	11.75	4.96	2.36	0.69	15.00	10.62
Liver	2.68 ±0.02	2.70 ±0.03	12.82 ±0.04	12.77 ±0.01	4.52 ±0.03	4.49 ±0.04
Cv	1.43	2.12	0.76	1.72	1.26	2.14
Branchia	0.50 ±0.02	0.56 ±0.02	12.38 ±0.04	12.36 ±0.06	0.64 ±0.01	0.62 ±0.01
Cv	7.10	7.47	0.79	1.09	4.66	4.23
Rate No. 5 BEGS IGB NASU						
Muscles	0.40 ±0.01	0.39 ±0.01	13.85 ±0.04	13.95 ±0.23	0.32 ±0.01	0.34 ±0.01
Cv	4.14	5.82	0.68	3.64	9.88	9.31
Liver	2.28 ±0.03	2.43 ±0.03	12.71 ±0.08	12.75 ±0.16	4.44 ±0.02	4.47 ±0.02
Cv	3.29	2.88	1.44	2.88	1.25	1.03
Branchia	0.49 ±0.01	0.45 ±0.01	12.27 ±0.05	12.23 ±0.05	0.59 ±0.01	0.60 ±0.01
Cv	9.82	6.65	0.85	0.85	4.48	3.33

The average glycogen values in most organs and tissues of biennials of the hybrid of silver and bighead carp caught from winter ponds in 2018 and 2019 were about and above 0.5% (Figure 10). Exceptions were glycogen content in the liver of bighead carp wintering ponds: No. 119 about 3%; No. 1 more than 2.5%, and No. 11 more than 2% in both years of research. As a result, a higher level of glycogen was found in the liver of biennials than its presence in the muscles and gills of the youth of the year hybrids of silver and bighead carp.

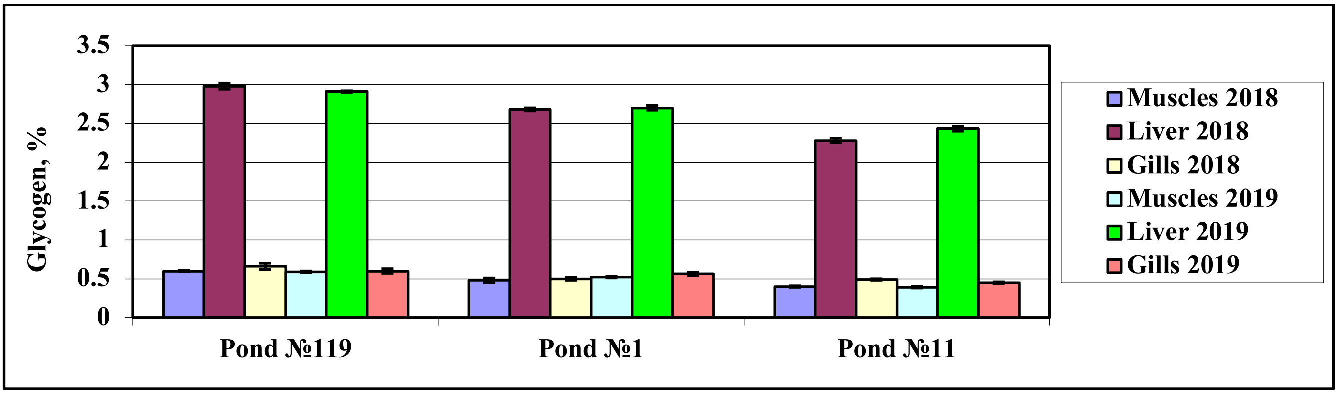

Figure 10 The average glycogen (%) values in the organs and tissues of biennials of the hybrid of silver and bighead carp from wintering ponds in 2018 and 2019.

The average values of the proportion of protein in most organs and tissues of biennials of the hybrid of silver and bighead carp caught from winter ponds in 2018 and 2019, as well as biennials, fluctuated markedly over the years and in particular organs and tissues: in muscle levels of 14 – 14.5%; in the liver at the level of 12.5 – 14% (the highest pond fish No. 2); in gills at least at the level of less than 12.5 – 13% (Figure 11).

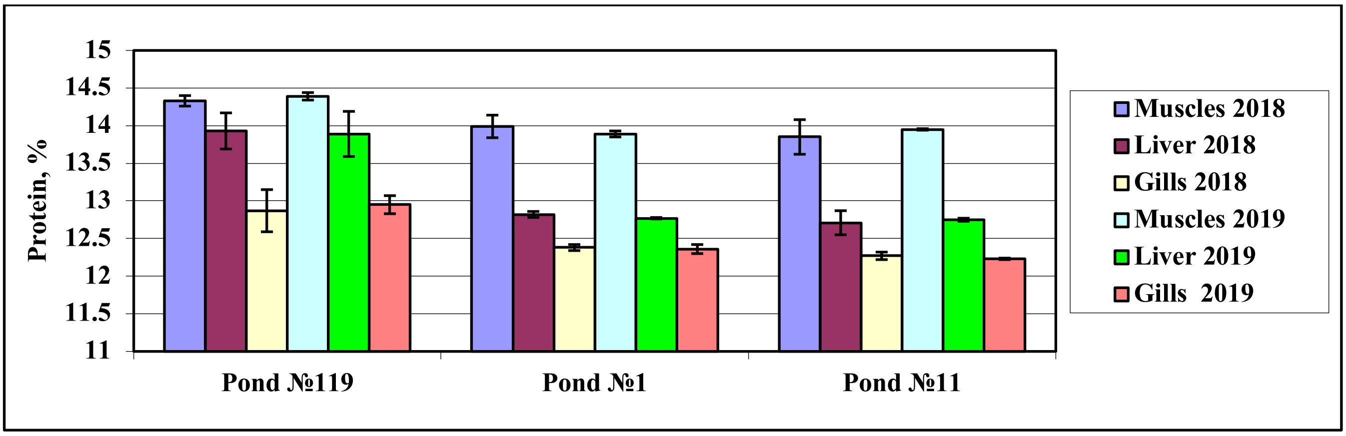

Figure 11 The average values of protein (%) in the organs and tissues of biennials of the hybrid of silver and bighead carp from wintering ponds in 2018 and 2019.

The average lipids values in most organs and tissues of biennials of the hybrid of silver and bighead carp caught from winter ponds in 2018 and 2019, as in biennials, were at 1% and below (Figure 12).

In the muscles of the Clarias gariepinus, as well as in the hybrid of silver and bighead carp, there is a low content of total lipids, which is 1.26%, which indicates that they belong to the group of fish with low-fat content (less than 5%) and confirmed by literature data **[39]**. Other literature sources should note that catfish (along with Atlantic wolffish, spotted wolffish, many carps, some Salmonidae, and most flounders) are medium-fat fish and the content of lipids in muscle is from 2 – 6% **[40]**.

Exceptions were the indicators of lipid content in the liver of bighead carp from winter ponds: in pond No. 119, above 5%; in ponds No. 1 and No. 11, more than 4% in both years of research. Thus, higher lipids are found in the liver of biennials and annuals and biennials, compared to its content in the muscles and gills of the youth of the year of hybrid silver and bighead carp.

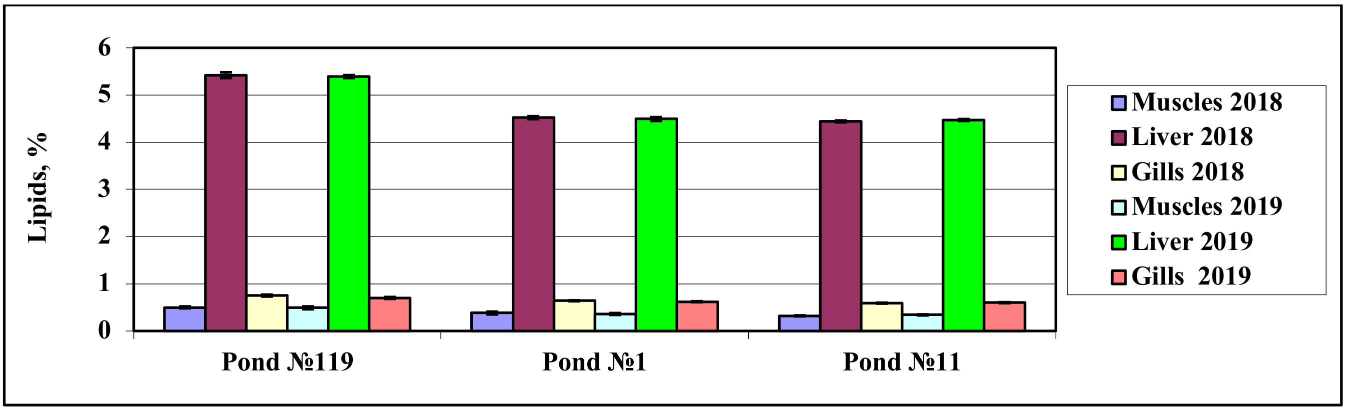

Figure 12 The average lipids (%) values in the organs and tissues of biennials of the hybrid of silver and bighead carp from wintering ponds in 2018 and 2019.

The results of the biennials of the hybrid of silver and bighead carp in 2018 and 2019 indicate that their physiological state at the time of the study was within the physiological norm. Table 4 shows the data on the general metabolism of different groups of white hybrids with variegated silver carp caught from the Kosiv Reservoir.

Table 4 General metabolism indicators of different groups of white hybrids with variegated silver carp caught from the Kosiv Reservoir, M ±m, n = 5.

Cloth	One-year	One-year	Three-year-olds (stocking in 2018)	Three-year-olds (stocking in 2018)
	Spring, 2018	Spring, 2019	Autumn, 2019	Autumn, 2019
		Glycogen, %		
Muscles	0.53 ±0.03	0.38 ±0.02	0.76 ±0.02	0.80 ±0.03
Cv	10.99	14.15	6.22	8.29
Liver	2.19 ±0.02	2.10 ±0.01	5.38 ±0.06	5.51 ±0.04
Cv	2.20	1.51	2.50	1.65
Branchia	0.40 ±0.02	0.37 ±0.02	0.82 ±0.03	0.86 ±0.01
Cv	9.71	13.49	7.02	3.68
		Albumen, %		
Muscles	13.48 ±0.07	13.41 ±0.05	20.04 ±0.21	22.16 ±0.11
Cv	1.24	0.82	2.42	1.14
Liver	12.67 ±0.08	12.59 ±0.04	18.00 ±0.19	18.56 ±0.21
Cv	1.35	0.68	2.38	2.52
Branchia	12.20 ±0.16	11.86 ±0.20	13.44 ±0.15	15.50 ±0.22
Cv	2.95	3.70	2.57	3.17
		Lipids, %		
Muscles	0.40 ±0.01	0.36 ±0.01	0.70 ±0.02	0.82 ±0.02
Cv	7.91	9.23	4.98	6.80
Liver	3.11 ±0.03	2.91 ±0.07	5.79 ±0.04	7.26 ±0.09
Cv	2.16	5.49	1.65	2.63
Branchia	0.32 ±0.01	0.28 ±0.02	1.19 ±0.01	1.36 ±0.05
Cv	9.92	12.39	2.82	7.71

The average glycogen values in most organs and tissues of annuals and triennials of the hybrid of silver and bighead carp of the Kosiv Reservoir in 2018 and 2019 were up to 0.5% and 1%, respectively (Figure 13). Exceptions were glycogen content in the liver of bighead carp of the Kosiv Reservoir, which was slightly more than 2% in one-year-olds and 5 – 5.5% in triennials in both years of research. Thus, a higher level of glycogen in the liver of annual fish is evident compared to its presence in the muscles and gills of the youth of the year of the hybrid of silver and bighead carp.

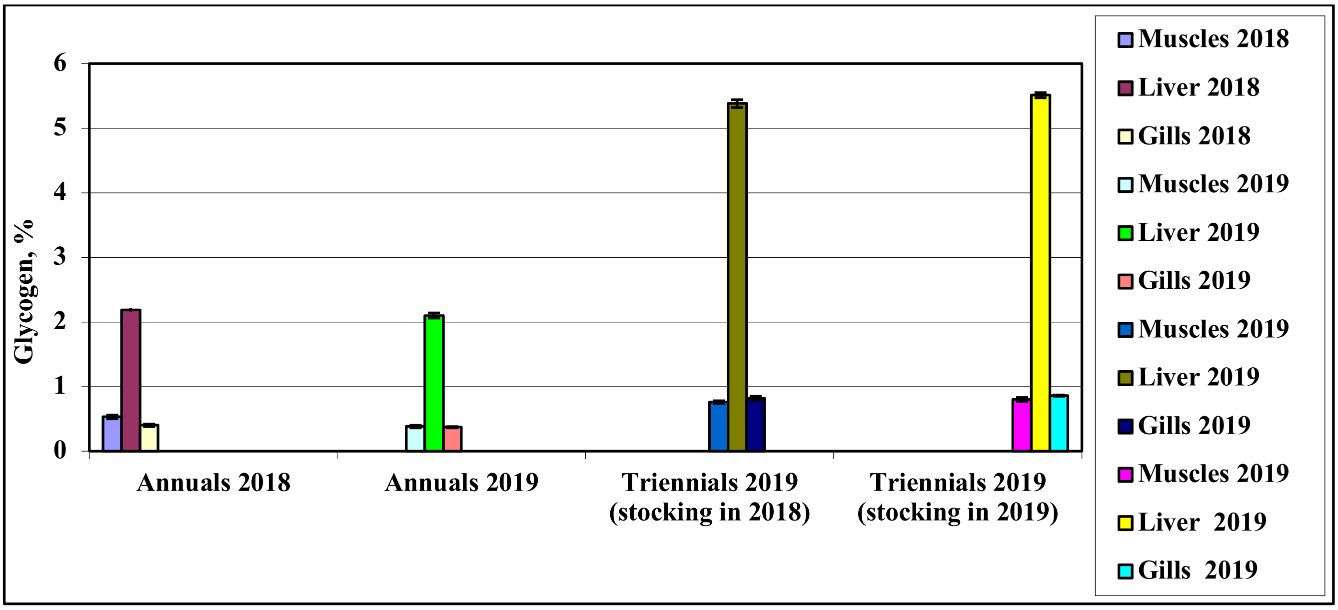

Figure 13 The average glycogen (%) values in the organs and tissues of annuals and triennials of the hybrid of silver and bighead carp from the Kosiv Reservoir during 2018 and 2019.

The average values of protein content in most organs and tissues of annuals and triennials of the hybrid of silver and bighead carp from the Kosiv Reservoir in 2018 and 2019 were lower in some organs and tissues in annuals, 12.5%, and higher in triennials, 15 – 22% (Figure 14). Moreover, the highest content was in the muscles of all age groups of fish, and the lowest protein content was in the gills.
The average lipid content in the muscles and gills of annuals and triennials of the hybrid of silver and bighead carp from the Kosiv Reservoir in 2018 and 2019 was at the level of annuals less than 0.5% and triennials about 1% or more (Figure 15). The lipid content in the liver was much higher: in annual fish, they were about 3%, and in triennials from stocking in 2018, about 6%, and from stocking in 2019, more than 7%.

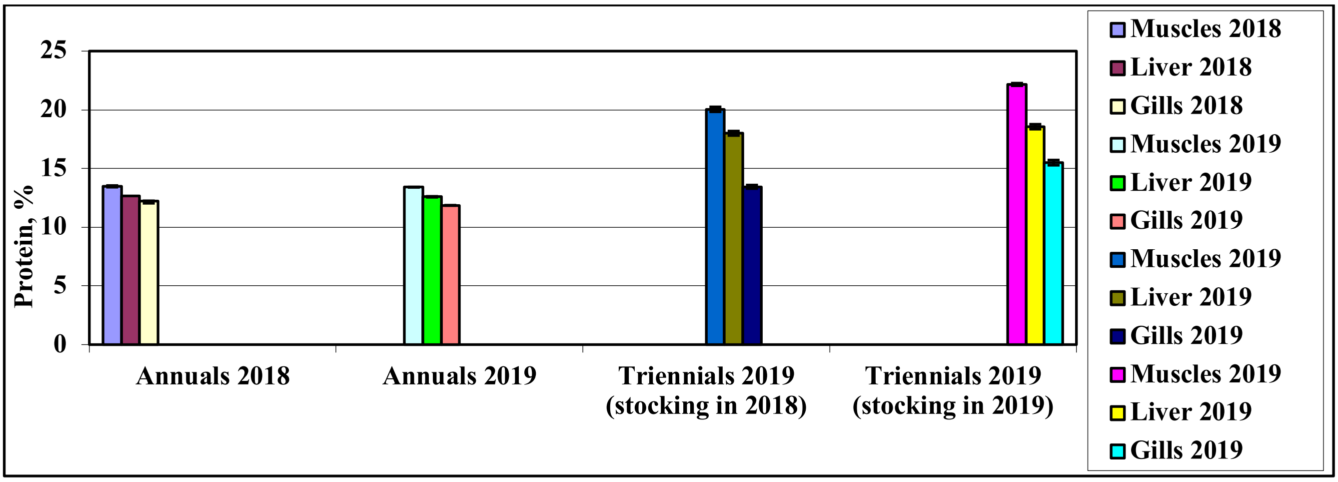

Figure 14 The average values of protein (%) in the organs and tissues of annuals and triennials of the hybrid of silver and bighead carp from the Kosiv Reservoir during 2018 and 2019.

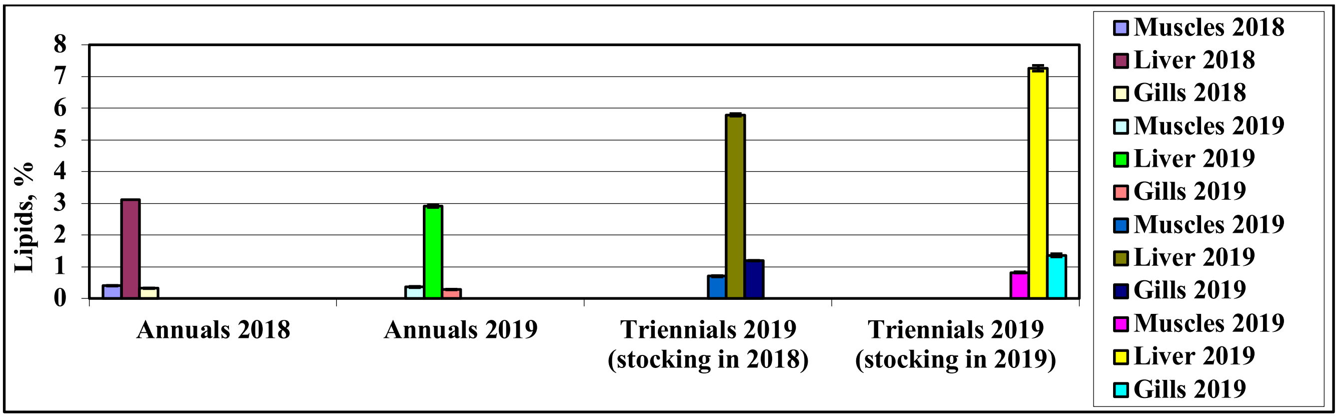

Figure 15 The average values of lipids (%) in the organs and tissues of annuals and triennials of the hybrid of silver and bighead carp from the Kosiv Reservoir during 2018 and 2019.

The results of the annuals of the hybrid of silver and bighead carp in 2018 and 2019 indicate that their physiological state at the time of the study was within the physiological norm.

The studied triennial hybrids of silver and bighead carp from the Kosiv Reservoir in 2019 were marked by significant fluctuations in total protein content in muscles (20.04 – 22.16%), liver (18.00 – 18.56%), and gills (13.44 – 15.50%). The amount of glycogen in the liver (1.79 – 1.84 times) and lipids in the gills (1.19 – 1.36 times) increased slightly. The obtained data can be explained by a certain heterogeneity of the general physiological state of fish. This can be caused by hereditary factors that determine a certain diversity of fish composition in reservoirs and the conditions of fish keeping.

Other scientists have shown that the total protein content in the white skeletal muscles of bream, roach, and zander in the fall significantly exceeded the protein content in the liver. 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 roach muscles by 147.1%, and in zander muscles by 16.1%, and in perch muscles by 111.7%. Comparing the protein content in the liver of almost all species of fish, it can be noted that the fish from the Kremenchuk reservoir contains less protein than in Kyiv. First of all, this indicates that the conditions of this reservoir are favorable for the accumulation of lipids and glycogen in the liver **[41]**.

When comparing the lipid content of roach, bream, and zander, it is noticeable that at lower temperatures of the reservoir (Kyiv Reservoir), they accumulate fewer lipids in the liver but more in the muscles **[42]**.

The highest glycogen content in the liver was in zander and perch (10.8 – 11.0 %) and the muscles (5.6 – 6.2 %) of fish inhabiting the Kyiv Reservoir. Bream and roach from this reservoir contained 8.5 – 9.6 % liver and 3.6 – 5.1% muscles **[43]**. The general metabolism indicators of different groups of white hybrids with variegated silver carp, which were caught from the Velykoburlutsky reservoir, are presented in Table 5.

Table 5 General metabolism indicators of different groups of white hybrids with variegated silver carp caught from Velykoburlutsky reservoir, M ±m, n = 5.

Cloth	Autumn 2017 ye.	Autumn2018 ye.	Summer2019 ye.	Autumn 2017 ye.	Autumn 2018 ye.	Summer 2019 ye.	Autumn 2017 ye.	Autumn 2018 ye.	Summer 2019 ye.
	Age group								
	0+	1+	2+	0+	1+	2+	0+	1+	2+
	Glycogen, %			Albumen, %			Lipids, %		
Muscles	0.46 ±0.04	0.56 ±0.01	0.79 ±0.03	12.62 ±0.27	14.28 ±0.06	17.20 ±0.12	0.31 ±0.01	0.53 ±0.02	1.21 ±0.03
Cv	18.95	4.77	9.08	4.79	0.95	1.59	9.72	7.80	5.05
Liver	2.04 ±0.02	2.78 ±0.02	3.65 ±0.04	12.17 ±0.08	12.82 ±0.06	15.99 ±0.23	3.12 ±0.04	5.33 ±0.19	6.16 ±0.09
Cv	2.15	1.64	2.31	1.48	1.00	3.22	4.02	7.91	3.37
Branchia	0.81 ±0.04	0.93 ±0.02	1.20 ±0.02	11.90 ±0.25	12.37 ±0.04	13.80 ±0.29	0.33 ±0.01	0.81 ±0.02	1.47 ±0.08
Cv	13.97	3.68	3.20	4.65	0.67	4.72	10.05	4.61	11.70

The average values of glycogen in most organs and tissues of the youth of the year of the hybrid of silver and bighead carp of the Velykoburlutsky Reservoir in 2017, 2018, and 2019 increased from annuals to triennials and were, respectively, at the level: in muscle up to 0.5% or more; in gills 0.8% and more; the highest in the liver - 2.0 – 3.5% and more (Figure 16).

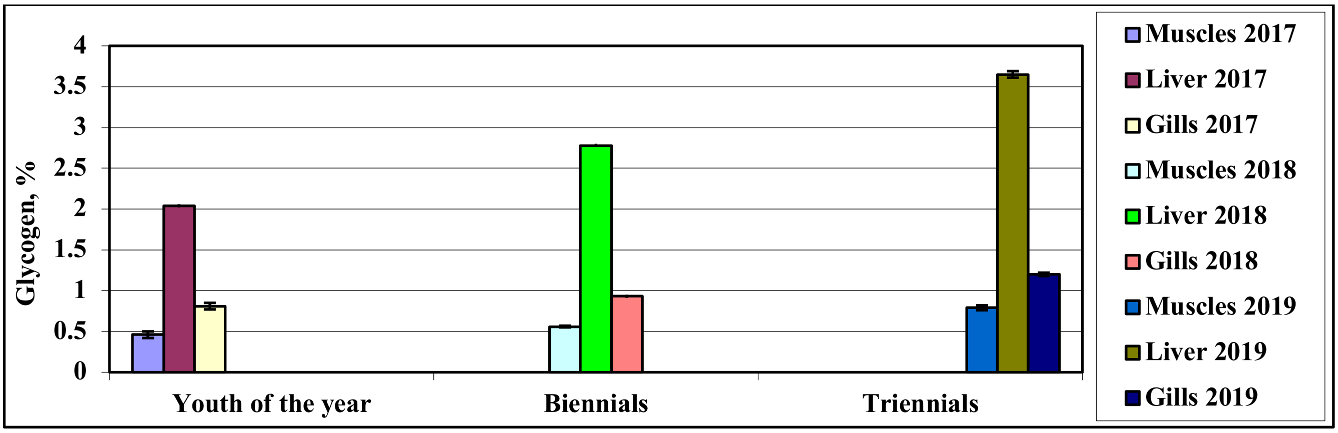

Figure 16 The average glycogen values in the organs and tissues of the youth of the year, biennials and triennials hybrid of silver and bighead carp from the Velykoburlutsky Reservoir during 2017, 2018, and 2019.

The average values of protein content in most organs and tissues of the youth of the year, biennials, and triennials of hybrid of silver and bighead carp from Velykoburlutsky Reservoir in 2017, 2018, 2019 also increased with age and fluctuated in some organs and tissues of fish: in the highest muscles more than 12 – 17%; in the gills 12 – 14%; in the liver 12 – 16% (Figure 17).

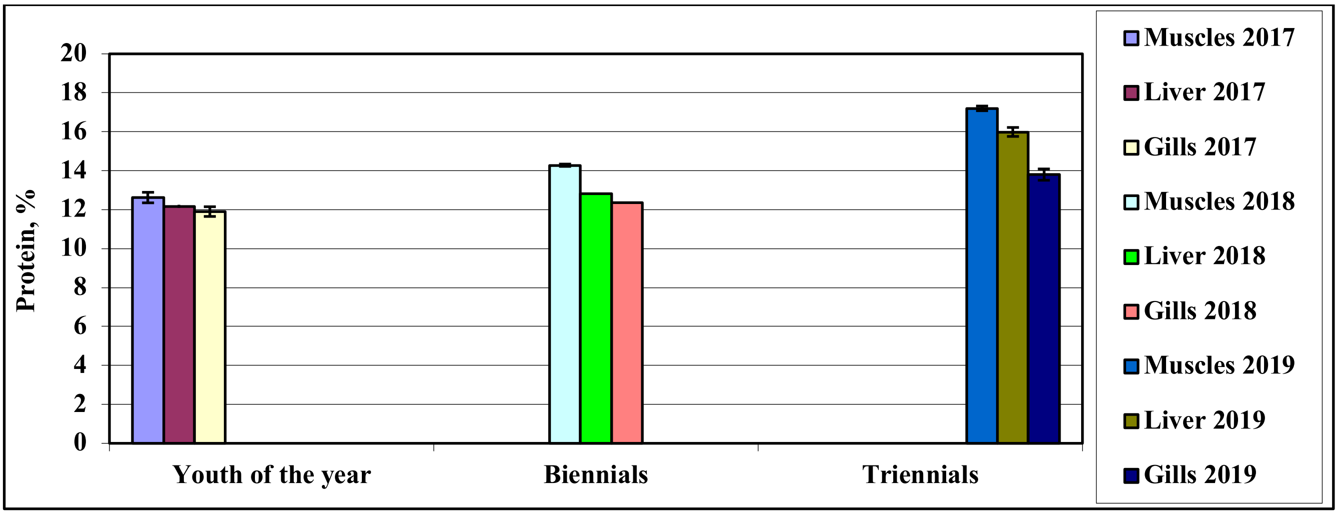

Figure 17 The average protein values in the organs and tissues of the youth of the year, biennials and triennials hybrid of silver and bighead carp from the Velykoburlutsky Reservoir during 2017, 2018, and 2019.

The average lipid content in the liver, muscles, and gills of the youth of the year, biennials, and triennials hybrid of silver and bighead carp from the Velykoburlutsky Reservoir in 2017, 2018, and 2019 gradually increased from the youth of the year to triennials. It was at a lower level in muscle 0.2 – 1.2% and gills 0.2 – 1.5% (Figure 18). Lipid content in the liver was much higher: in the year's youth, it was more than 3%, in biennials, 5.3%, and in triennials, more than 6%.

Satisfactory levels of essential nutrients were characteristic of the experimental groups from the Velykoburlutsky Reservoir, not considering the slight excess of glycogen content in the gills of triennials.

In the study of freshwater fish in the Kremenchuk reservoir, it was found that the protein content in the meat of bighead carp, on average from 16% to 18.7% (autumn catch), and carp meat was from 16% to 18.8% (spring catch), bream meat from 17% to 21.7% (autumn catch). Total fat content from 3.1% to 8%. These data indicate that this raw material is characterized by high protein content and medium fat **[45]**, **[46]**

In the Samarska bay of the Zaporizhzhya Reservoir, the protein content in the muscles of perch, zander, and roach decreased, but only 14% had a significant deviation of 14%. In the gills, a significant decrease in protein content was observed in predatory fish by 24% in perch and 13% in zander. In the liver, the protein content was significantly reduced in all species of fish, perch by 30%, zander by 27%, bream by 30%, and roach by 34% **[47]**, **[48]**. Decreased protein content in fish may indicate the presence of stressors stimulating its proteolysis.

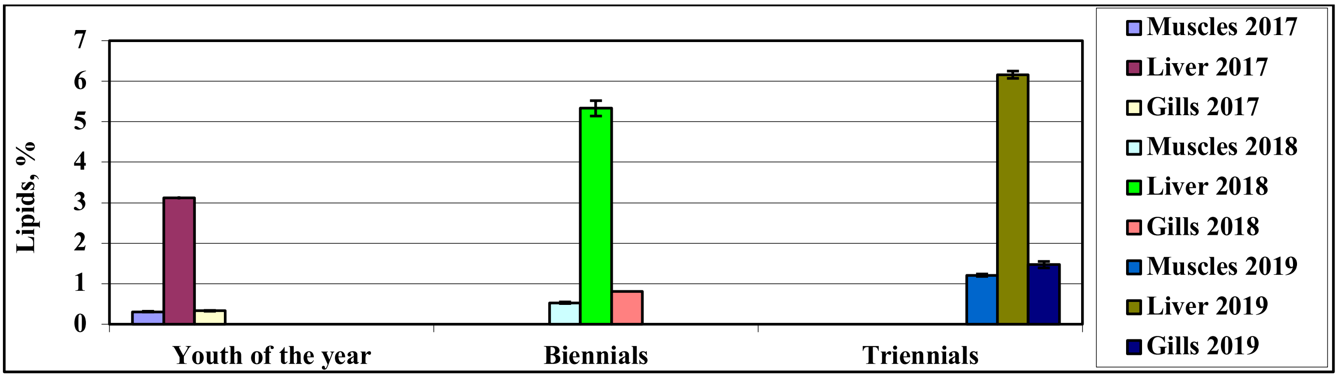

Figure 18 The average values of lipids in the organs and tissues of the year's youth, biennials, and triennials hybrid of silver and bighead carp from the Velykoburlutsky Reservoir during 2017, 2018, and 2019.

Fish from Samarska bay showed a significant increase in muscle lipid content: roach by 70%, bream by 67%, perch by 31%, and zander by 75%. Elevated levels of lipids in the muscle tissue of fish in Samarska bay may indicate a violation of metabolism due to unfavourable living conditions in this area **[49]**, **[50]**.

Reduced fat content was found in fish liver. Significant deviations were found in nonpredatory fish, in bream - by 18%, and in roach by 12%. A significant decrease in total fat content in the liver of the studied fish selected in the Samarska bay, indicates a general violation of lipid metabolism and the accumulation of reserve lipids.

In industrial fish species caught in the Samarska bay of the Zaporizhzhya Reservoir, there is a predominant decrease in glycogen content in the studied tissues and organs. Glycogen decreased by 20% in perch muscle and 29% in zander muscle. In the liver of perch, glycogen content decreased by 35%, in the liver of zander by 36%, roach by 20% **[44]**.

Thus, for the studied, different size and weight groups of the hybrid of silver and bighead carp, caught from ponds and reservoirs are mainly characterized by satisfactory values of overall metabolic rates.

CONCLUSION

It has been found that the liver of annuals and biennials of fish has a higher level of glycogen and lipids than its presence in the muscles and gills of bighead carp. In most organs and tissues of annual fish in 2017 and 2018, the average values of the specific weight of protein were almost evenly distributed in them, were at the level of 10 – 14%. The exceptions were the protein content in the winter pond No. 5 was lower (about 10%) and fluctuated depending on age. In 2017 and 2018, the average values of the specific weight of muscle protein varied between 14 – 14.5%; in the liver ,12.5 – 14% (the highest in fish pond No. 2); in the gills - less than 12%. In biennials caught from winter ponds in 2018, and 2019, muscle protein content was 14 – 14.5%; in the liver - at the level of 12.5 – 14% (the highest rates in fish caught from pond No. 2); in gills - at least at the level of less than 12.5 – 13%. The study of reservoirs showed significant fluctuations in protein content in the muscles (20.04 – 22.16%), liver (18.00 – 18.56%), and gills (13.44 – 15.50%) of fish. In the bighead carp triennials, the glycogen content in the liver (1.79 – 1.84 times) and lipids in the gills (1.19 – 1.36 times) was recorded.

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In accordance with the protocols № 3/2017, № 5/2018 and № 1/2019 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 № 1/3, 2/5 and 1/1 ie in the process of catching hybrid hypophthalmichthys (*Hypophthalmichthys spp.)* "all norms of the current legislation of Ukraine according to DSTU 2284:2010 are observed".
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