ARTICLE IN PRESS

Marine Pollution Bulletin xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Baseline

Seasonal changes in stress biomarkers of an exotic coastal species – *Chaetopleura angulata* (Polyplacophora) – Implications for biomonitoring

Diana Madeira[a,b,*], Catarina Vinagre[c,*], Vanessa Mendonça[c], Mário Sousa Diniz[a]

[a] UCIBIO - Requimte, Department of Chemistry, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[b] Department of Biology & CESAM, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[c] MARE – Marine and Environmental Sciences Centre, Faculdade de Ciências da Universidade de Lisboa, Campo Grande 1749-016, Lisboa, Portugal

ARTICLE INFO

Keywords:
Environmental quality assessment
Cellular stress response
Environmental change
Biomonitoring
Intertidal

ABSTRACT

Knowledge on baseline values of stress biomarkers in natural conditions is urgent due to the need of reference values for monitoring purposes. Here we assessed the cellular stress response of the chiton *Chaetopleura angulata in situ*. Biomarkers commonly used in environmental monitoring (heat shock protein 70 kDa, total ubiquitin, catalase, glutathione-S-transferase, superoxide-dismutase, lipid peroxidation) were analyzed in the digestive system, gills and muscle of *C. angulata*, under spring and summer conditions in order to assess seasonal tissue-specific responses. Season had an effect on all targeted organs, especially affecting the digestive system which displayed clear seasonal clusters. The respective Integrated Biomarker Response (IBR) showed a 7.2-fold seasonal difference. Muscle and gills showed similar IBRs between seasons making them appropriate organs to monitor chemical pollution as they were less responsive to seasonal variation. The most stable biomarkers in these organs were ubiquitin and superoxide-dismutase thus being reliable for monitoring purposes.

Anthropogenic disturbance of aquatic communities is a major environmental issue that affects ecosystem services provided to society. To estimate the impact of human activities (pollution, land use, marine traffic, global change) on aquatic ecosystems, tools have been developed in order to improve ecological risk assessment (ERA). Such tools include the assessment of biological end-points measured in relevant species, termed as biomarkers. Biomarkers may be any physiological, biochemical or molecular measurement which can elucidate the effects of chemical, physical or biological hazards (World Health Organization, 1993; Hook et al., 2014). Biomarkers provide information on ecosystem's health that can be used to support environmental management (Adams et al., 2001; Bucheli and Fent, 1995), and can be divided into three sub-classes, following the World Health Organization (1993): biomarkers of exposure, biomarkers of effect and biomarkers of susceptibility.

The most commonly applied biomarkers to monitor ecosystem health include heat shock proteins, metallothionein enzymes, antioxidant enzymes, mixed function oxygenases and bile metabolites (Adams et al., 2001). Heat shock proteins are part of the cellular stress response (CSR); they play a primary role in intra-cellular defense (Csermely and Yahara, 2003) as they have a molecular chaperone function that prevents protein damage when organisms are exposed to stress, including temperature, hypoxia, Reactive Oxygen Species (ROS), pollution, UV radiation, viral and bacterial infections, and osmotic stress, among others (Das et al., 2014; Köhler et al., 2001; Madeira et al., 2012; Rola et al., 2014; Tiedke et al., 2014; Tine et al., 2010). Ubiquitin is another important biomarker in the protein quality control system, being involved in the selective tagging of proteins for proteasomal degradation (Hershko and Ciechanover, 1992). As such, it has been used as a proxy for irreversible protein damage in the cells (Hofmann and Somero, 1995). Antioxidant defense is also an important component of the stress response in marine organisms. Oxidative stress occurs when the production and accumulation of reactive oxygen species (ROS) exceeds the organisms' antioxidant defenses. Reactive oxygen species are chemically reactive molecules containing oxygen that form as a natural by-product of normal metabolism (Cadenas,

Abbreviations: BSA, bovine serum albumin; CAT, catalase; CDNB, 1-Chloro-2,4-dinitrobenzene; CSR, cellular stress response; DNA, deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; ERA, ecological risk assessment; GST, glutathione-S-transferase; Hsc, Heat shock constitutive protein; Hsp70, Heat shock protein 70 kDa; IBR, Integrated Biomarker Response; LPO, lipid peroxidation; MDA, malondialdehyde bis(dimethylacetal); mtHsp70, mitochondrial Heat shock protein 70 kDa; NBT, nitroblue tetrazolium; PBS, Phosphate Buffered Saline; PCA, Principal Components Analysis; ROS, Reactive Oxygen Species; SDS, sodium dodecyl sulfate; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TUb, Total ubiquitin; XOD, xanthine oxidase

* Corresponding authors.
E-mail addresses: dianabmar@gmail.com, d.madeira@campus.fct.unl.pt (D. Madeira), cmvinagre@fc.ul.pt (C. Vinagre), vsmendonca@fc.ul.pt (V. Mendonça), mesd@fct.unl.pt (M.S. Diniz).

http://dx.doi.org/10.1016/j.marpolbul.2017.05.005
Received 6 March 2017; Received in revised form 26 April 2017; Accepted 1 May 2017

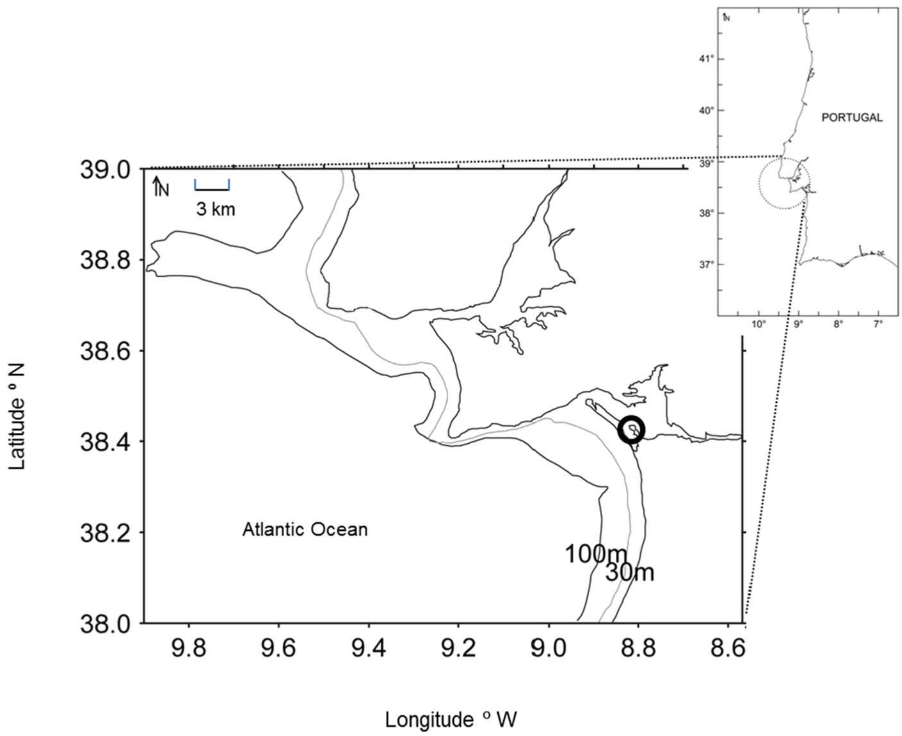

Fig. 1. Location of the sampling area in the Portuguese coast (circle indicates the sampling area - 38°29′ N; 8°53′ W). Bathymetric lines of 30 and 100 m are marked as grey and black lines, respectively.

1989), yet environmental stress can cause its levels to increase dramatically (Abele et al., 2002; Heise et al., 2006). This can damage lipids, proteins and deoxyribonucleic acid (DNA). The reaction of ROS with lipids and the consequent destruction of the cellular membrane are regarded as major mechanisms of cellular damage (Halliwell and Gutteridge, 1999). Under most physiological states, ROS production is closely matched by enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST), which form an important part of the antioxidant response (Lesser, 2006; Regoli and Winston, 1999). Biomarkers of oxidative stress have been commonly used in habitat quality assessments, as indicators of cellular stress resulting from environmental contamination originated by anthropogenic pollutants (Ferreira et al., 2005; Tsangaris et al., 2011). However, the application of biomarkers in the ERA framework may be limited by their variability due to natural environmental fluctuations (*e.g.* seasonal changes) and intrinsic biotic factors (Nahrgang et al., 2013; Schmidt et al., 2013; Shaw et al., 2004). Recent work with fish, crustaceans and molluscs has shown that both the protein quality control system and oxidative stress biomarkers are very sensitive to other environmental factors (*e.g.* elevated temperature) and can change seasonally, potentially confounding pollution biomonitoring (Dissanayake et al., 2011; Madeira et al., 2014a, 2014b, 2014c, 2013; Mieiro et al., 2011; Padmini and Vijaya Geetha, 2009; Vinagre et al., 2014; Vinagre et al., 2012; Zanette et al., 2006). This may be especially relevant in temperate regions, which have large seasonal variations. Temperature, reproductive cycle and differential feeding have been appointed as three main factors affecting biomarker responses, consequently affecting environmental monitoring (Cuevas et al., 2015; Dalzochio and Gehlen, 2016). Therefore, the sampling period should be standardized and confounding factors identified before elaborating the monitoring plan. On site research is thus needed to examine biomarkers' response to natural variation. This will provide baseline reference values that can be compared to those obtained in the future. Furthermore, another issue that remains scarcely studied is the differential response among different body tissues, in the same organism. Due to their different functions and location within the body, different tissues may have different coping strategies and responses to cellular stress (Freire et al., 2011; Madeira et al., 2014c; Madeira et al., 2016b; Rodrigues et al., 2012; Vinagre et al., 2014).

The present study aims to investigate the type of response of several biomarkers in a shallow water species, the chiton *Chaetopleura angulata* (Polyplacophora), exposed to natural environmental variation in order to improve biomonitoring related decision making. We aim to establish a set of baseline data for biomarkers commonly used in monitoring plans and identify the most suitable biomarkers and organs to be targeted. In its native range in South America, this species occurs from the tropical coasts of Brazil (Cabo Frio) to the cold coasts of Chile (Cape Horn). It also occurs in the temperate-subtropical Atlantic coasts of the Iberian Peninsula, possibly introduced in the past by Portuguese and Spanish trading ships (Besteiro et al., 2011). It is highly abundant in Portuguese coasts (Mendonça, 2012) and inhabits both the subtidal and intertidal areas and during the ebb tide it remains on the shore, out of water, highly exposed to terrestrial conditions. As grazers, chitons have been used as bioindicator species in monitoring studies, representing the benthic environment (Burger et al., 2006). Accordingly, it is very important to understand the natural variation in cellular biomarkers that may be useful for environmental biomonitoring. Such studies fulfill the need for the validation and application of biomarkers within the ERA framework (Adams et al., 2001). Therefore, we aim to determine (i) if season influences the levels and activity of biomarkers commonly used in environmental monitoring, (ii) which organs could be used for monitoring purposes, (iii) which biomarkers would be reliable to monitor in each organ.

All procedures included in this work are in accordance with national and international laws on animal welfare (Directive 2010/63/EU). Three of the authors (CV, DM, MSD) have a level C certification issued by the Federation of European Laboratory Animal Science Associations.

Specimens were collected in the Portuguese West coast (38°29′ N; 8°53′ W) on the 30th of April (spring) and on the 28th of June of 2013 (summer), in a sheltered estuarine bay of the Sado estuary (Fig. 1), which has been classified as not-polluted in a recent assessment by Caeiro et al. (2009). These seasons were chosen because the sampling