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Effect of temperature in multiple biomarkers of oxidative stress in coastal shrimp

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ABSTRACT

Various studies in captivity and in the wild have pointed to the effect of season, and temperature in particular, in the levels of the oxidative stress biomarkers currently used for environmental quality assessment. However, knowledge on how temperature affects the oxidative stress response is unavailable for most species. This study investigated the effect of increasing temperature on lipid peroxidation, catalase activity, superoxide dismutase and glutathione-S-transferase in the shrimps, *Palaemon elegans* and *Palaemon serratus*. It was concluded that increasing temperatures significantly affect all the biomarkers tested in both species, with the exception of superoxide dismutase in *P. serratus* which was not affected by temperature. The oxidative stress response was more intense in *P. elegans*, than in *P. serratus*, producing higher peaks of all biomarkers at temperatures between 22 °C and 26 °C, followed by low levels at higher temperatures. It was concluded that monitoring of ecosystems using oxidative stress biomarkers should take into account the species and thermal history of the organisms. Sampling should be avoided during heat waves and immediately after heat waves.

1. Introduction

Significant efforts have been made in the integration of multiple response indicators for the biological monitoring of aquatic ecosystems and in the assessment of habitat quality (Patrício et al., 2009; Borja et al., 2009; Martinez-Crego et al., 2010). The ecological indices produced for this purpose range from community indices (Deegan et al., 1997; Ramm, 1990; Borja et al., 2000; Smith et al., 2001) to indices based on sub-organism biomarkers like the Health Assessment Index (Adams et al., 1993), the Bioeffect Assessment Index (Broeg et al., 2005), the Integrated Biomarker Response Index (Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006) and the Biomarker Response Index proposed for application within the European Commission's Water Framework Directive (Hagger et al., 2008).

Some of these indices and various habitat quality studies have used biomarkers of oxidative stress, namely lipid peroxidation, catalase activity, superoxide dismutase and glutathione-S-transferase. These biomarkers have been used as indicators of cellular stress resultant from environmental contamination originated by agricultural, industrial and urban pollution, such as heavy metals, PCBs and PAHs (e.g. Ferreira et al., 2005; Amado et al., 2006; Dissanayake et al., 2011; Serafim et al., 2012; Carreira et al., 2013).

Frequently, such studies are conducted over wide areas, at different depths and/or aim to compare different aquatic systems (e.g. Fitzpatrick et al., 1997; Padmini et al. 2009; Lukyanova et al., 2010; Dissanayake et al., 2011; Serafim et al., 2012; Carreira et al., 2013). This kind of approach dismisses the potential effect of temperature on oxidative stress biomarkers. However, recent experimental studies, with fish and crabs, indicated that temperature may be a confounding variable when comparing oxidative stress biomarkers in sites and/or species exposed to different thermal regimes (Vinagre et al., 2012a; Madeira et al. 2013,2014). Some field studies also reported an important effect of season on oxidative stress responses, warning to the role of temperature as a potential confounding variable (Zanette et al., 2006; Cravo et al., 2009; 2012; Guimarães et al., 2009; Padmini et al., 2009; Dissanayake et al., 2011; Mieiro et al., 2011).

Given that heat waves and other extreme climatic events are predicted to become more frequent and prolonged due to climate change (IPCC, 2001), the effect of the recent thermal history of the

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species under analysis for the assessment of habitat quality should be taken into consideration. Frequently, monitoring of aquatic systems is carried out in summer, when biodiversity and abundances are higher. However, if done during a prolonged heat-wave, temperature may be a confounding variable, originating higher oxidative stress levels than those caused solely by environmental contamination.

Other factors may also be at play, such as salinity (Rodrigues et al., 2012), which is particularly important in estuaries, due to its wide daily and seasonal variation. This way, the effect of temperature on the oxidative stress response is better isolated in experimental studies done in captivity. Yet, such studies are lacking for most species. The present work contributes to the investigation of the effects of temperature on the oxidative stress response of aquatic species, which may be used in environmental assessment given their high abundance, wide distribution and easy capture. It investigates the oxidative stress response of two common shrimp species, *Palaemon elegans* and *Palaemon serratus*, exposed to increasing temperatures, in captivity.

2. Materials and methods

2.1. Specimens collection and acclimation conditions

Specimens were collected in the Portuguese West coast (38°71′ N; 9°48′ W) in a rocky exposed shore, in November of 2012. Individuals were collected using hand nets in six representative tidal pools. Temperature in the tidal pools at the time of capture was 20 ± 1 °C. This temperature was chosen for the acclimation of the specimens to the laboratory conditions. Capturing the individuals in autumn ensured that they had not been exposed to heat-waves in the weeks prior to their collection, thus assuring that their thermal history did not include recent exposure to high temperatures.

Tidal pools were surveyed monthly for 2 years (2011–2012) to determine their temperature fluctuations throughout the year. Temperature was measured with a multi-parameter probe. The pools mean area was 12.7 m^2 and mean depth was 41 cm.

After capture, the 50 individuals of *P. elegans* and 23 of *P. serratus* were immediately transported to the laboratory facilities and housed in a re-circulating system with aquaria with a capacity of 70 l, with aerated sea water, a constant temperature of 20 ± 1 °C and salinity 35‰. The water dissolved O_2 level varied between 95% and 100%. The organisms were acclimated for 1 week, being fed ad libitum with commercial food pellets, twice a day. They were starved for 24 h before the experiments.

2.2. Experimental setup

The individuals were placed in a thermostatized bath, at 20 °C. The experiment was only started when the animals did not show any agitated behaviour indicative of handling stress. Previous experiments with another crustacean, using the same methodology, indicate that handling, confinement and crowding is not significant (Vinagre et al. 2012b). The experimental containers consisted of white plastic boxes of $60 \times 42 \times 10$ cm^3 (10 individuals per box), with a plastic lid with a central perforation for the passage of an aeration tube. These containers were placed in a water bath thermostatically controlled by digital MultiTemp III (Pharmacia Biotech) heater/refrigerated circulator. During the experiment, a constant rate of water–temperature increase of 1 °C h^{-1} was applied. Organisms were observed continuously until they reached the end-point. Samples of dorsal muscle of the abdomen from 6 individuals (in *P. elegans*) and 3 individuals (in *P.serratus*) were taken every 2 °C until the end-point was reached (at this point, nine individuals of *P. elegans* and seven individuals of *P. serratus* were sampled) and immediately frozen in liquid nitrogen.

The end-point was loss of equilibrium (Mora and Ospina, 2001). The temperature at which each animal reached its end-point was measured with a digital thermometer, recorded and then the Critical Thermal Maximum (CTMax), which is defined as the "arithmetic mean of the collective thermal points at which the end-point is reached" (Mora and Ospina, 2001) and its standard deviation was calculated for both species. Shrimp were measured with a slide calliper at the end of the trial.

2.3. Lipid peroxides assay

The lipid peroxides assay was adapted from the thiobarbituric acid reactive substances (TBARS) protocol (Uchiyama and Mihara 1978). 5 μL of each sample, already processed as previously described were added to 45 μL of 50 mM monobasic sodium phosphate buffer. Then 12.5 μL of SDS 8.1%, 93.5 μL of trichloroacetic acid (20%, pH=3.5) and 93.5 μL of thiobarbituric acid (1%) were added to each microtube. To this mixture, 50.5 μL of Milli-Q grade ultrapure water were added and the eppendorfs were put in a vortex for 30 s. The eppendorfs' lids were punctured with a needle and the eppendorfs were incubated in boiling water for 10 min. Straight after, they were placed in ice for a few minutes to cool and 62.5 μL of Milli-Q grade ultrapure water and 312.5 μL of n-butanol pyridine (15:1, v/v) were added. Then the eppendorfs were placed in a vortex and centrifuged at $10000 \times g$ for 5 min. Duplicates of 150 μL of the supernatant of each reaction were put into a 96-well microplate and absorbance was read at 530 nm. To quantify the lipid peroxides, an eight-point calibration curve (0–0.3 μM TBARS) was calculated using malondialdehyde bis (dimethylacetal) (from Merck) standards.

2.4. Determination of enzymes activity

The enzymatic assay of Catalase (EC 1.11.1.6) was carried out according to the procedures described previously (Beers 1952, Aebi 1983, Li and Schellhorn 2007). Activity from a standard bovine catalase solution of 1523.6 U mL^{-1} was used as a positive control. Catalase activity was calculated using a molar extinction coefficient (at 240 nm) for H_2O_2 of 0.04 ε^{mM}.

The enzymatic assay of *glutathione S-transferase* (*GST*) *activity* (*EC* 2.5.1.18), using the substrate CDNB (1-Chloro-2,4-dinitrobenzene), was carried out according to Habig et al. (1974). After reading the absorbance at 340 nm GST activity was calculated using a molar extinction coefficient for CDNB of 0.0053 ε^{mM}.

The enzymatic assay of superoxide dismutase (SOD) activity, using nitroblue tetrazolium (NBT) and xanthine oxidase (XOD), was carried out according to Sun et al. (1988). After reading the absorbance at 560 nm, SOD activity was calculated using the equation for the %inhibition

$((Abs_{560}/min\ negative\ control - Abs_{560}/min\ sample)/(Abs_{560}/min\ negative\ control)) \times 100$

For normalization purposes, the results were divided by the total amount of protein, calculated through the Bradford method (Bradford 1976), to obtain enzymes activity in nmol min^{-1} mg^{-1}.

For the SOD assay a negative control (NC) was performed. It included all components of the assay except SOD or sample (Trevigen, 2010). In this case, the increase in absorbance due to generation of superoxide radical proceeds maximally. The NC is then used in the following equation: (Abs/min CN–Abs/min/sample)/(Abs/min CN) × 100=% inhibition