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Changes in antioxidant defense system in gills of *Capoeta umbla* caught from Uzuncayir Dam Lake, Turkey

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ABSTRACT

The aim of this work was to determine the seasonal changes in the activities of selected biomarkers in *Capoeta umbla* (Heckel, 1843) caught from Uzuncayir Dam Lake (Tunceli, Turkey) and to evaluate the effects of environmental factors on these activities. Fish were sampled on seasonal basis, and superoxide dismutase, catalase, glutathione peroxidase activities and levels of glutathione and malondialdehyde in gills were determined. Significant variations of oxidative stress biomarkers were observed between seasons and sites. The results of this study show that seasonal variations of oxidative stress responses and lipid peroxidation in gills of *C. umbla* are sensitive to the contaminants present in water of Uzuncayir Dam Lake and selected parameters are in valuable biomarkers for monitoring of water systems, since they give an early warning signal of effects of xenobiotics on aquatic organisms at molecular levels which help to prevent their effects at organismal level.

1. Introduction

In aquatic animals, many natural and anthropogenic factors (xenobiotics) can induce an imbalance between the production of reactive oxygen species (ROS) and their removal, and as a result, oxidative stress occurs (Halliwell and Gutteridge, 2007; Yonar et al., 2014). Antioxidant defenses serve as an excellent scavenging potential against oxidative stress. Antioxidant defenses form from both enzymatic and non-enzymatic parameters which commonly use as biomarkers in environmental monitoring studies (Sheehan and Power, 1999). Biomarkers are cellular, biochemical, molecular, or physiological changes that are measured in cells, body fluids, tissues, or organs of an organism and are indicators of xenobiotic exposure and/or their effects (Lam and Gray, 2003). Among enzyme biomarkers, the measurement of the activities of superoxide dismutase (SOD) and catalase (CAT), glutathione (GSH) concentration and malondialdehyde (MDA) formation has become a promising tool for the biomonitoring of aquatic systems (Livingstone, 2001; Yildirim and Ergin, 2013). Antioxidants such as CAT, SOD and GSH

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have been proposed as biomarkers of contaminant or seasonally mediated oxidative stress in a variety of marine and freshwater organisms, and their induction reflects a response to pollutants (Borkovic et al., 2005). Nevertheless, environmental variables such as temperature, dissolved oxygen and food availability via their influence on metabolism and reproduction are known to affect oxidative stress responses (Sheehan and Power, 1999).

It is well established that the most important antioxidant enzymes are SOD, which detoxifies $O_2^{\bullet -}$, catalase, which reduces H_2O_2, glutathione peroxidase (GSH-P_X), which reduces both H_2O_2 and organic peroxides by a glutathione-dependent reaction, and glutathione reductase (GR), which catalyzes the NADPH-dependent regeneration of GSH from the oxidized form (GSSG) generated by GSH-P_X (Halliwell and Gutteridge, 2007). Malondialdehyde is one of the lipid peroxidation (LPO) products deriving from oxidative attack on cell membrane phospholipids and circulating lipids, and its level directly reflects the degree of oxidative damage induced by contaminants (Yonar and Yonar, 2010; Banerjee et al., 1999).

It is known that dam lakes especially cause some changes on chemical structure of water systems and create some ecotoxicological impacts on ecosystem. This pollution not only affecting negatively on the livings in the water but also these adversely affecting reaches to human-being through food chain (Yildirim and Danabas, 2014). The release of pollutants into the aquatic environment is known to cause detrimental effects to the environment and to the living organisms, and to give an increasing interest to the studies of oxidative stress responses in aquatic organisms induced by toxicants (Soares et al., 2008; Yonar, 2013).

Fish, being poikilotherms, are strongly influenced by water temperature; therefore they continuously adjust their bodies according to environmental conditions. They are widely used in biomonitoring studies (Aleshko and Lukyanova, 2008). *Capoeta umbla* is one of the most popular fish in Tunceli and has an economical importance in city (Yildirim and Ergin, 2013). The gills are efficient tools for biomonitoring of potential impacts (Oliveira-Ribeiro et al., 2005), because of their large area in contact with the water and high permeability (Vigliano et al., 2006), and environmental impacts caused by pollutants may affect fish gill tissues (Schwaiger et al., 1997). In this study, the suitability and sensitivity of the oxidative stress responses in gill tissues of *C. umbla* collected from different regions of research area for the early detection of the health of the freshwater ecosystem were evaluated.

2. Materials and methods

Activities of SOD, GSH-P_X, and CAT, and levels of GSH and MDA were measured in gill samples of *C. umbla*. In totally, 400 male *C. umbla* were used as indicator organism in this study.

2.1. Sampling sites

Fish samples were collected by local fishermen using fishing nets in March and September in 2011 and 2012. These terms could indicate possible seasonal swings in pollution and different biological activities of the fish.

Fishes (10 fish from each sites) were sampled from ten sites. These sites (Fig. 1) were determined considering the main locations of contaminant inputs into the system on pre-dam, Dam Lake and postdam points at Munzur and Pulumur Rivers and Uzuncayir Dam Lake (Fig. 1). The dam lake is only used for hydropower generation.

2.2. Water collection and physico-chemical analysis

The general physicochemical parameters of the water were measured at each sampling site during each fish-sampling period. The pH, temperature and the dissolved oxygen (DO) were measured with the handheld multiparameter (YSI Professional Plus).

2.3. Preparation of samples

Captured fishes were placed in plastic bags, and anesthetized immediately with 0.7 g L^{-1} benzocaine dissolved in ethyl alcohol (Sardella et al., 2004) and anesthesia of fish being observed as deep sedation, loss of swimming actions and partial loss of equilibrium (Altun and Danabas, 2006). They were transported to laboratory in freezer bags with ice and dissected purposed tissues. The blood in the tissues was removed by treating them with 0.9% of NaCl then, tissues are divided into two groups to determine the levels of enzymatic activity and MDA. Antioxidative enzyme analyses have been determined in the first group of tissues. For this reason, firstly the samples have been weighed and homogenized by adding PBS buffer (salt solution buffered with phosphate) at a rate of 1/5 w/v and using homogenizer with ice. The homogenized samples have been centrifuged for 15 min in a refrigerated centrifuge at 17,000 rpm the supernatants obtained were immediately put in deep freeze at −70 °C and kept there until their analyses. The second group of tissues was used for MDA analyses. Tissues were homogenized in 1.15% of KCI (potassium chloride) at a rate of 1/10 w/v and the homogenates obtained were kept in deep freeze at −70 °C until their analysis was done.