BIOCELL
2016, 40(1): 35-38

ISSN 1667-5746
ELECTRONIC

Oxidative stress in the hydrophilic medium of algae and invertebrates

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Key words: algae, ascorbyl/ascorbate ratio, invertebrates, oxidative stress

ABSTRACT: The harmful effects of the reactive species may be due to the increase in their steady state concentration either by the enhancement of their production rates and/or the decrease of their consumption rate by antioxidant activity. The ascorbyl radical ($A^{\bullet}$) can be considered as a final product of radical oxidative transformations of ascorbate (AH^-). The ratio $A^{\bullet}$ content/AH^- content ($A^{\bullet}/AH^-$) has been widely used as an interesting tool to estimate mild to moderate oxidative transformations, providing a quick and simple method of diagnosis of stress in the hydrophilic cellular medium. The aim of this work was to summarize studies on the cellular oxidative condition in algae and invertebrates by assessing the $A^{\bullet}/AH^-$ ratio under environmentally changing conditions. The use of indices of oxidative stress increasingly sensitive and, somewhat more specific, can bring a new light to the still unknown world of oxidative responses in marine organisms.

The harmful effects of the reactive oxygen species (ROS) and the reactive nitrogen species (RNS) may be due to the increase in their steady state concentration either by the enhancement of their production rates and/or the decrease of their consumption rate by antioxidant activity. Electron Paramagnetic Resonance (EPR) has been applied for detecting biologically important radicals in marine communities. The ascorbyl radical ($A^{\bullet}$) can be considered as a final radical product from the oxidative transformations of the ascorbate (AH^-). The $A^{\bullet}$ can be detected by EPR (Buettner GR, Jurkiewicz, 1993), and the AH^- content by HPLC (Kutnink *et al.,* 1987). The ratio $A^{\bullet}$ content/AH^- content ($A^{\bullet}/AH^-$) has been widely described as an interesting tool to estimate mild to moderate oxidative transformations, providing a quick and simple method of diagnosis of stress in the hydrophilic cellular medium (Malanga *et al.,* 2012; 2015a). Thus, cellular oxidative condition in algae and invertebrates exposed to physiological and anthropogenic generated stress situations were analyzed by assessing the $A^{\bullet}/AH^-$ ratio.

Both, natural and anthropogenic environmental changes can affect phytoplankton productivity and produce modifications in the food web by causing oxidative stress (Torres *et al.,* 2008). Microalgae are one of the major contributors to global carbon fixation and represent 40% of total primary production in the ocean (Sarthou *et al.,* 2005). Algae in their natural environment, regardless of their origin, seem to keep the cellular $A^{\bullet}/AH^-$ ratio in a narrow range, indicating that single-cell photosynthetic organisms are able to adapt themselves to the habitat by using different mechanisms to limit oxidative damage. Moreover, microalgae from extreme environmental conditions, such as Antarctic (e.g. *Chlorella* sp., *Navicula* sp.) and sub-Antarctic algae (e.g. *Asteromonas* sp.) showed a lower $A^{\bullet}/AH^-$ ratio than

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Received: October 30, 2015. Accepted: April 16, 2016

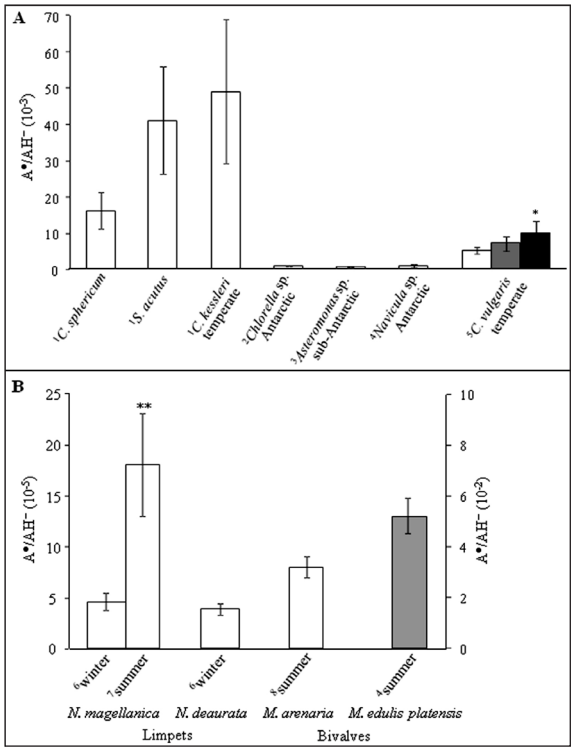

FIGURE 1. A•/AH- ratio in algae and invertebrates. **A.** Oxidative ratio was measured in the ceparium algae *C. sphericum* (CCAP217/2, SAG217/2) and *S. acutus* (CCAP276/3a, SAG276/3a). The rest of the algae were taken from their natural environments. *C. vulgaris* algae were cultured under: i) natural environmental conditions (□), ii) exposure to 30 kJ/m^2 UV-B (□), and iii) exposure to 500 μM Fe-EDTA (■) in the culture medium. **B.** Oxidative ratio in invertebrates. Right y axis is used for data in open bars (□) and left y axis for data in grey bars (■).
*significantly different to natural condition samples (ANOVA, $p < 0.05$).
**significantly different to winter samples (ANOVA, $p < 0.05$).
Taken and modified from [1]Malanga *et al.* (2001), [2]Estevez *et al* (2001b), [3]Hernando *et al.* (2008), [4]González *et al.* (2013), [5]Estevez *et al* (2001a), [6]Malanga *et al.* (2004), [7]Malanga *et al.* (2007) and [8]González *et al.* (2008).

algae from temperate habitats (e.g. *Coelastrum sphaericum, Scenedesmus acutus, Chlorella kessleri, Chlorella vulgaris*) probably due to a lower oxidative metabolism rate (Figure 1A).

As a strategy to increase the food chain primary production in the South Atlantic Ocean, *in situ* Fe fertilization was performed to regulate the production of CO_2 in the atmosphere (Aumont and Bopp, 2006). The alteration in the Fe content in the medium can significantly affect the oxidative condition in *C. vulgaris* cultures (Figure 1A) by increasing the A•/AH- ratio by 2-fold compared to controls. However, the exposure to UV-B (30 kJ/m^2) did not significantly affect the ratio in relation to non-exposed cultures (Figure 1A). Thus, oxidative stress in the hydrophilic cellular medium could contribute to cell damage by inhibiting the growth rate and eventually leading to cell death. These data are consistent with previous measurements using cultures of *C. vulgaris* algae supplemented with 500 μM Fe that pointed out that although the Fe is an essential metal for the normal development of the phytoplankton, an excess in its availability affects the growth of the algae (Estevez *et al.,* 2001a). Oxidative condition by the effect of added Fe was also evaluated under laboratory conditions in culture media of *Navicula* sp. Fe supplementation to a concentration of 100 μM produced an increase in *Navicula* sp. biomass, suggesting that this Fe concentration can be considered as an optimal feature for growth (Malanga *et al.,* 2015b).

The physiological complexity, location and seasonality affect the magnitude of the A•/AH- ratio in invertebrates (Figure 1B). The digestive gland (DG) of the limpets *Nacella magellanica* and *N. deaurata* from the sub-Antarctic region, showed a non-significant difference for the A•/AH- ratio when animals were collected during winter time (Figure 1B). However, the A•/AH- ratio in DG from *N. magellanica* collected during summer was significantly higher than the values measured in winter, suggesting that temperature may be a relevant factor affecting the generation of oxidative stress in the hydrophilic medium of limpets (Figure 1B).

Bivalve molluscs have been extensively used as indicators of the effect of anthropogenic environmental contamination or of natural changes in marine organisms (Zhou *et al.,* 2008). The A•/AH- ratio was determined in the DG of the bivalves *Mya arenaria* (from the North Sea) and *Mytilus edulis platensis* (from the east coast of southern South America). In *M. arenaria* this ratio was in the same order of magnitude as compared to the values measured in limpets (Figure 1B). In *M. edulis platensis*, an important resource used as food, the A•/AH- ratio in DG was similar in winter and summer, and was higher than those observed in other Antarctic and sub-Antarctic invertebrates, such as limpets and sea urchins, under physiological conditions (Malanga *et al.,* 2015b). These data suggested that seasonality does not seem a critical factor to alter oxidative stress condition in the hydrophilic medium in *M. edulis platensis.*

Red tide results in fish killing, poisoning marine mammals and humans through ingestion of contaminated molluscs with toxins from the phytoplankton (mollusc food web). The diatom *Pseudo-nitzschia sp.* is known for the production of domoic acid, a tricarboxylic acid with neurotoxic properties. This bloom usually occurs in spring and is favored by high levels of nutrients, such as Fe^{3+}, phosphates and nitrates in the water (Fisher *et al.,* 2006). The toxin is concentrated in the molluscs, especially in *M. edulis platensis,* without causing the death of the invertebrates. The study of oxidative condition through the measurement of the A•/AH- ratio, evaluated in the GD of *M. edulis platensis*

samples isolated during the time of the red tide, showed a significant increased (50%) as compared to the data on samples isolated in summer (Malanga *et al.,* 2015b).

Hemocytes are the immune cells in bivalves and are able to recognize foreign elements and to generate oxidative burst reactions against them by secreting and producing humoral factors (Husmann *et al.,* 2011). Hemocytes were obtained from *M. edulis platensis* collected in the San Matías Gulf, Rio Negro, Argentina during winter and spring (red tide). The rate of oxidation of 2', 7' dichlorodihydrofluorescein diacetate, used as an indicator of the generation of active species (González *et al.,* 2010), was significantly increased during the red tide burst, as compared to the winter samples (60%). These data are in agreement with the increase in the $A^{\bullet}/AH^{-}$ ratio observed in the DG (Malanga *et al.,* 2015b) and suggest that even though the red tide toxins do not provoke the death of molluscs, they do provoke oxidative stress to the animals.

Based on the obtained results it can be concluded that each organism maintains its own oxidative condition, dependent on a set of intrinsic and extrinsic factors. Both natural (e.g. UV-B radiation, seasonal changes, red tide) and anthropogenic challenges (e.g. contamination, Malanga and Puntarulo, 2014) are able to modify the steady state concentration of the active species and to compromise cellular integrity. The strategy of decreasing the concentration of deleterious components by the antioxidant capacity ensures the survival of the species, and is the basis of the adaptive phenomena. On the other hand, in this complex scenario mild changes in the oxidative condition were recently postulated as critical factors to trigger cell signaling mechanisms to protect several organisms against further damage. However, experimental precautions must be maximized as multiple factors act simultaneously and even individuals within a population may respond differently, depending on various factors including temperature, genetic variability, nutritional status and other stressful joint actions (Depledge *et al.,* 1993). The use of indices of oxidative stress increasingly sensitive and, somewhat more specific, can bring a new sight to the still unknown world of oxidative responses in marine organisms. However, future studies on the signaling pathways are still essential to characterize the mechanism of cellular processes triggered by oxidative changes.

Acknowledgement

This study was supported by grants from the University of Buenos Aires (20020130100383BA), National Agency of Science and Scientific Promotion (ANPCYT PICT 00845) and National Council for Science and Technology (CONICET PIP 0697). G.M., P.M.G. and S.P. are career investigators from CONICET, and J.M.O. is a fellow from ANPCyT.

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