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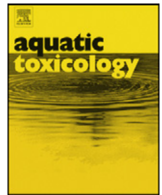

Review

Environmentally induced oxidative stress in aquatic animals

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ABSTRACT

Reactive oxygen species (ROS) are an unenviable part of aerobic life. Their steady-state concentration is a balance between production and elimination providing certain steady-state ROS level. The dynamic equilibrium can be disturbed leading to enhanced ROS level and damage to cellular constituents which is called "oxidative stress". This review describes the general processes responsible for ROS generation in aquatic animals and critically analyses used markers for identification of oxidative stress. Changes in temperature, oxygen levels and salinity can cause the stress in natural and artificial conditions via induction of disbalance between ROS production and elimination. Human borne pollutants can also enhance ROS level in hydrobionts. The role of transition metal ions, such as copper, chromium, mercury and arsenic, and pesticides, namely insecticides, herbicides, and fungicides along with oil products in induction of oxidative stress is highlighted. Last years the research in biology of free radicals was refocused from only descriptive works to molecular mechanisms with particular interest to ones enhancing tolerance. The function of some transcription regulators (Keap1–Nrf2 and HIF-1α) in coordination of organisms' response to oxidative stress is discussed. The future directions in the field are related with more accurate description of oxidative stress, the identification of its general characteristics and mechanisms responsible for adaptation to the stress have been also discussed. The last part marks some perspectives in the study of oxidative stress in hydrobionts, which, in addition to classic use, became more and more popular to address general biological questions such as development, aging and pathologies.
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Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; AMT, 3-amino-1,2,4-triazole; DDC, diethyldithiocarbamate; DPXs, DNA–protein crosslinks; GSH, GSSG, reduced and oxidized glutathione; GST, glutathione-S-transferase; HCB, hexachlorobenzene; OP, organophosphate; RBC, red blood cells; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

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1. Introduction

The presence of free radicals in biological systems was discovered over five decades ago and in fact immediately was implicated to diseases and aging (Harman, 1956). The description of enzymatic function for erythrocuprein as superoxide dismutase (SOD) in 1969 by McCord and Fridovich (1969) demonstrated the potential of living organisms to detoxify free radicals. It became clear that deleterious effects of free radicals may be controlled by specific antioxidant systems. The abovementioned and other researches paid attention to free radicals as occasionally generated and damaging species, and their negative effects were seen to be counterbalanced by antioxidant systems. Therefore, the interaction of free radicals with components of living organisms was supposed as clearly negative and one that should be avoided. However, followed physiological research discovered that the factor of muscle relaxation was also a free radical—nitric oxide (Palmer et al., 1987). In 1990th the attention of scientists was attracted also to deciphering regulatory pathways in which free radicals are involved (Wu and Weiss, 1991). To date, many of these pathways are investigated in details, and some of them are bacterial *OxyR* and *SoxRS* regulons; yeast Yap1, Skn7, and Msn2/4p stimulons; plant and animal Nrf2/Keap1, Ap-1, and MAP-kinase pathways (Scandalios, 2005; Jacob et al., 2006; Veal et al., 2007; Winterbourn and Hampton, 2008). It is absolutely clear, that although free radicals possess injuring potential and they realize it in living organisms, their level is under strict control to prevent damage. However, the delicate balance in some cases may be disturbed leading to perturbations of redox status. Many free radicals are sensed by specific systems and may be involved in regulation of redox status via feedback mechanisms. Moreover, in many cases free radicals and their derivatives regulate many different processes, like hormonal response in plants (Apel and Hirt, 2004; Chagué et al., 2006) and animals (Murphy et al., 2005; Winterbourn and Hampton, 2008). Being relatively non-specific, free radicals may be involved in particular pathways due to the specificity of especially designed sensor molecules and signal transducers (Winterbourn and Hampton, 2008). Recently it was recognized that spatiotemporal pattern, i.e. specific localization of the signal transduction and realization along with its time course, is also as an important player in the described processes.

Aquatic organisms also possess systems for generation and degradation of free radicals (Winston, 1991; Winston and Di Giulio, 1991; Kelly et al., 1998; Valavanidis et al., 2006). Water bodies receive increased number of agricultural and industrial chemicals which being taken up by organisms may perturb free radical processes. The uptake of these pollutants by hydrobionts can occur from water, sediments, suspended particulate matter, and food sources. The current knowledge and recent advances in general toxicology and particularly in toxicology of hydrobionts provide a fertile field for aquatic toxicology studies. In addition, aquatic organisms may serve as model systems in investigation of basic processes of cellular damage and protection by free radicals, development of tissue injury and followed physiological consequences like diseases, and aging.

This review article describes the mechanisms of generation and elimination of reactive species, essential methods for evaluation of oxidative stress development, some of known mechanisms of induction of oxidative stress in hydrobionts by broad set of environmental factors and pathways leading to up-regulation of antioxidant potential in aquatic organisms.

2. Reactive oxygen species and oxidative stress

The metabolism of free radicals in biological systems has been a hot point during last half century. Free radicals are atoms, molecules, or ions with unpaired electrons on an otherwise open shell configuration (Halliwell and Gutteridge, 1989). These unpaired electrons are usually highly reactive due to which radicals are likely to take part in chemical reactions. Very often they are confused with reactive oxygen species (ROS) such as molecular and singlet oxygen, superoxide anion, hydrogen peroxide, hydroxyl radical and some their derivatives. Although hydrogen peroxide (H_2O_2) is not a radical, it is a reactive species because has higher activity than molecular oxygen. The transformation and relationships between different ROS species are given in Fig. 1. ROS are products of partial reduction of molecular oxygen. Usually molecular oxygen is reduced via four-electron mechanism by mitochondrial electron-transport chain resulting in water formation. However, sequential addition of a single electron to O_2 gives superoxide anion ($O_2^{\bullet -}$) further reduced to hydrogen peroxide, and finally to hydroxyl radical ($^{\bullet}OH$) and hydroxyl anion. The chain is finished by water formation after electron and proton addition to $^{\bullet}OH$. The ways of ROS generation in biological systems will be described below, and here we only underline that they are continuously produced in these systems either as side products of aerobic metabolism, or products of specialized systems, designed to produce ROS. As abovementioned, ROS can be

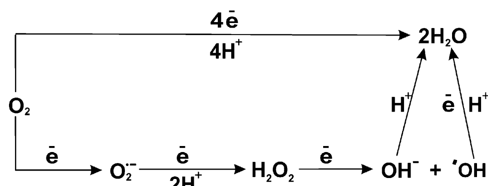


Fig. 1. Routes of oxygen metabolism in organisms. The upper part of the scheme demonstrates four-electron oxygen reduction with water formation. The lower one shows one-electron reduction sequence, leading to the formation of reactive oxygen species, namely superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$), and ended by reduction of $^{\bullet}OH$ to water.

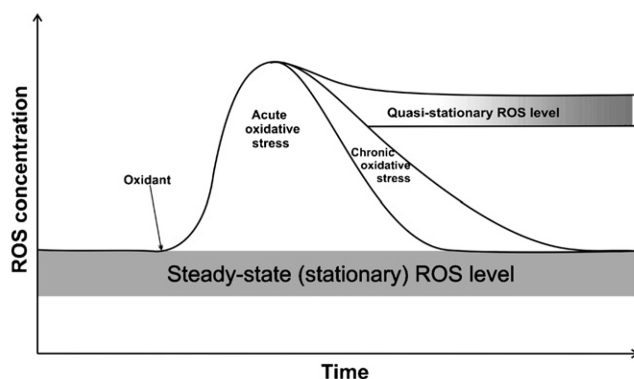


Fig. 2. The dynamics of oxidant-induced perturbations of level of reactive oxygen species in living organisms. A steady-state ROS level is a dynamic parameter reflecting the balance between their production and elimination. At oxidative boots, the ROS level is enhanced and if counterbalanced by antioxidants, quickly returns to initial stationary range and these events are called "acute oxidative stress". However, if the efficiency of antioxidant system is not high enough to decrease ROS level to initial stationary concentration, the enhanced ROS level can be maintained for longer periods (called "chronic oxidative stress") and the only increase of efficiency of antioxidant system may return the system into initial corridor. Under some circumstances, the perturbed system does not return to initial ROS steady-state level and it is stabilized at increased ROS level called "quasi-stationary".

bound or detoxified by different types of antioxidants or can interact with cellular/extracellular components. The metabolism of ROS due to their high damaging capacity and biological activity is under fine cellular control and their concentrations usually do not exceed 10^{-8} M (Halliwell and Gutteridge, 1989).

Under some circumstances, ROS concentrations may be changed. Because ROS are continuously generated and eliminated, it should be remembered, that *ROS concentration is a dynamic parameter*, i.e. we have to talk about *steady-state ROS concentrations*. This means that usually the amount of ROS produced is virtually equal to the eliminated one. However, due to some reasons ROS concentration may be changed leading to disturbance of redox status that has been called as oxidative or reductive stress. *Oxidative stress is a situation when steady-state ROS concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents.* "Reductive stress" may be defined in similar way with the only difference that steady-state ROS concentration is decreased. Oxidative stress development, cellular ROS sources and antioxidant systems will be analyzed below. The schematic presentation of oxidative stress is given at Fig. 2. Under normal conditions, the ROS level is stabilized in certain corridor provided by the balance between ROS production and elimination. Oxidative insult enhances the ROS steady-state level and, if the antioxidant potential is high enough, ROS level returns into initial corridor and a transient increase of ROS level is called "acute oxidative stress". When the efficiency of antioxidant system does not counterbalance enhanced ROS production quickly, the system may recover from the stress longer which is called "chronic oxidative stress". However, the system may be stabilized at new ROS steady-state level, higher than the initial one and that can be called "quasi-stationary ROS level".

3. Production and elimination of reactive oxygen species

3.1. Generation of reactive oxygen species in biological systems

There are plural mechanisms of ROS generation in biological systems. In most cases, they are produced as side-products of oxygen metabolism. Over 90% of oxygen consumed by organisms is used via four-electron mechanisms by electron-transport chains related to energy production (Papa and Skulachev, 1997). In eukaryotes, there is a mitochondrial system, while in prokaryotes the chains are localized in plasmatic membrane. Less than 10% of consumed oxygen is reduced via one-electron scheme giving rise to ROS. Coenzyme Q and complex III are believed to be the main places of mitochondrial electron-transport chain where electrons "escape" it and interact with molecular oxygen giving $O_2^{\bullet -}$ (Demin et al., 1998). Electron-transport chain of endoplasmic reticulum is the second most important ROS source (Malhotra and Kaufman, 2007). The catabolism of cellular and foreign chemicals by cytochromes P450 includes the redox steps and is responsible for ROS production in endoplasmic reticulum.

Certain amounts of ROS are produced in cytosol and peroxisomes by different oxidases. For example, tryptophan dioxigenase (Li et al., 2007), xanthine oxidase (Shmarakov and Marchenko, 2008; Kelley et al., 2010), and cytochrome P450 reductase (Cederbaum, 1989) mainly produce $O_2^{\bullet -}$, while such enzymes as oxidases of amino acids and glucose mainly generate H_2O_2 (Bonnefont-Rousselot, 2002).

Autooxidation of certain cellular components and xenobiotics (their oxidation that occuring in open air or in presence of oxygen and/or UV radiation and forms peroxides and hydroperoxides) may be responsible for the production of substantial ROS amounts. Catecholamines and some other compounds naturally occurring in organisms can be important ROS producers under specific physiological states, leading to diseases and aging (McAnulty et al., 2003). In according with the goal of this review, foreign compounds, i.e. xenobiotics, should be considered especially. Again, more details on their effect on ROS metabolism will be given below, and therefore, here we will only mention the main groups. They are well known pollutants: metals, aromatic hydrocarbons, pesticides, polychlorinated biphenyls, dioxins and many others. The underlining mechanisms throughout they lead to ROS production may be very different, but the production of reactive species combines them together (Bauer and Bauer, 1999; Valko et al., 2005, 2007; Lushchak, 2008). It should be noted here that the development of oxidative stress is a common component in any substantial stress.

Most organisms possess specifically designed systems to produce certain reactive species in finely controlled manner. Firstly described in leucocytes as cyanide-insensitive oxidative burst (Dri et al., 1978, 1979), similar processes were found later in many other cell types of animals and further in plants (Asai et al., 2008; Foyer and Noctor, 2009). The molecular mechanisms on this delicately regulated system of ROS production rely on enzymatic oxidation of NADPH by NADPH-oxidase. This system is used to attack invading microorganisms and, probably, to control cellular ROS level. Interestingly, very similar systems of immune defense were found in both, animals and plants. The second, well characterized system of reactive species production was found to generate nitric oxide by specific NO-synthase (Agnisola, 2005). The latter is responsible for the regulated cleavage of amino acid arginine resulting in $^{\bullet}$NO formation which further may either directly interact with cellular targets, or do that after combining with other compounds. As abovementioned, reactive species of oxygen or nitrogen are low specific ones, but specificity of their effects may be reached via interaction with certain cellular components. There is plenty of information on the involvement of specific sensors in the realization of ROS and RNS effects via certain sensors like Keap1/Nrf2, NF-kB, MAP-kinase, HIF-1α and other regulatory pathways.

3.2. Elimination of reactive oxygen species

The antioxidant system in aquatic animals comprises both—low molecular mass and high molecular mass antioxidants (Livingstone, 2001). Low molecular mass antioxidants

described to date include water-soluble compounds such as reduced glutathione, ascorbic acid (vitamin C), and lipid-soluble ones such as carotenoids (including β-carotene), retinol (vitamin A), α-tocopherol (vitamin E). They usually operate as free radical scavengers. However, other mechanisms can be implicated here. For example, glutathione may serve as a cofactor for antioxidant enzymes such as glutathione-dependent peroxidases, or glutathione-S-transferases, a second phase detoxification enzyme. High molecular mass antioxidant group consists of specific or non-specific proteins. A specific group includes antioxidant enzymes superoxide dismutases (EC 1.15.1.1), catalases (EC 1.11.1.6), Se-dependent glutathione peroxidases (GPx, EC 1.11.1.9), DT-diaphorase (EC 1.6.99.2) and associated ones providing needed cofactors—glutathione reductase (GR, EC 1.6.4.2), glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49). Non-specific high molecular mass antioxidants are represented by proteins that prevent ROS-induced damage by binding to transition metal ions (mainly iron and copper) such as metallothioneins and ferritin.

The steady-state level of antioxidants is provided by the balance between their absorption/synthesis, transport, metabolization, inactivation, and excretion. Some antioxidants like tocopherol and carotenoids are obtained by aquatic animals with food, while most are produced. The latter ones include glutathione, ascorbic acid along with high molecular mass ones. Interestingly, the production of antioxidants usually corresponds to needs of organisms and is subjected to active regulation. To date, many mechanisms of up-regulation of antioxidant potential have been deciphered and some of them will be discussed below.

The modern ideas on the balance between production and elimination of reactive oxygen species are given at Fig. 3 with concominant relationship with potential biological effects and resulting in the changes in organism functions.

4. Critical analysis of oxidative stress markers

The evaluation of oxidative stress markers is a key question in the investigation of oxidative stress in organisms. In some cases, ROS level may be monitored by direct or nondirect methods. Although the direct registration of ROS is a very useful approach, it is virtually impossible to perform *in vivo* due to technical reasons. Instead of that the monitoring of products of ROS-induced modification of cellular constituents or specific introduced compounds is more common approach to evaluate the stress. Due to low concentrations and high instability of ROS, it would be debatable to state that there is some ideal method or group of methods which let to characterize all aspects of oxidative stress. Many precautions should be taken into account dealing with oxidative stress.

4.1. ROS registration

Radical species possess unpaired electrons that can be detected by electron paramagnetic resonance (EPR) method. This technique was successfully applied to register reactive species *in vivo* in bacteria (Macomber et al., 2007; Ren et al., 2009) and yeast (Teixeira et al., 2004; Poljsak et al., 2005). Very few works are known on the use of this technique to monitor the ROS levels in aquatic organisms (Luo et al., 2008; Sun et al., 2008). A fluorescent technique is the second and more popular than previous approach which is broadly used to monitor ROS level in cells. In this case, special compounds are introduced into the cell. Here they undergo oxidative modification due to which become fluorophores. The intensity of fluorescence is supposed to be proportional to ROS level. Dichlorofluorescein is one of the commonly applied fluorophores from this class. The acetate ether of dichlorofluorescein is uncharged molecule due to which it easily crosses biological membranes. In the cell, it is cleaved by

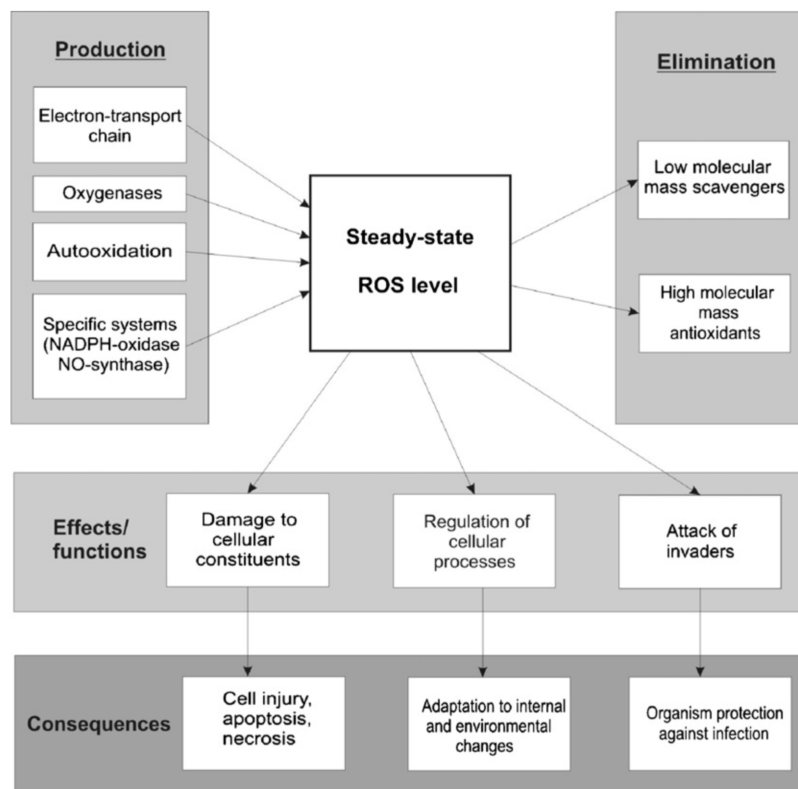


Fig. 3. Balance between production and elimination of ROS and their potential biological effects. The steady-state ROS level is provided by the balance between ROS generation and elimination. However, under any conditions some ROS portion escapes defense systems and affects biological systems—damage cellular components, induce and modify regulatory cascades, and attack invaders. The latter three events lead to cellular injury via different mechanisms, adaptation to changeable conditions and host protection against infections.

intracellular esterases and became charged ion and, therefore, is locked in the cell. The oxidation of dichlorofluorescein results in the formation of fluorophore (Pereira et al., 2003; Poljsak et al., 2005). Despite low specificity to ROS, with some precautions it is used for registration of intracellular ROS levels, generally applied to monitor ROS levels in cell suspensions or individual cells. The use of this approach to determine ROS levels in multicellular organisms, particularly, to aquatic animals is scarce (Laville et al., 2004; Amado et al., 2009).

The induction of oxidative stress usually is monitored via registration of products of ROS-induced modification of cellular constituents. ROS-modified lipids, proteins and nucleic acids along with low and high molecular mass antioxidants and antioxidant potential is a battery of indices commonly used to describe oxidative stress. But never one of the parameters should be used to describe oxidative stress, and instead some battery of techniques should be applied.

4.2. ROS-induced modification of lipids, proteins and nucleic acids

It seems that oxidation of lipids is the most commonly used approach in free radical research field because many organisms, especially aquatic ones, contain high amounts of lipids with polyunsaturated fatty acid residues, a substrate for oxidation. Since lipids are oxidized usually through the formation of peroxides, the process of their formation has been called "lipid peroxidation". Again, in this case as well as in described below ROS-induced modification of proteins and nucleic acids, the parameters monitored most likely reflect the increase in steady-state ROS concentrations, rather than turnover of damaged molecules, and this may underestimate the real level. Very often products of lipid peroxidation are unstable and to specify this they have been called "primary", "secondary" and "end" products (Janero, 1990). Primary products include shortly lived species frequently of radical nature. Under model experimental conditions they can be measured by EPR technique. The second group, secondary products, consists of lipid peroxides, dienic conjugates, and ketodienes. They are more reliably measured that the first group. For example, in our works we used iron/xylenol orange method developed by Hermes-Lima and colleagues to evaluate the level of lipid peroxides (Hermes-Lima et al., 1995). It let to characterize oxidative stress development in several fish species (Lushchak et al., 2005a,b, 2007), and diene conjugates were successfully measured in goldfish tissues (Lushchak et al., 2001). These methods can be recommended as reliable, reproducible, nonexpensive and easy to perform for monitoring of oxidative stress in aquatic organisms.

However, the most frequently used methods to monitor lipid peroxidation are based on measuring of the end products. Among other they are represented by malonic dialdehyde (MDA), and 4-hydroxynonenal. MDA is of particular interest because in commonly used assay it is measured with thiobarbituric acid (TBA). It should be noted that TBA reacts also with many types of compounds, such as different aldehydes, amino acids, and carbohydrates. Therefore, in this case it is not correct to refer to MDA measurement, but rather to TBA-reactive substances (TBARS). Taking into account the low specificity of this method we mainly omitted it from our experiments. But MDA is really one of the end products of lipid peroxidation and it is very attractive to monitor its concentration. Some relatively new approaches to measure the end products of lipid peroxidation were proposed recently. They are HPLC and immune techniques (Claeson et al., 2001), which are more specific than at TBARS measurement and can be recommended for research with aquatic organisms.

It should be again underlined, that so-called "end products of lipid peroxidation" are dynamic parameter because they can be further either catabolized, or interact with other cellular components, for example, with proteins. The lipid adducts with proteins also may be determined by immune techniques (Toroser et al., 2007), which potentially may be applied for hydrobionts.

ROS-induced modification of proteins became a popular measure of oxidative stress. This is partially due to introduction of rather simple spectrophotometric technique to evaluate the content of protein carbonyl groups with dinitrohenylhydrazine (Levine et al., 2000). This method has been extensively used (Hansen et al., 2006a,b; Ivanina et al., 2008; Falfushynska et al., 2008a,b, 2009; Lushchak et al., 2008, 2009a,b,c) to evaluate the intensity of free radical processes in aquatic animals. The parameter is reliable and can be recommended for a broad usage. At least three issues should be taken into account in this case. Firstly, proteins always contain carbonyl groups and ROS-induced oxidation only adds new ones. Secondly, it is again a dynamic parameter because proteins, especially oxidized ones, can be catabolized. However, in some cases heavily oxidized proteins can be accumulated in the cell (Widmer et al., 2006; Dunlop et al., 2009). Thirdly, at long term studies the set of cellular proteins with different carbonyl content may be changed leading to apparent change in protein oxidation intensity. In addition to measurement of protein carbonyl groups as a marker of oxidative stress, several other techniques can be applied to reveal the intensity of ROS-induced damage to proteins and interested readers can be addressed to several reviews (Dean et al., 1997; Stadtman and Levine, 2003; Lushchak, 2007).

Reactive species induce plural changes in DNA. Because of critical DNA importance for the cell, it is very attractive to have a relevant method to evaluate its ROS-induced DNA modifications. Several techniques have been developed to address the issue. The formation of oxidized bases, particularly 8-oxoguanine (8-OG), gives a powerful tool to evaluate this process. It can be measured by HPLC (Kelly et al., 2008) or immune (Bespalov et al., 1999) techniques. The measurement of 8-OG was applied to aquatic animals (Malins et al., 1996; Gielazyn et al., 2003; Grygoryev et al., 2008). The Comet assay, which in different modifications may reflect variety of DNA damages was successfully applied to fish (Toyoizumi et al., 2008; Cavalcante et al., 2008; Caliani et al., 2009) and other aquatic animals (Petridis et al., 2009; Binelli et al., 2009). For more details on the usage of Comet assay, readers should refer to several reviews (Jha, 2008; Valverde and Rojas, 2009).

Summarizing this section, it should be underlined that due to specific nature of reactive species, there are no "ideal markers" of oxidative stress. At least several indices should be used to characterize oxidative stress development. In model experiments, the induction of oxidative stress has to be evaluated in two measures: (*i*) dynamics of the process, and (*ii*) concentration effects. The dynamics is very important because different indices demonstrate varied time-courses.

4.3. Antioxidant potential

The antioxidant potential is a very complicated parameter which depends on many circumstances. There are several approaches to register it. They are usually divided for low and high molecular mass antioxidants. The first group – low molecular mass antioxidants – is represented by ascorbic and uric acids, tocopherol, glutathione and others, while the second one is represented by antioxidant enzymes and some specific proteins/enzymes like ferritin and metallothioneins. The measurement of activities of antioxidant enzymes which is a sum of activities of many enzymes, in fact is not good way to get some integrative parameter. The monitoring of activities of individual antioxidant enzymes may give some clues to evaluate total antioxidant potential, but in many cases it is complicated to interpret the results. That is because the activities are measured *in vitro*, but results should be discussed from the point of their *in vivo* operation. The activity of

antioxidant enzymes was measured in many aquatic organisms and usually is an element of monitoring of free radical processes (Timofeyev et al., 2006; Valavanidis et al., 2006; Falfushynska and Stolyar, 2009). Very similar ideology is used for antioxidant potential measurement which is mainly based on monitoring the levels of low molecular mass antioxidants such as glutathione, tocopherol, ascorbic and uric acids, and has been applied to hydrobionts (Wilhelm Filho, 1996; Sayeed et al., 2003; Wang et al., 2009). Again, usually individual compounds are monitored either as total content, or in some cases both, reduced and oxidized forms. The second approach, particularly with glutathione allows to calculate cellular redox potential (Jones, 2006; Heise et al., 2006a; Philipp et al., 2008) or at least the ratio of the concentrations of reduced and oxidized forms (Lushchak et al., 2009a,c) and may give some clues to characterize the redox status of intracellular milieu.

The use of specific compounds which are readily oxidized and monitored is also used to evaluate a total antioxidant potential. In this case, a delay of induced oxidation of certain compounds by cell extracts is evaluated. Several systems are used for these purposes and scavenging the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation ($ABTS^+$) generated in the aqueous phase in combination with Trolox calibration gives satisfying results for low molecular mass antioxidants (Mira et al., 1999).

5. Environmentally induced oxidative stress

Up to now, it has been clearly established that actually any strong stress usually is accompanied by oxidative stress. It seems to be true for hydrobionts as well. It can be assumed that oxidative stress may be responsible for adaptation of organisms to a broad range of environmental stressors. In this section we will analyze the environmental conditions known to be inducers of oxidative stress in hydrobionts.

5.1. Temperature

Temperature is known to affect all living organisms, and considering that in this review we focus on ectothermic animals, the temperature critically influences this group. Different mechanisms are responsible for induction of oxidative stress by increase and decrease of environmental temperature. The increase in temperature stimulates all metabolic processes in according with known thermodynamic principles. For example, it enhances oxygen consumption and, therefore, may increase ROS production as side products of intensified metabolism resulting in oxidative stress. The enhanced environmental temperature induced oxidative stress in frogs (Bagnyukova et al., 2003), fish (Parihar and Dubey, 1995; Heise et al., 2006a,b; Lushchak and Bagnyukova, 2006; Bagnyukova et al., 2007a,b) and other aquatic animals (Verlecar et al., 2007; Bocchetti et al., 2008). In according with abovementioned intensification of oxidative metabolism one might expect decrease in the risk of oxidative stress induction at decreased temperature. Generally it is true. But under some circumstances the decrease in environmental temperature also may cause oxidative stress in fish (Malek et al., 2004) and barnacle *Balanus balanoides* (Niyogi et al., 2001). One may speculate that at least two reasons lead to this: (*i*) temperature decrease weakens the systems of ROS elimination, and/or (*ii*) enhances ROS production. Unfortunately, there is no information on the mechanisms involved.

5.2. Oxygen level

Both, temperature along with oxygen availability are critical parameters for hydrobiont distribution. It is logically suggested that increased O_2 levels increase ROS generation due to enhanced probability of electrons escaped from electron-transport chains to combine with molecular oxygen. However, organisms possess specific adaptive mechanisms to prevent negative consequences of high environmental oxygen levels. At behavior level they may avoid the areas oversaturated by oxygen, whereas at organism level, they may reduce their capability to extract environmental oxygen.

The exposure to hyperoxia induced oxidative stress in different fish species such as goldfish *C. auratus* (Lushchak et al., 2005a), Atlantic salmon *Salmo salar* (Olsvik et al., 2005), and senegal sole *Solea senegalensis* (Salas-Leiton et al., 2009). Similar results were reported with Antarctic scallop *Adamussium colbecki* and Mediterranean scallop *Pecten jacobaeus* (Viarengo et al., 1995) and sea anemone *Anthopleura elegantissima* (Dykens et al., 1992). Therefore, it is clear that the increased level of external oxygen is an inducer of oxidative stress in aquatic animals.

The decreased oxygen concentration can decrease the chance of production of free radicals in the organisms. That is true in many cases, but here we analyze the induction of oxidative stress. It may look strange, but surprisingly the decrease in environmental oxygen concentrations also can induce oxidative stress. In goldfish, the exposure to anoxia increased SOD and catalase activities in liver (Lushchak et al., 2001) which was used as a demonstration of "preparation to oxidative stress" idea (Hermes-Lima et al., 1998). That hypothesis states that in organisms evolutionary adapted to transitions between normal and extreme external conditions, an exposure to extreme ones induces the adaptive response which helps them to survive at recovery. For example, when these organisms are exposed to oxygen deficiency, they enhance the antioxidant potential in order to prevent the development of oxidative stress when oxygen supply is restored (so-called oxygen paradox when the tissues are injured due to restoration of transient oxygen limitation). One may suggest that the mechanisms of up-regulation of antioxidant enzymes could involve the response to enhanced ROS level at transitions from normoxia via hypoxia to anoxia. Later we found that hypoxia also increased the activities of SOD and catalase in liver of common carp *Cyprinus carpio* (Lushchak et al., 2005b), which was also discussed in frames of "preparation to oxidative stress" idea. Finally, exploiting another fish model, we clearly demonstrated oxidative stress development in rotan *Percottus glenii* under hypoxic conditions (Lushchak and Bagnyukova, 2007). Recently, hypoxia-induced oxidative stress was partly confirmed on medaka *Oryzias latipes* (Oehlers et al., 2007) where hypoxia increased the level of glutathione-S-transferase. In other work, on the freshwater clam *Corbicula fluminea* hypoxia increased the activities of catalase and glutathione peroxidase (Vidal et al., 2002). The transcription of antioxidant enzymes was up-regulated in disk abalone *Haliotis discus* under hypoxic exposure (De Zoysa et al., 2009).

The mechanisms of hypoxia-induced oxidative stress have not been established yet. However, several possibilities on those can be suggested. The first, under hypoxic conditions the carriers of electron-transport chains are more reduced due to limited oxygen availability. Therefore, there are more electrons to escape from the chains and join oxygen molecules. The second of principally known mechanisms, may be related with operation of xanthine reductase/xanthine oxidase system. Under hypoxic conditions the first enzyme can be theoretically converted to the second via limited proteolysis or oxidation and be transformed in efficient ROS producer.

Oxygen in ozone form may appear in water due to solar irradiation. Although in this case high ozone concentrations are not supposed to be reached, the local concentration may be high enough to induce oxidative stress. Hydrobionts may be exposed to ozone at water treatment stations. In model experiments, the exposure of red blood cells (RBC) of rainbow trout (*Oncorhynchus mykiss*) to ozone induced hemolysis, formation of methemoglobin,

and RBC membrane lipid peroxidation (Fukunaga et al., 1999). The incubation with ozone enhanced the generation of H_2O_2 which was supposed to play a critical role in mediating RBC damage. The authors concluded that neither ozone, nor its derivatives, directly attacked the cell from the outside, but ozone that penetrated through the membrane was suggested to stimulate ROS production by intracellular hemoglobin. The effects of ozone on rainbow trout (*O. mykiss*) could be also connected with the stimulation of ROS production (Hébert et al., 2008).

5.3. Salinity

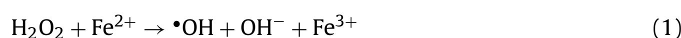
In aquatic organisms, salinity change causes a variety of physiological responses such as plasma enhanced stress-related hormones, stimulation of energy metabolism, and disturbation of electrolyte equilibrium. The stress induced by salinity change has been associated with enhanced ROS generation, causing oxidative damage (Liu et al., 2007). For example, when shrimp *Litopenaeus vannamei* were subjected to acute salinity change (30‰ vs 5, 15, 30, and 50% of that) for 24 h, the activities of SOD, catalase, glutathione peroxidase and Na^+/K^+-ATPase were changed. The diet supplementation with vitamin E in moderate doses enhanced the resistance of shrimp to acute changes in salinity, while its higher doses were not effective. These results demonstrated that change in salinity might be toxic due to induction of oxidative stress and vitamin E can be potentially useful to prevent dangerous scenario under these conditions (Liu et al., 2007). Choi et al. (2008) exposed olive flounder *Paralichthys olivaceus* for 48 h to seawater (35‰) and 17.5, 8.75, 4 and 0‰ by adding the underground water and studied the expression of glutathione peroxidase and glutathione-S-transferase by qPCR technique and some other markers of fish physiological state. They concluded that both studied enzymes played an important role in detoxification of ROS, and thereby these might serve as indicators of oxidative stress responses to salinity changes in olive flounder (Choi et al., 2008).

At gradual acclimation of sturgeons *Acipenser naccarii* from freshwater to full seawater (35‰ salinity), catalase, glutathione peroxidase and superoxide dismutase activities and lipid peroxide levels were measured in blood, liver and heart (Martínez-Alvarez et al., 2002). After 20 days at 35‰ salinity, plasma osmolality, erythrocyte constants and muscle water content all returned to values usual for low environmental salinity, indicating that osmoregulatory processes have achieved their objective. However, cortisol values, antioxidant enzyme activities in the blood, lipid peroxidation in plasma, and hepatic proteins did not return to initial values, showing that osmoregulatory processes caused substantial physiological changes in the fish. The antioxidant defense had the ability to augment under increasing environmental salinity showing that free radical processes disturbed under transition to seawater indicated their active role in adaptation. The described at this subsection data clearly show that perturbation of environmental salinity is accompanied by modification of free radical processes in hydrobionts.

5.4. Transition metal ions

Metal ions are well known inducers of oxidative stress. They can stimulate ROS production via two different mechanisms. The first one is related with the interference of metal-related processes and the second one with the generation of free radicals by ions with changeable valence. Actually, the second mode also may interfere with the first, but usually the main attention is paid to effects of metal ions with changeable valence. Therefore, in this subsection we will focus on ions with changeable valence, or ions of transition metals.

The information on generation of oxidative stress in hydrobionts by ions of transition metals is abundant. Here we will give a systematic analysis of this question with special attention to the mechanisms involved. The capability of these ions to change valence state is actually the most important property of this group of ions in addition to their metallic ones. It has many consequences and we will analyze their capability to induce oxidative stress via reversible oxidation in aquatic animals. Due to limited space we will concentrate here on the most important and studied ions—iron, copper, chromium, mercury and arsenic. The first three ions can donate electrons to H_2O_2 yielding hydroxyl radical and anion in Fenton reaction:

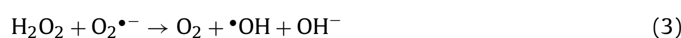


$$H_2O_2 + Fe^{2+} \rightarrow {}^{\bullet}OH + OH^- + Fe^{3+} \quad (1)$$

Oxidized iron ion (III) may be reduced via a reaction with $O_2^{\bullet-}$ to form molecular oxygen in according with equation:

$$O_2^{\bullet-} + Fe^{3+} \rightarrow O_2 + Fe^{2+} \quad (2)$$

The net of reactions described above give the reaction:



$$H_2O_2 + O_2^{\bullet-} \rightarrow O_2 + {}^{\bullet}OH + OH^- \quad (3)$$

that for the first time was postulated by F. Haber and T. Weiss in 1934 and has become known later as Haber–Weiss reaction. In addition to iron and copper, this reaction can be catalyzed by ions of chromium, cobalt, titanium, vanadium and their complexes.

As mentioned above here we will describe only effects of ions of iron, copper, chromium, mercury and arsenic on free radical processes in aquatic animals.

5.4.1. Iron

Up to date, the metabolism of iron in eukaryotes is rather well studied (Aisen et al., 2001; Ponka, 1999). In fact, all studied organisms depend on iron for survival. The paradox of iron-related life is connected with the hazards of both, iron deficiency and iron overload, each with serious or even fatal consequences. It is assumed that iron metabolism and its function in aquatic animals is similar to other ones. For example, fish obtain iron from water by uptake across the gill epithelium and by intestinal uptake from food (Bury et al., 2003). Iron metabolism in rainbow trout at normal and iron deficient diets have been studied in details (Walker and Fromm, 1976; Carriquiriborde et al., 2004). It is well established that iron is essential element involved in many living processes. However, because it undergoes redox cycle it is involved also in initiation and propagation of free radical processes, but the information on free radical processes induced by this element in hydrobionts is limited.

In a series of experiments, the African catfish *Clarias gariepinus* was fed with normal and iron enriched diets during five weeks (Baker et al., 1997). Ingestion of the higher dietary iron ration suppressed fish growth indicating that the metal was supplied at sub-lethal, but toxic levels. The tissue iron concentrations were unaffected by dietary regimen. However, the concentrations of malonic dialdehyde, a product of free radical lipid peroxidation, in liver and heart increased concomitantly with dietary ion dose. At the same time, fat-soluble antioxidant α-tocopherol (vitamin E) was significantly depleted in liver of fish fed with high iron diet. The dynamic of malonic dialdehyde and α-tocopherol, demonstrated the development of oxidative stress induced by high iron level in food (Baker et al., 1997).

The effects of waterborn iron on free radical processes in goldfish *C. auratus* liver and kidney were studied in our laboratory (Bagnyukova et al., 2006). The treatment increased the levels of protein carbonyl groups, a marker of oxidative modification of proteins, but decreased the concentrations of lipid peroxides. At the same time, the concentrations of TBARS were increased

in liver and kidney of fish treated with 500 μM of iron sulfate. The described changes demonstrate the development of oxidative stress in goldfish tissues. A strong positive correlation between lipid peroxidation products and the activities of catalase in liver and glutathione reductase in kidney indicated the possible up-regulation of the enzymes by these products (Bagnyukova et al., 2006).

In mussel *Mytilus galloprovincialis*, the treatment with iron ions increased the production of hydroxyl radical (Viarengo et al., 1999). Interestingly, whole organisms treated with iron ions demonstrated lower survival under anoxic conditions which indicated the interplay between oxygen availability and iron metabolism.

5.4.2. Copper

Copper homeostasis in aquatic animals entails on regulated uptake, transport and excretion similarly to mammals. However, there is some specificity of these processes in hydrobionts related with possibility of branchial uptake along with intestinal. Both ways are efficient, but depend on many factors and are highly regulated processes (Kamunde et al., 2002).

The oxidative stress was demonstrated to be induced by both dietary and waterborne exposure to high copper, or even by copper injection. At oral copper exposure of grey mullet *Chelon labrosus*, hepatic α-tocopherol concentrations were 63% lower, while malondialdehyde increased by 304% when fish were fed a high copper diet for 67 days, which along with other parameters led to conclusion that the diet high in copper could induce oxidative stress in this fish (Baker et al., 1998). In gilthead seabrams *Sparus aurata* injection with $CuCl_2$ increased the concentrations of thiobarbituric reactive substances and oxidized proteins (Pedrajas et al., 1995). The treatment also increased the specific activity of superoxide dismutase and resulted in appearance of two new Cu,Zn-SOD isoforms.

The 40 day exposure of goldfish *C. auratus* to 0.005–0.025 mg/l copper reduced catalase and 0.05–0.05—selenium dependent glutathione peroxidase activities, whereas 0.0025–0.01 mg/l increased glutathione-S-transferase activity (Liu et al., 2006). The modification of activities of antioxidant enzymes also demonstrated that copper injection induced oxidative stress in goldfish tissues. In warm water African catfish *C. gariepinus* dietary copper exposure also elevated copper concentration in the intestine, liver and gills in tissue-specific manner (Hoyle et al., 2007). The treatment with copper significantly increased TBARS concentrations in the gills and intestine, and total glutathione content in the intestine was doubled.

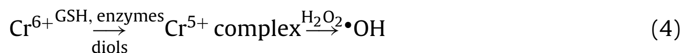
Hansen et al. (2006a,b) investigated the effects of water contamination on the activities and steady-state concentrations of mRNA of certain antioxidant enzymes in brown trout tissues. The general conclusions, which may be drawn from their studies are: (*i*) fish exposure to metal ions, particularly to copper, enhances the activities of primary (SOD, catalase, glutathione peroxidase) and secondary (glutathione reductase, metallothionein) enzymes/proteins; (*ii*) the level of mRNA do not always correspond to respective protein level and (*iii*) metallothioneins does not necessary are up-regulated by the addition of metal ions like copper.

Copper treatment of rainbow trout gill cells resulted in a dose-dependent elevation in cytotoxicity and enhanced ROS formation (Bopp et al., 2008). It significantly increased DNA strand breaks, but did not affect lipid peroxide level. The exposure of zebrafish to copper increased protein carbonyl concentrations, the activity of superoxide dismutase, while suppressed catalase activity and enhanced gene expression of cytochrome c oxidase subunit 17 (COX-17) (Craig et al., 2007). The variable changes in the activities of COX and citrate synthase indicated the possible alterations in cell oxidative capacity. In fresh water flea *Daphnia magna*, exposure to copper ions enhanced the level of TBARS and lipofucsin as well as activities of SOD, catalase, glutathione peroxidase, glutathione-S-transferase (Barata et al., 2005). In blue crab *Callinectes sapidus*, copper exposure did not affect intracellular levels of glutathione and oxidized protein, and catalase activity, but the levels of oxidized lipids were significantly higher in copper-exposed crabs (Brouwer and Brouwer, 1998).

5.4.3. Chromium

Chromium occurs predominantly in two valence states—hexavalent (Cr^{6+}) and trivalent (Cr^{3+}). Hexavalent chromium compounds are used widely in diverse industries and trivalent chromium salts, for example, chromium picolynate, chromium chloride and niacin-bound chromium are used as micronutrients and dietary supplements. The direct or indirect bioaccumulation in organisms in contaminated waters may be significant and, therefore, it may affect not only individual organisms, but also the food chains.

Chromium exists in several states of oxidation, ranging from +2 to +6, and its forms +3 and +6 are the most stable in the environment and biologically important. Being an element with changeable valence (II, III, IV, V and VI) it can enter Haber–Weiss-type reaction resulting in generation of •OH radical and at least several types of this reaction may be found in the literature (Halliwell and Gutteridge, 1989; Shi and Dalal, 1990; Valko et al., 2005; Lushchak, 2008).



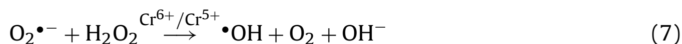
$$Cr^{6+} \xrightarrow[\text{diols}]{\text{GSH, enzymes}} Cr^{5+}\ \text{complex} \xrightarrow{H_2O_2} {}^{\bullet}OH \quad (4)$$

The given above reactions may be presented in another form:

$$Cr^{6+} + O_2{}^{\bullet -} \rightarrow Cr^{5+} + O_2 \quad (5)$$

$$Cr^{5+} + H_2O_2 \rightarrow Cr^{6+} + {}^{\bullet}OH + OH^{-} \quad (6)$$

and overall



$$O_2{}^{\bullet -} + H_2O_2 \xrightarrow{Cr^{6+}/Cr^{5+}} {}^{\bullet}OH + O_2 + OH^{-} \quad (7)$$

Two aspects should be noted here: (*i*) cellular reduction is needed for generation of •OH in reaction (4), and (*ii*) chromium may play a role of catalyst being able to enter reversible oxidation (reactions (5)–(7)). Therefore, it is commonly accepted that biological effects of chromium, at least partially, are connected with ROS generation.

Most results on chromium effects on living organisms have been reported on mammals and their effects on hydrobionts are very limited. However, the question cannot be ignored with aquatic animals. Chromium has both beneficial and deleterious effects on organisms being essential trace element involved in regulation of broad array of biological processes, particularly in glucose metabolism. In experiments with guppies *Poecilia reticulata* Perez-Benito (2006) found that low concentrations (<10^{-4} M) of Cr^{6+} increased the maximum lifespan in both mails and females. The toxicity of chromate was substantially decreased by antioxidant D-mannitol. The latter might indicate the ROS involvement in described effects of chromate (Perez-Benito, 2006). The exposure to potassium dichromate clearly induced oxidative stress in gills and kidney of European eel *Anguilla anguilla* L. (Ahmad et al., 2006). In gills, 1 mM dichromate did not affect catalase and glutathione-S-transferase activities, but increased glutathione peroxidase activity and decreased glutathione (GSH) concentrations. Lipid peroxidation, assessed as thiobarbituric acid reactive substances (TBARS), was intensified in kidney, but no other changes were found in this tissue. DNA integrity, evaluated as DNA strand breaks, was lower in both tissues of dichromate-treated animals (Ahmad et al., 2006).

Chromium exposure activated lipid peroxidation in tissues of Chinook salmon *O. tshawytscha* and high chromium concentrations significantly impaired fish health (Farag et al., 2006). Kidney was the target organ during chromium exposure—it had gross and microscopic lesions (e.g. necrosis of cells lining kidney tubules)

and, levels of products of lipid peroxidation were elevated. These changes were associated with increased chromium concentrations in kidney, and reduced fish growth and survival. Authors proposed that accumulated chromium induced the lipid peroxidation pathway where fatty acid oxidation and DNA damage (expressed as chromosome changes) occurred and caused cell death and tissue damage (Farag et al., 2006).

Another approach to evaluate free radical-induced damage to DNA was used by Kuykendall and colleagues (2006). They studied the formation of DNA–protein crosslinks (DPXs) in erythrocytes of largemouth bass *Microproterus salmonoides*, and fathead minnows *Pimephales promelas* exposed to waterborne and dietary hexavalent chromium. Fathead minnow exposure to 2 ppm Cr^{6+} led to significant DPX formation in erythrocytes, with over 140–200% increase above background at 3–4 days. Largemouth bass exposed similarly was found to have 62% elevation of DPX levels after 4 days. When largemouth bass was fed a diet of minnows injected with 20 mM Cr^{6+} for 5 days, a significant increase of DPXs in erythrocytes was observed. Therefore, the authors concluded that both waterborne and high dose dietary exposure to Cr^{6+} can lead to DPX formation in erythrocytes of predatory fish species such as bass (Kuykendall et al., 2006).

We also investigated the effects of Cr^{6+} and Cr^{3+} on free radical processes in goldfish tissues (Lushchak et al., 2008, 2009a,b; Vasylkiv et al., 2010; Kubrak et al., 2010). It was shown that both ions induced oxidative stress in this organism. We compared the influence of these chromium forms and found that the effects depended on the valence state. The possible involvement of glutathione system in detoxification of Cr^{6+} was proposed (Lushchak et al., 2008). Interestingly, fish mucus can reduce Cr^{6+} and Cr^{3+} and this mechanism was proposed to be involved in Cr^{6+} detoxification (Arillo and Melodia, 1990).

Chromium compounds also affected free radical processes in *D. magna* (De Coen and Janssen, 2003) as well as in freshwater field crab, *Barytelphusa guerini* (Sridevi et al., 1998). All data analyzed in this subsection clearly demonstrate that the toxicity of chromium compounds to big extend may rely on the stimulation of ROS production.

5.4.4. Mercury

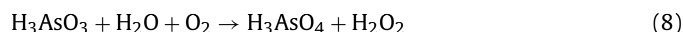
Mercury exists as a cation with an oxidation state +1 (mercurons) and +2 (mercuric). In the environment, mercury may be found in methylmercury form, produced mainly as the result of methylation of inorganic (mercuric) forms by microorganisms in soil and water (Valko et al., 2007). The biological effects of inorganic or organic mercury are related to their interaction with sulfhydryl-containing residues (Rooney, 2007). Mercuric conjugates of cysteine and glutathione are transportable species at the site of the organic anion transporters. Because of high affinity of mercury to glutathione, the first can deplete intracellular GSH pool and directly or indirectly cause oxidative stress.

Fish as other animals may accumulate high mercury levels (Salonen et al., 1995; Guallar et al., 2002). In experiments with Atlantic salmon *Salmo salar parr* exposed for four months to mercuric chloride, methyl mercury was accumulated significantly in brain and did not cause mortality or growth reduction (Berntssen et al., 2003). But it significantly increased levels of lipid peroxidation products (evaluated as TBARS) and decreased the activities of SOD and glutathione peroxidase. Comparing with other organs, brain was particularly susceptible for dietary mercury, while kidney and liver were less sensitive. It should be also noted that low dietary concentrations of mercury induced protective redox defenses in the brain evidenced by the induction of antioxidant enzyme SOD (Berntssen et al., 2003). Recently, several works on the mercury effects on free radical processes in fish were published. Mieiro et al. (2010) found that mercury exposure depleted antioxidant in feral golden grey mullet (*Liza aurata*). The total glutathione content and the activities of catalase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase significantly decreased. At the same time, fish exhibited unaltered lipid peroxidation levels, pointing out a higher propensity of mercury to inhibit enzymes than to oxidatively damage lipids. The authors concluded that mercury increased susceptibility of the fish brain to oxidative challenges. The exposure to $HgCl_2$ of freshwater fish matrinxã, *Brycon amazonicus* to sub-lethal concentration for 96 h of mercury chloride for 96 h in a static system increased the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase and glutathione reductase (Monteiro et al., 2010). If in liver and gills mercury increased reduced glutathione level, in the white muscle it was decreased. Mercury treatment enhanced lipid peroxidation and protein carbonyl content in all tissues studied. Collectively, these data suggest that oxidative stress in response to mercury exposure could be the main pathway of its toxicity induced by this metal in fish.

5.4.5. Arsenic

The most common oxidation numbers of arsenic are +5, +3 and −3. It can form both inorganic and organic compounds in the environment and cells. Inorganic arsenic includes arsenite (As^{3+}) and arsenate (As^{5+}). The inorganic arsenics can be either methylated (monomethylarsonic acid, MMA), or dimethylated (dimethylarsinic acid—DMA) *in vivo* (Valko et al., 2005).

Several ROS and RNS are known to be involved into generation of dimethylarsinic peroxyl ($(CH_3)_2AsOO^\bullet$) and dimethylarsinic ($(CH_3)_2As^\bullet$) radicals as well as some intermediary arsine reactive species (Rin et al., 1995). It is interesting to note that the oxidation of As^{3+} to As^{5+} under physiological conditions results in H_2O_2 formation (Valko et al., 2005):



$$H_3AsO_3 + H_2O + O_2 \rightarrow H_3AsO_4 + H_2O_2 \quad (8)$$

Therefore, arsenic compounds generating both ROSs and RNSs may be involved in oxidation of cellular components particularly lipids, DNA and proteins.

In two cell lines, TF (fin cells of *Therapon jarbua*) and TO-2 cells (ovary cells of tilapia) treated with sodium arsenite time- and concentration-dependent line-specific cell death was found (Wang et al., 2004). The DNA-fragmentation analysis and the flow cytometric analysis of cell cycle progression demonstrated clearly that most cells were killed via apoptosis. Since antioxidants, *N*-acetylcysteine and dithiothreitol, significantly prevented apoptosis in TF cells, it was concluded that ROS were involved in arsenite-induced apoptotic cell death (Wang et al., 2004).

Schlenk et al. (1997) studied the effects of arsenite, arsenate and herbicide monosodium methyl arsonate on hepatic metalloprotein expression and lipid peroxidation in channel catfish. They found dose-dependent increase in metallothionein levels, while hepatic lipid peroxidation (determined as TBARS) and glutathione concentrations were unaltered by any of the arsenical treatments. It demonstrates the lack of correlation between metallothionein expression and oxidative stress.

We also investigated the effects of arsenite treatment on oxidative stress markers and antioxidant defense in goldfish liver (Bagnyukova et al., 2007b). The treatment during 1–4 days did not affect concentrations of TBARS and protein carbonyls, but it increased the oxidation of glutathione, total glutathione concentration and lipid peroxides clearly showing the development of oxidative stress. The activities of the main antioxidant enzymes—superoxide dismutase, catalase and glutathione peroxidase, were also enhanced demonstrating an antioxidant response.

Taking into account the described above effects of arsenic compounds on fish, it is easy to understand, that this element is able to enter cellular metabolism and in some cases it enhances the pro-

duction of free radicals. The latter can modify virtually all cellular constituents, including membranes. The chain may be ended by cell death via necrosis or apoptosis and the discrimination between these both ways depends on many circumstances.

This section clearly shows that ions of transition metals both, abovementioned and some others, may realize their toxic effects via stimulation of ROS production. That can cause plural adverse effects culminating in cell death via apoptosis and/or necrosis.

5.5. Pesticides

Pesticides are physical, chemical or biological agents intended to kill an undesirable plant and animal pests. Major classes of pesticides are: insecticides, herbicides and fungicides. It is important to note that most pesticides are synthetic agents, new to the environment and humans and, therefore, their effects on biological systems are poorly predictable.

This group of toxicants may induce oxidative stress via several mechanisms: (*i*) being capable to enter redox cycles (reversible oxidation) accepting/donating electrons to cellular constituents they may increase ROS level, (*ii*) at cellular metabolism some of pesticides may need involvement of reductants, such as glutathione, exhaust their reserves and result in decreased antioxidant potential, (*iii*) certain pesticides may inactivate antioxidant and associated enzymes leading to decreased antioxidant potential, (*iv*) interference with energy-providing processes may decrease supplement for metabolism and detoxification, and, finally, (*iv*) modification of core vital processes, such as transcription and translation, in non-direct way may enhance steady-state ROS level. Below we will describe some examples of pesticide-induced oxidative stress in hydrobionts.

5.5.1. Insecticides

This group includes not only insecticides, but also acaricides. Most insecticides are organophosphate (OP) compounds used to control insects on crops, household, stored products, and treat external parasitic infections of farmed fish, livestock, and domestic animals. The known mechanism of action is related with the inhibition of the enzyme acetylcholinesterase (AChE), which is responsible for terminating the transmission of the nerve impulse. That is the primary effect of OPs in vertebrates and invertebrates. They realize their effects by blocking the hydrolysis of the neurotransmitter acetylcholine (ACh) at the central and peripheral neuronal synapses and leading to excessive accumulation of ACh and activation of ACh receptors (Shih and McDonough, 1997). The toxic effects of OPs are believed to be largely due to the hyperactivity of the cholinergic system as a result of the accumulation of ACh at the synaptic cleft. The break of neuronal and endocrine signaling leads to intracellular influx of Ca^{2+}, triggering the activation of proteolytic enzymes, nitric oxide synthase, and the generation of free radicals (Beal, 1995) resulting in oxidative stress. Since the metabolism of OPs (particularly, dichlorvos) in the liver is connected with glutathione consumption, this also may initiate oxidative stress.

Several works have described the induction of oxidative stress in fish under treatment with OPs. For example, dichlorvos was found to induce oxidative stress in the carp *C. carpio* and catfish *Ictalurus nebulosus* (Hai et al., 1997b), and in European eels *A. anguilla* (Peña-Llopis et al., 2003), trichlorfon in Nile tilapia (Thomaz et al., 2009), Folisuper 600 BR (methyl parathion) in the freshwater characid fish matrinxã *Brycon cephalus* (Monteiro et al., 2009), fenthion in *Oreochromis niloticus* (Piner et al., 2007), and malathion in gilthead seabream (Rosety et al., 2005).

The mechanisms of toxic effects of other insecticide group the chlorinated hydrocarbons including lindane and DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane), seem to involve the induction of oxidative stress in cell line from a skin tumour of carp *C. carpio* (Ruiz-Leal and George, 2004) and hepatocytes from *Hoplias malabaricus* (traíra) (Filipak Neto et al., 2008). The mechanisms implicated here may be related with the generation of free radicals after metabolic activation with enzyme induction of P450 by DDT and resulting product(s) may enter reversible oxidation (Harada et al., 2003). The induction of oxidative stress may be secondary to metabolic activation and can be a key factor in hepatocarcinogenesis initiated by DDT and may play an important role in tumor promotion and progression (malignant transformation).

5.5.2. Herbicides

Two groups of herbicides will be analyzed here: the first is known to be directly responsible for the enhancement of free radical generation, like paraquat (*N*,*N*′-dimethyl-4,4′-bipyridinium dichloride) known also as a viologen, entering redox cycles and constantly generating ROS, and the second, inactivate antioxidant enzymes like aminotriazole and dithiocarbamates.

Paraquat was shown to induce oxidative stress in *Channa punctata* (Parvez and Raisuddin, 2006), zebrafish (Bretaud et al., 2004), and rainbow trout (Stephensen et al., 2002). Similarly diquat also stimulates ROS production, for example, in established carp cell line (Wright et al., 2000), and in rainbow trout, *O. mykiss* tissues (Hook et al., 2006).

The second group of herbicides consists of several classes of compounds known to be mainly inhibitors of antioxidant enzymes, such as SOD and catalase, for example. Hai et al. (1997a) found that although diethyldithiocarbamate (DDC) modified pro/antioxidant system in tissues of common carp, it also serve as antioxidant due to the presence of thiol groups in its structure. We clearly demonstrated that DDC induced oxidative stress in goldfish tissues (Lushchak et al., 2007). The mechanism believed to be responsible for the induction of oxidative stress by DDC is connected with the extraction of copper ions from the active center of Cu,Zn-containing SOD. Other thiocarbamate herbicide molinate also induced oxidative stress in European eel *A. anguilla* (Peña-Llopis et al., 2001). Since molinate is detoxified via sulfoxidation by endoplasmic reticulum oxygenase system followed by conjugation with glutathione and depleting reserves of the latter, it may lead to the induction of oxidative stress in animals exposed to it. Aminotriazole (3-amino-1,2,4-triazole, AMT), a heterocyclic organic compound, is nonselective systemic herbicide broadly used. It is a competitive inhibitor of imidazoleglycerol-phosphate dehydratase, but also it inhibits catalase via binding of iron atom in its active site (Lushchak et al., 2003). The exposure to aminotriazole was found to induce oxidative stress in rainbow trout *O. mykiss* (Dorval and Hontela, 2003) and goldfish (Bagnyukova et al., 2005a,b).

It is important to add that herbicides may also exert their biological activity via induction of oxidative stress. For example, glyphosate (N-phosphoromethyl glycine) is a nonselective herbicide that inhibits plant growth through interference with the production of essential aromatic amino acids by inhibiting the enzyme enolpyruvylshikimate phosphate synthase. This enzyme is responsible for the biosynthesis of chorismate, an intermediate in phenylalanine, tyrosine, and tryptophan biosynthesis in plants. In goldfish, Roundup, a glyphosate-based herbicide, induced oxidative stress, although it was rather weak (Lushchak et al., 2009c). That confirmed previous data on the induction of oxidative stress by Roundup in other fish species—pava *Leporinus obtusidens* (Glusczak et al., 2006) and in silver catfish *Rhamdia quelen* (Glusczak et al., 2007).

5.5.3. Fungicides

Probably, different copper-containing compounds are among the most important fungicides, but the effects of copper ions were described above. Therefore, we will describe below the

only publications on organic fungicides. Several works are known on the matter. For example, hexachlorobenzene (HCB), or perchlorobenzene, which is a chlorocarbon, was found to induce oxidative stress in liver and brain of common carp *C. carpio* (Song et al., 2006). Although HCB use has been banned globally under the Stockholm Convention on persistent organic pollutants, it is one of the most widespread persistent organic pollutants because it is still released into the environment as a by-product in many industrial processes. HCB was suggested to stimulate ROS production due to two causes (Song et al., 2006). Firstly, as a lipid-soluble chemical, HCB can bind to cytochromes and it is not readily metabolized, thus uncoupling the electron-transport chain from mono-oxygenase activity, and consequently favoring the production of reactive species. Secondly, pentachlorophenol, one of HCB major metabolites, is a potent source of ROS during its metabolism.

The group of azole fungicides, particularly, prochloraz (*N*-propyl-*N*-(2-(2,4,6-trichlorophenoxy) ethyl)imidazole-1-carboxamide) is a broad spectrum contact fungicides. Similarly to AMT, and other imidazole compounds, nonspecific strong interaction of prochloraz with the iron atom of cytochrome P450 and other proteins such as catalase should be especially mentioned here. Prochloraz can generate adverse effects to aquatic organisms, particularly, modulating cytochrome P450 enzyme activities. It induced a transient increase of antioxidant enzymes and depleted glutathione in three-spined stickleback *Gasterosteus aculeatus* (Sanchez et al., 2008). The hydroxylation of heterocyclic ring also may be responsible for the induction of oxidative stress because the products formed may enter redox cycles.

5.6. Oil and related pollutants

More and more substantial part of the oil consumed globally derives from off shore oil fields and, therefore, the pollution either due to technologies used or incidents related with oil production or transportation is increasing concern. The main pollutants from this activity include polycyclic aromatic hydrocarbons (PAH), alkylphenols, and hydrocarbons (Sturve et al., 2006). They may affect aquatic organisms in many ways and the oxidative stress is one of the key elements of their toxicity. PAH are primarily metabolized via hydroxylation, and thereby detoxified, by enzymes in the cytochrome P450 system, mainly CYP1A (Goksøyr and Förlin, 1992), and this metabolism leads to the formation of compounds entering redox cycles. Two potential mechanisms may be involved in increase of ROS generation by the products analyzed in this section. The first one is the conjugation of products of metabolism with glutathione, resulting in consumption of GSH and decrease the defense potential leading to oxidative stress. The second one is also related with metabolites, but ones that are capable to enter redox cycles. For example, benzo[a]pyrene can be metabolized by CYP1A to benzo[a]pyrene diones that have the ability to form ROS through the abovementioned process (Lemaire et al., 1994). Exposure to several PAH caused oxidative stress in aquatic organisms (Winston and Di Giulio, 1991; Livingstone, 2001). It has also been shown with Atlantic cod *Gadus morhua* that the exposure to alkylphenols elevated glutathione reductase activities and total glutathione levels, possibly as a result of the induced oxidative stress (Hasselberg et al., 2004a,b). Nonylphenol depleted GSH in Atlantic cod (Hasselberg et al., 2004a,b), in largemouth bass *Micropterus salmoides* (Hughes and Gallagher, 2004) and in rainbow trout *Onchorhynchus mykiss* (Uguz et al., 2003). In addition, in the last work it was found that GST activity increased during the first week of nonylphenol exposure, but further decreased and to the third week the GST activity was less than that seen in the controls. The depletion in total glutathione levels could also be due to the excretion of its oxidized form, and the increase in oxidized glutathione level could result from oxidative inactivation of glutathione reductase (DeLeve and Kaplowitz, 1991).

6. Transcriptal regulation of antioxidant enzymes

The up-regulation of activity of antioxidant enzymes is mainly realized through the synthesis of new molecules. The oxidative stress signal is received by specific molecular sensors and transduced to transcriptional/translational machinery. Finally, that results in the synthesis of new molecules of the enzymes. Up to date, several common with mammals pathways involved in up-regulation of antioxidant enzymes were found in hydrobionts. However, the only two of those systems, namely Keap1–Nrf2 and Hif-1α, are well characterized in this group of animals, while others are underway. Therefore, here we will concentrate on the abovementioned systems, and the rest will be only mentioned.

6.1. Keap1–Nrf2 signalling

Nrf2 (NF-E2—Related Factor 2) is a transcription factor of the leucine zipper family, and Keap1 (Kelch-like ECH-associated Protein 1) is its specific repressor. These two proteins in concert mediate cellular response to electrophilic xenobiotics and oxidants (Kobayashi and Yamamoto, 2005; Osburn and Kensler, 2008). The transcription factor Nrf2, has emerged as the central protein that binds to the antioxidant response element (ARE) to activate gene transcription. It has been shown to regulate the expression of a group of genes encoding cytoprotective enzymes in response to cell exposure to certain toxicants, including ROS, electrophiles, as well as dithiolethiones, isothiocyanates, and triterpenoids. The target genes include ones encoding such enzymes as antioxidative, catalyzing electrophile conjugation, glutathione homeostasis, producing reducing equivalents, proteasome function and other. Therefore, Keap1–Nrf2 pathway is suggested to be a key player in coordinating cell adaptation to abovementioned compounds.

Fig. 4 schematically demonstrates possible events leading to the realization of Keap1–Nrf2 signaling. Under normal (unstressed) conditions, transcription factor Nrf2 is bound to Keap1. The latter one forms homodimer and is anchored to F-actin. Keap1 also specifically binds Cullin 3 (Cul3), a subunit of the E3 ligase complex. This interaction promotes the ubiquitination of Nrf2 in cooperation with the Cul3–Roc1 complex. It is appreciated that Keap1 functions as an adaptor of Cul3-based E3 ligase. Ubiquitination of Nrf2 leads to its proteasomal degradation (Nguyen et al., 2009). Cell exposure to electrophiles and oxidants results in oxidation of specific cysteine residues of Keap1 and formation of disulfide crosslinks between protein monomers. The formation of crosslinked Keap1 homodimers changes the conformation and it is not able longer to bind Nrf2 protein. Therefore, it is released from the complex and in this way escapes ubiquitination followed by proteasomal degradation. This results in increase of Nrf2 concentration. Further Nrf2 migrates into the nucleus, where it together with proteins Mat and CBP/P300 binds to cis-element called ARE (Antioxidant Response Element) or electrophile response element (EpRE) within the regulatory region of target genes.

Keap1–Nrf2 system also may be activated by phosphorylation (Kobayashi and Yamamoto, 2005). PKR-like endoplasmic reticulum kinase (PERK) and protein kinase C (PKC) can phosphorylate Nrf2 directly *in vitro* and *in vivo*. The phosphorylation of Nrf2 triggers its dissociation from the complex with Keap1 preventing the ubiquitination and degradation of Nrf2. It was suggested that the protein kinases, PERK and/or PKC, or their upstream signaling molecules may be candidate sensor molecules of oxidative stress and electrophiles (reviewed in Kobayashi and Yamamoto, 2005). The next protein kinases are supposed to be possible

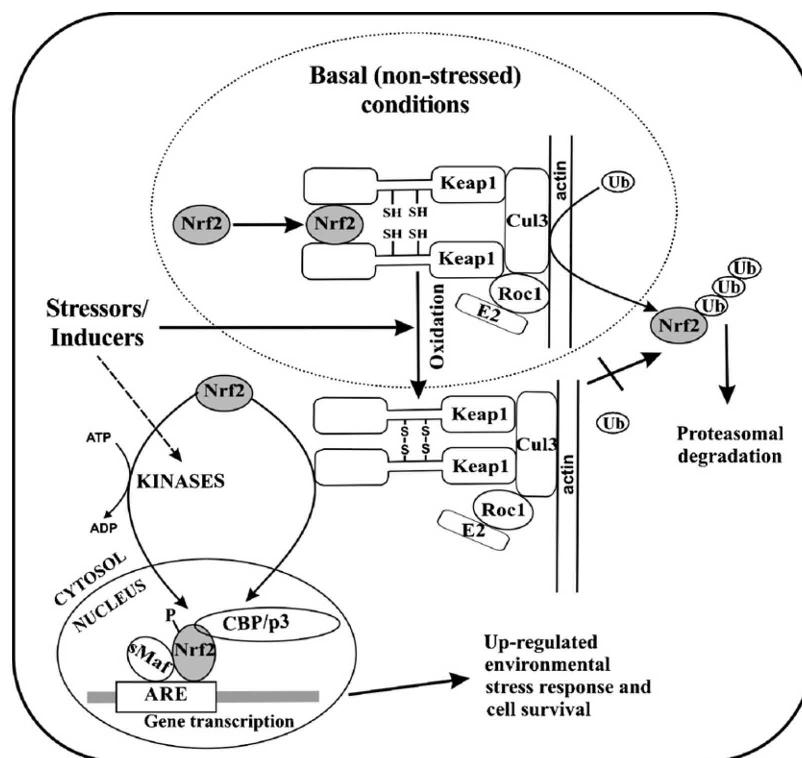


Fig. 4. Regulation of antioxidant enzyme activity by Nrf2/Keap1 system. Under unstressed conditions, Nrf2 protein interacts with dimeric Keap1 protein and is subjected to ubiquitination followed by proteasomal degradation. The thiol groups of certain cysteine residues in Keap1 are oxidized under oxidative stress which leads to impossibility to bind Nrf2 protein and its diffusion into nucleus Nrf2 in concert with sMaf and CBp/p3 proteins interacts with antioxidant response element (ARE) of target genes stimulating transcription.

candidates for sensing and transduction of the abovementioned signals: extracellular signal-regulated kinases (ERK), p38 mitogen-activated protein kinase (MARK), MARK/ERK kinase-1, MEK kinase, and phosphatidylinositol 3-kinase.

Keap1–Nrf2 system being a key in cellular response to electrophiles and oxidants may affect/interact with other pathways sensing these types of signals. These are particularly nuclear factor-κB (NF-κB), cJun, peroxisome proliferator-activated receptor (PPAR), CCAT/enhancer-binding protein βCC/EBPβ which also may sense the compounds via covalent modification of specific cysteine residues (Gong et al., 2002; Banning and Brigelius-Flohé, 2005; Gopalakrishnan and Tony Kong, 2008). The interplay between all these and probably undiscovered yet regulatory pathways might let cell delicately regulate the efficiency of cytoprotective mechanisms in according to type and intensity of hazard stress.

Kobayashi et al. (2002) discovered the Keap1–Nrf2 system in zebrafish. They systematically cloned zebrafish cytoprotective enzyme cDNAs and found their expression to be efficiently induced by electrophilic agents. The identification of Nrf2 and Keap1 in zebrafish demonstrated conservative character of this regulatory pathway. The operation of Nrf2 was suppressed by Keap1, exactly as it was found in mice and human cells. The domains responsible for the interaction between Nrf2 and Keap1 also were found to be similar in mammalian and fish cells. Therefore, the authors concluded that the Keap1–Nrf2 pathway is highly conserved among vertebrates and that the interface between Nrf2 and Keap1 forms an important molecular basis of this regulatory system (Kobayashi et al., 2002).

Two years after the description of Keap1–Nrf2 in fish, the same Japanese group described one more component of the system in zebrafish (Takagi et al., 2004). They found small Maf proteins, involved in the formation of heterodimeric complex with Nrf2 which binds to ARE/EpRE sequence in *cis*-element of target genes resulting in their up-regulation. They possess a bZip motif mediating the DNA binding and dimer formation in common, but lack any recognizable transcriptional effector domains. Therefore, the interaction with CNC proteins, particularly with Nrf2, provides specificity in binding to DNA. In higher vertebrates three small Maf proteins were described to date, namely Maf F, Maf G and Maf K, and they shared redundant functions. The only Maf G and Maf K were found in zebrafish, but also a new small Maf protein was identified in this organism which was named Maf T. The latter one bound MARE sequence as a homo- or heterodimer with Nrf2 and regulated the expression of certain target genes in zebrafish embryos. It led to conclusion that fish possess all components of Keap1–Nrf2 system of gene regulation in response to stresses. Interestingly, a gene encoding highly homologous to Maf T protein was found in the genomic DNA database of the fish *Tetradon nigroviridis* knows as fugu, but not in human, mice or other vertebrate databases, implying that this subfamily was specific for teleosts. Because of that, the new member of small Maf proteins was named Maf T (small Maf in Tallest).

Two genes of glutathione-S-transferase of Pi class, gstp1 and gstp2, were identified in zebrafish genome (Suzuki et al., 2005). Their expression was enhanced at fish exposure to electrophiles. In regulatory regions the genes gstp1 and gstp2 contained ARE-like element, which specifically bound Nrf2-MafK heterotrimer. This work clearly described the realization of full signaling pathway Keap1–Nrf2 way of adaptive defense response—from signal sensing by Keap1, its transduction by liberated Nrf2 with migration into nucleus, forming heterodimer with Maf proteins and up-regulation of target genes with GST in this specific case.

Further clarification of operation of Keap1–Nrf2 pathway in fish came in 2008 (Li et al., 2008), when it was found that in opposite to man, mice and hen, zebrafish contains two types of Keap1

proteins, named as Keap1a and Keap1b. Interestingly they differed in highly conservative in higher vertebrates cysteine residues Cys-288 and Cys-273. They contained only one of two mentioned residues—Cys-288 in Keap1a and Cys-273 in Keap1b. Both these proteins facilitated the degradation of Nrf2 protein and repressed Nrf2-mediated target gene activation. The mutation of any cysteine residues mentioned above disrupted the ability of Keap1 to repress Nrf2. This indicated that the presence of either Cys-273 or Cys-288 was sufficient to maintain full activity of fish Keap1. It is supposed, therefore, that a presence one of the cysteine residues might be enough to provide correct response to inducers. It was proposed that that under oxidation Cys-288 of Keap1a and Cys-273 of Keap1b, they form intermolecular disulfide bridge resulting in impossibility to bind Nrf2 protein. Interestingly, the fact that Keap1 was found to bind zink ions raised the idea on the involvement of this ion in operation of Keap1. However, the real role of zink should be clarified in future. The genes encoding Keap1-related proteins were identified in other fish—fugu *Tetradon nigroviridis*, and medaka (Li et al., 2008).

Recently M. Kobayashi (personal communication) reported that an Elephant shark has two Keap1 homolog genes. One encodes Keap1a and the other does vertebrate-type Keap1 (like from frog, chick and mammals which possess both Cys273 and Cys288). There is no Keap1b. Therefore, he hypothesizes that (*i*) invertebrate-type Keap1 diverge into Keap1a and vertebrate-type Keap1 in chondrichthyes and then (*ii*) in teleost (at least in some including zebrafish, medaka and fugu) vertebrate-type Keap1 changed to Keap1b and lost their Keap1a activity.

The systematic analysis of molecular mechanisms of up-regulation of cytoprotective proteins and careful selection of model compounds let Kobayashi et al. (2009) to categorize inducers in six classes. The proposed system was based mainly on the involvement of different reactive cysteine residues of Keap1 in sensing process. However, classic molecular biology approaches along with isolation of zebrafish mutants defective in their response to certain compounds called for necessity of involvement of the third factor a gene it275 in addition to Nrf2 and Keap1. The authors proposed so-called a "cysteine code" hypothesis that concerts a set of cysteine modification into specific biological effects (Kobayashi et al., 2009). They suppose that breaking of the cysteine code for each Nrf2-activating compound will serve to improve our understanding of its therapeutic and/or toxic effects.

6.2. Hypoxia and role of HIF-1α protein

In 1995 Wang and Semenza reported the discovery of the protein factor which activated the expression of erythropoietin (Epo) gene in Hep 3B cells subjected to hypoxia or cobalt chloride treatment (Wang and Semenza, 1995). The level of this factor was increased under the mentioned above conditions and, therefore, was called "hypoxia-inducible factor 1" (HIF-1). Its binding to DNA also was induced by hypoxia or cobalt in not EPO-producing cells, which suggested a general role for HIF-1 in hypoxia signal transduction and transcriptional regulation. These authors also found that active HIF-1 binds to DNA as heterodimer composed of two different subunits: 120 kDa HIF-1α and 91–94 kDa HIF-1β (Wang and Semenza, 1995). Up to now, the operation of HIF-1α/HIF-1β system is rather well studied and is a subject of many reviews and fundamental works (for example, Ke and Costa, 2006; Hoogewijs et al., 2007a,b). Molecular mechanisms and principles of operation are well characterized. Therefore, here we will not describe the system in details, but rather will give a brief overview. Actually, HIF-1α does not sense O_2 directly. The latter is sensed by two specific enzymes, called "prolyl hydroxylase domain" proteins (PHD) and "asparaginyl hydroxylase" called "factor inhibiting HIF" (FIH). The latter enzymes are 2-oxoglutarate dependent iron dioxygenases, which use one of the atoms of O_2 molecule to hydroxylate prolyl or asparaginyl residues of HIF-1α, respectively. The second atom of O_2 molecule is used to convert 2-oxoglutarate to carbon dioxide and succinate. Iron is maintained in reduced form (Fe^{2+}) and is reduced by ascorbate serving here as a cofactor. Hydroxylated by two hydroxylases HIF-1α is recognized by the von Hippel-Lindau (vHL) protein in the multiprotein E3 ubiquitin ligase complex. Followed ubiquitination marks the complex for degradation by proteasome. This system efficiently operates under normoxic conditions. When oxygen concentration is decreased, the hydroxylation reactions are inhibited resulting in enhanced level of HIF-1α. Under hypoxia HIF-1α migrates from cytosol into the nucleus where it meets other protein, HIF-1β. Heterodimer HIF-1α/HIF-1β recognizes so-called hypoxia response element (HRE) of DNA and in concert with CBP/p300 protein regulates the expression of target genes.

One may ask on possible relationship between operation of HIF-1α/HIF-1β system and free radical processes. Really, it seems that ROS are not directly involved in the described pathway. Although it looks paradoxally, under some circumstances it was found that hypoxia may lead to increased ROS production and oxidative stress (Clanton, 2007; Lushchak and Bagnyukova, 2007; Shukla et al., 2009). We are not going to analyze the highlighted problem here, but rather only mark it and search for possible relationship between operation of HIF-1α pathway and its possible involvement in cellular response to hypoxia-induced oxidative stress. The interested reader may be addressed to several recent publication on this topic (Clanton, 2007; Wang et al., 2008; Pialoux et al., 2009a,b; Ruchko et al., 2009).

In 2001 Nikinmaa and colleagues described HIF-1α in fish (Soitamo et al., 2001). They found that in rainbow trout and chinook salmon cells HIF-1α level was increased in response to hypoxia and HG-132, a proteasome inhibitor, could enhance its concentration under normoxia. Later, hypoxia-inducible factor was found in many other fish species. For example, both, zebrafish (Kajimura et al., 2006) and Atlantic croaker *Micropogonias undulates* (Rahman and Thomas, 2007), contain at least two HIF-1α genes—HIF-1α and HIF-2α. Hypoxia-inducible pathway is established in early embryogenesis of fish (Kajimura et al., 2006; Rojas et al., 2007).

Up to now, in this section we described the operation of HIF-1α pathway mainly in mammalian tissues and its presence in fish. The question is: if this system is responsible for cell adaptation to hypoxia, how can it be connected with oxidative stress in fish? There are several potential mechanisms: (*i*) oxidative stress may result in oxidative inactivation of prolylhydroxylases (PHD); (*ii*) hypoxia development under some conditions may lead to oxidative stress and in this case both, HIF-1α system and oxidative stress, operate simultaneously; (*iii*) fish possess specific forms of HIF-1α, which are sensitive to ROS-induced modification. Further we will discuss all three listed above issues.

Firstly, PHD may be inactivated by ROS (Aragonés et al., 2009). This may result in accumulation of HIF-1α and, consequently, induce hypoxic adaptive response. The stimulation of glycolysis in concert with functioning mitochondria due to high oxygen supply may provide enough energy to cope with oxidative stress and provide reducing equivalents like NADPH and GSH to protect against ROS. Secondly, it became more and more clear that in many instances paradoxally, hypoxia may lead to the development of oxidative stress (Aragonés et al., 2009) and in fact this situation was documented in fish (Lushchak et al., 2001, 2005b; Lushchak and Bagnyukova, 2007).

Because HIF-1α is a master regulator of hypoxic adaptive response, one may suggest that this pathway can be involved at least in up-regulation of antioxidant enzymes as it was discussed by us earlier (Lushchak and Bagnyukova, 2006). Thirdly, the efforts of teams led by M. Nikinmaa in Finland, U.O. Portner and D. Abele

in Germany and G. Nilsson in Norway gave broad understanding of HIF-1α pathway operation in fish. Here we will not analyze their works because that has been done in several excellent reviews (Nikinmaa and Rees, 2005; Sollid et al., 2006; Hoogewijs et al., 2007b). Briefly, these works demonstrated: (*i*) a broad distribution of HIF-1α among fishes (Heise et al., 2006a,b; Rytkönen et al., 2008), (*ii*) concominant changes in HIF-1α levels with metallothionein level and temperature variation (van Heerden et al., 2004; Rissanen et al., 2006; Heise et al., 2006a,b, 2007), (*iii*) clear relationship between oxidative stress parameters, HIF-1α levels and expression of heat shock proteins (Heise et al., 2006a,b). That may stimulate ideas on possible involvement of HIF-1α in fish response to oxidative stress. The structural peculiarities of fish HIF-1α proteins and their susceptibility to oxidative modification were given recently (Hoogewijs et al., 2007a,b). Although direct evidence on the involvement of HIF-1α in fish response to oxidative stress is not available, several relationships, partially described here suppose the important role of this multitasking regulator in metabolism and clearly promise new discoveries.

Hif-1 factor was found also in invertebrates, particularly in aquatic ones. For example two shrimp species, white shrimp *L. vannamei* (Soñanez-Organis et al., 2009) and grass shrimp *Palaemonetes pugio* (Li and Brouwer, 2007) were found to response to hypoxia in Hif-dependent manner. More detail information is available on the operation of the system in crustacean, water flea *D. magna* (Gorr et al., 2004). In this organism hypoxia-induced synthesis of hemoglobin depended on hypoxia-inducible factor.

7. Conclusions and perspectives

The production of free radicals is an inevitable part of aerobic life. They are continuously produced and eliminated which creates the basis for maintaining of certain steady-state concentrations. Due to some reasons, either enhanced production, or decreased elimination, or both these processes, the steady-state ROS level may be enhanced leading to disruption of core metabolic and regulatory processes and this state was called “oxidative stress”. If earlier ROS were supposed to be damaging species, now they are seen also as regulatory and protective ones.

The metabolism of ROS in aquatic animals possesses, in fact, all characteristics found in other groups, because in this review we focused on ectotherms, the generation of ROS substantially increases at temperature growth. The increase in environmental oxygen level induces oxidative stress in this group, and although unexpectedly, hypoxia also was among inducers of this state. Many xenobiotics, such as pesticides, oil products, and metal ions also were found to be inducers of oxidative stress. Therefore, it can be concluded that virtually all strong enough stresses are accompanied by oxidative stress. Interestingly, even principal mechanisms of adaptive response to oxidative stress, i.e. up-regulation of antioxidant enzymes found in mammalian Keap1–Nrf2 system were found in aquatic animals. That makes them not only interesting group to study, but may help to clarify some general principles of operation of systems of ROS metabolism.

The different aspects of ROS metabolism are investigated to different extend. Most published works are mainly descriptive ones and they are in need further to understand basic principles of generation and elimination of ROS. That may open the window to manage the processes and to avoid negative consequences related to oxidative stress. Even more, pre-exposure to low intensity oxidative stress, no matter very much how the stress was induced, may enhance tolerance to followed higher intensity stress boots. That may be so-called preadaptation or cross-adaptation approach.

The quality of water may be a key determinant for future development of hydrobiology. Biological diversity and the physiological state are direct indices of water quality. Free radical approach is one of the most commonly used to develop markers of environmental pollution and in addition, it can be used in model experiments to evaluate effects of natural and polluted waters, as well as potential and virtual pollutants.

It should be noted that the parameters of oxidative stress in fish in many cases are age-related (Rudneva et al., 2010; Lushchak et al., not published) which demonstrates the need for the careful biological analysis of the material to be accounted at interpretation of data received.

At field studies, which are carried out in many cases to characterize their pollution and effects on biota, the markers of oxidative stress in hydrobionts are frequently used. For example, the series of works of O. Stolayr with colleagues used multiple approaches to evaluate the effects of human-born pollution on several aquatic animals. They used mussels *Anodonta cygnea* (Falfushynska et al., 2009), frogs *Rana ridibunda* (Falfushynska et al., 2008a,b,c) and fish *C. carpio* (Falfushynska and Stolyar, 2009). The extensive use of mathematic and statistic approaches (two-way factorial Anova, discriminant functional analysis and principal component analysis were used to determine the statistical significance of individual biochemical variables (discriminant Analysis) or individual specimens (PCA namely centroid grouping analysis)) showed that this way may let to discriminate between statistically important and non-important factors. More simple correlation analysis also can be applied in this case. But the statistical treatments must be supported by careful biological analysis because in many cases instead statistically important relationships simple coincidence can be the reason for found correlations. In some cases, the situation may be clarified by the model experiments in laboratory.

Finally, as we have seen, aquatic animals possess all mechanisms to oxidative response found in terrestrial ones. However, in many cases they provide better and cheaper models for broad screening of certain compounds of animal response to oxidative stress.

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References

Agnisola, C., 2005. Role of nitric oxide in the control of coronary resistance in teleosts. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 142, 178–187.

Ahmad, I., Maria, V.L., Oliveira, M., Pacheco, M., Santos, M.A., 2006. Oxidative stress and genotoxic effects in gill and kidney of *Anguilla anguilla* L. exposed to chromium with or without pre-exposure to β-naphthoflavone. Mutat. Res. 608, 16–28.

Aisen, P., Enns, C., Wessling-Resnick, M., 2001. Chemistry and biology of eukaryotic iron metabolism. Int. J. Biochem. Cell Biol. 33, 940–959.

Amado, L.L., Garcia, M.L., Ramos, P.B., Freitas, R.F., Zafalon, B., Ferreira, J.L., Yunes, J.S., Monserrat, J.M., 2009. A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: application to evaluate microcystins toxicity. Sci. Total Environ. 407, 2115–2123.

Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55, 373–399.

Aragonés, J., Fraisl, P., Baes, M., Carmeliet, P., 2009. Oxygen sensors at the crossroad of metabolism. Cell Metab. 9, 11–22.

Arillo, A., Melodia, F., 1990. Protective effect of fish mucus against Cr (VI) pollution. Chemosphere 20, 397–402.

Asai, S., Ohta, K., Yoshioka, H., 2008. MAPK signaling regulates nitric oxide and NADPH oxidase-dependent oxidative bursts in *Nicotiana benthamiana*. Plant Cell 20, 1390–1406.

Bagnyukova, T.V., Chahrak, O.I., Lushchak, V.I., 2006. Coordinated response of goldfish antioxidant defenses to environmental stress. Aquat. Toxicol. 78, 325–331.

Bagnyukova, T.V., Danyliv, S.I., Zin'ko, O.S., Lushchak, V.I., 2007a. Heat shock induces oxidative stress in rotan *Perccottus glenii* tissues. J. Therm. Biol. 32, 255–260.

Bagnyukova, T.V., Luzhna, L.I., Pogribny, I.P., Lushchak, V.I., 2007b. Oxidative stress and antioxidant defenses in goldfish liver in response to short-term exposure to arsenite. Environ. Mol. Mutagen. 48, 658–665.

Bagnyukova, T.V., Storey, K.B., Lushchak, V.I., 2003. Induction of oxidative stress in *Rana ridibunda* during recovery from winter hibernation. J. Therm. Biol. 28, 21–28.

Bagnyukova, T.V., Storey, K.B., Lushchak, V.I., 2005a. Adaptive response of antioxidant enzymes to catalase inhibition by aminotriazole in goldfish liver and kidney. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 142, 335–341.

Bagnyukova, T.V., Vasylkiv, O.Y., Storey, K.B., Lushchak, V.I., 2005b. Catalase inhibition by amino triazole induces oxidative stress in goldfish brain. Brain Res. 1052, 180–186.

Baker, R.T.M., Handy, R.D., Davies, S.J., Snook, J.C., 1998. Chronic dietary exposure to copper affects growth, tissue lipid peroxidation, and metal composition of the grey mullet, *Chelon labrosus*. Mar. Environ. Res. 45, 357–365.

Baker, R.T.M., Martin, P., Davies, S.J., 1997. Ingestion of sub-lethal levels of iron sulphate by African catfish affects growth and tissue lipid peroxidation. Aquat. Toxicol. 40, 51–61.

Banning, A., Brigelius-Flohé, R., 2005. NF-kappaB, Nrf2, and HO-1 interplay in redox-regulated VCAM-1 expression. Antioxid. Redox Signaling 7, 889–899.

Barata, C., Varo, I., Navarro, J.C., Arun, S., Porte, C., 2005. Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran *Daphnia magna* exposed to redox cycling compounds. Comp. Biochem. Physiol. C 140, 175–186.

Bauer, V., Bauer, F., 1999. Reactive oxygen species as mediators of tissue protection and injury. Gen. Physiol. Biophys. 18, 7–14.

Beal, M.F., 1995. Aging, energy, and oxidative stress in neurodegenerative diseases. Ann. Neurol. 38, 357–366.

Berntssen, M.H.G., Aatland, A., Handy, R.D., 2003. Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behaviour in Atlantic salmon (*Salmo salar*) parr. Aquat. Toxicol. 65, 55–72.

Bespalov, I.A., Bond, J.P., Purmal, A.A., Wallace, S.S., Melamede, R.J., 1999. Fabs specific for 8-oxoguanine: control of DNA binding. J. Mol. Biol. 293, 1085–1095.

Binelli, A., Cogni, D., Parolini, M., Riva, C., Provini, A., 2009. In vivo experiments for the evaluation of genotoxic and cytotoxic effects of triclosan in *Zebra mussel* hemocytes. Aquat. Toxicol. 91, 238–244.

Bocchetti, R., Lamberti, C.V., Pisanelli, B., Razzetti, E.M., Maggi, C., Catalano, B., Sesta, G., Martuccio, G., Gabellini, M., Regoli, F., 2008. Seasonal variations of exposure biomarkers, oxidative stress responses and cell damage in the clams. *Tapes philippinarum*, and mussels, *Mytilus galloprovincialis*, from Adriatic sea. Mar. Environ. Res. 66, 24–26.

Bonnefont-Rousselot, D., 2002. Glucose and reactive oxygen species. Curr. Opin. Clin. Nutr. Metab. Care 5, 561–568.

Bopp, S.K., Abicht, H.K., Knauer, K., 2008. Copper-induced oxidative stress in rainbow trout gill cells. Aquat. Toxicol. 86, 197–204.

Bretaud, S., Lee, S., Guo, S., 2004. Sensitivity of zebrafish to environmental toxins implicated in Parkinson's disease. Neurotoxicol. Teratol. 26, 857–864.

Brouwer, M., Brouwer, T.H., 1998. Biochemical defense mechanisms against copper-induced oxidative damage in the blue crab, *Callinectes sapidus*. Arch. Biochem. Biophys. 351, 257–264.

Bury, N.R., Walker, P.A., Glover, C.N., 2003. Nutritive metal uptake in teleost fish. J. Exp. Biol. 206, 11–23.

Caliani, I., Porcelloni, S., Mori, G., Frenzilli, G., Ferraro, M., Marsili, L., Casini, S., Fossi, M.C., 2009. Genotoxic effects of produced waters in mosquito fish (*Gambusia affinis*). Ecotoxicology 18, 75–80.

Carriquiriborde, P., Handy, R.D., Davies, S.J., 2004. Physiological modulation of iron metabolism in rainbow trout (*Oncorhynchus mykiss*) fed low and high iron diets. J. Exp. Biol. 207, 75–86.

Cavalcante, D.G., Martinez, C.B., Sofia, S.H., 2008. Genotoxic effects of Roundup on the fish *Prochilodus lineatus*. Mutat. Res. 655, 41–46.

Cederbaum, A.I., 1989. Oxygen radical generation by microsomes: role of iron and implications for alcohol metabolism and toxicity. Free Radic. Biol. Med. 7, 559–567.

Chagué, V., Danit, L.V., Siewers, V., Schulze-Gronover, C., Tudzynski, P., Tudzynski, B., Sharon, A., 2006. Ethylene sensing and gene activation in *Botrytis cinerea*: a missing link in ethylene regulation of fungus-plant interactions? Mol. Plant-Microbe Interact. 19, 33–42.

Choi, C.Y., An, K.W., An, M.I., 2008. Molecular characterization and mRNA expression of glutathione peroxidase and glutathione S-transferase during osmotic stress in olive flounder (*Paralichthys olivaceus*). Comp. Biochem. Physiol. A 149, 330–337.

Claeson, K., Thorsen, G., Karlberg, B., 2001. Methyl malondialdehyde as an internal standard for the determination of malondialdehyde. J. Chromatogr. B 751, 315–323.

Clanton, T.L., 2007. Hypoxia-induced reactive oxygen species formation in skeletal muscle. J. Appl. Physiol. 102, 2379–2388.

Craig, P.M., Wood, C.M., McClelland, G.B., 2007. Oxidative stress response and gene expression with acute copper exposure in zebrafish (*Danio rerio*). Am. J. Physiol. 293, R1882–R1892.

De Coen, W.M., Janssen, C.R., 2003. A multivariate biomarker-based model predicting population-level responses of *Daphnia magna*. Environ. Toxicol. Chem. 22, 2195–2201.

De Zoysa, M., Whang, I., Lee, Y., Lee, S., Lee, J.S., Lee, J., 2009. Transcriptional analysis of antioxidant and immune defense genes in disk abalone (*Haliotis discus discus*) during thermal, low-salinity and hypoxic stress. Comp. Biochem. Physiol. B 154, 387–395.

Dean, R.T., Fu, S., Stocker, R., Davies, M.J., 1997. Biochemistry and pathology of radical-mediated protein oxidation. Biochem. J. 324, 1–18.

DeLeve, L.D., Kaplowitz, N., 1991. Glutathione metabolism and its role in hepatotoxicity. Pharmacol. Ther. 52, 287–305.

Demin, O.V., Kholodenko, B.N., Skulachev, V.P., 1998. A model of O2.-generation in the complex III of the electron transport chain. Mol. Cell. Biochem. 184, 21–33.

Dorval, J., Hontela, A., 2003. Role of glutathione redox cycle and catalase in defense against oxidative stress induced by endosulfan in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*). Toxicol. Appl. Pharmacol. 192, 191–200.

Dri, P., Bellavite, P., Berton, G., Rossi, F., 1979. Interrelationship between oxygen consumption, superoxide anion and hydrogen peroxide formation in phagocytosing guinea pig polymorphonuclear leucocytes. Mol. Cell. Biochem. 23, 109–122.

Dri, P., Bisiacchi, B., Cramer, R., Bellavite, P., de Nicola, G., Patriarca, P., 1978. Oxidative metabolism of chicken polymorphonuclear leucocytes during phagocytosis. Mol. Cell. Biochem. 22, 159–166.

Dunlop, R.A., Brunk, U.T., Rodgers, K.J., 2009. Oxidized proteins: mechanisms of removal and consequences of accumulation. IUBMB Life 61, 522–527.

Dykens, J.A., Shick, J.M., Benoit, C., Buettner, G.R., Winston, G.W., 1992. Oxygen radical production in the sea anemone *Anthopleura elegantissima* and its endosymbiotic algae. J. Exp. Biol. 168, 219–241.

Falfushynska, H.I., Delahaut, L., Stolyar, O.B., Geffard, A., Biagianti-Risbourg, S., 2009. Multi-biomarkers approach in different organs of *Anodonta cygnea* from the Dnister Basin (Ukraine). Arch. Environ. Contam. Toxicol. 57, 86–95.

Falfushynska, H., Loumbourdis, N., Romanchuk, L., Stolyar, O., 2008a. Validation of oxidative stress responses in two populations of frogs from Western Ukraine. Chemosphere 73, 1096–1101.

Falfushynska, H.I., Romanchuk, L.D., Stolyar, O.B., 2008b. Different responses of biochemical markers in frogs (*Rana ridibunda*) from urban and rural wetlands to the effect of carbamate fungicide. Comp. Biochem. Physiol. C 148, 223–229.

Falfushynska, H.I., Romanchuk, L.D., Stolyar, O.B., 2008c. Seasonal and spatial comparison of metallothioneins in frog *Rana ridibunda* from feral populations. Ecotoxicology 17, 781–788.

Falfushynska, H.I., Stolyar, O.B., 2009. Responses of biochemical markers in carp *Cyprinus carpio* from two field sites in Western Ukraine. Ecotoxicol. Environ. Saf. 72, 729–736.

Farag, A.M., May, T., Marty, G.D., Easton, M., Harper, D.D., Little, E.E., Cleveland, L., 2006. The effect of chronic chromium exposure on the health of Chinook salmon (*Oncorhynchus tshawytscha*). Aquat. Toxicol. 76, 246–257.

Filipak Neto, F., Zanata, S.M., Silva de Assis, H.C., Nakao, L.S., Randi, M.A., Oliveira Ribeiro, C.A., 2008. Toxic effects of DDT and methyl mercury on the hepatocytes from *Hoplias malabaricus*. Toxicol. In Vitro 22, 1705–1713.

Foyer, C.H., Noctor, G., 2009. Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. Antioxid. Redox Signaling 11, 861–905.

Fukunaga, K., Nakazono, N., Suzuki, T., Takama, K., 1999. Mechanism of oxidative damage to fish red blood cells by ozone. IUBMB Life 48, 631–634.

Gielazyn, M.L., Ringwood, A.H., Piegorsch, W.W., Stancyk, S.E., 2003. Detection of oxidative DNA damage in isolated marine bivalve hemocytes using the comet assay and formamidopyrimidine glycosylase (Fpg). Mutat. Res. 542, 15–22.

Glusczak, L., dos Santos Miron, D., Crestani, M., Braga da Fonseca, M., de Araújo Pedron, F., Duarte, M.F., Vieira, V.L., 2006. Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic and hematological parameters in piava (*Leporinus obtusidens*). Ecotoxicol. Environ. Saf. 65, 237–241.

Glusczak, L., dos Santos Miron, D., Moraes, B.S., Simxes, R.R., Schetinger, M.R., Morsch, V.M., Loro, V.L., 2007. Acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*). Comp. Biochem. Physiol. C 146, 519–524.

Goksøyr, A., Förlin, L., 1992. The cytochrome P450 system in fish, aquatic toxicology and environmental monitoring. Aquat. Toxicol. 22, 287–312.

Gong, P., Stewart, D., Hu, B., Li, N., Cook, J., Nel, A., Alam, J., 2002. Activation of the mouse heme oxygenase-1 gene by 15-deoxy-Delta(12,14)-prostaglandin J(2) is mediated by the stress response elements and transcription factor Nrf2. Antioxid. Redox Signaling 4, 249–257.

Gopalakrishnan, A., Tony Kong, A.N., 2008. Anticarcinogenesis by dietary phytochemicals: cytoprotection by Nrf2 in normal cells and cytotoxicity by modulation of transcription factors NF-kappa B and AP-1 in abnormal cancer cells. Food Chem. Toxicol. 46, 1257–1270.

Gorr, T.A., Cahn, J.D., Yamagata, H., Bunn, H.F., 2004. Hypoxia-induced synthesis of hemoglobin in the crustacean *Daphnia magna* is hypoxia-inducible factor-dependent. J. Biol. Chem. 279, 36038–36047.

Grygoryev, D., Moskalenko, O., Zimbrick, J.D., 2008. Non-linear effects in the formation of DNA damage in medaka fish fibroblast cells caused by combined action of cadmium and ionizing radiation. Dose Response 6, 283–298.

Guallar, E., Sanz-Gallardo, M.I., van't Veer, P., Bode, P., Aro, A., Gomez-Aracena, J., Kark, J.D., Riemersma, R.A., Martin-Moreno, J.M., Kok, F.J., 2002. Heavy metals and myocardial infarction study group. Mercury, fish oils, and the risk of myocardial infarction. N. Engl. J. Med. 347, 1747–1754.

Hai, D.Q., Varga, S.I., Matkovics, B., 1997a. Effects of diethyl-dithiocarbamate on antioxidant system in carp tissue. Acta Biol. Hung. 48, 1–8.

Hai, D.Q., Varga, S.I., Matkovics, B., 1997b. Organophosphate effects on antioxidant system of carp (*Cyprinus carpio*) and catfish (*Ictalurus nebulosus*). Comp. Biochem. Physiol. C 117, 83–88.

Halliwell, B., Gutteridge, J.M.C., 1989. Free Radicals in Biology and Medicine, second ed. Clarendon Press, Oxford, UK.

Hansen, B.H., Rømma, S., Garmo, Ø.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. Comp. Biochem. Physiol. C 143, 263–274.
Hansen, B.H., Rømma, S., Søfteland, 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 65, 1707–1714.
Harada, T., Yamaguchi, S., Ohtsuka, R., Takeda, M., Fujisawa, H., Yoshida, T., Enomoto, A., Chiba, Y., Fukumori, J., Kojima, S., Tomiyama, N., Saka, M., Ozaki, M., Maita, K., 2003. Mechanisms of promotion and progression of preneoplastic lesions in hepatocarcinogenesis by DDT in F344 rats. Toxicol. Pathol. 31, 87–98.
Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11, 298–300.
Hasselberg, L., Meier, S., Svardal, A., 2004a. Effects of alkylphenols on redox status in first spawning Atlantic cod (*Gadus morhua*). Aquat. Toxicol. 69, 95–105.
Hasselberg, L., Meier, S., Svardal, A., Hegelund, T., Celander, M.C., 2004b. Effects of alkylphenols on CYP1A and CYP3A expression in first spawning Atlantic cod (*Gadus morhua*). Aquat. Toxicol. 67, 303–313.
Hébert, N., Gagné, F., Cejka, P., Bouchard, B., Hausler, R., Cyr, D.G., Blaise, C., Fournier, M., 2008. Effects of ozone, ultraviolet and peracetic acid disinfection of a primary-treated municipal effluent on the immune system of rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. C 148, 122–127.
Heise, K., Puntarulo, S., Nikinmaa, M., Abele, D., Pörtner, H.O., 2006a. Oxidative stress during stressful heat exposure and recovery in the North Sea eelpout *Zoarces viviparus* L. J. Exp. Biol. 209, 353–363.
Heise, K., Puntarulo, S., Nikinmaa, M., Lucassen, M., Pörtner, H.O., Abele, D., 2006b. Oxidative stress and HIF-1 DNA binding during stressful cold exposure and recovery in the North Sea eelpout (*Zoarces viviparus*). Comp. Biochem. Physiol. A 143, 494–503.
Heise, K., Estevez, M.S., Puntarulo, S., Galleano, M., Nikinmaa, M., Pörter, H.O., Abele, D., 2007. Effects of seasonal and latitudinal cold on oxidative stress parameters and activation of hypoxia inducible factor (HIF-1) in zoarcid fish. J. Comp. Physiol. B 177, 765–777.
van Heerden, D., Vosloo, A., Nikinmaa, M., 2004. Effects of short-term copper exposure on gill structure, metallothionein and hypoxia-inducible factor-1alpha (HIF-1alpha) levels in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 69 (3), 271–280.
Hermes-Lima, M., Storey, J.M., Storey, K.B., 1998. Antioxidant defenses and metabolic depression. The hypothesis of preparation for oxidative stress in land snails. Comp. Biochem. Physiol. B 120, 437–448.
Hermes-Lima, M., Willmore, W.G., Storey, K.B., 1995. Quantification of lipid peroxidation in tissue extracts based on Fe(III)xylenol orange complex formation. Free Radic. Biol. Med. 19, 271–280.
Hoogewijs, D., Geuens, E., Dewilde, S., Vierstraete, A., Moens, L., Vinogradov, S., Vanfleteren, J.R., 2007a. Wide diversity in structure and expression profiles among members of the *Caenorhabditis elegans* globin protein family. BMC Genomics 8, 356.
Hoogewijs, D., Terwilliger, N.B., Webster, K.A., Powell-Coffman, J.A., Tokishita, S., Yamagata, H., Hankeln, T., Burmester, T., Rytknen, K.T., Nikinmaa, M., Abele, D., Heise, K., Lucassen, M., Fandrey, J., Maxwell, P.H., Phlman, S., Gorr, T.A., 2007b. From critters to cancers: bridging comparative and clinical research on oxygen sensing. HIF signaling, and adaptations towards hypoxia. Integr. Comp. Biol. 47, 552–577.
Hook, S.E., Skillman, A.D., Small, J.A., Schultz, I.R., 2006. Gene expression patterns in rainbow trout. *Oncorhynchus mykiss*, exposed to a suite of model toxicants. Aquat. Toxicol. 77, 372–785.
Hoyle, I., Shaw, B.J., Handy, R.D., 2007. Dietary copper exposure in the African walking catfish, *Clarias gariepinus*: transient osmoregulatory disturbances and oxidative stress. Aquat. Toxicol. 83, 62–72.
Hughes, E.M., Gallagher, E.P., 2004. Effects of 17-[beta] estradiol and 4-nonylphenol on phase II electrophilic detoxification pathways in largemouth bass (*Micropterus salmoides*) liver. Comp. Biochem. Physiol. C 137, 237–247.
Ivanina, A.V., Sokolova, I.M., Sukhotin, A.A., 2008. Oxidative stress and expression of chaperones in aging mollusks. Comp. Biochem. Physiol. B 150, 53–61.
Jacob, C., Knight, I., Winyard, P.G., 2006. Aspects of the biological redox chemistry of cysteine: from simple redox responses to sophisticated signalling pathways. Biol. Chem. 387, 1385–1397.
Janero, D.R., 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic. Biol. Med. 9, 515–540.
Jha, A.N., 2008. Ecotoxicological applications and significance of the comet assay. Mutagenesis 23, 207–221.
Jones, D.P., 2006. Extracellular redox state: refining the definition of oxidative stress in aging. Rejuvenation Res. 9, 169–181.
Kajimura, S., Aida, K., Duan, C., 2006. Understanding hypoxia-induced gene expression in early development: in vitro and in vivo analysis of hypoxia-inducible factor 1-regulated zebra fish insulin-like growth factor binding protein 1 gene expression. Mol. Cell. Biol. 26, 1142–1155.
Kamunde, C., Clayton, C., Wood, C.M., 2002. Waterborne vs. dietary copper uptake in rainbow trout and the effects of previous waterborne copper exposure. Am. J. Physiol. 283, 69–78.
Ke, Q., Costa, M., 2006. Hypoxia-inducible factor-1 (HIF-1). Mol. Pharmacol. 70, 1469–1480.
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. Environ. Health Perspect. 106, 375–384.
Kelley, E.E., Khoo, N.K., Hundley, N.J., Malik, U.Z., Freeman, B.A., Tarpey, M.M., 2010. Hydrogen peroxide is the major oxidant product of xanthine oxidase. Free Radic. Biol. Med. 48 (4), 493–498.
Kelly, M.C., White, B., Smyth, M.R., 2008. Separation of oxidatively damaged DNA nucleobases and nucleosides on packed and monolith C18 columns by HPLC-UV-EC. J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 863, 181–186.
Kobayashi, M., Itoh, K., Suzuki, T., Osanai, H., Nishikawa, K., Katoh, Y., Takagi, Y., Yamamoto, M., 2002. Identification of the interactive interface and phylogenic conservation of the Nrf2-Keap1 system. Genes Cells 7, 807–820.
Kobayashi, M., Li, L., Iwamoto, N., Nakajima-Takagi, Y., Kaneko, H., Nakayama, Y., Eguchi, M., Wada, Y., Kumagai, Y., Yamamoto, M., 2009. The antioxidant defense system Keap1–Nrf2 comprises a multiple sensing mechanism for responding to a wide range of chemical compounds. Mol. Cell. Biol. 29, 493–502.
Kobayashi, M., Yamamoto, M., 2005. Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. Antioxid. Redox Signaling 7, 385–394.
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. Comp. Biochem. Physiol., C 152, 360–370.
Kuykendall, J.R., Miller, K.L., Mellinger, K.N., Cain, A.V., 2006. Waterborne and dietary hexavalent chromium exposure causes DNA–protein crosslink (DPX) formation in erythrocytes of largemouth bass (*Micropterus salmoides*). Aquat. Toxicol. 78, 27–31.
Laville, N., Aït-Aïssa, S., Gomez, E., Casellas, C., Porcher, J.M., 2004. Effects of human pharmaceuticals on cytotoxicity, EROD activity and ROS production in fish hepatocytes. Toxicology 196, 41–55.
Lemaire, P., Matthews, A., Forlin, L., Livingstone, D.R., 1994. Stimulation of oxyradical production of hepatic microsomes of flounder (*Platichthys flesus*) and perch (*Perca fluviatilis*) by model and pollutant xenobiotics. Arch. Environ. Contam. Toxicol. 26, 191–200.
Levine, R.L., Wehr, N., Williams, J.A., Stadtman, E.R., Shacter, E., 2000. Determination of carbonyl groups in oxidized proteins. Methods Mol. Biol. 99, 15–24.
Li, T., Brouwer, M., 2007. Hypoxia-inducible factor, gsHIF, of the grass shrimp *Palaemonetes pugio*: molecular characterization and response to hypoxia. Comp. Biochem. Physiol. B 147, 11–19.
Li, J.S., Han, Q., Fang, J., Rizzi, M., James, A.A., Li, J., 2007. Biochemical mechanisms leading to tryptophan 2,3-dioxygenase activation. Arch. Insect Biochem. Physiol. 64, 74–87.
Li, L., Kobayashi, M., Kaneko, H., Nakajima-Takagi, Y., Nakayama, Y., Yamamoto, M., 2008. Molecular evolution of Keap1. Two Keap1 molecules with distinctive intervening region structures are conserved among fish. J. Biol. Chem. 283, 3248–3255.
Liu, H., Wang, W., Zhang, J.F., Wang, X.R., 2006. Effects of copper and its ethylenediaminetetraacetate complex on the antioxidant defenses of the goldfish, *Carassius auratus*. Ecotoxicol. Environ. Saf. 65, 350–354.
Liu, Y., Wang, W.N., Wang, A.L., Wang, J.M., Sun, R.Y., 2007. Effects of dietary vitamin E supplementation on antioxidant enzyme activities in *Litopenaeus vannamei* (Boone, 1931) exposed to acute salinity changes. Aquaculture 265, 351–358.
Livingstone, D.R., 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Pollut. Bull. 42, 656–666.
Luo, Y., Sui, Y.X., Wang, X.R., Tian, Y., 2008. 2-Chlorophenol induced hydroxyl radical production in mitochondria in *Carassius auratus* and oxidative stress—an electron paramagnetic resonance study. Chemosphere 71, 1260–1268.
Lushchak, V.I., 2007. Free radical oxidation of proteins and its relationship with functional state of organisms. Biochemistry (Moscow) 72, 809–827.
Lushchak, V.I., 2008. Oxidative stress as a component of transition metal toxicity in fish. In: Svensson, E.P. (Ed.), Aquatic Toxicology Research Focus. Nova Science Publishers Inc., Hauppaug, NY, USA, pp. 1–29.
Lushchak, V.I., Bagnyukova, T.V., 2006. Effects of different environmental oxygen levels on free radical processes in fish. Comp. Biochem. Physiol. B 144, 283–289.
Lushchak, V.I., Bagnyukova, T.V., 2007. Hypoxia induces oxidative stress in tissues of a goby, the rotan *Perccottus glenii*. Comp. Biochem. Physiol. B 148, 390–397.
Lushchak, O.V., Bagnyukova, T.V., Lushchak, V.I., 2003. Effect of aminotriazole on the activity of catalase and glucose-6-phosphate dehydrogenase of two frog species, *Rana ridibunda* and *R. esculenta*. Ukr. Biochem. J. 75, 45–50.
Lushchak, V.I., Bagnyukova, T.V., Husak, V.V., Luzhna, L.I., Lushchak, O.V., Storey, K.B., 2005a. Hyperoxia results in transient oxidative stress and an adaptive response by antioxidant enzymes in goldfish tissues. Int. J. Biochem. Cell Biol. 37, 1670–1680.
Lushchak, V.I., Bagnyukova, T.V., Lushchak, O.V., Storey, J.M., Storey, K.B., 2005b. Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (*Cyprinus carpio*) tissues. Int. J. Biochem. Cell Biol. 37, 1319–1330.
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. Chem. -Biol. Interact. 170, 1–8.
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. Aquat. Toxicol. 87, 108–114.
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. Aquat. Toxicol. 93, 45–52.

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 76, 932–937.

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 75, 56–62.

Lushchak, V.I., Lushchak, L.P., Mota, A.A., Hermes-Lima, M., 2001. Oxidative stress and antioxidant defenses in goldfish *Carassius auratus* during anoxia and reoxygenation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 280, 100–107.

Macomber, L., Rensing, C., Imlay, J.A., 2007. Intracellular copper does not catalyze the formation of oxidative DNA damage in *Escherichia coli*. J. Bacteriol. 189, 1616–1626.

Malek, R.L., Sajadi, H., Abraham, J., Grundy, M.A., Gerhard, G.S., 2004. The effects of temperature reduction on gene expression and oxidative stress in skeletal muscle from adult zebrafish. Comp. Biochem. Physiol. C 138, 363–373.

Malhotra, J.D., Kaufman, R.J., 2007. Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? Antioxid. Redox Signaling 9, 2277–2293.

Malins, D.C., Polissar, N.L., Garner, M.M., Gunselman, S.J., 1996. Mutagenic DNA base modifications are correlated with lesions in nonneoplastic hepatic tissue of the English sole carcinogenesis model. Cancer Res. 56, 5563–5565.

Martínez-Alvarez, R.M., Hidalgo, M.C., Domezain, A., Morales, A.E., García-Gallego, M., Sanz, A., 2002. Physiological changes of sturgeon *Acipenser naccarii* caused by increasing environmental salinity. J. Exp. Biol. 205, 3699–3706.

McAnulty, S.R., McAnulty, L.S., Nieman, D.C., Morrow, J.D., Utter, A.C., Henson, D.A., Dumke, C.L., Vinci, D.M., 2003. Influence of carbohydrate ingestion on oxidative stress and plasma antioxidant potential following a 3 h run. Free Radic. Res. 37, 835–840.

McCord, J.M., Fridovich, I., 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244, 6049–6055.

Mieiro, C.L., Ahmad, I., Pereira, M.E., Duarte, A.C., Pacheco, M., 2010. Antioxidant system breakdown in brain of feral golden grey mullet (*Liza aurata*) as an effect of mercury exposure. Ecotoxicology 19 (6), 1034–1045.

Mira, L., Silva, M., Rocha, R., Manso, C.F., 1999. Measurement of relative antioxidant activity of compounds: a methodological note. Redox Rep. 4, 69–74.

Monteiro, D.A., Rantin, F.T., Kalinin, A.L., 2009. The effects of selenium on oxidative stress biomarkers in the freshwater characid fish matrinxã, *Brycon cephalus* (Günther, 1869) exposed to organophosphate insecticide Folisuper 600 BR (methyl parathion). Comp. Biochem. Physiol. C 149, 40–49.

Monteiro, D.A., Rantin, F.T., Kalinin, A.L., 2010. Inorganic mercury exposure: toxicological effects, oxidative stress biomarkers and bioaccumulation in the tropical freshwater fish matrinxã, Brycon amazonicus (Spix and Agassiz, 1829). Ecotoxicology 19 (1), 105–123.

Murphy, B.J., Sato, B.G., Dalton, T.P., Laderoute, K.R., 2005. The metal-responsive transcription factor-1 contributes to HIF-1 activation during hypoxic stress. Biochem. Biophys. Res. Commun. 337, 860–867.

Nguyen, T., Nioi, P., Pickett, C.B., 2009. The nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J. Biol. Chem. 284, 13291–13295.

Nikinmaa, M., Rees, B.B., 2005. Oxygen-dependent gene expression in fishes. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288, R1079–R1090.

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. Mar. Environ. Res. 52, 13–26.

Oehlers, L.P., Perez, A.N., Walter, R.B., 2007. Detection of hypoxia-related proteins in medaka (*Oryzias latipes*) brain tissue by difference gel electrophoresis and de novo sequencing of 4-sulfophenyl isothiocyanate-derivatized peptides by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Comp. Biochem. Physiol. C 145, 120–133.

Olsvik, P.A., Kristensen, T., Waagbø, R., Rosseland, B.O., Tollefsen, K.E., Baeverfjord, G., Berntssen, M.H., 2005. mRNA expression of antioxidant enzymes (SOD, CAT and GSH-Px) and lipid peroxidative stress in liver of Atlantic salmon (*Salmo salar*) exposed to hyperoxic water during smoltification. Comp. Biochem. Physiol. C 141, 314–323.

Osburn, W.O., Kensler, T.W., 2008. Nrf2 signaling: an adaptive response pathway for protection against environmental toxic insults. Mutat. Res. 659, 31–39.

Palmer, R.M., Ferrige, A.G., Moncada, S., 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327, 524–526.

Papa, S., Skulachev, V.P., 1997. Reactive oxygen species, mitochondria, apoptosis and aging. Mol. Cell. Biochem. 174, 305–319.

Parihar, M.S., Dubey, A.K., 1995. Lipid peroxidation and ascorbic acid status in respiratory organs of male and female freshwater catfish *Heteropneustes fossilis* exposed to temperature increase. Comp. Biochem. Physiol. C 112, 309–313.

Parvez, S., Raisuddin, S., 2006. Effects of paraquat on the freshwater fish *Channa punctata* (Bloch): non-enzymatic antioxidants as biomarkers of exposure. Arch. Environ. Contam. Toxicol. 50, 392–397.

Pedrajas, J.R., Peinado, J., López-Barea, J., 1995. Oxidative stress in fish exposed to model xenobiotics. Oxidatively modified forms of Cu,Zn-superoxide dismutase as potential biomarkers. Chem. -Biol. Interact. 98, 267–282.

Peña-Llopis, S., Ferrando, M.D., Peña, J.B., 2003. Increased recovery of brain acetylcholinesterase activity in dichlorvos-intoxicated European eels *Anguilla anguilla* by bath treatment with N-acetylcysteine. Dis. Aquat. Organ. 55, 237–245.

Peña-Llopis, S., Peña, J.B., Sancho, E., Fernandez-Vega, C., Ferrando, M.D., 2001. Glutathione-dependent resistance of the European eel *Anguilla anguilla* to the herbicide molinate. Chemosphere 45, 671–681.

Pereira Ede, J., Panek, A.D., Eleutherio, E.C., 2003. Protection against oxidation during dehydration of yeast. Cell Stress Chaperones 8, 120–124.

Perez-Benito, J.F., 2006. Effects of chromium (VI) and vanadium (V) on the lifespan of fish. J. Trace Elem. Med. Biol. 20, 161–170.

Petridis, P., Jha, A.N., Langston, W.J., 2009. Measurements of the genotoxic potential of (xeno-) oestrogens in the bivalve mollusc *Scrobicularia plana*, using the Comet assay. Aquat. Toxicol. 94 (1), 8–15.

Philipp, E.E., Schmidt, M., Gsottbauer, C., Sänger, A.M., Abele, D., 2008. Size- and age-dependent changes in adductor muscle swimming physiology of the scallop *Aequipecten opercularis*. J. Exp. Biol. 211, 2492–2501.

Pialoux, V., Brugniaux, J.V., Fellmann, N., Richalet, J.P., Robach, P., Schmitt, L., Coudert, J., Mounier, R., 2009a. Oxidative stress and HIF-1alpha modulate hypoxic ventilatory responses after hypoxic training on athletes. Respir. Physiol. Neurobiol. 167, 217–220.

Pialoux, V., Mounier, R., Brown, A.D., Steinback, C.D., Rawling, J.M., Poulin, M.J., 2009b. Relationship between oxidative stress and HIF-1 alpha mRNA during sustained hypoxia in humans. Free Radic. Biol. Med. 46, 321–326.

Piner, P., Sevgiler, Y., Uner, N., 2007. In vivo effects of fenthion on oxidative processes by the modulation of glutathione metabolism in the brain of *Oreochromis niloticus*. Environ. Toxicol. 22, 605–612.

Poljsak, B., Gazdag, Z., Jenko-Brinovec, S., Fujs, S., Pesti, M., Bélagyi, J., Plesnicar, S., Raspor, P., 2005. Pro-oxidative vs antioxidative properties of ascorbic acid in chromium(VI)-induced damage: an in vivo and in vitro approach. J. Appl. Toxicol. 25, 535–548.

Ponka, P., 1999. Cellular iron metabolism. Kidney Int. Suppl. 69, S2–S11.

Rahman, M.S., Thomas, P., 2007. Molecular cloning, characterization and expression of two hypoxia-inducible factor alpha subunits, HIF-1alpha and HIF-2alpha, in a hypoxia-tolerant marine teleost, Atlantic croaker (*Micropogonias undulatus*). Gene 396, 273–282.

Ren, B., Duan, X., Ding, H., 2009. Redox control of the DNA damage-inducible protein DinG helicase activity via its iron-sulfur cluster. J. Biol. Chem. 284, 4829–4835.

Rin, K., Kawaguchi, K., Yamanaka, K., Tezuka, M., Oku, N., Okada, S., 1995. DNA-strand breaks induced by dimethylarsinic acid, a metabolite of inorganic arsenics, are strongly enhanced by superoxide anion radicals. Biol. Pharm. Bull. 18, 45–48.

Rissanen, E., Tranberg, H.K., Sollid, J., Nilsson, G.E., Nikinmaa, M., 2006. Temperature regulates hypoxia-inducible factor-1 (HIF-1) in a poikilothermic vertebrate, crucian carp (*Carassius carassius*). J. Exp. Biol. 209, 994–1003.

Rojas, D.A., Perez-Munizaga, D.A., Centanin, L., Antonelli, M., Wappner, P., Allende, M.L., Reyes, A.E., 2007. Cloning of hif-1alpha and hif-2alpha and mRNA expression pattern during development in zebrafish. Gene Expr. Patterns 7, 339–345.

Rooney, J.P.K., 2007. The role of thiols, dithiols, nutritional factors and interacting ligands in the toxicology of mercury. Toxicology 234, 145–156.

Rosety, M., Rosety-Rodríguez, M., Ordonez, F.J., Rosety, I., 2005. Time course variations of antioxidant enzyme activities and histopathology of gilthead seabream gills exposed to malathion. Histol. Histopathol. 20, 1017–1020.

Ruchko, M.V., Gorodnya, O.M., Pastukh, V.M., Swiger, B.M., Middleton, N.S., Wilson, G.L., Gillespie, M.N., 2009. Hypoxia-induced oxidative base modifications in the VEGF hypoxia-response element are associated with transcriptionally active nucleosomes. Free Radic. Biol. Med. 46 (3), 352–359.

Rudneva, I.I., Skuratovskaya, E.N., Kuzminova, N.S., Kovyrshina, T.B., 2010. Age composition and antioxidant enzyme activities in blood of Black Sea teleosts. Comp. Biochem. Physiol. C 151, 229–239.

Ruiz-Leal, M., George, S., 2004. An in vitro procedure for evaluation of early stage oxidative stress in an established fish cell line applied to investigation of PHAH and pesticide toxicity. Mar. Environ. Res. 58, 631–635.

Rytkönen, K.T., Ryynänen, H.J., Nikinmaa, M., Primmer, C.R., 2008. Variable patterns in the molecular evolution of the hypoxia-inducible factor-1 alpha (HIF-1alpha) gene in teleost fishes and mammals. Gene 420 (1), 1–10.

Salas-Leiton, E., Cánovas-Conesa, B., Zerolo, R., López-Barea, J., Cañavate, J.P., Alhama, J., 2009. Proteomics of juvenile senegal sole (*Solea senegalensis*) affected by gas bubble disease in hyperoxygenated ponds. Mar. Biotechnol. 11, 473–487.

Salonen, J.T., Seppanen, K., Nyyssonen, K., Korpela, H., Kauhanen, J., Kantola, M., Tuomilehto, J., Esterbauer, H., Tatzber, F., Salonen, R., 1995. Intake of mercury from fish, lipid peroxidation, and the risk of myocardial infarction and coronary, cardiovascular, and any death in eastern Finnish men. Circulation 91, 645–655.

Sanchez, W., Piccini, B., Porcher, J.M., 2008. Effect of prochloraz fungicide on biotransformation enzymes and oxidative stress parameters in three-spined stickleback (*Gasterosteus aculeatus* L.). J. Environ. Sci. Health Part B 43, 65–70.

Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Haque, R., Raisuddin, S., 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. Ecotoxicol. Environ. Saf. 56, 295–301.

Scandalios, J.G., 2005. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. Braz. J. Med. Biol. Res. 38, 995–1014.

Schlenk, D., Wolford, L., Chelius, M., Steevens, J., Chan, K.M., 1997. Effect of arsenite, arsenate, and the herbicide monosodium methyl arsonate (MSMA) on hepatic metallothionein expression and lipid peroxidation in channel catfish. Comp. Biochem. Physiol. C 118, 177–183.

Shi, X., Dalal, N.S., 1990. Evidence for a fenton-type mechanism for the generation of.OH radicals in the reduction of Cr (VI) in cellular media. Arch. Biochem. Biophys. 281, 90–95.

Shih, T.M., McDonough, J.H., 1997. Neurochemical mechanisms in soman-induced seizures. J. Appl. Toxicol. 17, 255–264.

Shmarakov, I.O., Marchenko, M.M., 2008. Xanthine oxidase activity in the rat liver tissue in the process of oncogenesis. Ukr. Biokhim. Zh. 80, 86–91.

Shukla, D., Saxena, S., Jayamurthy, P., Sairam, M., Singh, M., Jain, S.K., Bansal, A., Ilavazaghan, G., 2009. Hypoxic preconditioning with cobalt attenuates hypobaric hypoxia-induced oxidative damage in rat lungs. High Alt. Med. Biol. 10, 57–69.
Soitamo, A.J., Rabergh, C.M., Gassmann, M., Sistonen, L., Nikinmaa, M., 2001. Characterization of a hypoxia-inducible factor (HIF-1alpha) from rainbow trout. Accumulation of protein occurs at normal venous oxygen tension. J. Biol. Chem. 276, 19699–19705.
Sollid, J., Rissanen, E., Tranberg, H.K., Thorstensen, T., Vuori, K.A., Nikinmaa, M., Nilsson, G.E., 2006. HIF-1alpha and iNOS levels in crucian carp gills during hypoxia-induced transformation. J. Comp. Physiol. B 176, 359–369.
Soñanez-Organis, J.G., Peregrino-Uriarte, A.B., Gómez-Jiménez, S., López-Zavala, A., Forman, H.J., Yepiz-Plascencia, G., 2009. Molecular characterization of hypoxia inducible factor-1 (HIF-1) from the white shrimp *Litopenaeus vannamei* and tissue-specific expression under hypoxia. Comp. Biochem. Physiol. C 150, 395–405.
Song, S.B., Xu, Y., Zhou, B.S., 2006. Effects of hexachlorobenzene on antioxidant status of liver and brain of common carp (*Cyprinus carpio*). Chemosphere 65, 699–706.
Sridevi, B., Reddy, K.V., Reddy, S.L., 1998. Effect of trivalent and hexavalent chromium on antioxidant enzyme activities and lipid peroxidation in a freshwater field crab, *Barytelphusa guerini*. Bull. Environ. Contam. Toxicol. 61, 384–390.
Stadtman, E.R., Levine, R.L., 2003. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids 25, 207–218.
Stephensen, E., Sturve, J., Förlin, L., 2002. Effects of redox cycling compounds on glutathione content and activity of glutathione-related enzymes in rainbow trout liver. Comp. Biochem. Physiol. C 133, 435–442.
Sturve, J., Hasselberg, L., Fälth, H., Celander, M., Förlin, L., 2006. Effects of North Sea oil and alkylphenols on biomarker responses in juvenile Atlantic cod (*Gadus morhua*). Aquat. Toxicol. 78, 73–78.
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. Ecotoxicol. Environ. Saf. 71, 446–453.
Suzuki, T., Takagi, Y., Osanai, H., Li, L., Takeuchi, M., Katoh, Y., Kobayashi, M., Yamamoto, M., 2005. Pi class glutathione S-transferase genes are regulated by Nrf 2 through an evolutionarily conserved regulatory element in zebrafish. Biochem. J. 388, 65–73.
Takagi, Y., Kobayashi, M., Li, L., Suzuki, T., Nishikawa, K., Yamamoto, M., 2004. MafT, a new member of the small Maf protein family in zebrafish. Biochem. Biophys. Res. Commun. 320, 62–69.
Teixeira, M.C., Telo, J.P., Duarte, N.F., Sá-Correia, I., 2004. The herbicide 2,4-dichlorophenoxyacetic acid induces the generation of free-radicals and associated oxidative stress responses in yeast. Biochem. Biophys. Res. Commun. 324, 1101–1107.
Thomaz, J.M., Martins, N.D., Monteiro, D.A., Rantin, F.T., Kalinin, A.L., 2009. Cardio-respiratory function and oxidative stress biomarkers in Nile tilapia exposed to the organophosphate insecticide trichlorfon (NEGUVON). Ecotoxicol. Environ. Saf. 72, 1413–1424.
Timofeyev, M.A., Shatilina, Z.M., Kolesnichenko, A.V., Kolesnichenko, V.V., Steinberg, C.E., 2006. Specific antioxidant reactions to oxidative stress promoted by natural organic matter in two amphipod species from Lake Baikal. Environ. Toxicol. 21, 104–110.
Toroser, D., Orr, W.C., Sohal, R.S., 2007. Carbonylation of mitochondrial proteins in *Drosophila melanogaster* during aging. Biochem. Biophys. Res. Commun. 363, 418–424.
Toyoizumi, T., Deguchi, Y., Masuda, S., Kinae, N., 2008. Genotoxicity and estrogenic activity of 3,3′-dinitrobisphenol A in goldfish. Biosci. Biotechnol. Biochem. 72, 2118–2123.
Uguz, C., Iscan, M., Erguven, A., Isgor, B., Togan, I., 2003. The bioaccumulation of nonyphenol and its adverse effect on the liver of rainbow trout (*Onchorynchus mykiss*). Environ. Res. 92, 262–270.
Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Saf. 64, 178–189.
Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 39, 44–84.
Valko, M., Morris, H., Cronin, M.T., 2005. Metals, toxicity and oxidative stress. Curr. Med. Chem. 12, 1161–1208.
Valverde, M., Rojas, E., 2009. Environmental and occupational biomonitoring using the Comet assay. Mutat. Res. 681, 93–109.
Vasylkiv, O.Yu., Kubrak, O.I., Storey, K.B., Lushchak, V.I., 2010. Cytotoxicity of chromium ions may be connected with induction of oxidative stress. Chemosphere 80, 1044–1049.
Veal, E.A., Day, A.M., Morgan, B.A., 2007. Hydrogen peroxide sensing and signaling. Mol. Cell 26, 1–14.
Verlecar, X.N., Jena, K.B., Chainy, G.B., 2007. Biochemical markers of oxidative stress in *Perna viridis* exposed to mercury and temperature. Chem. -Biol. Interact. 167, 219–226.
Viarengo, A., Burlando, B., Cavaletto, M., Marchi, B., Ponzano, E., Blasco, J., 1999. Role of metallothionein against oxidative stress in the mussel *Mytilus galloprovincialis*. Am. J. Physiol. 277, 1612–1619.
Viarengo, A., Canesi, L., Garcia Martinez, P., Peters, L.D., Livingstone, D.R., 1995. Pro-oxidant processes and antioxidant defence systems in the tissues of the Antarctic scallop (*Adamussium colbecki*) compared with the Mediterranean scallop (*Pecten jacobaeus*). Comp. Biochem. Physiol. B 111, 119–126.
Vidal, M.L., Bassères, A., Narbonne, J.F., 2002. Influence of temperature, pH, oxygenation, water-type and substrate on biomarker responses in the freshwater clam *Corbicula fluminea* (Müller). Comp. Biochem. Physiol. C 132, 93–104.
Walker, R.L., Fromm, P.O., 1976. Metabolism of iron by normal and iron deficient rainbow trout. Comp. Biochem. Physiol. A 55, 311–318.
Wang, G.L., Semenza, G.L., 1995. Purification and characterization of hypoxia-inducible factor 1. J. Biol. Chem. 270, 1230–1237.
Wang, X., Li, C., Chen, Y., Hao, Y., Zhou, W., Chen, C., Yu, Z., 2008. Hypoxia enhances CXCR4 expression favoring microglia migration via HIF-1alpha activation. Biochem. Biophys. Res. Commun. 371, 283–288.
Wang, Y., Chen, J., Lin, J., Wang, Z., Bian, H., Cai, X., Hao, C., 2009. Combined experimental and theoretical study on photoinduced toxicity of an anthraquinone dye intermediate to *Daphnia magna*. Environ. Toxicol. Chem. 28, 846–852.
Wang, Y.C., Chaung, R.H., Tung, L.C., 2004. Comparison of the cytotoxicity induced by different exposure to sodium arsenite in two fish cell lines. Aquat. Toxicol. 69, 67–79.
Widmer, R., Ziaja, I., Grune, T., 2006. Protein oxidation and degradation during aging: role in skin aging and neurodegeneration. Free Radic. Res. 40, 1259–1268.
Wilhelm Filho, D., 1996. Fish antioxidant defenses—a comparative approach. Braz. J. Med. Biol. Res. 29, 1735–1742.
Winston, G.W., 1991. Oxidants and antioxidants in aquatic animals. Comp. Biochem. Physiol. C 100, 173–176.
Winston, G.W., Di Giulio, R.T., 1991. Prooxidant and antioxidant mechanisms in aquatic organisms. Aquat. Toxicol. 19, 137–161.
Winterbourn, C.C., Hampton, M.B., 2008. Thiol chemistry and specificity in redox signaling. Free Radic. Biol. Med. 45, 549–561.
Wright, J., George, S., Martinez-Lara, E., Carpene, E., Kindt, M., 2000. Levels of cellular glutathione and metallothionein affect the toxicity of oxidative stressors in an established carp cell line. Mar. Environ. Res. 50, 503–508.
Wu, J., Weiss, B., 1991. Two divergently transcribed genes, soxR and soxS, control a superoxide response regulon of *Escherichia coli*. J. Bacteriol. 173, 2864–2871.