



Assessment of Ecosystem Pressure and Impact on Fish Population in the Channel Studencista (Lake Ohrid) trough Haematological and Histopathological Biomarkers

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Abstract: The Channel Studencista provides the water supply from Biljana's Sources and is closely connected with the Studencista Wetland and Lake Ohrid. This channel is an important winter habitat for the endemic *Alburnus scoranza* Heckel & Kner, 1858 (Cyprinidae). In the last decade, the channel has been intensely used as a marina, which has caused major changes in the aquatic ecosystem. Similarly, in recent years, when the water level of the lake and the channel was lowered, fish pestilence was often recorded. We present data on histological and haematological studies of population of *A. scoranza* from the Channel Studencista. Our results suggest good health condition of fish population. The oxygen saturation of the water is a crucial factor for fish health during the winter 2017 and 2018.

Key words: Channel Studencista, Lake Ohrid, histology, haematology, *Alburnus scoranza*.

Introduction

The Channel Studencista is a part of the wetland system Studencista located near the city of Ohrid. This channel is a small tributary of Lake Ohrid, which is filled with water from natural springs (Biljana's Sources) coming from the karstic mountain Galicica. This tributary is a natural refuge for numerous cyprinid fishes during winter. In addition, it is used as a marina for numerous motor boats and is exposed to constant anthropogenic pressure.

Physiological and pathological changes in fish can be easily monitored by evaluating the haematological characteristics of their blood. Haematological studies contribute to understanding the effects of the habitat quality on the blood characteristics and the adaptability of the species to environmental factors (FAZIO et al. 2013). The oxygen-binding capac-

ity of the blood is dependent on the concentration and properties of the haemoglobin in the red blood cells. Haemoglobins are particularly important for the fish adaptation as they constitute an interface between the organism and the environment (DE SOUZA & BONILLA-RODRIGUEZ 2007).

Various human activities emit pollutants into the environment and their magnitude makes the assessment of the environmental impact a subject of an utmost interest. The study of organisms as bioindicators of pollutants has several advantages over the chemical analyses of abiotic parameters (FERNANDEZ et al. 2007). Organisms can only accumulate the biologically available forms of pollutants that are always present in the environment, thus enabling continuous monitoring of pollutants (RAJESHKUMAR et al. 2015). Fishes are used as sentinel species for the biomonitoring of land-based pollution because they

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may accumulate organic compounds in their tissues directly from water, sediment and/or through their diet. An increasing amount of research is now incorporating histopathological biomarkers in practical ecological risk assessment methodologies (WESTER et al. 2002). Histopathological changes are often a result of the integration of a large number of interacting physiological processes (VAN DER OOST et al. 2003).

In winter, during periods with small quantity of rainfall, a rapid reduction of the water quantity has been recorded in the Channel Studencista. In some cases, it coincides with fish pestilence, probably as a result of the intense eutrophication processes and hypoxic conditions in the aquatic environment. The aim of the present study was to perform a histological and haematological analysis on the endemic fish species *Alburnus scoranza* Heckel & Kner, 1858 (Cyprinidae) from the Channel Studencista in order to estimate the ecological pressure on the fish population and outline the reasons for the negative impact of the environmental conditions on this species.

Materials and Methods

A total of 28 specimens of *A. scoranza* were caught in the Channel Studencista. The sampling of the fishes was conducted in February 2017 and November 2018, when an overpopulation and fish pestilence of *A. scoranza* occurred in the tributary. Our analyses were conducted on alive specimens. The fish were caught by hand net and anaesthetised and prepared for further analyses. Firstly, the total length and weight of all specimens were measured using a calliper (to the nearest 1 mm) and a digital scale (to the nearest 0.1 g), respectively. Using the values for the total length and weight the Fulton condition factor was calculated (RICKER 1975). Blood was collected after severance of the caudal peduncle (HESSER 1960) and then stored in tubes with EDTA. The haemoglobin concentration in the blood was determined using the cyanmethaemoglobin method (BLAXHALL & DAISLEY 1973).

A piece of the liver and the gill of each specimen of *A. scoranza* were dissected for histological analysis. Samples were quickly removed from the fishes and fixed in 4% neutral buffered formaldehyde solution (pH=7.0). The tissues were dehydrated through a graded alcohol series and embedded in paraffin wax. Tissues sections were stained with haematoxylin and eosin. Histological preparations were explored under a microscope and the registered tissue lesions were photographed.

Results

The sampled specimens of *A. scoranza* had an average (avg. \pm SD) length and weight of 115.77 ± 3.85 mm and 10.56 ± 1.61 g, respectively. The Fulton condition factor was 0.68 ± 0.06 . The analyses of the haemoglobin blood concentration resulted in an average value of 11.01 ± 1.46 g/dl.

We recorded relatively normal histological findings in the liver and gill tissues. The tissue of some gills displayed presence of progressive changes such as hypertrophy of the lamellar epithelium and lamellar disorganisation, while circulatory and inflammatory changes in the hepatocellular parenchyma of the liver of some specimens were detected with low prevalence. Only 3.5% of all studied fishes from the Channel Studencista showed evidence of steatosis and, in 14%, we detected hypertrophy of the lamellar epithelium as well as lamellar disorganisation.

Discussion

The Fulton condition factor of the sampled specimens had low values compared to previous data. TALEVSKI & MILOSEVIC (2016) recorded average values of 0.98 for *A. scoranza* in Lake Ohrid, which is much higher than the values obtained by the present study. This means that the specimens studied by us were in poor condition at the time of the sampling. As the condition factor is a measure of how the mass of the fish is distributed per unit volume (ONI et al. 1983), the loss of weight is the likely cause of this poor condition of fish. This loss of weight could be the result of the decreased food availability due to the crowding and overpopulation, which by itself could lead to starvation. In addition, in winter, fishes feed with lower intensity or do not feed at all. Consequently, this would result in weight loss and lower values of the condition factor. As for the blood haemoglobin concentration, compared to published records for *A. scoranza* in Lake Shkodra (GOLEMI et al. 2013) and our unpublished data, the values presented here are slightly higher. Elevated haemoglobin concentration occurs in fish subjected to stress related with high-density populations (MONTERO et al. 1999, TRENZADO et al. 2009, WITESKA et al. 2015). This represents an adaptive mechanism for increasing the oxygen-carrying capacity of the blood in situation of constant stress and decreased oxygen availability in the environment.

The presence of steatosis in certain samples could be common during the spawning period. The high fat and glycogen contents in hepatocytes



Fig. 1. Steatosis in the hepatocellular parenchyma (H&E, x 100).

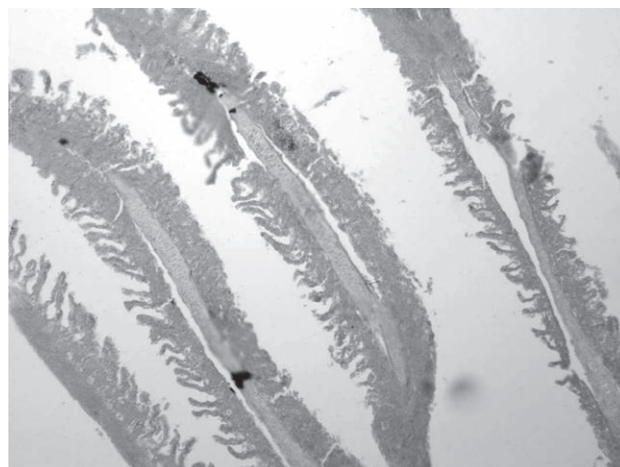


Fig. 2. Lamellar disorganisation, fusion of some lamellae and hypertrophy of the lamellar epithelium (H&E, x 100).

is a common and normal finding in fishes such as *Abramis brama* (L.) and *Carassius auratus gibelio* (Bloch) in the course of the spawning period and is considered as a result of the normal hepatic metabolic activity (TALIKINA 1985). Compared to mammals, fish have greater quantity of vacuolated hepatocytes due to high glycogen or lipid contents and an uniform vacuolisation in these cells is usual (SHIMADA et al. 2014). Although histopathology has been used to evaluate the direct and indirect effects of toxic agents, the structural changes are not specific. Steatosis is common in fish exposed to toxic agents (SAELE et al. 2013) such as polyaromatic hydrocarbons, heavy metals (CASTRO et al. 2014) and sodium perchlorate (BURCU et al. 2009).

Several events of fish pestilence have been recorded during drought periods in the Trebenista Reservoir, which belongs to Lake Ohrid watershed. The analysis of the histological preparations from liver tissues of barbell *Barbus meridionalis petenyi* Heckel (syn. *Barbus peloponnesius* Valenciennes in Cuvier & Valenciennes, 1842) from this locality indicated the presence of haemorrhaging in hepatocellular parenchyma. Moreover, the pigment haemosiderin was recorded, probably as a result of the internal bleeding in the liver tissue of barbells. Apart from these changes, there was also presence of parasites, most probably nematodes (VELKOVA-JORDANOSKA & KOSTOSKI 2005).

Lake Ohrid is an oligotrophic water body but certain localities within the littoral zone display loading with contaminants from the ground, especially in regions where rivers inflow into the lake. Histopathological analyses on liver from barbells caged in these localities evidenced some structural

lesion. Microscope analysis of histological preparation of barbell liver tissue displayed the presence of cholangiofibrosis in the hepatic bile duct with high prevalence in such localities (VELKOVA-JORDANOSKA 2002). At the level of the hepatocellular parenchyma, histopathological analysis revealed granulomatous inflammation and presence of eggs of the nematode parasite *Capillaria* sp., which has been the cause of this type of lesions (SIMA et al. 1996, ROGANOVIC-ZAFIROVA et al. 2003). Light microscopy examination of liver tissue of roach *Rutilus rubilio ohridanus* Karaman, 1924 revealed presence of inflammatory cells infiltrating in the liver parenchyma and accumulating very frequently around the bile ducts and the blood vessels. In addition, the obtained results revealed bile duct proliferation and bile duct epithelium necrosis (VELKOVA-JORDANOSKA, 2005). In liver tissue of *A. scoranza* from the Channel Studencista, parasites and histopathological changes such as granulomatous inflammation and cholangiofibrosis were not found.

The gills participate in many important functions in fishes such as respiration, osmoregulation and excretion. They remain in close contact with the external environment and are sensitive to changes in the quality of the water; thus, they are considered as a primary target of contaminants (POLEKSIC & MITROVIC-TUTUNDZIC 1994). Alterations like hypertrophy and hyperplasia of epithelial cells, besides partial fusion of some secondary lamellae, are examples of defence mechanisms since, in general, these result in the increase of the distance between the external environment and the blood and, serving as a barrier to the entrance of contaminants (MALLAT 1985).

Conclusions

The results presented in this study clearly show the stressful effects of crowding and high-density population in confined areas (e.g., small tributaries) on the fish health. The related reduced food and oxygen availability have impact on the physiological status of the fish, which is evident in the values of the examined parameters. Histopathological and haematological biomarkers can be indicators for the effects of various anthropogenic pollutants on organisms and represent reflection of the overall health of the entire population in the ecosystem.

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