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# ECOLOGICAL STATUS OF SERBIAN RIVERS BASED ON AN ICHTHYOLOGICAL ASSESSMENT

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## ABSTRACT
Anthropogenic influences on fish populations in river ecosystems can be studied at the individual level, as well as through the assessment of fish fauna composition. This study presents research that was focused at the individual level, such as the analysis of genotoxic effects (Comet assay), assessment of the heavy metal accumulation in fish liver, gills, intestine and muscle, histopathological analysis of liver, gills and skin, presence of parasites and the level of parasite infestation, hepatosomatic index and the condition factor. At the level of fish fauna composition, monitoring of the changes caused by dam construction was conducted through the use of modified index of biotic integrity (IBI). The aim of this work was to analyze all above listed parameters as potential bioindicators that can be utilized to assess the extent of disturbance of river systems in Serbia by anthropogenic impacts.
**Keywords:** Comet assay, histopathology, heavy metal, condition factor, fish fauna composition

## INTRODUCTION

Society gains immeasurable benefits from rivers, but at the same time it changed rivers dramatically through irrigation, power utilities, drinking-water utilities, recreational fisheries and anglers (Karr 1999). Beside the physical changes, industrial and communal waste waters and the agricultural surface runoff waters also have a significant negative impact on the state of rivers. As a result, there is nowadays a growing need to preserve and restore the physical, chemical and biological integrity of rivers (Tafangenyasha and Dube, 2008). Industrial waste waters (partly treated or untreated) are commonly discharged into sewage systems, or directly into receiving waters (Teodorovic, 2009). According to recent estimates (SWRDMP, 2002) less than 10% of all waste waters generated in Serbia are treated at all, which means that they still present an actual hazard to aquatic environment. Beside the water pollution, state of rivers in Serbia is also influenced by the dam construction, river regulation and the gravel exploitation.

Ecotoxicological research, which should have been seen as a helping hand for competent authorities, has been ignored in Serbia for years and therefore remained isolated and restricted to a small scale laboratory and field monitoring (Teodorovic, 2009). Status of rivers can be investigated through the analysis of microbiological, hydrobiological and biochemical parameters (Matavuly et al., 2007), as well as through the phytoplankton composition assessment (Nemes et al., 2007). Furthermore, the Water Framework Directive (WFD) states that fish represent one of the main Biological Quality Elements to be used for the river ecological status assessment (Berge and Dahl-Hansen, 2005). This was the reason to initiate toxicological research on fish in Serbian waters, and to evaluate their importance as indicators of river health.

This paper summarizes investigation on fish from Serbian rivers and explains possibility of their use as indicators of river status.

## MATERIAL AND METHODS

Majority of the work presented in this paper was based on the peer-reviewed articles, published in international journals (Lenhardt et al., 2009*a*; Lenhardt et al., 2009*b*; Poleksic et al., 2010; Visnjic-Jeftic et al., 2010). Methods applied in these studies have been thoroughly presented in mentioned articles.

Comet assay was performed on erythrocytes of sterlet (*Acipenser ruthenus* L.) and barbel (*Barbus barbus* L.), caught in the Danube River near Belgrade. Images of randomly selected cells/fish were analyzed with fluorescence microscope Leica and image analysis software (Comet Assay IV Image Analysis system, PI, UK). Fifty nuclei were analyzed per experimental point, and the tail moment was scored as a reflection of DNA damage.

## RESULTS

The negative impact of different pollutants and physical changes in water ecosystem on fish can be monitored on a number of different levels of organization. While it is possible to investigate the impact of pollution from the molecular level to the level of the whole organism, physical changes have more pronounced impact on community composition (Figure 1.)

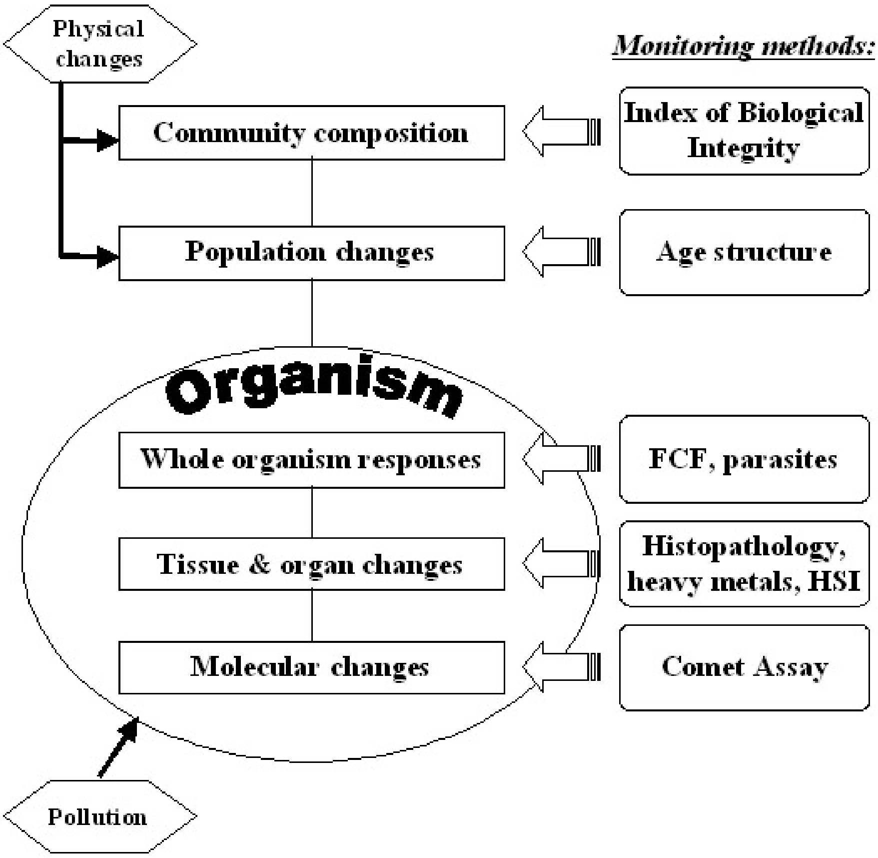


Fig. 1 Different levels of organization and related monitoring methods, used for the assessment of river ecological status.

***Genotoxic activity***

A number of techniques for detecting DNA damage have been developed to identify substances with genotoxic activity, as opposed to the biological effects (e.g. micronuclei, mutations, structural chromosome aberrations) that result from the DNA damage,. The most useful approach for assessing DNA damage is the single-cell gel (SCG) or the Comet assay. Investigation on barbel and sterlet from the Serbian part of the Danube River revealed more pronounced genotoxic effects in barbel than in sterlet, even though both species are bottom feeding fish (Figure 2).

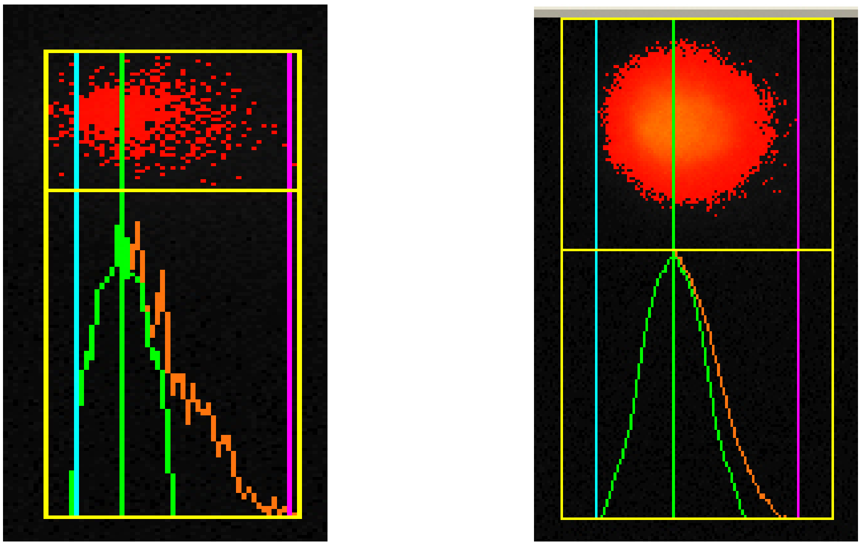

Fig. 2 The tail moment was higher in barbel (left, 15.5) than in sterlet (right, 3.28)


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*Histopathological changes*

Investigation of histopathological changes in sterlet included three organs: liver, gills and skin. Dominant change on the gills, found in 92.5% analyzed specimens, were distended tips of the secondary lamellae The second most frequently encountered change, found on 47.5% of the examined gills, was hypertrophy of respiratory epithelial cells (Figure 3). This is also a mild and reparable change, regularly found in presence of increased heavy metal concentrations in aquatic environment.

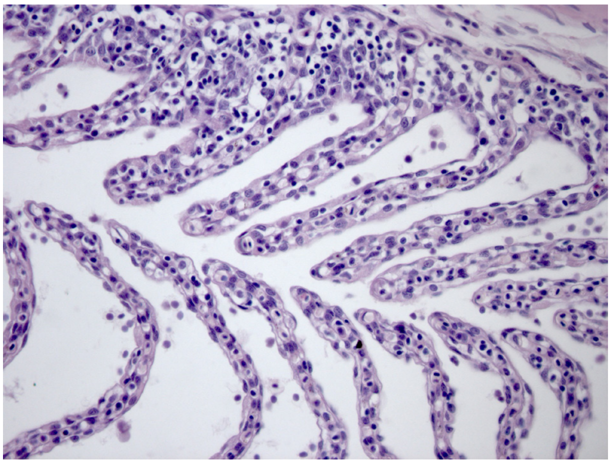

Figure 3. Hypertrophy of respiratory epithelial cells

Other changes, found on more than 10% of the examined gills, were focal hyperplasia and fusion of several primary lamellae (Figure 4.). Focal hyperplasia is most commonly result of a local, most often mechanical irritation, either from suspended material or from parasites.

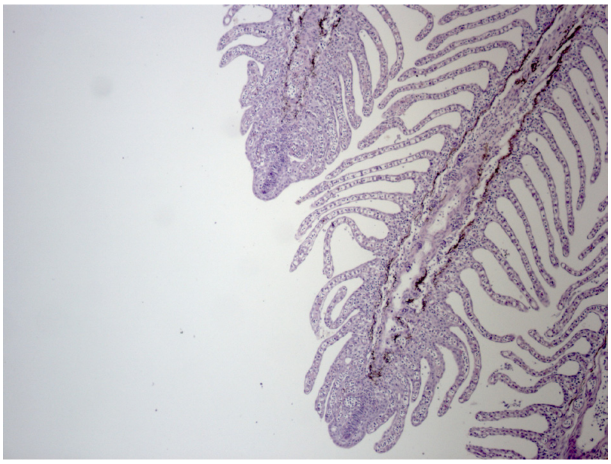

Figure 4. Fusion of the tips of primary lamellae

The most frequently found change on sterlet liver samples was fibrosis (Figure 5). This increase of connective tissue follows or is related to lymphocytic inflammation, manifested by infiltration of leucocytes and vascular congestion.

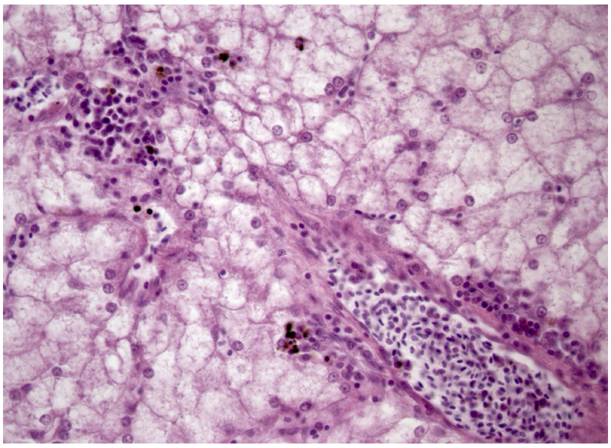

Figure 5. Fibrosis, leucocytes infiltration, stasis in blood vessels and vacuolization.

Melano-macrophage centers appear when fish store lipids in the liver, but they can also occur in toxic conditions or in case of vitamin deficiency. Other, more severe changes noted were signs of cirrhosis (Figure 6) and, finally, necroses (Figure 7), which represent terminal alterations leading to a cell death, and were noted on 46.4 % of livers of sterlet from Serbia.

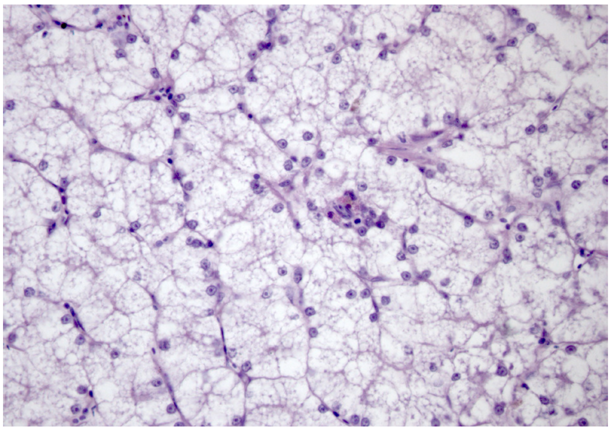

Figure 6. Fatty degeneration

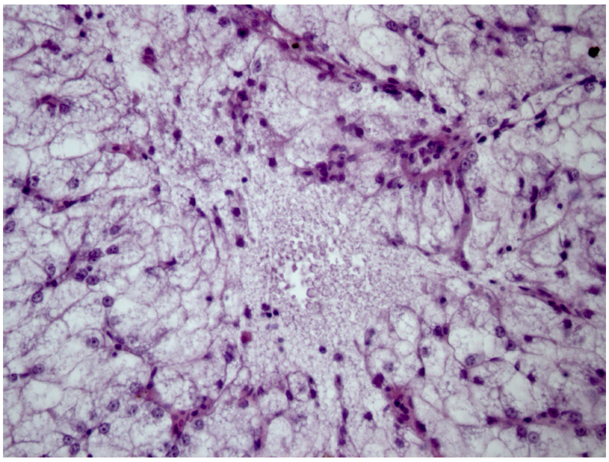

Figure 7. Necrosis


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Examined samples of sterlet skin have revealed changes only at the level of the epidermis. No major changes in the dermis and hypodermis were found. One common feature of the majority of samples was erosion and desquamation of epithelium and rupture of parts of epidermis (Figure 8).

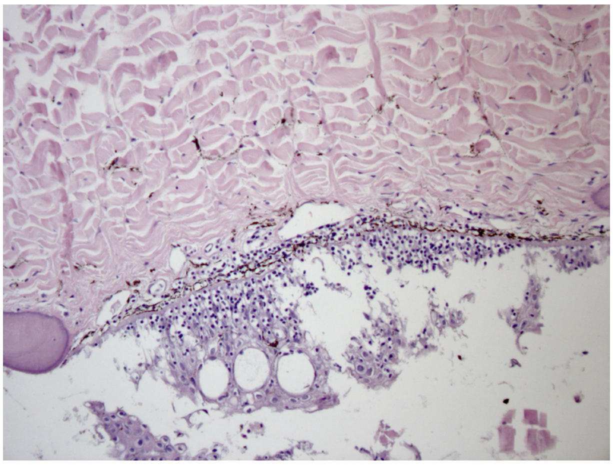

Figure 8. Skin epidermis erosion and excoriation, leucocyte infiltration.

Major changes found in analyzed skin samples were hyperplasia of the epidermal cells and hyperplasia of mucous cells (Figure 9).

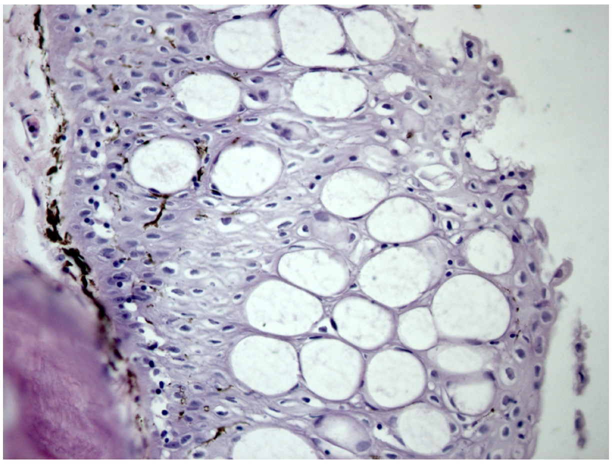

Figure 9. Mucous cells hyperplasia, nuclear picnosis.

***Heavy metal concentration in fish tissues***

Analyses of heavy metal accumulation in Pontic shad (*Alosa immaculata* Bennet) and sterlet (Poleksic et al., 2010; Visnjic-Jeftic et al., 2010) have revealed significant differences of heavy metal concentrations among different tissues. In general, liver was the centre of accumulation of the majority of analyzed heavy metals and microelements, with maximum concentrations of Cd, Cu, Zn, Fe, Ni, B and Pb. Gills had the maximum concentrations of Al, Sr, Ba, Mg, Mn and Li, while the muscle had the lowest concentrations of most of the analyzed heavy metals, except for As in Pontic shad. Sex dependent differences of heavy metal and trace element concentrations in analyzed tissues were also observed. Detected heavy metal concentrations can be probably attributed to a response to presence of these pollutants in the environment. Accumulation of heavy metals in Pontic shad muscle tissue above maximum acceptable concentrations (Cd and As) may be considered as an important warning signal for both fish health and human consumption (Visnjic-Jeftic et al., 2010).

***Hepatosomatic index (HSI), Fulton condition factor (FCF) and parasitism***

HSI, which is associated with liver energetic reserves, had a maximum value in investigated sterlet in Serbia during October and November. The minimum values were recorded in March, and statistical analysis found significant differences in HSI among different months throughout the year.

Although the condition factor should be interpreted with caution, body condition is very commonly used in fisheries biology because it is inexpensive and non-lethal alternative to a common tissue analysis. FCF showed significant changes in sterlet during the year, with the lowest values recorded in December and March.

In the search for indicators to monitor the impact of pollutants, there are good reasons for focusing on parasites, especially due to their complex life history. However, as in the case of HSI and FCF, prevalence and intensity of parasite infection showed significant changes among different months throughout the year. This fact must be kept in mind when using these parameters as biomarkers, and any biomarker sampling on different locations has to be performed within a short time frame, in order to avoid differences generated by confounding factors.

*Index of Biological Integrity (IBI)*

IBI represents a relatively new approach for measuring river condition by multimetric biological indices. Karr (1981) proposed the index of biotic integrity (IBI), while the European Fish Index (EFI) was developed within the project Fish-based Assessment Method for the Ecological Status of European Rivers (FAME 2005, http://fame.boku.ac.at). Our investigations of reservoirs in Serbia (Lenhardt et al., 2009*a*) were based on the modified IBI metrics with the assignment of fish species based on the data developed for the EFI, as well as on the other resources and our own observations. There was a significant negative correlation between the IBI and the reservoir sediment deposition rate.


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## DISCUSSIONS

The major problem encountered when parameters presented in this study are being applied is the lack of knowledge on reference values of these parameters. This is further complicated by a lack of knowledge on their relationship with the age, maturity and the gender of analyzed specimens. There are also significant variations in studied parameters among different seasons of the year, which are also related to the water temperature and the photoperiod.

The Comet assay has proved to be highly suitable for aquatic genotoxicity monitoring, due to its simplicity and a high sensitivity (Kim and Hyun, 2006). Its combined use with other biomarkers, as well as standardization and inter-laboratory calibration, are recommended to further strengthen its use in environmental assessment studies (Frenzilli et al., 2009). There is a lack of studies that are focused on establishing reference values for heavy metal concentrations in different fish tissues, so there is a present need for additional research that would determine these values in fish tissues (liver, gills and muscle).

Froese (2006) showed the presence of seasonal and reproductive variations in condition with growth stanzas between juveniles and adults, as well as changes in condition with fish size. Therefore, it is important to determine presence of such variations in FCF for each fish species before condition becomes commonly used as a biomarker, or to follow guidelines for data collection and analysis presented by Froese (2006).

In case of the complex anthropogenic influence on aquatic ecosystems, assessment of the extent of human impact can be only conducted through multimetric analyses and the establishment of standard and pathological values for studied parameters for a number of different fish species. This paper presents overview of a number of parameters assessed on fish in Serbia, conducted mostly on sterlet, and additional research on barbel and ichthyofaunistic analyses of accumulations on rivers Zapadna Morava and Drina.

## CONCLUSIONS

Based on a number of studies conducted on fish populations on the territory of Serbia, it can be concluded that presented parameters have a good potential to be utilized as bioindicators of the state of aquatic ecosystems. While the Comet assay can be used for the monitoring of genotoxic effects, histopathological analyses can reveal sublethal changes on vital organs as a result of pollution. Some of the observed pathological changes can be explained by increased heavy metal levels that were registered in some fish organs. Some parameters, such as HSI, FCF and parasitism, will require establishment of reference values for each studied species, in order to be efficiently utilized as bioindicators. Besides being an indicator of pollution levels in aquatic ecosystems, IBI can be utilized as a regular indicator of physical changes in river ecosystems.

## ACKNOWLEDGEMENTS

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