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Oxidative stress biomarkers in liver and gill tissues of 
spotted barb (*Capoeta barroisi* Lortet, 1894) living in the river 
Ceyhan, Adana, Turkey

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Received: 19.12.2008

**Abstract:** This study was carried out in an agricultural, industrial, domestic, and slaughterhouse area that is also a discharging region of the river Ceyhan just under the crest of the Aslantaş dam. Levels of pollution indicator parameters of the water were observed and their effects on various oxidative stress biomarkers in gill and liver tissues of spotted barb (*Capoeta barroisi* Lortet, 1894) were investigated. The oxidative stress biomarkers analyzed included superoxide dismutase (SOD), catalase (CAT), and glucose-6-phosphate dehydrogenase (G6PD). Levels of reduced glutathione (GSH) and lipid peroxidation (LPO) were also evaluated. High levels of CAT, G6PD, GST, and GSH activity were found in the liver tissues of fish collected from the river Ceyhan discharging region; it was determined that the region was polluted. Substantially high levels of SOD and LPO (P < 0.05 in gill and liver) were observed.

The findings of the present investigation will provide a rational use for oxidative stress biomarkers in aquatic ecosystem pollution biomonitoring.

**Key words:** Spotted barb (*Capoeta barroisi* Lortet, 1894), oxidative stress biomarkers, river Ceyhan, Aslantaş dam, pollution

**Ceyhan nehri (Adana-Türkiye)’nde yaşayan benekli siraz (*Capoeta barroisi* Lortet, 1894)’larda solungaç ve karaciğer dokudaki oksidatif stresin biyolojik göstergeleri**

**ÖZET:** Araştırmanın Ceyhan Nehri’nin tarımsal, sanayi, mezbaha ve evsel atıklarının deşarj olduğu bölge (Büyükmangıt köyü) ile aynı nehir üzerinde kurulu olan Aslantaş Barajı kret altı bölgesinde (Osmaniye) yapılmıştır. Çalışmada kirilik indikatörü su parametre değerleri gözlenmiş ve bunların benekli sirazda karaciğer ve solungaç dokudaki çeşitli oksidatif stres biomarkerleri üzerine olan etkileri incelenmiştir. Superoksid dismutaz (SOD), katalaz (CAT) ve glikoz-6-fosfat dehidrogenaz (G6PD)’i içeren oksidatif stres biomarkerleri analiz edilmiştir. Glutatyon (GSH) ve lipid peroksidsasyon (LPO) seviyeleri de ayrıca değerlendirilmiştir. Kirli olarak tayin edilen deşarj bölgesinde toplanan baıkların karaciğer dokularında CAT, G6PD, GST ve GSH aktivitesi yüksek seviyelerde bulunmuştur. SOD ve LPO miktarları da (solungaç ve karaciğer için P < 0.05) deşarj bölgesinde oldukça yüksek seviyelerde gözlenmiştir.

Bu çalışmaların bulguları, sucul ekosistem kirliliğinin biyolojik gözeleminde, oxidative stress biomarkerlerine rasyonel bir kullanım sağlayacaktır.

**Anahtar sözcükler:** Benekli Siraz (*Capoeta Barroisi* Lortet, 1894), Oksidatif stresin biyolojik göstergeleri, Ceyhan nehri, Aslantaş barajı, kirilik
Introduction

The aquatic environment is subject to temporal and spatial variations in quality due to internal and external factors. The indiscriminate dumping and release of wastes containing the above-mentioned hazardous substances into rivers might lead to environmental disturbance that could be considered a potential source of stress to the biotic community (1). Recent data indicate that the pollution toxicity in aquatic organisms may be associated with increased production of “reactive oxygen species” (ROS), leading to oxidative stress. In the aquatic environment, despite the presence of constitutive or enhanced antioxidant defense systems, increased levels of oxidative damage will occur in organisms exposed to contaminants that stimulate the production of ROS (2). The increased ROS production and subsequent oxidative stress has been associated with a pollutant-mediated mechanism of toxicity in fish liver. The use of biochemical or physiological measurements as indicators of toxicity is under constant development and has the advantage of delineating effects prior to the manifestation of diseases (3).

This study was intended to evaluate the relationships between polluted and unpolluted sites. The study also looked at the *C. barroisi* oxidative stress response modulation in fish liver and gill focussing on the various antioxidants that counter peroxidative damage. Moreover, an evaluation was made regarding the suitability and sensitivity of *C. barroisi* oxidative stress biomarkers in early detection for the health of the freshwater ecosystem.

Materials and methods

Specifications of the sampling areas

Ceyhan is the most important town in the study area and an important industrial center for multiple sectors including textile, maize drying, food, agriculture, and oil and cotton-processing. The Baku-Ceyhan Crude Oil Pipeline also adds importance to the strategic and industrial significance of the Ceyhan area.

The present study was carried out in the summer because that is the season in which amounts of pollution peak in the river Ceyhan (4). During the summer (June, July, August), water and fish were sampled monthly in triplicate. In this study, water and fish samples were obtained from 2 regions along the river Ceyhan. The regions were chosen considering their agricultural and industrial activities. The first region was just under the crest of the Aslantaş Dam. This location was thought to be less affected by pollution and so was considered the control region (Sampling Region I (SR I): control region). The second region was in the discharging area and assumed to be highly polluted (Sampling Region II (SR II): polluted region) (Figure 1).

Physico-chemical analysis

Water samples were transported to the Water Quality and Chemistry Laboratory in the Faculty of Fisheries, University of Çukurova in labeled, dark colored bottles (1 L) in a carrying case at +4 °C. Then water samples were analyzed for physico-chemical characteristics such as temperature, pH, nitrite (NO₂⁻ N), nitrate (NO₃⁻ N), ammonia (NH₃–N), soluble reactive phosphorus (SRP), and chemical oxygen demand (COD) using procedures described in APHA (1998) (5). Water quality parameters were evaluated according to the criteria of the WHO (1996) (6).

Experimental sampling

A total of 180 fish were caught alive under the crest of Aslantaş Dam Lake (SR I) and from the Ceyhan location of the river (SR II) using fishing lines and extension nets. Then fish were transported to the Fish Diseases Laboratory in the Faculty of Fisheries, University of Çukurova, in a conveying tank (270 L) reinforced with oxygen. Body weight and total length values were measured (with a Toledo scale, 0.01 sensitivity; millimetric ruler). During measuring the fish were anaesthetized using quinaldine sulphate (Sigma Chemical Company, Germany) at a dose of 20 mL/L (7).

Physiological indexes

Once the fish arrived at the laboratory, the body weight and length were determined and the liver and gills were excised. The condition factor (CF) was calculated as CF = body weight (g) · [(length (cm)³)⁻¹ × 100 (8). The liver somatic index (LSI) (or liver–body weight rate) was calculated as LSI = [liver weight (g) · body weight (g)⁻¹] × 100 (9).
Preparation of liver and gill homogenates

Fish liver and gill tissues were rapidly removed and frozen in a dry ice-refrigerated container and kept until examination. Prior to the analysis, tissue samples were unfrozen. Then they were weighed, perfused with 1.15% ice-cold KCl, minced, and homogenized in 5 volumes (w/v) of the same solution, using a Heidolph 50110 R2R0 homogenizer. Antioxidant systems and LPO assays were performed on the supernatant preparation in a Sorvall RC-2B centrifugation of the homogenate at 14,000 rpm for 30 min at +4 °C (10).

Biochemical parameters

G6PD activity was analyzed with Beutler’s method (1975). Again, using Beutler’s method (1975), CAT activities were determined by measuring the decrease in hydrogen peroxide concentration at 230 nm (11). SOD activity was measured according to the method described by Fridovich (12). GST activity was measured by the method described by Mannervik and Guttenberg (13). Enzyme activity was calculated using a molar extinction coefficient of $9.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$. G6PD activity, CAT activities, SOD activity, GST activity, and enzyme activity were all expressed as U/mg protein. GSH levels were determined using Beutler’s method by measuring a highly coloured yellow anion formed by the reduction of DTNB [5, 5’-Dithiobis (2-nitrobenzoic acid)] with nonprotein sulfhydryl compounds of tissue samples (11). The levels of GSH were calculated as μmol/mg protein. The LPO level in the tissue samples was expressed as malondialdehyde (MDA) (14). Total protein contents were determined using the method by Lowry (15), using bovine serum albumin (Merck, Darmstadt, Germany) as a standard.

Statistical analysis

The statistical analysis of data was done using an unpaired, one-tailed Student’s 't' test. The significance of the results was ascertained at $P < 0.05$. 
Results

The mean body weight and total length of the *C. barroisi* was measured as 137.92 ± 18.37 g and 24.77 ± 1.78 cm, respectively, for SR I; and 110.97 ± 5.22 g and 19.21 ± 1.47 cm, respectively, for SR II.

Some physico-chemical characteristics of the water are given in Table 1. The level of COD in SR I was found to be 8.81 mg/L, whereas in SR II it was found to be 37.75 mg/L. In SR II, almost 2- or 3-fold higher mean values of SRP, NO₃-N, NH₃-N, and NO₂-N were observed when compared to the SR I values. Moreover, the mean values of temperature and pH of SR II were found to be higher than SR I.

Enzyme profile

The mean activities of antioxidant enzymes are shown in Tables 2 and 3. The mean SOD activities of SR II were found to be significantly higher (P < 0.05 in liver and gill) when compared to the mean activities in the fish from SR I. The SOD activity was maximum in liver. The CAT activity was found to be higher in the liver tissues of fish collected from SR II compared with data from the fish from SR I. However, the activity of gill CAT was found to be significantly lower in SR II (P < 0.05). The CAT activity was maximum in liver.
The mean activities of G6PD in liver and gill are also given in Tables 2 and 3. The G6PD enzyme values were significantly higher in the liver the tissue in SR II when compared with the ones in the SR I. The activity of G6PD was found to be significantly higher in the liver (P < 0.05) of fish in SR II than SR I, whereas the activity of G6PD in the gill of fish from SR II was lower (P < 0.05) when compared with the values of fish from SR I. The levels of GST activity in fish tissues collected from both regions are shown in Tables 2 and 3. When compared, the GST activity of the livers of fish collected from SR II showed significantly higher values (P < 0.05). Regarding GST activities in the gill, values were lower in the fish collected from SR II (P < 0.05). The percentage difference in GST activity in the liver from SR II over SR I was found to be approximately 444%.

Reduced glutathione level (GSH)

The levels of GSH in various tissues of fish sampled at SR I and SR II are shown in Figure 2. The values of GSH in liver of the fish from SR II were observed to be significantly higher (P < 0.05) when compared with values of fish from SR I. As regards GSH levels in gill, GSH values were lower in the fish collected from SR II (P < 0.05) when compared with the values in fish collected from SR I.

Lipid peroxidation levels (LPO)

Levels of LPO in fish tissues are shown in Figure 3. The mean values of LPO in liver and gill of the C. barroisi fish collected from SR II were found to be higher than the respective values from the SR I samples. Particularly in gill tissue, LPO level was found to be about 6 times higher in SR II (P < 0.05).

Physiological indices

In this study, CFs were determined in fish collected from both regions. The CF of fish living in the SR II area (0.92 ± 0.21 g/cm³) was lower than that of the fish from the SR I area (1.63 ± 0.43 g/cm³). Furthermore, LSI was determined in fish collected from both regions. The LSI of fish living in the SR II area (1.50 ± 0.37) was significantly higher than that in their SR I counterparts (0.61 ± 0.10).

Discussion

Physico-chemical analysis has long been employed to assess water quality. In the current study, water quality parameters are, in general, within acceptable levels considering criteria given in APHA (5). COD is often used as a measurement of the pollution load in wastewater and natural waters. The levels of COD were found to be higher in SR II, suggesting the presence of high levels of organic and inorganic pollutants in that region. The water pH was considerably higher in SR II, which can be assumed to be an indicator of pollution. Nitrite levels indicated a moderately polluted state in SR II. Nitrate and ammonia values found for SR II were similar with the results of Yılmazer and Yaman (4). Both the ammonia and nitrate values found in our study may be the...
indicators of negative impacts on the water quality of river Ceyhan as a result of agricultural and industrial activities. The high phosphorus concentrations in the SR II samples may point to the input of domestic and industrial waste-waters and fertilizers. The resulting water quality measurements taken from the river Ceyhan, with the exception of SR I, showed that the river is affected by agricultural and industrial activities as well as discharge from domestic sources. These pollutants may promote the production of superoxide anion radicals by redox cycling, while transition metals such as iron catalyze the reaction of superoxide anion radicals and hydrogen peroxide to produce hydroxyl radicals through Fenton reactions (16).

The SOD-CAT system provides the first defense against oxygen toxicity. SOD catalyzes the dismutation of the superoxide anion radical into water and hydrogen peroxide, which is detoxified by CAT activity. Usually a simultaneous induction response in the activities of SOD and CAT is observed when exposed to pollutants (17). In the present study such a relationship was observed. In our study, the activities of SOD and CAT were found to be high in the liver tissue of fish collected from the polluted environment. The SOD activity was reported by several workers to be higher in fish from the polluted site (18), indicating a high production of superoxide anion radicals. The high levels of CAT in the liver tissue could be attributed to high production of peroxide radicals. Increased SOD and CAT activities in the liver may be a response to oxidative stress. As a contributory factor in water pollutant induced stress, the occurrence of high nitrite levels can be an important source of pro-oxidants for fish (19), leading to the production of nitric radicals (nitrosative stress) as demonstrated in mammals by Lijima et al. (20). In this way, there may be a correlation between the antioxidant responses observed in the SR II CAT induction in liver and the nitrite levels detected in SR II.

In this study, SOD activity in the gill tissue was found to be higher whereas the CAT activity in gill tissue was found to be lower in fish collected from the polluted environment. The significant CAT inhibition in the gill samples could be attributed to a high production of superoxide anion radicals by the SOD enzyme, which has been reported to inhibit CAT activity in case of excess production (21). In addition, as previously reported by Dimitrova, the presence of heavy metals and their role in the decrease of CAT activity should also be considered (17), since Ahmad et al. reported a CAT decrease due to a high concentration of copper (22). As a result, we thought that while SOD-CAT systems in the liver tissues from the polluted area may be a response to oxidative stress these systems in gill tissue may not respond to oxidative stress. Therefore, increased oxidative stress in the gill tissue may lead to oxidative damage.

The induction of GST in liver tissue was observed in SR II, suggesting an activation of the liver detoxification processes probably due to the presence of organic contaminants. This statement is in partial agreement with the nitrite data (20) since the higher nitrite values were measured in SR II rather than SR I. However, the decrease in GST activity in gill tissue displayed in SR II suggests a significant reduction in fish capacity to detoxify and rid themselves of chemicals. Although GST induction has been widely demonstrated following exposure to some organic contaminants (23), its inhibition has also been reported as a non-specific response to chemical challenge (24). In our study, the higher hepatic glutathione concentration observed in C. barroisi living in a polluted environment at the SR II site indicates an adaptive and protective role for GSH against oxidative stress induced by chemical contaminants. Similarly, DiGiulio et al. observed high levels of GSH in catfish exposed to polluted sediment (25). However, the decrease in GSH content observed in the gill tissues of fish exposed in SR II may be due to insufficient glutathione regeneration.

Fish tend to adapt to oxidative conditions to which they are exposed. The increased G6PD activity in liver tissue demonstrates increased production of NADPH used in the detoxification process. This probably reflects an adaptation to oxidative conditions to which fish have been exposed. However, low gill G6PD observed in C. barroisi in SR II indicates that the low G6PD activity in the pollution may aggravate oxidative stress. Therefore, oxidative damage in gill tissue may be present. LPO activity is regarded as one of the best biomarkers for ecological risk assessment (26); its importance lies in initiating the cell membrane dissolution process, leaving cells exposed to...
to xenobiotic factors (27). Many environmental pollutants and their metabolites have shown to exert toxic effects associated with oxidative stress, producing free radicals that initiate the LPO and cause damage to membrane proteins (28). In this study, the measurement of LPO was provided as an indicator of pollution. Increased LPO levels in liver and gill tissues were accepted as indicative of oxidative stress.

Physiological index parameters are often used in field toxicological studies to assess the general condition of fish. CF is a physiological index parameter indicative of the health status of the whole body of fish related to the environmental availability of food. In our study, the CF of fish living at SR II was lower than that of the fish living at SR I. This result indicates that C. barroisi may be able to adjust to low oxygen levels and poor food environments, although polluted water would not allow fish to access sufficient nutritional status. Furthermore, one should also take into account the considerable increase in LSI values displayed by the exposed fish. Van der Oost et al. observed an increase in LSI values in fish caught in contaminated sites; our results strongly support these observations (26). The increase in the LSI of fish from SR II may be interpreted as a consequence of the liver enlargement secondary to exposure to pollutants due to compensatory proliferation processes (29).

This study showed that oxidative stress biomarkers and physiologic index parameters may be used to determine the response of organisms to environmental pollution. The use of both response types contributes to ecological risk assessment and sets adequate conservation strategies for the species most affected by pollutants.

## Conclusion

The determination of biomarkers on fish populations reflects whether they are subject to stress. The findings of the present investigation suggest that oxidative stress biomarkers, especially the estimation of antioxidant systems in fish, could provide a useful indicator of pollution in bodies of water. The induction of antioxidant systems (as observed in liver), as well as their inhibition (as observed in gill) should be considered a clear indication of the presence of pollution and environmental health degradation. In this study, the measurement of LPO, which has been described in several other studies as a biomarker of the effect of pollution, was also revealed to be a useful indicator of pollution load. Furthermore, physiological index parameters such as CF and LSI may be biomarkers sensitive enough to measure environmental stress.

## Acknowledgement

We would like to express our sincere thanks to Prof. Dr. Yakup Gümüşalan for his linguistic assistance in reviewing the manuscript.

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