7

Environmental Pollution and Oxidative Stress in Fish

Oksana B. Stoliar[1] and Volodymyr I. Lushchak[2]
[1]*Ternopil National Pedagogical University*
Named after Volodymyr Hnatiuk
[2]*Precarpathian National University*
Named after Vassyl Stefanyk
Ukraine

1. Introduction

Living systems encounter a variety of stresses during their continuous interaction with environment. Environmentally-induced stresses frequently activate the endogenous production of reactive oxygen species (ROS), most of which are generated as side products of tissue respiration. Hence, constant exposure to stressors may enhance ROS-mediated oxidative damage. Increased number of agricultural and industrial wastes enter aquatic environment and being taken up by aquatic organisms induce plural changes. Some of them directly enhance ROS formation whereas others act indirectly, for example, by binding with cellular thiols and reducing antioxidant potential. Fish are particularly threatened by water pollution. The use of sentinel species in biomonitoring needs to be discussed due to different level of their vulnerability by environmental toxicants.

Oxidative stress is defined as a situation when steady-state ROS concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents (Lushchak, 2011). The activation of oxidative manifestations leads to the response of antioxidants, activation of expression of genes encoding antioxidant enzymes, elevation of the concentration of ROS scavengers. Nevertheless, there are considerable gaps in our knowledge on response to oxidative stress, particularly in the feral animals. Indeed, in field studies, wide spectrum of inter-site differences (higher, equal or lower activities of various antioxidant enzymes with tissue peculiarities and disbalance) have been observed in polluted compared to clean areas reflecting both mild stress conditions of the location or strong oxidative damage. Different models of the aquatic animal response, therefore, need to be analysed before conclusions can be drawn. In any case, the integrated approach with the appreciation of balance between prooxidant manifestations and antioxidant defence (enzymatic and nonenzymatic) in biological systems needs to be a control point to assess toxic effects under stressful environmental conditions.

In field investigations, there are problems on selection of reference sites even in the cases when these sites were selected by generally appreciated criteria. It is clear that, given the spontaneous human activities, we will not be able to find a true reference site in some areas.

The practical use of oxidative stress markers in fish is also connected to significant difficulties, because of their considerable seasonal variation. Moreover, animals can adapt to low pollution conditions and, under these circumstances, seasonal factors might affect biomarker responses to a greater extent than pollution variations. Therefore, with the aim to standardize the results and avoid the effect of adaptation to chronically polluted environment, caged organisms, including fish are used for biomonitoring. However, for fish, the responses of caged specimens are studied sparsely.

This chapter summarizes current knowledge on oxidative stress responses of fish in field conditions and their potential for environmental toxicology studies and biomonitoring.

2. Peculiarities of field pollution as a stressful factor

Unlike model oxidative stress that is usually caused by singular substance acting under controlled laboratory conditions (concentration, period) (Bagnyukova et al., 2005, 2006, 2007; Kubrak et al., 2010; Lushchak et al., 2007, 2008, 2009a,b,c; Yi et al., 2007; Sun, et al., 2008), environmental impact is usually developed according to multiple stressor effects. Indeed, ecosystems are under the pressure of complex mixtures of contaminants released in the environment due to various human activities. They may originate from miscellaneous sources such as chemical and drug manufacture, domestic sewage, polymer and petrochemical-based industries, oil refineries, mining, glass blowing, battery manufacture and many others. Hydrological changes, hydromorphological degradation and invasive species also can contribute to the set of stressing factors (Amado et al., 2006; Sureda et al., 2006; dos Anjos et al., 2011). According to origin, two primary routes of pollution can be selected: (1) point-source pollution and (2) non-point-source pollution. **Point-source pollution** originates from discrete sources whose inputs into aquatic systems can often be defined in a spatially explicit manner. Examples of point-source pollution include industrial effluents (pulp and paper mills, steel plants, food processing plants), municipal sewage treatment plants and combined sewage-storm-water overflows, resource extraction (mining), and land disposal sites (landfill sites, industrial impoundments). In opposit, n**on-point-source pollution** originates from diverse poorly defined, diffuse sources that typically occur over broad geographical scales. Examples of non-point-source pollution include agricultural runoff (pesticides, pathogens, and fertilizers), storm-water and urban runoff, and atmospheric deposition (wet and dry deposition of persistent organic pollutants such as polychlorinated biphenyls (PCBs) and mercury) (Ritter et al., 2002). Basically the most prevalent xenobiotics arising out of agricultural and industrial activities are pesticides and trace metal ions.

Two examples will illustrate the complexity of natural water pollution that gives very poor prediction of its impact on biota. The first one is connected with industrial area, in the estuary near the city Göteborg, at the Swedish Western coast. The analyses of the sediments in this area showed high concentrations of anthropogenic compounds, such as polycyclic aaromatic hydrocarbons (PAHs), PCBs, tributyl tin, and dioxin, as well as transition metal ions. Toxicological analyses of the sediments in the Göteborg harbor area indicated that the levels of pollutants high enough to exert harmful effects on the ecosystem. To make the harbor more accessible and to secure future oil imports to Sweden, the dredging of the fairways Göteborg harbor was completed during 2003 (Sturve et al., 2005). In result, biomarker responses in the eelpout (*Zoarces viviparus*) sampled both before and during the

dredging indicated that fish were chronically affected by pollutants compared to those in a reference area. However, the results during the dredging activities clearly show that fish were even more affected by remobilized pollutants. The second example illustrates the composition of three aquatic bodies in generally low industrially disturbed area in Western Ukraine during three seasons. However, spontaneous pollution together with the use of collapse of water purification systems constitutes further pressures for the aquatic environment.

The represented example of physico-chemical analyses of the water from three typical field sites in Western Ukraine, forestry site F near the spring of the river, agricultural site A in the lower part of the river, and forestry site N on the bank of the cooling pond of Nuclear Power Plant, showed that run-off and sewage discharges, industrial processes could be important sources of phosphates, phenol, nitrites, ammonium to surface waters at sites F and A and trace metal ions, particularly Cd and Cu, are typical pollutants at site N. While site A is proved as the most polluted river site in the region due to information from the Public Administration of the Environmental Protection, the high level of anthropogenic impact at site F near the municipal water inlet of the city was unexpected. High pollution caused by Cu and Cd at site N, where agricultural activity is low, may be explained by specific composition of the sewages from Nuclear Power Plant. The results indicate that the levels of Cu, Cd, nitrite, and phosphate even exceed environmental quality levels (EU Council Directive 98/83/EC) (http://www.emwis.org/IFP/law_EU.htm) (Table 1). In any case, studied chemical parameters in general terms confirm a plurality of compounds which mutual effect could probably induce toxic effects to aquatic organisms. Moreover, the relativity of the concept of the reference site is clear, even though the reference site was selected by generally accepted criteria. Page: 133 This needs to be taken into consideration at examination of environmental impacts.

Hence, aquatic environment is a sink for many environmental contaminants which can be absorbed by aquatic organisms leading to disturbing of antioxidant/prooxidant balance in fish (Lackner, 1998; Livingstone, 2001, Lushchak, 2011). That may cause oxidative stress, determined as a state when antioxidant defenses are overcomed by prooxidant forces (Livingstone, 2001, 1991; Livingstone, 2001,). Moreover, dependently on the source of pollutant, steady-state ROS concentration can be enchanced transiently or chronically, disturbing cellular metabolism and its regulation and damaging cellular constituents (Lushchak, 2011). Synergistic or antagonistic effects of mixtures of pollutants are hardly interpreted and predicted exclusively from the chemical analyses; some contaminants are substantially accumulated in specific tissues without recorded toxic effects (Viarengo & Nott, 1993), while others demonstrate high toxicity even at low levels. So, oxidative stress response of fish cannot be predicted using data on the level of certain pollutants in their tissues. In some cases, the correspondence between these characteristics was reported, for example, the 22-fold increase in PCB concentrations in white muscle of brown bullhead (*Ameriurus nebulosus*) was accompanied by disturbance of antioxidant defence in the tissues of this fish compared to fish from the nonpolluted site (Otto & Moon, 1996). On the other

hand, Machala and colleagues (1997; 2001) did not find any correlation between markers of oxidative stress in liver of chub (*Leuciscus cephalus*) and concentrations of specific contaminants, namely organochlorine compounds, PAHs, and metals in several sampling sites of a river with various pollution types and rates. So only the direct determining of the

stress response, namely oxidative stress markers and may be some others, in biological systems has become the most adequate tool for early warning in environmental toxicology studies (Valavanidis et al., 2006).

Parameter	Site	Spring	Summer	Autumn
Phosphates, µM	F	16.7±2.1*	5.1±0.4^{b*}	20.7±2.2^{b*}
	A	15.1±1.7*	10.1±0.9^{a,b*}	24.5±2.5^{b*}
	N	1.0±0.1^{a}	3.9±0.3^{a,b}	1.9±0.1^{a,b}
Nitrites, mg $N\text{-}NO_2^{-}\cdot L^{-1}$	F	2.2±0.2*	6.4±0.5^{b*}	4.1±0.4^{b*}
	A	0.4±0.04^{a}	1.4±0.2^{a,b*}	1.4±0.1^{a,b*}
	N	0.4±0.04^{a}	1.4±0.1^{a*}	0.9±0.1^{a,b*}
Nitrates, mg $N\text{-}NO_2^{-}\cdot L^{-1}$	F	2.0±0.2	0.3±0.0^{b}	0.1±0.0^{b}
	A	2.9±0.3^{a}	12.8±1.2^{a,b}	3.0±0.4^{a}
	N	0.9±0.1^{a}	0.1±0.0^{a,b}	0.1±0.0^{b}
NH_4^{+}, $mg\cdot L^{-1}$	F	6.4±0.8*	2.9±0.3^{b*}	3.6±0.3*
	A	2.8±0.3^{a*}	1.0±0.1$^{a.b*}$	0.1±0.0^{a,b}
	N	1.6±0.2^{a*}	1.9±0.2^{a*}	0.1±0.0^{a,b}
Oxidisability, mg $O_2\cdot L^{-1}$	F	47.8±2.1*	24.8±2.2^{b*}	17.7±2.1^{b*}
	A	30.1±3.1^{a*}	8.5±0.5^{a,b*}	8.9±0.8^{a,b*}
	N	11.4±1.2^{a*}	41.2±3.2^{a,b*}	17.0±1.6^{b*}
Hardness, mM $CaCO_3$	F	1.1±0.1	1.1±0.1	0.5±0.1^{b}
	A	1.3±0.1^{a*}	1.7±0.2^{a,b*}	1.0±0.1^{a,b}
	N	0.5±0.1^{a}	0.8±0.1^{a,b}	0.9±0.1^{a,b}
Phenol, $\mu g\cdot L^{-1}$	F	3.6±0.4*	0.9±0.1^{b}	1.5±0.2^{b*}
	A	0.8±0.1^{a}	1.7±0.2^{a,b*}	4.9±0.5*
	N	0.6±0.1^{a}	0.7±0.1^{a}	0.8±0.1
Cu, $\mu g\cdot L^{-1}$	F	2.5±0.2	1.3±0.1^{b}	2.1±0.2^{b}
	A	3.5±0.3^{a*}	1.9±0.2^{a,b}	6.3±0.6^{a,b*}
	N	7.5±0.8^{a*}	5.3±0.5^{a,b*}	5.1±0.5^{a,b*}
Cd, $\mu g\cdot L^{-1}$	F	2.2±0.2	4.3±0.4^{b}	3.6±0.4^{b}
	A	2.9±0.3^{a}	4.1±0.4^{b}	3.4±0.3
	N	2.8±0.3^{a}	8.9±0.7^{a,b*}	6.3±0.6^{a,b*}

Table 1. Physico-chemical parameters of water in three seasons, M±SD, n=3 (from Falfushynska et al., 2010c with permission): *Exceeding of maximum permitted concentration allowed for the protection of freshwater aquatic life. The values are expressed as the mean ± SD; ^{a}Significantly different from spring value at the same site with $P < 0.05$; ^{b}significantly different from site F value in the same season with $P < 0.05$.

Xenobiotic-induced stress responses can be broadly categorized as intoxication and detoxication signals. Intoxication signals manifest debilitating phenomena while the detoxication signals are adaptive in nature and provide protection to the biological systems when affronted with toxic xenobiotics (Bhattachary, 2001). Dependently on the intensity and duration of toxicant effect and resistance of the studied organism, different manifestations of the oxidative stress can be expected. However, there are considerable gaps in our understanding of oxidative stress response mechanisms in the feral animals (Valavanidis et al., 2006). The long-term effect of pollutants, typical for chronically and heavily polluted areas, the enhancement of ROS level and perturbation of antioxidant efficiency often

prelude the onset of significant alterations like protein and DNA damage, lipid peroxidation (LPO) and enzyme inhibition (Winston & Di Giulio, 1991). Fish are particularly threatened by aquatic pollution, and the environmental stress they face may help to shape their ecology, evolution, or biological systems (Padmini, 2010).

3. Oxidative manifestations: Reactive oxygen species, damage to lipids, proteins and DNA

When the effect of environmental pollution on the antioxidant defence is elucidated, the exceeding of the resiliency of this system, and consequently, oxidative stress could be approved only basing on the elevations of the rate of oxidative manifestations. The expression of specific lesions known to arise specifically at oxidative stress, e.g. lipid peroxidation (membrane damage), oxidized bases in DNA and accumulation of lipofuscin pigments were found in many aquatic animals exposed to contaminants (Winston, 1991). However, whilst in the laboratory a wide spectrum of these indices is measured, only single parameter is often explored in Environmental Risk Assessment (ERA). In any case, the rate of oxidative damage is the control point of the effective adaptation to oxidative stress.

Lipid peroxidation or oxidation of polyunsaturated fatty acids, measured usually as a level of thiobarbituric acid reactive substances (TBARS), has been used most frequently to analyse the effect of pollutants (Livingstone, 2001; Lushchak et al., 2007, 2008, 2009 a, b, c, 2011). The elevated LPO in fish from heavily polluted field sites was observed (Ferreira et al., 2005; Farombi et al., 2007; Sanchez et al., 2007). For example, in the African catfish (*Clarias gariepinus*) from the Ogun River located close to major industries in the South Western part of Nigeria, TBARS levels of *C. gariepinus* were significantly higher in the liver, kidney, gills and heart by 177%, 102%, 168% and 71% respectively compared to that from fish farm which was considered as a reference site (Farombi et al., 2007). Elevated levels of LPO products were indicated in the blood of three cichlid fish species (*Oreochromis niloticus*, *Tilapia rendalli*, and *Geophagus brasiliensis*) from metal-contaminated site (Bonafé et al., 2008). Dorval et al. (2005) demonstrated higher level of hepatic LPO products in white sucker (*Catostomus commersoni*) from the river sites in Québec (Canada), impacted by agricultural chemicals. The killifish (*Fundulus heteroclitus*) inhabiting a creosote-polluted inlet of the Elizabeth River also exhibited higher LPO as compared to the reference population (Bacanskas et al., 2004). Differences of the level of TBARS in a liver of common carp (*Cyprinus carpio*) were also detected between fish from rural and industrial sites in relatively low polluted area in Western Ukraine (Falfushynska & Stoliar, 2009). In this study, the gills demonstrated significantly lower level of TBARS than the liver. Moreover, the correlation between TBARS levels and $O_2^{\bullet-}$ production was detected, confirming the conclusion on potential mechanisms of oxidative damage in fish. In crucian carp (*Carassius carassius)* from the similar two areas of comparison, in Western Ukraine (basin of the river Dnister) the differences in TBARS concentration were also observed. Especially high level of TBARS was observed in fish from industrial site in summer (Falfushynska et al., 2010).

Other examples demonstrate the absence of differences in TBARS concentration between fish from polluted and clean areas. In the study of Pandey et al. (2003), the differences of a broad set of antioxidants in gills, kidney and liver tissues in the Indian freshwater fish Wallago attu (Bl. & Schn.) from clean and polluted river sites were showed. But LPO

intensity assessed as TBARS level did not differ between two sites. Similar results were obtained by Huang et al. (2007) in the hepatopancreas of carp from polluted site, unlike the responses of other studied tissues, kidney and intestine. Despite differences in the activities of superoxide dismutase (SOD), glutathione transferase (GST) and glutathione peroxidase (GPx), the level of LPO was the same in the fish from two sites, indicating a stronger antioxidant capacity of this organ. In the series of materials devoted to the consequences of a dredging campaign in Göteborg harbor, Sweden, to eelpout (*Z. viviparous)*, as a sentinel species, TBARS did not show inter-site differences (Almroth et al., 2005). Similarly, in the liver of labrid fish (*Coris julis*) despite the variations in the antioxidant enzyme activities, there was no significant difference in TBARS concentrations (Sureda et al., 2006).

The end-products of LPO can be accumulated in lysosomes as insoluble granules containing autofluorescent pigments and are usually referred as lipofuscins. The indication of these pigments in the lysosome vacuolar system of fish hepatocytes also can be used for the assessment of the level of membrane LPO (Viarengo et al., 2007). The authors even recommend evaluation of lipofuscin levels as more valid characteristic of damage to lipids than TBARS. However, the corresponding studies with feral fish are scant and connected solely histological studies that do not permit to assess the oxidative stress response accurately. For example, histopathologic biomarkers in feral freshwater fish populations, namely redbreast sunfish (*Lepomis auratus*) and largemouth bass (*Micropterus salmoides*), showed the signs of lipofuscin accumulation only in polluted sites (Teh et al., 1997). The comparison of fish, barbels (*Barbus graellsii*) and bleaks (*Alburnus alburnus*) from areas located upstream and downstream of a mercury cell chlor-alkali plant on the Cinca River (NE Spain), demonstrated that the prominent elevation of the concentration of mercury in the tissues of fish sampled downstream of the plant (10- and 30-times higher in the muscle and liver of barbels downstream of the factory) was accompanied by significantly higher prevalence of liver pathologies consistent with the prooxidant effect of trace metals (Raldúa et al., 2007). Fifty paddlefish (*Polyodon spathula*) collected from two sites on the Ohio River, USA, demonstrated significantly higher organochlorine concentrations that even exceeded the Food and Drug Administration's action limit for chlordane (0.30 µg/g) than the fish from Cumberland River as a reference site. That was accompanied by the presence of hepatic hemosiderosis (Gundersen et al., 2000). However, concerning the signs of oxidative stress, these results represent only initial stage of study.

The formed free radicals cause various kinds of genotoxicity, particularly modifications to DNA bases. Most of the analytical assays have been focused on measuring of products of guanosine hydroxylation, namely 8-OHdG or 8-oxodG, and its free base 8-hydroxyguanine, in urine as an indirect method for oxidative damage by free radicals (Shigenaga & Ames, 1991). In the studies of feral fish these methods are presented scanty. A study with the fish (*Sparus aurata)* found that 8-oxodG determination in chromosomal DNA was a potentially useful biomarker of oxidative stress caused by urban and industrial environmental pollution (Rodriguez-Ariza et al., 1999). However, the proof of oxidative stress as a reason for genotoxicity is usually explored only in model studies, but not in ERA.

Proteins are considered to be important targets of free radical attack in cells (Eustace & Jay 2004; Almroth et al., 2008b; Lushchak, 2011) and thus compromise antioxidant defense, cellular function, and survival (Padmini, 2010). Therefore, protein oxidation, often under

investigation in proteomic studies, has been recently proposed as a biomarker of oxidative stress (Sheehan, 2006; Lushchak, 2011). In flounders, living in contaminated waters with xenobiotics, increased levels of oxidised proteins were reported (Fessard & Livingstone, 1998). Studies on dynamics showed that proteins can be oxidized before lipids or DNA in ROS-exposed cells (Du & Gebicki, 2004**)**. At the same time, many other factors can influence cell cycle and correspondingly, injury of proteins, related particularly to their oxidative damage. In any case, protein carbonyls (PC), so successfully explored in the studies of model oxidative stress in short-term laboratory experiments (Parvez & Raisuddin, 2005; Kubrak et al., 2010; Lushchak, 2011), are very seldomly used in the field studies for the assessment of environmental effects on fish. In the set of studies devoted to the consequences of a dredging campaign in Göteborg harbor, Sweden, to fish on the example of eelpout (*Z. viviparous*), as a sentinel species, monitor the impact of these events, the formation of additional carbonyl groups in proteins was studied (Almroth et al., 2005; 2008a). They confirmed that unlike LPO, PC, measured using an ELISA method, show differences between the reference and polluted sites in the field, as well as differences between time periods (before and during dredging and following the oil spill detected in this area were found. Particular results were reported for the fish from spontaneously polluted area. In the study with *C. carassius* from two field sites, significant differences of PC were indicated. However, lower level, particularly in the gills was found in fish from industrial site. The inter-site differences were opposite to that of the concentrations of GSH and metallothionein-related thiols (Falfushynska et al., 2010b). These data were interpreted from high tolerance of fish of genus Carassius to adverse conditions in the industrial site. On the other hand, for *C. carpio* higher levels of PC were detected in the liver and gills in two seasons in industrial site and only in summer the inter-site difference was opposite (Falfushynska et al., 2009).

Direct studies of intensity of ROS production in the field works are limited. The measurement of superoxide anion radical ($O_2^{\bullet-}$) production in the liver and gills of *C. carpio* from rural and industrial areas in Western Ukraine showed that the $O_2^{\bullet-}$ production was elevated at the industrial site in the majority of samples (Falfushynska & Stolyar, 2009). In this study, the negative correlation between Mn-SOD activity and $O_2^{\bullet-}$ production was observed and production of $O_2^{\bullet-}$ and TBARS correlated positively. In the compared groups, coherent changes of PC and $O_2^{\bullet-}$ levels were also detected in the liver and gills. In the study of *C. carassius* from two field sites, the significant difference of PC corresponded to variations in $O_2^{\bullet-}$ production, particularly in gills (Falfushynska et al., 2009b).

4. Non-enzymatic antioxidants: Glutathione and other scavengers

Non-enzymatic antioxidants are represented by ROS scavengers (both hydrophilic such as low-molecular mass thiols, glutathione (GSH), metallothioneins (MTs), ascorbic and uric acids, as well as lipophilic ones such as vitamin E and carotenoids (Viarengo et al., 2007). In the field studies, GSH is the most frequently studied scavenger. The hepatic ratio of oxidized to reduced glutathione (GSSG/GSH), a value used as an indicator of the "redox status" of the cell, may be appropriate biomarker for oxidative stress. However both GSH and GSSG levels have only been measured in a limited number of field studies (Van der Oost et al., 2003). It should be noted, that GSH can be involved in diverse processes different from related to free radical metabolism, within the cell and its variability can not be considered entirely in connection to oxidative stress.

In moiety of field studies, the elevated level of GSH was indicated in fish from polluted areas (Van der Oost et al., 2003). English sole (*Pleuronectes vetulus*) sampled from the Duwamish Waterway, a contaminated urban site in Puget Sound, Washington, showed increased GSH concentrations. The findings also indicated that induction of GSH synthesis from L-*Cys* was not a major factor in the increase of hepatic GSH in contaminant-exposed fish whereas it was not accompanied by changes in either L-Cys concentrations or gamma-glutamylcysteine synthetase activity (Nishimoto et al., 1995). A population of killifish (*F. heteroclitus*) inhabiting a creosote-polluted inlet of the Elizabeth River demonstrated higher total glutathione concentrations in adult hepatic tissue as compared to the reference population (Bacanskas et al., 2004). Study on the Indian freshwater fish *Wallago attu* (Bl. & Schn.) collected from two sites along the river Yamuna demonstrated that GSH in liver, kidney and gills was found to be substantially higher in the fish collected from more polluted site (Pandey, 2003). In the African catfish (*C. gariepinus*) from the Ogun River located close to major industries in the South Western part of Nigeria, GSH concentration was higher by 81%, 83% and 53% in the liver, kidney and heart, respectively, compared to that from the reference site (Farombi et al., 2007). Opposite response of GSH in the gills (lower by 44% in the fish from polluted area) was indicated in this study. At the same time, some field studies of contaminated sites did not detect differences in GSH level with the fish from the reference site (Jenner et al., 1990; Eufemia et al., 1997) or detected decreases (Otto & Moon, 1996; McFarland et al., 1999), and one study found an initial decrease followed by a sustained elevation (Steadman et al., 1991). Dorval et al. (2005) demonstrated that in hepatic and adrenal tissues of white sucker (*C. commersoni*) from a river that drains an agricultural region, GSH level was higher in reference site compared to fish from contaminated sites. Also, the three-spined stickleback (*Gasterosteus aculeatus* L.) sampled from heavily contaminated stream in France exhibited decreased liver GSH levels (Sanchez et al., 2007).

The increase of the ratio of GSSG/GSH in fish due to either direct scavenging of radicals or increased peroxidase activity can be expected. However, increases in total glutathione without increases in the percent of GSSG have been observed in channel catfish under the effects of sediments from polluted site (Di Giulio et al., 1993), in larvae of killifish (*F. heteroclitus*) inhabiting a Superfund site on the Elizabeth River (VA, USA) (Meyer et al., 2003). The levels of both, GSH and GSSG, in *C. carpio* from the river in Western Ukraine were higher in industrial site than in rural site and were more sensitive to spatial peculiarities in liver than in gills (Falfushynska & Stolyar, 2009). In the gills, GSH redox status was in the range 0.77−0.97, but in summer, it decreased to 0.54, with no difference between the sites. On the other hand, in *C. carassius* tissues high GSH levels and redox state of GSSG/GSH couple (particularly in the gills), were indicated in polluted site, which was expected to confer some advantages to this higly tolerant to environmental stresses fish (Falfushynska et al., 2010). Nevertheless, after 21 days in captivity, the fish from these two sites demonstrated opposite difference in GSH and GSSG levels (Falfushynska et al., 2011).

Some general relationships between Redox Index of GSH (RI GSH) calculated as the ratio of content GSH/(GSH+2GSSG) and other markers of oxidative stress were observed in field studies of fish (Falfushynska et al., 2010a), that can be explained by versatility of glutathione functions. The examples of discrepancies between RI GSH, LPO products and the activities of antioxidant enzymes in the field studies on fish were analysed in a review by Kelly et al. (1998). However, in any case, the elevation of GSH level and RI GSH can possess benefit to

fish in its ability to survive in polluted environment, while glutathione depletion is usually associated to enhancing of peroxidation processes in the cell membrane and leads to stress and can prominently contribute in hepatotoxicity (Viarengo et al., 2007).

Metallothioneins (MTs) are low molecular mass intracellular cysteine-enriched proteins that are suggested to be related to oxidative stress response. They constitute a diverse family of thermostable intracellular low molecular mass proteins, which are enriched in cysteines and bind metal ions in metal-thiolate clusters. Now they are considered to participate in the storage and detoxification of metal ions such as zinc, copper, and cadmium, and in the scavenging of ROS in diverse living organisms (Viarengo et al., 2007; Fernandes et al., 2008). However till now, despite a lot of publications devoted to MTs, their biological functions, relationship and necessity for the organism are discussed and adjusted. Some recent data demonstrate MTs induction in fish by other than metal pollutants, particularly in connection with oxidative stress (Paris-Palacios et al., 2000; 2003). However, several metals which are not essential for MTs (ferrum and nickel, for example) and also endocrine-disrupting chemicals have been known to be inhibitors of the MT gene transcription (Rhee et al., 2009; Lee et al., 2010).

Since their low redox potential, the metal-thiolate clusters of MTs can be easily reduced or oxidized *in vitro* and *in vivo* with concomitant binding/release of metal ions (Maret & Valee, 1998). It was found that MT levels in mammalian tissues under physiological conditions could be rather high to harbour important implications for MTs operation in Zn and redox metabolism (Capdevila et al., 1997; Capasso et al., 2005; Kelly et al., 2006). Different substances besides metals, such as fungicides fenhexamid, mancozeb, and also hydrogen peroxide, induce the elevation of MT content in fish (Viarengo et al., 1999; Cavaletto et al., 2002; Paris-Palacios et al., 2003; Mosleh et al., 2005; Kang, 2006). Inter-relation of elevated MT level with other stress proteins (catalase, GST) and negative relation to LPO products was confirmed for *C. carassius* over three seasons in a mixed polluted area characterized by spontaneous agricultural activities (Falfushynska et al., 2010a). On the other hand, studies on the *C. carpio,* have demonstrated the inability of their MTs to maintain high level of antioxidant defense, but elevated metal-binding capacity at the industrial site polluted by metals (Falfushynska & Stolyar, 2009; Falfushynska & Stoliar, 2009).

The participation of MTs in antioxidant defense can be explained by the high content of thiols and the particular metal binding/release dynamics intrinsic to these proteins (Atif et al., 2006; Monserrat et al., 2007; Viarengo et al., 2007). However, the effect of pollution on the relations between the metal binding and antioxidant functions of the MTs in aquatic animals has not been clarified (Chesman et al., 2007).

The participation of MTs in antioxidant defence can be also indirectly connected to the distribution of metal ions within the cell in deposited form and unbound, potentially toxic form. Complex field pollution can decrease the metal-binding function of MTs and promote the metal-related generation of ROS. With a view to include MTs in biomonitoring programs, simultaneous studies of the response of their expression, metal-binding capacity and thiol concentration in the field conditions must be undertaken. Metal-keeping function and possible participation in the antioxidant defense, expressed by concentrations of complexes of MT with metal ions (MT-Me) and total MTs (MT-SH), can be alternative/complementary characteristics of MTs in aquatic animals in complex field pollution.

Main discovery in the study of MTs expression is the distinguishing of basal and related emergency gene products both in vertebrate and invertebrate aquatic animals. In general, it seems that constitutive MT isoforms represented a primary action under 'less stressful' or 'sublethal' conditions whereas the activation of other isoforms became important under 'more stressful' or 'lethal' circumstances (Bargelloni et al., 1999; Lee et al., 2010). However, some limitations are still evident demonstrating that MTs are regulated at translational and transcriptional levels. The concentration MT proteins and their multiplicity do not appear to correlate always with constitutive expression of MTs. For example, two icefish species show the same number of MT genes despite a lack of expressed MTs at the protein level. In brown trout (*Salmo trutta*) from some polluted areas, the MT content was not elevated even when transcription of MT genes was enhanced (Hansen et al., 2006a, b). Therefore, with the aim to understand the importance of MTs in the response under oxidative stress, the study needs to combine the determination of gene expression at all levels including their properties.

There is limited evidence of induction of MTs due to exposure to environmental trace organic contaminants, and thus they usually are not discussed in corresponding literature. Moreover, they respond not only to anthropogenic pollution but also to physical stress and other natural factors. This makes them extremely difficult to be used as "stand-alone" biomarkers (Lam & Wu, 2003).

Studies addressed other potential ROS scavengers in the feral fish under stress conditions are very scarce. In a large-scale field study in Sweden, perch inhabiting water bodies contaminated with bleached kraft pulp mill effluents consistently displayed higher ascorbate concentrations than fish from the reference site (Andersson et al., 1988). α-Tocopherol (vitamin E), a lipid-soluble antioxidant, that is synthesized by plants, but required in the diets of animals, appears to play a major role in protecting of cell membranes from LPO (Stegeman et al., 1992). Measurements of these substances in tissues of feral fish are expected to be useful for ERA, especially within the set of oxidative stress indices.

5. Antioxidant enzymes

Antioxidant enzymes are included in the environmental pollution assessment because of their inducibility under conditions of mild oxidative stress and their potential role in adaptation to pollutant-induced stress. It is expected that they may be more sensitive at detecting of initial insults than such markers as histopathologies, changes in growth rates, etc. (Adams & Greeley, 2000). Laboratory studies confirmed that the measurement of changes in the expression of a large number of specific genes or activities of certain enzymes of antioxidant defence can be explored in an early warning system of toxicant exposure (Livingstone, 2001; Lushchak, 2011). However, if in the model studies, the enzyme response to disposable effect of toxic chemicals can depend on duration of pollutant influence, showing a bell-shaped relationship, in the field studies the results often indicate that antioxidant enzyme responses are transient and variable for different species, enzymes and chemicals (Viarengo et al., 2007). Obviously, the early warning can be used when temporal effect of pollution is expected. In the field studies, fish is frequently subjected to long-term exposure of number of factors. Therefore, the observed difference in the activity of antioxidant enzymes between two sites may be attributed both to their activation under mild stress conditions of the location or to their suppression due to strong oxidative damage. Different models of the aquatic animal response, therefore, need to be analysed

before conclusions can be drawn. Changes in gene expression may occur relatively quickly during an exposure, but the effect of long-term exposure on the expression may differ (Bagnyukova et al., 2005, 2006, 2007; Kubrak et al., 2010; Lushchak et al., 2007, 2008, 2009a, b, c). Additionally, contaminant-independent reference expression patterns should include natural fluctuations of the level. Indeed, in field studies, higher, equal or lower activities of various antioxidant enzymes have been observed in polluted compared to cleaner areas (Narbonne et al., 1999; Bonafé et al., 2008).

Typically, the battery of oxidative stress parameters in feral fish includes usually the activities of either SOD, or catalase. At least one of GSH-related enzymes is also often included in the study. Due to easily carried out and low-cost enzymatic tests, the assessment of catalase and GST activities has most often been used in biomonitoring programmes for fish (Romeo et al., 2000; Viarengo et al., 2007).

Whereas the field studies mostly belong to biomarker-type studies, the specification of antioxidant enzymes in them is limited. In according to this, it should be noted that including in the study the assessment of only one antioxidant enzyme and (desirable) its measurement only in single tissue/organ facilitates nominally the discussion of obtained data. In this case, the final conclusion concerning the indication of adverse effect is derived from the initial knowledge concerning the relative level of pollution (site in the lower stream of the river or situated close to the certain point of pollution). In any case, indication of changing enzyme activity comparing to selected reference site is considered as a sign of pollution effect. For example, significant differences in SOD, GPx, and catalase activities in the blood of three cichlid fish (*O. niloticus*, *T. rendalli*, and *G. brasiliensis*) taken during two seasons from site polluted by industrial effluents compare to reference site was used as an evidence of pollution in the area (Bonafé et al., 2008).

It is more difficult to give the multiple explanations of obtained results when a set of biomarkers includes several enzymes and they are studied in more than one tissue. In this case, discrepancy between different activities is usual attribute of oxidative stress in fish. In general, the elevated enzyme activity in fish from polluted areas is considered to be the main feature of compensatory response within its tolerance range and the lower its activity witness about the exceed of the resilience of this response.

Many biomarker-type studies have identified increases in antioxidant defenses in aquatic organisms (Collier & Varanasi, 1991; Stein et al., 1992; Rodriguez-Ariza et al., 1993; Livingstone et al., 1995; van der Oost et al., 1996; Eufemia et al., 1997; Stephensen et al., 2000). That is probably the result of both, physiological acclimation and/or genetic adaptation in the populations (Meyer et al., 2003). This activation is mainly connected to SOD, particularly in fish from sites contaminated with persistent organic substances (Buet et al., 2006). A field study at the Elizabeth River polluted by creosot demonstrated elevated Cu,Zn-SOD activity in feral spot (*Leiostomus xanthurus*) (Roberts et al., 1987).

The elevated SOD activity can be combined with the decrease or stable activity of the second main antioxidant enzyme, catalase. For example, in the field study *C. carpio* were collected in two sites of the upper Yellow River, and the results showed that in polluted site, SOD and GST activities were higher and catalase and also GPx activities were lower in almost each case of comparison (activities were determined in hepatopancreas, kidney and intestine) (Huang et al., 2007). The concerted elevation of SOD and GPx activities was indicated in

liver of sterlet (*Acipenser ruthenus* L.) collected from the Danube-oil refinery site compared to that from the reference site, while no differences were found in other studied enzymes (catalase, GST, the same as the enzymes of biotransformation in liver, aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transferase in serum) (Stanic et al., 2006). In the study of three populations of brown trout (*S. trutta*) exposed to elevated Cd and Zn or Cu levels in their natural environment, both metal-exposed groups had higher activities of SOD in liver compared to unexposed trout from reference site, and catalase activity in the liver was the same in all tree populations (Hansen et al., 2006a).

Disbalansed antioxidant activities were shown in the various oxidative stress biomarkers in the Indian freshwater fish *Wallago attu* (Bl. & Schn.) (Pandey et al., 2003). In this study, the fish from polluted river site possessed higher activities of SOD and xanthine oxidase (in liver, kidney and gills), glutathione reductase (GR) in liver and gills whilst catalase activity in both liver and kidney was found to be significantly lower when compared with values in tissues of fish collected from clean site.

In the African catfish (*C. gariepinus*) from the Ogun River located close to major industries in the South Western part of Nigeria, SOD activity was higher by 61% in the liver, 50% in the kidney and in the heart by 28 % compared to that from Agodi fish farm. The levels of GST activities in the liver, kidney and heart of this fish was higher by 62%, 72% and 37%, respectively. Only in the gills of fish from polluted area, a significantly lower SOD (44%) and GST (41%) activities were observed. On the contrary, there was 46%, 41%, 50% and 19% lower catalase activity in the liver, kidney, gills and heart, respectively (Farombi et al., 2007).

Low intensity, but prolonged effect of spontaneous sources of pollution can deplete SOD activity in fish tissues. That is examplified by very few cases stydied to date (Bacanskas et al., 2004, Pandey et al., 2008; Falfushynska & Stolyar, 2009; Falfushynska et al., 2010a). The decreased SOD activity along with increased $O_2^{\bullet-}$ levels suggests the weakness of antioxidant defences in common carp *C. carpio* at the chronically polluted industrial site (Falfushynska & Stolyar, 2009). Mn-SOD in *C. carpio* was both more abundant and more sensitive to local influence than Cu,Zn-SOD. Moreover, the importance of Mn-SOD was supported in this study by the showing the negative correlation between Mn-SOD activity and $O_2^{\bullet-}$ production. Similar site-related difference was obtained for SOD (total activity) of relatively more tolerant fish, *C. carassius*, from the same area (Falfushynska et al., 2010a).

The increase in catalase activity is often observed in the model experiments and also can occurre without relation to SOD responses and due to high pollutant impact (Üner et al., 2005; Moraes et al., 2007; Lushchak, 2011). The higher catalase activity was reported for the liver subcellular fractions of red mullet *Mullus barbatus* collected along the Western Mediterranean coast (the Northern Iberian Shelf). Moreover, only catalase activity was well related to pollution in the area and showed about doubled activities in four most contaminated sites in comparison with the reference sites. For SOD activity, significant difference among sampling sites were found in this study, but they had no clear relationship to the levels of studied pollutants. Additionally, no pollution- or site-related difference was observed for GPx activities (Se-dependent and total) (Porte et al., 2002). On the other hand, the study of fish *M. barbatus* from a coastal marine area of Salento Peninsula (Italy) indicated that catalase activity did not show any significant variation between animals sampled from

urbanized and conditionally uncontaminated sites, whilst acetylcholinesterase indicated the neurotoxicity of environment (Lionetto et al., 2003).

The depletion of catalase activity or its stability along with increment of SOD activity were reported (Pandey et al., 2003; Stanic et al., 2006; Huang et al., 2007), and even although catalase mRNA levels were higher in the exposed fish (Hansen et al., 2006a). Dorval and colleagues (2005) in hepatic and adrenal tissues of white sucker (*C. commersoni*) from a river Yamaska that drains an agricultural region in Québec (Canada) found that in fish from the contaminated sites catalase and GPx activities were lower than those in the fish from reference site.

In recent study (Falfushynska & Stolyar, 2009), the low catalase activity in *C. carpio* was attributed to high production of $O_2^{\bullet -}$, which has been reported to inhibit catalase in the case of excess of production (Kono & Fridovich, 1982). However, the negative correlations between them were not regular in three seasons. Two-fold higher catalase activity was found in the liver and gills of carps from industrially polluted site as compare to reference site. Principal component analysis showed that catalase activity was not included in the significantly important set of markers unlike other oxidative stress indexes (Fig. 1).

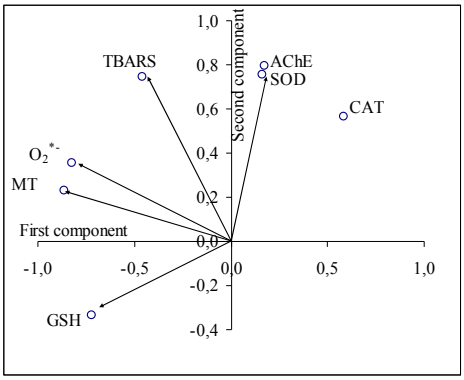


Fig. 1. Principal component analysis of the parameter data set in the gills of common carp from rural (and industrial sites from the river Seret in Western Ukraine. Parameters: activity of SOD, catalase; AChE, level of MT, GSH, TBARS, and $O_2^{\bullet -}$ production. The arrows indicate biomarkers having significant factorial weights > 0.7 (from Falfushynska & Stolyar, 2009 with pemission).

It seems that catalase activation can be considered as a last refuge of antioxidant defense in the feral fish. The particular catalase role in the antioxidant defense of feral fish was grounded by Porte et al. (2002), basing on the information on its activation by H_2O_2 at high concentrations. They suggested that catalase normally plays a relatively minor role in H_2O_2 catabolism at low rates of peroxide generation, but it becomes indispensable when the rate of H_2O_2 production is enhanced, for example, at oxidative stress. Comparatively higher stability of GPX activity reported in this and other studies can result from its dependence on both H_2O_2 and lipid peroxides, as substrates of decomposition. Therefore, it is possible that GPX activity would maintain normal cell functions, whereas catalase would form part of a stress-response mechanism (Janssens et al., 2000). Godin & Garnett (1992) found

compensatory relationship between catalase and GPX activities: low GPX activity was combined with high catalase activity.

Coordinated activation of antioxidant enzymes in reported sparse can be explained by the possible genetic adaptation to specific aquatic environment. Higher activities of GST, GPx and GR were detected in liver of teleost *C. julis* from two stations with seagrass Caulerpa species that produce toxic methabolite Caulerpenyne, as compare to fish from the area where Caulerpa species were absent. At that, no statistical difference was found in catalase activity between the groups (Sureda et al., 2006). However, in the case of worsening of environmental conditions caused by dredging of contaminated sediments, high activities of catalase, GR and GPx in red mullet *M. barbatus* sampled at a disposal site for dredged sediments was demonstrated by Regoli and colleagues (2002).

Including of different forms of enzymes in the study demonstrated variability of their responses to the environmental pollution. This is mostly connected with SOD (Roberts et al., 1987) and GST (Porte et al., 2002) and is obvious in relation to different genetic origin, location within the cell and function of individual/specific enzyme isoforms. Meyer et al. (2003) stressed that the study of spot (*L. xanthurus*) (Roberts et al., 1987) and killifish (larvae of *F. heteroclitus*) from the same polluted area demonstrated different species-specific sensitivity of Mn-SOD and Cu,Zn-SOD activation. Connecting tissue specificity of enzymes studied in field experiments, the enzymes of liver are considered to be less sensitive to some kinds of pollution than other tissues, because of the best antioxidant adaptation in the liver of fish, since despite the variations in the antioxidant enzyme activities, there was no significant difference in malondialdehyde concentration (Sureda et al., 2006).

Only a few field studies described the modulation of fish GR activity by the chemical stress. In the study of Machala et al. (1997), the GR activity was significantly higher and appeared to be a relevant biochemical marker of exposure to persistent chlorinated contaminants. However, Bainy and colleagues (1996) reported a decrease in hepatic GR activity in Nile tilapia collected at a PCB- and hexachlorocyclohexane-contaminated sampling site.

Sometimes, the attempts to find the relation between the accumulation of toxic substances in the tissues and antioxidant enzyme activities are successful. For example, muscle concentrations of PCB compounds as well as biliary levels of PAH metabolites showed that catalase activity, but not other ones, was well related to PCB body burden (Porte et al., 2002). Relatinship between the level of PCB in white muscle of brown bullhead (*A. nebulosus*) and cytosolic SOD activity in the kidney of fish from the polluted site was reported (Otto & Moon, 1996). However, catalase activity in the kidney, GPx activity in the red and white muscle, and total glutathione in the liver, kidney, and white muscle were decreased relative to fish from the nonpolluted site. The measuring of CAT activity in liver subcellular fractions together with markers of biotransformation, namely, 7-ethoxyresorufin *O*-deethylase (EROD) and UDP-glucuronosyltransferase (UGT), in two different fish species, the four-spotted megrim (*Lepidorhombus boscii*) and the pouting (*Trisopterus luscus*) collected along the Northern Iberian coast, showed a good positive correlation with the amount of alkylphenols and 1-naphthol accumulated in the tissues for EROD and UGT but not for catalase activities (Fernandes et al., 2008). In the study of Machala et al. (1997) the activities of a set of GSH-related enzymes in common carp from several field sites was analysed in concert with the chemical analysis of organic contamination in five sampling sites in ponds.

In the ponds polluted mainly by PAHs and PCBs, the activation of glutathione-dependent enzymes, namely cytosolic GR and GST toward 1-chloro-2,4-dinitrobenzene, ethacrynic acid and 1,2-epoxy-3-(p-nitrophenoxy) propane, and microsomal GST was detected even when these substances were not revealed in the tissues of fish.

Special attention needs to be paid to GST, the GSH-dependent enzyme that promotes the reactions of conjugation in the II phase of the biotransformation of toxic substances, and also participates in the antioxidant defence due to the dependence on GSH and reduction of some peroxides. It is recommended as the biomarker of oxidative stress in fish (Viarengo et al., 2007). Indeed, among all connected antioxidant defence enzymes, GST is frequently activated at pollution. In the study of three species of cyprinids, barbel (*B. plebejus*), chub (*L. cephalus*), and Italian nase (*Chondrostoma söetta*), from two sites of the River Po, located upstream and downstream from the confluence of one of its middle-reach polluted tributaries, the River Lambro, with the exception of a higher GST enzyme activity of barbel from the downstream site, no significant modification was evident in GR, and GPx activities, despite the difference in specific markers of pollution by PAHs and PCB (Vigano et al., 1998). In the study of Machala et al. (1997) GST toward 1-chloro-2,4-dinitrobenzene, ethacrynic acid and 1,2-epoxy-3-(p-nitrophenoxy) propane, and microsomal GST demonstated higher activity in common carp from several field ponds polluted even to small extent (registered in sediments but not in muscle tissue) by PAHs and PCBs. However it can indicate its involvement in the processes of biotransformation more than in the detoxification of oxygen radicals. The toxicity of many exogenous compounds can be modulated by increased activity of GSTs. Effects of inducing agents on total hepatic GST activity have been observed in several fish species (Armstrong, 1990; George, 1994; Commandeur et al., 1997).

However, in several studies no significant differences or decrease of its activity were observed in fish from polluted sites. The effects of the extensive dredging in Göteborg harbor, Sweden on eelpout (*Z. viviparus*) sampled along a gradient, both before and during the dredging, indicated that eelpout were exposed to increased levels of pollutants indicated by elevated EROD activities, cytochrome P4501A levels and MTs gene expression. The prominent increase in GR activity in eelpout from the inner harbour during dredging was indicated, but no difference was observed in GST activities between the sites (Sturve et al., 2005).

The direct measurement of changes in the expression of a large number of genes related to the markers of specific kinds of pollution, namely vitellogenin, cytochrome P450, hsp-related genes began to be explored for early warning of pollution, for example, by xeno-estrogenes or oil in recent years (Tom & Auslander, 2005; dos Anjos et al., 2011). However, at the moment, among piscine genes which expression is increasingly utilized as environmental biomarkers, very few literature data are devoted to antioxidant defence enzymes in feral fish (Nikinmaa & Rees, 2005; Hansen et al., 2006a, b) despite in laboratory studies a successes in the study of corresponding genes were achieved (Cho et al., 2008; Woo et al., 2009; Lee et al., 2010).

The comparison of expression of specific genes (mRNA level) and the activities or protein levels of corresponding antioxidant enzymes did not confirm the relation between them. When three populations of brown trout (*S. trutta*) exposed to different metal ion levels in the natural environments were compared, the data indicated that chronic exposures to Cd, Zn

and/or Cu did not involve maintenance of high activities of SOD and CAT in gills, although SOD mRNA levels were higher in the Cd/Zn-exposed trout (Hansen et al., 2006a, b). Further, in livers, mRNA levels of SOD, CAT and GPx were higher in the metal-exposed trout, but only for SOD enzyme activity was higher in liver compared to the unexposed reference trout. That could result from posttranscriptional modifications. Based on these observations, the necessity to combine studies on transcript and protein levels in the evaluation of antioxidant response in feral fish seems to be desirable approach for field studies. To date, only the highest concentrations of Cd, Zn, Cr, corresponding to LC_{50} 96h (1000 ppb) for Japanese medaka (*Oryzias javanicus*), provoked the activation of transcription of SOD whereas Cu (0.1, 10, 100 ppb) did not demonstrate this effect after exposure for 24 h (Woo et al., 2009).

6. Effect of seasons and abiotic factors on the biomarkers of oxidative stress

There are many factors that may influence the response of fish antioxidant system to exposure to field contaminants. The main criticisms that have been presented against the biomarker approach is connected to high seasonal variability that is frequently found in field studies based on biomarkers, particularly of oxidative stress markers. Season-related biotic regularities include biotic factors, such as reproductive and metabolic status of fish and environmental conditions, such as food availability, oxygen level, temperature of water, salinity, photoperiod, etc. (Parihar et al., 1997; Buet et al., 2006; Da Rocha et al., 2009). Inter-species differences in antioxidant responses depend on the quantitative distribution of antioxidant defenses in the different tissues and sub-cellular compartments. Toxic and organ-specific ROS responses can be related to the anatomical localization, exposure routes and distribution of pollutants, as well as to defense capacity (Ahmad et al., 2006; Ruas et al., 2007; Da Rocha et al., 2009). Fasting conditions (Ferreira et al., 2005), location in the trophic chain affect bioaccumulation of toxic substances (Solé et al., 2009). Fish species that develop different mechanisms of tolerancy to environmental conditions are of particular interest. Some benefits of gills related to high level of GSH known for fish of genus Carassius were expected to supply enhanced antioxidant capacity of fish from chronically polluted site (Falfushynska et al., 2010a). The effect of these factors must be considered in the field studies including this phenomenon in diverse aquatic animals (Winston & Di Giulio, 1991; Lushchak, 2011). Species differences in the efficiency of antioxidant defenses may partly explain prevalence of pathological lesions observed with certain fish species (Vigano et al., 1998). In experiments with hepatocytes of male and female flounder, it was demonstrated that many responses to oxidative stress were sex-related (Winzer et al., 2001). Particularly, increased LPO was showed to be related to a variety of insults other than exposure to xenobiotics causing oxidative stress (Kappus, 1987).

Besides all pointed peculiarities, the combinations of site and season related dependences in aquatic animals have been studied rather extensively. Several studies with fish aimed to distinguish between the local pollution, site-related effects of abiotic factors, and common seasonal regularities. Sometimes they demonstrated that seasonal variation was stronger than relation to site (Niyogi et al., 2001; Almroth et al., 2005; Gorbi et al., 2005). For example, the measuring of a set of biomarkers of antioxidant defense, including catalase, GPx, GR, and GST activities, total glutathione concentration and Total Oxyradical Scavenging

Capacity (TOSC-assay) in the field study of the European eel (*Anguilla anguilla*) and the striped mullet (*M. cephalus*) in Mediterranean lagoons on a seasonal basis suggest that natural variation of responses were associated with seasone variation of both environmental and biological factors, mainly temperature and reproductive cycle which, however, differently affected these two species (Gorbi et al., 2005). Striped mullets exhibited the strongest variation in October at spawning, whereas eels were not influenced by a seasonal sexual maturation and showed more marked changes during summer, likely related to the elevated seawater temperature and light irradiance in the lagoon. Obviously, fish can adapt to low pollution conditions and, under these circumstances, seasonal factors might affect biomarker responses to a greater extent than induced by pollution.

Common seasonal regularities of GSH-related parameters (decreased GSH total concentration and increased GR activities from early to late summer, as well as after maintaining in the laboratory) was demonstrated for killifish (*F. heteroclitus*) from two populations, wild caught in reference and polluted sites of Elizabeth River, USA (Bacanskas et al., 2004). A clear seasonality was found for gill GSH levels of all studied species (*Micropogonias furnieri*, *Pimelodus pintado*, *Loricariichthys anus* and *Parapimelodus nigribarbis*) from Southern Brazil, with higher concentration during spring (Da Rocha et al., 2009).

Seasonal variations of the wide set of indices in digestive tissue of barnacle, *Balanus balanoides*, from polluted and non-polluted populations have been evaluated by Niyogi and colleagues (2001). As a general trend in barnacles from polluted and non-polluted populations, maximum antioxidant enzyme, including GST, activities were detected in summer followed by a gradual decrease during the autumn with a minimum in the winter. Microsomal LPO exhibited an almost reverse trend of seasonal variation to that of antioxidant enzyme activities indicating an enhanced susceptibility of barnacle to oxidative stress. Among the environmental parameters, only water temperature seemed to have a significant effect on observed variations of the activities of antioxidant enzymes and GST. However, this pattern was similar to tissue concentrations of PAHs, resulting in significant positive correlation with the activities of antioxidant enzymes, mainly catalase and SOD. So, the seasonal dependence was not clear different from the seasonal level of pollution.

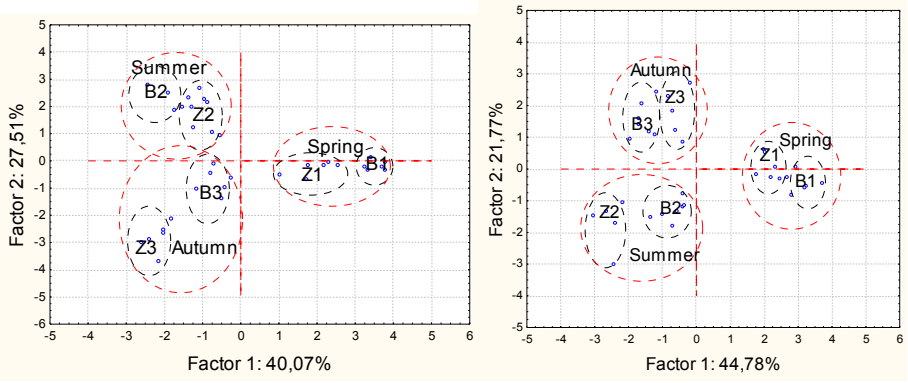
Comparison of flounder (*Platichthys flesus*) collected from nine stations once a month over whole year at Sobieszewo (Gulf of Gdańsk) demonstrated strong month (attributed mainly to spawning, and less to pollution) and geographical (attributed to pollution) variations in biomarker activities, as well as gender difference (Kopecka & Pempkowiak, 2008). In this work correlations between GST and catalase activities with abiotic properties of the environment were less important.

In the study of Da Rocha et al. (2009), the biomarkers of freshwater and estuarine fish species from Southern Brazil were compared in terms of seasonal variation and in three organs (muscle, liver and gills) for the four fish species (*M. furnieri*, *P. pintado*, *L. anus* and *P. nigribarbis*) in order to perform an environmental diagnosis. Obtained results showed that liver of *L. anus* and gills of *M. furnieri* presented higher total antioxidant capacity against peroxyl radicals during autumn. In terms of oxidative damage (TBARS), liver of *M. furnieri* and gills of *P. nigribarbis* showed higher TBARS levels during fall, whereas *P. pintado* showed the lowest TBARS value. Finally, a conspicuous seasonal effect was observed for

purified enzymes of the GST family as well as for non-purified GST, where minimum values were registered during fall, pointing to this season as one where fish species were less competent to perform detoxifying reactions (Da Rocha et al., 2009).

However, majority of season-related studies confirms that the continuous environmental press may affect the seasonal dynamics of biomarker states (Gorbi et al., 2005). When spatial and temporal dependences are compared, statistical approaches need to be applied to distinguish these determinants. For example, in the study of common carp (*C. carpio*) from two sites, despite of the priority of the season effect for the majority of markers, a similarity in the seasonal patterns between two sites was evident only for $O_2^{\bullet -}$, MT and TBARS (Falfushynska & Stoliar, 2009). This disparity as well as Two-factor interaction and the high classification rate between sites according to Discriminant Analysis reflect the impact of the effects of pollution on the seasonal regularity. However, in the areas that are characterised by spontaneous pollution, temporary inter-site differences can occur. Spatial effects could have seasone-related manner due to the specificity of agricultural activity, for example, or oxygen consumption and temperature that can be particularly important for the animals from cooling ponds of nuclear power plants. Therefore, the native "reference site" can be considered as the relative concept even if this site seems to be nearby unaffected. In this study, a battery comprising SOD, GSH and MTs in the liver as well as SOD, catalase and $O_2^{\bullet -}$ in the gills among several examined biomarkers was found to discriminate adequately fish from areas with varied water quality during three seasons.

In the study of more tolerant that *C. carpio* species, *C. carassius*, from the similar two field sites, Two variant ANOVA and the centroid grouping analysis allowed to distinguish the fish according to season more than to site, confirming the effect of season on all studied indices of oxidative stress except catalase activity in liver (Falfushynska et al., 2010a). The centroid grouping analysis of the separate specimens indicated clearly the general temporal dependence for fish from both sites (Fig. 2). However, in each season, especially in summer for gills and in autumn for liver, distinct spatial separation was found. Regarding the separate indices, the typical temporal difference in enzyme activities involved the elevation of values from spring to summer/autumn, probably due to increase in metabolic rates and accumulation of energy reserves was found (Chellappa et al., 1995). In some cases, different temporal dependence (for SOD and catalase activities in the liver) was observed in the groups from industrially polluted and reference sites. Obviously, this distortion of the general patterns could result from local adverse effects. In each season, especially in summer for gills and autumn for liver, distinct spatial separation was found. Temporal dependence with an elevation in summer was shown for the activities of SOD, catalase and concentration of GSSG in both tissues at reference site and only for the activities of SOD in the gills and GSSG in liver and gills at polluted site. The decrease from spring to autumn was typical for TBARS. The inter-site difference was especially high in summer, when lower actitivities of SOD (in the liver) and catalase and elevated TBARS levels were observed in the tissues of fish from polluted site. In autumn, the higher catalase activity in both tissues, and the higher SOD activity and lower TBARS levels in the gills indicated more efficient antioxidant defense in this group of fish. Only glutathione system showed relatively constant differences between fish from the two sites with higher GSH and Redox Index of GSH levels (particularly in the gills) and lower GSSG levels for the fish from industrial site in comparison to the other site.



A B

Fig. 2. Centroid grouping analysis of *C. carassius* from two sites, industrial (B) and rural (Z) parameter data set in the liver (A) and gills (B) in spring (1), summer (2) and autumn (3) (from Falfushynska et al., 2010a with permission).

To summarize, there is no doubts in the seasonal variation of antioxidant defence in fish. Different oxidative manifestations may have different dynamics. Environmental pollution, particularly spontaneous pollution, can interfere with seasonal dynamics and disturb it. However, fish can adapt to pollution in the areas with constant impact and do not show particular response compare to clean areas. So, the evidence of common seasonal difference in fish from polluted and clean areas can not be estimated as an absence of the effects of pollution. Integral statistic approach is effective in the discrimination between seasonal physiological regularities and spatial effects, particularly casual effects of non-pointed sources of pollution on fish.

7. Poisoning and adaptation: Caged fish

The main failure in application of markers of oxidative stress with feral fish is expected adaptation to the environmental conditions that leads to compensatory homeostasis occuring in antioxidant system (Barja de Quiroga et al., 1990; Regoli & Principato, 1995; Reynders et al., 2008). The choice of end points in the field studies of fish may be complicated by a history of exposure to xenobiotics causing oxidative stress. For instance, grey mullet (*Mugil* sp.) collected from an estuary polluted with metal ions, PAHs, PCBs, and pesticides demonstrated evidence of oxidative stress as indicated by Redox Index of GSH. However, these fish did not show elevated levels of LPO products while showing elevated activities of antioxidant enzymes (GPx, SOD, catalase, and GR). It is possible that an adaptive response occurred and repair of LPO mignt take place (Rodriquez-Ariza et al., 1993). Therefore, with the aim to standardize the results and avoid the effects of adaptation to chronically polluted environment, transplantation of caged organisms, including fish, to the sites of interest on the time suitable for the response, is recommended as an adequate step for the ERA. Caging studies often utilize farmed fish with known age and nutritional background, though wild captured fish can also be used. Caging allows the exposure of individual fish to conditions at a certain site, for known time (Almroth, 2008). In this case,

the early warning of toxic effect can be expected. The transferring of fish in new conditions can critically change their antioxidant profile. However, unlike with molluscs (Viarengo et al., 2007), for fish, the studies on the response of caged specimens are scarce.

For the appreciation of measurable oxidative stress response, 48 h exposure was recommended (Ahmad et al., 2004). This exposure strategy was adapted for different aquatic ecosystems biomonitoring using *A. anguilla* and other fish species. In the study of Ahmad and colleagues (2006), *A. anguilla* was plunged at five study sites located at increasing distances from the entrance point of the main source of contamination, originated from the introduction of agricultural chemicals, trace metal ions, domestic wastes, as well as eutrophication and incorrect utility of resources resulted in an increased water pollution. Inducing trend for total antioxidant enzyme activites (catalase, GPx and GST) was observed in gills of fish caged in the polluted area. In liver and kidney, the exposure typically induced significant decrease in the activity of the abovementioned enzymes. However, each studied parameter displayed a particular pattern in each site. Hepatic GSH concentration was increased, whereas LPO was decreased in individuals from polluted sites (Ahmad et al., 2006). Despite the authors assured that these findings provided a rational use of oxidative stress biomarkers in pollution biomonitoring of freshwater ecosystems, the discrepancy and variability of changes in each polluted site, did not allow to confirm the self-sufficience of this approach. No clear relationship could be established between gill oxidative stress responses and the distance to the main source of pollution in this study. Additionally, no gill LPO induction in this study may be explained by an effective antioxidant action. In general, in this study all organs studied revealed a similar resistance to peroxidative damage, suggesting that the antioxidants are more responsive biomarkers than LPO for short-term exposure. Besides the activation of antioxidant enzymes (as observed in gills), their inhibition (as observed in kidney and liver) should also be considered as a clear marker of pollutant presence and environmental degradation (Ahmad et al., 2006).

The utility of model studies was demonstrated in English sole (*P. vetulus*) exposed in laboratory and in their natural environment to an organic-solvent extract of sediment. Exposure provoked significant increase of hepatic GSH concentrations with a dose-dependent increase. Similarly, fish sampled directly from the polluted site showed higher GSH concentrations compared to fish from a reference site (Nishimoto et al., 1995).

Another period of time, usually used for caging experiments, is about 14-15 days. It is suitable for the indication of the effect of pollution related to the accumulation of specific pollutant/s. The transplantation of brown trout from a river with low levels of metal ions (the Stribekken River) to a river with high levels of Cd and Zn (the Naustebekken River) for up to 15 days allowed to demonstrate difference in the transcription and activities of central antioxidant enzymes and proteins in an environmental setting. This time was sufficient for significant uptake of both Cd and Zn in gills. Moreover, Cd levels were found to correlate significantly with transcript levels of MT, Cu,Zn-SOD, GPx, and GR. The activities of SOD and catalase increased in gills after transfer, but MT protein levels decreased. In liver, SOD activity and MT protein levels increased, while in kidney only MT protein concentrations were elevated after transfer. The detection of a general lack of consistency between mRNA transcription and specific enzyme activities, indicating that these proteins and enzymes are not solely under transcriptional control was very important result of this study (Hansen et al., 2006a).

The transfer of brown trout to a Cu-contaminated river in the Roros region in Central Norway provoked significant increase of MT-A, SOD and GR transcription along with uptake of Cu in gills, while only transcription of MT-A was found to respond in liver and kidney during the exposure. At that, no increase in MT protein levels were observed in gills. The levels of SOD and catalase enzymes were affected in all tissues during the exposure. A negative correlation between SOD and catalase activities was observed in gills indicating that the activities of these enzymes were influenced not only through transcription. The transcript levels of GPx and GR transcript levels correlated positively with each other in gills and liver, indicating their shared function in GSH-turnover (Hansen et al., 2006b).

The response of antioxidant system after depuration of feral fish, mullet, *M. cephalus*, and flounder, *P. flesus*, from the polluted site was expressed as the decrease of the activities of SOD and catalase only in mullet liver (Ferreira et al., 2005). Oxidative damage in liver, evaluated by estimating LPO and PC, increased in both species in most cases. This effect was explained by the decreased antioxidant defence after oxidative stress insults in natural environment.

The initial health status of fish can significantly affect the ability to form stress-related response. Some results in this direction were obtained when animals from two sites were compared under the modelling effect in laboratory (Hasspieler et al., 1994; Meyer et al., 2003; Falfushynska et al., 2011). Up-regulated stress-related parameters in the animals from chronically polluted sites at additional loading was revealed in the set of comparative studies of Di Gulio and colleagues on killifish *F. heteroclitus* inhabiting site polluted by creosote (Meyer et al., 2003; Bacanskas et al., 2004), and in the study of cadmium-acclimated rainbow trout (Chowdhury et al., 2004), whereas the fish from reference site were not able to activate the enzymes of antioxidant defense under the exposure. The comparison of the effects of prooxidant copper (Cu^{2+}, 0.005 and 0.050 mg·L^{-1}) or manganese (Mn^{2+}, 0.17 and 1.7 mg·L^{-1}) on *C. auratus gibelio* from polluted and unpolluted sites after exposure for 14 days indicated that fish from the polluted site showed lower activities of SOD (Cu,Zn- and Mn-SOD) and GST in the liver and gills. The oxidative stress response was more efficient in fish from the polluted site (Falfushynska et al., 2011) due to the activation of Cu,Zn- and Mn-SOD. The interference of the ability of the antioxidative defence and the origin of fish (pre-exposure to chronic pollution) was found in this study. In general, gibel carp from the polluted site demonstrated a highly effective response of the antioxidant system particularly with SOD activity in liver and gills. In this study site-related differences in the level of LPO and GSH between the two groups were mainly maintained in all groups.

Vega-López and colleagues (2008) studied the responses of antioxidant system (LPO, SOD and catalase activities) in fish *Girardinichthys viviparus* after exposure to water from PCBs contaminated habitants and from other site, expected to be suitable place for the re-introduction of this endangered species. Water enriched by PCB was also inspected in that study during 1, 2, 4, 8 and 16 days of exposure. Four types of responses were observed dependently on the composition of water and sex of fish: (1) increased lipid peroxidation intensity, depressed SOD and increased catalase activities; (2) an increase in all three biomarkers; (3) decreased LPO product levels, unchanged SOD and increased catalase activities; (4) increased LPO intensity and depressed SOD and catalase activities. At that, the only PCB addition to the natural water of fish resulted in decreased LPO, whilst the exposure no native water depressed both studied enzymes in concert with the

intensification of LPO. Evaluation of these responses is rather complicated and cannot provide a clear conclusion on the consequence of each response for fish health status. These results also let to conclude that in the field investigations, the selection of the reference site is important to understand the response of fish. The relativity of the concept of the reference site is sometimes indicated even though this site was selected by generally accepted criteria.

8. Biomarkers of oxidative stress in the multi-marker approach: Integrated data analysis

Performed analysis has shown that each of separate markers of oxidative defense alone cannot provide the conclusion on the nature of observed difference between compared groups. Indeed, in the field studies, wide spectrum of inter-site differences (higher, equal or lower activities of various antioxidant enzymes with tissues peculiarities and disbalance) have been observed in polluted compared to clean areas reflecting both mild stress conditions of the location or strong oxidative damage. Therefore, different models of the aquatic animal response need to be analysed before conclusions can be drawn. Only the evaluation of the final effect of oxidative impact can provide the conclusion on the response of organism. All these possibilities and their combinations have been reported (Winston & Di Guilio, 1991; Lushchak, 2011) and this complexity of antioxidant responses to pollutants often leads to a controversy on the use of oxidative stress markers in ecotoxicological studies.

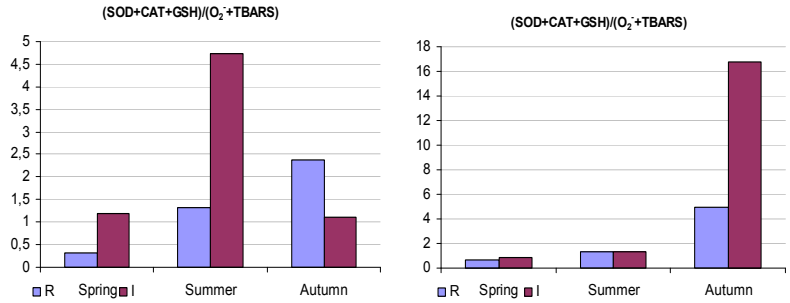
Two approaches for the appreciation of the severity of stress are proposed. The first one is connected to the measuring of the integrated state of the antioxidant capacity which was successfully applied by Regoli and colleagues (Regoli, 2000; Regoli et al., 2002a, b). The total oxyradical scavenging capacity (TOSC) quantifies the capability of the whole antioxidant system to neutralize oxyradicals, allowing to discriminate between different forms of ROS, thus providing useful indications to predict oxyradical-mediated adverse effects under certain physiological conditions of organisms (Regoli, 2000). Appropriate assay conditions have been standardized in which different ROS induced a comparable prooxidant force quantified by the oxidation of the substrate α-keto-γ-methiolbutyric acid. Thus, the efficiency of antioxidants toward various reactive species can be better compared by their ability to inhibit an oxidative pressure induced by specific oxidants. The validity of this approach was confirmed in different field studies. Particullarly, integration of measurement of individual antioxidants with TOSC analysis increased the evaluation of oxidative responses to pollutants in ecotoxicological studies (Regoli et al., 2005). The field study of an Antarctic silverfish (*Pleuragramma antarcticum*), that is developed in the environment with strong pro-oxidant characteristics, revealed particularly prompt responses for GSH metabolism which, however, did not prevent high intensity of LPO. From the analysis of TOSC, the overall efficiency to neutralize peroxyl radicals remained almost constant while slightly lower TOSC values were obtained toward hydroxyl radicals at the end of sampling period (Gorbi & Regoli, 2003; Regoli et al., 2005). The analysis of TOSC revealed that the overall capacity of specific tissues of red mullet (*M. barbatus*) in the area of dredging to absorb various oxidants was not substantially compromised when challenged with increased prooxidant pressures (Regoli, 2002). In the study of Meyer et al. (2003), offsprings of killfish from polluted site showed higher basal TOSC value that was balanced by higher GSH concentrations, and Mn-SOD protein levels.

In general, it is considered that individual antioxidants are useful as ''response biomarkers'' indicating a varied prooxidant challenges and potentially important early warning signals. Variations of individual antioxidants are useful for understanding the mode of action of a chemical stressor and the possible molecular targets with specific responses. However, their value is more limited for understanding of the biological effects in terms of health conditions of the organisms. The value of TOSC is a less sensitive marker than individual characteristics of antioxidant defence, but it provides more holistic picture of susceptibility to oxidative stress. It is an ''biomarker'' effect with predictive value since varied capability to counteract oxyradical efects can induce alterations at other levels of biological organization. In conclusion, the combination of measurement of individual antioxidants with TOSC analysis seems to improve the evaluation of oxidative responses to pollutants in ecotoxicological studies (Regoli, 2000).

The second approach to give an integrated assessment of antioxidant response is based on the multi-marker approach and calculation of the balance between prooxidant endogenous and exogenous factors (i.e., environmental pollutants) and antioxidant defenses (enzymatic and nonenzymatic) in biological systems. It gives so-called coefficient - the Integrated Oxidative Stress (IOS) index, calculated as the ratio of antioxidant factors, A, and prooxidant manifestations, O, after data standardization (Falfushynska et al., 2008). The basis of standardization of each factor taking into consideration mean values and standard deviations for each group was described by Leiniö and Lehtonen (2005). The antioxidant factors (A) may include the activities of SOD and catalase, GSH level, and oxidative damage manifestations (O) represented by TBARS and $O_2^{\bullet -}$ levels. They also can include PC and GSSG if they were detected. Lower IOS value in the counterpart implies the weakness of antioxidant defence and higher one − its suppression. In the tissues of common carp from two field sites, all measured markers of oxidative stress contributed to the integrated index. For example, in the polluted area, season-related difference between two groups can be detected (Fig. 3). In most cases, the balance between A and O was found, particularly in gills. However, in spring, the IOS in the liver suggested the unbalance, or presence of oxidative stress in the fish at the reference site. The particularly high overbalancing appeared in the groups for the polluted site for the antioxidant systems of liver and gills in summer and autumn, respectively. In the gills, the highest antioxidant capacity is reflected in autumn according to the calculated IOS. Indeed, the elevation of TBARS level at the R-site in spring and at the I-site in autumn reveals that the variations in activities of different antioxidant enzymes and levels of nonenzymatic antioxidants were unable to prevent oxidative damage in fish liver. In contrast, the gills were significantly more tolerant to oxidative damage. Additionally, this integrative approach applied in different seasons confirmed that the fish in relatively low polluted area can form adaptive response in the particular cases, especially in autumn, and possesses the weakness of this response in spring after the winter metabolic depression.

Generally, it needs to be pointed out that the measuring of oxidative stress markers in the feral fish is almost obligate constituent of the evaluation of its health status. Obviously, as it was shown above, their response are higly variable. Based on this variability, correspondent articles are traditionally finalized by the suggestion that selected parameters are suitable biomarkers of pollution, and studied certain species is suggested to be potential bioindicator organism against environmental pollution. However, the classification of the interrelation of

the changes in different tissues, of separate characteristics and their relation to the ability of fish to withstand pollution have not been represented yet. The intercalibration and generalization of results and based on this issue classification of oxidative stress response remains to be very actual technology in ERA.



A B

Fig. 3. Integrated Oxidative Stress index of liver (A) and gills (B) of common carp from relatively clean (R) and chronically polluted (I) field sites in three seasons, calculated from their SOD and catalase activities, GSH level, superoxide anion production and TBARS levels (from Falfushynska et al., 2009 with permission).

9. Conclusions and perspectives

Oxidative stress in fish is a general consequence of the environmental pollution. Information on antioxidant defense in fish is meagre despite that fish are constantly exposed to a myriad of environmental stresses including oxidant-induced ones. In the feral organisms, deleterious effects of environment are often difficult to evaluate since many of these effects tend to manifest only after longer periods of time and organisms tend to adapt to them (van der Oost et al., 2003). Early warning of the toxic effects of pollutants, particularly in spontaneously polluted areas can be predicted only using a biomarker approach, including oxidative stress manifestations and adaptive responses.

Antioxidant defense system in fish is very sensitive to environmental conditions. However, in different studies with fish from mixed polluted field sites, the enzymes of antioxidant defence demonstrated case-dependent difference. Whereas, apparently, the study of the frame of resilience of antioxidant enzymes in the fish from polluted and pristine sites needs to be the object of specific study. When several enzymes were studied, their responses were frequently imbalanced, or their transcriptional level and enzyme activity trends might be different. In some studies, the absence of the changes of their activities was observed when biomarkers of specific pollution confirmed toxicity of environment. Studies addressed potential ROS scavengers in the feral fish under stress conditions are very scarce. Free radical-related processes and ROS production were found to be responsible for a variety of oxidative damages leading to adverse health effects and diseases in the feral fish even from comparatively indisturbed areas. Moreover, it is clear that, given spontaneous human activities, nobody will be able to find a true reference site in some areas.

The integrated approach with the appreciation of balance between prooxidant manifestations and antioxidant defences in biological systems needs to be a control point to assess toxic effects under stressful environmental conditions.

10. Acknowledgment

The authors wish to thank Dr. H. Falfushynska for comments on the manuscript and Ph.D. student L. Gnatyshyna for technical assistance with the preparation of the manuscript.

11. References

Adams, S.M. & Greeley, M.S. (2000). Ecotoxicological Indicators of Water Quality: Using Multi-response Indicators to Assess the Health of Aquatic Ecosystems. *Water, Air and Soil pollution*, Vol.123, pp. 103–115, ISSN 0049-6979

Ahmad, I.; Pacheco, M. & Santos M.A. (2004). Enzymatic and Non-enzymatic Antioxidants as an Adaptation to Phagocyte-induced Damage in *Anguilla anguilla* L. Following in Situ Harbor Water Exposure. *Ecotoxicology and Environmental Safety*, Vol.3, No.57, pp. 290–302, ISSN 0147-6513

Ahmad, I.; Pacheco, M. & Santos, M.A. (2006). *Anguilla anguilla* L. Oxidative Stress Biomarkers: An in Situ Study of Freshwater Wetland Ecosystem (Pateira de Fermentelos, Portugal). *Chemosphere*, Vol.65, Issue 6, (November 2006), pp. 952–962, ISSN 0045-6535.

Almroth, B.C. (2008). Oxidative Damage in Fish Used as Biomarkers in Field and Laboratory Studies. Department of Zoology/Zoophysiology, University of Gothenburg, Sweden, ISBN 978-91-628-7420-9

Almroth, B.C.; Albertsson, E., Sturve, J. & Förlin, L. (2008a). Oxidative Stress, Evident in Antioxidant Defences and Damage Products, in Rainbow Trout Caged Outside a Sewage Treatment Plant. *Ecotoxicology and Environmental Safety*, Vol.70, No.3, pp. 370–378, ISSN 0147-6513

Almroth, B.C.; Sturve, J., Berglund, Å. & Förlin, L. (2005). Oxidative Damage in Eelpout (*Zoarces viviparus*), Measured as Protein Carbonyls and TBARS, as Biomarkers. *Aquatic Toxicology*, Vol.73, pp. 171–180, ISSN 0166-445X

Almroth, B.C.; Sturve, J., Stephensen, E., Holth, T.F. & Förlin, L. (2008b). Protein Carbonyls and Antioxidant Defences in Corkwing Wrasse (*Symphodus melops*) from a Heavy Metal Polluted and a PAH Polluted Site. *Marine Environmental Research*. Vol.66, No.2, (April 2008), pp.271–277, ISSN 0141-1136

Amado, L.L.; Robaldo, R.B., Geracitano, L., Monserrat, J.M. & Bianchini, A. (2006). Biomarkers of Exposure and Effect in the Brazilian Flounder *Paralichthys Orbignyanus* (Teleostei: Paralichthyidae) from the Patos Lagoon Estuary (Southern Brazil). *Marine Pollution Bulletin,* Vol.52, No.2, (December 2005), pp. 207–213, ISSN 0025-326X.

Andersson, T.; Forlin, L., Hardig, J. & Larsson, A. (1988). Physiological Disturbances in Fish Living in Coastal Waters Polluted with Bleached Kraft Pulp Mill Effluents. *Canadian Journal of Fisheries and Aquatic Sciences*, Vol. 45, pp. 1525–1536, ISSN 0706-652X.

Armstrong, D.T. (1986). Environmental Stress and Ovarian Function. *Biology of Reproduction*. Vol.34, No.1, (February 1986), pp. 29–39, ISSN 0006-3363.

Atif, F.; Kaur, M., Yousuf, S. & Raisuddin, S. (2006). *In vitro* Free Radical Scavenging Activity of Hepatic Metallothionein Induced in an Indian Freshwater Fish, *Channa punctata* Bloch. *Chemico-Biological Interactions*, Vol.162, (June 2006), pp. 172–180, ISSN 0009-2797.

Bacanskas, L.R.; Whitaker, J. & Di Giulio, R.T. (2004). Oxidative Stress in Two Populations of Killifish (*Fundulus heteroclitus*) with Differing Contaminant Exposure Histories. Marine Environmental Research, Vol.58, No.2-5, (August-December 2004), pp. 597–601, ISSN 0141-1136.

Bagnyukova, T.V.; Chahrak, O.I. & Lushchak, V.I. (2006). Coordinated Response of Goldfish Antioxidant Defenses to Environmental Stress. *Aquatic Toxicology*, Vol. 78, pp. 325–331, ISSN 0166-445X.

Bagnyukova, T.V.; Luzhna, L.I., Pogribny, I.P. & Lushchak, V.I. (2007). Oxidative Stress and Antioxidant Defenses in Goldfish Liver in Response to Short-term Exposure to Arsenite. *Environmental and Molecular Mutagenesis*, Vol. 48, pp. 658–665, ISSN 0893-6692.

Bagnyukova, T.V.; Storey, K.B. & Lushchak, V.I. (2005). Adaptive Response of Antioxidant Enzymes to Catalase Inhibition by Aminotriazole in Goldfish Liver and Kidney. *Comparative Biochemistry and Physiology,* Vol. 142 B, pp. 335–341, ISSN 0305-0491.

Bainy, A.C.D.; Saito, E., Carvalho, P.S.M. & Junquieira, V.B.C. (1996). Oxidative Stress in Gill, Erythrocytes, Liver and Kidney of Nile Tilapia (*Oreochromis Niloticus*) from a Polluted Site. *Aquatic Toxicology*, Vol.34, (February 1996), pp. 151–/162, ISSN 0166-445X.

Bargelloni, L.; Scudiero, R., Parisi, E., Carginale, V., Capasso, C. & Patarnello, T. (1999). Metallothioneins in Antarctic fish: Evidence for Independent Duplication and Gene Conversion. *Molecular Biology and Evolution*, Vol.16, (July 1999), pp. 885–897, ISSN 0737-4038

Barja de Quiroga, G.; Pérez-Campo, R. & López-Torres, M. (1990). Changes on Cerebral Antioxidant Enzymes, Peroxidation, and the Glutathione System of Frogs after Aging and Catalase Inhibition. *The Journal of Neuroscience.* Vol.26, No.3, (July 1990), pp. 370-376, ISSN 0270-6474

Bhattacharya, S. (2001). Stress Response to Pesticides and Heavy Metals in Fish and Other Vertebrates. *Proceedings of the Indian Academy of Sciences*. B67, No.5, (May 2001), pp. 215–246, ISSN 0073-6600.

Buet, A.; Banas, D., Vollaire, Y., Coulet, E. & Roche, H. (2006). Biomarker Responses in European Eel (*Anguilla anguilla*) Exposed to Persistent Organic Pollutants. A Field Study in the Vaccarès Lagoon (Camargue, France). *Chemosphere*. Vol.65, No.10, (December 2006), pp. 1846–1858, ISSN 0045-6535.

Capasso, C.; Carginale, V., Crescenzi, O., Di Maro, D., Spadaccini, R., Temussi, P.-A. & Parisi, E. (2005). Structural and Functional Studies of Vertebrate Metallothioneins: Cross-talk between Domains in the Absence of Physical Contact. *Biochemical Journal*, Vol.391, pp. 95–103, ISSN 0264-6021.

Capdevila, M.; Cols, N., Romero-Isart, N., Gonzalez-Duarte, R., Atrian, S. & Gonzalez-Duarte, P. (1997). Recombinant Synthesis of Mouse Zn3-beta and Zn4-alpha Metallothionein 1 Domains and Characterization of Their Cadmium (II) Binding Capacity. *Cellular and Molecular Life Sciences*, Vol.53, pp. 681–688, ISSN 1421-682X

Cavaletto, M.; Ghezzi, A., Burlando, B., Evangelisti, V., Ceratto, N. & Viarengo, A. (2002). Effect of Hydrogen Peroxide on Antioxidant Enzymes and Metallothionein Level in

the Digestive Gland of Mytilus galloprovincialis. *Comparative Biochemistry and Physiology*, Vol.131 C, pp. 447–455, ISSN 0742-8413

Chellappa, S.; Huntingford, F.A., Strang, R.H.C. & Thomson, R.Y. (1995). Condition Factor and Hepatosomatic Index as Estimates of Energy Status in Male Three-spined Stickleback. *Journal of Fish Biology*, Vol.47, pp. 775–787, ISSN 0022-111

Chesman, B.S.; O'Hara, S., Burt, G.R. & Langston, W.J. (2007). Hepatic Metallothionein and Total Oxyradical Scavenging Capacity in Atlantic cod *Gadus morhua* Caged in Open Sea Contamination Gradients. *Aquatic Toxicology*, Vol.84, pp. 310–320, ISSN 0166-445X

Cho, Y.S.; Lee, S.Y. Kim, K.-Y., Bang, I.C., Kim, D.S. & Nam, Y.K. (2008). Gene Structure and Expression of Metallothionein During Metal Exposures in *Hemibarbus mylodon*. *Ecotoxicology and Environmental Safety*, Vol. 71, pp.125–137, ISSN 0147-6513

Collier, T.K. & Varanasi, U. (1991). Hepatic Activities of Xenobiotic Metabolizing Enzymes and Biliary Levels of Xenobiotics in English Sole (*Parophrys vetulus*) Exposed to Environmental Contaminants. *Archives of Environmental Contamination and Toxicology,* Vol.20, pp. 462–473, ISSN 0090-4341

Commandeur, J.N.M.; Hermanns, R.C.A., Meerman, J.H.N., Van Baar, B.L.M. & Vermeulen, N.P.E. (1997). Simultaneous Determination of Eight Lipid Peroxidation Degradation Products in Urine of Rats Treated with Carbon Tetrachloride Using Gas Chromatography with Electroncapture Detection. *Journal of Chromatography*, Vol.694, pp. 277–288, ISSN 0021-9673

Da Rocha, A.M.; Salomão de Freitas. D.P., Burns, M., Vieira, J.P., de la Torre, F.R. & Monserrat, J.M. (2009). Seasonal and Organ Variations in Antioxidant Capacity, Detoxifying Competence and Oxidative Damage in Freshwater and Estuarine Fishes from Southern Brazil. *Comparative Biochemistry and Physiology*, Vol.150 C, pp.512–520, ISSN 0742-8413

Di Giulio, R.T.; Habig, C. & Gallagher, E.P. (1993). Effects of Black Rock Harbor Sediments on Indices of Biotransformation, Oxidative Stress, and DNA Integrity in Channel Catfish. *Aquatic Toxicology*, Vol.26, pp.1–22, ISSN 0166-445X

Dorval, J.; Leblond, V., Deblois, C. & Hontela, A. (2005). Oxidative Stress and Endocrine Endpoints in White Sucker (*Catostomus commersoni*) from a River Impacted by Agricultural Chemicals. *Environmental Toxicology and Chemistry*, Vol.24, No.5, pp. 1273–1280, ISSN 0730-7268

dos Anjos, N.A; Schulze, T., Brack, W., Val, A.L., Schirmer, K. & Scholz, S. (2011). Identification and Evaluation of Cyp1a Transcript Expression in Fish as Molecular Biomarker for Petroleum Contamination in Tropical Freshwater Ecosystems. *Aquatic Toxicology*, Vol.103, No.(1-2), (May 2011), pp. 46–52, ISSN 0166-445X

Du, J. & Gebicki, J.M. (2004). Proteins are Major Initial Cell Targets of Hydroxyl Free Radicals. *The International Journal of Biochemistry & Cell Biology*, Vol.36, No.11, (November 2004), pp. 2334–2343, ISSN 1357-2725

EU Council Directive 98/83/EC), Retrieved from <http://www.emwis.org/IFP/law_EU.htm>

Eufemia, N.; Collier, T.K., Stein, J.E., Watson, D.E. & Di Giulio, R.T. (1997). Biochemical Responses to Sediment-associated Contaminants in Brown Bullhead (*Ameriurus nebulosus*) from the Niagara River Ecosystem. *Ecotoxicology*, Vol.6, pp. 13–34, ISSN 0963-9292

Eustace, B.K. & Jay, D.G. (2004). Extracellular Roles for the Molecular Chaperone, Hsp90. *Cell Cycle*, Vol. 3, pp. 1098–1100, ISSN 1538-4101

Falfushinska, H.; Loumbourdis, N., Romanchyk, L. & Stolyar, O. (2008). Validation of Oxidative Stress Responses in Two Populations of Frogs from Western Ukraine. *Chemosphere*, Vol. 73, pp. 1096–1101, ISSN 0045-6535

Falfushinska, H.I.; Goch, I.V., Bugera, L.I. & Stoliar, O.B. (2009). Metallothioneins and the Indices of Oxidative Damage in the Tissues of Carp *Cyprinus carpio* as the Biomarkers of the Environmental Pollution. Studia Biologica, Vol.3, No.2, pp. 99–108, ISSN 1996-4536

Falfushynska, H. & Stolyar, O. (2009). Responses of Biochemical Markers in Carp *Cyprinus carpio* from Two Field Sites in Western Ukraine, *Ecotoxicology and Environmental Safety*, Vol.72. pp. 729–36, ISSN 0147-6513

Falfushynska, H.; Gnatyshyna, L., Priydun, Ch., Stoliar, O. & Nam, Y.K. (2010a). Variability of Responses in the Crucian Carp *Carassius carassius* from Two Ukrainian Ponds Determined by Multi-marker Approach, *Ecotoxicology and Environmental Safety*, Vol.73, pp. 1896–1906, ISSN 0147-6513

Falfushynska, H.; Gnatyshyna, L., Shymkiv, T. & Stoliar, O. (2010b). Reactivity of Metallothioneins and the Efficiency of their Metal-keeping and Antioxidative Functions in the Adaptation of Fish *Carassius carassius* to Field Pollution. *Proceeding of the Shevchenko Scientific Society. Chemistry & Biochemistry*, Vol. 25, pp. 229–242, ISSN 1563-3569.

Falfushynska, H.I. & Stoliar, O.B. (2009). Function of Metallothioneins in Carp *Cyprinus carpio* from Two Field Sites in Western Ukraine. *Ecotoxicology and Environmental Safety*, Vol.72, pp. 1425–1432, ISSN 0147-6513.

Falfushynska, H.I.; Gnatyshyna, L.L., Farkas, A., Vehovszky, Á., Gyori, J. & Stoliar, O.B. (2010c). Vulnerability of Biomarkers in the Indigenous Mollusk *Anodonta cygnea* to Spontaneous Pollution in a Transition Country. *Chemosphere*, Vol. 81, Issue 10, (November 2010), pp. 1342–1351, ISSN 0045-6535

Falfushynska, H.I.; Gnatyshyna, L.L., Stoliar, O.B. & Nam, Y.K. (2011). Various Responses to Copper and Manganese Exposure of *Carassius auratus gibelio* from the Two Populations. *Comparative Biochemistry and Physiology*, Vol. 154, No. 3, pp. 242–253, ISSN 1532-0456

Farombi, E.O.; Adelowo, O.A. & Ajimoko, Y.R. (2007). Biomarkers of Oxidative Stress and Heavy Metal Levels as Indicators of Environmental Pollution in African Catfish (*Clarias gariepinus*) from Nigeria Ogun River. *International Journal of Environmental Research and Public Health*, Vol.4, pp. 158–165, ISSN 1660-4601

Fernandes, D.; Andreu-Sánchez, O., Bebianno, M.J. & Porte, C. (2008). Assessment of Pollution Along the Northern Iberian shelf by the Combined Use of Chemical and Biochemical Markers in Two Representative Fish Species. *Environmental Pollution*, Vol.155, pp. 327–335, ISSN 0269-7491

Fernandes, D.; Bebianno, M.J. & Porte, C. (2008). Hepatic Levels of Metal and Metallothioneins in Two Commercial Fish Species of the Northern Iberian Shelf, Science of the Total Environment, Vol.391, pp.159–167, ISSN 0048-9697

Ferreira, M.; Moradas-Ferreira, P. & Reis-Henriques, M.A. (2005). Oxidative Stress Biomarkers in Two Resident Species, Mullet (*Mugil cephalus*) and Flounder

(*Platichthys flesus*), from a Polluted Site in River Douro Estuary, Portugal. *Aquatic Toxicology*, Vol.71, pp. 39–48, ISSN 0166-445X

Fessard, V. & Livingstone, D.R. (1998). Development of Western Analysis of Oxidized Proteins as a Biomarker of Oxidative Damage in Liver of Fish. *Marine Environmental Research,* Vol. 46, pp. 407–410, ISSN 0141-1136

George, S.G. (1994). Enzymology and Molecular Biology of Phase II Xenobiotic-conjugating Enzymes in Fish. In: Malins, D.C., Ostrander, G.K. (Eds.). *Aquatic Toxicology*; Molecular, Biochemical and Cellular perspectives. Lewis Publishers, CRC press, pp. 37–85, ISSN 0166-445X

Godin, D.V. & Garnett, M.E. (1992). Species-related Variations in Tissue Antioxidant Status-I. Differences in Antioxidant Enzyme Profiles. *Comparative Biochemistry and Physiology*, Vol.103 B, No. 3, pp. 737–742, ISSN 1879-1107

Gorbi, S. & Regoli, F. (2003). Total Oxyradical Scavenging Capacity as an Index of Susceptibility to Oxidative Stress in Marine Organisms. *Comment on Toxicology*, Vol.9, pp 303–322, ISSN 0886-5140

Gorbi, S.; Baldini, C. & Regoli, F. (2005). Seasonal Variability of Metallothioneins, Cytochrome P450, Bile Metabolites and Oxyradical Metabolism in the European eel *Anguilla anguilla* L. (Anguillidae) and Striped mullet Mugil cephalus L. (Mugilidae). *Archives of Environmental Contamination and Toxicology*. Vol.49, pp. 62–70, ISSN 0090-4341

Gundersen, D.T.; Miller, R., Mischler, A., Elpers, K., Mims, S. D., Millar, J. G. & Blazer, V. (2000). Biomarker Response and Health of Polychlorinated Biphenyl- and Chlordane-contaminated Paddlefish from the Ohio River Basin, USA. *Environmental Toxicology and Chemistry*, Vol.19, pp. 2275–2285, ISSN 1552-8618

Hansen, B.H.; Romma, S., Garmo, O.A., Olsvik, P.A. & Andersen, R.A. (2006a). Antioxidative Stress Proteins and Their Gene Expression in Brown Trout (*Salmo trutta*) from Three Rivers with Different Heavy Metal Levels. *Comparative Biochemistry and Phisiology*, Vol.143 C, pp. 263–274, ISSN ISSN 1532-0456.

Hansen, B.H.; Romma, S., Softeland, L.I., Olsvik, P.A. & Andersen, R.A. (2006b). Induction and Activity of Oxidative Stress-related Proteins during Waterborne Cu-exposure in Brown Trout (*Salmo trutta*). *Chemosphere*, Vol.65, pp. 1707–1714, ISSN 0045-6535

Hasspieler, B.M.; Behar, J.V., Carlson, D.B., Watson, D.E. & Di Giulio, R.T. (1994). Susceptibility of Channel Catfish (*Ictalurus punctatus*) and Brown Bullhead (*Ameriurus nebulosus*) to Oxidative Stress: a Comparative Study. *Aquatic Toxicology*, Vol.28, pp. 53–64, ISSN 0166-445X.

Huang, D.J.; Zhang, Y.M., Song, G., Long, J., Liu, J.H. & Ji, W.H. (2007). Contaminants-induced Oxidative Damage on the Carp *Cyprinus carpio* Collected from the Upper Yellow River, China. *Environmental Monitoring and Assessment*. Vol.128, pp. 483–488, ISSN 0167-6369.

Janssens, B.J.; Childress, J.J., Baguet, F. & Rees, J.F. (2000). Reduced Enzymatic Antioxidative Defense in Deep-sea Fish. *Journal of Experimental Biology*, Vol. 203, No.24, pp. 3717–3725, ISSN 0022-0949

Jenner, N.K.; Ostrander, G.K., Kavanagh, T.J., Livesey, J.C., Shen, M.W., Kim, S.C. & Holmes, E.H. (1990). A Flow Cytometric Comparison of DNA Content and Glutathione Levels in Hepatocytes of English sole (*Parophyrs vetulus*) from Areas of Differing Water Quality. *Archives of Environmental Contamination and Toxicology,* Vol.19, pp. 807–815, ISSN 0090-4341

Kang, Y.J. (2006). Metallothionein Redox Cycle and Function. *Experimental Biology and Medicine*, Vol.231, pp. 1459–1467, ISSN 0037-9727

Kappus, H. (1987). A Survey of Chemicals Inducing Lipid Peroxidation in Biological Systems. *Chemistry and Physics of Lipids*, Vol.45, pp. 105–115, ISSN 1873-2941

Kelly, E.; Duncan, R., Ngu, T.T., Chan, J., Salgado, M.T., Merrifield, M.E. & Stillman, M.J. (2006). Peptide Folding, Metal-binding Mechanisms, and Binding Site Structures in Metallothioneins. *Experimental Biology and Medicine,* Vol.231, pp. 1488–1499, ISSN 1535-3702.

Kelly, K.A.; Havrilla, C.M., Brady, T.C., Abramo, K.H. & Levin, E.D. (1998). Oxidative Stress in Toxicology: Established Mammalian and Emerging Piscine Model Systems. Environmental Health Perspectives, Vol. 106, pp. 375–384, ISSN 0091-6765.

Kono, Y. & Fridovich, I. (1982). Superoxide Radical Inhibits Catalase. *The Journal of Biological Chemistry*, Vol. 257, pp**.** 57–51, ISSN 0021-9258

Kopecka, J.J.P. & Pempkowiak, J. (2008). Temporal and Spatial Variations of Selected Biomarker Activities in Flounder (*Platichthys flesus*) Collected in the Baltic Proper. *Ecotoxicology and Environmental Safety*, Vol.70, Issue 3, (July 2008), pp. 379–391, ISSN 0147-6513

Kubrak, O.I.; Lushchak, O.V., Lushchak, J.V., Torous, I.M., Storey, J.M., Storey, K.B. & Lushchak, V.I. (2010). Chromium Effects on Free Radical Processes in Goldfish Tissues: Comparison of Cr(III) and Cr(VI) Exposures on Oxidative Stress Markers, Glutathione Status and Antioxidant Enzymes. *Comparative Biochemistry and Physiology*, Vol. 152 C, pp. 360–370, ISSN 1532-0456.

Lackner, R. (1998). ''Oxidative stress'' in Fish by Environmental Pollutants. In: Braunbeck, T., Hinton, D.E., Streit, B. (Eds.), *Fish Ecotoxicology*. Birkhauser Verlag, Basel, pp. 203–224.

Lam, P. K. S. & Wu, R. S. S. (2003). Use of Biomarkers in Environmental Monitoring. *STAP Workshop on the use of bioindicators, biomarkers and analytical methods for the analysis of POPs in developing countries*, (December 2003), pp. 1–77.

Lee, S.Y.; Stoliar, O. & Nam, Y.K. (2010). Transcriptional Alteration of Two Metallothionein Isoforms in Mud Loach *Misgurnus Mizolepis* fry During Acute Heavy Metal Exposure. *Fisheries and Aquatic Science*, Vol.13, pp. 112–117, ISSN 1303-2712

Leiniö, S. & Lehtonen, K.K. (2005). Seasonal Variability in Biomarkers in the Bivalves *Mytilus edulis* and *Macoma balthica* from the Northern Baltic Sea. *Comparative Biochemistry and Physiology*, Vol.140 C, pp. 408–421, ISSN 1532-0456

Lionetto, M.G.; Caricato, R., Giordano, M.E., Pascariello, M.F., Marinosci, L. & Schettino, T. (2003). Integrated Use of Biomarkers (Acetylcholinesterase and Antioxidant Enzymes Activities) in *Mytilus galloprovincialis* and *Mullus barbatus* in an Italian Coastal Marine Area. *Marine Pollution Bulletin*, Vol. 46, No.3, (March 2003), pp. 324–330, ISSN 0025-326X

Livingstone, D.R. (2001). Contaminant-stimulated Reactive Oxygen Species Production and Oxidative Damage in Aquatic Organisms. *Marine Pollution Bulletin,* Vol. 42, pp. 656–666, ISSN 0025-326X

Livingstone, D.R.; Lemaire, P., Matthews, A., Peters, L.D., Porte, C., Fitzpatrick, P.J., Forlin, L., Nasci, C., Fossatto, V., Wootton, N. & Goldfarb, P. (1995). Assessment of the Impact of Organic Pollutants on Goby (*Zosterisessor ophicephalus*) and Mussel

(*Mytilus galloprovincialis*) from the Venice Lagoon, Italy: Biochemical Studies. Marine Environmental Research, Vol.39, pp. 235–240, ISSN 0141-1136

Lushchak, V.I. (2011). Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology: CBP*, Vol. 153, No. 2, pp. 175-190. ISSN 1532-0456

Lushchak, O.V.; Kubrak, O.I., Lozinsky, O.V., Storey, J.M., Storey, K.B. & Lushchak, V.I., (2009a). Chromium (III) Induces Oxidative Stress in Goldfish Liver and Kidney. *Aquatic Toxicology*, Vol. 93, pp. 45–52, ISSN 0166-445X

Lushchak, O.V.; Kubrak, O.I., Nykorak, M.Z., Storey, K.B. & Lushchak, V.I. (2008). The Effect of Potassium Dichromate on Free Radical Processes in Goldfish: Possible Protective Role of Glutathione. *Aquatic Toxicology*, Vol. 87, pp. 108–114, ISSN 0166-445X

Lushchak, O.V.; Kubrak, O.I., Storey, J.M., Storey, K.B., Lushchak, V.I. (2009b). Low Toxic Herbicide Roundup Induces Mild Oxidative Stress in Goldfish Tissues. *Chemosphere*, Vol. 76, pp. 932–937, ISSN 0045-6535.

Lushchak, O.V.; Kubrak, O.I., Torous, I.M., Nazarchuk, T.Y., Storey, K.B. & Lushchak, V.I. (2009c). Trivalent Chromium Induces Oxidative Stress in Goldfish Brain. *Chemosphere*, Vol. 75, pp. 56–62, ISSN 0045-6535.

Lushchak, V. (2011). Environmentally Induced Oxidative Stress in Aquatic Animals. *Aquatic Toxicology,* Vol. 101, pp. 13–30, ISSN 0166-445X

Lushchak, V.I.; Bagnyukova, T.V., Lushchak, O.V., Storey, J.M. & Storey, K.B. (2007). Diethyldithiocarbamate Injection Induces Transient Oxidative Stress in Goldfish Tissues. Chemico-Biological Interactions, Vol. 170, pp. 1–8, ISSN 0009-2797.

Machala, M.; Dusek, L., Hilscherova, K., Kubinova, R., Jurajda, P., Neca, J., Ulrich, R., Gelnar, M., Studnickova, Z. & Holoubek, I. (2001). Determination and Multivariate Statistical Analysis of Biochemical Responses to Environmental Contaminants in Feral Freshwater Fish *Leuciscus Cephalus*, L. *Environmental Toxicology and Chemistry*, Vol. 20, No.5, (May 2001), pp. 1141–1148, ISSN 1552-8618

Maret, W. & Vallee, B. (1998). Thiolate Ligands in Metallothionein Confer Redox Activity on Zinc Clusters. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.95, pp. 3478–3482, ISSN 0027-8424

McFarland, V.A.; Inouye, L.S., Lutz, C.H., Jarvis, A.S., Clarkem, J.U. & McCant, D.D. (1999). Biomarkers of Oxidative Stress and Genotoxicity in Livers of Field-collected Brown Bullhead, *Ameriurus nebulosus*. *Archives of Environmental Contamination and Toxicology*, Vol.37, pp. 236–241, ISSN 0090-4341

Meyer, J.N.; Smith, J.D., Winston, G.W. & Di Giulio, R.T. (2003). Antioxidant Defenses in Killifish (*Fundulus heteroclitus*) Exposed to Contaminated Sediments and Model Prooxidants: Short Term and Heritable Responses. *Aquatic Toxicology,* Vol.65, pp. 377–395, ISSN 0166-445X

Monserrat, J.M.; Martínez, P.E., Geracitano, L.A., Amado, L.L., Martins, C.M.G., Pinho, G.L.L., Chaves, I.S., Ferreira-Cravo, M., Ventura-Lima, J. & Bianchini, A. (2007). Pollution Biomarkers in Estuarine Animals: Critical Review and New Perspectives. *Comparative Biochemistry and Physiology*, Vol.146 C, pp. 221–234, ISSN 1532-0456

Moraes, B.S.; Loro, V.L., Glusczak, L., Pretto, A., Menezes, C., Marchezan, E. & de Oliveira Machado, S. (2007). Effects of Four Rice Herbicides on Some Metabolic and Toxicology Parameters of Teleost Fish (*Leporinus obtusidens*). *Chemosphere*, Vol.68, pp.1597–1601, ISSN 0045-6535

Mosleh, Y.Y.; Paris-Palacios, S., Couderchet, M., Biagianti-Risbourg, S. & Vernet, G. (2005). Effects of the Herbicide Isoproturon on Metallothioneins, Growth, and Antioxidative Defenses in the Aquatic Worm *Tubifex tubifex* (Oligochaeta, Tubificidae). *Ecotoxicology*, Vol.14, pp. 559–571, ISSN 0963-9292

Narbonne, J.F.; Daube`ze, M., Cle´randeau, C. & Garrigues, P. (1999). Scale of Classification Based on Biochemical Markers in Mussels: Application to Pollution Monitoring in European Coasts. *Biomarkers*, Vol. 4, pp. 415–424, ISSN 1875-3183.

Nikinmaa, M. & Rees, B. B. (2005). Oxygen-dependent gene expression in fishes. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, Vol.288, Issue 5, pp. R1079-R1090, ISSN: 0363-6119.

Nishimoto, M.; Le Eberhart, B.T., Sanborn, H.R. Krone, C., Varanasi, U. & Stein, J.E. (1995). Effects of a Complex Mixture of Chemical Contaminants on Hepatic Glutathione, L-cysteine and {gamma}-glutamylcysteine Synthetase in English Sole (*Pleuronectes vetulus*). *Environmental Toxicology and Chemistry*, Vol.14, Issue 3, pp. 461–469, ISSN 1552-8618

Niyogi, S.; Biswas, S., Sarker, S. & Datta, A.G. (2001). Seasonal Variation of Antioxidant and Biotransformation Enzymes in Barnacle, Balanus balanoides, and their Relation with Polyaromatic Hydrocarbons. Marine Environmental Research, Vol.52, pp. 13–26, ISSN 0141-1136

Otto, D.M.E. & Moon, T.W. (1996). Phase and 11 Enzymes and Antioxidant Responses in Different Tissues of Brown Bullheads from Relatively Polluted and Non-polluted Systems. *Archives of Environmental Contamination and Toxicology*, Vol.31, pp.141–147, ISSN 0090-4341

Padmini, E. (2010). Physiological Adaptations of Stressed Fish to Polluted Environments: Role of Heat Shock Proteins. *Reviews of Environmental Contamination and Toxicology*, Vol. 206, pp. 1–27, ISSN 0179-5953

Pandey, S.; Parvez, S., Sayeed, I., Haque, R., Bin-Hafeez, B. & Raisuddin, S. (2003). Biomarkers of oxidative stress: a comparative study of river Yamuna fish *Wallago attu* (Bl. & Schn.). *Science of the Total Environment*, Vol.309, pp. 105–115, ISSN 0048-9697

Parihar, M.S.; Javeri, T., Hemnani, T., Dubey, A.K. & Prakash, P. (1997). Responses of Superoxide Dismutase, Glutathione Peroxidase and Glutathione Antioxidant Defenses in Gills of the Freshwater Catfish (*Heteropneustes fossilis*) to Short-term Elevated Temperature. *Journal of Thermal Biology*, Vol.22, pp. 151–156, ISSN 0306-4565

Paris-Palacios, S.; Biagianti-Risbourg, S. & Vernet, G. (2003). Metallothionein Induction Related to Hepatic Structural Perturbations and Antioxidative Defences in Roach (*Rutilus rutilus*) Exposed to the Fungicide Procymidone. Biomarkers, Vol.8, pp. 128–141, ISSN 1354-750X

Paris-Palacios, S.; Biagianti-Risbourg, S., Fouley, A. & Vernet, G. (2000). Metallothioneins in Liver of Rutilus Rutilus Exposed to $Cu2^{+}$. Analysis by Metal Summation, SH Determination and Spectrofluorimetry. *Comparative Biochemistry and Phisiology*, Vol.126 C, pp. 113–122, ISSN 1532-0456

Parvez, S. & Raisuddin, S. (2005). Protein Carbonyls: Novel Biomarkers of Exposure to Oxidative Stress-Inducing Pesticides in Freshwater Fish *Channa punctata* (Bloch). *Environmental Toxicology and Pharmacology*, Vol.20, No.1, (December 2004), pp. 112–117, ISSN 1382-6689

Porte, C.; Escartín, E., de la Parra, L.M.G., Biosca, X. & Albaigés, J. (2002). Assessment of Coastal Pollution by Combined Determination of Chemical and Biochemical Markers in *Mullus barbatus*. *Marine Ecology Progress Series,* Vol.235, pp. 205–216, ISSN 0171-8630.

Raldúa, D.; Díez, S., Bayona, J.M. & Barceló, D. (2007). Mercury Levels and Liver Pathology in Feral Fish Living in the Vicinity of a Mercury Cell Chlor-Alkali Factory. *Chemosphere*, Vol.66, Issue 7, (January 2007), pp. 1217–1225, ISSN 0045-6535

Regoli, F. & Principato, G. (1995). Glutathione, Glutathione-dependent and Antioxidant Enzymes in Mussel, *Mytilus galloprovincialis*, Exposed to Metals in Different Field and Laboratory Conditions: Implications for a Proper Use of Biochemical Biomarkers. *Aquatic Toxicology*, Vol. 31, pp. 143–164, ISSN 0166-445X.

Regoli, F. (2000). Total Oxyradical Scavenging Capacity (TOSC) in Polluted and Translocated Mussels: a Predictive Biomarker of Oxidative Stress. *Aquatic Toxicology*, Vol.50, pp. 351–361, ISSN 0166-445X.

Regoli, F.; Gorbi, S., Frenzilli, G., Nigro, M., Corsi, I., Focardi, S. & Winston, G.W. (2002a). Oxidative Stress in Ecotoxicology: from the Analysis of Individual Antioxidants to a More Integrated Approach, *Marine Environmental Research*, Vol.54, pp. 419–423, ISSN 0141-1136

Regoli, F.; Nigro, M., Benedetti, M., Fattorini, D. & Gorbi, S. (2005). Antioxidant Efficiency in Early Life Stages of the Antarctic Silverfish, *Pleuragramma antarcticum*: Responsiveness to Pro-Oxidant Conditions of Platelet Ice and Chemical Exposure. *Aquatic Toxicology,* Vol.75, No.1, pp. 43–52, ISSN 0166-445X.

Regoli, F.; Pellegrini, D., Winston, G.W., Gorbi, S., Giuliani, S., Virno-Lamberti, C. & Bompadre, S. (2002b). 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*). *Marine Pollution Bulletin,* Vol.44, No.9, (September 2002), pp. 912–922, ISSN 0025-326X.

Reynders, H.; Bervoets, L., Gelders, M., De Coen, W.M. & Blust, R. (2008). Accumulation and Effects of Metals in Caged Carp and Resident Roach Along a Metal Pollution Gradient. *Science of the Total Environment*, Vol.391, No.1, (December 2007), pp. 82–95, ISSN 0048-9697

Rhee, J.S.; Raisuddin, S., Hwang, D.S., Lee, K.W., Kim, I.C. & Lee, J.S. (2009). Differential Expression of Metallothionein (MT) Gene by Trace Metals and Endocrine-Disrupting Chemicals in the Hermaphroditic Mangrove Killifish, Kryptolebias marmoratus. *Ecotoxicology and Environmental Safety*, Vol.72, pp. 206–212, ISSN 0147-6513.

Ritter, L.; Solomon, K., Sibley, P., Hall, K., Keen, P., Mattu, G. & Linton, B. (2002). Sources, Pathways, and Relative Risks of Contaminants in Surface Water and Groundwater: a Perspective Prepared for the Walkerton Inquiry. *Journal of Toxicology and Environmental Health*. Vol. 65, No.1, (January 2002), pp. 1-142, ISSN 1528-7394

Roberts, M.H.; Sved, D.W. & Felton, S.P. (1987). Temporal changes in AHH and SOD Activities in Feral Spot from the Elizabeth River, a Polluted Sub-estuary. *Marine Environmental Research,* Vol.23, pp. 89–101, ISSN 0141-1136.

Rodriguez-Ariza, A.; Alhama, J., Diaz-Mendez, F. M. & Lopez-Barea, J. (1999). Content of 8-oxodG in Chromosomal DNA of *Sparus aurata* Fish as Biomarker of Oxidative Stress

and Environmental Pollution. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, Vol.438, No.2, (January 1999), pp. 97-107, ISSN 1383-5718

Rodriguez-Ariza, A.; Peinado, J., Pueyo, C. & Lopez-Barea, J. (1993). Biochemical Indicators of Oxidative Stress in Fish from Polluted Littoral Areas. *Canadian Journal of Fisheries and Aquatic Sciences*, Vol.50, pp. 2568–2573, ISSN 0706-652X

Romeo, M.; Bennani, N., Gnassia-Barelli, M., Lafaurie, M. & Girard, J.P. (2000). Cadmium and Copper Display Different Responses Towards Oxidative Stress in the Kidney of the Sea Baas Dicentrarchus Labrax. *Aquatic Toxicology*, Vol.48, pp. 185–194, ISSN 0742-8413

Ruas, C.B.; Carvalho, C.D., de Araújo, H.S., Espíndola, E.L. & Fernandes, M.N. (2007). Oxidative Stress Biomarkers of Exposure in the Blood of Cichlid Species from a Metal-contaminated River. *Ecotoxicology and Environmental Safety,* Vol.71, Issue 1, (September 2008), pp. 86–93, ISSN 0147-6513.

Ruas, C.B.G.; Carvalho, C.S., de Araújo, H.S.S., Espíndola, E.L.G. & Fernandes, M.N. (2006). Oxidative Stress Biomarkers of Exposure in the Blood of Cichlid Species from a Metal-contaminated River. *Ecotoxicology and Environmental Safety*, Vol. 71, Issue 1, (September 2008), pp. 86–93, ISSN 0147-6513.

Sanchez, W.; Aït-Aïssa, S., Palluel, O., Ditche, J.M. & Porcher, J.M. (2007). Preliminary Investigation of Multi-biomarker Responses in Three-spined Stickleback (*Gasterosteus aculeatus* L.) Sampled in Contaminated Streams. *Ecotoxicology*, Vol.16, pp. 279–287, ISSN 0963-9292

Sheehan, D. (2006). Detection of Redox-based Modification in Two-dimensional Electrophoresis Proteomic Separations. *Biochemical and Biophysical Research Communications*, Vol.349, Issue 2, (October 2006), pp.455–462, ISSN 0006-291X.

Shigenaga, M.K. & Ames, B.N. (1991). Assays for 8-hydroxy-20-deoxyguanosine: A Biomarker of *in vivo* Oxidative DNA Damage. *Free Radical Biology and Medicine*. Vol.10, pp. 211–216, ISSN 0891-5849.

Solé, M.; Rodríguez, S., Papiol, V., Maynou, F. & Cartes, J.E. (2009). Xenobiotic Metabolism in Marine Fish with Different Trophic Strategies and their Relationship to Ecological Variables. Comparative Biochemistry and Physiology, Vol. 149 C, pp. 83–89, ISSN 0742-8413

Stanic, B.; Andric, N., Zoric, S., Grubor-Lajsic, G. & Kovacevic, R. (2006). Assessing Pollution in the Danube River Near Novi Sad (Serbia) Using Several Biomarkers in Sterlet (*Acipenser ruthenus* L.). *Ecotoxicology and Environmental Safety,* Vol. 65, pp. 395–402, ISSN 0147-6513.

Steadman, B.L.; Farag, A.M. & Bergman, H.L. (1991). Exposurerelated Patterns of Biochemical Indicators in Rainbow trout Exposed to No. 2 fuel oil. *Environmental Toxicology and Chemistry*, Vol.10, pp. 365–374., ISSN 1552-8618

Stegeman, J.J.; Brouwer, M., Richard, T.D.G., Forlin, L., Fowler, B.A., Sanders, B.M. & van Veld, P.A. (1992). Molecular Responses to Environmental Contamination: Enzyme and Protein Systems as Indicators of Chemical Exposure and Effect. In: Huggett, R.J., Kimerly, R.A., Mehrle, P.M., Jr, Bergman, H.L. (Eds.), *Biomarkers: Biochemical, Physiological and Histological markers of Anthropogenic Stress*. Lewis Publishers, Chelsea, MI, USA, pp. 235–335.

Stein, J.E.; Collier, T.K., Reichert, W.L., Casillas, E., Hom, T. & Varanasi, U. (1992). Bioindicators of Contaminant Exposure and Sublethal Effects: Studies with Benthic

Fish in Puget Sound, Washington. *Environmental Toxicology and Chemistry*, Vol. 11, pp. 701–714, ISSN 1552-8618

Stephensen, E.; Svavarsson, J., Sturve, J., Ericson, G., Adolfsson-Erici, M. & Forlin, L. (2000). Biochemical Indicators of Pollution Exposure in Shorthorn Sculpin (*Myoxocephalus scorpius*), Caught in Four Harbors on the Southwest Coast of Iceland. *Aquatic Toxicology*, Vol.48, pp. 431–442, ISSN 0166-445X

Sturve, J.; Berglund, Å., Balk, L., Broeg, K., Böhmert, B., Massey, S., Savva, D., Parkkonen, J., Stephensen, E., Koehler, A. & Förlin, L. (2005). Effects of Dredging in Göteborg Harbor, Sweden, Assessed by Biomarkers in Eelpout (*Zoarces viviparus*). *Environmental Toxicology and Chemistry*, Vol.24, No.8, pp. 1951–1961, ISSN 1552-8618

Sun, Y.; Yin, Y., Zhang, J., Yu, H., Wang, X., Wu, J. & Xue, Y. (2008). Hydroxyl Radical Generation and Oxidative Stress in *Carassius auratus* Liver, Exposed to Pyrene. *Ecotoxicology and Environmental Safety*, Vol.71, pp. 446–453, ISSN 0147-6513.

Sureda, A.; Box, A., Enseñat, M., Alou, E., Tauler, P., Deudero, S. & Pons, A. (2006). Enzymatic Antioxidant Response of a Labrid Fish (*Coris julis*) Liver to Environmental Caulerpenyne. *Comparative Biochemistry and Physiology*, Vol. 144 C, Issue 2, (October 2006), pp. 191–196, ISSN 0742-8413

Teh, S.J.; Adams, S. M. & Hinton, D.E. (1997). Histopathologic Biomarkers in Feral Freshwater Fish Populations Exposed to Different Types of Contaminant Stress. *Aquatic Toxicology*, Vol.37, Issue 1, (January 1997), pp. 51–70, ISSN 0166-445X

Tom, M. & Auslander, M. (2005). Transcript and Protein Environmental Biomarkers in Fish—a Review. *Chemosphere*, Vol. 59, Issue 2, (April 2005), pp. 155–162, ISSN 0045-6535

Üner, N.; Oruç, E. & Sevgiler, Y. (2005). Oxidative Stress-related and ATPase Effects of Etoxazole in Different Tissues of *Oreochromis niloticus. Environmental Toxicology and Pharmacology*, Vol.20, pp. 99–106, ISSN 1382-6689

Valavanidis, A.; Vlahogianni, Th., Dassenakis, M. & Scoullos, M. (2006). Molecular Biomarkers of Oxidative Stress in Aquatic Organisms in Relation to Toxic Environmental Pollutants. *Ecotoxicology and Environmental Safety*, Vol. 64, Issue 2, (June 2006), pp. 178–189, ISSN 0147-6513

van der Oost, R.; Beyer, J. & Vermeulen, N.P.E. (2003). Fish Bioaccumulation and Biomarkers in Environmental Risk assessment: a Review. *Environmental Toxicology and Pharmacology*, Vol. 13, Issue 2, (February 2003), pp. 57–149, ISSN 0926-6917.

van der Oost, R.; Goksoyr, A., Celander, M., Heida, H. & Vermeulen, N.P.E. (1996). Biomonitoring of Aquatic Pollution with Feral Eel (*Anguilla anguilla*). II. Biomarkers: Pollution Induced Biochemical Responses. *Aquatic Toxicology*, Vol.36, pp. 189–222, ISSN 0166-445X.

Vega-López, A.; Jiménez-Orozco, F.A., García-Latorre E.& Domínguez-López, M.L. (2008). Oxidative Stress Response in an Endangered Goodeid Fish (*Girardinichthys viviparus*) by Exposure to Water from its Extant Localities. *Ecotoxicology and Environmental Safety*, Vol.71, Issue 1, (September 2008), pp. 94–103, ISSN 0147-6513

Viarengo, A. & Nott, J.A. (1993). Mechanisms of Heavy Metal Cation Homeostasis in Marine Invertebrates. *Comparative Biochemistry and Physiology*, Vol.104 C, pp. 355–372, ISSN 0742-8413

Viarengo, A.; Burlando, B., Cavaletto, M., Marchi, B., Ponzano, E. & Blasco, J. (1999). Role of Metallothionein Against Oxidative Stress in the Mussel *Mytilus galloprovincialis*.

American Journal of Physiology – Regulatory, Integrative and Comparative Physiology, Vol.277, pp. 1612–1619, ISSN 0363-6119

Viarengo, A.; Lowe, D., Bolognesi, C., Fabbri, E. & Koehler, A. (2007). The Use of Biomarkers in Biomonitoring: a 2-tier Approach Assessing the Level of Pollutant-induced Stress Syndrome in Sentinel Organisms. *Comparative Biochemistry and Physiology*. Vol.146 C, pp. 281–300, ISSN 0742-8413

Vigano, L.; Arillo, A., Melodia, F., Arlanti, P. & Monti, C. (1998). Biomarker Responses in Cyprinids of the Middle Stretch of the Rover Po, Italy. *Environmental Toxicology and Chemistry*, Vol.17, pp. 404–411, ISSN 1552-8618

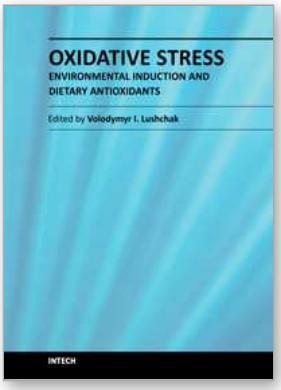
Winston, G.W. (1991). Oxidants and Antioxidants in Aquatic Animals. *Comparative Biochemistry and Physiology,* Vol.100 C, pp. 173–176, ISSN 0742-8413

Winston, G.W. & Di Giulio, R.T. (1991). Prooxidant and Antioxidant Mechanisms in Aquatic Organisms. *Aquatic Toxicology*, Vol.19, pp. 137–161, ISSN 0166-445X

Winzer, K.; Winston, G.W., Becker, W., Van Noorden, C.J.F. & Kohler, A. (2001). Sex-related Responses to Oxidative Stress in Primary Cultured Hepatocytes of European flounder (*Platichthys flesus* L.). *Aquatic Toxicology,* Vol. 52, pp. 143–155, ISSN 0742-8413

Woo S.; Yum, S., Kim, D.-W. & Park H.-S. (2009). Transcripts Level Responses in a Marine Medaka (*Oryzias javanicus*) Exposed to Organophosphorus Pesticide. *Comparative Biochemistry and Physiology*, Vol.149 C, No.3, pp. 427–443, ISSN 0742-8413

Yi, X.; Ding, H., Lu, Y., Liu, H., Zhang, M. & Jiang, W. (2007). Effects of Long-term Alachlor Exposure on Hepatic Antioxidant Defense and Detoxifying Enzyme Activities in Crucian Carp (*Carassius auratus*). *Chemosphere*. Vol.68, pp.1576–1515l, ISSN 0045-6535



Oxidative Stress - Environmental Induction and Dietary Antioxidants

Edited by Dr. Volodymyr Lushchak

ISBN 978-953-51-0553-4
Hard cover, 388 pages
Publisher InTech
Published online 02, May, 2012
Published in print edition May, 2012

This book focuses on the numerous applications of oxidative stress theory in effects of environmental factors on biological systems. The topics reviewed cover induction of oxidative stress by physical, chemical, and biological factors in humans, animals, plants and fungi. The physical factors include temperature, light and exercise. Chemical induction is related to metal ions and pesticides, whereas the biological one highlights host-pathogen interaction and stress effects on secretory systems. Antioxidants, represented by a large range of individual compounds and their mixtures of natural origin and those chemically synthesized to prevent or fix negative effects of reactive species are also described in the book. This volume will be a useful source of information on induction and effects of oxidative stress on living organisms for graduate and postgraduate students, researchers, physicians, and environmentalists.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821