

**PARAMETERS OF OXIDATIVE STRESS  
IN LIVER AND WHITE MUSCLE  
OF HAKE (*Merluccius merluccius* L.) FROM THE ADRIATIC SEA**

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**ABSTRACT:** Fish are the most important organisms in the biomonitoring of aquatic ecosystems. A number of pollutants may cause the oxidative stress in the organism of fish. The monitoring of parameters of oxidative stress in tissues of fish is a good biomarker in the assessment of the condition of environment.

Catch of commercially important species of fish, hake (*Merluccius merluccius* L.) was carried out in front of sea-port Bar (South Adriatic) at the end of May 2005. The parameters of oxidative stress: concentrations of lipid peroxides (LP) and reduced glutathione (GSH) were determined in liver and white muscle. Physical-chemical parameters (salinity, concentration of dissolved oxygen and temperature) as well as the concentrations of nitrites, nitrates and detergents in the water of investigated locality were determined.

The obtained results showed the tissue specificity that was the consequence of different metabolic and antioxidative activities. In the liver of hake, the increased concentration of LP was found in comparison to the white muscle, whereas in white muscle the increased concentration of GSH was recorded in comparison to the liver. These results suggest that the intensity of oxidative stress was higher in the liver in comparison to the white muscle, which is the consequence of increased presence of pollutants in water of the investigated locality.

**Key words:** hake, Adriatic Sea, lipid peroxidation, reduced glutathione

## INTRODUCTION

Harmful anthropogenic influence is exerted on the biological reserves of coastal sea area due to uncontrolled fishing and increased pollution of sea, which is prominent in the areas of bigger urban and industrial centers [1].

Fish species used in the biomonitoring are chosen according to their reactions to a changed quality of environment and according to their exposition to toxic substances. Fish play an important role in food chain, as well [2].

In fish, as well as in other aerobic organisms, an antioxidative defense system matches the toxic action of prooxidative system. However, when the balance is disturbed in direction to prooxidants [3], the oxidative stress occurs, which causes oxidative damage of cells. Harmful effects of toxic action of redox active substances and oxidative stress elicit an adaptive response of the organism and the increased activity of enzymes of antioxidative defense system. There are also secondary consequences, such as the oxidation of proteins, lipids and nucleic acids, as well as the changed cell redox status [4]. The enzymes involved in detoxification of xenobiotics and their metabolites are the most frequently studied biomarkers in fish. They are the biotransformation enzymes and enzymes of antioxidative defense system. The most frequently used organ in such studies is the liver of fish, which is metabolically the most active tissue, and in which the majority of biotransformation changes occur [5].

The best-studied harmful consequence of action of ROS on living organisms is lipid peroxidation (LP), i.e. series of chain free radical reactions, leading to breakdown of polyunsaturated fatty acids that cause destruction of cell membranes and death of cells [6].

Glutathione (GSH), being a component of the mechanism of non-enzymatic antioxidative protection, is the first linker in counteracting against oxidative processes in cell.

The aim of this study was to determine the concentrations of GSH and LP in liver and white muscle of hake (*Merluccius merluccius* L.) in front of sea-port Bar, South Adriatic. Physical-chemical parameters (salinity, concentration of dissolved oxygen and temperature) as well as the concentrations of nitrites, nitrates and detergents in the water of investigated locality were determined.

## MATERIALS AND METHODS

In late spring (May 2005), a day tour by boat was organized in front of sea-port Bar (coast of Montenegro, the south Adriatic),(Figure 1).

After trawling, fish samples were transferred in tanks together with seawater and their identification was made. Weight and length of each sample were measured, and individuals of similar length and weight were chosen to provide uniformity of samples. Average weight of hake was 80-120 grams. Liver and white muscles were isolated in the laboratory. Before the isolation of liver, perfusion with ice-cold saline for poikilotherms (0.65%) was carried out through portal vein to remove residual blood. White muscle was isolated from lateral side of all examined fish. Samples of tissue for analysis were stored in deep freeze chamber (-85° C) until examination.

Measurements of environmental parameters (salinity, temperature and oxygen concentration of sea water) were performed with a WTW (Wissenschaftlich-technische Werkstätten, Dr Karl Slevogt straÙe, Weilheim, Germany) multilab system. They were made at the time of fish sampling at 40 m depth (spot measurements, late spring - May, 2005). Spectrophotometric determination of the nitrite concentration was performed by

using  $\alpha$ -naphthyl amine and sulphanic acid [7], the nitrate level by using sodium salicylate [8] and detergents (the determination of anionic surfactants) by using methylene blue after chloroform extraction [9].

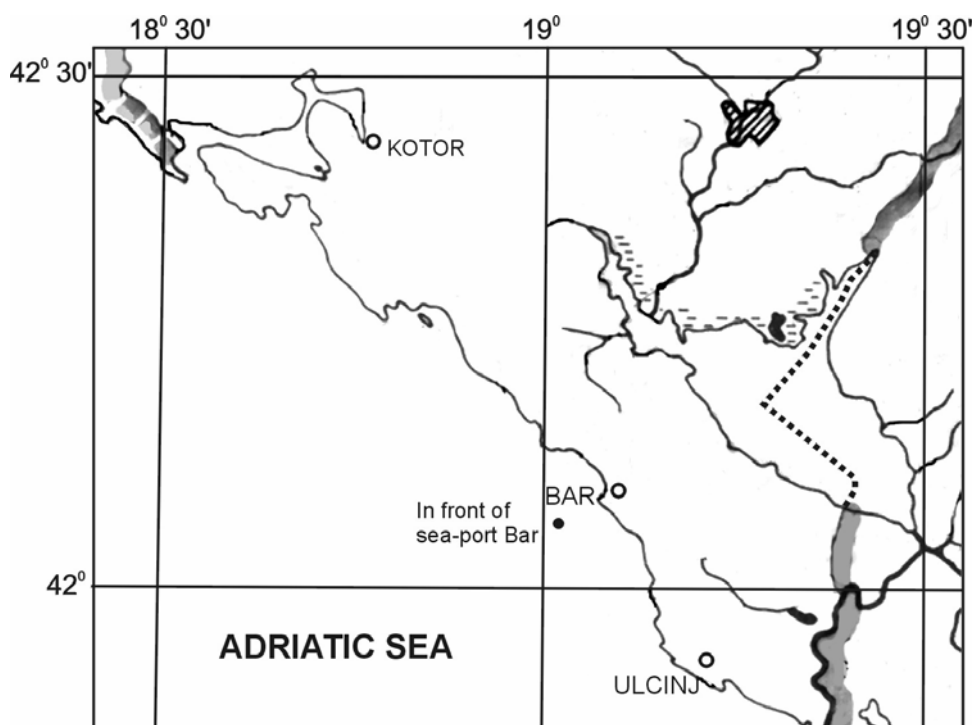


Figure 1. The geographical position of location in front of the sea-port Bar in the south areas of the Adriatic Sea.

The tissues were dissected, thoroughly washed with ice-cold saline, weighed, minced and homogenized with a Thomas Sci Co. glass homogenizer (Teflon pestle) at 0-4°C (10% w/v) using 1.15% KCl for lipid peroxides (LP) determination. The concentration of LP measured as thiobarbituric acid reactive substances (TBARS) in the tissues of fish was assayed by the method of Ohkawa et al. [10] using thiobarbituric acid (TBA) as reagent. In this reaction, the colored complex was formed and absorbance was determined spectrophotometrically at 530 nm. LP was expressed as nmol TBARS/mg tissue using a molar extinction coefficient for MDA of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .

Tissue samples for glutathione (GSH) assays were homogenised on ice with 20 volumes of precipitating solution (1.5 mL 100 mmol/L Na-phosphate / 5 mmol/L EDTA buffer, pH 8.0 and 0.4 mL 25% metaphosphoric acid). Total homogenate was centrifuged at 4°C at 100,000 g for 30 minutes to obtain supernatants for the assay of GSH. The concentration of GSH in tissues were measured by standard method of Beutler [11]. The assay mixture contained 0.3 mL of supernatant, 0.75 mL of Na-phosphate puffer (0.2 M, pH 7.4), 0.1 mL DTNB and 0.04 mL NaOH. The reaction product optical density was read immediately at 412 nm. GSH was expressed as nmol GSH/mg protein.

Protein contents in samples were determined by the method of Lowry et al. [12] using Folin's reagent and bovine serum albumin (BSA) as standard.

The data are presented as mean  $\pm$  S.D. values. Numbers of animals per group are stated in the table or figure legends. The statistical analysis of data was done using one-way analysis of variance (ANOVA). The significance of the results was ascertained at  $p < 0.05$ .

## RESULTS

Examination of basic physical and chemical parameters of water at the locality in front of sea-port Bar (Table 1) shows that the temperature of sea surface was 22.9°C, which is significantly higher in comparison to the bottom (17.5°C). The highest concentration of dissolved oxygen was recorded in the middle layer of water at the depth of 30 m.

Table 1. Hydrographic data (salinity, oxygen (O<sub>2</sub>) and temperature) at the locality in front of sea-port Bar.

Depth (m)	Salinity (‰)	O <sub>2</sub> (mg/L)	T (°C)
0	37.70	7.0	22.9
30	37.90	7.7	17.7
65	38.30	6.7	17.5

Results of nitrite, nitrate and detergent concentrations in seawater at the locality in front of sea-port Bar are shown in Table 2. The highest concentration of nitrites and nitrates are recorded at the depth of 30 m, whereas the highest concentration of detergents was recorded at the surface (0m).

Table 2. Concentrations of nitrites, nitrates and detergents at the locality in front of sea-port Bar.

Depth (m)	Nitrites (mg/L)	Nitrates (mg/L)	Detergents (mg/L)
0	0.351	2.389	0.069
30	1.164	2.552	0.030
65	0.202	1.768	0.042

Concentration of LP in liver was  $14.36 \pm 0.63$ , which was significantly higher in comparison to white muscle of hake ( $0.38 \pm 0.07$ ). These results are shown in Figure 2.

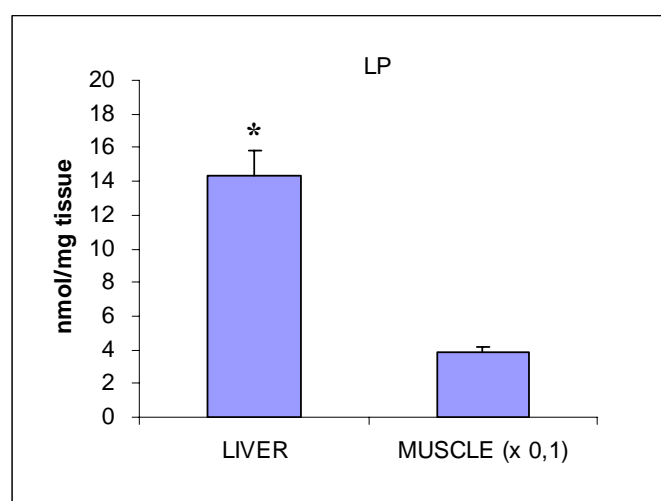


Figure 2. Concentration of lipid peroxides (LP) in liver and white muscle of hake (*Merluccius merluccius* L.) at the locality in front of sea-port Bar.

Results are expressed as mean  $\pm$  SD (n = 8). \*p<0.05, differences from tissues.

Concentration of GSH is significantly lower in liver of hake ( $429.48 \pm 44.73$ ), in comparison to white muscle ( $p < 0.05$ ), where the concentration of GSH is  $758.60 \pm 55.30$  (Figure 3).

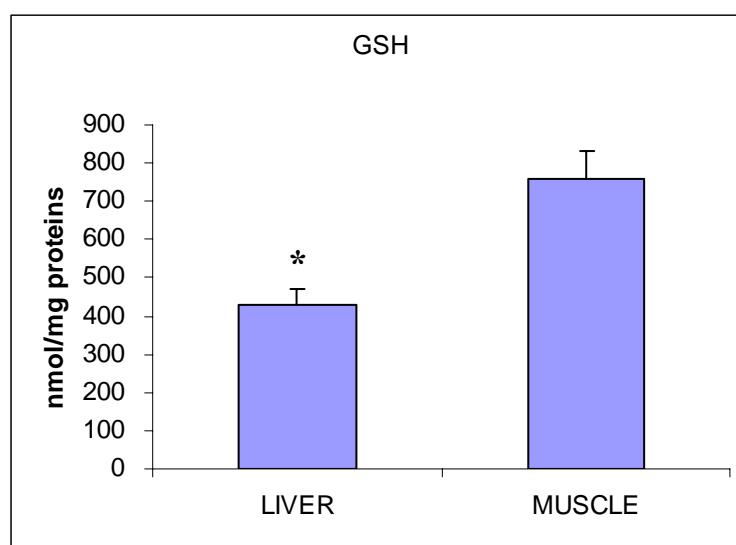


Figure 3. Concentration of glutathione (GSH) in liver and white muscle of hake (*Merluccius merluccius* L.) at the locality in front of sea-port Bar. Results are expressed as mean  $\pm$  SD (n = 8). \* $p < 0.05$ , differences from tissues.

## DISCUSSION

Numerous investigations concerning antioxidative defense system in fish provide data on the organization of this system depending on factors of environment such as nutrition, seasonal variations of environmental conditions etc [13]. The environment of fish is very unstable in respect to physical-chemical characteristics and the fish are exposed to diurnal and seasonal changes of temperature, concentration of dissolved oxygen in water and presence of certain pollutants [14]. Parameters of aquatic environment, such as temperature and concentration of oxygen, represent changeable variables that may lead to oxidative stress in non-polluted environment, as well [15].

One of important factors that affect the antioxidative damage in fish is the availability of oxygen that is not equally distributed in seawater [16]. Many regions of the world sea are characterized by higher concentration of oxygen in eutrophic zone and fall of its concentration with depth.

Our results showed that the concentration of dissolved oxygen was not distributed uniformly in seawater (Table 1). The highest concentration was recorded at the depth of 30 m, which suggested the increased seasonal production of phytoplankton.

The system of defense from oxidative damage in fish depends on oxygen availability and oxygen consumption because the production of ROS in mitochondria is linearly dependent on oxygen partial pressure. Results of some authors show that the fish that are more active possess better antioxidative defense than less active species [17]. Hake is a very active fish, predator, with muscular and spindle-shaped body and it migrates to upper layers of water daily. In our experiments, significantly increased concentration of GSH (Figure 3) and decreased level of LP (Figure 2) were found in white muscle in comparison to liver.

Liver is metabolically very active tissue in which the biotransformation of harmful pollutants occurs, which leads to generation of reactive oxygen and nitrogen species. Prooxidative-antioxidative metabolism is significantly increased in liver, where significantly increased concentrations of LP were recorded in comparison to white muscle [18].

The increased concentrations of nitrites and nitrates are a clear index of the increased rate of organic pollution, while the increased presence of detergents at the surface points to an increased anthropogenic influence (vicinity of seaport and town), (Table 2). It may be the direct cause of oxidative stress, decreased concentration of GSH and increased level of LP in the liver of hake (Figures 2 and 3). Reciprocal relation of decrease of GSH concentration and increase of lipid peroxidation may be explained by oxidative-antioxidative mechanism. The increase of LP points to oxidative stress and the decrease of GSH concentration points to its role in the prevention from oxidative injuries [19].

The increased level of GSH in white muscle and LP in liver of hake suggests that there is the increased anthropogenic influence and moderate pollution of sea at the investigated locality. Van der Oost et al. [17] showed that the ratio of reduced and oxidised GSH represented significant biomarker of oxidative stress in the fish exposed to pollutants, thus being a significant bioindicator of environmental pollution. It was established that the level of LP was also increased in the environment with anthropogenic and industrial pollution.

## CONCLUSIONS

According to our results, it can be concluded that the level of investigated parameters of oxidative stress (concentrations of GSH and LP) in tissues of hake represents the significant and well-established biomarker in the biomonitoring of environmental pollution. Tissue differences of oxidative stress parameters were established due to different metabolic and antioxidative activities, presence of pollutants and changes of physical-chemical parameters of the environment (the concentration of dissolved oxygen and water temperature). Levels and characteristics of antioxidants show that the system of defense from oxidative injury in liver is a good ecological and ecotoxicological index of biomonitoring, whereas muscles are important in the monitoring of influence of environmental factors on consumer value.

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