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Hepatic and branchial xenobiotic biomarker responses in *Solea* spp. from several NW Mediterranean fishing grounds

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ABSTRACT

The common sole, *Solea solea* and the Senegalese sole, *Solea senegalensis* are two important commercial benthic species that coexist in the NW Mediterranean Sea. Several common biomarkers of chemical exposure were measured in two organs (liver and gills) involved in a different degree in biotransformation and detoxification processes. These parameters were: phase I cytochrome P450 CYP1A-dependent ethoxyresorufin *O*-deethylase and carboxylesterase activities, phase II glutathione *S*-transferase activity and the enzymatic antioxidants: catalase, glutathione reductase and glutathione peroxidase. Principal Component Analysis (PCA) considering biometric variables (size and weight) and all liver and gill biomarkers discriminated at a certain extent individuals of both species collected at the different fishing grounds. Esterase inhibition by the organophosphorus pesticides dichlorvos and diazinon was also compared *in vitro* in muscle, liver and gill of the two species revealing a differential sensitivity. The use of benthic sole in pollution monitoring of Southern Europe is discussed as local sentinel in respect to other benthic fish from more Northern latitudes.

1. Introduction

Benthic fish, and particularly Solea species (*Solea solea* and *Solea senegalensis*), are important economic resources that coexist in the Mediterranean region and are highly appreciated for human consumption (Imsland et al., 2003). Thus, presence of significant levels of toxic chemicals in these species can not only affect their physiological performance but also negatively affect consumer's health. A few studies in the Mediterranean have already reported on the presence of pesticides, metals and persistent organic pollutants in these two fish species (Ben Ameur et al., 2013; Dierking et al., 2009; Trisciani et al., 2011; Sànchez-Nogué et al., 2013; Siscar et al., 2013). In particular, the use of *S. senegalensis* as sentinel species, amply exceeds that of *S. solea* and it corresponds mostly to studies conducted in Atlantic waters (Costa et al., 2009, 2010; 2012; Fonseca et al., 2011a, 2011b; Oliva et al., 2010, 2012; Gonçalves et al., 2013, 2014). By contrast, the use of *S. solea* as sentinel species in Southern Europe is more restricted (Trisciani et al., 2011; Ribeco et al., 2012; Jebali et al., 2013; Siscar et al., 2013). In addition, most of these field studies have focussed on juvenile specimens of *S. senegalensis* from estuarine areas, as little mobility habits have been demonstrated in this age group (Vinagre et al., 2013). However, studies with adults of *S. solea* have also validated their adequacy as sentinels. Tissue chemical analysis of adult fish could discriminate between different populations, suggesting little mobility habits also during adulthood; in spite of the annual reproductive migrations to more coastal waters (Dierking et al., 2009). From recent laboratory exposures, there is also growing evidence that Solea spp. are responsive to pollutants (Costa et al., 2009; López-Galindo et al., 2010a; b; Wessel et al., 2010; Solé et al., 2014). All these field and laboratory studies point out that *S. senegalensis* and *S. solea* could serve as good local sentinels in relation to other flatfish species (e.g. *Platichthys flesus, Parophrys vetulus, Pleuronectes* spp., *Scophthalmus maximus* and *Limanda limanda*) encountered in Northern latitudes (Akcha et al., 2003; Kopecka and Pempkowiak, 2008; Lehtonen et al., 2006; Lerebours et al., 2014; Napierska and Podolska, 2005).

Biomarkers of chemical exposure such as cytochrome P450 CYP1A1-related ethoxyresorufin O-deethylase (EROD) activity, carboxylesterase (CbE), conjugation glutathione *S*-transferase (GST) and the antioxidant defences: catalase (CAT), glutathione reductase

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(GR) and glutathione peroxidase (GPX) are of widespread use in pollution monitoring studies (Van der Oost et al., 2003). Responses to pollutants can be measured in liver, as the main biotransformation organ, but also in gill as the first target tissue for water borne chemicals and site for presystemic metabolism (Barron et al., 1999; Regoli et al., 2011). The approach, using both tissues, has been applied in laboratory exposures with *S. senegalensis* (López-Galindo et al., 2010a; b) and also recently addressed in a field study with *S. solea* (Jebali et al., 2013). In the actual context of climate change, and in an anthropogenic-impacted environment, a greater adaptability of one species in respect to a sympatric one, such is the case of both sole species, would be of great ecological significance. Recent studies have revealed a great adaptability of *S. senegalensis* to temperature variations (Arjona et al., 2010; Siscar et al., 2014; Solé et al., 2015) and different *in vitro* pesticide sensitivity in juveniles and adults of both species (Sànchez-Nogué et al., 2013; Koenig et al., 2013).

The pesticides dichlorvos and diazinon were selected to contrast muscle acetylcholinesterase (AChE) sensitivity and the potential protective role of CbEs (Wheelock et al., 2008) in both sole. In *S. senegalensis*, AChE (EC 3.1.1.7) is predominant in brain and muscle and CbE (EC 3.1.1.1) is more abundant in liver (Solé et al., 2012). AChE is mainly involved in neural transmission and CbE catalyses the hydrolysis of a wide range of xenobiotic esters, amides and thioesters. Dichlorvos commercialisation has been banned in the EU (ECC directive 1376/07 (07/387)) due to its toxic action over non-target species. However, the pesticide diazinon, because of its higher lipophilic character and current use, it is found in the environment and bioaccumulated in sole from estuarine and marine areas (Sànchez-Nogué et al., 2013; Ben Ameur et al., 2013). Moreover, dichlorvos does not require metabolic activation to be toxic, whereas diazinon needs to be metabolised to the oxon form (by cytochrome P450 enzymes) to be able to inhibit AChE.

The aims of the present study were: (1) to contrast the enzymatic biomarker responses in liver and gill in two sole species sampled at several Mediterranean fishing grounds considered from low to moderately polluted, (2) to contrast *in vitro* pesticide sensitivity in several tissues of sole adults and (3) to discuss the response of the two sole species to environmental pollutants, their use as sentinels and the influence of biological traits in xenobiotic biomarker responses.

2. Material and methods

2.1. Study area and sample collection

Six fishing grounds located in the Catalan coast, NW Mediterranean, were selected for the study. The sites were from North to South: Costa Brava, Maresme, Garraf, Vendrell, Tarragona and Cambrils (Table 1 for coordinates). Fish sampling was carried out between January and March 2011 using gillnets at the six fishing areas with the collaboration of local artisanal fishermen. After sampling, the fish were immediately transported alive to a nearby laboratory in a refrigerated and aerated recipient container, and once there, biological parameters (total length and total weight) were recorded. Immediately after sacrifice, fish were dissected and their sex determined. Sample tissues of muscle, liver and gills, were fast frozen in liquid N_2 and maintained at −80 °C for biomarker analysis. Handling of the fish was done according to national and institutional regulations of the Spanish Council for Scientific Research (CSIC) and the European Directive 2010/63/EU. Site characterisation was based on organic pollutants present in the local sediment (Solé et al., 2013) and metals measured in kidney of these same species as well as in the local sediment (Siscar et al., 2013).

2.2. Sample preparation

A portion of individual liver and gills (≈0.2–0.3 g) were homogenised in ice-cold 100 mM buffer phosphate (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) at a 1:4 (w:v) ratio using a polytron® blender. The homogenate was centrifuged at 10,000 g × 30′ at 4 °C. The supernatant obtained (S10) was used for the enzymatic determinations.

2.3. Analysis of hepatic and gill biomarkers

Assay conditions were kept similar and only the sample volume was changed in order to achieve linearity in the enzymatic measurements. All assays were carried out in triplicate at 25 °C, except EROD which was at 30 °C, in 96-wellplates using a TECAN Infinite M200 microplate reader.

Carboxylesterase (CbE) activity was measured in the S10 fraction, either 5-fold (gills) or 20-fold (liver) diluted. In each case, 25 μl of sample and 200 μl of αNA as substrate (250 μM final concentration in well) were measured during 5 min at 235 nm ($\varepsilon = 23.4\ mM^{-1} * cm^{-1}$) as described in Mastropaolo and Yourno (1981). Activity was expressed in nmol/min/mg protein.

7-ethoxyresorufin O-deethylase (EROD) activity was measured using 50 μl of undiluted liver and gill homogenate samples (S10) and incubated at 30 °C with a reaction mixture containing: 0.2 mM NADPH, 3.3 μM 7-ethoxyresorufin (ER) in 100 mM phosphate buffer pH 7.4 (Burke and Mayer, 1974). The reaction was followed over resorufin formation for 10 min in a 96-well plate using the fluorescence mode set at 537 nm excitation and 583 nm emission. A six-point standard curve of resorufin (0–160 nM) was used to measure activity as pmol/min/mg protein. A good agreement between S10 and microsomal EROD activity determinations was formerly identified ($r = 0.951$; $n = 12$).

Glutathione *S*-transferase (GST) activity was measured in 25 μl of diluted S10 5-fold (gill) and 20-fold (liver), using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The final reaction mixture contained 1 mM CDNB and 1 mM reduced glutathione (GSH).

Table 1
Sampling site and coordinates of the NW Mediterranean fishing grounds. Characteristics of the fish (*Solea solea* and *Solea senegalensis*) sampled in winter 2011 (January–March). Fish weight and length are indicated as mean ± SD.

Fishing site	Coordinates	Species sampled	Sex (M:F)	Fish weight (g)	Fish length (cm)
Costa Brava	42° 01, 505′ N/03° 14,676′ E	*S. senegalensis*	7:5	548 ± 160	38.8 ± 3.8
Maresme	41° 25, 497′ N/02° 18, 765′ E	*S. solea*	1:10	631 ± 179	39.3 ± 3.3
Garraf	41° 10, 953′ N/01° 54, 716′ E	*S. solea*	3:7	600 ± 193	38.9 ± 3.6
		S. senegalensis	7:2	415 ± 83.6	35.9 ± 2.1
Vendrell	41° 08, 056′ N/01° 27, 398′ E	*S. solea*	4:9	511 ± 254	36.6 ± 5.0
Tarragona	41° 05, 705′ N/01° 18, 782′ E	*S. solea*	3:1	273 ± 59.2	32.6 ± 1.9
Cambrils	41° 00, 511′ N/01° 01, 498′ E	*S. solea*	6:3	262 ± 102	30.8 ± 3.4