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Impact of chemical exposure on the fish *Pomatoschistus microps* Krøyer (1838) in estuaries of the Portuguese Northwest coast

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Abstract

Juveniles of the estuarine fish *Pomatoschistus microps* were collected from autumn 2001 to summer 2002 in five stations along the Portuguese Northwest coast with different types and/or levels of environmental contamination: two reference sites with low levels of contamination (R1 and R2) and three differently impacted areas with higher levels of contamination. UI is located in an estuary under the influence of urban and industrial effluents, AA in a channel that receives intensive agriculture run-off and IE in a highly impacted industrial area. The activity of the enzymes acetylcholinesterase (AChE), lactate dehydrogenase (LDH), 7-ethoxyresorufin *O*-deethylase (EROD) and glutathione S-transferases (GST) were used as environmental biomarkers on *P. microps*. A significant seasonality effect on all the enzymatic activities was found, lower levels being registered in winter and spring on AChE, in autumn on LDH, and in winter on GST and EROD. The battery of biomarkers used was capable of discriminating sites with different types and/or levels of contamination, R1 and UI being the highest discriminated (91.7% and 66.7%, respectively). At R1 significantly lower levels of AChE and LDH were found, and EROD was significantly inducted at UI. Furthermore, IE presented higher levels of GST, and R2 and AA an inhibition of AChE in winter and spring. The results indicated that the battery of biomarkers used in this study seems to be a useful tool to distinguish between different types of environmental contamination in estuarine systems, and that *P. microps* is a suitable species to be used as bioindicator.

Keywords: Estuaries; *Pomatoschistus microps*; Seasonal variation; AChE; LDH; EROD; GST; Environmental contamination

1. Introduction

In recent years, the levels of contaminants in estuaries and coastal zones increased as a consequence of anthropogenic activities (Kennish, 1992). Estuaries are productive natural habitats, where large phytoplankton populations support a variety of other organisms, including many commercially important marine fish and crustacean species that use them as nursery grounds (Cattrijsse et al., 1994). For this reason, methods to evaluate the degree of exposure and contamination of natural populations to minimise the impact of human activities are needed.

Biomarkers have been widely used in ecotoxicology as early warning signals of chemical effects, offering the possibility of anticipating severe alterations potentially induced at a population level (Peakall, 1992). They are particularly important in the case of contamination by complex mixtures of chemicals where determinations of the presence of individual agents by chemical analysis provide limited information regarding the effects induced on organisms. Among the biomarkers potentially available for use, inhibition of acetylcholinesterase (AChE), alterations of lactate

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dehydrogenase activity (LDH), as well as the induction of glutathione S-transferases (GST) activity and monooxygenase enzymes of the P450 system (P450) have been shown to be appropriate for use in a large variety of species and real scenarios (Galgani et al., 1992; Wu and Lam, 1997; Sanchez-Hernandez et al., 1998; Doyotte et al., 2001; Niyogi et al., 2001; Porte et al., 2001; Ferreira et al., 2006).

Inhibition of AChE activity has been widely used to diagnose the exposure to anticholinesterase compounds, such as organophosphorous (OP) and carbamate (CB) pesticides (Fulton and Key, 2001). More recently, several studies indicate that this biomarker is also sensitive to other compounds, including some metals and surfactants (Gill et al., 1990; Labrot et al., 1996; Guilhermino et al., 1998). Furthermore, field studies have been showing the suitability of this biomarker for use in freshwater and marine environments that apparently are not contaminated by pesticides (Galgani et al., 1992; Payne et al., 1996).

LDH is the terminal enzyme of anaerobic glycolysis, therefore being of crucial importance to the muscular physiology, particularly in conditions of chemical stress when high levels of energy may be required in a short period of time (De Coen et al., 2001). Alterations of normal LDH activity pattern have already been found in several fish species collected or *in situ* exposed in polluted areas (Castro et al., 2004; Gül et al., 2004), in fish exposed to crude oil (Gagnon and Holdway, 1999) or under hypoxia conditions (Cooper et al., 2002).

GSTs are a family of enzymes that catalyse the conjugation of reduced glutathione (GSH) with a variety of both endogenous and foreign compounds, being of crucial importance in the detoxification of several xenobiotics (George, 1994). They also have an important function in preventing lipid peroxidation. GSTs have been used as a biomarker in field studies with both vertebrates and invertebrates (Lenartova et al., 1997; Castro et al., 2004; Moreira et al., 2004; Moreira and Guilhermino, 2005).

EROD (7-ethoxyresorufin *O*-deethylase) activity may be used as indicative of the cytochrome P4501A1 enzyme system function, which is responsible for the Phase I biotransformation of ubiquitous environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs) and several dioxin-like compounds (PCDD/Fs, polychlorinated dibenzo-*p*-dioxin and furans, and PCBs, polychlorobyphenyls) (Stegeman and Hahn, 1994; Bucheli and Fent, 1995). EROD activity in liver has been recognized as an useful biomarker in various species of fish exposed to these compounds (Goksoyr and Forlin, 1992; Bucheli and Fent, 1995; Sanchez-Hernandez et al., 1998).

The main objective of this study was to investigate the annual variation of the activity of AChE, LDH, GST and EROD in wild *Pomatoschistus microps* collected in five sites of three estuaries in the Portuguese Norwest coast with different types and/or levels of environmental contamination.

P. microps (Krøyer, 1838), an epibenthic and euryhaline fish, was selected as bioindicator organism in this study due to its position as an intermediate predator in estuarine food-webs, wide geographical distribution, and abundance in estuaries and near-shore waters, which are potentially contaminated environments (Miller, 1986; Arruda et al., 1993). Furthermore, previous laboratorial studies with *P. microps* AChE, LDH and GST have already been performed (Monteiro et al., 2005, 2006).

2. Material and methods

2.1. Study area

Five sampling sites were selected for this study (Fig. 1): R1 (41°53′27.28″N; 8°49′30.81″W) – located in the Minho river estuary and having low levels of environmental contamination; it was used as a reference site in our previous studies (Cairrão et al., 2004); UI (41°08′3.45″N; 8°39′43.91″W) – located in the Douro river estuary, near Porto city and under the influence of urban and industrial effluents; R2 (40°38′32.89″N; 8°44′09.81″W) – located in the Aveiro lagoon near to the artificial connection of the lagoon to the sea; it has low levels of environmental contamination; AA (40°34′14.97″N; 8°45′23.66″W) – located in the Mira channel of the Aveiro lagoon; this channel receives intensive agriculture run-off, therefore, this site might be under the influence of pesticides and other chemicals used in crop fields. IE (40°43′45.75″N; 8°39′03.01″W) – located in the Aveiro lagoon, in an area known as Laranjo bay which is described as polluted with metals (Monterroso et al., 2003) and other chemicals used in local industries.

2.2. Biological material

Twenty juvenile fish (length ranging from 22 to 30 mm) were seasonally collected (Autumn 2001, Winter 2002, Spring 2002, Summer 2002) at each sampling site, using a landing net during low tide. Fish were transported alive to the laboratory and sacrificed by decapitation, after being measured. Head, muscle, gills, and liver were isolated and used for AChE, LDH, GST and EROD determinations, respectively.

Head was homogenised in 1 ml of cold potassium phosphate buffer (0.1 M, pH = 7.2) on ice and homogenates were stored at −20 °C until being further used. Just prior to enzymatic determinations, homogenates were centrifuged (4 °C, 4629*g*, 3 min) in a centrifuge (Beckman Centrifuge Avanti™ J-25I). The supernatants were collected and used for AChE activity determinations.

Dorsal muscle tissue was homogenised in 1 ml of cold Tris-NaCl buffer (0.1 M, pH 7.2) on ice, and were stored at −20 °C until being used for LDH analysis. Just prior to enzymatic determinations, samples were centrifuged (4 °C, 4629*g*, 3 min) and the supernatants were collected and used to determine LDH activity.

Samples for GST activity determinations were prepared by homogenisation of a pair of gills in 0.5 ml of potassium phosphate buffer (0.1 M, pH 6.5). Homogenates were