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ECOLOGY

Effects of Temperature and pH on the Oxidative Stress of Benthic Marine Invertebrates[1]

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Abstract—The impacts that environmental conditions generate on the physiological performance of benthic marine invertebrates are specific and involve several levels of structural organization. The central component is related to the perception of temperature, which in turn produces the dissipation of oxygen in the mitochondria and triggers oxidative stress. Several studies have evaluated the effect of temperature on oxidative stress, however, few studies have examined their interaction with other environmental factors. This review analyzes the results of antioxidant activity that have been reported for benthic marine invertebrates, considering the combined effects of temperature and pH. The evidence shows that the activity increases with the reduction of pH and that the differences between the optimal values and the exposure define the scope of the responses. The results are used to suggest a generalized pattern of the mechanism and discuss the hypotheses that have been proposed to explain the interaction of temperature and pH on stress oxidative response.

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INTRODUCTION

Marine invertebrates respond to temperature by adjusting mitochondrial electron transport (Handy et al., 2009). The response generates superoxide anions ($O_2^{-\bullet}$) as minors by-products (Davidson and Schiestl, 2001), while the antioxidants influx in the mitochondria stimulate the protection of proteins, and avoid lipid peroxidation (Candas and Li, 2014). The enzymatic antioxidant activity increase as response of imbalances of production and scavenging of reactive oxygen species (ROS). This response is the indirect form in which the effect of the environmental condition over oxidative stress has been investigated (Tomanek, 2015).

The ability to adjust the production of antioxidants enzymes to avoid the increase of ROS in response to acute local temperature fluctuations is not exclusive of marine invertebrates as it generally occurs in all eukaryotic cells (Davidson and Schiestl, 2001). It is known that the enzyme activity gradually increases as environment temperature does, and as the exposure to high temperature continues, the rate of enzymatic reaction falls rapidly due to heat energy denatures or inactivates the enzymes. The reaction which also occurs in a significant temperature reduction (Tattersall et al., 2012), becomes complex when interaction between oxidant species and energy metabolism is considered (Quijano et al., 2016).

It stands to reason that tricarboxylic acid cycle (TCA), enzyme α-ketoglutarate dehydrogenase and glycerophosphate deshidrogenase are important sources of ROS (Tomanek, 2015), and that even when ROS production are part of normal cell metabolism, the overproduction of $O_2^{-\bullet}$ and H_2O_2 contribute to cellular DNA damage and promote apoptosis (Handy et al., 2009). The cellular mechanism that reduce the levels of ROS are the antioxidant enzymes, which eliminate hydrogen peroxide, and thus the cellular response is regulated and maintain in homeostasis. Antioxidant activity occurs by dismutation of $O_2^{-\bullet}$ to H_2O_2, and the reduction of H_2O_2 to H_2O happens by activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalases (CAT), and thioredoxins-peroxiredoxin (Trx-Prx) (Vives-Bauza et al., 2007, Tomanek, 2015).

The pH of environment also plays a key role in the control of antioxidant production in aquatic organisms, particularly when they are exposed to changing temperatures (Wang et al., 2009; Chen et al., 2015; Ramírez-Duarte et al., 2016). The importance of pH in the dynamic interaction of antioxidants and the effects of prolonged exposure to acidic conditions have been reported (Wang et al., 2009; Chen et al., 2015). The generalized pattern with the exception of CAT is the increase in the activity of antioxidant enzymes as

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the pH decreases. It is possible that the increase of GPx activity in reduced pH scenarios is not only related to oxidative stress, but also to other metabolic pathways that may be affected in acidic conditions

Overexpression of GPx could attenuate protein kinase B a serine/threonine-specific protein that plays a key role in multiple cellular processes such as glucose metabolism, apoptosis, cell proliferation, transcription and cell migration (Handy et al., 2009). Even when increasing the production of the enzymes at low pH might constitute a mechanism to cope with stress, which becomes important to avoid oxidative and microbial impairment (Wang et al., 2009; Chen et al., 2015), the possibility that such response might play a first step towards apoptosis brings the need for a new interpretation of the results of thermal tolerance and oxidative stress under the combined effects of pH.

Some authors have addressed both, temperature and pH effects on the antioxidant activity on marine animals. Matozzo et al. (2013) reported that gills and digestive gland of the clam *Chamelea gallina* and the mussel *Mytilus gallopronvincialis* exposed to 22°C at pH 7.7 had higher activity of SOD and glutathione S-transferase (GST) than those of pH 8.1. Recently, Gullian and Terrats (2017) described a negative correlation between GPx and SOD of coelomic fluid of *Isostichopus badionotus* exposed to suboptimal temperatures and low pH values; they reported that GPx activity was maximal at the extremes of the cold and warm temperatures, and that the activity of SOD increased between 28 and 30°C. These results showed that in the short time, pH rather than temperature were more important controlling the activity of the antioxidants, and that multiple process could be involved. To distinguish these effects, it is necessary to generalized single and combined impacts of these parameters, and to establish generalized patterns for the description of both.

MATERIALS AND METHODS

The generalized pattern that temperature and pH exert on the antioxidant activity of marine benthic organisms was obtained by comparing experiments that have been carried out. The direction and magnitude of the antioxidant responses were determined in relation to the values recorded for optimum pH and temperature. Whenever possible, the data were modeled with different mathematical expressions and their adjustments were defined by residual analysis (Ratkowsky, 2004). If possible, regression analyses of the transformed data were run to determine correlations among antioxidants. All analyses were performed at $p < 0.05$, using the package STATISTICS (Statsoft Inc.). For parsimony, antioxidants ratios were verified by generalized linear regression models supported on the relation of O^{2-} and H_2O_2. The differences between enzymatic activities obtained with different pH values were analyzed to verify structural changes of slopes using the test of Chow (Gujarati and Porter, 2009).

RESULTS

The effects that the reduction of pH produced in the antioxidant activity of octocorals, ascidians, crabs, prawns, bivalves and sea cucumbers were significant. In all cases, the activity of SOD and GPx increased with the reduction of pH. The antioxidant activity of SOD at low pH values was adjusted to a quadratic model with vertex in the maximum antioxidant activity. In the parabola, the antioxidant activity reduced as the temperature increased above the optimum (26°C). The same pattern was observed when temperature reduced below this point. Unlike the low pH, the quadratic relationship did not occur in the alkaline treatments, where the antioxidant activity did not show significant differences as the temperature moved away from the optimum (Table 1, Fig. 1). Opposed to the SOD activity and considering again a low pH environment, the GPx activity presented an inverted parabola. There was greater GPx activity in treatments whose temperatures differed considerably from the optimal temperature (Table 1, Fig. 1). In most cases, the maintenance of constant values of temperature over time, from any tangent of the parabola, determined the behavior that was best adapted to the physiological condition of the organism. In this sense, if the tangent was found to be well below the physiological optimum, decadent expressions of antioxidant activity could be observed; while tangents close to the physiological optimum, were prone to produce oscillating synodal responses of antioxidant activity (Table 1). In comparison with SOD and GPx patterns, CAT activity was less consistent and did not present a defined scheme. In some species, the decrease in temperature increased the activity of the enzyme (*Palaemon* spp.) and presumably *I. badionotus*; while in other organisms the antioxidant activity of CAT did not present modifications that could be associated with changes in temperature and pH. Previous studies have shown that the interaction between the antioxidant activities of GPx and SOD were significantly affected by pH and time of exposure. GPx activities were negatively associated with the activities of SOD (Table 1, Fig. 2). For the specific case of *I. badionotus*, the level of SOD explained 48% of the activity of GPx under alkaline condition, and reducing the pH increased the predictor power of SOD up to 58% (Fig. 2).

DISCUSSION

It was observed that the change of temperature from optimal values (24 to 28°C) to extremes (16 and 36°C) increased the antioxidant activity of SOD and GPx; and that these effects were greater when the pH was reduced. The results were reported for the oyster *Crassostrea virginica* (Tomanek et al., 2011), shrimp

Table 1. Effects of temperature and pH on the antioxidant activity of superoxide dismutase (SOD), glutathione peroxidase (GPx)–glutathione S-transferase (GST), and catalase (CAT)

Specie	Starting, °C	Rate of change, °C day^{-1}	Treatments, °C	Exposure, hours	pH	SOD	CAT	GST–GPx	Ref.
A	20.33 26.80				8.5 8.2	Without distinction of pH, the rachis and the peduncle have the same activity in both tissues and temperatures			1
B	16.3 16.4 17.7			528	8.2 8.6 8.4	Activity decreases according to the reduction of temperature and pH	Activity decreases according to the reduction of the pH but not of the temperature		2
C	22.0	2.0	3, 6, 9, 12, 15	672	7.6	Regardless of tissue, activity increases from 22 to 9°C and then decreases to 6 and 3°C			3
D	22.0		5, 27	168	7.6	Activity decreases with the reduction of temperature		Same activity regardless of temperature	4
E	25.0		16, 20, 22, 27, 30, 32	456		Activity increased from 20 to 25°C and then decreased to 32°C without being lower than 20°C			5
F	20.0	1.0	22, 24, 26, 28, 30, 32, 34	2, 4, 6, 8, 10, 12, 14		[1] Activity reduced from 22 to 32°C, and [2] it did not change with time	[1] Activity increased from 20 to 26°C and then decreased to 28 to 34°C, and [2] it remained the same at all temperatures	[1] Increased from 20 to 26°C and then decreased from 28 to 34°C, and [2] increased from 20 to 28°C and then dropped from 30 to 34°C	6
G	17.0		11, 23, 28	0, 1, 24, 72		While at 23 and 28°C it increased after one hour of exposure and subsequently, from 24 to 72 h, it was reduced; at 11°C it remained unchanged			7
H	22.0	2.0	28	168	8.1 7.7 7.4	[1] Without distinction of temperature, in both tissues, the activity is maintained with the reduction of pH; [2] the activity of the digestive gland increased with the reduction of pH	[1] Gill activity but not the gland remained the same in relation to the reduction of temperature and pH; while controls of digestive gland were lower than the rest of the pH values; [2] in both tissues, gill activity increased with the reduction of pH	[1] Gill activity increased with the reduction of pH, while the activities of digestive gland were the same; [2] the activity of both tissues increased with the reduction of pH	8

Table 1. (Contd.)

Specie	Starting, °C	Rate of change, °C day^{-1}	Treatments, °C	Exposure, hours	pH	SOD	CAT	GST–GPx	Ref.
I	25.0	1.0	30	24, 72, 168, 336	8.1 7.7 7.3	Gill activity at 25°C increased with exposure and with the reduction of pH, while 30°C and extreme values of pH reduced it. Regardless of pH and time of exposure, the digestive gland tends to had the same activity at 25°C, while 30°C produced higher enzymatic activities at low values of pH			9
J	16.0	0.5	26	0, 240, 480, 720, 960		Raising temperature from 16 to 26°C increased antioxidant activity up to 30 days, after that, it sharply decreased. Maintenance of such temperature for a longer period of time rapidly reduced the activity in the respiratory tree and intestine, and it slowly decreased the activity in the body wall			10
K	16.0		20, 25	0, 72, 168		Raising temperature from 16 to 20°C or 25°C rapidly increased activity in the body wall, while increasing exposure reduced the activity of the same			11
L	25.0			0, 8640, 17280		Increasing exposure rise the activity to maximum and after that it drops to minimum			12
M	26.0	1.0	16, 20, 24, 28, 30, 34, 36	48	7.70 8.17	At a low pH, the activity decreased as the temperature moved away from the optimum, while at normal pH, there were no changes in the antioxidant activity. Enzyme activity decreased with exposure	At normal pH the activity of coldest temperature was higher than the warmest treatments, the changes from optimum temperature increased the activity within the first three degree of differences, and activity decreased to the extreme treatments. Furthermore, enzyme activity decreased with exposure	At a low pH, activity increased from optimum to 24 and 30°C, and then it remains unchanged, while at normal pH activity still at the same level at all temperatures. Enzyme activity increased with exposure	13

A *Veretillum cynomorium* (raquis and peduncle); B *Botryllus schlosseri* (homogenized tissue); C (*Portunus trituberculatus* (muscle and hepatopancreas); D *Carcinus maenas* (hepatopancreas); E *Macrobachium nipponense* (muscle); F *Palaemon elegans*[1] y *P. serratus*[2] (muscles); G *Chlamys farreri* (Haemolymph); H *Chamelea gallina*[1] y *Mytilus galloprovincialis*[2] (Gill and digestive gland); I *Mytilus coruscus* (Gill and digestive gland); J *Apostichopus japonicus* (body wall, intestine, respiratory tree); K *Apostichopus japonicus* (body wall); L *Isostichopus badionotus* (coelomic fluid); M *Isostichopus badinotus* (coelomic fluid); 1 Madeira et al. (2015); 2 Taselli et al. (2017); 3 Meng et al. (2014); 4 Rodríguez et al. (2015); 5 Wang et al. (2006); 6 Vinagre et al. (2014); 7 Chen et al. (2007); 8 Matozzo et al. (2013); 9 Hu et al. (2015); 10 Ji et al. (2008); 11 Shao et al. (2015); 12 Gullian (2013); 13 Gullian-Terrats (2017).

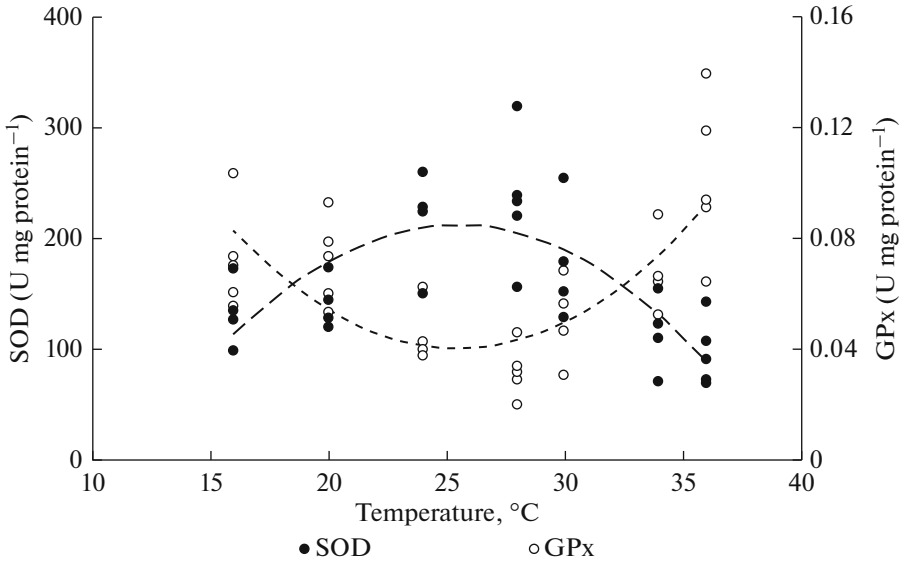

Fig. 1. Adjustment quadratic models for antioxidant activity of SOD and GPx considering simple and combined effects of pH and temperature. Data were taken from Gullian and Terrats (2017). SOD $f(x) = -1.1089x^2 + 56.434x - 506.35$, $R^2 = 0.5683$, $F_{(1,35)} < 6.27$, $p = 0.02$; GPx $f(x) = 0.005x^2 - 0.024x + 0.3457$, $R^2 = 0.5212$, $F_{(1,35)} < 7.80$, $p = 0.00$.

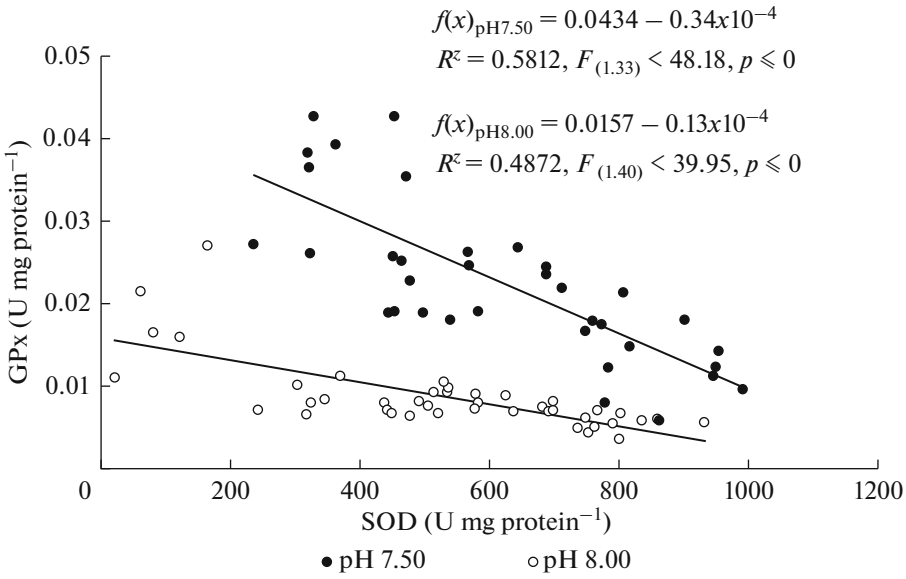

Fig. 2. Antioxidant activity interaction of superoxide dismutase (SOD) and glutathione peroxidase (GPx) based on catalyzed dismutation of $O_2^{-\bullet}$ to H_2O_2, and the reduction of H_2O_2 to H_2O. Lineal regression models adapted from Gullian and Terrats (2017). Significant with R^2 at $p \leq 0.05$. Residuals normality for pH 8.00 and pH 7.50: $W = 0.973$, $p \geq 0.74$, and $W = 0.948$, $p \geq 0.09$.

Litopenaus vannamei (Wang et al., 2009), clam *C. gallina* (Matozzo et al., 2013), mussels *M. gallopronvincialis* (Matozzo et al., 2013) and *M. coruscus* (Hu et al., 2015), and sea cucumber *I. badionotus* (Gullian and Terrats, 2017). The hypothesis that has been proposed to explain the increased antioxidant activity at low pH, includes, the direct or indirect effect of pH via the Fenton reaction or by CO_2 (Trujillo et al., 2008). Ramirez-Duarte et al. (2016) observed an increased antioxidant activity in *Oryzias latipes* which were exposed to low pH, and considered three possible causes for this response: (i) a decreased hemoglobin affinity for oxygen at low pH, (ii) enhanced iron-mediated ROS production, and (iii) an increased metabolism. Another hypothesis suggests the effect of temperature as a mechanism of perception with oxidative stress. The increased temperatures could activate fluxes of calcium (Ca^{2+}), which acts as a mediator in

the mechanism of thermal detection in eukaryote cells (Clapham and Miller 2011; Sengupta and Garrity, 2013). If that occurred, then the rise of Ca^{2+} within the cell, could sustain for a longer period the ionic balance under hypercapnia conditions, which under the reduction of pH, would results in a higher tolerance to temperature at the costs of increased activity of enzymatic antioxidant. Furthermore, since GPx activity relates poorly with SOD, the increased action of the first need to be related with additional mechanisms to respond to acidity.

Using the data of Gullian and Terrats (2017) to calculated the interaction SOD-GPx, we could identify low coefficients of determination (generally no greater than $R^2 = 0.58$). The low relationship between SOD and GPx, agreed with Kalyanaraman (2013), in the sense that no strong interaction occurred between these enzymes. Thus other types of antioxidants, rather than GPx could be more important in the antioxidant response (Cox et al., 2010; Tomanek, 2015). Increased GPx activity at pH 8.00, even in the absence of SOD, could indicate that peroxides from lipid degradation could have been incorporated into the action of the enzymatic activity (Nikolic et al., 2006). Therefore, such increase, instead of being considered as an antioxidant response related with temperature, should be considered as a cell death signaling.

Another feature of the experiments was the fact that a high variability of antioxidant activity occurred even among the same tissues of organisms of the same species. Madeira et al. (2015) found a high variability in antioxidant activity in octocorals collected at different seasons (Table 1). The response has also been reported in bivalves exposed to thermal stress at different pH (Matozzo et al., 2013), and in sea cucumbers exposed to different temperatures and pH (Gullian and Terrats, 2017). It is possible that the differences on activity could indicate that another antioxidant system might be involved in the removal of H_2O_2 (Tomanek et al., 2015). That correlates with the fact that 90% of ROS produced in mitochondria are eliminated by the reaction of thioredoxin-peroxiredoxin (Cox et al., 2010), which is congruent with the low correlation that we identified between SOD and GPx.

Finally, changing temperature from optimum and increasing time of exposure could affect the energy of the enzymes and shifted protein aminoacids towards abnormal action kinetic (Tattersall et al., 2012). It is known that values above and below optimum reduce the use of energy, and that such response is critical when time of exposure increased (Dong et al., 2006; An et al., 2007; Lavitra et al., 2010). Therefore, the longer of the activity depends on the difference that exists between optimum and tested conditions.

CONCLUSIONS

All species exhibited a broad antioxidant activity when subjected to the simple and combined effects of pH and temperature. The appearance of greater enzymatic activities in the presence of suboptimal temperatures and reduced pH conditions could be related to the perception of temperature and the control of hypercapnia conditions. The increase of GPx activity and the reduction of the action of SOD constituted a deleterious condition that could affect the survival of these organisms.

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