Arch. Biol. Sci., Belgrade, 60 (4), 601-607, 2008 DOI:10.2298/ABS0804601O

CONCENTRATION OF ANTIOXIDANT COMPOUNDS AND LIPID PEROXIDATION IN THE LIVER AND WHITE MUSCLE OF HAKE (*MERLUCCIUS MERLUCCIUS* L.) IN THE ADRIATIC SEA

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Abstract — Specimens of a marine freshwater fish — hake (*Merluccius merluccius* L.) — were collected at the localities of Platamuni and Valdanos (Montenegro, South Adriatic) in the spring of 2003. Our results showed higher concentrations of LPO, Vit C, and Vit E in the liver in comparison with white muscle. The concentration of LPO in both tissues was higher, while that of Vit E was lower at Valdanos compared to Platamuni. These differences in parameters of oxidative stress are partly due to differences in temperature and the concentrations of nitrites, nitrates, and detergents in the waters of Valdanos compared to Platamuni.

Key words: Vitamin C, vitamin E, lipid peroxidation, hake, Montenegro, Adriatic Sea

UDC 612:597.555.5(262.3)

INTRODUCTION

Contamination of the aquatic environment occurs due to growth of the human population and technological development. Changes in the chemical composition of natural aquatic ecosystems can affect biochemical processes in aquatic organisms (Van der Oost, 2003). Fish are sensitive and ecologically relevant bioindicators of aquatic ecosystems owing to their tendency to accumulate different contaminants. Xenobiotics may accumulate in fish through different mechanisms: via direct uptake from the water by gills or skin, via uptake of suspended particles, and via consumption of contaminated food. In aquatic organisms, many xenobiotics may cause oxidative stress and lead to the generation of reactive oxygen species (ROS) and alterations in the antioxidant defense system (AOS) (Livingstone, 2001). Contaminant-stimulated ROS are associated with different pathologic processes involved in the etiology of many fish diseases and may also be a mechanism of toxicity in aquatic organisms exposed to pollutants (Livingstone, 2001). Reactive oxygen species such as the superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radical ($^{\bullet}OH$) can affect nucleic acids and disturb their native conformation, react with membrane lipids causing lipid peroxidation, and disturb the structure of proteins (Matés, 2000).

Lipid peroxidation results from oxidative stress and has been used to analyze the effect of pollutants in aquatic organisms (Fessard and Livingstone, 1998). The process of lipid peroxidation is the result of a chain reaction and, as in the case of redox cycling, demonstrates the ability of a single radical species to propagate a number of deleterious biochemical reactions. Numerous studies have demonstrated enhancement of lipid peroxidation in various tissues of fish species exposed *in vivo* to polyacyclic aromatic hydrocarbons and polychlorinated biphenyls (Solé, 2000; Livingstone, 2001), t-butyl hydroperoxide and heavy metals (Livingstone, 1998), and anthracene and UV-light (Kappus, 1987). Oxyradical generation can influence the redox status of cells by imposing a drain on intra-

cellular reducing equivalents, potentially affecting a variety of metabolic processes (Van der Oost et al., 2003).

Under physiological conditions, overproduction of ROS and their neutralization is prevented by the AOS, which contains enzymatic and non-enzymatic components such as glutathione (GSH), vitamin C (Vit C), vitamin E (Vit E), vitamin A (Vit A) and coenzyme Q (Livingstone, 2001). Ascorbate (Vit C) is an important water-soluble antioxidant and may additionally serve as a cofactor for the enzymes involved in collagen biosynthesis or neurotransmitter conversions. Studies addressing the feasibility of ascorbate as a biomarker are very scarce. α-Tocopherol (Vit E) is a lipid-soluble antioxidant that is synthesized by plants, but required in the diets of animals (Halliwell and Gutteridge, 2007). This compound appears to play a major role in the protection of membranes from LPO.

Symptoms of oxidative stress and altered concentrations of non-enzymatic components (Vit C and Vit E) of the AOS in fish represent significant biomarkers in assessing the status of the environment (Regoli et al., 2002).

The aim of this study was to explore the influence of physical and chemical parameters, as well as concentrations of nitrites, nitrates, and detergents in the water on concentrations of antioxidant compounds such as Vit C, Vit E, and LPO in the liver and white muscle of hake (*Merluccius merluccius* L.) in the waters of Valdanos compared to Platamuni.

MATERIAL AND METHODS

Hake *(Merluccius merluccius* L.) is a commercially utilized marine species. Specimens of this species were obtained by trawling at two localities (Platamuni and Valdanos) in the South Adriatic Sea (Montenegro) in the spring (May) of 2003. The geographical locations are shown in Fig. 1.

After collection, the fish were immediately transferred to seawater tanks, where they were identified. Fish of the same size weighing 80 - 100 g were selected to ensure uniformity of samples. Fish were killed on-board by severing the spinal cord and dissected within three minutes on ice. Samples of liver and white muscle were isolated, washed in ice-cold 0.65 % NaCl, and then frozen in liquid nitrogen (-196 °C) before storage at –80 °C.

The tissues were dissected, thoroughly washed with ice-cold saline, weighed, minced, and homogenized with a Thomas Sci Co. glass-type homogenizer (Teflon pestle) at 4°C (10% w/v) using 1.15% KCl for lipid peroxide (LPO) determination. The concentration of LPO measured as thiobarbituric acid reactive substances (TBARS) in the tissues of fish was assayed by the method of Ohkawa et al. (1979) using thiobarbituric acid (TBA) as reagent. In this reaction, a colored complex was formed and absorbance was determined spectrophotometrically (UV/VIS Spectrophotometer, Janway, GBR) at 530 nm. Lipid peroxidation was expressed as nmol TBARS/mg tissue using a molar extinction coefficient for MDA of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Liver and white muscle were minced and homogenized (10%, w/v) separately in ice-cold saline with sucrose buffer (0.25 M sucrose, 1 mM EDTA, and 0.05 M Tris-HCl, pH 7.4) in a Thomas glass-type

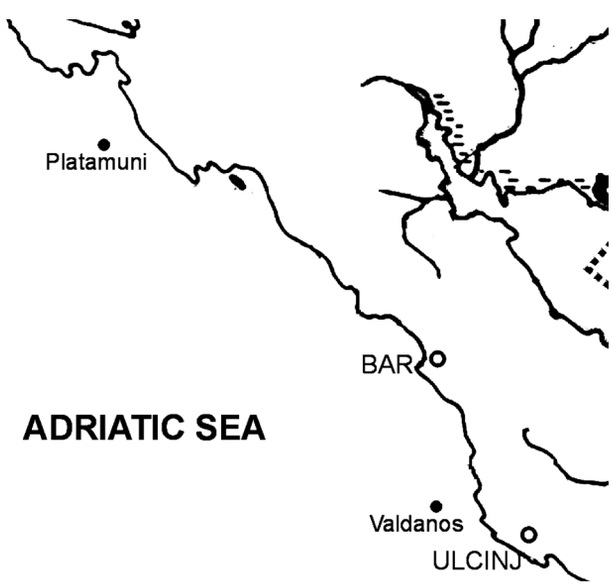

Fig. 1. Geographical position of the localities of Platamuni and Valdanos in the South Adriatic Sea (Montenegro).

homogenizer (Teflon pestle). Tissue homogenates were used for determination of vitamins C and E.

The concentration of vitamin C (Vit C) was determined spectrophotometrically by the dinitrophenyl-hydrazine method at 530 nm (Omaye et al., 1979). The method is based on the oxidation of ascorbic acid to dehydroascorbic acid, which spontaneously converts to diketogulonic acid. In the presence of 2,4-dinitrophenyl-hydrazine, diketogulonic acid forms bis-2,4-dinitrophenyl-hydrazone. Final color development was achieved with 85% sulfuric acid. The content was expressed as mg/100 g tissue (mg %).

The concentration of vitamin E (Vit E) was measured by the method of Desai (1984) based on the reduction of Fe^{3+} in Fe^{2+} in the presence of tocopherol and production of a colored complex with bathophenanthroline. Absorbance of the produced complex was measured spectrophotometrically at 535 nm, and the concentration was expressed as μg/g tissue.

Measurements of environmental parameters (salinity, temperature, and oxygen concentration of seawater) were performed with a WTW (Wissenschaftlich-technische Werkstatten, Dr Karl Slevogt Straβe, Weilheim, Germany) multilab system. They were made at the time of fish sampling at 30-m depth (spot measurements, spring — May 2003). Spectrophotometric determination of nitrite concentration was performed by using α-naphtyl amine and sulphanilic acid (Rump, 1992). The nitrate level was determined using sodium salicylate (Rump, 1992). Detergents (anionic surfactants and detergents) were determined using methylene blue after chloroform extraction (Rump, 1992).

The data are presented as means ± S.D. values. Numbers of fish per group are stated in the figure legends. Statistical analysis of the data was done employing one-way analysis of variance (ANOVA). Significance of the results was ascertained at $p < 0.05$.

Table 1. Values of salinity, temperature, and oxygen (O_2) at Platamuni and Valdanos.

	Depth (m)	Salinity (‰)	T (^{0}C)	O_2 (mg/L)
Platamuni	0	38.4	21.2	7.3
	40	38.0	17.6	7.6
Valdanos	0	36.5	20.1	7.1
	30	38.3	16.8	7.7

Table 2. Concentrations of nitrites, nitrates, and detergents at Platamuni and Valdanos.

	Depth (m)	Nitrites (mg/L)	Nitrates (mg/L)	Detergents (mg/L)
Platamuni	0	0.158	0.368	0.044
	40	0.152	0.501	0.029
Valdanos	0	0.288	1.511	0.238
	30	0.278	2.766	0.100

RESULTS

The levels of salinity, temperature, and saturation with oxygen are shown in Table 1, while concentrations of nitrites, nitrates, and detergents at the Platamuni and Valdanos localities in spring are presented in Table 2. The salinity of Valdanos seawater is lower in comparison with Platamuni. Our results showed that the concentration of dissolved oxygen was not uniform, higher values being recorded at depths of 40 m (Platamuni) and 30 m (Valdanos). The concentration of dissolved oxygen decreases with increase of water temperature toward the surface (0 m) at both localities (Table 1).

Concentrations of nitrites, nitrates and detergents were significantly higher in seawater of Valdanos in comparison with Platamuni (Table 2). This indicated that the Valdanos locality had higher water pollution.

The concentration of LPO in the liver and white muscle of fish at both localities is shown in Fig. 2. It was significantly higher in the liver than in white muscle. In addition, the concentration of LPO was higher in the liver and white muscle at Valdanos than at Platamuni ($p<0.05$).

The concentration of Vit C in the liver and white muscle of fish at Valdanos and Platamuni is present-

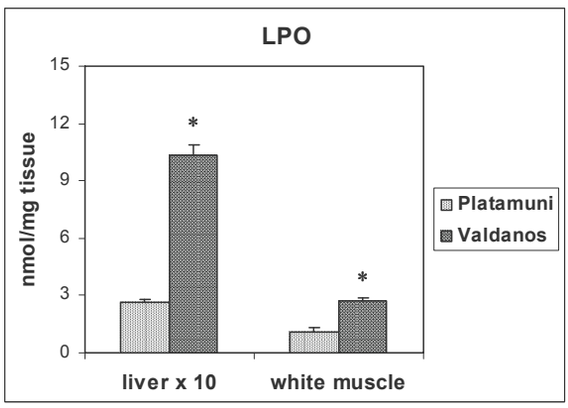

Fig. 2. Concentrations of lipid peroxides (LPO) in the liver and white muscle of hake (*Merluccius merluccius* L.) at Platamuni and Valdanos. The results are expressed as means ± SD (n = 8). *p<0.05, difference between localities.

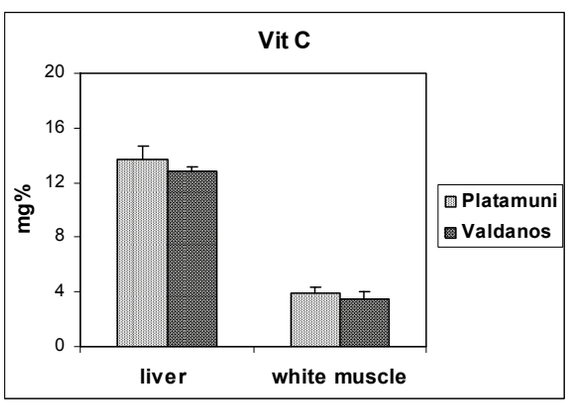

Fig. 3. Concentration of vitamin C (Vit C) in the liver and white muscle of hake (*Merluccius merluccius* L.) at Platamuni and Valdanos. The results are expressed as means ± SD (n = 8). *p<0.05, difference between localities.

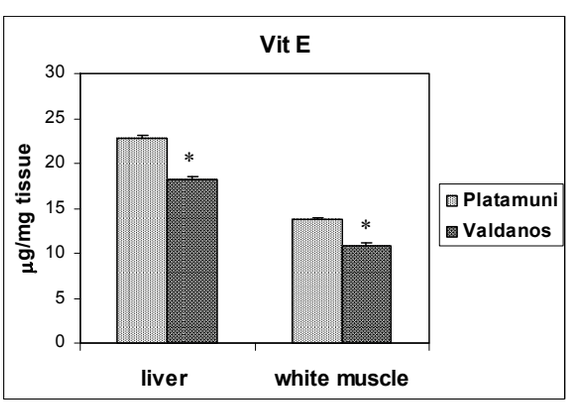

Fig. 4. Concentration of vitamin E (Vit E) in the liver and white muscle of hake (*Merluccius merluccius* L.) at Platamuni and Valdanos. Results are expressed as means ± SD (n = 8). *p<0.05, difference between localities.

ed in Fig. 3. Significantly lower values were recorded in white muscle than in the liver at both localities.

As for Vit E, its concentration was lower in white muscle than in the liver at both localities (Fig. 4). In contrast to LPO, the concentration of Vit E in both tissues was lower at Valdanos than at Platamuni ($p<0.05$).

DISCUSSION

Numerous investigations on aquatic organisms have been performed in order to provide data for comparative studies and establish environmental effects or influence of different pollutants on the AOS (Winston and Di Giulio, 1991). Monitoring of oxidative stress parameters in tissues of aquatic organisms is a well-established procedure in assessing the status of the environment (Van der Oost et al., 2003).

Many environmental pollutants are capable of inducing oxidative stress in aquatic animals, including fish. Oxidative stress resulting from the production of ROS has become a subject of considerable interest in the field of ecotoxicology (Kappus, 1987).

Production of ROS, oxidative processes, and the AOS in aquatic organisms are directly linked with anthropogenic influences and the effects of abiotic factors (salinity, concentration of oxygen in the water, and environmental temperature) or with the metabolic activity of some tissues. Water is a very unstable environment in respect to physico-chemical characteristics, and fish are exposed to diurnal and seasonal changes as well (Filho et al., 1993; Van der Oost et al., 2003).

Our results showed that the concentration of dissolved oxygen was not uniform, higher values being recorded at depths of 40 m (Platamuni) and 30 m (Valdanos) owing to increased seasonal production of phytoplankton. The concentration of dissolved oxygen decreased with increase of water temperature toward the surface (0 m) (Table 1).

It is known that oxygen is not uniformly distributed in seawater (Janssens et al., 2000). Many

regions of the world ocean are characterized by higher concentration of oxygen in the eutrophic zone and concentration decrease with depth. Numerous deep-sea aerobic organisms are faced with the problem of living in conditions of lower oxygen concentration. They solve this problem with different physiological adaptations, such as decrease in capacity of the AOS with depth increase (Childress, 1995; Childress and Siebel, 1998). Temperature also decreases with depth, being lower in deeper layers of water than in upper layers. On the other hand, *in vitro* experiments showed that the concentration of LPO decreased with elevation of water temperature. Most studies involving alteration of antioxidant defenses in aquatic animals have focused on stress induced by salinity changes, temperature fluctuations, hypoxia, etc. (Van der Oost et al., 2003; Žikić et al., 2006).

Concentrations of nitrites, nitrates and detergents in the water were higher at Valdanos than at Platamuni. This indicated increased water pollution at the of Valdanos locality (Table 2).

In an aquatic environment, despite the presence of the constitutive AOS or one that is enhanced, increased levels of oxidative damage will occur in organisms exposed to contaminants that stimulate the production of ROS (Livingstone, 2001). This increased ROS production and subsequent oxidative damage has been associated with pollutant-mediated mechanisms of toxicity in the liver of fish (Förlin et al., 1995). Environmental contaminants may enhance oxidative stress in aquatic organisms, e.g., highly elevated rates of ideopathic lesions and neoplasia among fish inhabiting polluted environments are increasingly related to oxidative stress associated with environmental pollution (Winston and Giulio, 1991; Livingstone, 2001).

Lipid peroxidation or the oxidation of polyunsaturated fatty acids has been observed and used to analyze the effect of pollutants (Hageman et al., 1992; Rudneva, 1997). Indeed, increased LPO levels have been recorded in the liver of organisms exposed to contaminants such as PAHs, PCBs, and others (Baumard et al., 1998; Solé, 2000; Livingstone, 2001).

Our results show that all the investigated oxidative stress parameters were significantly higher in the liver than in white muscle (Figs. 2-4). Such results may be a consequence of higher metabolic and antioxidative activity in the liver than in white muscle.

Our results showed that the concentration of LPO was higher in the liver than in white muscle of hake. These differences of oxidative stress parameters point to tissue specificity, which is a consequence of different metabolic and antioxidative activities. Processes of biotransformation of exogenous agents (harmful pollutants) also occur in the liver, which leads to the production of ROS and reactive nytrogen species (RNS) as side products of degradation and increased oxidative stress. That is the reason why prooxidative/antioxidative metabolism is significantly higher in the liver.

Our results confirm findings of other researchers indicating tissue differences in the AOS (Filho, 1996; Van der Oost et al., 2003; Žikić et al., 2006).

The higher concentrations of nitrites, nitrates, and detergents in the water of Valdanos (Table 2) may have been a direct result of oxidative stress and loading of LPO in tissues of hake (Fig. 2). The concentrations of Vit C and Vit E were significantly lower in white muscle than in the liver at both localities (Figs. 3 and 4). At the same time, decrease of Vit E concentration in contrast to increase of LPO may be attributable to an oxidative-antioxidative mechanism. In fact, as a liposoluble antioxidant, Vit E is the first step in the prevention of oxidative processes in the lipid bilayer of cell membranes and other lipid constituents of organisms. By accepting free electrons from chain reactions during lipid peroxidation, Vit E is converted into the α-tocopherol radical, which is reduced in reaction with Vit C, coenzyme Q, or GSH (Halliwell and Gutteridge, 2007). Thus, increase of LPO concentration points to oxidative stress, while decrease in the concentrations of Vit C and Vit E indicates their involvement in the prevention of oxidative damage. These differences of oxidative stress parameters point to tissue specificity, which is a consequence of different metabolic

and antioxidative activity. Our results showed that oxidative stress in the examined tissues was greater in the liver than in white muscle.

Our results also showed variation in oxidative stress parameters due to locality. The LPO concentration was higher (and the Vit E concentration lower) in the liver of fish caught at Valdanos compared to specimens collected at Platamuni. Greater oxidative stress in tissues of the Valdanos fish could have been caused by higher concentrations of nitrates, nitrites, and detergents in the seawater in comparison with the seawater at Platamuni (Table 2). These results point to greater pollution of the seawater at the Valdanos locality. Elevated concentrations of nitrates and nitrites indicate an increased level of organic pollution, which could be the reason for the increased rate of oxidative stress in the analyzed tissues of fish at Valdanos.

Many classes of environmental contaminants (or their metabolites) are known to enhance the intracellular formation of ROS, causing oxidative damage to biological systems. The uptake of these pollutants by fish can occur via sediments and particulate material suspended in the water-column, as well as through food sources. The major routes of uptake depend on the dietary and ecological life-styles of particular organisms (Livingstone, 1998, 2001). Nitrite is formed from ammonia and may be accumulated in aquatic systems as a result of an excess of nitrifying activity of the bacteria *Nitrosomonas* sp. and *Nitrobacter* sp. High levels of nitrite in water are potential factors that trigger stress in aquatic organisms (Wang et al., 2004).

Different LPO and Vit E concentrations in the tissues of hake at the selected localities could have been a consequence of oxidative-antioxidative metabolism as part of the mechanism of lipid peroxidation, i.e., antioxidative activities of nonenzymatic components of the AOS.

In conclusion, it can be asserted that concentrations of the investigated antioxidant compounds (Vit C and Vit E) and LPO in the tissues of hake are good markers for pollution biomonitoring and follow-up analysis of environmental conditions. Differences in oxidative stress parameters in the tissues were caused by different metabolic and antioxidative activities and can be considered functional responses to the variations in physical and chemical parameters of the environment and the presence of pollutants.

Acknowledgments — This study was supported by the Ministry of Science of the Republic of Serbia, Grant No. 143035B. The authors thank Radmila Paunović-Štajn for proofreading the manuscript.

REFERENCES

Baumard, P., Budzinski, H., and *P. Garrigues* (1998). Polycyclic aromatic hydrocarbons in sediments and mussels of the western Mediterranean Sea. *Environ. Toxicol. Chem.* **17,** 765-776.

Childress, J. J. (1995). Are there physiological and biochemical adaptations of metabolism in deep-sea animals. *Trends Ecol. Evol.* **10**, 30-36.

Childress, J. J., and *B. A. Siebel* (1998). Life at stable oxygen levels: adaptations of animals to oceanic oxygen minimum layers. *J. Exp. Biol.* **201,** 1223-1232.

Desai, I. D. (1984). Vitamin E analysis methods for animal tissues. *Methods Enzymol.* **105,** 138-147.

Fessard, V., and *D. R. Livingstone* (1998). Development of western analysis of oxidized proteins as a biomarker of oxidative damage in liver of fish. *Mar. Environ. Res.* **46,** 407-410.

Filho, D. W. (1996). Fish antioxidant defenses–a comparative approach. *Braz. J. Med. Biol. Res.* **29**, 1735-1742.

Förlin, L., Lemaire, P., and *D. R. Livingstone* (1995). Comparative studies of hepatic xenobiotic metabolizing and antioxidant enzymes in different fish species. *Mar. Environ. Res.* **39,** 201-204.

Hageman, J. J., Bast, A., and *N. P. E. Vermeulen* (1992). Monitoring of oxidative free radical damage *in vivo*: analytical aspects. *Chem. Biol. Interact.* **82,** 243-293.

Halliwell, B., and *J. M. C. Gutteridge* (2007). Free Radicals in Biology and Medicine, Fourth Edition, Oxford University Press.

Janssens, B. J., Childress, J. J., Baguet, F., and *J. F. Rees* (2000). Reduced enzymatic antioxidative defence in deep-sea fishes. *J. Exp. Biol.* **203**, 3717-3725.

Kappus, H. (1987). Oxidative stress in chemical toxicity. *Arch. Toxicol.* **60**, 144–149.

Livingstone, D. R. (1998). The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in biotransformation by invertebrates and fish. *Comp. Biochem. Physiol.* **120**, 43-49.

Livingstone, D. R. (2001). Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Mar. Pollut. Bull.* **42,** 656-666.

Matés, M. (2000). Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology* **153**, 83-104.

Ohkawa, H., Okishi, N., and *K. Yagi* (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351-358.

Omaye, S. T., Turnbull, J. D., and *H. E. Sauberlich* (1979). Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. *Methods Enzymol.* **62,** 3-14.

Regoli, F., Pellegrini, D., Winston, G. W., Gorbi, S., Giuliani, S., Virno-Lamberti, C., and *S. Bompadre* (2002). Application of biomarkers for assessing the biological impact of dredged materials in the Mediterranean: the relationship between antioxidant responses and susceptibility to oxidative stress in the red mullet (*Mullus barbatus*). *Mar. Pollut. Bull.* **44**, 912-922.

Rudneva, I. I. (1997). Blood antioxidant system of Black Sea elasmobranch and teleosts. *Comp. Biochem. Physiol.* **118C,** 255-260.

Rump, H. H., and *H. Krist* (1992). Nitrate, In: *Laboratory Manuel for the Examination of Water, Waste Water, and Soil* (Eds. H. H. Rump and H. Krist), p. 116. VCH Verlagsgesellschaft, Weinheim.

Rump, H. H., and *H. Krist* (1992). Nitrite, In: *Laboratory Manuel for the Examination of Water, Waste Water, and Soil* (Eds. H. H. Rump and H. Krist), p. 117. VCH Verlagsgesellschaft, Weinheim.

Rump, H. H., and *H. Krist* (1992). Surfactants, In: *Laboratory Manuel for the Examination of Water, Waste Water, and Soil* (Eds. H. H. Rump and H. Krist), p. 132. VCH Verlagsgesellschaft, Weinheim.

Solé, M. (2000). Assessment of the results of chemical analyses combined with the biological effects of organic pollution on mussels. *Trends Anal. Chem.* **19,** 1-9.

Van der Oost, R., Beyer, J., and *N. P. E. Vermeulen* (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Pharm.* **13,** 57-149.

Winston, G. W., and *R. T. Di Giulio* (1991). Prooxidant and antioxidant mechanisms in aquatic organisms. *Aquat. Toxicol.* **19,** 137-161.

Wang, W. N., Wang, A. L., Zhang, Y. J., Li, Z. H., Wang, J. X., and *R. Y. Sun* (2004). Effects of nitrite on lethal and immune response of *Macrobrachium nipponense*. *Agriculture* **232**, 679-686.

Žikić, R. V., Ognjanović, B. I., Marković, S. D., Pavlović, S. Z., Mihajlović, R. P., Saičić, Z. S., and *A. Š. Štajn* (2006). Lipid peroxidation and level of antioxidant compounds (vitamins E and C) in the liver and white muscle of red mullet (*Mullus barbatus*) from the Adriatic Sea. *Period. Biol.* **108,** 139-143.

КОНЦЕНТРАЦИЈА АНТИОКСИДАЦИОНИХ КОМПОНЕНТИ И ЛИПИДНИХ ПЕРОКСИДА У ЈЕТРИ И МИШИЋИМА ОСЛИЋА *(MERLUCCIUS MERLUCCIUS* L.) ИЗ ЈАДРАНСКОГ МОРА

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Током 2003. године узорковане су јединке морске рибе - ослића (*Merluccius merluccius* L.) из вода са локалитета Платамуни и Валданос (Црна Гора, јужни Јадран) у пролећном периоду. Добијени резултати су показали веће концентрације LP, Vit C и Vit E у јетри ослића у односу на бело мишићно ткиво. Концентрација LP је била већа, а Vit E мања у оба испитивана ткива ослића у водама локалитета Валданос у поређењу са Платамуни. Овакве промене параметара оксидационог стреса последица су промена температуре, као и повећаних концентрација нитрита, нитрата и детерцената у водама локалитета Валданос у односу на Платамуне.