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Assessing pollution in the Danube River near Novi Sad (Serbia) using several biomarkers in sterlet (*Acipenser ruthenus* L.)

Bojana Stanic, Nebojsa Andric, Sonja Zoric, Gordana Grubor-Lajsic, Radmila Kovacevic*

Department of Biology and Ecology, 2 Dositeja Obradovica Square, Faculty of Sciences, University of Novi Sad, 21000 Novi Sad, Serbia and Montenegro

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Abstract

The response of wild fish to pollutants was studied in sterlet (*Acipenser ruthenus* L.) collected in 2001 and 2002 at two sampling sites in the Danube River near Novi Sad (Serbia): in the vicinity of the oil refinery and at the Danube–Begec, remote from the oil refinery and considered a reference site. The following biomarkers were measured in sterlet collected from these two sites: the activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), and glutathione *S*-transferase and the induction of CYP1A1 in liver and the activities of aspartate aminotransferase, alanine aminotransferase, and γ-glutamyl transferase in serum. The results demonstrated increase in the activity of SOD and GSH-Px in sterlet collected from the Danube-oil refinery (DOR) compared to that from the reference site, while no differences were found in other enzymes. In conclusion, the overall results suggest that an alteration in the activity of SOD and GSH-Px during the observed period reflects the presence of certain prooxidative compounds that can lead to oxidative stress in the liver of sterlet at the DOR site.

Keywords: Fish; *Acipenser ruthenus*; Biomarkers; CYP1A1; EROD activity; Antioxidant enzymes; Transaminases

1. Introduction

The impact of contaminants on aquatic ecosystems can be assessed by measurement of biochemical parameters in fish that respond specifically to the degree and type of contamination (Petrivalsky et al., 1997). Despite their limitations, such as relatively high mobility, fish are generally considered to be the most feasible organisms for pollution monitoring in aquatic ecosystems (Van der Oost et al., 2003). Fish employ several enzyme systems in biotransformation of various xenobiotics (Van der Oost et al., 2003). Induction of hepatic mixed-function oxidase enzymes of phase I, especially CYP1A1 and associated ethoxyresorufin *O*-deethylase (EROD) activity, is considered a common indicator of exposure of fish to environmental pollutants, such as polycyclic aromatic hydrocarbons (PAHs) (Stephensen et al., 2003) and polychlorinated biphenyls (PCBs) (Hugla and Thome, 1999). In their recent paper, Van der Oost et al. (2003) reviewed data on induction of CYP1A1 in fish after exposure to different pollutants and suggested that this enzyme can be both a useful tool for the assessment of exposure and an early warning sign for potentially harmful effects of many organic trace pollutants. In addition to enzymes that participate in the phase I of elimination of xenobiotics, most fish possess a second group of biotransformation enzymes referred to as conjugation or phase II enzymes, which are also induced by exposure to different type of pollutants (Tate, 1988; Palace et al., 1996; Petrivalsky et al., 1997; Van der Oost et al., 1996, 2003). As compared with phase I systems, the induction responses of phase II enzymes are generally less pronounced, so that they can be masked by natural variability factors, such as sex, maturity, nutrition, season, temperature, etc. (Van der Oost et al., 2003). Nevertheless, both groups of enzymes are responsible for metabolism of lipophilic chemicals such as PAHs, PCBs, and dioxins to more readily excreted water-soluble forms (Palace et al., 1996; Fenet et al., 1998).

Many environmental pollutants or their metabolites are capable of inducing oxidative stress in aquatic organisms including fish (Ahmad et al., 2000; Almeida et al., 2002;

*Corresponding author. Fax: +381 21 450 620.
E-mail address: radmilak@ib.ns.ac.yu (R. Kovacevic).

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Sayeed et al., 2003). To neutralize toxic effects of reactive oxygen species (ROS) fish, like mammals, possess well-developed antioxidant defense systems (Palace et al., 1996; Almeida et al., 2002; Ahmad et al., 2004; Pandey et al., 2003; Sayeed et al., 2003; Zhang et al., 2004a, b). This system includes antioxidant enzymes (AOEs) such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione *S*-transferase (GST), and glutathione reductase. Also, numerous low-molecular-weight antioxidants such as glutathione, β-carotene (vitamin A), ascorbate (vitamin C), and α-tocopherol (vitamin E) can participate in the process of eliminating oxyradicals (Van der Oost et al., 2003). Several field studies have shown alterations in the activity of AOE in liver of fish that were caught from polluted sites (Rodriguez-Ariza et al., 1993; Van der Oost, 1996; Ahmad et al., 2000, 2004; Pandey et al., 2003; Sayeed et al., 2003). It has been demonstrated that the response of AOEs can be a valuable tool in studies of oxidative stress in fish (Stephensen et al., 2002; Pandey et al., 2003; Zhang et al., 2004a).

Furthermore, the leakage of specific enzymes (e.g., transaminases) into the blood can be used in the diagnosis of damage caused by pollutants in various fish tissues such as liver, muscle, and gills (de la Torre et al., 2000). However, hematological parameters are nonspecific in their response toward chemical stressors. Nevertheless, they may provide important information in effect assessment studies, e.g., by providing an indication as to the general physiology and health status of the organism under investigation (Van der Oost et al., 2003; Li et al., 2004).

To obtain relevant results from the field studies, it is absolutely necessary to choose a common fish species that enables the measurement of both biochemical and physiological responses to pollutants. The sterlet (*Acipenser ruthenus* L.) is a very common sturgeon fish (*Acipenseridae*) in the middle and upper Danube. Sturgeon populations have declined steadily in European freshwaters for at least 100 years. In the past, sterlet regularly occurred in the Danube River up to Vienna and even up to Ulm (Hensel and Holcik, 1997). In the Serbian stretch of the Danube River, the most abundant populations of sterlet occur near Belgrade and in the upstream sections in Vojvodina, near Novi Sad (Hensel and Holcik, 1997). The life history of sterlet may leave them particularly vulnerable to the effects of bioaccumulative pollutants. Because they are benthic organisms with high lipid content and a relatively long age to sexual maturity, sterlet have the potential to accumulate lipophilic contaminants that may contribute to their declining number.

According to our previous research in 1999, we suggested a possible correlation between the altered activity of AOEs in the liver of sterlet and the adverse effects of the destroyed oil refinery spills on the Danube ecosystem near Novi Sad (Grubor-Lajsic et al., 2000). The aim of the present study was to investigate whether the consequences of environmental damage that occurred 3 years ago were still present in the Danube ecosystem and to monitor the general status of the environment and overall pollution in the vicinity of Novi Sad. Therefore, we investigated the activities of AOEs and the possible induction of CYP1A1 in fish liver. In addition, the levels of transaminases and γ-glutamyl transferase (γ-GT) were measured in sterlet caught in the Danube River at two sampling sites: near the Novi Sad oil refinery and at an upstream reference site.

2. Materials and methods

2.1. Chemicals

All of the chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA), unless indicated otherwise.

2.2. Animals

Sterlet (*A. ruthenus* L.) were collected in July 2001 and 2002 from two sampling sites near the town of Novi Sad (Serbia): in the vicinity of the mouth of the Danube–Tisa–Danube (DTD) irrigation canal system in the Danube River near the Novi SAD oil refinery and at the Danube–Begec (DB), an upstream location, remote from the oil refinery and treated as a reference site (locations shown in Fig. 1). According to our knowledge, the sampling site DB is free of any major industrial sources that could cause pollution in this location. There are no industrial or any other facilities in the surrounding area or upstream of the DB site that could be affecting the biochemical responses of the control fish. After the destruction of Zezelj Bridge in Novi Sad in spring 1999, huge concrete blocks fell into the Danube River, forming very fast river currents and large whirlpools, which present a sort of physical barrier for various fish including sterlet. This barrier divided sterlet populations and enabled us to obtain relevant results from the present study. In both years, 15 specimens from each sampling site were caught using fishing nets. The body weight of sterlet from DB site ranged 160–285 g, whereas total lengths were between 34.7 and 41.2 cm. Fish from the Danube-oil refinery (DOR) had body weights in the range 90–320 g and total lengths between 31.0 and 43.6 cm. Age was determined using pectoral fin spine sections according to the method of Stevenson and Secor (1999). All specimens were immature (0^+ and 1^+ age classes), so possible differences between juveniles and adults were excluded.

2.3. Serum enzyme assays

Fish were sacrificed in the field by a sharp blow to the head and blood samples were obtained immediately by cardiac puncture. Fish and blood samples were transported on ice into the laboratory within 30 min. Sera obtained after centrifugation were used for measuring the enzyme activities. The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined spectrophotometrically using kinetic UV test ("Alfapanon" kit, DGKC method; Novi Sad, Serbia and Montenegro). The enzyme activities were estimated indirectly by the rate of NADH oxidation at 365 nm for 4 min. The activity of γ-GT was determined using L-γ-glutamyl-3-carboxy-4-nitroanilide as a substrate

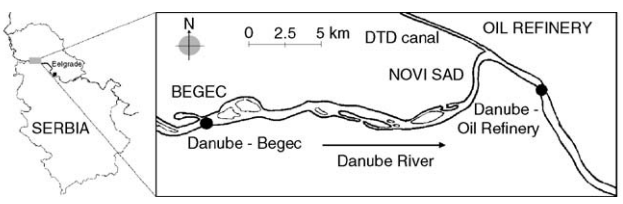

Fig. 1. Sampling site map.