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OXIDATIVE STRESS IN FISH AS A METHOD FOR DETECTION OF ECOSYSTEM DISTURBANCES

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Abstract
Oxidative stress is an early indicator of deterioration in the health of aquatic organisms when exposed to harmful environmental factors. In fish, oxidative stress results from a delicate balance between the production of reactive oxygen species and the capacity of antioxidant defence systems. Developing a scientific review of the biochemical mechanisms involved in oxidative stress in fish is essential for consolidating and integrating current knowledge in the field of aquatic physiology. Although the number of studies on oxidative stress has increased significantly in recent decades, the information remains scattered across various species, experimental conditions, and ecological contexts. A thorough review enables the organisation of this data, facilitating the identification of general trends, knowledge gaps, and future research directions. Fish are a particularly valuable biological model for studying oxidative stress due to their direct exposure to contaminants and environmental variations. Understanding the biochemical reactions associated with reactive oxygen species (ROS) production, antioxidant defence mechanisms, and oxidative stress markers is fundamental for assessing the health of aquatic organisms and interpreting pollution impacts on ecosystems [1]. Conducting a thematic review not only ensures a comprehensive synthesis of existing data but also provides a critical analysis of the methodologies employed, the relevance of proposed biomarkers, and their integration into modern ecotoxicological monitoring programmes. Consequently, this work holds dual value: scientific, by consolidating the theoretical basis of oxidative stress in fish, and practical, by supporting efforts to conserve biodiversity and manage aquatic resources sustainably.

Keywords: ***oxidative stress in fish, biomarkers, aquatic toxicology, antioxidant defense mechanisms***

Introduction

Oxidative stress represents an imbalance between the production of reactive oxygen species, hereinafter referred to as ROS, and the ability of antioxidant systems to neutralize them. In fish, this phenomenon has major implications for cellular homeostasis, physiological performance, and survival, especially under environmental stressors such as hypoxia, pollution, thermal variations, salinity, and exposure to heavy metals or pesticides [1]. Due to their ecological and economic importance, fish are essential model organisms for the study of oxidative stress in aquatic environments. Under stress conditions, decreased electron transport efficiency causes electrons to leak to oxygen, forming the superoxide radical. Excess ROS can cause protein oxidation, lipid peroxidation, and damage to mitochondrial and nuclear DNA. Studying the biochemical mechanisms underlying oxidative stress in fish is a major area of interest in the context of ecotoxicity and aquatic physiology. Fish, as ectothermic organisms, are constantly subjected to the variable action of environmental stressors, such as organic and inorganic contaminants, dissolved oxygen fluctuations, ultraviolet radiation and temperature changes.

These disturbances can generate an imbalance between the production of reactive oxygen species (ROS) and the ability of antioxidant systems to neutralize them, thus leading to the onset of oxidative stress. A detailed understanding of the biochemical mechanisms underlying oxidative stress is essential for elucidating the processes of fish adaptation and resistance to stressors [2,3].

In fish, ROS are predominantly generated in mitochondria, especially at complexes I and III of the respiratory chain [4]. Analysis of the activity of antioxidant enzymes (such as superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidant compounds (vitamin E, glutathione, ascorbic acid) provides valuable information on the physiological state of the body and its ability to maintain redox homeostasis [3,5]. The development of this synthesis aims to present, in an integrative manner, the main biochemical mechanisms involved in the generation and control of oxidative stress in fish, while highlighting the importance of redox biomarkers in assessing the impact of pollution on aquatic ecosystems [6]. By addressing this topic, we aim to contribute to a better understanding of the role of oxidative stress as an indicator of fish health and as a tool in modern ecotoxicology studies.

Materials and methods

This paper represents a review-type synthesis based on the analysis and integration of data available in the specialized scientific literature regarding the biochemical mechanisms of oxidative stress in fish. The information used was collected from academic sources published between 2022 and 2025, available in the main international databases, such as ScienceDirect, PubMed, Scopus and SpringerLink. The selection of articles was based on relevant keywords, such as "oxidative stress in fish", "antioxidant defense mechanisms", "reactive oxygen species (ROS)", "aquatic toxicology" and "biomarkers of oxidative stress". Original papers, review articles and experimental studies presenting data on oxidative processes, antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and non-

enzymatic antioxidant compounds involved in the physiological response of fish were included. The inclusion criteria focused on thematic relevance, methodological rigor, and the impact of the journals in which the articles were published. Papers presenting incomplete data, lack of information on experimental conditions, or statistically unvalidated results were excluded. Data analysis was carried out through a comparative and integrative approach, aiming to highlight the main biochemical mechanisms involved, interspecific variations and ecotoxicological implications. The methodological goal of the review was to synthesize current knowledge in a coherent form, which would facilitate the understanding of the relationship between oxidative stress, antioxidant response and fish health in aquatic ecosystems.

Results and discussion

The article is structured to provide an integrated analysis of the biochemical processes involved in oxidative stress in fish, the environmental factors that induce it, and practical management strategies in aquaculture.

3.1. Biochemical mechanisms of oxidative stress in fish

To counteract the harmful effects of ROS, fish have a complex antioxidant system, composed of enzymatic and non-enzymatic components [3]. The enzymatic antioxidant components are represented by :

• Superoxide dismutase (SOD) which catalyzes the conversion of superoxide to hydrogen peroxide. There are mitochondrial (Mn-SOD) and cytosolic (Cu/Zn-SOD) isoforms.

• Catalase (CAT) which is located in peroxisomes and breaks down hydrogen peroxide into water and oxygen.

• Glutathione peroxidase (GPx), which reduces lipid peroxides using reduced glutathione (GSH) as an electron donor.

• Glutathione reductase (GR), which regenerates GSH from its oxidized form (GSSG), thereby maintaining the intracellular redox balance..

Non-enzymatic antioxidant components include molecules that directly scavenge free radicals, such as [6]:

• Glutathione (GSH), a tripeptide with an essential function in detoxification and maintaining redox potential.

• Vitamin E (α-tocopherol) which inhibits membrane lipid peroxidation.

• Vitamin C (ascorbic acid) which causes the regeneration of oxidized vitamin E.

• Carotenoids and flavonoids, derived from food, have a complementary antioxidant role.

Oxidative stress can be assessed by a series of biochemical markers that reflect the level of oxidative damage to lipids, proteins, and DNA. These markers are [6,7]:

• Malondialdehyde (MDA) and the TBARS test. MDA is a byproduct of lipid peroxidation and is determined by the TBARS (Thiobarbituric Acid Reactive Substances) test. Elevated MDA levels indicate damage to cell membranes and increased oxidative stress.

• GSH/GSSG ratio. The ratio of reduced (GSH) to oxidized (GSSG) glutathione is a sensitive indicator of intracellular redox status. A low ratio signals depletion of antioxidant defenses.

• Total antioxidant capacity (TAC). TAC provides an integrated picture of the tissue's overall antioxidant potential. It is determined by spectrophotometric methods (e.g. ABTS, FRAP) and allows comparison between species or experimental conditions.

• Protein carbonylation (figure 1). Determines the degree of protein oxidation as a result of free radical attack. It is a stable and easily quantifiable marker of chronic oxidative stress.

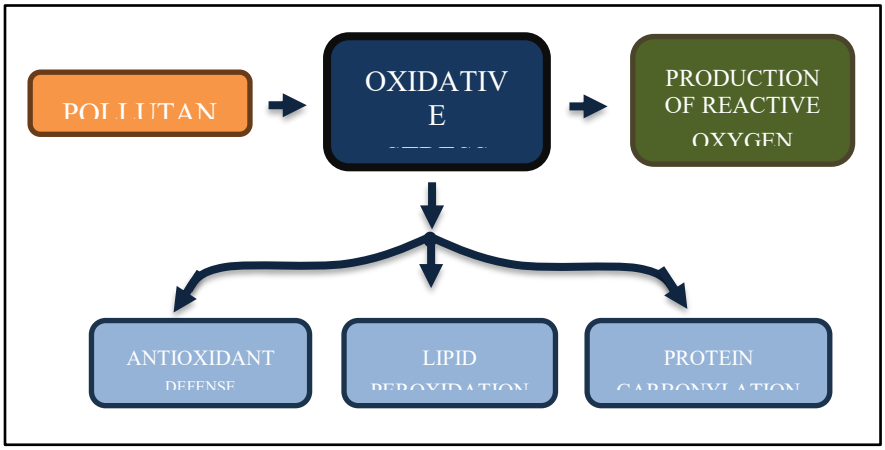

Fig. 1. Oxidative stress induced by aquatic pollutants in fish

Antioxidant enzyme activity and biochemical markers are mainly determined in the liver, gills, kidneys, muscles or brain [6,7]. The samples are homogenized in cold buffer media, and the activity is expressed in specific units (U/mg protein). Determinations are usually performed spectrophotometrically at 25°C, using standardized substrates for each enzyme. For MDA, the absorbance is measured at 532 nm. For interpretation, increased antioxidant enzyme activity suggests an adaptive response to oxidative stress, while their decrease associated with high MDA levels indicates cellular damage [7].

In biomonitoring, the combination of SOD–CAT–GPx–MDA provides a complete picture of the oxidative status. These parameters are used to assess water quality, exposure to contaminants, and the effectiveness of antioxidant treatments in aquaculture. Physiological and ecotoxic implications are:

• damage to cell membranes through lipid peroxidation (indicator: increased levels of malondialdehyde — MDA);

•damage to muscle proteins and metabolic enzymes, reducing energy performance;

•liver and kidney lesions, observed through histopathological changes;

•disruption of reproductive function and embryonic development;

• altered immune response and increased susceptibility to infections.

3.2. The main aquatic pollutants and their mode of action

In Table 1 we have conducted an analysis of the main pollutants involved in the induction of oxidative stress: heavy metals (such as cadmium, lead and mercury), organophosphorus and organochlorine pesticides, microplastics and pharmaceutical residues. Each category is presented in relation to the specific mode of action on oxidative metabolism and the cumulative effects on fish health.

Thus, each category of pollution acts through specific mechanisms (enzymatic inhibition, direct generation of radicals, chemical interactions) that lead to disruption of the redox balance and the triggering of oxidative stress. Comparative table of aquatic pollutants and their mechanisms of inducing oxidative stress in fish.

Table 1. Comparative table of aquatic pollutants and their mechanisms of inducing oxidative stress in fish

Pollutant category	Specific examples	Main biochemical mechanisms	Effects on antioxidant systems	Frequently used biomarkers	Affected organs/ tissues	Physiological/ ecotoxic consequences
Heavy metals – Cadmium (Cd)	$CdCl_2$, $CdSO_4$	Induces ROS indirectly by inhibiting antioxidant enzymes. binds the –SH groups of GSH and proteins; disrupts oxidative phosphorylation	↓GSH, ↓CAT/GPx, ↑SOD (compensator, ↑MDA metallothionein induction	SOD, CAT, GPx, GSH/GSSG, MDA, MT	Liver, kidneys, gills	Metabolic dysfunctio, liver damage, systemic oxidative stress
Heavy metals – Copper (Cu)	$CuSO_4$, $CuCl_2$	Participates in Fenton-like reactions (Cu^{2+}/Cu^{+}) → formation of •OH; activates NADPH oxidase; disrupts mitochondria	SOD/CAT disorders, MDA increase, GSH impairment	SOD, CAT, GPx, MDA, GSH	Liver, gills, brain	Oxidative stress, cell apoptosis, neuro-oxidative dysfunction [7]
Heavy metals – Mercury (Hg, MeHg)	Methylmercury, $HgCl_2$	Binds to –SH groups; reduces GSH; affects Ca^{2+} homeostasis; alters mitochondrial function	↓GSH, ↓GPx, ↓CAT, ↑MDA; inducție MT	GSH, GPx, MDA, MT	Brain, liver, kidneys	Neurotoxicity, redox and histopathological disorders
Microplastics (MP/NP)	Polyethylene, Polystyrene, PVC	Physical damage, local inflammation, metal/pesticide adsorption, additive release (BPA, phthalates), mitochondrial dysfunction [8,9]	↓GSH, ↓CAT, ↓GPx, ↑MDA, ↑ROS	SOD, CAT, GPx, MDA, GSH, ROS	Liver, intestine, gills	Chronic inflammation, systemic oxidative stress, endocrine disorders
Pesticides – Organophosphates (OP)	Malathion, Chlorpyrifos	Inhibits AChE; mitochondrial dysfunction → increased ROS; NF-κB activation and inflammation	↓CAT, ↓GPx, ↑SOD (compensator), ↑MDA	AChE, SOD, CAT, GPx, MDA	Brain, liver, gills	Neurotoxicity, lipid peroxidation, neuronal apoptosis [10,11]
Pharmaceutical residues	Diclofenac, Paracetamol, Ciproflox acin, Fluoxetine	Reactive metabolites; inhibit GPx and CAT; affect mitochondrial respiration; generate ROS through hepatic metabolism [12]	↓GSH, ↓GPx/CAT, ↑MDA, ↑8-OHdG	SOD, CAT, GPx, GSH/GSSG, 8-OHdG	Liver, brain, kidneys	Hepatic oxidative stress, genotoxicity, alteration of redox homeostasis
Mixtures (MP + metals/pesticides)	MP + Cd, MP + OP	Synergistic effects; vectorization and bioaccumulation; amplifies ROS and lipid peroxidation	↓GSH, ↓CAT, ↑MDA, ↑protein carbonyls	SOD, CAT, GPx, MDA, carbonyls	Liver, gills, intestine	Amplification of oxidative stress, cumulative ecotoxic effects

3.3. Evaluation of the antioxidant response in different fish species used in aquaculture

The antioxidant response in fish has been intensively studied internationally in the context of exposure to pollutants (heavy metals, pesticides, microplastics, PFAS), abiotic stress (temperature, hypoxia) or nutritional interventions (antioxidant supplements). The most commonly used biomarkers include the enzymes SOD, CAT, GPx, GR; redox reserves (GSH/GSSG); lipid peroxidation products (MDA/TBARS) and integrated measures of total antioxidant capacity (TAC) [13]. Most studies show that the effect is strongly dependent on species, tissue, dose and duration: the liver generally displays the strongest response, followed by the intestines, kidneys, brain and gills, but there are numerous exceptions related to ecology and specific adaptations. Commonly used models are zebrafish (*Danio rerio*) for mechanistics/toxicology, tilapia (*Oreochromis spp.*), trout (*Oncorhynchus mykiss*), sea bream/seabass (*Sparus aurata / Dicentrarchus labrax*) and polar or extremophile species for special adaptations.

Factors that induce changes in the antioxidant response are exposures to pollutants (metals, pesticides, microplastics, PFAS), temperature/oxygen variations, hypoxia, diet/nutraceutical adjuvants and nanoparticles. The response is strongly dependent on dose, exposure time and tissue analyzed (figure 2).

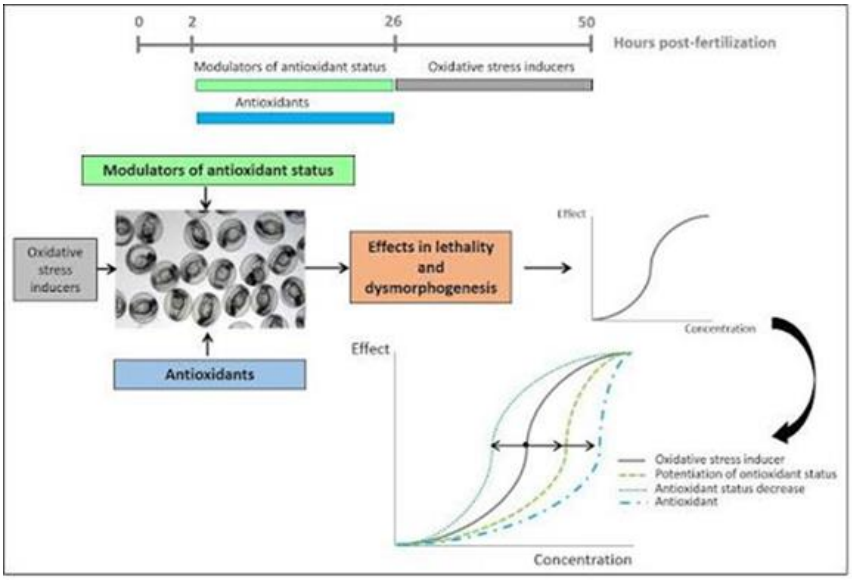

Fig. 2. The fish response to aquatic pollutants

The most studied species to date are [10,11]:

• *Danio rerio* (zebrafish): reference model for mechanistics, embryo/larval testing and compound screening; advantage — genetic homology and reproducibility.

•*Oreochromis spp.* (tilapia): applied ecotoxicology and aquaculture studies; frequently used in studies on microplastics and pesticides.

•*Oncorhynchus mykiss* (rainbow trout), *Salmo spp*.: sensitive to cold water contaminants; used in metal studies.

• Commercial marine species (*Sparus aurata, Dicentrarchus labrax*): studies related to coastal pollutants and nutritional interventions in culture.

• Polar/extremophile species: exhibit special antioxidant adaptations; useful for comparative studies and understanding the plasticity of the antioxidant response.

Antioxidant capacity differs among fish species, depending on habitat, diet, and evolutionary adaptations. For example, zebrafish (*Danio rerio*) is the primary model for oxidative toxicology studies due to its well-known genetics and sensitivity to pollutants [14]. Fish from polluted areas, such as *Oreochromis niloticus* (tilapia) or *Oncorhynchus mykiss* (rainbow trout), develop antioxidant responses induced by chronic exposure to metals or pesticides. In contrast, polar species (e.g. *Trematomus spp.*) exhibit adaptive antioxidant mechanisms for low temperatures and reduced oxidative stress.

Conclusions

Oxidative stress is a complex process embedded in fish physiology and influenced by numerous external factors. Understanding the biochemical mechanisms involved, the role of antioxidant enzymes, and related gene regulation enables the development of effective strategies to protect aquatic biodiversity and enhance fish health in aquaculture. It is crucial to standardise methodologies and expand comparative studies across different species. Enzymatic biomarkers (SOD, CAT, GPx) and MDA are the most commonly used and can indicate both activation and depletion of antioxidant systems. Zebrafish remains the primary model for mechanistic research and screening of antioxidant or toxic compounds due to its homology with higher vertebrates and reproducibility. The differences between species and tissues (usually liver > kidney > brain > gills) are consistent: the liver typically exhibits the strongest response to pollutants. Exposure to metals and organic compounds results in significant increases in ROS and alterations in antioxidant enzyme activity, though responses may vary between induction or inhibition depending on dose and exposure time. Dietary interventions (for example, natural antioxidants like curcumin and polyphenols) have been shown in recent studies to restore or enhance antioxidant capacity in fish. Recent research indicates that chemical residues (such as pharmaceuticals, pesticides, heavy metals, etc.) induce oxidative stress in fish, which can serve as a signal of ecosystem disruption. In light of increasing pollution and the variable physicochemical conditions of aquatic environments, monitoring oxidative stress in fish offers a valuable method for detecting ecosystem disturbances.

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