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# Oxidative effects of the harmful algal blooms on primary organisms of the food web

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**Abstract:** Degraded water quality from nutrient pollution, physical, biological, and other chemical factors contributes to the development and persistence of many harmful algal blooms (HABs). The complex dynamics of the HABs is a challenge to marine ecosystems for the toxic effects reported. The consequences include fish, bird, and mammal mortality, respiratory or digestive tract problems, memory loss, seizures, lesions and skin irritation in many organisms. This review is intended to briefly summarize the recent reported information on harmful marine toxin deleterious effects over the primary organisms of the food web, namely algae, zooplankton and invertebrates. Special focus is made on oxidative stress status of cells and tissues. Even though *in situ* field research is less controlled than laboratory studies, in which the organisms are directly exposed to the toxins under consideration, both types of approaches are required to fully understand such a complex scenario. On top of that, the contribution of the increasing water temperatures in the sea, as a consequence of the global climate change, will be addressed as a topic for further studies, to evaluate the effect on regulating algal growth, species composition, trophic structure, metabolic stress and function of aquatic ecosystems.

## Introduction

In the marine environments, certain microalgae (diatoms, dinophyceae, rhodophyte, dinoflagellates), ciliates and cyanobacteria (Reguera, 2002) species synthetize toxins, among other compounds. These elements are available to other organisms (from plankton to humans) directly from the water or through the trophic transfer, and their cellular metabolism could be altered. These effects could be severe when a high bloom is accomplished in events call harmful algal blooms (HABs). The complex scenario of HABs dynamics is a challenge for the analyses of their toxic effects *in situ* over marine ecosystems. Heisler *et al.* (2008) summarized the principal features of HABs appearance and promotion stating that: i) increased nutrient pollution promotes the development, persistence and expansion of many HABs; ii) the nutrient pool composition impacts HABs; iii) the availability of exogenous nutrients is required to maintain high-biomass blooms, and iv) HAB progress is promoted by either chronic or episodic changes of nutrient distribution. However, degraded water quality from nutrient pollution is not the only factor implicated in HABs. Physical, biological, and other chemical factors also contribute to the growth and persistence of many HABs (Sellner *et al.*, 2003; Vadstein *et al.*, 2004). Moreover, actual changes in environmental conditions, mainly by anthropogenic causes, could potentially lead to yet larger toxic events increasing global occurrence of HAB. The growing human population associated to nutrient pollution promotes variations in coastal nutrient loads from lands runoff (Seitzinger *et al.*, 2010; Bergamaschi *et al.*, 2012). Environmental modifications may also be due to altered abiotic parameters or physical dynamics, such as warmer ocean water sand stratification that are caused by climatic changes (Glibert *et al.*, 2011; Wells *et al.*, 2015). In this regard, the microalgae community was reported to be changed when they were exposed *in situ* to higher temperatures. Hernando *et al.* (2015); Li *et al.*, (2009) observed that the proportion of small phytoplankton cells increased while larger ones decreased under meltwater conditions and episodic inputs of large river runoff, either in Antarctic or Arctic waters, respectively. Since temperature, oxygen ($O_2$) consumption, availability of food, endogenous rhythms and HABs fluctuate seasonally, these factors might be potential stressors for aquatic organisms. The most severe consequences of HAB include effects on fish, bird, and mammal mortalities, respiratory or digestive tract problems, memory loss, seizures, lesions and skin irritation (Sellner *et al.*, 2003). This review is intended to briefly summarize the recent published information on harmful marine toxin deleterious effects over the primary organisms of the food

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web, such as algae, zooplankton and larger invertebrates. Special focus will be made on oxidative stress status on cells and tissues. Even though *in situ* field research is less controlled than laboratory studies in which the organisms are directly exposed to the toxins under consideration, both types of approaches are required to fully understand such a complex scenario. Fig. 1 was designed to connect the interaction between the external factors (biotic and abiotic) that regulate the HAB, and the objective of this work in terms of analyzing the effect of the marine toxins on oxidative metabolism in marine organisms.

**Briefly Overview on Oxidative Metabolism**

Molecular $O_2$, that represents 21% of the composition of the atmosphere, is essential to life, but it is also toxic because reactive $O_2$ species (ROS) are constantly formed as respiration by-products and by other pathways. Even more, ROS formation is a well established event in aerobic cells. The term ROS include free radicals, such as superoxide anion ($O_2^{\bullet-}$) and hydroxyl radical ($^{\bullet}OH$) and non-radical reactive species such as hydrogen peroxide ($H_2O_2$), and singlet $O_2$ ($^1O_2$) (Boveris, 1998). When $O_2$ accepts one electron ($e^-$) the primary product is $O_2^{\bullet-}$. The reception of the second $e^-$ leads to the formation of $H_2O_2$, and with the incorporation of another $e^-$, the molecule decomposes to generate $^{\bullet}OH$. The subcellular structures such as, the mitochondria, the endoplasmic reticulum, the peroxisomes, the plasma membrane, and several enzyme activities are responsible for ROS generation by partial reduction of $O_2$ (Robello *et al.*, 2016). Nevertheless, as nature has been exposed to ROS for two billion years, selected mechanisms have been developed to allow biological systems, not only to live but also to use them, in multiple functions. Thus, ROS toxicity depends on their steady state concentration and the cellular scenario in which they are produced (Nunn, 1985). To be able to regulate hazardous reactive species at low steady state concentrations, the presence of an antioxidant system and radicals scavenging biochemical reactions are required. The antioxidants might be enzymatic (e.g., catalase, CAT; superoxide dismutase, SOD; glutathione-S-transferase, GST; glutathione peroxidase, GPx; etc.) or non-enzymatic, such as hydrophilic (e.g., ascorbic acid, $AH^-$) and lipophilic (e.g., α-tocopherol, α-T) compounds.

On the other hand, nitrogen ($N_2$) represents 79% of the composition of atmospheric air. The radical nitric oxide (NO) is formed when $N_2$ combines with $O_2$ and the a NO reacts with the $O_2^{\bullet-}$ producing peroxynitrite ($ONOO^-$) a radical potencially more oxidative than the first one. Reactive $N_2$ species (RNS), as well, could lead to cellular damage by nitration, nitrosylation and finally to lipid peroxidation (Robello *et al.*, 2016). Thus, ROS and RNS were known for decades to play important roles in mediating cytotoxicity through alterations in protein, lipid, and nucleic acid structure and function with resultant disruption of cellular homeostatic mechanisms. These noxious consequences are often due to markedly elevated (orders of magnitude) steady-state concentrations or rates of production of reactive species. In addition, recently it has become apparent that more subtle changes in rates of production of reactive species may critically impact cellular homeostasis and may serve physiological roles in initiating signaling cascades. Fig. 2 shows the actual understanding of the role of the magnitude of the steady state concentration of ROS and RNS in relation to cellular damage/protection status in marine organisms.

Detection of ROS and RNS in biological systems is often problematic. Sensitive, specific, and reliable methods to detect changes in reactive species are essential to the understanding of the roles of these substances. Low intracellular steady-state concentrations of these species occur as a result of the balance between the basal rates of generation, scavenging, and the extracellular release of small proportions of the intracellularly formed molecules. However, oxidative stress ratios (damage/protection), such as ascorbyl radical ($A^{\bullet}$)/$AH^-$ content and lipid radical ($LR^{\bullet}$)/α-T content were proved to provide a useful tool for stress diagnosis (Puntarulo *et al.*, 2004; González *et al.*, 2013). The possibility of marine toxins to induce toxicological effects through an oxidative and/or a nitrosative pathway was assessed for several researches worldwide at different taxonomic levels.

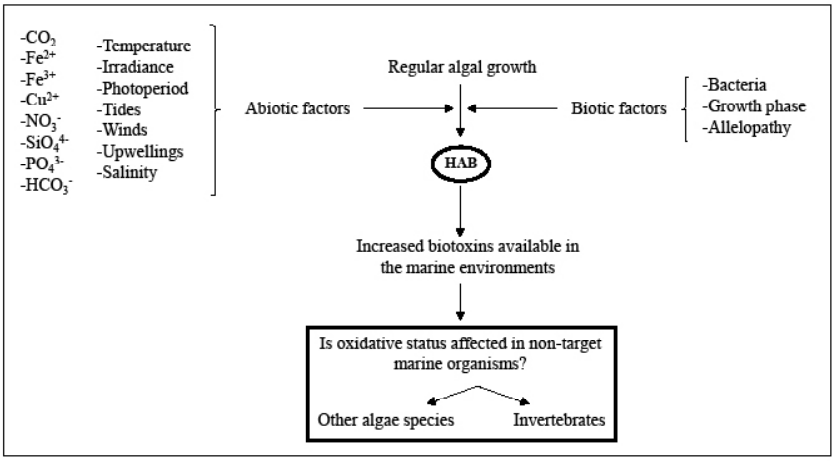


**FIGURE 1**. Schematic synopsis of the aspects to be analyzed on the light of the main biotic and abiotic factors which promote HAB subsequently affecting oxidative metabolism in marine organisms.

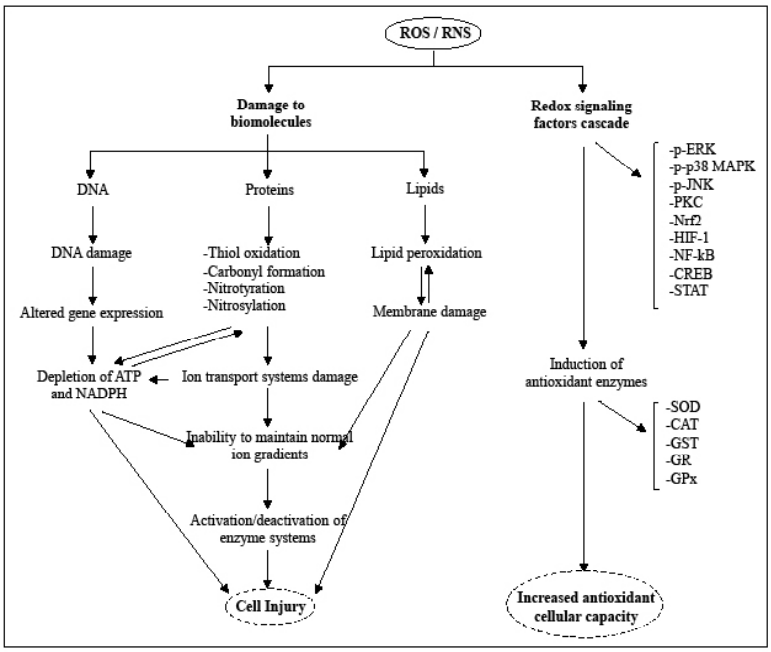


**FIGURE 2**: Brief summary of the two edges of the action of ROS and RNS in relation to the cellular damage/protection processes. Phosphorylated extracellular regulated kinase (p-ERK), phosphorylated p38 mitogen-activated protein kinase (p-p38 MAPK), phosphorylated c-Jun N-terminal kinase (p-JNK), protein kinase C (PKC), nuclear factor erythroid 2-related factor 2 (Nrf2), hypoxia inducible factor-1 (HIF-1), nuclear factor-kB (NF-kB), cAMP-response element binding (CREB) and signal transducer and activator of transcription (STAT).

**Oxidative Stress Generation in Algae by Exposure to Marine Biotoxins**

Even though physiological and ecological roles for some marine toxins produced during HAB were postulated, the matter has not yet been fully elucidated. Dring (2006) suggested that instant responses of marine plants to adverse environmental conditions involve excess production of ROS. Phytoplankton is the most significant primary producer (50% of global primary production; Falkowski and Raven, 2007) in the ocean sustaining the pelagic food chains in the aquatic ecosystems. Phytoplankton is also responsible for the substantial sink for $CO_2$ in marine ecosystems. Then, if these organisms are adversely affected, then the surrounding ecosystem may also feel the effects, either directly or indirectly, from the lack of a food source (Wang and Zheng, 2008). The oxidative stress and damage induced by saxitoxin (STX) in the green phytoplankton alga *Chlamydomonas reinhardtii* was reported by Melegari *et al.* (2012). Quantification of the malondialdehyde (MDA) content, as an indicator of lipid peroxidation, showed no significative differences between algae exposed to STX and control ones. However, some tested antioxidant enzymes showed different profiles upon the exposure to the toxin, since 3 nM STX lead to a decrease in CAT and GST activities, meanwhile the ascorbate peroxidase (APX) and glutathione reductase (GR) activities were significantly higher as compared to control cells. The authors concluded that high concentrations of STX can affect the algal defense system, breaking its oxidative balance.

Some of the harmful species of the phytoplankton found in the Argentinean Sea are *Alexandrium tamarense*, *Gymnodinium catenatum*, *Dinophysis acuminate*, *Pseudo-nitzschia australis*, *P. pseudodelicatissima* (Gayoso, 2001; Reguera, 2002; Vinuesa and Varisco, 2007). Particularly, *Pseudo-nitzschia australis* produces the toxin domoic acid (DA), that was reported to lead the amnesic shellfish poison (ASP) (Pulido, 2008). Several hypotheses explain the potential roles for DA in the algae: i) it could serve as an osmolyte under conditions of increasing salinity (Doucette *et al.*, 2008; Jackson *et al.*, 1992), ii) it may act as a provident against the action of consumer's organism like copepods (Tammilehto *et al.*, 2015), iii) it could be a binding ligand for trace nutrients like transition metals (Trick *et al.*, 2010; Rue and Bruland, 2001) and iv) it may have an allelopathic effect in other members of the phytoplankton, stimulating changes in the dynamics and composition of this algae community (Xu *et al.*, 2015). Moreover, the overall mechanism involved in the interaction between DA and microalgae species include the generation of oxidative stress (Pulido, 2008). Since it is not clear the effect of DA on non-target aquatic organisms, the oxidative condition in the pennate diatom *Phaeodactilum tricornutum*, which does not produce toxins, was studied during the exposition to DA. Cells in exponential (EXP) phase were incubated during 12 min either in the presence of 64 µM DA (Sigma D6152) or absence of DA (controls). The generation of active species was measured with a fluorometric assay following the oxidation rate of 2′,7′ dichlorofluorescein diacetate (DCFH-DA) in a fluorometer (F-3010 Hitachi) at $\lambda_{ex}$ = 488 nm and $\lambda_{em}$ = 525 nm (Zhu *et al*, 1994). The F/2 medium (Guillard, 1975; Guillard and Ryther, 1962) containing the diatom was centrifuge and washed. The supernatant was removed and the pellet was sonicated and incubated with 10 µM of DCFH-DA during 10, 30, 45 and 60 min, at 18°C. After this incubation, the samples were centrifuged, and the rate of oxidation of DCFH-DA was measured following the protocol according to Hernando *et al.* (2012). Data in Fig. 3 show a linear increase in the production of reactive species from both, control and exposed algae homogenates. A significantly higher increased in DCFH-DA oxidation rate was observed in homogenates incubated in the presence of DA as compared to control homogenates in all the tested conditions (Fig. 3).

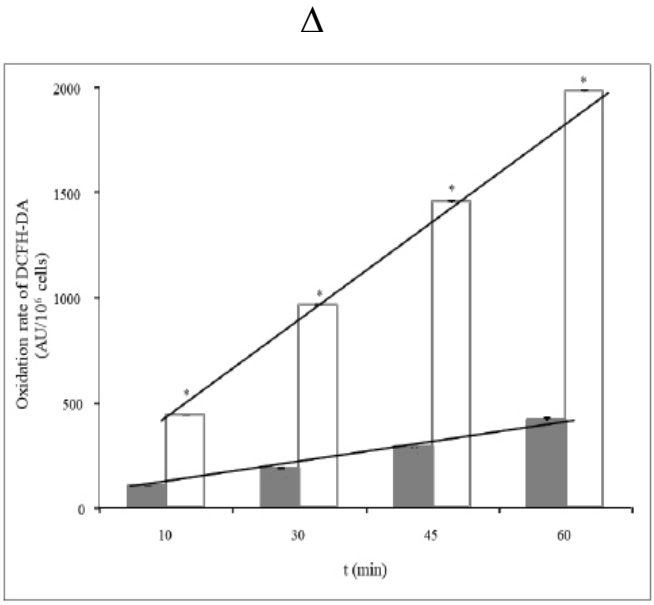


**Figure 3**: Production of reactive species in *P. tricornutum* cells during the EXP phase at different times of incubation with DCFH-DA. Samples exposed to 64 μM DA, $R^2$ = 0.995, m = 30.77 (□); and not exposed samples, $R^2$ = 0.967, m = 6.263 (■). *significantly different to samples unexposed to DA; Anova, ($p < 0.001$).

**TABLE 1**
**Effect of the exposure to scavengers on the oxidation rate of DCFH-DA by P. tricornutum homogenates**

| Oxidation rate of DCFH-DA (AU/$10^6$ cells) | | | | |
|---|---|---|---|---|
| | -DA | + DA | Δ Oxidation rate | Inhibition |
| Basal | 427 ± 1 | 735 ± 3$^{*,***}$ | 308 ± 4 | |
| SOD (300 U/ml) | 387 ± 4$^{*}$ | 551 ± 9$^{*,***}$ | 164 ± 9$^{*}$ | 47% |
| CAT (500 U/ml) | 376 ± 5$^{*}$ | 472 ± 10$^{*,**}$ | 96 ± 6$^{*}$ | 69% |
| DMSO (50 mM) | 419 ± 4$^{*}$ | 652 ± 13$^{*,***}$ | 233 ± 10$^{*}$ | 24% |
| GSH (5 mM) | 262 ± 4$^{*}$ | 371 ± 2$^{*,***}$ | 109 ± 6$^{*}$ | 65% |
| DF (50 μM) | 379 ± 2$^{*}$ | 484 ± 6$^{*,***}$ | 105 ± 8$^{*}$ | 66% |

Cells of *P. tricornutum* from three axenic algal cultures were collected during the EXP phase and were incubated during 45 min with DCFH-DA (10 μM). Where indicated (+ DA) samples were exposed to 64 μM DA. The experiment was made taking three replicas of each algal culture.

Percentage of inhibition by scavengers on the change in the oxidation rate of DCFH-DA by the addition of DA was calculated as the decrease in the Δ Oxidation rate after incubation with each agent.

*Significantly different from the DCFH-DA oxidation rate of the cellular homogenate in the absence of scavengers; ANOVA, ($p < 0.0001$).

**Significantly different from the DCFH-DA oxidation rate of the cellular homogenate of algae incubated in the absence of DA; ANOVA, ($p < 0.0001$).

***Significantly different from the DCFH-DA oxidation rate of the cellular homogenate of algae incubated in the absence of DA; ANOVA, ($p < 0.001$).

The DCFH-DA oxidation rate is a general indicator of the cellular oxidative metabolism (McDowell *et al.*, 2013), thus to assess the possible contribution of the different reactive species produced by the algae cells, the reaction rate was measured in the presence of scavengers. In the previously described assay, after the pellet sonication the samples were incubated with DCFH-DA in the presence of different scavengers during 45 min at 18°C. Then, the homogenate was centrifuged and the fluorometric measurement was performed in a microplate reader (Varioskan Lux Thermo Scientific). The effect of the enzymes SOD (300 U/ml) and CAT (500 U/ml), that scavenges $O_2^{\bullet-}$ and $H_2O_2$, respectively; dimethylsulfoxide (DMSO, 50 mM, that binds $^{\bullet}OH$); the antioxidant glutathione (GSH 5 mM, a general antioxidant) and deferoxamine (DF, 50 μM, that binds Fe, inhibiting the production of $^{\bullet}OH$) were tested. In the presence of DA the oxidation rate of DCFH-DA showed a 72% increase, as compared to samples incubated in the absence of DA in a time-dependent manner (Tab. 1). In the absence of DA, except for GSH, all the tested scavengers showed low percentages of inhibition (9%, 12%, 2%, 39%, and 11%, for SOD, CAT, DMSO, GSH and DF, respectively). When the cells where exposed to DA the effect of the scavengers was significantly higher than in the absence of the DA (25%, 36%, 11%, 49%, and 34%, for SOD, CAT, DMSO, GSH and DF, respectively). These results suggested a similar contribution of several molecules (such as $H_2O_2$, Fe, and $O_2^{\bullet-}$) in the increase of the generation of active species in the diatoms faced to

DA. The relatively lower inhibition by DMSO could be due to the lack of specificity of the scavenger or the small life time of $^{\bullet}OH$. When the cells where exposed to DA the effect of the scavengers was significantly higher than when they were incubated in its absence.

Cyanobacterial toxins, that may be able to promote oxidative stress, play a major role in seaweed affecting oxidative metabolism (Collén and Davison, 1997, 1999a,b). Since macroalgae are key factors in the marine coastal ecosystem, any metabolic alteration may have a long-term ecological significance. Pflugmacher *et al.* (2007) evaluated the effects on the promotion of oxidative stress of a cyclic pentapeptide hepatotoxin nodularin (NOD)-containing crude cell extract of the cyanobacterium *Nodularias spumigena* on the most abundant Baltic Sea brown algae, the bladder wrack *Fucus vesiculosus*. Lipid peroxidation was measured assessing the content of MDA and 4-hydroxynonenal (4-HNE) assays. The authors reported that the concentration of both metabolites increased during the experimental period, but the concentration of MDA was always 10-fold lower than the concentration of 4-HNE. They also showed that the activities of the antioxidant enzymes SOD and CAT, were significantly elevated after 4 h of NOD exposure. Moreover, Collén and Davison (1999b) suggested that antioxidant enzymes in fucoids are a major factor to determine the resistance against oxidative stress, and since the total antioxidant status of *F. vesiculosus* showed a significant increase after 6 h of NOD exposure, it is probably that several antioxidants were co-operating among them. Pflugmacher *et al.* (2010) studied the promotion of an oxidative stress response in the red macrophyte *Furcellaria lumbricalis* after the exposure to a NOD-containing crude extract of *N. spumigena*. The toxins induced the antioxidant defense system since SOD, GPx, GST and GR enzymes were significantly elevated after the NOD exposure, as compared to control algae. However, since GPx and GST use GSH for their catalytic reactions, a decrease in GSH content within the cells exposed to NOD was measured. One possibility to recover GSH was through the activation observed of GR activity. Based on these observations, the authors proposed that *F. lumbricalis* could be used as a bioindicator of cyanotoxin exposure in field conditions due to the prominent antioxidant enzymatic responses reported.

**Oxidative Stress Generation in Invertebrates**

For marine zooplankton, the major link between pelagic primary producers and invertebrates and fish (Mauchline, 1998), the dietary uptake of biotoxins from phytoplankton can be a dominant route for entry of marine toxins and the nexus to other levels of the food web. Cyanobacterial blooms have the potential to produce cyanotoxins, including hepatotoxic microcystins (MCs), e.g., MC-Leucine Arginine (MC-LR), the most toxic of all MC analogues. The ability of MCs to trigger oxidative stress in cells and tissues has been documented in marine animals (Gonçalves-Soares *et al.*, 2012; Kim *et al.*, 2017). Min *et al.* (2018) studied the marine mysid, *Neomysis awatschensis*, which is a relatively small crustacean commonly found in brackish, estuarine, coastal, and oceanic regions as a part of the zooplankton with potential use in environmental monitoring. Given the ecological importance of mysids, the age-dependent modulatory effects of MC-LR on the lipid peroxidation and antioxidant defense mechanism were studied. After the exposure to 10 μg/l of MC-LR, elevated levels of MDA and GSH were observed during the late (days 5 and 7) exposure and early (days 1 and 3) depuration periods in juveniles, while adults showed a peak on day 7 of exposure. Age-specific responses were also observed in the enzymatic activities of GST, CAT, SOD, GPx, and GR. Juvenile mysids showed a significant elevation in all enzymatic activities, but only CAT and SOD enzymes showed significant changes in adults during the exposure and/or depuration phase of MC-LR.

Vehmaa *et al.* (2013) evaluated the oxidative status (antioxidant capacity, oxidative damage, and oxidative balance expressed as a ratio between the antioxidant capacity and the oxidative damage) in the adult (copepodite stage VI) calanoid copepod *Acartia bifilosa* from the Baltic Sea. These organisms were fed with diets consisting of green algae mixed with the toxic cyanobacteria *N. spumigena*. The authors observed that the presence of cyanobacteria promoted antioxidative defenses (measured by the intracellular soluble antioxidant capacity using the $O_2$ radical absorption capacity (ORAC assay; Prior *et al.*, 2003) and decreased lipid peroxidation levels (thiobarbituric acid reactive substances, TBARS), thus contributing toward the maintenance of the redox-state and oxidative balance of the copepods.

One of the top consumers of phyto- and small zooplankton are marine bivalves, who they also constitute a major taxonomic group in estuarine and coastal regions, and are key players in the community structure and the ecosystem functioning (Dumbauld *et al.*, 2009). Even more, many bivalve species are particularly important for the local economy in several regions as a product of their commercialization for human consumption. These mollucs are also considered biomarkers organisms for environmental monitoring, including during HABs, based on their economic importance, wide geographical distribution, abundance, sedentarism, tolerance to environmental alterations, their ability to concentrate toxins, their levels of metabolizing enzyme activities of organic contaminants, the nature of the populations, the life span, the body size, and the potential to survive in laboratory and field studies in cages (González *et al.*, 2018). Even though, when confronted to HABs these organisms usually survive, oxidative and/or nitrosative metabolism is altered. In addition, some cases of bivalve's mass mortalities were observed (Anderson *et al.*, 2000). Considering that biotoxins are accessible directly from the water and also through the food, gills and digestive glands (DG) may serve as the first target, accumulation and detoxification organs. González and Puntarulo (2016) collected the mussels *Mytilus edulis platensis* from the Argentinean Sea in the presence of harmful planktonic toxins (spring), and in its absence (winter and summer). During the HAB season, the bivalves were facing oxidative stress, as indicated by the increases measured on the hydrophilic ($A^{\bullet}/AH^{-}$ content) and lipophilic ($LR^{\bullet}$/α-T content) cellular media indexes of redox balance and on DCFH-DA oxidation rate measured in the DG. Meanwhile, under normal physiological conditions during winter and summer, these biomarkers appeared to be adequately controlled to keep steady state concentrations of damaging reactive species distant from

hazardous levels.

The ability of the bivalve antioxidant defense system to effectively respond to a broad variety of environmental stressors has been widely reported (Lima *et al.*, 2007; Regoli and Giuliani, 2014; Oliveira *et al.*, 2015). In this context, Prego-Faraldo *et al.* (2017) studied the transcriptional expression levels and biochemical activities of antioxidant enzymes in different tissues of the mussel *Mytilus galloprovincialis* during experimental exposures to diarrheic shellfish poisoning (DSP) toxins produced by the dinoflagellate *Prorocentrum lima*. Results were consistent with the presence of a compensatory mechanism in the mollusc gills and DG, involving a down-regulation in the expression of specific genes encoding for several enzymes. The significant changes observed in the activities of SOD, CAT, GPx and GST enzymes in both tissues were consistent with the implication of the antioxidant system during early responses to the biotoxin exposure. A reduced lipid peroxidation in both tissues also supported the role of DSP toxins to increase the protection against oxidative stress in this organism.

In other study, *M. galloprovincialis* and the scallops *Patinopecten yessoensis* were faced to the paralytic shellfish toxin (PST) produced by the dinoflagellate *Alexandrium tamarense* (Qiu *et al.*, 2013). The results, on the muscle and DG, presented a rapid ROS generation followed by a disappearance during the toxin accumulating and depurating periods, respectively. However, the response of the antioxidant enzymes differed in the two mollusc species. The SOD, CAT, and GPx enzyme activities were stimulated to avoid oxidative damage in the mussel, but only GPx activity was induced in the scallop tissues. Recently, Cao *et al.* (2018) identify whether distinct sensitivity exists between the oyster *Crassostrea gigas* and the scallop *Chlamys farreri* under the same amount of STX exposure. It was reported that, even though not being lethal to oysters and scallops, STX exposure induced oxidative stress, cellular damage, and immunotoxicity indiscriminately in both organisms; with a slightly higher sensitivity of scallops over oysters.

Invertebrates are limited to an innate immune response to pathogens, parasites, and physical injury (Janeway, 1994). This response is mediated by specialized cells (hemocytes) circulating throughout the body, and humoral factors such as antimicrobial peptides (Cheng 1996; Hine 1999; Bachère *et al.*, 2004), lysozymes, lectins and an alternative complement pathway (Medzhitov and Janeway, 2002). Hemocytes recognize and attempt to eliminate these invasive particles within an open vascular system and tissues, but they cannot "remember" a prior experience with a harmful agent to be able to efficiently protect an individual for subsequent exposures (Hégaret *et al.*, 2007a). But, when pathogens encounter the external protective barrier of the mollusc, the host recognizes their specific molecular pattern (pathogen-associated molecular patterns) and then, initiates hemocyte-mediated responses such as phagocytosis and oxidative burst to accomplish complete elimination of the intruder (Hégaret *et al.*, 2011). Among the environmental agents that may activate or modulate the immune system of bivalves are harmful or toxic microalgae (Hégaret and Wikfors, 2005a,b; Hégaret *et al.*, 2007b,c; da Silva *et al.*, 2008; Galimany *et al.*, 2008a,b; Haberkorn *et al.*, 2010).

Núñez-Acuña *et al.* (2013) analyzed *Mytilus chilensis* hemolymph after the injection of *Alexandrium catenella,* which produces STX. The expression of 13 candidate genes associated with cellular stress and immune response was evaluated and higher gene transcription levels was observed in the bivalves injected with the toxin compared to control organisms. High levels of differential gene expression were observed for SOD, CAT, ferritin and heat-shock protein genes. Gorbi *et al.* (2013), exposed *M. galloprovincialis* mussels to the benthic dinoflagellate *Ostreopsis cf. ovate,* which produces palytoxin-like compounds represented by ovatoxins (more than a 90%) and a putative-palytoxin (approximately 1%; Ciminiello *et al.*, 2008; Ciminiello *et al.*, 2012). The antioxidant parameters measured in the hemocytes revealed a limited role of *O. cf. ovata* to induce oxidative stress, excepting for a certain increase of CAT, GR and GPx activities, and a significantly higher capability to neutralize peroxyl radicals in mussels exposed for 14 days. Bianchi *et al.* (2019) reported that *Mytilus edulis* exposed to a PST strain of *Alexandrium* showed a not clearly evident oxidative burst response (enhanced intracellular and/or extracellular ROS), since the intracellular DCFH-DA oxidation in hemocytes was lower in mussels fed 3 days with the dinoflagellate than in mussels from control groups. Then, these animals display adaptive fitness traits to survive and maintain the immune capacity upon prolonged exposure to environmentally relevant concentrations of PST. Other studies were performed in hemocytes of different oyster species. For example, juvenile *C. gigas* exposed to *A. catenella* induced higher ROS production of circulating hemocytes (Lassudrie *et al.*, 2016). However, when Hégaret *et al.* (2007a) exposed *Crassostrea virginica* to bloom concentrations of the dinoflagellate *Alexandrium fundyense* and *C. gigas* to *A. catenella*, no statistically-significant effect on hemocyte parameters such as ROS production in the three hemocyte cell populations (granulocytes and small and large hyalinocytes) were found.

MCs have been found to accumulate also in crustacean species (Chen and Xie, 2005). Lightner (1975) postulated that shrimp mortality by toxin bioaccumulation was related to the presence of a marine cyanobacteria. Gonçalves-Soares *et al.* (2012) evaluated in the hepatopancreas, the responses of the white shrimp *Litopenaeus vannamei* GST isoforms and CAT after the injection with a sub-lethal level of the MC type [D-Leu1] call MC-LR. MCs caused up-regulation for GST Ω, μ and a MAPEG isoforms, by 12-, 2.8- and 1.8-fold, respectively, and increases in the total GST and CAT enzyme activities. Thus, it was suggested that the GSH conjugation can be an important MC's detoxification mechanism in shrimp, similarly to what has been observed in mammals and other invertebrates. Other crustacean, the grapsoid crab *Neohelice granulate*, is a dominant species in estuarine intertidal environments of Brazil, Uruguay and Argentina (Boschi, 1964; Botto and Irigoyen, 1979). This crab specie is exposed to ingest toxic cyanobacteria during cyanobacterial blooms. The possible oxidative stress produced by MC-LR in the hepatopancreas of *N. granulate* was evaluated through the measurement of SOD and GST activities, and the levels of GSH and lipid peroxidation (Sabatini *et al.*, 2015). The

different pattern of response over time in TBARS levels, GSH content, SOD and GST activities suggested that the oxidative damage was limited and reversed partially by antioxidant mechanisms which were evidently activated at 3-4 weeks after the start of the intoxication.

## Final Remarks

Many studies were undertaken to examine the influence of marine toxins on contaminated seafood toxicity to humans with an association to economic losses. However, other critical aspect is to examine the influence of the biotoxins on ecological damage when the toxins are released from the species that generate them to the environment. In this regard, different responses can be observed when micro and macroalgae, zooplankton, mussels and crustaceans were exposed either *in situ* or in laboratory studies to biotoxins. These compounds may affect physiological pathways, including cell functionality, membrane stability, antioxidant enzymes activities, gene expression and cell signaling, depending on the studied organism. Generation of reactive species that were detected in many of these aquatic organisms after the exposure to biotoxins could be both, responsible for cellular deterioration by their deleterious actions or promotors of the activation of certain antioxidant enzymes. A deeper biochemical analysis of the transcriptional levels and enzymatic activity of identified proteins may help to the understanding of the mechanisms of metabolism and elimination of the toxin in the marine ecosystem and the significance in the food web. Moreover, this knowledge would allow the finding of strategies that could avoid, or at least limit, the drastic effects of global climate changes in this complex scenario. HABs showed a global increase in the frequency, magnitude, and geographic extent over the last two decades. The detection of these effects is the result of the increased awareness and active research performed by the scientific community. A strong correlation between the number of HABs with the degree of coastal pollution, the global warming and aquaculture procedures were reported. It also appears likely that toxic algal species have spread within regions over spatial scales of hundreds of kilometers, moving with major water currents and storms (John *et al.*, 2005; Klöpper *et al.*, 2003). Thus, oxidative/nitrosative actions are not the only way biotoxins lead to deleterious alterations on the organisms. Integrated studies are required to clarify these effects and could be the key for future advances in this field.

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