EFFECT OF DIFFERENT ENVIRONMENTAL CONDITIONS ON LIPID PEROXIDATION LEVEL IN *Rutilus rutilus* (ACTINOPTERYGII: CYPRINIDAE)

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Abstract

The present study aimed to assess the lipid peroxidation (LPO) level under the different environmental conditions on the common roach (Rutilus rutilus). Lipid peroxides were measured in fish collected within the Danube River at two points: Veliko Ratno ostrvo (P1), which was considered a reference point, and Višnjica (P2), the point with compromised water quality. A significant difference is revealed between the points suggesting signs of stress in fish from the Višnjica point. Concerning that Višnjica experiences intense environmental pressure, we noticed an increased concentration of lipid peroxides in the fish liver, compared to those individuals from the P1 ($p < 0.05$). A significantly increased level of LPO in fish from P2 can be the indication of the presence of pro-oxidant stressors that through oxidative stress lead to oxidation of cell lipids in fish. These results give a good base for R. rutilus to be a bioindicator of a disturbed environment.

Keywords: Danube River, lipid peroxidation, *Rutilus rutilus*

INTRODUCTION

Fish are an important bioindicator species and play an increasingly important role in the monitoring of water pollution because they respond with great sensitivity to changes in the aquatic environment [1]. The effects of fish exposure to sub-lethal levels of pollutants can be measured in terms of their biochemical, physiological, or histological responses. Environmental contaminants that are accumulated in the tissues of fish may catalyze reactions that generate reactive oxygen species (ROS). When the production and accumulation of ROS are beyond the organism's capacity to deal with these reactive species oxidative stress occurs [2]. This can damage lipids, proteins, and deoxyribonucleic acid (DNA). Some ROS can initiate lipid peroxidation, a self-propagating process in which a peroxyl radical is formed when a ROS has sufficient reactivity to abstract a hydrogen atom from an intact lipid which may generate DNA alterations and peroxidation of membrane lipids initiating the cellular degenerative process [3].

In the aquatic environment, biota can be subjected to a multipollutant state in the presence of a mixture of pollutants. Chemical analyses of pollutants are expensive and it is not feasible to measure all classes of chemicals likely to be found in an aquatic environment given the complex mixture. Biomarkers represent toxicant-induced changes in biological systems and

serve as links between environmental contamination and its effects, providing relevant data about the possible pathological process in fish [4].

The liver is an organ that is widely used in biomarker approach studies on fish health assessment and is associated with detoxification processes, due to its function, position, and blood supply. It is also one of the most affected organs in contaminated waters but also plays an important role in fish physiology. In addition, the parenchymal liver tissue in fish has important physiological functions, such as the detoxification of chemical contaminants [5]. Lipid peroxidation (LPO) is one of the main manifestations of oxidative damage induced by various compounds, including metals hence, it has been used as a biomarker of pollution [6].

This study aimed to explore whether the untreated wastewaters of Belgrade city could induce oxidative stress and damage to aquatic organisms in the Danube River. To evaluate the effects of environmental pressure, levels of malondialdehyde (MDA) in the liver tissue of the *Rutilus rutilus* collected from Veliko Ratno ostrvo and Višnjica points were analyzed.

MATERIALS AND METHODS

Sampling points

Fish samples were caught from two points at the Serbian part of the Danube River in the April of 2021. The first point Veliko Ratno ostrvo (P1, Lat. 44.8289559, Long. 20.4297724), devoid of any industrial facilities that could cause pollution, is considered a reference point. The second point, Višnjica (P2, Lat. 44.8295251, Long. 20.5498088) suffers anthropogenic pressure resulting from the presence of a marina for recreational boats (Figure 1).

Figure 1 *The geographical position of the sampling points on the Danube River: Veliko Ratno ostrvo and Višnjica*

At the time of sampling, environmental parameters, including water temperature, pH, and dissolved oxygen were measured *in situ* at both points (Table 1).

Table 1 *Values of physicochemical parameters at Veliko Ratno ostrvo (P1), and Višnjica (P2) in situ on the day of fish collection*

Physicochemical parameters	P1	P2
Temperature (°C)	9.3	10
pH	8.1	8.3
Dissolved O_2 (mg^{-1})	9.7	9.8

The adult fish (n = 10 per sampling point) were transported to the laboratory in ice-cold containers (0–4 °C) on the same day. The fishes were dissected and the livers were quickly removed and stored at -80 °C until further analysis.

Determination of malondialdehyde (MDA)

Lipid peroxidation was determined as described by Rehncrona *et al*. [7]. Tissue (100 mg from each liver) was minced and homogenized in 10 volumes of 0.05 M TRIS-EDTA buffer (pH 7.4) using a Janke & Kunkel IKA-Werk Ultra-Turrax homogenizer and then sonicated for 15 s at 10 kHz on ice [8]. The lipid peroxides concentration was measured as the absorbance change at 532 nm in the reaction of sample malondialdehyde (MDA) with the thiobarbituric acid as the reagent. Malondialdehyde was quantitated by using $\Sigma = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Statistical analyses

All variables were tested for normal distribution using the Kolmogorov-Smirnov test and for homogeneity of variance among groups using Levene's test. Significant differences were analyzed using one-way ANOVA and post-hoc Tukey honest significant difference (HSD) multiple-comparison test. $p < 0.05$ was considered significant. All the statistics were carried out in SAS 9.1.3 software (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Aquatic systems are the main recipients of almost all anthropogenic discharges. Metals are major pollutants of aquatic ecosystems due to disposal of industrial effluents, or via direct dumping in the river of waste material such as sewage sludge [9]. They are usually toxic at high levels, may accumulate in the aquatic organisms, and trigger a range of oxidative damages [10]. Malondialdehyde is one of the LPO products deriving from an oxidative attack on cell membrane phospholipids and circulating lipids, and its level directly reflects the degree of oxidative damage induced by contaminants [11]. Several studies have already shown enhanced LPO in aquatic organisms exposed to high concentrations of inorganic contaminants [12–14].

The MDA levels (an index of LPO) in the liver of *Rutilus rutilus* samples from P1 and P2 are shown in Figure 2. MDA was significantly higher in the fish liver (~ 60%) caught at P2 than in fish from the reference point P1. Fish inhabiting the Višnjica region of the Danube River are exposed to a complex of different mixtures of pollutants. A marina for recreational boats sited at the Višnjica location is occasionally subjected to accidental spillage of fuel/oil into the surrounding water. Diesel fuel is considered to be highly toxic due to its high content of polycyclic aromatic hydrocarbons, the most toxic component of petroleum hydrocarbons.

Because of its widespread use in recreational vessels, diesel fuel represents a potentially significant contaminant to aquatic environments [15]. Dramatically increased utilization of Cu-based antifouling coatings on vessel hulls has led to elevated Cu concentrations in those marinas and harbors where substantial numbers of small craft or large vessels are berthed [16]. Most antifouling paints prevent the attachment and growth of aquatic organisms by continually leaching biocides such as Cu into the water [17]. In addition, abiotic factors may influence biomarker responses to pollutants, but in our study physicochemical parameters varied in a narrow range between two sampling points (Table 1).

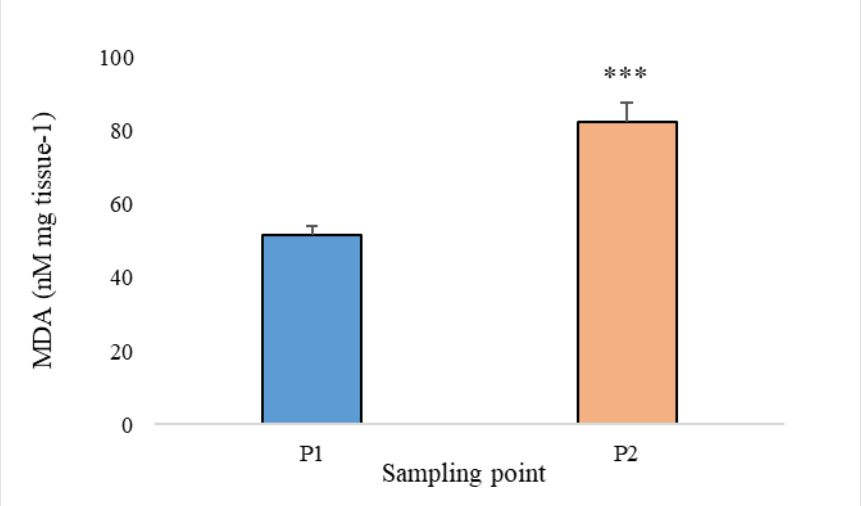

Figure 2 *The levels of malondialdehyde (MDA) in the liver of Rutilus rutilus from the Veliko Ratno ostrvo (P1) and Višnjica (P2). Values are mean + SE of 10 fishes. The significant difference between P1 and P2 were calculated by one-way ANOVA and marked with an asterisk,*** $p < 0.001$*

Our results suggest that the elevated MDA level could be considered a result of oxidative stress from xenobiotics at Višnjica point. Similar results were obtained for the specimen of freshwater fish (Cyprinidae), as demonstrated by other authors [18,19]. These researchers reported that the MDA level was increased in the liver of fish collected from polluted areas. As measurement of MDA content provides a relative measure of the potential for pollutants to cause oxidative injury [20,21], significantly elevated levels of MDA in the liver tissue of *R. rutilus* in response to toxicants indicate that some cell damage might have occurred.

CONCLUSION

The present study confirmed that the aquatic environment at Višnjica point with its current environmental pressure is responsible for oxidative stress in the liver tissue of *Rutilus rutilus*. This state of oxidative stress fish reflects through drastically increase in MDA levels. The findings of the present investigation suggest that the presence of certain prooxidative pollutants that can lead to oxidative stress in the fish at the Višnjica point and oxidative stress biomarkers may be important to evaluate the effects of untreated waste on living organisms in the Danube River.

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