Marine Environmental Research 101 (2014) 101–114

Contents lists available at ScienceDirect

Marine Environmental Research

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

Integrated biomarker responses of an estuarine invertebrate to high abiotic stress and decreased metal contamination

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A R T I C L E I N F O

Article history:
Received 23 July 2014
Received in revised form
20 September 2014
Accepted 1 October 2014
Available online 2 October 2014

Keywords:
Metals
Early-warning biomarkers
Extreme climate events
Integrated chemical biological effects monitoring
Estuaries
Contamination history

A B S T R A C T

An integrated chemical–biological effects monitoring was performed in 2010 and 2012 in two NW Iberian estuaries under different anthropogenic pressure. One is low impacted and the other is contaminated by metals. The aim was to verify the usefulness of a multibiomarker approach, using *Carcinus maenas* as bioindicator species, to reflect diminishing environmental contamination and improved health status under abiotic variation. Sampling sites were assessed for metal levels in sediments and *C. maenas*, water abiotic factors and biomarkers (neurotoxicity, energy metabolism, biotransformation, anti-oxidant defences, oxidative damage). High inter-annual and seasonal abiotic variation was observed. Metal levels in sediments and crab tissues were markedly higher in 2010 than in 2012 in the contaminated estuary. Biomarkers indicated differences between the study sites and seasons and an improvement of effects measured in *C. maenas* from the polluted estuary in 2012. Integrated Biomarker Response (IBR) index depicted sites with higher stress levels whereas Principal Component Analysis (PCA) showed associations between biomarker responses and environmental variables. The multibiomarker approach and integrated assessments proved to be useful to the early diagnosis of remediation measures in impacted sites.

1. Introduction

Concern around water quality and its sustainable management has increased throughout the years. In 2000 the European Commission adopted policies to protect and manage superficial and groundwater. The Water Framework Directive (WFD, Directive 2000/60/EC) established an innovative approach based on natural boundaries, the river basins (Laane et al., 2012). Following issuing of the directive, initial assessments were performed and restoration measures were implemented for several aquatic systems. Despite this, data on follow-up and routine monitoring, providing information on spatial and temporal scales, is still required. Particularly, there is a need for data that may improve prediction on responses to restoration measures in multiple stressor contexts (Hering et al., 2010).

Recovery of biotic communities is the ultimate goal. Though, it shows limited interest as parameter to assess potential indications of amelioration. Recent evidence indicates that functional recovery of aquatic ecosystems disturbed by human action may be achieved in a feasible temporal horizon but is usually a long process showing average times of 10–20 years (Jones and Schmitz, 2009). Moreover, although different levels of biological organisation would be expected to respond on different time scales, both community– and ecosystem-level variables appear to operate on contemporary time scales (Jones and Schmitz, 2009). Hence, other biological effect parameters that may respond in shorter time scales and could anticipate the suitability of management actions and the potential success of recovery should be sought and incorporated in follow-up programmes.

Biomarkers reflect the integrated impact of natural and man-made chemical stressors to which the organisms may be exposed, giving crucial information on health status of species. They provide early warning signals of exposure and potential adverse outcomes

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http://dx.doi.org/10.1016/j.marenvres.2014.10.001

that may reflect to population-level effects (Chapman et al., 2013). As biological effects measured at the sub-individual or individual levels (*e.g.*, molecular, cellular, metabolic, physiological, behavioural) they should respond on a much shorter time scale than community-level variables. Furthermore, batteries of cost-effective specific biomarkers may be combined in integrated multibiomarker response indices useful to identify sites where organisms are under higher or lower stress (Marigómez et al., 2013; Rodrigo et al., 2013). They also give valuable contribution to assess exposure to and effects of the complex mixture of anthropogenic contaminants found in ecosystems, providing added information on their bioavailability. For these reasons, they have been considered as a strategic tool in assessment of environmental quality of coastal waters (Allan et al., 2006; Picado et al., 2007). Under these considerations they have been recommended for inclusion in Portuguese monitoring programmes (Picado et al., 2007).

Contamination by metals and its impact in estuarine organisms is a continuous priority due to their persistence in environment and abiding life-threatening effects. The estuary of the Lima River, in NW Iberia Peninsula, shows high susceptibility to human influence (Ferreira et al., 2003). It is under important industrial pressure (*e.g.*, harbour, shipyard, paper mill), and also receives wastewaters of husbandry and livestock origin. Its sediments were previously shown to be contaminated by metals, and to a lesser extent with polycyclic aromatic hydrocarbons (PAHs), which were associated to reduced health status of local relevant fish species, such as *Anguilla anguilla* and *Pomatoschistus microps* (Guimarães et al., 2009, 2012). Hence, further investigation of sediments contamination by metals and their effects in key structuring species was needed. In recent years, in addition to some protection measures and in consequence of generally hard economic conditions, several industries have diminished (including the shipyard) or ceased their activity. Moreover, intense temperature variation and drought events have been reported in the area. This provided thus an interesting case study to investigate temporal and spatial trends in metal contamination of sediments and how multibiomarker responses would reflect such changes in anthropic activity and abiotic variation.

This study was, therefore, intended to carry out an integrated chemical–biological effects evaluation in two sites located in the terminal part of Lima estuary and two sites located in the terminal part of Minho estuary, which is under low pollution pressure (Guimarães et al., 2012), during the years of 2010 and 2012. The aim was to investigate the usefulness of a multibiomarker approach, using *Carcinus maenas* as bioindicator species, to reflect diminishing environmental contamination and potential improvement of stressor effects in Lima estuary.

C. maenas is widely used in ecotoxicological studies because it is sensitive to a wide range of aquatic pollutants and its biological responses are linked to exposure concentrations (Rodrigues and Pardal, 2014 and references herein). Given its important ecological role and widespread geographical distribution the species was recommended for biomarker measurements in Portuguese monitoring programmes (Picado et al., 2007). Additionally, there is a need for information on the effects of Lima contamination in benthic invertebrates with key role in its trophic chains. Hence, the study sites were chosen according to life history characteristics of *C. maenas* (Queiroga, 1996) and to be comparable as much as possible. Assessments of metal levels in sediments and *C. maenas*, water abiotic factors and biomarkers were performed in two key seasons in each year. The set of biomarkers selected was previously employed in resident fish species: acetylcholinesterase (AChE, neurotoxicity), lactate (LDH) and $NADP^{+}$-dependent isocitrate (IDH) dehydrogenases (anaerobic and aerobic energy production, respectively), glutathione *S*-transferases (GST, phase II biotransformation), glutathione peroxidase (GPx), glutathione reductase (GR) and total glutathiones (TG) (as anti-oxidant defences), and lipid peroxidation (LPO, as measure of oxidative damage to macromolecules). The Integrated Biomarker Response (IBR) index was used to depict sites with different stress levels and spatial and temporal evolution of integrated responses to environmental change. Principal Component Analysis (PCA) allowed investigating patterns of biomarker responses and their association to heavy metal contamination and abiotic stress.

2. Material and methods

Minho and Lima estuaries (NW Iberian coast) were selected as different contamination scenarios. The Minho estuary is located approximately 20 km to the north of the Lima estuary. In each estuary sampling sites, one upstream and another downstream, were selected to be at approximately the same distance from the mouth of the estuary (Fig. 1). Minho estuary sites were located in Seixas (upstream, M_U) and Moledo (downstream, M_D) villages. Lima estuary sites were located in Porto Velho (upstream, L_U) and Cabedelo (L_D) villages. The selection of these sites in each estuary was related to life history of the species. Younger crabs are expected to occupy upstream regions with lower salinity, whereas older crabs inhabit the mouth of the estuary where reproduction takes place (Queiroga, 1996). Sampling, and all animal experiments were conducted in compliance with ethical guidelines of the European Union Council (Directive 2010/63/EU of 22nd September) for the protection of animals used for experimentation and other scientific purposes.

2.1. Chemicals

The reagents used were of analytical grade or suprapur® grade (65% nitric acid) and were purchased from Sigma–Aldrich Chemical (Steinheim, Germany), except the Bio–Rad protein assay dye reagent that was purchased from Bio–Rad Laboratories, Inc. Metal standard solutions (zinc, copper, lead, nickel, chromium, cadmium) were prepared with the 1000 mg L^{-1} stock solutions (Panreac) and ultrapure water.

2.2. Water, sediments and crab sampling

In each sampling campaign, water physico–chemical parameters (*i.e.* temperature, salinity, conductivity, pH, dissolved oxygen) were measured, in triplicate, with a multiparametric sea gauge WTW multi 340i with appropriate probes (pH Sen Tix 41 and Tetracon 325) (Table 1). Water samples were collected, in triplicate, and stored at −20 °C until further analysis. These samples were used for later determination of nitrites, nitrates, ammonium, phosphates, iron, silica and hardness by colourimetric methods using a Hanna 200 photometer. Sediment samples were collected, also in triplicate, from the top layer to determine metals concentrations.

Samplings were carried out over three days in each location, during the colder and warmer seasons in mid-February and mid-August of 2010 and 2012 at the predicted time to high tide. Intermoult green male crabs (n = 16–22 per site and season), with complete appendices, were captured using trawl nets in the four sampling sites. Male crabs with similar size, colour morph and moult stage were selected to control for gender-related sources of variability. Once in the laboratory, crabs were allowed to deplete gut contents for 24 h. After this period, crabs were ice–anaesthetised, measured (length, width and weight) (Table 2) and sacrificed. Sub-samples of muscle (AChE, LDH, IDH) and digestive gland (GST, GPx, GR, TG, LPO) were collected for biomarkers determination and stored at −80 °C until further analysis.