Albanian j. agric. sci. 2017; (Special Edition) *Agricultural University of Tirana*

RESEARCH ARTICLE **(Open Access)**

Pathological changes in liver morphology of Crucian carp (*Carassius carassius*) from Seferani Lake in Dumrea region

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Abstract

The present study was conducted to determine the effect of stress in liver of *Carassius carassius*, a freshwater fish that normally dwell in the bottom layer of the waters of lakes, rivers and reservoirs. It is a sedentary fish that naturally feeds on zoo benthos and plant components and thanks to its sensitivity to the changes in surrounding medium is an ideal animal for indication of the health of freshwater aquatic ecosystems. It is susceptible for the teleost fish liver to be disturbed by numerous, stressful factors that influence in their health. Liver pieces of 30 individuals collected from Seferani Lake, in Dumrea region (Elbasan, Albania), were excised and processed for standard histopathological analysis. The result revealed pathological changes in liver tissue including heterogeneity of tissue parenchyma, irregular hepatocyte cells and their nuclei. Massive vacuolization of liver cells and their nuclei, necrotic foci, karyolysis and karyopicnosis were also observed. Our findings imply that histopathological evaluation can be used effectively as biomarker of fish physiological stress response and health status.

Key words: histopathology, liver, necrosis, physiological stress response, fish health.

1. Introduction

The environment is continuously loaded with foreign organic chemicals released by urban communities and industries [19]. Agricultural, industrial and domestic effluents generally contain a wide variety of organic and inorganic pollutants, such as solvents, oils, heavy metals, pesticides, fertilizers and suspended solids [13] invariably, are discharged into lakes, rivers and streams, without proper treatment. Nowadays more frequently many thousands of organic trace pollutants, such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzofurans (PCDFs) and dibenzop-dioxins (PCDDs) have been produced and, in part, released into the environment becoming potential risks for aquatic ecosystems [19]. Such contaminants change water quality and may cause many problems to fish, such as diseases and structural alterations [3]. Fish liver appears as does the liver of other vertebrates as a key organ which controls many life function and plays a vital role in fish physiology, both in anabolism (proteins, lipids and carbohydrates) and catabolism (nitrogen, glycogenolysis detoxification) and acts as storage center for many substances, mainly glycogen [20, 1]. Fish liver is an important model for the study of interaction between environmental factors and hepatic structures and functions [22, 10]. Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to stress factors [24]. One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs, especially liver, that is responsible for vital functions, such as excretion, accumulation and biotransformation of xenobiotics in the fish [5]. Furthermore, the alterations found serve as warning signs of damage to animal health [7]. Histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism. For example, the mechanism of action of several xenobiotics could initiate the formation of a specific enzyme that causes changes in metabolism, further leading to cellular intoxication and death, at a cellular level, whereas this manifests as necrosis, i. e. histopathological biomarker on a tissue level. As well as from chemical insult, histopathological lesions may arise from infectious diseases and parasites, provoking necrotic and degenerative alterations to which the organism responds with an inflammatory, defensive reaction [23, 17]. The Dumrea region is a very important area from the biological and economical point of view. Belshi, Merhoja and Seferani Lakes are the most important between 80 carstic lakes which are situated in this plateau. The number of inhabitants in the surrounding areas of these lakes has increased in the last 20 years. These processes are followed by damages caused to the vegetation around the lakes, the discharge of many different kinds of organic and inorganic pollutants into the lakes including some organic fish food [9]. The aim of the present investigations was to perform a histological analysis in liver tissue of *Carassius carassius*, exposed in different stress factors.

2. Material and Methods

2.1. Sample collection**:** Thirty live adult fish, *Carassius carassius* (total body length 15-22 cm) were collected from Seferani Lake at Dumrea region, Belsh. Fishes were used without sexual distinctions, after their identification, the body cavity were opened through a midventral incision and the liver was immediately fixed in formaldehyde solution.

2.2 Light Microscopy**:** After fixation, samples of liver were dehydrated in an ethanol series, cleared in xylene and embedded in paraffin wax and sectioned at 5µm. After dewaxing with xylene and hydration in ethanol series of descending concentration, sections were stained for general histological purposes with haematoxylin and eosin stain. At the end the evaluation of quality and amount of hepatic tissue is done for every sample such as transparency of the tissue,the vitality of the cells and the ratio of nucleus and citoplasmatic diameters.

3. Results and Discussion

Histopathological evaluation

Table 1 contain data of the characteristics of cells in liver tissues of fishes used in the experiment. Characteristics such as tissue density, transparency, cell vitality, nucleoplasmic ratio etc.

Tissue Transparency (T.T) and Cell Membrane (C.M) are expressed with number from 0-3 and 0-1 respectively:

0 –solid tissue	0-continued membrane
1-easily transparent	1-interrupted membrane
2-middle transparent	
3-very transparent	

Using the data collected we built the dependency of tissue transparency, percentage of vital cells as well as nucleoplasmic ratio to tissue density.

Observing these dependencies we come to the conclusion that when the density increases the transparency of the tissue is higher, which means the damage is bigger (Fig. 1). Whereas the dependency of vital cells to the density becomes smaller (Fig. 2).

The ratio created between Nucleoplasmic Ratio and Density shows us that, when the density decreases the ratio becomes ½ which means citoplasm is the double of the nucleus (Fig 3).

By microscopic observations we notice other characteristics which tell us about the damage of tissue liver. These damages include heterogenous parenchyma of tissues and vacuolisation is also noticed (Fig. 4), non-regular forms of hepatocytes and their nucleus, as well as massive necrotic ingles (Fig. 5), nuclear degeneration karyolysis and karyopyknosis is also observed (Fig. 6).

Table 1. L.W liver weight, L.V liver volume, T.D tissue density, T.T tissue transparency, C.M cell membrane V.C vital cells, N.D nucleus diameter, C.D cytoplasm diameter, NP.R nucleoplasmic ratio

Fish Nr.	*L.W g*	*L.V ml*	*T.D g/cm^3*	*T.T*	*C.M*	*V.C %*	*N.D µm*	*C.D µm*	*NP.R*
1	2	4	0.5	1	0-1	55	45.3	162.4	0.28
2	3	5.4	0.6	0	0	95	77.4	156.8	0.49
3	4	3.3	1.2	3	1	25	32.6	196.5	0.17
4	1.7	3.5	0.5	1	0-1	65	65.7	136.4	0.48
5	3	6	0.5	1	0	80	64.5	146.7	0.44
6	2.4	7.8	0.3	0	0	98	59.7	184.6	0.32
7	3.3	4.8	0.7	2	1	35	38.5	176.3	0.22
8	3	3	1.0	3	1	30	34.3	188.4	0.18
9	5.2	7.5	0.7	2	0-1	60	40.4	145.8	0.28
10	3.4	4.6	0.7	2	1	40	36.9	188.4	0.20
11	4	4.5	0.9	3	1	20	32.3	193.6	0.17
12	2	4.7	0.4	1	0	85	76.5	187.8	0.41
13	3	7	0.4	0	0	90	65.8	124.8	0.53
14	4.1	5	0.8	3	1	35	34.7	175.8	0.20
15	2.6	6.3	0.4	1	0	75	67.8	127.5	0.53
16	4.4	8	0.6	2	0	55	58.4	164.8	0.35
17	3.6	5.6	0.6	1	0-1	35	42.4	128.5	0.33
18	4	3.7	1.1	3	1	30	39.5	183.7	0.22
19	3	5.4	0.6	1	0-1	45	56.9	137.9	0.41
20	2.5	4.8	0.5	2	0	50	64.8	153.2	0.42
21	1.8	3.6	0.5	2	0-1	45	44.5	173.9	0.26
22	4.2	7	0.6	1	1	35	37.6	173.5	0.22
23	3	6.2	0.5	2	0	65	53.5	158.3	0.34
24	4	7	0.6	2	1	50	54.3	185.3	0.29
25	5	6.7	0.7	3	1	35	47.2	191.7	0.25
26	3.6	5.8	0.6	1	0-1	80	68.4	136.5	0.50
27	2.8	4	0.7	2	1	45	45.9	168.3	0.27
28	2.4	3.6	0.7	2	1	50	40.5	143.6	0.28
29	3	6	0.5	0	0-1	65	56.4	137.5	0.41
30	3.1	5.9	0.5	1	0	80	72.5	187.1	0.39

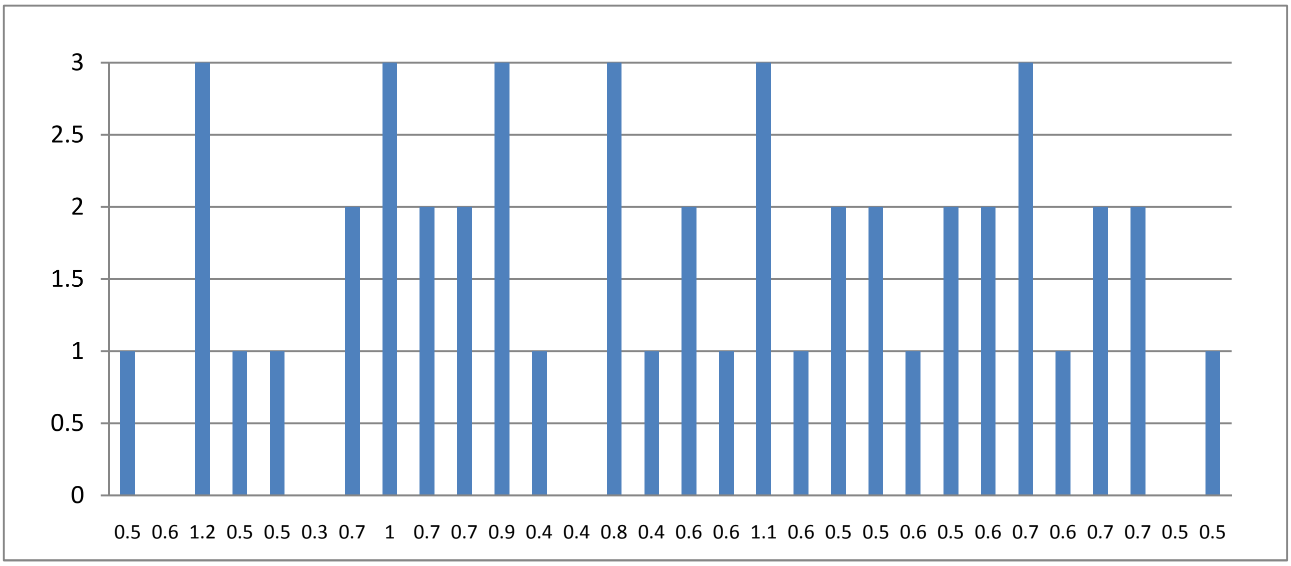

Figure 1. Dependency between tissue transparency T.T and tissue density T.D

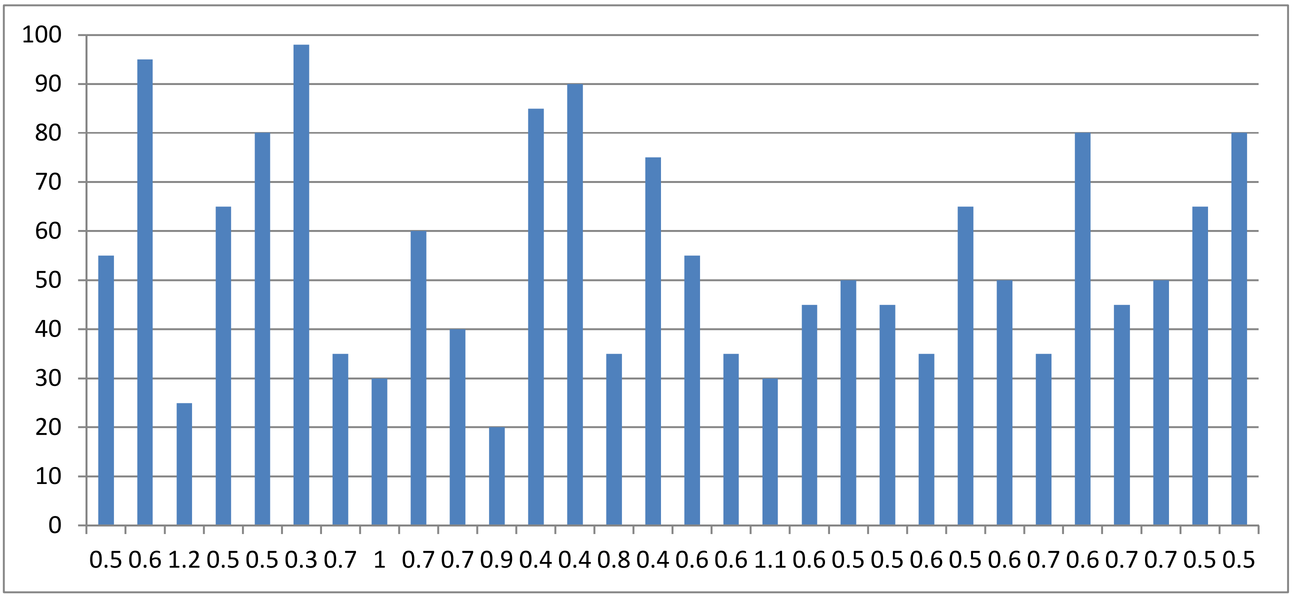

Figure 2. Dependency between vital cells V.C and tissue density T.D

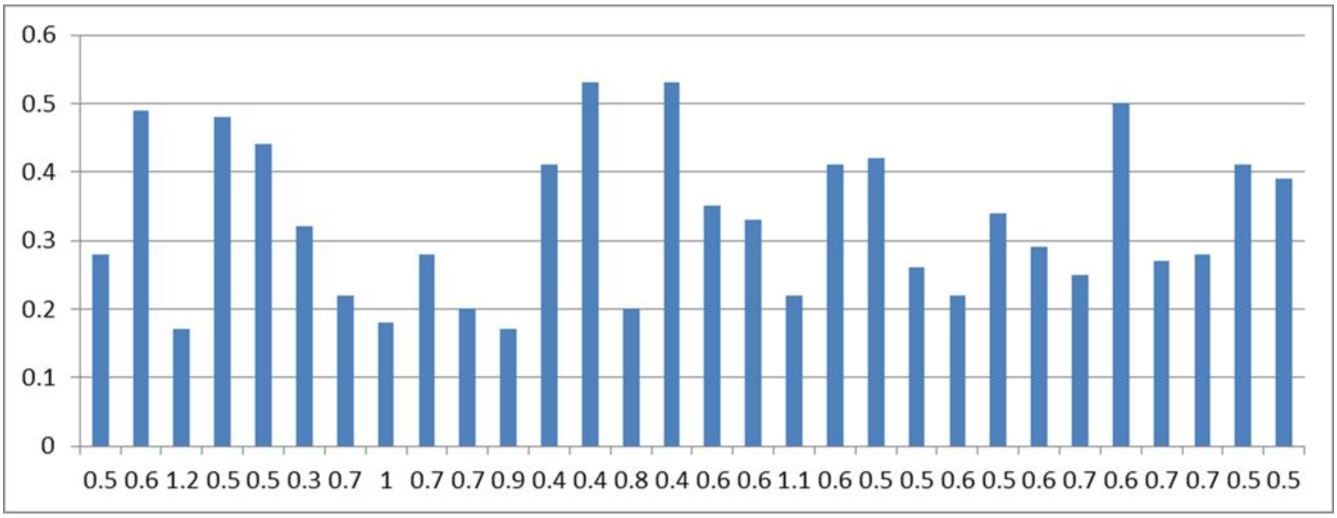

Figure 3. Dependency between nucleoplasmic ratio NP.R and tissue density T.D

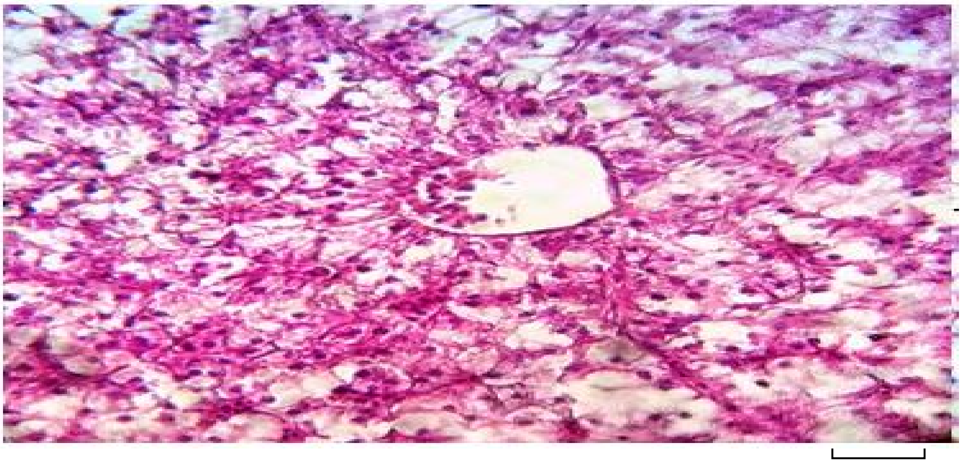

Figure 4. View of hepatic tissue that shows heterogenic parenchyma and cytoplasmic vacuolisation

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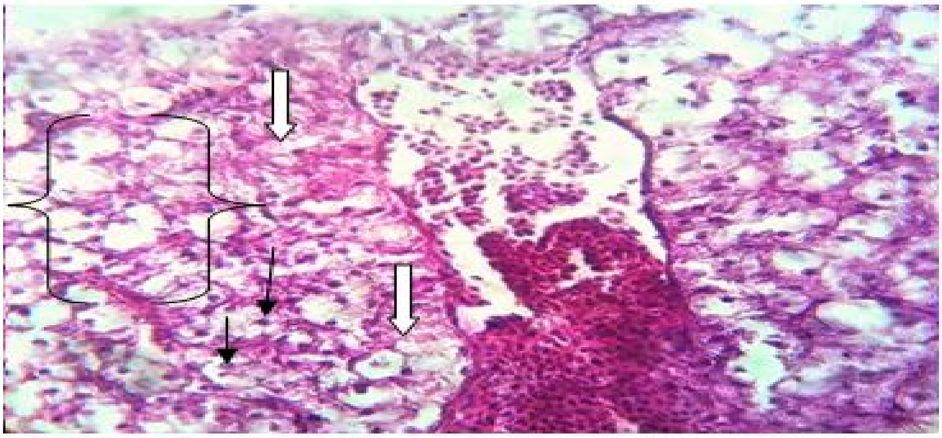

Figure 5. Cellular and nuclear polymorphism. White arrows show a change of the shape of cells. Black arrows show a reduction of the nuclei. Brackets show cellular necrotic ingles.

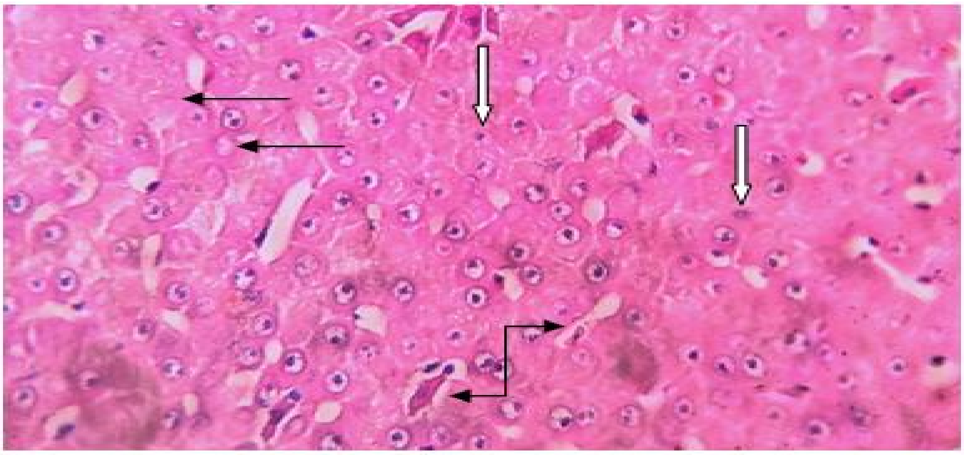

Figure 6. Nuclear degeneration and sinusoidal capillaries. Arrows show nuclear degeneration, black arrows show karyolysis, white arrows show karyopyknosis, elbow double-arrow show sinusoidal capillaries.

The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, position and blood supply [21] it is also one of the organs most affected by contaminants in the water [16]. Anomalies such as irregular shaped hepatocytes, cytoplasmic vacuolation and nucleus in a lateral position, close to the cell membrane, were also described [4]. Vacuoles in the cytoplasm of the hepatocytes can contain lipids and glycogen, which are related to the normal metabolic function of the liver. Depletion of the glycogen in the hepatocytes is usually found in stressed animals [7, 25], because the glycogen acts as a reserve of glucose to supply the higher energetic demand occurring in such situations [14]. [12] described increased vacuolisation of the hepatocytes as a signal of degenerative process that suggests metabolic damage, possibly related to exposure to contaminated water. Change in the diameters of the nuclei of hepatocytes can be considered a response to a stressor, and is indicative of activation of liver function as a result of increased metabolic activity against adverse conditions [8, 18, 2]. On the other hand, a decrease in the size of the nuclei can be related to the possible multiplication of hepatocytes during the replacement of damaged cells [6]. This type of change is indicative of the cell degeneration process, since it starts with the condensation and reduction of the diameter of nuclei (pyknosis), suggesting an early apoptosis event [11, 15]. An increased number of sinusoids can be signs of stress in fish, which may be caused by the presence of chemical agents [12, 26].

4. Conclusions

The histological changes observed in the liver of the fish, *Carassius carassius* in the present study indicate that the fish were responding to the direct effects of the stress factors. The analysis variation in the histological parameters confirms that histopathological alterations are good biomarkers for field assessment, in particular in urban lake areas that are naturally subject to a multiplicity of environmental variations. It must be emphasized that histopathology is able to evaluate the early effects and the responses to acute exposure to chemical stressors. In conclusion the present study showed that histopathology is a useful biomarker for environmental contamination. The liver showed to be the organ most affected by the type of stressors to which the fish were subjected. The teleost fish species, C. carassius, was shown to be appropriate for in situ tests and environmental monitoring.

5. References

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