DOI: 10.2478/cjf-2022-0016
CODEN RIBAEG ISSN 1330-061X (print)
1848-0586 (online)

COMPARATIVE ANALYSIS OF SELECTED TISSUES OF BROWN TROUT FROM THE FISH POND AND RIVER PLIVA, BOSNIA AND HERZEGOVINA

Radojka Pajčin[1], Rajko Roljić[1], Zoran Ružić[2], Vera Nikolić[1,3*], Zoran Marković[4], Smiljana Paraš[1]

[1] Department of Biology, Faculty of Natural Sciences and Mathematics, University of Banja Luka dr Mladena Stojanovića 2, 78000 Banja Luka, Bosnia and Herzegovina
[2] Faculty of Agriculture, Department of Veterinary Medicine, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[3] Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Beograd, Serbia
[4] Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Zemun, Serbia

*Corresponding Author: vera@bio.bg.ac.rs

ARTICLE INFO

Received: 21 June 2022
Accepted: 18 July 2022

ABSTRACT

Brown trout is a common type of fish grown for consumption in open and flowing fish ponds on the rivers of Bosnia and Herzegovina. We conducted this study during three seasons (spring, summer and autumn) to obtain a complete picture of the morphological and histological characteristics of selected trout tissues. The physicochemical characteristics of the water from the Pliva River near Pljeva and the fish pond where intensive breeding of trout is carried out were analyzed. These analyses have shown that there are no major deviations in water quality and that the water in the pond is of excellent quality. A total of 90 trout were harvested during all three seasons and their morphometric characteristics were determined by analyzing ten parameters. After the dissection of all individuals, histological and stereological analysis was performed to compare the tissues of the liver, stomach, gills and muscles. These analyses showed significant differences in the histoarchitecture of selected trout tissues from the pond compared to the river. Liver tissue had altered hepatocyte shape and presence of adipocytes; stomach tissue had stronger mucosa and weaker muscle layer; gill tissue had lower respiratory lamellae; while muscle tissue had a significant presence of adipose tissue in brown trout living in the fish pond compared to those in the river. All these changes are due to the quality of commercial feed and the inability to move freely.

Keywords:
Brown trout
Morphometry
Histology, stereology
Pliva River
Water quality parameters

How to Cite

Pajčin, R., Roljić, R., Ružić, Z., Nikolić, V., Marković, Z., Paraš, S. (2022): Comparative analysis of selected tissues of brown trout from the fish pond and River Pliva, Bosnia and Herzegovina. Croatian Journal of Fisheries, 80, 151-164. DOI: 10.2478/cjf-2022-0016.

INTRODUCTION

The farming of trout *Salmo trutta m. fario* has been steadily increasing worldwide due to increased market demand. The meat of brown trout is of exceptional quality due to its high protein content and low-fat content. Open cold-water trout ponds are built in the upper parts of river courses, as these fish require very clean, unpolluted, cold and well-oxygenated water (Cantonati et al. 2006). Springs of mountain rivers such as the Pliva are fed by small surface aquifers, while the zones above are often composed of large rocks with high porosity and easy contact between groundwater and the atmosphere, so mountain sources are well-oxygenated (Camargo et al.2011). Open ponds imply that part of the river flow is partitioned by wire barriers, access to the river is concreted, while the bottom remains unconcreted and stocked with only one fish species (Cantonati et al. 2011). Trout are fed commercial, balanced and pelleted feed in pond systems, as well as macrozoobenthos present in the river. Anthropogenic impact on rivers is a constant threat to the water quality of mountain rivers in Bosnia and Herzegovina, especially during periods of low flow. The negative impact on water quality is caused mainly by the intake of fecal matter and wastewater from individual households, as well as the leaching of pesticides from fields (Boaventura et al.1997). Declining water quality in rivers also directly affects the quality of the environment in which brown trout live (Bartoli et al. 2007). The physicochemical composition of the waters of all rivers is changing in the direction of increasing the concentration of soluble and insoluble phosphate (Pulatsu et al. 2004), increasing the content of nitrogen in the form of NH_4 as well as organically bound nitrogen (Selong et al. 1998), reducing the concentration of dissolved oxygen (Oberdorff et al. 1994), and the accumulation of suspended particles from the river bottom (Foy et al. 1991) All these support the trend of decreasing the quality of waters where brown trout are farmed and the production of trout meat is of poorer quality (Maire et al. 1994).

The aim of this study was to show the differences in morphometric, histological and stereological conditions of the selected tissues of trout from natural and open pond systems.

Trout liver tissue is an indicator of environmental conditions in which it lives; it first responds by changing cytoarchitecture and adipogenesis (Jenabi et al. 2019). Changing the diet from active hunting to taking processed feed changes the histological structure of the mucous membrane and muscle layer of the intestine in trout (Barišić et al. 2018). The most important indicator of water quality in which trout live, both in the wild and in the pond system, is the gill tissue. This tissue is very sensitive to the change in oxygen concentrations and the degree of water pollution in which trout live (Perola et al. 2019). The quality and volume density of the adipose tissue in trout muscles are an indicator of their lifestyle and thus the quality of the meat. An active lifestyle leads to the formation of a smaller amount of adipose tissue between the muscle fibers, while a more passive lifestyle in the pond systems causes fat to build up and increases the calorific value of the meat per gram (Tayfun et al. 2021). The total size of trout farms in Bosnia and Herzegovina is 91,026 m^2 with an average production of about 5,000 tonnes. In addition to the control of histological parameters of trout organs, it is also necessary to control the composition and quantity of commercial fish feed used.

MATERIAL AND METHODS

Site description

A sampling of 90 individuals of trout was carried out in 2015 on the Pliva River at the Pljeva site and the fish pond during the spring, summer and autumn.

Physicochemical analysis of water

Water samples for physicochemical analysis were taken three times for each season separately. Physical and chemical properties of water from the open and flowing fish pond and the Pliva River were analyzed in the Laboratory for Analytical Chemistry at the Department of Chemistry of the Faculty of Natural Sciences and Mathematics, University of Banja Luka.

Fish sampling

Brown trout samples were taken either by fly fishing or net fishing in the early morning and late evening hours. For each of the seasons, 15 fish from the Pliva and 15 from the pond were caught. Immediately after the catch, the fish were transported in refrigerators to the Laboratory of Physiology, Department of Biology at the Faculty of Natural Sciences and Mathematics, University of Banja Luka.

Morphometric analysis of trout

Determination of all 90 trout was performed using the keys for determining the species of river fish according to Vuković and Ivanovic (Vuković et al. 1971). All individuals analyzed in this study were treated in a fresh state, immediately after fishing. To analyze the morphometric characteristics of the trouts, the following parameters were determined: TL = total length, SL = standard length, H = height, M = mass, TGL= length of the digestive tract, TGM = mass of the digestive tract, MH = mass of the liver, MGF = mass of female gonads, MGM = mass of male gonads and FK = Fulton's factor for each trout. The measurement of the total and standard body length and height, as well as the length of the digestive tract of individuals, was carried out with a tape measure with up to 1 mm accuracy. Measurement of the body mass of individuals, as well as the mass of the digestive tract, liver and gonads, was performed on a technical scale with 0.01 g accuracy. Based on the measured morphometric parameters, Fulton's condition factor of all trouts was determined according to the formula:

$$FCF = \frac{W}{T^3}$$

where:

W - weight, T - total length (Riker, 1975).

Fish dissection was performed in the Laboratory at the Faculty of Natural Science and Mathematics, University of Banja Luka. During the dissection of each trout, a medioventral incision was made, which allowed access to the internal organs. The digestive tract, liver, gills and gonads of all 90 trout were isolated.

Histological and stereological analysis

During the dissection of all ninety trout, their livers, gasters, gills and parts of muscle tissue were removed from the tail region. After emptying the digestive tract of its contents, the central part of the gaster was taken for histological analysis. All organs and tissues were removed and immersed in Bouin's solution for 24 h. For better absorption, tissues were further cut into smaller parts and then immersed into fresh Bouin's solution. Subsequently, all dissected organs and tissues were prepared for light microscopy using the standard tissue preparation procedure. Samples of tissues of all organs were embedded in paraffin and were cut in a frontal plane on a Leica rotary Microtome RM 2165 (Leica Microsystems, Wetzlar, Germany) in 5-µm thick serial sections. Slices of all tissues were stained with Hematoxylin-Eosin (H&E), Mallory-Azan trichrome, Toluidine blue and violet stain (all stains by Merck KGaA, Darmstadt, Germany). The qualitative histology analysis of the liver, gastric, gill and muscle tissue slides was performed using a Leica DM8000 light microscope with a MEGA VIEW camera and digital image transfer system (Wetzlar, Germany) and magnification of 50x, 100x, 250x and 400x. For quantitative, stereological analysis, micrographs of all organ tissues were acquired in the RGB layout and converted to binary format. Measurements were made using a stereo-universal test system according to Cavalier's principle, using a 16.0 point-counting system (3.0.0. Version, MBF Bioscience, Williston, VT, USA), with a P2 spacing grid at the maximum 400x magnification. Stained tissue sections of the liver, gaster, gills and muscles were used to measure the number and volume density (Vv) of their cells. Volume density was calculated by the following formula: Vv=Pf/Pt (mm^0), where Pf represents the number of the desired phase on the test system and endothelia cells represent the total number of points of the test system (Paraš et al. 2019). The numerical density (Nv) of all cells was calculated based on the cell count (Q) in the volume of analyzed tissues (Vo). The volume of analyzed tissues was evaluated as the product of the number of counted frames (ΣPi), the space of counted frames (a=25002) and the height factor (h) (the histological section), as presented in the following formula (Santos et al. 2009):

$$\mathrm{Nv} = \frac{Q}{Vo} = \frac{Q}{\sum_{i=1}^{n} Pi \times a \times h}\ (\mathrm{mm}^{-3})$$

Statistical analysis

Measured values were presented as mean ± standard deviation. Group comparisons were performed by using parametric (two-way ANOVA, one-way ANOVA followed by Dunnett's test and t-test) or nonparametric tests (Kruskal-Wallis and Mann-Whitney U-test), depending on data distribution, with significance set at $p<0.05$. Statistical software SPSS 20.0 (IMB corp., Armonk, NY, USA) was used for data processing.

RESULTS

Physicochemical analyses of water

An analysis of the physicochemical parameters of the water from two localities on the Pliva River, the pond and the free-flowing river where trout were caught during all three seasons, showed that there was a difference in their values (Table 1). The water temperature in the pond was 2.7-3.7°C higher than in the free-flowing river. The value of dissolved oxygen was significantly lower in the pond than in the river, while the value of BOD_5 was higher in the pond water. The water in the pond in the spring contained nitrites and ammonia nitrogen, as well as the water in the pond in the summer.

Morphometric analysis of trout

Morphometric analysis was performed for all ninety brown trout (Table 2), also by season. In spring and summer pond trout were longer than those living in natural conditions, while in autumn river trout were longer. The weight of trout from the pond and all organs studied was higher than that of fish from the free-flowing Pliva River in all three seasons. FK through all three observed seasons was higher in trout from the river compared to those from the pond.

Fulton's condition factor values

Analyzing the individually obtained Fulton's condition factor values by season, it is observed that the average value ranges from 0.90 in spring to 1.16 in autumn (Table 3). According to the results obtained, Fulton's nutritional factor values are relatively uniform in all seasons. There are no statistically significant differences in the value of Fulton's index between the sexes.

Stereological and histological analysis of trout tissues

1. Liver tissue

Stereological analysis of the liver tissue of trout indicates changes in all parameters in the form of a decrease in the volume and numerical densities of hepatocytes at the expense of an increase in the same parameters of adipose tissue in trout from the pond compared to those from the Pliva River in all three seasons (Table 4). Volume densities of trout adipose tissue from the pond in the summer and autumn seasons are statistically significantly higher compared to the trout from the river.

Table 1. Physicochemical parameters of water by annual seasons from both sites: the Pliva River (river) and the open trout pond on the Pliva River (pond) (mean ±SD)

Season	Spring		Summer		Autumn	
Parameter/site	**River (n=15)**	**Fish pond (n=15)**	**River (n=15)**	**Fish pond (n=15)**	**River (n=15)**	**Fish pond (n=15)**
Temperature (C°)	8.0	11.0	8.2	12.0	8.3	11.0
pH	7.25	6.98	7.3	7.19	8.0	7.1
Dissolved O_2 (mg/l)	11.2	7.5	9.0	7.1	9.0	7.0
Electrical conductivity (µS/cm)	317	328	332	334	380	330
BOD_5 (mg/l)	1.4	1.5	1.5	1.6	1.6	1.8
Nitrites (mg/l)	0.01	3.0	0.01	2.0	0.01	3.0
Nitrates (mg/l)	0	0	0	0	0	0
Ammonia nitrogen (mg/l)	0	0.2	0	0	0	0
Phosphates (mg/l)	0.5	0	1.2	0.1	1.0	0

Table 2. Morphometric parameters of fish by annual seasons from both sites: the Pliva River (river) and the open trout pond on the Pliva River (pond) (mean ±SD)

Season	Spring (n=30)		Summer (n=30)		Autumn (n=30)	
Parameter/site	**River (n=15)**	**Fish pond (n=15)**	**River (n=15)**	**Fish pond (n=15)**	**River (n=15)**	**Fish pond (n=15)**
TL (cm)	23.265±2,288	23.954±0.878	23.212±3.265	23.384±0.704	23.222±1.753	23.167±1.034
SL (cm)	20.623±1.812	21.599±0.907	20.267±2.086	20.768±0.919	20.993±1.985	20.187±1.038
H (cm)	5.311±0.612	6.833±0.363	5.173±1.092	6.065±0.526	5.212±0.9524	6.392±0.643
M (g).	113.223±30.198	159.076±17.436	120.701±34.777	163.125±12.346	140.233±23.265	167.111±11.485
TGL (cm)	19.234±2.166	23.396±2.249	19.753±2.815	20.765±1.229	19.829±1.588	20.913±1.089
TGM (g)	5.055±1.025	13.978±0.465	9.379±4.507	11.588±2.687	8.182±3.929	8.707±1.818
MH (g)	1.098±0.477	2.255±0.675	1.696±0.728	2.833±0.568	1.445±0.294	2.443±0.201
MGF (g)	0.814±0.611	1.155±0.285	2.263±2.803	2.277±1.642	2.058±1.285	5.251±2.151
MGM (g)	0.412±0.092	1.125±0.322	2.085±1.695	2.95±2.022	2.066±0.793	4.388±2.134

Table 3. Fulton's condition factor values by season; M- male, F-female

			FCF - River		
	Mean	Min	Max	Std. Dev.	CV
Spring (M)	0.90	0.66	1.03	0.15	17.05
Spring (F)	0.91	0.75	0.99	0.08	9.18
Summer (M)	1.03	0.83	1.11	0.09	8.67
Summer (F)	1.01	0.91	1.16	0.08	8.09
Autumn (M)	1.04	0.89	1.17	0.11	10.15
Autumn (F)	1.16	0.87	1.44	0.20	17.34
			FCF - Pond		
	Mean	Min	Max	Std. Dev.	CV
Spring (M)	1.15	0.95	1.40	0.12	10.1
Spring (F)	1.17	1.08	1.26	0.08	6.88
Summer (M)	1.33	1.24	1.41	0.06	4.30
Summer (F)	1.25	1.10	1.49	0.13	10.32
Autumn (M)	1.42	1.21	1.62	0.15	10.35
Autumn (F)	1.27	1.14	1.32	0.06	4.91

Table 4. Stereological parameters of trout liver from the three studied seasons and from natural and artificial conditions (mean ±SD)

Season	**Spring**		**Summer**		**Autumn**	
Parameter/site	**River (n=15)**	**Fish pond (n=15)**	**River (n=15)**	**Fish pond (n=15)**	**River (n=15)**	**Fish pond (n=15)**
Hepatocyte volume density (mm^0)	0.455±0.054	0.334±0.038	0.472±0.044	0.294±0.012	0.390±0.032	0.257±0.025
Reticular connective tissue volume density (mm^0)	0.225 ±0.018	0.219±0.023	0.218±0.022	0.222±0.024	0.229±0.022	0.214±0.025
Blood vessel volume density (mm^0)	0.275±0.029	0.269±0.031	0.294±0.022	0.281±0.028	0.349±0.031	0.303±0.033
Volume density of adipose tissue (mm^0)	0.045±0.009	0.178±0.019	0.016±0.007	0.203±0.043*	0.032±0.008	0.226±0.027*
Hepatocyte area (μm^2)	133.9±14.2	97.4±9.9	135.6±15.1	95.1±9.2	121.7±13.4	94.3±10.1
Hepatocyte nuclei area (μm^2)	58.3±5.4	39.8±4.4	55.7±5.8	35.2±4.5	52.6±5.1	34.7±4.9
Hepatocyte NCR	0.354±0.055	0.224±0.047	0.323±0.053	0.212±0.039	0.341±0.042	0.201±0.034
Numerical density of hepato-cytes (mm^{-3})	65671.2±5794.3	55203.2±4099.1	64319.7±4971.5	52633.7±5408.8	65189.3±4790.5	51048.6± 3565.4
Hepatocyte number	257144.6±12835.7	198202.4±13112.2	244892.3±12418.4	187564.2±11900.6	251921.4±12077.4	180012.1±13115.5

Histological analysis of the liver tissue of trout shows the position and greater representation of adipose cells as well as the occlusion of capillaries in trout from the pond compared to those from the free-flowing river in all seasons (Figure 1).

2. Stomach tissue

Stereological analysis of the stomach tissue of trout indicates changes in all parameters in the form of a decrease in the volume and numerical density of muscle tissue at the expense of an increase in the same parameters of epithelial and connective tissue in trout from the pond compared to those from the Pliva River in all three seasons (Table 5).

Histological analysis of the stomach tissue of trout shows a smaller thickness of muscle layer and a wider epithelial layer of the stomach wall in trout from the pond compared to those from the free-flowing river in all seasons (Figure 2).

3. Gill tissue

Stereological analysis of the gill tissue of trout indicates changes in all parameters in the form of a decrease in the volume and numerical density of the respiratory epithelium at the expense of an increase in the same parameters of elastic connective tissue, cartilage and blood vessels in trout from the pond compared to those from the Pliva River in all three seasons (Table 6).

Histological analysis of the trout gill tissue shows a lower density of respiratory epithelium, as well as stronger and more voluminous cartilage in trout from the pond compared to those from the free-flowing river in all seasons (Figure 3).

4. Muscle tissue

Stereological analysis of the tail muscles of trout indicates changes in all parameters in the form of a decrease in the volume and numerical densities of myocytes at the expense of an increase in the same parameters of connective and adipose tissue, as well as blood vessels in the muscles of trout from the pond compared to those from the Pliva River in all three seasons (Table 7).

Histological analysis of the tail muscles of trout shows the appearance of adipose tissue in the trout from the pond compared to those from the free-flowing river in which adipose tissue was not even represented (yellow arrows in Figure 4) in all seasons.

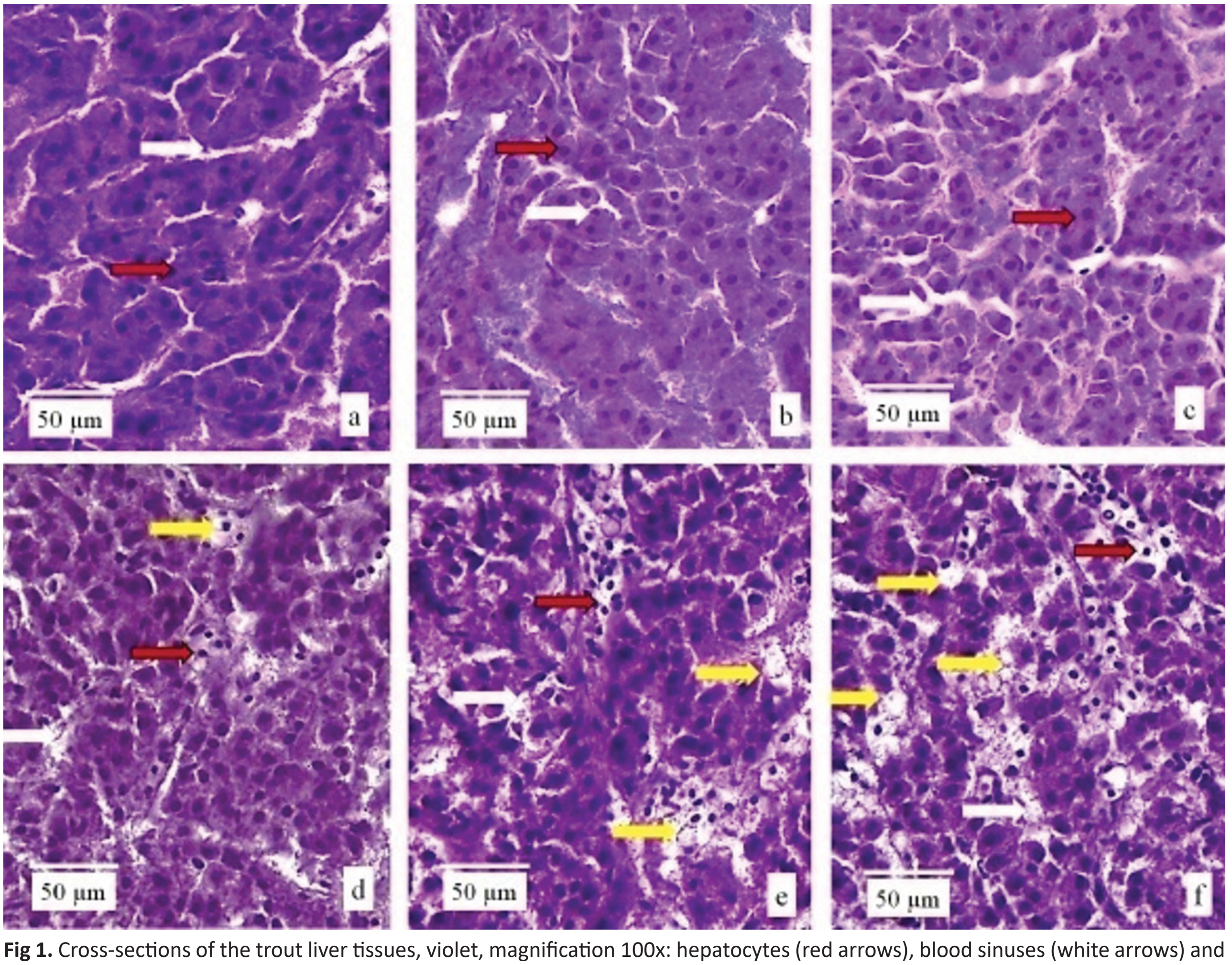

Fig 1. Cross-sections of the trout liver tissues, violet, magnification 100x: hepatocytes (red arrows), blood sinuses (white arrows) and adipocytes (yellow arrows); a. spring, river, b. summer, river, c. autumn, river, d. spring, pond, e. summer, pond, and f. autumn, pond.

Table 5. Stereological parameters of trout stomach from the three studied seasons and from natural and artificial conditions (mean ±SD)

Season	Spring		Summer		Autumn	
Parameter/site	**River (n=15)**	**Fish pond (n=15)**	**River (n=15)**	**Fish pond (n=15)**	**River (n=15)**	**Fish pond (n=15)**
Enterocyte volume density (mm^0)	0.149±0.023	0.175±0.022	0.104±0.019	0.143±0.023	0.146±0.025	0.199±0.022
Connective tissue volume density (mm^0)	0.216±0.029	0.335±0.039	0.232±0.029	0.351±0.037	0.228±0.027	0.391±0.035
Blood vessel volume density (mm^0)	0.162±0.015	0.115±0.011	0.188±0.014	0.125±0.010	0.178±0.015	0.127±0.013
Volume density of stratum muscularis circulare (mm^0)	0.336±0.041	0.214±0.026	0.347±0.044	0.216±0.023	0.339±0.038	0.201±0.025
Volume density of stratum muscularis longitudinale (mm^0)	0.137±0.014	0.161±0.015	0.129±0.011	0.165±0.013	0.109±0.010	0.082±0.011
Enterocyte area (μm^2)	121.5±10.5	118.5±9.9	128.3±11.3	115.5±10.8	124.9±10.2	113.7±10.9
Enterocytes nuclei area (μm^2)	43.4±5.6	44.2±5.5	45.2±5.7	43.4±5.7	43.1±5.5	41.6±5.5
Enterocyte NCR	0.347±0.055	0.366±0.055	0.342±0.046	0.368±0.058	0.346±0.059	0.369±0.055
Numerical density of enterocytes (mm^{-3})	4971.8±537.2	5523.1±566.6	4732.3±513.7	5634.6±525.3	4782.5±518.4	5822.1±541.4
Enterocyte number	21376.9±1465.5	29357.7±1554.9	22304.1±1218.3	31021.5±1691.2	21949.61297.5±	34176.2±1327.4
Width of muscle layer (µm)	213.6±25.5	127.7±13.6	254.7±27.5	115.9±12.2	222.2±24.8	109.4±11.6

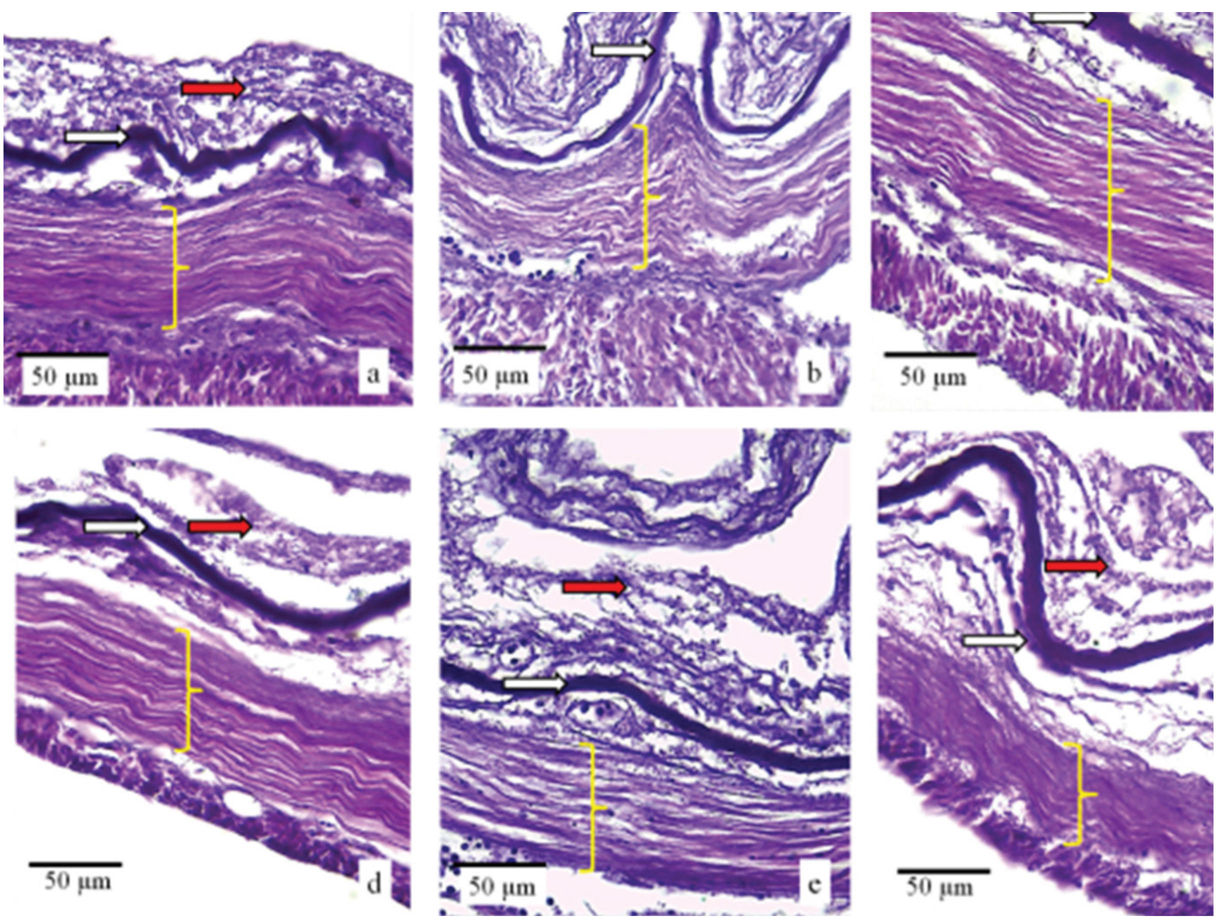

Fig 2. The tissue cross-sections of the trout stomach, Mallory Azan, magnification 100x: enterocytes (white arrows), stratum mucosae (red arrows) and stratum circulare muscularis (yellow bracket); a. spring, river, b. summer, river, c. autumn, river, d. spring, pond, e. summer, pond, and f. autumn, pond

Table 6. Stereological parameters of trout gill from the three studied seasons and from natural and artificial conditions (mean ±SD)

Season	Spring		Summer		Autumn	
Parameter/site	**River (n=15)**	**Fish pond (n=15)**	**River (n=15)**	**Fish pond (n=15)**	**River (n=15)**	**Fish pond (n=15)**
Respiratory epithelial volume density (mm^0)	0.207±0.018	0.155±0.011	0.211±0.011	0.162±0.012	0.197±0.012	0.159±0.012
Elastic connective tissue volume density (mm^0)	0.127±0.010	0.195±0.016	0.119±0.010	0.214±0.012	0.123±0.011	0.184±0.012
Cartilage volume density (mm^0)	0.135±0.011	0.257±0.017	0.148±0.013	0.283±0.017	0.139±0.012	0.264±0.016
Blood vessel volume density (mm^0)	0.167±0.014	0.245±0.019	0.183±0.017	0.279±0.018	0.199±0.016	0.262±0.019
Respiratory epithelial cell surface area (μm^2)	91.9±7.8	87.6±7.7	90.5±6.9	88.3±7.9	92.9±7.8	87.5±7.7
Respiratory epithelial cell nuclei surface area (μm^2)	36.4±4.5	35.9±4.2	36.2±4.4	35.8±4.5	35.5±4.5	35.9±4.7
NCR of respiratory epithelial cells	0.349±0.046	0.356±0.049	0.357±0.052	0.362±0.041	0.331±0.044	0.348±0.049
Numerical density of respiratory epithelial cells (mm^{-3})	2014.2±235.1	1663.6±209.4	2242.5±211.8	1525.5±148.3	2205.5±196.2	1637.2±141.7
Respiratory epithelial cell count	17924.5±1275.9	12963.8±1003.3	18232.1±1207.5	12423.5±1103.7	18603.7±1570.2	12006.1±945.8
Height of gill septum (µm)	35.3±2.4	26.5±3.1	36.1±2.5	25.8±2.4	35.5±3.7	25.1±2.1

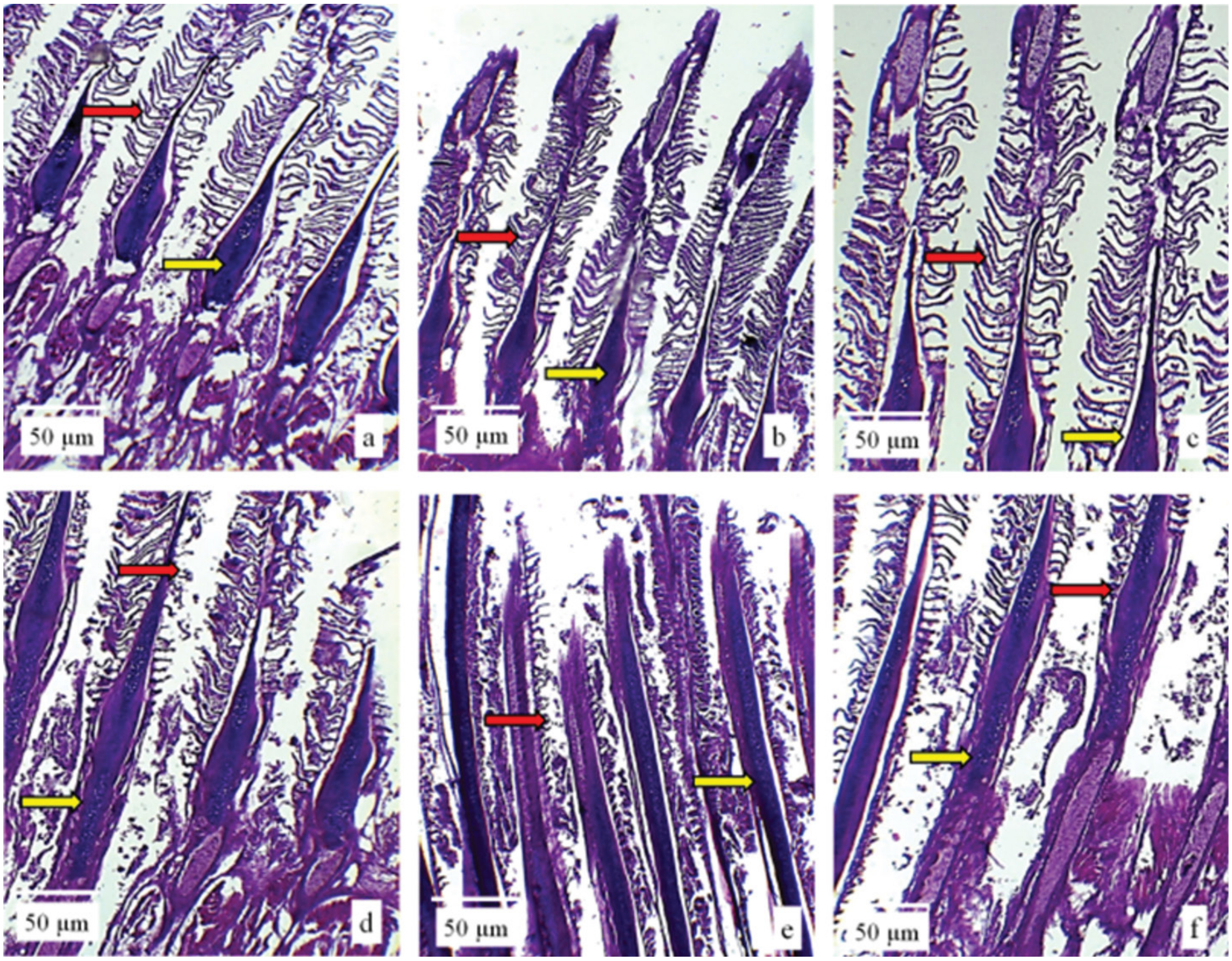

Fig 3. The tissue cross-sections of trout gills, Mallory Azan, magnification 100x: respiratory epithelial cells (red arrows) and cartilage (yellow arrows); a. spring, river, b. summer, river, c. autumn, river, d. spring, pond, e. summer, pond, and f. autumn, pond

Table 7. Stereological parameters of trout muscle from the three studied seasons and from natural and artificial conditions (mean ±SD)

Season	Spring		Summer		Autumn	
Parameter/site	River (n=15)	Fish pond (n=15)	River(n=15)	Fish pond (n=15)	River (n=15)	Fish pond (n=15)
Respiratory epithelial volume density (mm^0)	0.207±0.018	0.155±0.011	0.211±0.011	0.162±0.012	0.197±0.012	0.159±0.012
Elastic connective tissue volume density (mm^0)	0.127±0.010	0.195±0.016	0.119±0.010	0.214±0.012	0.123±0.011	0.184±0.012
Cartilage volume density (mm^0)	0.135±0.011	0.257±0.017	0.148±0.013	0.283±0.017	0.139±0.012	0.264±0.016
Blood vessel volume density (mm^0)	0.167±0.014	0.245±0.019	0.183±0.017	0.279±0.018	0.199±0.016	0.262±0.019
Respiratory epithelial cell surface area (μm^2)	91.9±7.8	87.6±7.7	90.5±6.9	88.3±7.9	92.9±7.8	87.5±7.7
Respiratory epithelial cell nuclei surface area (μm^2)	36.4±4.5	35.9±4.2	36.2±4.4	35.8±4.5	35.5±4.5	35.9±4.7
NCR of respiratory epithelial cells	0.349±0.046	0.356±0.049	0.357±0.052	0.362±0.041	0.331±0.044	0.348±0.049
Numerical density of respiratory epithelial cells (mm^{-3})	2014.2±235.1	1663.6±209.4	2242.5±211.8	1525.5±148.3	2205.5±196.2	1637.2±141.7
Respiratory epithelial cell count	17924.5±1275.9	12963.8±1003.3	18232.1±1207.5	12423.5±1103.7	18603.7±1570.2	12006.1±945.8
Height of gill septum (μm)	35.3±2.4	26.5±3.1	36.1±2.5	25.8±2.4	35.5±3.7	25.1±2.1

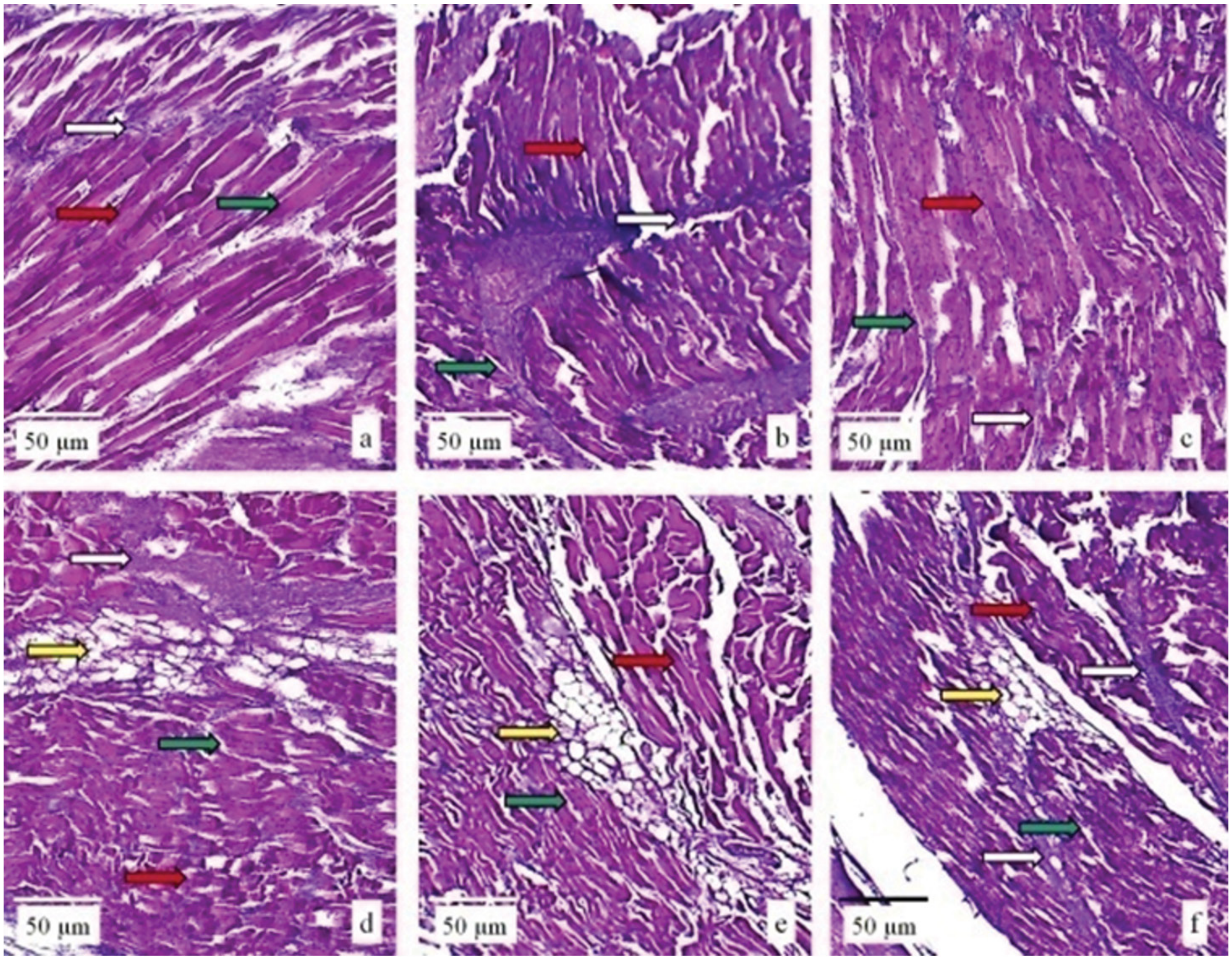

Fig 4. The tissue cross-sections of the trout tail muscles, H&E, magnification 100x: myocytes (red arrows), adipocytes (yellow arrows), connective cells (white arrows) and capillaries (green arrows); a. spring, river, b. summer, river, c. autumn, river, d. spring, pond, e. summer, pond, and f. autumn, pond

DISCUSSION

In our study, the physicochemical parameters of water quality from both localities had satisfactory characteristics (Annonimous, 2001). The analysis of the physical and chemical parameters of water showed the expected differences in quality: the water in the pond is warmer and contains less oxygen and more suspended matter, which is a consequence of reduced aeration due to partitioning, as well as a higher load of organic matter from pelleted feed (Sidoruk et al. 2018). Water quality is also negatively affected by the density of the trout population in the pond.

According to the composition of the gastrointestinal contents, satisfactory nutritional status with a condition factor greater than 1 was found. The nearness of the K value to 1.0 indicated the suitability of the environment in the Pliva River for the good growth of the fish (Arslan et al. 2004).

The analysis of morphological characteristics showed that trout from the pond were longer, higher and heavier, and the deviation in the last season of the survey was due to fishing from the pond when the largest specimens were caught first. It is advised to enforce the breeding of the native Danubian lineage of brown trout in fish farms and to use native populations for stocking purposes (Špelić et al. 2018).

A different diet and lifestyle of trout from the pond and the wild affect the changes in the structure of their livers. Fibrosis and liver cystinosis were not present, but liver adiposes were present in trout from the pond. During the dissection of trout from the pond system, large accumulations of adipose tissue were observed around all organs, which also affected their histological structure. The livers of all trout in the pond were between 69% and 105% heavier than those living in the river. Adipocytes are present in groups, individually and in addition to blood capillaries in liver tissue. They led to capillary obstruction which aggravated the liver function of trout from the pond (Xu et al. 2016). The mean surface area of hepatocytes and their nuclei are significantly lower in trout from the pond. However, there was also a change in the shape of the trout hepatocytes from the pond because of very small hepatocytes with large nuclei and very large ones with a small nucleus, with a completely atypical appearance. Large hepatocytes with a small nucleus are characteristic of liver tissue that has a high production of metabolic enzymes, while small hepatocytes with a large nucleus are characteristic of regenerating liver tissue (Sun et al. 2019). These are indicators of the difficult function of trout livers in fish pond systems from our study both because of the increased function and the significantly increased presence of adipose tissue. The increased percentage of lipids in fish feed has less effect on the presence of adipose tissue in their liver than the increased percentage of carbohydrates in the feed, which leads to the genesis of adipose cells in the liver parenchyma of fish (Li et al. 2020).

In our study, the intestinal wall of the trout from the free-flowing river was thicker than the intestinal wall of the trout from the pond. The reason for this is a much thicker layer of muscle in trout from the free-flowing river which is always active in the food digestion process and indirectly active through the movement of fish while swimming. The mucous membranes with enterocytes at the top are thinner in the fish from the river because they take less food but more often, the food is not retained in their digestive system for long and their digestive system is not dilated due to overeating as in trout from the pond. Food retention, slower digestion due to the intake of heavier digestible substances, as well as a passive lifestyle, lead to the thickening of the mucous membrane which was under inflammation in some places (Bowyer et al. 2012). The cleanliness of the access ramps to the ponds is very important because the retention of feed on them can lead to the deterioration of the feed and its contamination with bacteria and fungi, which additionally causes problems for the breeders when such feed ends up in the pond as a meal for fish (Rašković et al. 2011). The volume density of the gastric wall mucosa is thinner in trout from the free-flowing river than in those from the pond, which is a consequence of the diet and lifestyle of these fish. The feed that trout are fed in the pond is more difficult to digest because it is made from nutrients that take a longer time to digest, such as milled corn, soybean meal or animal protein. Protein prevalence in the commercial fish feed fed to trout is high, ranging from 40% to over 50%, and it originates very often from mammals and birds that are not otherwise natural food for fish (Caimi et al. 2020). Fish from the natural environment take proteins originating from invertebrates and fishes, and these are much more easily digestible proteins and adapted to the digestive system of trout (Liu et al. 2018). The feed in the ponds was available in large quantities and was given all at once during the day, causing the fish to eat too much food at once. The food taken in this way is harder to digest than the food fish take from the free-flowing river throughout the day, while eating a little. The difference in length, and especially in weight of the digestive tract of trout from the pond, was also evidence of this assumption, which was greater than of those from the free-flowing river. The method of taking food is completely different; fish from the pond take feed without struggling to catch it and passively take it from the water while the pellets sink, or from the surface, depending on whether the nutrients sink or swim. On the other hand, trout from the river are hunters and predators, i.e. they hunt their food and stimulate the digestive system during the hunt with swimming movements and so improve digestion. These are all characteristics of the lifestyle of trout from two different environments that condition both steric and histological differences in the structure of the intestinal wall.

The reduction of the epithelial cell surface area by about 10% and the volume density by about 33% of the respiratory epithelium of the gills, as well as the reduction of the gill septum height by about 39%, are indicators of the degraded state of the gills in trout living in the pond systems compared to those from the river. The stereological and histological analysis of the characteristics of the gills in trout directly indicates the quality of the water in which the fish live. Polluted and oxygen-poor water, as well as warm water, is not conducive to trout growth and development (Velmurugan et al. 2007). Analysis of the histoarchitecture of trout gills is an indicator of the environment in which they live, i.e. the state of water of the Pliva River at the examined site. However, the changed way trout live in the pond compared to the river also affected the condition of their gills. Trout in ponds are much less active and do not hunt and swim constantly in the same area, while trout from the river are constantly on the move during the day. The health of fish gills is most affected by the amount of dissolved oxygen in the water, regardless of whether they are in the pond or free-flowing system. The activity and mobility of trout depend on oxygen because it directly gives them the energy to move. If the quantity is not satisfactory, trout move around the river looking for better conditions and more oxygenated water (Pajcin et al. 2018). Compensation of the lower representation of the respiratory epithelium on the gill lamellae was histologically determined by elastic and cartilage connective tissue because in this way the lamellae become firmer. In this way, adapted gills allow trout to have open gill covers for longer, which they normally do when there is not enough oxygen in the water (Lujić et al. 2013).

Stereological and histological analysis of the muscle tissue of trout tails from the pond system showed a large difference compared to the fish from the river. Trout in the free-flowing river did not at all have the presence of adipose tissue between muscle fibers, while those from the pond had the presence of small and large clusters of adipose tissue. Stereological analysis showed the presence of adipose in the muscular tissue of trout tails from the fish pond system in the range of 10-15%. The presence of adipose tissue in the muscular tissue of the pond trout reduces the quality of its meat when used for consumption, as was the case with the fish from the pond in our study. Adipose deposits occur due to poor diet, reduced activity and the inability of trout to swim due to limited space in the pond. A large amount of unbalanced commercial fish feed, and even the addition of pure milled maize, leads to the fattening of trout in ponds (Caballero et al. 1999). Fish feed in ponds must be of good quality, with vitamins and minerals, and adapted to the age of the fish (Caballero et al. 2002). Histological parameters show a decrease in the number and size of myocytes, which is an indicator of the deterioration of muscle tissue by atrophy in trout from the pond system. Atrophy of the strongest muscles of fish is not uncommon when they are farmed in ponds due to limited living space, as it is known that trout swim several kilometers a day in search of food under natural conditions (Trenzado et al. 2018). The space of atrophied muscle fibers is usually occupied by connective and adipose tissue when nutrient intake is increased in trout from the pond system.

CONCLUSIONS

Research of the condition of selected trout tissues from two different ecosystems that differ in the physicochemical characteristics of the water has shown significant differences in many parameters. As a consequence of the available concentrated food, the trout from the pond showed higher values of all morphometric characters. Ingestion of a larger amount of nutritionally unbalanced food results in an enlargement of the liver and its difficult operation. The mucous membrane of the intestine is thinner in fish from the river because the food is not retained for a long time and their digestive system is not dilated. Slower digestion due to the intake of concentrated food leads to the thickening of the mucous membrane, which is often under inflammation. The decrease in the respiratory surface area of epithelial gills, as well as the decrease in the height of the gill septum, are indicators of the degraded state of the gills of trout from the pond. The trout in the river had no adipose tissue between the muscle fibers, unlike the ones from the pond. The presence of adipose tissue significantly reduces the quality of the meat. Based on all of the above, the results from these studies have both a theoretical and a practical contribution to trout fisheries.

UPOREDNA STUDIJA ODABRANIH TKIVA POTOČNE PASTRVE IZ RIBOGOJILIŠTA I RIJEKE PLIVE, BOSNA AND HERCEGOVINA

SAŽETAK

Potočna pastrva česta je vrsta ribe koja se uzgaja u otvorenim, protočnim ribogojilištima u konzumne svrhe na rijekama Bosne i Hercegovine. Lokalitet Pljeva na rijeci Plivi u srednjoj Bosni idealan je za uzgoj pastrve te uzgajivači ovih područja imaju dugu tradiciju uzgoja u otvorenim - protočnim ribogojilištima. U cilju sagledavanja morfoloških i histoloških obilježja pastrva iz ovih ribnjaka u usporedbi s onima izvan ribnjačkog uzgoja provedena je ova studija kroz tri sezone: proljeće, ljeto i jesen, kako bi se vidjela potpuna slika stanja odabranih tkiva i kondicije potočnih pastrva. Analizirana su fizičko-kemijska obilježja vode iz rijeke Plive kod Pljeve i iz ribogojilišta koji se bavi intenzivnim uzgojem potočnih pastrva. Ove analize su pokazale da ne postoje velika odstupanja u kvaliteti vode i da je voda u ribogojilištu dobre kvalitete. Izlovljeno je ukupno 90 jedinki kroz sve tri sezone i analizirane su morfometrijske karakteristike na deset parametara. Nakon disekcije provedena je histološka i stereološka analiza s ciljem komparacije tkiva jetre, želuca, škrga i mišića. Ove analize su pokazale značajne razlike u histoarhitekturi odabranih tkiva pastrva iz ribnjaka u odnosu na rijeku. Tkivo jetre bilo je s izmijenjenim oblikom hepatocita i prisustvom adipocita; tkivo želuca bilo je s jačom mukozom i slabijim mišićnim slojem; tkivo škrga imalo je niže respiratorne lamele, dok je mišićno tkivo imalo znatno prisustvo masnog tkiva kod pastrva iz ribogojilišta u odnosu na one iz rijeke. Sve promjene su posljedica kvalitete komercijalne hrane kao i nemogućnosti slobodnog kretanja.

Ključne riječi: potočna pastrva, morfometrija, histologija, stereologija, Pliva, parametri kvaliteta vode

ACKNOWLEDGMENT

Work was granted by Ministry of Education, Science and Technological Development of the Republic of Serbia (Contract No. 451-03-68/2022-14/200178, for the University of Belgrade, Faculty of Biology, Contract No. 451-03-68/2022-14/200116, for the University of Belgrade, Faculty of Agriculture)

REFERENCES

Annonimous (2001). Službeni glasnik Republike Srpske, br. 42. Uredba o klasifikaciji voda i kategorizaciji vodotoka.

Arslan M, Yildirim A, Bektas S (2004): Length-weight relationship of brown trout, Salmo trutta L., inhabiting Kan stream, Coruh Basin, North-Eastern Turkey. Turk. J. Fish. Aquat. Sci. 4:45-48

Barišić J., Filipović M.V., Mijošek T., Čož-Rakovac R., Dragun Z., Krasnići N., Ivanković D., Kružlicova D., Erk M. (2018): Evaluation of architectural and histopathological biomarkers in the intestine of brown trout (*Salmo trutta* Linnaeus, 1758) challenged with environmental pollution, Science of the Total Environment,642,

Bartoli M., Nizzoli D., Longhi D., Laini A., Viaroli P. (2007): Impact of trout farm on the water quality of an Apennine creek from daily budgets of nutrients, Chemical Ecology, 23.

Boaventura R., Pedro M. A., Coimbra J., Lencastre E. (1997): Trout farm effluents: characterization and impact on the receiving streams, Environmental Pollutions,95.

Bowyer J. N., Qin G. J., Adams L. R., Thomson M. J. S., Stone D. A. J. (2012): The response of digestive enzyme activities and histology in yellowtail kingfish (*Seriola lalandi*) to dietary fish oil substitution at different temperatures, Aquaculture, 368-369, 19-28.

Caballero M. J., Calero-Lopez G., Socorro J., Roo J. F., Izquierdo M. S., Fernandez A. J. (1999): Combined effect of lipid level and fish meal quality on liver histology of glithead seabream (*Sparus aurata*), Aquaculture, 179(1-4), 277-290.

Caballero M. J., Obach A., Rosenlund G., Montero D., Gisvold M., Izquierdo M. S. (2002): Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*, Aquaculture, 214(1-4), 253-271.

Caimi C., Gasco L., Biasato I., Malfatto V., Varello K., Prearo M., Pastorino P., Bona C. M., Francese R. D., Schavone A., Concetta E. A., Ambrosius J. M. D., Gai F (2020): Could Dietary Black Soldier Fly Meal Inclusion Affect the Liver and Intestinal Histological Traits and the Oxidative Stress Biomarkers of Siberian Sturgeon (*Acipenser baerii*) Juveniles? Animals, 10(1), 155-165.

Camargo J. A., Gonzalo C., Alonso Á. (2011): Assessing trout farm pollution by biological metrics and indices based on aquatic macrophytes and benthic macroinvertebrates: A case study, Ecological Indicators, 11(3).

Cantonati M., Gerecke R., Bertuzzi E. (2006): Springs of the Alps-Sensitive Ecosystems to Environmental Change: From Biodiversity Assessments to Long-term Studies, Hydrobiology, 562.

Cantonati M., Gerecke R., Jüttner I., Cox E. J. (2011): The challenges of long-term ecological research in springs in the northern and southern Alps: indicator groups, habitat diversity, and medium-term change, Journal Limnology, 70(1).

Foy R. H., Rosell R. (1991): Fractionation of phosphorus and nitrogen loadings from a Northern Ireland fish farm, Aquaculture, 96.

Jenabi R., Haghparast K., Moghanlou M., Mohseni A., Imani I. (2019): Effect of dietary soybean lecithin on fish performance, hemato-immunological parameters, lipid biochemistry, antioxidant status, digestive enzymes activity and intestinal histomorphometry of pre-spawning Caspian brown trout (*Salmo trutta caspius*), Fish and Shellfish Immunology, 91.

Li X., Zheng S., Ma X., Cheng K., Wu G. (2020): Effects of dietary protein and lipid levels on the growth performance, feed utilization, and liver histology of largemouth bass (*Micropterus salmoides*), Amino Acids, 52, 1043-1061.

Liu X., Hegab I. M. M., Si J., Du X., Fan X., Zhang Q., Gao Y., Wang H. (2018): Effects of different durations of fasting/re-feeding bouts on growth, biochemical and histological changes in the digestive tract of Gansu golden trout (*Oncorhynchus mykiss*), Czech Journal of Animal Science, 63, 389-398.

Lujić J., Marinović Z., Miljanović B. (2013): Histological analysis of gills as an indicator of water pollution in the Tamiš River. Acta Agriculture Serbica, 36: 133-141

Maire R., Pomel S. (1994): Karst geomorphology and environment. International Gilbert Journal, Danielopol & J. Stanford, Groundwater Ecology, Academic Press, London.

Oberdorff Th., Percher J. P. (1994): An index of biotic integrity to assess biological impact of salmonid farm effluents on receiving waters, Aquaculture, 119.

Pajcin, R., Paras, S., Nikolić, S., Jelić, E. (2018): Histological analysis of gills of brown trout (*Salmo trutta morpha fario*) from the river Pliva and trout fish farm. 8th International Conference "Water & Fish" Conference Proceedings. Faculty of Agriculture, Belgrade-Zemun, Serbia June, 13 – 15. 2018. pp. 338-345.

Paraš S., Janković O., Trišić D., Čolović B., Mitrović-Ajtić O., Dekić R., Soldatović I., Živković-Sandić M., Živković S., Jokanović V. (2019): Influence of nanostructured calcium aluminate and calcium silicate on the liver: histological and unbiased stereological analysis, International Endodontic Journal, 52(8), 1162–1172.

Perola A., Floravanti M. L., Gustinelli A., Manfrin A., Dalla P. M., Lunelli F., Accini A., Quaglio F. (2019): Occurrence of nodular gill disease in farmed brown trout (*Salmo trutta* Linnaeus, 1758), Fishery Diseases, 42(9), 1315-1320.

Pulatsu S., Rad F., Köksal G., Aydin F., Benli K. C., Topcu A.(2004): The impact of rainbow trout farm effluents on water quality of Karasu tream, Turkey, Turkey Fisheries and Aquatic Sciences, 4.

Rašković B., Stanković M., Marković Z., Poleksić V. (2011): Histological methods in the assessment of different feed effects on liver and intestine of fish, Journal of Agricultural Sciences, 56(1), 87-100.

Ricker W. E. (1975): Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada 191: 1–382.

Santos M., Marcos R., Santos N., Malhão F., Monteiro R. A. F., Rocha E. (2009): An unbiased stereological study on subpopulations of rat liver macrophages and on their numerical relation with the hepatocytes and stellate cells, Journal of Anatomy, 214(5), 744–751.

Selong J. H., Helfrich A. L. (1998): Impacts of trout culture effluent of water quality and biotic communities in Virginia headwater streams, Progressive Fish Culturist, 60.

Sidoruk, M, Ireneusz C. (2018): Effect of Water Management Technology Used in Trout Culture on Water Quality in Fish Ponds. Water, 10, 9: 1264.

Sun S., Wu Y., Yu H., Su Y., Ren M., Zhu J., Ge X. (2019): Serum biochemistry, liver histology and transcriptome profiling of bighead carp *Aristichthys nobilis* following different dietary protein levels, Fish & Shellfish Immunology, 86, 832-839.

Špelić, I., Rezić, A., Simonović, P., Tošić, A., Maguire, I., Piria M. (2018): Morphometric and meristic characteristics of brown trout (*Salmo trutta m. fario* Linnaeus, 1758.) populations in north-west Croatia.VIII international conference Water & Fish, 13-15 June 2018, Belgrade, Serbia. Conference Proceedings, 59-61.

Tayfun K., Sukru O., Serkan Y. (2021): Effects of prolonged fasting on levels of metabolites, oxidative stress, immune-related gene expression, histopathology and DNA damage in liver and muscle tissues of rainbow trout (*Oncorhymchus mykiss*), Fish Physiology and Biochemistry,47.

Trenzado C. E., Carmona R., Merino R., Gallego-Garcia M., Furne M., Domezain A., Sanz A. (2018): Effect of dietary lipid content and stocking density on digestive enzymes profile and intestinal histology of rainbow trout (*Oncorhynchus mykiss*), Aquaculture, 497, 10-16.

Velmurugan B., Selvanayagam M., Cengiz E. I., Unlu E. (2007): Histopathology of lambda-cyhalothrin on tissue (gill, kidney, liver and intestine) of *Cirrhinus mrigala*, Environmental Toxicology and Pharmacology, 24(3), 286-291.

Vuković, T., Ivanović, B. (1971): Slatkovodne ribe Jugoslavije. Sarajevo: Zemaljski muzej BiH.

Xu H., Dong X., Zuo R., Mai K., Ai Q. (2016): Response of juvenile Japanese seabass (*Lateolabrax japonicus*) to different dietary fatty acid profiles: Growth performance, tissue lipid accumulation, liver histology and flesh texture, Aquaculture, 461(1), 40-47.

