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Assessment of seasonal and sex-related variability of biomarkers in carp (*Cyprinus carpio* L.) from Karakaya Dam Lake, Turkey

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ABSTRACT

This study examines seasonal changes in the activities of selected biomarkers in carp (*Cyprinus carpio* L.) from Karakaya Dam Lake and evaluates the influence of gender and environmental factors on those activities. Physicochemical characteristics of water were evaluated in the lakewater. Fish were sampled on seasonal basis, and liver ethoxyresorufin-O-deethylase (EROD), glutathione-S-transferase, glutathione reductase, plasma lactate dehydrogenase, aspartate and alanine aminotransferase, and brain acetylcholinesterase (AChE) activities were assayed. Plasma vitellogenin level and hepatosomatic index and condition factors were also determined. Strong seasonal variations were observed but there were no gender differences among selected markers. The highest vitellogenin level of male fish was detected as 606 ng/mL which represents the estrogenicity of water in the lake in September 2005. In addition, the seasonal changes of some biomarkers such as EROD and AChE showed that the lake may be at risk of pollution by some xenobiotics arising from agricultural and/or industrial activities.

1. Introduction

Biomarkers have been defined as measurable changes in biological responses that can be related to exposure to or toxic effects of environmental chemicals. They have been proposed as sensitive tools for the early detection of environmental exposure to pollutants and their adverse effects on aquatic organisms (Sanchez et al., 2008). However, there is no single biomarker that can unequivocally measure environmental degradation. Biomarker responses may also be masked by natural factors such as sex, maturity, nutrition, season, and temperature (van der Oost et al., 2003). Therefore, long-term and multibiomarker studies on sentinel species have been collectively defined as a more complete and realistic approach to evaluate the effects of complex mixtures of chemicals in the environment (Solé et al., 2008).

Biomarkers can be indicators of either exposure or effects. To monitor the effects of the exposure of xenobiotics on wildlife and aquatic organisms, a suite of biomarkers has been developed. From the variety of available biomarkers, a selected set of enzymes was used in this study. In this set, EROD activity in the liver, as a measure of CYP1A induction, is considered a sensitive and most accepted biomarker to detect hazardous effects before they potentially occur in aquatic organisms and important for deciding the presence

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of different kinds of toxicants in the aquatic ecosystem (van der Oost et al., 2003; Ferreira et al., 2006; Damasio et al., 2007). On the other hand, in fish, glutathione-S-transferase (GST) activity is widely used as a nonspecific biomarker of exposure to pollutants (Tlili et al., 2010). Glutathione peroxidase (GR) is an antioxidant enzyme that may be used as an oxidative stress parameter that is a reliable and widely used biomarker (Stephensen et al., 2002). Furthermore, inhibition of acetylcholinesterase (AChE) activity has also been widely used to diagnose the exposure to anticholinesterase compounds, such as organophosphorus (OP) and carbamate (CB) pesticides (Fulton and Key, 2001). Moreover, other biochemical parameters, such as plasma lactate dehydrogenase (LDH) and aspartate and alanine aminotransferase (ALT and AST, respectively), are thought to be good biomarkers for animals exposed to contaminants, such as pesticides and other xenobiotics (Malbrouck et al., 2003; Agrahari et al., 2007; Elumalai et al., 2007).

There is increasing evidence that anthropogenic xenobiotics can affect the endocrine status of wildlife and many xenobiotic chemicals can reduce the reproductive capacity of fish through disruption of the endocrine system (Kime, 1999; Versonnen et al., 2004). These chemicals, known as endocrine disrupters, can finally lead to alterations of growth, development, sexual differentiation and reproduction (Navas et al., 2005). Vitellogenin (VTG) is a complex phospholipoglycoprotein that is normally produced in the livers of oviparous females. In male fish, the VTG gene is present but it is normally not expressed. However, many environmental compounds can mimic the effects of natural estrogens and induce VTG synthesis in males in a manner similar to females (Solé et al., 2000). Therefore, VTG levels are being intensively studied as they can be influenced by estrogenic compounds (Moncaut et al., 2003).

Fish can be found virtually everywhere in the aquatic environment and they play a major ecological role in the aquatic food webs due to their function as a carrier of energy from lower to higher trophic levels (van der Oost et al., 2003). Therefore, fish are regularly used as valuable surrogates in monitoring programs (Whyte et al., 2000). The test organism in this study was common carp, *Cyprinus carpio* L. Carp is a widespread, robust fish species that resists highly polluted waters (Solé et al., 2002). It is found all year in lakes and the abundance of this species and its ease of capture were reasons why it was chosen for this study.

Damming up the Euphrates River in 1987 formed Karakaya Dam Lake (KDL), which is one of the most important freshwater reservoirs used for agriculture and fishery purposes in Eastern Anatolia (Turkey) (Fig. 1). The lake was stocked with about 3 million common carp from 1990 to 1999 (Ozmen et al., 2006). Fishable stocks have been estimated for *C. carpio* to be 56.4 kg/year (2.47 kg/ha) (Yuksel and Celayir, 2010). The surface area of the dam lake is about 268 km^2 and reservoir volume at normal water surface elevation is 9,580,000 h m^3.

Previous water chemistry analysis has shown that the water is quite polluted in some regions of KDL. Some metal accumulation has been determined in both KDL water and carp samples (Kucukbay and Orun, 2003; Ozmen et al., 2006). Wastewater discharges of textile dye processing and municipal sewage of the city of Malatya have been the main sources of pollution for many years.

The main objectives of this study were: (1) to investigate seasonal variation in some biochemical parameters proposed as selected biomarkers in carp in KDL, (2) to determine whether or not these enzymes could be used as markers in future studies in KDL and (3) to evaluate reliable and reproducible methods for monitoring inland water pollution and to determine the environmental quality of freshwater.

2. Materials and methods

2.1. Chemicals

Reagents for enzymatic reactions (resorufin, ethoxyresorufin, 1-chloro-2,4-dinitrobenzene (CDNB), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ACTI), reduced glutathione (GSH), bovine serum albumin (BSA) and Bradford reagent were purchased from Sigma (MO, USA). Phenylmethanesulphonylfluoride (PMSF) and ethyl-m-aminobenzoate methanesulfonate salt (MS222) were obtained from Merck (Germany). Heparin, oxidized glutathione (GSSG) and $NADPH^+$ were purchased from MP Biomedicals (USA). Carp Vitellogenin Assay Kits (VTG-103) were gained from Biosense Laboratories AS (Bergen, Norway). LDH, AST and ALT diagnostic kits were obtained from Biolabo (France).

2.2. Sampling and biometric indices

In total, 155 male and 131 female mature carps were sampled during the study and they were captured from November 2004 to April 2006. All of the captured fish were at least 2 years old. The fish were collected with gill nets having mesh sizes (stretched mesh) of 8–13 cm and the length of each gill net was 200 m. All the samples were collected from 4 important fishery localities: site 1 (38°28′48.55″N; 38°18′59.82″E), site 2 (38°29′21.61″N; 38°23′38.98″E), site 3 (38°31′0.57″N; 38°24′22.72″E) and site 4 (38°28′30.95″N; 38°26′41.17″E) (Fig. 1).

Fish were collected during different periods representing seasonal changes and possible effects of pollution. The nets were lifted and captured fish were transferred into a live box immediately. Each fish was anesthetized in a gallon containing 100 mg/L MS222 for bleeding and sacrificing. After anesthesia, approximately 2 mL × 2 mL samples of blood were taken from the caudal vein using a heparinized syringe in field conditions. Then the blood samples were immediately transferred into ice-cold heparinized tubes. The first tube contained PMSF (1 mM final concentration) for VTG analysis and the second was without PMSF for measuring plasma enzyme activities. Both tubes were centrifuged at 5000 rpm for 5 min in the field with a portable centrifuge powered by an electric generator. The plasma samples were separated into clean tubes and were transferred to the laboratory in iceboxes. The plasma samples were stored at −80 °C until VTG and enzyme assays were conducted.

After sacrificing, fish were also transferred to the laboratory in iceboxes within 2 h of being caught. They were weighed, measured, and examined for any observable morphological abnormalities or parasitic infestation. The fish were dissected, their livers quickly examined for any abnormalities, before all fish tissues were removed immediately. Samples were