Environmental pollution and natural populations: A biomarkers case study from the Iberian Atlantic coast

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Abstract

The degradation of estuaries is a result of human activities which overloads the environment with substances of both industrial and/or natural origins. Bioindicators have been consistently used to interpret effects of contaminants in the environment. In this study, the use of biomarkers (particular measurable characteristics of a bioindicator organism) was used to evaluate the contamination by xenobiotics of *Crangon crangon* natural populations. The central aim was to evaluate the capability of a battery of biomarkers to discriminate sites with different types of contamination. The activity of the enzymes cholinesterases (ChE), lactate dehydrogenase (LDH) and glutathione S-transferases (GST) were used as biomarkers. In addition, the ChE form(s) present in the cephalotorax of *C. crangon* were characterised. Organisms were seasonally sampled from winter 2001/2002 to autumn of 2002, at "reference" sites and at sites that receive agricultural, industrial and/or urban effluents. Results obtained in the characterisation of ChE with different substrates and selective inhibitors demonstrate that the form of ChE present in the cephalotorax of *C. crangon* shows proprieties of vertebrates' AChE and therefore it may be classified as true AChE-like ChE. The battery of biomarkers exhibited seasonal and local variations, apparently related to agricultural, industrial or urban effluent contamination. The tested biomarkers proved to be able to discriminate sources of environmental contamination, and confirms *C. crangon* as a sensitive species suitable to be used as a bioindicator.
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Keywords: *Crangon crangon*; Battery of biomarkers; Monitoring program; AChE; LDH; GST

1. Introduction

Human activity has overloaded estuaries with substances of both industrial and/or natural origins in an increasing rate, resulting in an accelerated degradation of the ecosystems. Estuaries in particular have been receiving a complex mixture of contaminants over decades of unmeasured usage: of agricultural fertilizers and pesticides, heavy metals and organic synthetic compounds from industry and shipping and/or urban effluents from human settlings (Kennish, 1992). As transition environments, estuaries act like an interface between marine and fresh water environments, presenting themselves as shelter, reproduction, nursery and recruiting areas for numerous species (Kennish, 1992). Due to their high biodiversity, the development of monitoring methods to evaluate their current status becomes urgent.

Crangon crangon (Linnaeus, 1758) is an epibenthic decapod (Arnott et al., 1998; Cattrijsse et al., 1997), euryhaline (Boddeke, 1996; Gelin et al., 2000), that performs tidal, nocturnal and seasonal migrations mainly related to salinity, temperature and food availability (Wolff and Zijlstra, 1981). Geographically, the species is widely distributed throughout estuarine and coastal waters of Europe from the Baltic (Gelin et al., 2000; Gelin et al., 2001; Wolff

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and Zijlstra, 1981) to the Mediterranean (Gelin et al., 2000; Wolff and Zijlstra, 1981). Wide distribution, yield capability, playing a key role in terms of benthic food chains are characteristics that have led to widespread use of *C. crangon* as a test organism (Smith et al., 1995). In addition, it was also a suitable species in a previous study using vitellogenin as biomarker (Matthiessen et al., 2002).

Normally, the tests used to evaluate toxicity in estuaries are costly. For this reason, laboratory assays are performed to interpret large scale events taking place in situ while simultaneously trying to predict the evolution and response of these sensitive ecosystems.

In the last few decades the use of biomarkers intensified as a tool to evaluate the toxicity of xenobiotics in natural populations, at sub-individual level, with the objective of extrapolating its implications to higher levels of biological organization (Stegeman et al., 1992; Walker et al., 2001).

The inhibition of cholinesterases (ChE) activity, the alteration of lactate dehydrogenase (LDH) activity and the induction of glutathione S-transferases (GST) activity of several species are tools known to be appropriate biomarkers for use in several environmental contexts (Peakall, 1992). ChE inhibition was initially used to detect toxic activity by organophosphate and carbamate pesticides in several organisms, including fish (Fulton and Key, 2001), copepods (Forget et al., 2003; Fulton and Key, 2001), molluscs (Escantín and Porte, 1997; Fulton and Key, 2001) and crustaceans (Fulton and Key, 2001; Varó et al., 2002). However, several studies recently revealed its sensitivity to other compounds such as some metals, surfactants and complex mixtures of pollutants in fish (Gill et al., 1990; Payne et al., 1996), crustaceans (Guilhermino et al., 2000) and molluscs (Moreira et al., 2004; Moreira and Guilhermino, 2005).

The existence of several forms of ChE in different tissues is well known in vertebrates (Sturm et al., 2000; Monteiro et al., 2005) and invertebrates, including crustaceans (Fulton and Key, 2001; Mayer et al., 1992). Different forms of ChE may have distinct sensitivity to different anti-cholinergic agents. This makes it important to evaluate the type of ChE present in the tissue to be studied. This can be achieved by the use of a set of selective inhibitors and substrates. Hence, in the first phase of the study, the biochemical characterisation of ChE present in the cephalotorax of *C. crangon* was performed. Cephalotorax was selected as it has a high content of nervous tissue where ChE presents a determinant physiological role and it has been found to be a suitable tissue for ChE determinations in preliminary assays (data not shown).

LDH is an enzyme with a fundamental role in the glycolytical chain, catalysing the reversible conversion of pyruvate to lactate (Mayer et al., 1992; Ribeiro et al., 1999). It has been used as a biomarker of hypoxia in mussels (Wu and Lam, 1997), and in situations of chemical stress, as in fish (Cohen et al., 2001; Gagnon and Holdway, 1999), *Daphnia* (Diamantino et al., 2001; Guilhermino et al., 1994) and isopods (Ribeiro et al., 1999) when organisms require additional energy.

GST is the enzyme responsible for the junction of reduced glutathione (GSH) with certain xenobiotic compounds, giving the enzyme a central role in the detoxification processes (George, 1994). In fish, the activity of this enzyme has been shown to be induced by exposure to aromatic polycyclic hydrocarbons (PAH), polychlorated byphenils (PCB) and to phenobarbital (George, 1994; Pedrajas et al., 1995; Stegeman et al., 1992). The central aim of this study was to evaluate the capability of a battery of biomarkers (AChE, LDH and GST) determined in natural populations of *C. crangon* to discriminate sites with different types of contamination and evaluate this species as a bioindicator. In order to use *C. crangon* ChE as an environmental biomarker, the form(s) present in the cephalotorax of this species were characterised using a biochemical approach.

2. Material and methods

2.1. Sampling stations

Five sampling stations were selected in the Iberian Atlantic coast (Fig. 1), and the different types of contamination are summarized in Table 1. The station located in Minho river estuary was considered a reference station (R1), since no significant levels of contamination were detected in this area (Cairrão et al., 2004). One station in Ria de Aveiro (R2) was considered as reference too, with low contamination levels (Cerqueira and Pio, 1999). A station with marked urban and industrial influence was located in the Douro river estuary (Ua). In the Ria de Aveiro at Mira channel (as well as the already mentioned R2) were located the sampling stations with agricultural influence (Aa), and industrial influences (Ia) were located in the Laranjo bay; this has been described as a heavy metals polluted site (Monterroso et al., 2003).

2.2. Biological material

To characterise the prevailing type of ChE, 45 individuals with a total length (distance between anterior region of the *rostrum* and the posterior region of the *telson*) of 20–27 mm were collected in the Minho river estuary (R1) with a 1 cm mesh net at 1.0 m maximum depth in ebb tide. Sampling was undertaken in the four seasons (winter 2001/2002, spring 2002, summer 2002 and autumn 2002). In each sampling station, 40 specimens were collected with the same size and in the same conditions. After collection, organisms were transported to the laboratory in local water, in 100 L containers. In the laboratory, the cephalotorax was separated from the abdomen and both anatomical parts were prepared for enzymatic analysis.

The cephalotorax was homogenised in 1 ml of cold potassium phosphate buffer (0.1 M, pH 7.2) and the homogenates (45 from Minho river Estuary, for ChE characterisation and 20 from each station, for the monitoring program) were stored at −20 °C until enzymatic analysis.