

# Cellular Oxidant/Antioxidant Network: Update on the Environmental Effects Over Marine Organisms

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**Abstract:** Aquatic organisms are exposed and adjust to a wide variety of environmental challenges including natural and anthropogenic factors. Natural sources are understood the effects of temperature, and saline fluctuations, oxygen availability, the relative abundance of chemical elements and pathogenic invasion. On the contrary, anthropogenic factors are considered the availability of heavy metals, the presence of hydrocarbons, industrial and urban wastes, and pesticides. Moreover, these organisms suffer, in order to maintain homeostasis, growth and reproduction, the effect of temporal and spatial variations. All the environmental changes (natural and non-natural) may cause a different degree of stress in aquatic organisms, via induction of disbalance between the generation and elimination of reactive oxygen species and reactive nitrosative species. A brief summary on the actual knowledge on the establishment, by environmental effects, of oxidative/nitrosative stress and the effect on the antioxidant system in marine organisms, is presented in this review to contribute to the deeper understanding of the complexity of the metabolic and physiological changes that aquatic organisms are constantly suffering.

**Keywords:** Anthropogenic environmental changes, natural environmental changes, marine organisms, nitrosative stress, oxidative stress, reactive oxygen species.

## 1. INTRODUCTION

Aquatic organisms are exposed and adjust to a wide variety of environmental challenges including natural and anthropogenic factors (Fig. 1). Water bodies and oceans receive increased number of pollutants that could be uptaken by different species from water, sediments, suspended particulate matter, and food sources [1]. Water pollution type and level can be accurately identified by analyzing the species, amounts, physiological and biological responses and residue contents. All the environmental changes (natural and non-natural) may cause a different degree of stress in aquatic organisms, via induction of disbalance between the generation and elimination of reactive oxygen species (ROS) and reactive nitrosative species (RNS).

Among the feasible organisms actually chosen for pollution monitoring, aquatic algae, molluscs and fishes are the mostly used. The uptake of xenobiotic by phytoplankton is the first step in the bioaccumulation in aquatic food webs. The aquatic algae, important elementary producers in marine and inland water, play a key role to the whole ecosystem, since the type of species and their amount can even reflect the water quality. Molluscs, like bivalves and limpets, are considered key organisms for environmental monitoring based on their wide geographical distributing, abundance, type of movement (sedentary or mobile), tolerance to

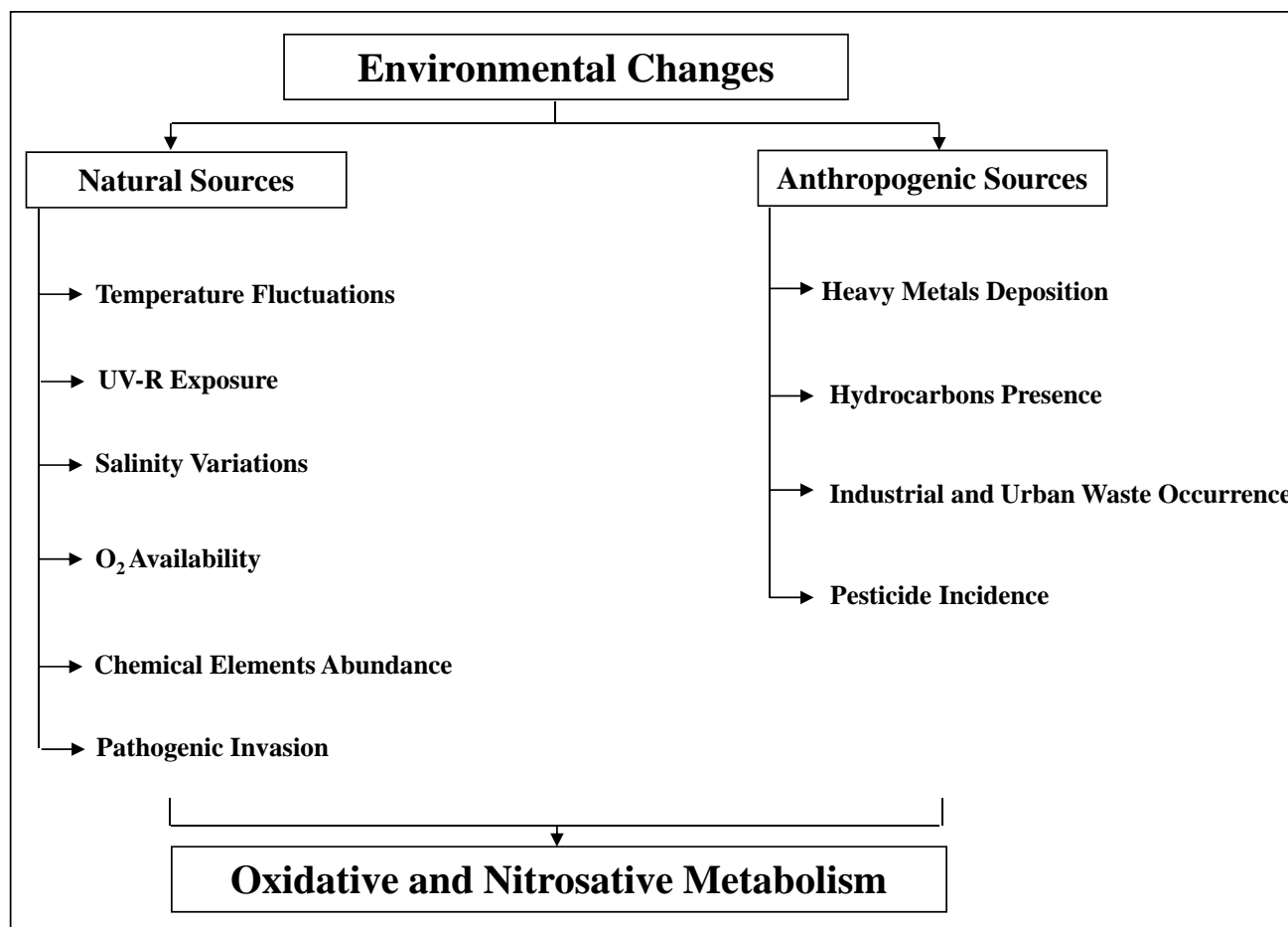
environmental (natural and contaminants) alterations, their ability to concentrate pollutants, the level of metabolizing enzyme activities of organic contaminants, the nature of the populations, the life span and size, and the potential to survive in laboratory and field studies in cages [2]. Fishes are generally used for pollution monitoring because not only can be found virtually everywhere in the aquatic environment but also play a major ecological role in aquatic food webs because of their function as carriers of energy from lower to higher trophic levels [3]. Among the wide array of functions selected to assess the effect of pollution in these organisms, oxidative status is one of the most studied.

A brief summary on the actual knowledge (see Pinto *et al.* [4] and Lushchak [1] for previous reviews on the subject) on the establishment in marine organisms of oxidative/nitrosative stress and antioxidant capacity associated to environmental effects, is presented in this review to contribute to a deeper understanding of the complexity of the metabolic and physiological changes that aquatic organisms are constantly suffering.

## 2. GENERAL FEATURES OF OXIDATIVE/NITROSATIVE METABOLISM AND ANTIOXIDANT CAPACITY IN MARINE ORGANISMS

Basically, pro- and antioxidant processes are the same across marine invertebrates and mammalian systems. However, the specific conditions for radical formation, a key feature in stress physiology and aging, are different and highly variable among aquatic cold blooded organisms [5]. ROS include superoxide anion ( $O_2^-$ ), hydroxyl radical ( $\cdot OH$ ),

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**Fig. (1).** General diagram showing the environmental changes, both natural and anthropogenic, influencing the settlement of oxidative and nitrosative stress condition in marine organisms.

hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), singlet oxygen ( $^1\text{O}_2$ ), and peroxy radicals. ROS are highly reactive oxidants and mainly regarded as hazardous species whose production in cellular and extracellular systems has to be tightly controlled by antioxidants and radical scavenging biochemical reactions. However, recently it has been recognized the importance of radical species in cellular signaling and in the maintenance of homeostatic conditions. Moreover, some radicals, such as the very short-lived and extremely hazardous  $\cdot\text{OH}$ , are still regarded as highly reactive and dangerous, but many other more stable species have been postulated as signaling molecules in cellular growth, or as oxidants that assure an appropriate oxidation state of cellular compartments and the biochemical structures and elements they contain [6]. According to the present view, a basal amount of ROS is formed at all times in all aerobic cells, and the steady state concentration of ROS in each cell (or compartment) depends on the formation rate of the radical (being mitochondria one of the most important intracellular radical sources), its reactivity and the concentration of available reaction partners. On the other hand, besides the bound Fe, there is a fraction of the total cellular Fe content called the labile Fe pool (LIP). The LIP is experimentally defined as a low-molecular-weight pool of weakly chelated Fe that is involved

in a dynamic flux of solutes passing rapidly through the cell. The LIP is assumed to consist of both forms of ionic Fe ( $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ ) bound to citrate and other organic ions, phosphates (ATP, AMP), carbohydrates and carboxylates, nucleotides and nucleosides, polypeptides, and phospholipids [7]. Besides the importance of this readily available Fe for cellular growth and metabolism, the LIP (also defined as the source for catalytically active available Fe in the cells) is held responsible for the generation of extremely reactive species, such as  $\cdot\text{OH}$  [8].

Nitric oxide (NO) is recognized both, as a signaling molecule that regulates many enzyme activities, but as a toxic agent as well. It has been found that NO is able to protect all cell types from oxidative damage resulting from  $\text{O}_2^-$ ,  $\text{H}_2\text{O}_2$  and alkyl peroxides by acting as a terminator of free radical chain reactions [9-12]. ROS and RNS interact through the reaction of  $\text{O}_2^-$  with NO, to generate peroxynitrite ( $\text{ONOO}^-$ ) at a velocity close to the diffusion rate.  $\text{ONOO}^-$  acts as both, a nitrating agent and a powerful oxidant capable of modifying proteins (formation of nitrotyrosine), lipids (lipid oxidation, lipid nitration) and nucleic acids (DNA oxidation and DNA nitration) [13]. Besides being a regularly occurring radical intermediate of

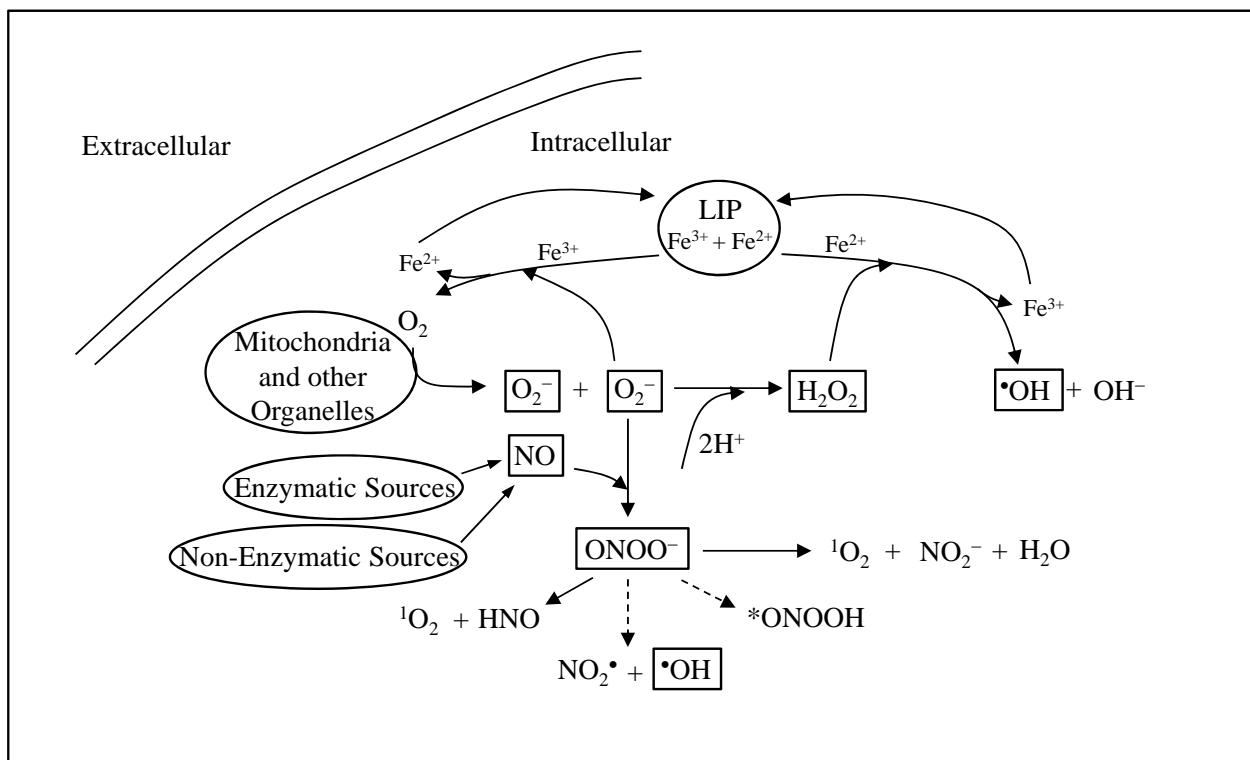


Fig. (2). Interaction between oxidative and nitrosative cellular networks.

many reactions, NO is also a molecule capable of binding Fe [14] and a highly diffusible second messenger that can elicit effects relatively far from its site of production. The concentrations, and the source of NO, are the major factors determining its biological effects [15]. NO is mainly generated in mammalian cells and tissues from L-arginine by the activity of the enzyme nitric oxide synthase (NOS, EC 1.14.13.39) [16]. NOS-like enzymatic activity has been detected in marine, freshwater and terrestrial molluscs, including three gastropod subclasses (rosobranchs, opisthobranchs and pulmonates) [17], and in the bivalve *Mya arenaria* (Linnaeus) [18]. Fig. (2) summarizes the interaction among these chemical species in a cell.

To be able to maintain dangerous reactive species at low steady state concentrations the presence of many components are required to act as an adequate antioxidant defense system. The mechanisms for the action of these antioxidants involve three levels: (1) deactivation of reactive species. These antioxidants are either enzymatic (e.g. catalase, CAT; superoxide dismutase, SOD; glutathione peroxidase, GPx; glutathione reductase, GR) or non-enzymatic compounds, such as hydrophilic (e.g. reduced glutathione, GSH and ascorbic acid, AH<sup>-</sup>) and lipophilic (e.g.  $\alpha$ -tocopherol,  $\alpha$ -T) compounds; (2) prevention of radical formation [19]. At this level, proteins such as ferritin (Ft) (chelating Fe) or ceruloplasmine (chelating Cu) are considered important examples; (3) repair of already performed damage, such as the activity of DNA repairing enzymes [19]. Besides their individual actions, all these mechanisms interact among them in the intracellular environment (Fig. 3).

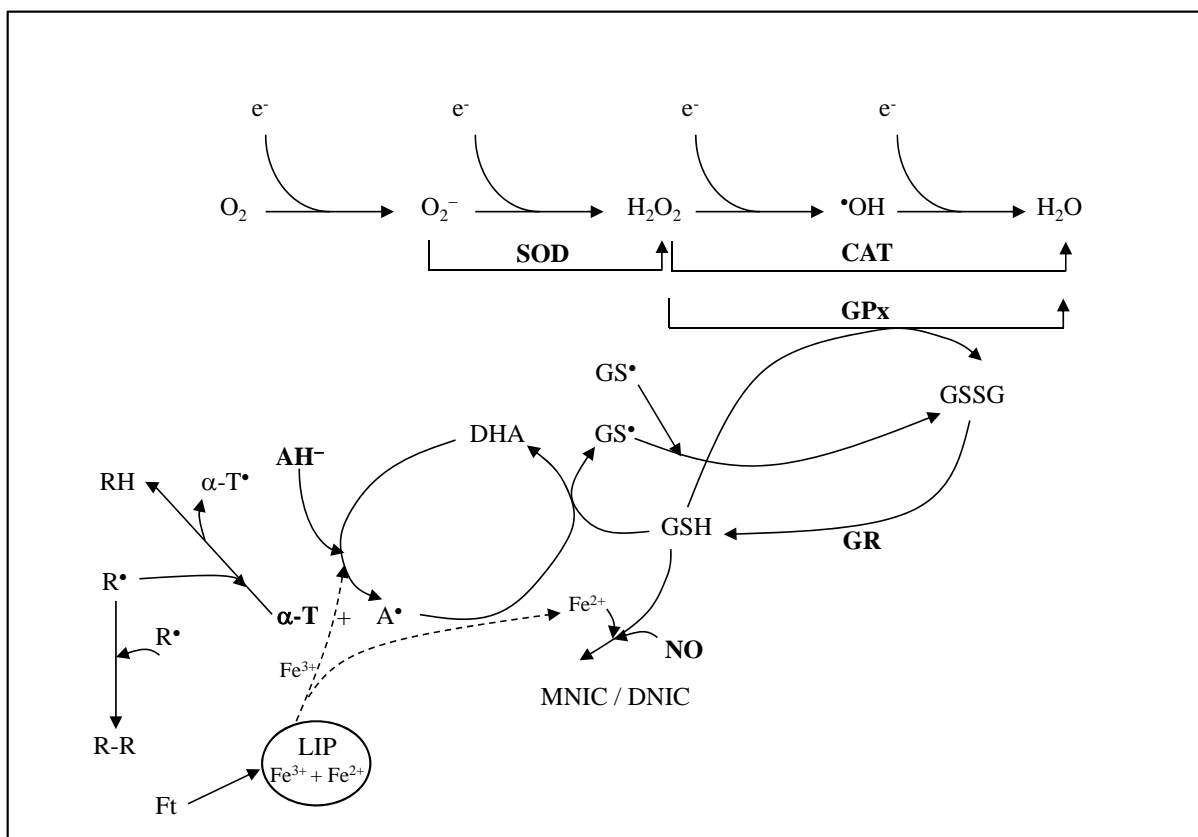
### 3. EFFECT OF ENVIRONMENTAL CHANGES ON OXIDATIVE/NITROSATIVE STATUS AND ANTIOXIDANT ABILITY OF MARINE ORGANISMS

#### 3.1. Natural Sources

Marine organisms withstand some of the harshest environmental conditions known, including freezing and heat stress, desiccation, salinity variations, carbon limitation, radiation in the intertidal regions. In addition, some areas suffer from O<sub>2</sub> availability and also high chemical elements abundance from natural sources. All these factors may act together adding potentially stress to the marine biota (e.g. UV-B or temperature and salinity fluctuations). The environmental extremes also contribute to the photo-inhibition of photosynthesis observed in ecologically important marine algae and to deleterious effects on the cellular metabolism [5].

##### 3.1.1. Temperature fluctuations

Temperature fluctuations are known to affect all living organisms. With increasing atmospheric CO<sub>2</sub> concentration, global temperature is expected to increase by the year 2100 in 2.5-6.4°C in the atmosphere [20], and by 2-3°C in the ocean surfaces [21]. Such changes will also have important effects on various organisms, since most physiological processes are temperature-dependent [22]. An increase of environmental temperature, which leads to metabolic activation, combined with an increase in O<sub>2</sub> consumption initiates oxidative stress [23]. Long-term adaptations to high



**Fig. (3).** General diagram of the cellular antioxidant network including enzymatic and non-enzymatic compounds. RH, membrane fatty acids;  $R^\cdot$ , lipid radicals;  $\alpha-T^\cdot$ , tocopheryl radical;  $A^\cdot$ , ascorbyl radical; DHA, dehydroascorbate;  $GS^\cdot$ , glutathione radicals. MNIC, mononitrosyl-Fe complexes; DNIC, dinitrosyl-Fe complexes.

temperatures result in crucial modifications of intermediary metabolism and cell membrane properties [24].

Ectotherms from permanently cold waters may be more susceptible to this stress and oxidative damage than organisms from other regions. This fact may be due to: i)  $O_2$  in cold waters and in corporal fluids presents more solubility than in temperate environments, and ii) invertebrates and polar fishes have lipidic membranes with a higher percentage of polyunsaturated fat acids that in the sustained life of free radicals at cold temperatures, may facilitate even more the propagation of lipid peroxidation reactions and extend the oxidative damage to tissues [25]. Elevated susceptibility of polar animals to oxidative stress would create a need to adjust antioxidant defense systems to function at low temperatures. A survey of antioxidant enzymes activities in polar and Mediterranean molluscs performed by Regoli *et al.* [26] supports this idea, since significantly higher SOD activities in gills of the Antarctic scallop *Adamussium colbecki* (Smith) were measured when compared to the Mediterranean bivalves *Mytilus galloprovincialis* (Lamarck) and *Pecten jacobaeus* (Linnaeus). These authors also found higher activities for other antioxidant enzymes including CAT, GR, and GPx. However, this conclusion may not be valid for all tissues, since Regoli *et al.* [26] and Viarengo *et al.* [27] with the same polar scallop stock showed lower SOD activities in digestive gland of *A. colbecki* as compared

to the Mediterranean scallops. Moreover, some enzymatic systems including antioxidant enzymes, like SOD, display temperature optimum curves with a maximal activity within the habitat temperature range in temperate ectotherms [28]. Snow and ice melt waters were shown to be extremely stressful for animals such as limpets, which clamp their shells down to the rock in order to avoid fresh water exposure [29]. Diversified responses to temperature fluctuations are expected in view of organism diversities in physiological pathways and ecological niches [30]. An essential adaptation to cold temperature involves biochemical compensation to the physiological functioning of cellular membranes. This adaptation is expressed by the production of low molecular weight lipids and the elaboration of higher levels of unsaturated and branched-chain fatty acids in the composition of cellular membranes [31]. However, achieving membrane functional homeostasis by increasing levels of lipid unsaturation occurs at the expense of enhancing the vulnerability of cellular membranes to oxidative damage. Thus, biochemical adaptation of cellular membranes to function at low-temperature affects a corresponding need for enhanced lipid-phase antioxidant protection, and this is demonstrated by the need for significantly dietary uptake of  $\alpha-T$  by coldwater teleost [32]. The biochemical selection for 'marine-derived' tocopherol (MDT) synthesis in cold-water marine producers may thus

evolved to provide enhanced antioxidant protection for metabolic adaptation to low temperature [33]. The occurrence of MDT in Antarctic notothenioids [34] is consistent with the finding of MDT in sub-polar fishes [33].

### 3.1.2. UV Radiation (UV-R) Exposure

Solar UV-R penetrates to ecological significant depths in aquatic systems and can affect from major biomass producers to consumers. Many factors influence the depth of penetration of radiation into natural waters including dissolved organic compounds, whose concentration and chemical composition are likely to be influenced by climate and UV-R variability. Organisms living in clear, shallow water are exposed to the damaging wavelengths of solar UV-R coincident with the longer wavelengths of photosynthetically available radiation (PAR). Solar UV-B (280-315 nm) radiation at the Earth's surface has been shown to increase due to the ozone depletion and its interplay with climate change [35]. UV-A (315-400 nm) + UV-B is known to inhibit growth and photosynthesis [36-39] and to damage proteins and DNA [40-42]. However, moderate UV-R levels were shown to increase photosynthetic carbon fixation [43, 44], with UV-A even driving photosynthetic carbon fixation in the absence of PAR [45]. In marine systems, the absorption of solar radiation, and especially of its UV-R wavelengths, by dissolved organic matter in seawater leads to the photochemical production of diverse reactive transient products, including ROS [46].

Hernando *et al.* [47] showed that photoprotection against UV-induced damage is characterized by short-term consumption of  $\alpha$ -T and long-term synthesis of mycosporine-like amino acids (MAAs) in an Antarctic diatom *Thalassiosira* sp. The UV-B damage/repair ratio during long-term exposure involves the combined action of several endogenous factors within the cell, with MAAs synthesis being the most effective factor. Besides vertical migration and UV screening, copepods rely on photorepair of UV-B-induced DNA damage [48] as shown in species from Patagonia, Argentina [49]. Even more, Obermüller *et al.* [50] investigated tolerance to UV-R in three amphipod species from the Arctic Kongsfjord, Spitsbergen: the herbivore *Gammarellus homari* (Fabricius), the strictly carnivore scavenger *Anonyx nugax* (Phipps) and the detritivore/carnivore *Onisimus edwardsi* (Krøyer). In both carnivore amphipods elevated sensitivity to experimental UV-R exposure went along with a degradation of tissue carotenoids and MAAs, and a decrease of the enzymatic antioxidant defense (SOD, CAT), which resulted in increased lipid peroxidation in exposed animals. In contrast, the herbivore *G. homari* seems well protected by high concentrations of MAAs absorbed from its algal diet, and no oxidative stress occurred under experimental UV-R exposure. The species-specific degree of UV tolerance correlates well with the animals' typical vertical distribution in the water column. Moreover, exposure to UV-R causes apoptosis in developing sea urchin embryos. Embryos of three sea urchin species from different habitats ranging from the Gulf of Maine to the Antarctic showed significant amounts of accumulated DNA damage in the form of cyclobutane pyrimidine dimers [51].

### 3.1.3. Salinity Variations

In marine waters the most common reported value for salinity is around 35 ups, but it could also vary from 10 to 70 ups as a result of evaporation or precipitation/freshwater influxes [52]. Osmotic stress most often resulting from fluctuating salinities exerts considerable oxidative stress in organism living in the intertidal zone. In aquatic organisms, salinity change causes a variety of physiological responses such as plasma enhanced stress-related hormones, stimulation of energy metabolism, and alteration of electrolyte equilibrium, growth and photosynthesis inhibition in algae [53, 54]. The stress induced by salinity change has been associated with enhanced ROS generation, causing oxidative damage [55]. Rijstenbil [53] showed that when the diatom *Cylindrotheca closterium* (Ehrenberg) was exposed to combined UV-B and salt stress (as in immersion) inhibition of growth was measured. Moreover, under these conditions higher activities of SOD, ascorbate peroxidase (APx) and monodehydroascorbate reductase (MDHAR) were found as compared to non-exposed diatoms. Studies employing the macroalga *Ulva fasciata* (Delile) exposed to salinity stress showed that a long-term exposure to hypo-saline and hyper-saline conditions inhibited growth rate and enhanced the availability of antioxidants and the activities of antioxidant enzymes to cope with the oxidative stress generated [56]. Also, a study from Kumar *et al.* [57] suggested that *Gracilaria corticata* (J. Agardh) regulates its antioxidant machinery to eliminate ROS under long term salt stress conditions. The ark shell *Scapharca broughtonii* (Schrenck) and the olive flounder fish *Paralichthys olivaceus* (Temminck and Schlegel) exposed to changes in salinity showed that the antioxidant enzymes played important roles in reducing oxidative stress [54, 58]. However, in the shrimp *Litopenaeus vannamei* (Boone) the experimental findings demonstrated that changes in salinity might be toxic due to a reduction in the antioxidant enzymes activities and the establishment of oxidative stress. Vitamin E (mainly  $\alpha$ -T) dietary supplementation can be potentially useful to prevent metabolic damage under the tested conditions [55]. Hamer *et al.* [59] found a close inverse relationship between salinity and susceptibility of DNA to oxidative damage in both, summer and winter, presumably indicating an imbalance of pro-oxidant/antioxidant status in the mussel gills of *M. galloprovincialis* depending on the magnitude of the hypo-saline stress. Thus, changes exert on the environmental salinity generate alterations in the oxidative metabolism, including the antioxidant defense systems and the oxidative damage.

### 3.1.4. O<sub>2</sub> Availability

Marine invertebrates preserve a high surface to volume ratio and, in contrast to air breathing animals, diffusive O<sub>2</sub> uptake over the body surface is an important factor affecting survival. Many aquatic invertebrates are oxyconformers, i.e. O<sub>2</sub> consumption varies as a function of the environmental O<sub>2</sub> partial pressure [60, 61]. Since some of these species are highly sensitive to higher environmental O<sub>2</sub>, they colonize sedimentary, low O<sub>2</sub> environments [62, 63]. However, maintenance of life, in the absence of water, requires a

complex and finely tuned set of mechanisms working in close coordination [64]. Also, in marine invertebrates from typically low O<sub>2</sub> sedimentary habitats, several forms of physiological stress, include critical warming, and lead to functional tissue hypoxia, as ventilation and circulation fail to cover tissue O<sub>2</sub> demand [65]. Either directly during the hypoxic state [66, 67] or following a hypoxic episode [68-70], ROS are released from ubiquinone during tissue re-oxygenation. Thus, marine ectotherms, which undergo frequent episodes of environmental and physiological hypoxia, are likely to receive elevated levels of ROS formation during or, on recovery from physiological stress. During low tides, tissues like foot and gills are the main targets of the oxidative damage, and as a consequence they may be better protected by the antioxidant enzymes than other tissues. The increase in the antioxidant defenses during physiological states where ROS production might be reduced (anoxia/hypoxia, freezing, hibernation), could be a preparatory mechanism to minimize the potential damage due to the oxidative stress during re-oxygenation (or unfreezing and excitation) when O<sub>2</sub> consumption is increased.

Weihe *et al.* [71] showed that the response to aerial exposure and hypoxia in the oxidative stress parameters differed in intertidal limpet *Nacella concinna* (Strebel), whereas the antioxidant enzyme activities in sub-littoral specimens responded in the same way to both forms of stress. Intertidal animals respond more rigorously to air exposure than sub-littoral limpets, which maintained shell water O<sub>2</sub> pressure at low levels independently of submergence state. The most obvious difference with respect to the antioxidants occurs in the gills. Intertidal limpets reduce SOD activities, whereas in sub-littoral limpets gill SOD activity increases under both forms of stress. These data support the idea that the first strategy of intertidal limpets to control cellular damage is to down regulate metabolism and save energy, including the synthesis of antioxidants, during air exposure/hypoxia [72]. In the stone or false king crab *Paralomis granulosa* (Hombron and Jacquinot) air exposure alters its oxidant/antioxidant status and triggers antioxidant responses of its enzymes. The oxidative status of *P. granulosa* is also significantly affected by emersion and re-submersion periods. Almost all analyzed tissues showed an increase of antioxidant enzyme activities after air exposure and re-submersion with the highest enzymatic activities found in gills [73].

### 3.1.5. Natural Abundance of Chemical Elements

The toxic effect of heavy metals appears to be related to production of ROS and the resulting unbalanced cellular redox status [4]. Fe is a Fenton reactant which, if not tightly bound to Ft, forms <sup>•</sup>OH [74]. Al is the most abundant metal in the earth crust, and although not a transition metal and Fenton reactant, exacerbates Fe catalyzed lipid peroxidation in animal tissues [75]. Likewise, Zn is not in itself a ROS producing metal, but when highly concentrated it can damage the mitochondria and induce high rates of O<sub>2</sub><sup>-</sup> release [76]. The prooxidant effect of the non-essential

element Cd is mainly via depletion of GSH and protein-bound sulfhydryl groups, resulting in enhanced production of ROS [77]. The responses on the oxidative metabolism of the organisms to high chemical elements abundance primary depends on the type, concentration, exposure period and excretion efficiency of these elements.

King-George Island (Antarctic) volcanic rock contains between 5 and 7% Fe [78], Al, Cu and Zn; sediment ablation from glacier melting, eroding the rock surface underneath the glaciers, can lead to metal enrichment through transport of lithogenically derived sediment particles in coastal areas [79, 80]. González and Puntarulo [81] showed that Fe, constantly taken up by the bivalve *Laternula elliptica* (King), is gradually incorporated into the Ft. The bivalve seems to be successfully adapted to its high Fe natural environmental conditions, being the antioxidant activity crucial. Even more, formation of NO-Fe complexes (such as mononitrosyl-Fe complexes, MNIC and dinitrosyl-Fe complexes, DNIC) could be one of the mechanisms involved in limiting the damage dependent on Fe exposure. Chronically high Fe content effects in the Antarctic species *L. elliptica* could be adequately controlled by this endogenous mechanism. NO generated in *L. elliptica* was suggested to contribute to restrict oxidative damage by a close link with Fe metabolism. Studies in sub-littoral *N. concinna* limpets digestive glands showed high content of heavy metals (Fe, Al, Zn) get from ingested sediments, and this fact was associated with higher rates of ROS formation as compared to intertidal animals. Also, ROS formation was accompanied by significantly higher SOD activity in the sub-littoral group [71]. The differences in the stress response of both limpet sub-groups indicate fundamental differences in the metabolic strategies, which may perhaps have a genetic background [82].

Hydrothermal vents also produce ROS. The abundance of hydrogen sulfide (H<sub>2</sub>S) and O<sub>2</sub> near vents leads to the oxidation of H<sub>2</sub>S in seawater and the production of both O<sub>2</sub> and sulfur-centered radicals [83]. In particular, electron paramagnetic resonance (EPR) spin-trapping has shown that sulfide oxidation produces O<sub>2</sub><sup>-</sup> [83]. High concentrations of O<sub>2</sub><sup>-</sup> near vents probably lead to H<sub>2</sub>O<sub>2</sub> production by O<sub>2</sub><sup>-</sup> dismutation being responsible for subsequent oxidative stress for vent fauna.

Vent worms (*Riftia pachyptila* Jones), vent clams (*Calyptogena magnifica* Boss and Turner), and their bacterial symbionts all express SOD and exhibit peroxidase activity [84]. Bebianno *et al.* [85] reported an interesting study developed in the gills of the mussel *Bathymodiolus azoricus* (Cosel and Comtet) collected from three contrasting Mid-Atlantic Ridge (MAR) vent fields. These fields have three different zones: Menez-Gwen, Lucky Strike and Rainbow areas. MAR vent sites not only contain high metal concentrations, but are also characterized by a mixture of toxic compounds (metals, H<sub>2</sub>S and radionuclide) that play an important role in the triggering of antioxidant defenses in marine organisms. Thus, a different profile of the antioxidant defense network was shown to be particularly active in the gills of the mussel isolated from each area. Total Oxyl radical

Scavenging Capacity (TOSC) (towards peroxy,  $\cdot\text{OH}$  radicals and  $\text{ONOO}^-$ ); cytosolic SOD and GPx activities; and CAT activity, respectively are the main antioxidant mechanisms observed in the mussels depending on the isolation zone. On the other hand, sulphur-amino acids may act as antioxidants in these organisms as well; since  $\text{H}_2\text{S}$  is known to react spontaneously with  $\text{O}_2$  to produce free radicals, such as  $\text{O}_2^-$  and sulphur-centered free radicals [83], during sulphide oxidation.

### 3.1.6. Pathogen Invasion

Parasitism influence in ecosystems is often considered to be a tool for regulating the dynamics of invertebrate benthic populations in coastal areas. Higher activity of antioxidant defenses and higher levels of oxidative cellular damage in coral larvae with the dinoflagellate zooxanthellae as compared with no pathogen invasion of the larvae suggest that oxidative stress, originated in the symbionts, is a possible cause of tissue damage in the host under heat stress [86]. Moreover, Neves *et al.* [87] studied the shrimp *Palaemonetes argentinus* (Nobili) infected by the isopod *Probopyrus ringueleti* (Verdi and Schuldt), a gill chamber parasite known for its capacity to cause host metabolic changes, including changes in  $\text{O}_2$  consumption rates. No significant differences were reported for either CAT or GPx activities; however, SOD activity was significantly reduced in infected shrimp. Even more, *Polydora* sp., a parasite of the oyster *Crassostrea gigas* (Thunberg), modifies the behavior and the respiratory physiology of the molluscs probably due to the existence of oxidative stress associated with parasitism characterized by an increase in expression level of the *sod1* [88].

## 3.2. Anthropogenic Sources

Regoli *et al.* [89] and Viarengo *et al.* [90] have reported that the toxicity of pollutants often depends on their capacity to increase the cellular levels of ROS, since physiological changes in organisms can be related to the effects of toxic chemicals in water that are incorporated and accumulated in tissues and cells. Marine microalgae are particularly promising indicator species for organic and inorganic pollutants because they are typically the most abundant life forms in aquatic environments and occupy the base of the food chain [91]. Oxidative stress is increasingly studied in marine invertebrates and fishes used as sentinel organisms for monitoring pollution in coastal as well as in more remote environments [3, 26, 79, 92-98].

### 3.2.1. Deposition of Heavy Metals from Human Activities

Environmental pollution by metals became extensive as mining and industrial activities increased. Chemical industries, disposal of waste metal, ports, boating activities, agricultural fungicide runoff, domestic garbage dumps, aeolian deposition of atmospheric dust from polluted areas may also bring metals to the marine ecosystem. Algae respond to heavy metals by induction of several antioxidants, including diverse enzymes such as SOD, CAT, GPx and

APx, and the synthesis of low molecular weight compounds such as carotenoids and GSH [4]. Cu has been observed to induce metallothionein gene expression in the seagrass *Posidonia oceanica* (Linnaeus) [99] and in the brown alga *Fucus vesiculosus* (Linnaeus) [100], and malondialdehyde (MDA) generation in the marine diatom *Phaeodactylum tricorutum* (Bohlin) [101]. In the marine dinoflagellate *Lingulodinium polyedrum* (F.Stein), heavy metals cause increased oxidation of proteins and lipids, levels of SOD, APx and  $\beta$ -carotene; and a decrease in GSH content [102].

The exposure to Fe of *M. galloprovincialis* [103] or *Perna perna* (Linnaeus) [104] was reported to lead to a significant increase in MDA levels in digestive glands. In both cases, the induction of phospholipid hydroperoxide-GPx activity following the exposure to Fe overload, was described as a potential biomarker of toxicity associated with contaminant exposure. More recently, González *et al.* [105] reported the effect of *in vivo* Fe exposure on the bivalve *M. arenaria* suggesting that induction of CAT activity could be operative to limit oxidative damage. In this study, the authors also showed in digestive gland the presence of NO by EPR, and the ability of this molecule to chelate Fe was proposed as a candidate to inhibit lipid peroxidation.

Giarratano *et al.* [106] employing the mussel *Mytilus edulis chilensis* (Hupé) reported that digestive glands were the main target tissue of accumulation of Fe and Cu, while gill accumulated the highest levels of Zn. Lately, studies in digestive glands from the ribbed mussel *Aulacomya atra atra* (Molina) showed higher induction of ROS production in samples isolated from animals living in control sites than those from considered impacted sites with high metal concentrations such as Fe, Zn, Cu, Cd and Pb, suggesting the possibility of some biochemical adaptation of the organisms [107]. On the other hand, Rivera-Ingraham *et al.* [108] analyzing the effect of trace metals on the endangered limpet *Cymbula nigra* (da Costa) in the Bay of Algeciras (Strait of Gibraltar) where high trace metals concentrations prevail due to industrial activity [109], showed no significant differences in the content of lipid radical in both digestive gland and gills from the limpets from low and high-polluted areas, reinforcing the idea of the ability of this endangered species to get tolerance to degraded environments. In vertebrates, like the silversides fishes *Odontesthes nigricans* (Richardson), Lattuca *et al.* [110] suggested that a higher content of Fe and Zn in Varela Bay, as compared to Golondrina Bay (Tierra del Fuego, Argentina), could be responsible for the increase on the lipid radical content and the antioxidant activities observed in the liver of the fish from Varela Bay.

### 3.2.2. Presence in Seawater of Hydrocarbons Due to Oil Manufacturing

The main pollutants dependent on oil activity include polycyclic aromatic hydrocarbons (PAH), alkylphenols, and hydrocarbons [111]. These compounds have low vapor pressures ( $\log K_{ow} > 5$ ); therefore, they are rapidly absorbed by particulate matter and by living organisms [112]. Exposure to several PAH causes oxidative stress in aquatic

organisms [113, 114]. Liping and Binghui [101] by laboratory studies employing the marine diatom *P. tricornutum* exposed to fluoranthene reported that MDA content of algal cells increased with time and with increasing exposure concentrations. Sureda et al. [115] showed that Glutathion-S-transferase (GST) and cytochrome P4501A activities and metallothionein gene expression in the digestive gland of wild mussels *M. galloprovincialis* were significantly increased one month after an oil spill accident (Don Pedro oil spill at Eivissa Island, Spain), returning to the basal values after six months. Even though, Camus et al. [116] showed that, following exposure to oil-contaminated sediment in an Arctic fjord, PAH were taken up by the bivalve *Mya truncata* (Linnaeus) and resulted in destabilization of the hemocytes membranes, ROS production was not affected. Moreover, Ansaldo et al. [117] measured lipid hydroperoxides content in digestive gland of the Antarctic limpet *N. concinna* intertidal population exposed up to 1% diesel and did not observed any significant difference with control animals up to 48 h. In spite of this, after longer time periods (168 h) animals in contact with 0.05% diesel showed significantly higher peroxide levels as compared to control animals.

Crude oil does not dissolve to the same extent at low compared with higher temperatures. Such differences in dissolution have important consequences for the bioavailability of oil components at different temperatures [118]. Consequently, the biological adaptation of cold-water organisms, together with the altered oil behavior at low temperature, may affect the typical temperate derived biomarker responses [116]. It has also been shown in the Atlantic cod *Gadus morhua* (Linnaeus) that the exposure to alkylphenols elevated GR activities and total GSH levels, possibly as a result of the induced oxidative stress; however, nonylphenol depleted the cells from GSH [119]. Regarding the production of RNS, Laffon et al. [120] observed in *M. galloprovincialis* that three months after the Prestige oil spill in the Galician coast, basal NO production by the hemocytes of mussels showed a progressive decrease ending in a stop, both in summer and winter. The loss in NO synthesis capacity paralleled the decrease in DNA stability in gill cells during the same period of study [120].

### 3.2.3. Industrial and Urban Wastes

Wastes from anthropogenic sources include industrial and domestic effluents, storm water runoff, streams, leaching from garbage, solid waste dumps and inputs related to maritime traffic. The major pollutants related to municipal effluents are organic matter, suspended solids, nutrients (nitrogen and phosphorus) and pathogenic microorganisms, while other pollutants such as heavy metals, petroleum and chlorinated hydrocarbons, may also be present [121]. The lack of a sewage treatment plant, the discharges from electronic assembling factories in the area and the intensive maritime traffic were reported as the main sources of pollutants [122, 123]. Oxidative stress biomarkers, measured in gills and digestive gland of the mussel *M. edulis chilensis* from the Beagle Channel, were successfully used to monitor

sites of a coastal marine area with urban influences, showing impact of heavy metals, inorganic nutrients and particulate organic matter [124]. In the mussel *M. galloprovincialis* different degrees of pollution were related to urban wastes, harbors, agriculture and tourism-derived activities. Moreover, antioxidant activities (CAT, GSH and SOD in gills, and CAT and GR in digestive gland) showed an adaptive response increasing in the polluted areas with significant differences as compared to non-polluted stations [125].

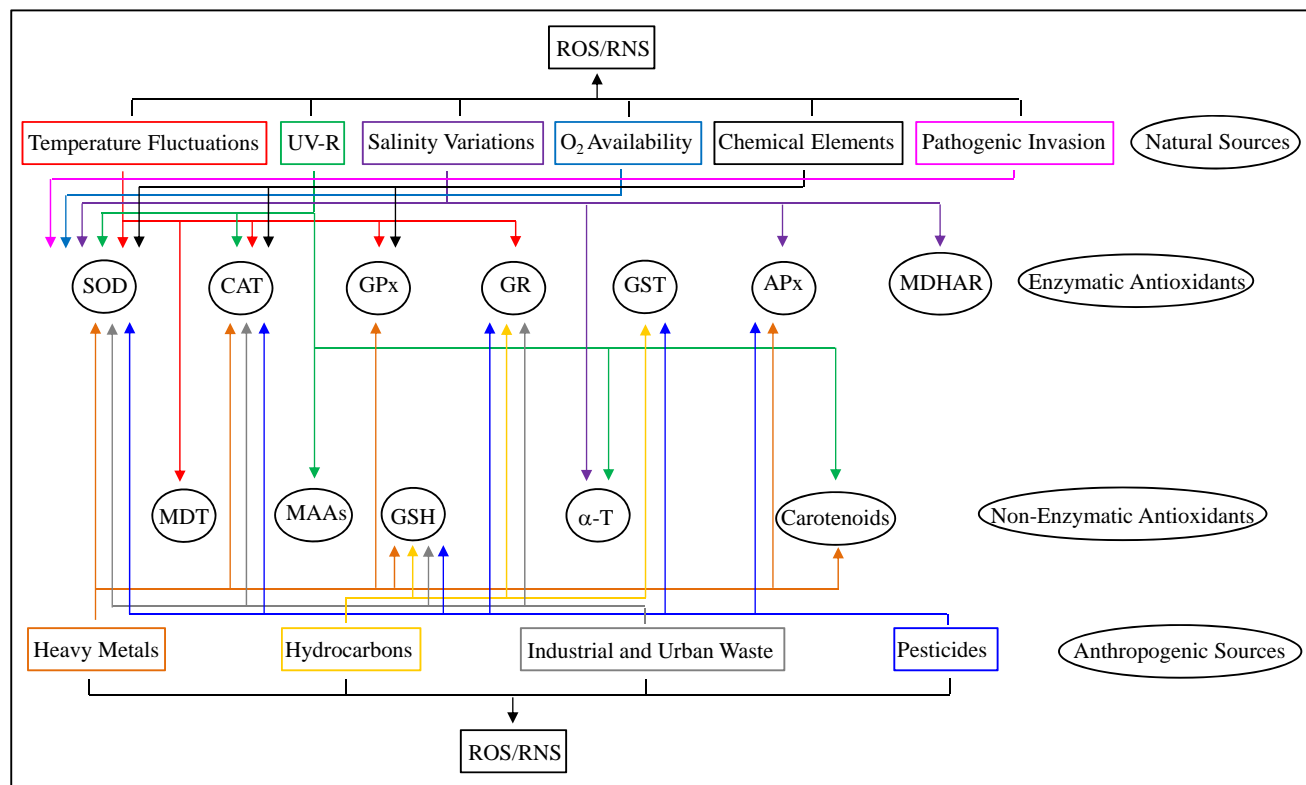
### 3.2.4. Pesticides Incidence

Pesticides are physical, chemical or biological agents intended to kill an undesirable plant or animal pests. 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. Intense agriculture activity, which can be a source of several toxic elements and compounds found in fertilizers and pesticides may be washed off from soils during heavy rain into the adjacent seawater, and that may cause some toxic effects to organisms inducing oxidative stress via several mechanisms [1]. Organophosphates were introduced as a replacement for the organochlorines and are generally considered non-toxic to plants and algae. However, Schweikert and Burritt [126] studied the impact of Coumaphos, a commonly used organophosphate, on the macrophyte *Ulva pertusa* (Kjellman). Exposure to Coumaphos (up to 0.01 mg/l) caused rapid increases in the levels of protein carbonyls and lipid hydroperoxides (markers of oxidative damage), increases in GSH levels and enhancement of SOD, CAT, GR, APx and GST activities. Recently, Gómez-Mendikute and Cajaraville [127] reported in the mussel *M. galloprovincialis* that hemocytes ROS production was increased in paraquat exposure experiments.

## CONCLUSION

It is widely accepted that both, ROS and RNS, possess injuring potential in living organisms, and that their level should be under strict control to prevent damage. However, the cellular delicate balance could be disturbed leading to perturbations of redox status. The increases in ROS and RNS steady state concentrations could be sensed by specific systems, and regulation mechanisms could be triggered. These responses may be responsible for adaptation of organisms to a broad range of natural environmental stressors [1]. The idea of “preparation to oxidative stress” [128] states that in organisms evolutionarily adapted to transitions between normal and extreme external conditions, an induction of adaptive response would help them to survive at recovery. However, several studies have demonstrated that, for example mussels exposed to different pollutants or stressors, are less tolerant to additional stress exposure and *vice versa* [129]. A quite different situation is established when the organisms are exposed to anthropogenic factors since there are not “programmed cellular mechanisms” to afford these extreme conditions.





**Fig. (4).** Brief summary on the effect of the different environmental sources on enzymatic and non-enzymatic antioxidants in the different marine organisms as mentioned in the text. The lines represent effect and not a specific increase on the content or activity.

The data briefly summarized here indicated that the antioxidant network play a key role facing both natural and anthropogenic challenges. The diagram shown in Fig. (4) points out the increase in the activity of the enzymatic antioxidants that was described in a wide array of marine organisms affected by environmental factors.

In an ecological context, marine organisms are exposed to the influence of several abiotic and biotic factors, the effects of multiple factors can greatly differ from simple combinations of single-factor responses [130], and i.e. variables can act in synergistic or antagonistic ways [131]. Thus, effects of environmental factors have to be considered in sampling strategies for monitoring programs to prevent false interpretation of results. A combination of natural stressors and anthropogenic pollution results in several adverse effects occurring under *in situ* conditions, and their interpretation requires further analysis. Moreover, a relatively new concept of understanding moderate oxidative and nitrosative stresses as a pre-conditioning challenge mean to trigger protection mechanisms to avoid further deeper damage, should be carefully studied in marine organisms. Among the enzymatic antioxidants, this summary suggests that SOD activity seems as the main candidate to act as the antioxidant with more relevance to afford protection, since this activity was increased in almost all the tested conditions. Future research should be responsible for answering this open question.

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflict of interest.

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