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Hepatic biotransformation and antioxidant enzyme activities in Mediterranean fish from different habitat depths

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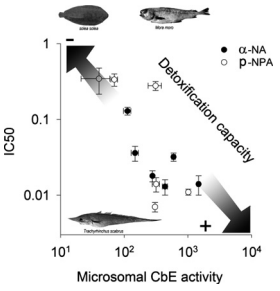
HIGHLIGHTS

- There was a negative trend between CbE activities and IC50 values for dichlorvos.
- UDPGT and some antioxidant defences decreased with habitat depth.
- CbE activities in deep-sea fish are mostly microsomal.
- Factor analysis of the hepatic biomarkers defined species differences.
- *T. scabrus* seemed to be the species better equipped for xenobiotic detoxification.

GRAPHICAL ABSTRACT



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ABSTRACT

Marine fish are threatened by anthropogenic chemical discharges. However, knowledge on adverse effects on deep-sea fish or their detoxification capabilities is limited. Herein, we compared the basal activities of selected hepatic detoxification enzymes in several species (*Solea solea*, *Dicentrarchus labrax*, *Trachyrhynchus scabrus*, *Mora moro*, *Cataetix laticeps* and *Alepocehalus rostratus*) collected from the coast, middle and lower slopes of the Blanes Canyon region (Catalan continental margin, NW Mediterranean Sea). The xenobiotic-detoxifying enzymes analysed were the phase-I carboxylesterases (CbEs), and the phase-II conjugation activities uridine diphosphate glucuronyltransferase (UDPGT) and glutathione *S*-transferase (GST). Moreover, some antioxidant enzyme activities, i.e., catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR), were also included in this comparative study. Because CbE activity is represented by multiple isoforms, the substrates α-naphthyl acetate (αNA) and ρ-nitrophenyl acetate (ρNPA) were used in the enzyme assays, and in vitro inhibition kinetics with dichlorvos were performed to compare interspecific CbE sensitivity.

Activity of xenobiotic detoxification enzymes varied among the species, following a trend with habitat depth and body size. Thus, UDPGT and some antioxidant enzyme activities decreased in fish inhabiting lower slopes of deep-sea, whereas UDPGT and αNA-CbE activities were negatively related to fish size. A trend between CbE activities and the IC50 values for dichlorvos suggested *S. solea* and *M. moro* as potentially more sensitive to anticholinesterasic pesticides, and *T. scabrus* as the most resistant one. A principal component analysis considering all enzyme activities clearly identified the species but this grouping was not related to habitat depth or phylogeny. Although these results can be taken as baseline levels of the main xenobiotic detoxification enzymes in Mediterranean fish, further research is needed to evaluate their response to environmental contaminant exposure.

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1. Introduction

Biotransformation of non-polar xenobiotics generally involves enzymatic systems (phase-I and phase-II enzyme systems) by which these compounds gain water solubility, and therefore can be more easily excreted. The phase-I includes mainly oxidation, reduction, hydrolysis and acetylation reactions, and cytochrome P450-dependent monooxygenases (CYP450s, EC 1.14.14.1) and carboxylesterases (CbE, EC 3.1.1.1) are the most common enzymes implicated in this first step. Phase-II reactions are characterised by the addition of endogenous polar compounds (e.g., glutathione or glucuronyl sugars) to metabolites formed during the phase-I reactions or directly to the original xenobiotic. Enzymes such as uridine-5-diphosphate glucuronyltransferase (UDPGT, EC 2.4.1.17) and glutathione *S*-transferase (GST, EC 2.5.1.18) have a pivotal role in these conjugation reactions. Biotransformation of xenobiotics may lead to an enhanced production of reactive oxygen species (ROS), which are counteracted by antioxidant enzymes such as catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GPX, EC 1.11.1.9) and glutathione reductase (GR, EC 1.8.1.7) (Livingstone, 2001). Exposure of organisms to environmental contaminants causes significant changes in these enzymatic processes, so they are commonly used as biomarkers of contaminant exposure (Whyte et al., 2000; van der Oost et al., 2003). Knowledge on normal variations of these enzyme activities may be useful to identify those species with higher detoxification capabilities and consequently more tolerant to environmental pollution.

Past studies have documented that coastal marine species from Mediterranean Sea are continuously exposed to low or moderate inputs of pollutants, mostly due to their proximity to anthropogenic sources (Gómez-Gutiérrez et al., 2007). Nonetheless, species inhabiting deep-sea areas are also threatened by pollution, particularly in sites of significant hydrodynamic events such as the marine phenomenon known as *dense shelf water cascading*, which takes place every 6–10 years (Canals et al., 2006). During this event, high inputs of organic matter, and their associated non-polar contaminants, are transported from the coast to deep-waters, representing a serious risk for fauna inhabiting deep-sea habitats (Company et al., 2008). Likewise, it has been long recognised that deep-sea environments are impacted by chemicals such as persistent organic pollutants, toxic metals, radio elements, pesticides, herbicides and pharmaceuticals, and deep-sea sediments represent a pollutant sink (Ramirez-Llodra et al., 2011). Indeed, many studies have proved that Mediterranean deep-sea fish may accumulate high levels of pollutants, in some cases even greater than coastal fish, without apparently compromising their health status (Borghi and Porte, 2002; Castro-Jimenez et al., 2013; Koenig et al., 2013a,b; Siscar et al., 2014; Storelli et al., 2009). Taken together, these studies point out the hypothesis that deep-sea fish species display efficient detoxifying mechanisms against environmental contaminant exposure.

Within the framework of this hypothesis, the aim of this study was to determine basal levels of key biotransformation enzyme activities involved in the metabolism of xenobiotics in six Mediterranean fish species, which are representative of three water depths. Two important species in fisheries (*Solea solea* and *Dicentrarchus labrax*) were selected in the coastal range (30–120 m), the species *Trachyrhynchus scabrus* and *Mora moro* were chosen as representative of the middle slope (900–1500 m), and *Alepocephalus rostratus* and *Cataetyx laticeps* as typical species in the deep-sea slope (1500–3000 m) (D'Onghia et al., 2004; Fanelli et al., 2013; Fernandez-Arcaya et al., 2013; Stefanescu et al., 1992). In a previous study, we investigated CYP450-isoform composition and their responses to common mammalian CYP450 inducers (Ribalta and Solé, 2014). However, studies on detoxifying enzymes other than monooxygenases are still limited (Koenig and Solé, 2012; Koenig et al., 2012, 2013a; Siscar et al., 2014). Results of this study should be useful in the field monitoring surveys of marine pollution using ecotoxicological biomarkers or in decision-making related to ecosystem management.

2. Materials and methods

2.1. Sampling and tissue preparation

The coastal species *S. solea* was obtained in the frame of the DEPURAMAR project (sampling coordinates 41°10′N; 1°53′E), whereas *D. labrax* was acquired from the laboratory facilities of our Institute (41°23′N; 2°11′E). Fish inhabiting the middle and lower deep waters were caught by trawling in the Blanes canyon region (NW Mediterranean, sampling coordinates 41°15′N; 2°50′E, PROMETEO project). All fish were collected during winter, so physical water parameters from the coast and the stable deep-slope were similar. Table 1 summarises some biological traits of the fish used in this study. Although most specimens were adults, we considered only juveniles of *D. labrax*. Enzyme activities were measured in both the microsomal and cytosolic fractions of livers. These organs (≈1.5 g) were homogenised in ice-cold 100 mM K-phosphate buffer (pH 7.4) containing 150 mM KCl, 1 mM dithiothreitol (DTT), 0.1 mM phenanthroline, 0.1 mg/ml trypsin inhibitor and 1 mM ethylenediaminetetraacetic acid (EDTA) in a 1:4 (w:v) ratio using a Polytron® blender. The homogenate was centrifuged at 10,000 *g* for 30 min and 4 °C, and the supernatant obtained was centrifuged at 100,000 *g* for 60 min and 4 °C. The pellet (microsomes) was suspended in the homogenisation buffer (2:1 w/v ratio) containing 20% (w/v) glycerol and used, together with the supernatant (cytosolic fraction), for enzymatic determinations.

2.2. Carboxylesterase activity

This esterase activity was measured in the cytosolic and microsomal fractions using the substrates α-naphthyl acetate (αNA) and ρ-nitrophenyl acetate (ρNPA). Hydrolysis of αNA was assayed according to Mastropaolo and Yourno (1981), adapted to microplate format. The reaction medium was composed by 100 mM K-phosphate buffer (pH 7.4), 200 μl of αNA (250 μM final concentration), and 25 μl of sample (appropriately diluted as to be linear over the kinetic assay). The reaction was monitored for 5 min at 235 nm. Hydrolysis of ρNPA was measured by the method of Hosokawa and Satoh (2002), with some modifications. The reaction mixture consisted of 100 mM K-phosphate buffer (pH 7.4), 200 μl of ρNPA as substrate (1 mM final concentration), and 25 μl of sample. The formation of ρ-nitrophenol was read for 5 min at 405 nm. Kinetics were measured in triplicate using a TECAN Infinite 200 microplate reader, and CbE activity was expressed as nmol/min/mg protein using the extinction coefficients of 23.4 mM^{-1} cm^{-1} and 18 mM^{-1} cm^{-1} for αNA and pNPA hydrolyses, respectively.

Carboxylesterases belong to a group of esterase enzymes called B-type esterases (Sogorb et al., 2007), which are inhibited by organophosphate (OP) insecticides. To ensure that hydrolyses of αNA and ρNPA were catalysed by true CbEs, liver microsomes were previously incubated in the presence of the OP dichlorvos (2,2-dichlorovinyl dimethyl phosphate, CAS no. 62-73-7,Sigma-Aldrich Química S.A., Madrid, Spain). Stock solution of dichlorvos was prepared in distilled water, and serial concentrations (1000 to 0.01 μM) were diluted in the reaction buffer. Inhibition kinetics were run at room temperature (23 °C) for 30 min in triplicate. After this period, the inhibition reaction was stopped by addition of the substrate (αNA and ρNPA), and the residual CbE activity was determined as described above.

2.3. Uridine-5-diphosphate glucuronyltransferase activity

This enzyme activity was measured in the microsomal fraction following the method by Collier et al. (2000). The reaction mixture contained 10 μl of microsomes, 0.1 mM 4-methylumbelliferone (4-MU) prepared in 0.1 M Tris–HCl buffer pH 7.4 (supplemented with 5 mM $MgCl_2$ and 0.05% bovine serum albumin), and 2 mM uridine-5′-diphospho-glucuronic acid (UDPGA). The activity was quantified as the decrease in fluorescence (4-MU consumption) recorded over