



May sediment contamination be xenoestrogenic to benthic fish? A case study with *Solea senegalensis*



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ABSTRACT

Within an environmental risk assessment framework of a moderately contaminated estuary (the Sado, SW Portugal), the present work intended to detect endocrine disruption in a flatfish, *Solea senegalensis* Kaup, 1858, and its potential relationship to organic toxicants. Animals were collected from two distinct areas in the estuary (industrial and rural) and from an external reference area. Hepatic vitellogenin (VTG) levels, cytochrome P450 (CYP1A) induction, ethoxyresorufin-O-deethylase (EROD) activity plus gonad histology were analysed. Males and females were sexually immature and showed no significant evidence of degenerative pathologies. However, hepatic VTG concentrations in males from the industrial area were higher than Reference, even reaching levels comparable to females, indicating low but measurable oestrogenic effects caused by the complex contaminant mixture in estuarine sediments. These individuals also presented elevated CYP1A induction and EROD activity, which is consistent with contamination by organic toxicants such as PAHs and other aryl hydrocarbon receptor (Ahr)-mediated toxicants.

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1. Introduction

The monitoring of environmental quality in transition ecosystems such as estuaries and coastal lagoons is deemed priority. Still, the complexity of these areas, combined with the multiple sources and types of polluting agents, hamper risk assessment strategies. Among the many types of toxicants affecting transition ecosystems, endocrine disruptor compounds (EDCs) are considered an emerging environmental threat and guidelines are being issued in industrialized countries to monitor these substances and their effects onto the aquatic milieu. In the European Union (EU), specifically, the Water Framework Directive (WFD, Directive 2000/60/EC) does not consider EDCs as class of environmental toxicants on its own right. In 2012 emerged a proposal for an amendment of the

WFD that included “emerging” pollutants, as EDCs, which should be implemented by 2014, meaning a revision of the Environmental Quality Standard for a few substances. However, the real causes and effects of the potentially wide array of chemicals with endocrine-active properties remain largely unknown.

Even though some metals and metallic compounds may interfere with the endocrine system, most acknowledged EDCs are organic substances, from industrial compounds such as bisphenol-A to some pesticides and personal care products (PCP) and their by-products, most of which are released to ecosystems via urban wastewater (e.g. Bergman et al., 2012; Iavicoli et al., 2009). Endocrine disruptor agents act by interfering in biosynthesis, transport, availability and metabolism of hormones, as well as by interacting with hormonal receptors (Lister and Van Der Kraak, 2001). When interfering with reproductive hormone signalling processes, EDCs may trigger effects that may have repercussions at population development level (Jobling et al., 1998). In fact, some studies already demonstrated the EDC-related effects onto the

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reproductive system in fish, including flatfish (see, for instance, Lye et al., 1997; Hashimoto et al., 2000; Vethaak et al., 2002; Kirby et al., 2004).

Many environmental risk assessment (ERA) approaches in coastal ecosystems of these programmes employ fish as sentinels, especially flatfish, due to their ecological and economical importance, sensitivity and close contact to sediment floors, in combination with a battery of biomarker responses (e.g. Kirby et al., 2007; Costa et al., 2012). For example, cytochrome P450 (CYP1A) induction and ethoxyresorufin-O-deethylase (EROD) activity are acknowledged biomarkers of exposure to some organic compounds (e.g. polycyclic aromatic hydrocarbons and dioxins). These biomarkers are based on the principle that, following exposure to this specific class of contaminants, the CYP1A gene is overexpressed, resulting in higher synthesis of CYP1A enzymes (including EROD) increasing global CYP mixed function oxidase activity, responsible for the biotransformation of many organic toxicants. In males, the determination of vitellogenin (VTG), which is a protein precursor of egg yolk in oviparous vertebrates, is considered one of the most sensitive biomarker of exposure to EDCs, specifically to oestrogenic compounds (van der Oost et al., 2003). It must also be noted that, like many organic and inorganic toxicants EDCs may be dissolved in water, bound to suspended matter or trapped in aquatic sediments. The latter thus acts as a storage compartment of xenobiotics, with emphasis on sediments of transition ecosystems, which are usually complex in respect to their geochemical composition and combination of toxicants, whose interactions may yield *in vivo* additive, synergistic or antagonist effects, further compromising the interpretation of results (see, e.g., Waring and Harris, 2005). Thus, reading ecotoxicological data from impacted transitional ecosystems is biased by many confounding factors, from the presence of mixed toxicants to the many noise variables that affect these ever-changing systems. As a consequence, studies attempting to link a specific effect, such as endocrine disruption, to a given set of stressors under such challenging scenarios are scarce.

In Portugal, formal biomonitoring programs are yet relatively incipient, in spite of the recommendations of the WFD and, more recently, the Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC). In order to develop the first biomonitoring approach for EDC-related effects in the Sado estuary, SW Portugal, the flatfish *Solea senegalensis* was chosen as sentinel species, being a common estuarine fish in SW Europe, holding high commercial value for both fisheries and aquaculture. This species typically occupies sandy-muddy bottoms of coastal waters (estuaries included) and it feeds on small invertebrates (Cabral and Costa, 1999). Its benthic behaviour makes *S. senegalensis* vulnerable to sediment contamination. This species has already been employed in risk assessment studies of coastal ecosystems in Portugal and other SW European countries (e.g. Gonçalves et al., 2013; Siscar et al., 2013), where, however, EDCs have yet to be surveyed and their effects validated as potential indicators of exposure. In fact, this particular ecosystem holds many constraints towards risk assessment strategies, in most part owing to its size, biogeographical variation and heterogeneity regarding sources and nature of environmental toxicants (see Costa et al., 2012). As such, the main objectives of the present work can be summarized as follows: a) to detect possible effects of endocrine disruption in juvenile male *S. senegalensis* collected from the Sado estuary b) to relate endocrine disruption with gonadal alterations in male and female soles c) to investigate a possible link between endocrine disruption and estuarine contamination by organic toxicants, through a biomarker approach (VTG production in males plus induction of CYP1A and EROD activity) contrasted to sediment contamination profiles and d) to compare two distinct areas of the estuary impacted by different sets of stressors, namely urban/industrial and rural/riverine, in

order to contribute to a risk assessment strategy in an ecosystem already classified as ecotoxicologically heterogeneous and moderately contaminated.

2. Material and methods

2.1. Study area and sampling

The Sado Estuary is the second largest estuary in Portugal ($\approx 240 \text{ km}^2$). The estuary holds high biogeographical diversity and high ecological importance, mainly due to the existence of spawning and nursery areas for many aquatic species (Fig. 1). The estuary comprises the city of Setúbal and several industrial units which form its large heavy-industry belt. The estuary is also important for tourism, fisheries, aquaculture, maritime transport and part is classified as a national reserve (see for instance Costa et al., 2012; Carreira et al., 2013). Besides urban and industrial sources, the agricultural activities along the banks of the Sado River may constitute an additional input of contaminants for the estuary, such as pesticides and fertilizers. Additionally, metals are also transported to the estuary through the river Sado, since the river crosses an important pyrite mining region (see Cortesão and Vale, 1995, 1996; Caeiro et al., 2009; Costa et al., 2012). Previous studies revealed the existence of contamination levels capable of inducing adverse effects to the biota, albeit the estuary being globally judged as moderately contaminated (e.g. Caeiro et al., 2009; Costa et al., 2012). With respect to EDCs, a single study has been performed in the estuary (Ribeiro et al., 2009), showing null or low-risk concentrations of oestrogenic toxicants in water samples. However, to date, no studies focused on EDC effects to the biota.

The contamination status of the Sado estuary was inferred from sediment samples analysed during previous research, which revealed the distinction between the northern (urban and industrial) and southern (rural and riverine) areas of the Sado estuary (Costa et al., 2011; Carreira et al., 2013). In brief: sediments were hitherto collected from five locations distributed within the northern (Sado 1) and southern (Sado 2) areas (both being acknowledged fishing grounds for flatfish in the estuary) plus a reference site, located at an oceanic beach off the Mira estuary (Fig. 1), located at the same biogeographical region, being one of the least impacted coastal areas in Portugal (e.g. Vasconcelos et al., 2007) and one of the most important coastal fishing areas in the SW Portuguese coast. Sediments were analysed for metals and metalloids; polycyclic aromatic hydrocarbons (PAHs) and organochlorines, besides standard physico-chemical parameters. The full results and procedural specifications and validation procedures are given in detail by Costa et al. (2011) and Carreira et al. (2013). Table 1 summarizes the main parameters and contamination profiles of the surveyed sediments. The contamination profiles revealed that the overall most contaminated sites were located off the estuary's heavy industry belt (Sado 1 area), followed by the area near the mouth of the river Sado (Sado 2). The differences between areas are mostly related to increased levels of sediment organic toxicants, especially PAHs, in Sado 1. The levels of metals were similar between the two areas, while the levels of organochlorines were overall low. The least contaminated sediments (sample Ss) of the estuary, located at a site with stronger oceanic influence, were found to hold the same characteristics of the reference area off the Mira estuary (sample M), with respect to contamination, low loads of organic matter and fine particles.

Fish were collected at the same time than sediment samples (except S₁ in the spring 2007). Specifically, during the fall/winter 2010 twenty-one and nineteen animals were collected from Sado 1 and off the Mira estuary (Reference), respectively (Fig. 1). Animals from Sado 2 could only be collected during the spring 2011 (ten

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