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A description of chloride cell and kidney tubule alterations in the flatfish *Solea senegalensis* exposed to moderately contaminated sediments from the Sado estuary (Portugal)

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ABSTRACT

The effects of sediment-bound contaminants on kidney and gill chloride cells were surveyed in juvenile *Solea senegalensis* exposed to fresh sediments collected from three distinct sites of the Sado Estuary (Portugal) in a 28-day laboratorial assay. Sediments were analyzed for metallic contaminants, polycyclic aromatic hydrocarbons and organochlorines as well as for total organic matter, redox potential and fine fraction. The potential for causing adverse biological effects of each surveyed sediment was assessed by comparison of contaminant levels to available guidelines for coastal sediments, namely the Threshold Effects Level (*TEL*) and the Probable Effects Level (*PEL*). The Sediment Quality Guideline Quotient indices (*SQGQ*) were calculated to compare the overall contamination levels of the three stations. A qualitative approach was employed to analyze the histo/cytopathological traits in gill chloride cells and body kidney of fish exposed to each tested sediment for 0, 14 and 28 days. The results showed that sediment contamination can be considered low to moderate and that the least contaminated sediment (from a reference site, with the lowest *SQGQ*) caused lesser changes in the surveyed organs. However, the most contaminated sediment (by both metallic and organic xenobiotics, with highest *SQGQ*) was neither responsible for the highest mortality nor for the most pronounced lesions. Exposure to the sediment presenting an intermediate *SQGQ*, essentially contaminated by organic compounds, caused the highest mortality (48%) and the most severe damage to kidneys, up to full renal necrosis. Chloride cell alterations were similar in fish exposed to the two most contaminated sediments and consisted of a pronounced cellular hypertrophy, likely involving fluid retention and loss of mitochondria. It can be concluded that sediment contamination considered to be low or moderate may be responsible for severe injury to cells and parenchyma involved in the maintenance of osmotic balance, contributing for the high mortality levels observed. The results suggest that sediment-bound organic contaminants such as PAHs (polycyclic aromatic hydrocarbons) and PCBs (polychlorinated biphenyls) may be very toxic to the analyzed organs, especially the kidney, even when present in low-risk concentrations.

1. Introduction

Osmotic regulation is a vital function in marine fish due to the hypertonic nature of their environment. Estuarine fish, on the other hand, require some plasticity of the mechanisms that maintain their internal osmotic balance due to the salinity fluctuations of their environment. In either case, the failure of osmotic balance structures implicates severe stress to fish and overall loss-of-fitness to their habitat. Kidneys and chloride cells are the most important structures for osmotic balance in fish, although with different functions: whereas the gill chloride cells in fish have long been described to maintain internal osmotic balance by actively excreting or uptaking ions (Keys and Wilmer, 1932); the secretory component of the renal system such as the body (trunk) kidney of fish is known to act as a primary system for the elimination of organic xenobiotic metabolites as part of ion-excreting processes (see Pritchard and Miller, 1997 for a review). Toxic metals, however, may only negligibly be excreted by the kidneys but are known to severely impair renal ion excretion

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functions (Leffler et al., 2000; Chowdhury and Wood, 2007). For these reasons, many authors have focused on the effects of environmental contaminants on the function and morphology of these structures (e.g. Triebskorn et al., 2002, 2004; Giari et al., 2007). Nevertheless, specific information regarding lesions and alterations to osmotic regulating structures is scarce, especially regarding the effects of complex mixtures of contaminants as in natural sediments and the differential toxicity of the various classes of contaminants.

Histopathological biomarkers have long been surveyed in benthic fish with the purpose of monitoring estuarine sediment contamination. Histopathology is frequently considered a more realistic tool than biochemical approaches to assess toxicity for directly reflecting fish health, thus are more effectively being extrapolated to community-level effects of contamination (Stentiford et al., 2003; Au, 2004). Despite the growing number of research on fish cyto/histopathology, this approach still suffers from many constraints, ranging from terminology discrepancies to the difficulties in establishing cause–effect relationships between environmental parameters and pathological traits. Conversely, while liver and overall gill structure have been widely surveyed, specific alterations of chloride cells and body kidney tubules still need further research.

The Senegalese sole, *Solea senegalensis* Kaup, 1858 (Pleuronectiformes: Soleidae), is a benthic teleost of important value for fisheries and aqualculture in Southern Europe. The species inhabits soft bottoms of coastal areas, especially estuaries, which function as breeding and nursing grounds, where it feeds on small invertebrates (Cabral and Costa, 1999; Cabral, 2000; Sá et al., 2003). Combined with its relative abundance, these characteristics contribute to the species' projected value as a sentinel for environmental contamination (Jiménez-Tenorio et al., 2007).

The Sado estuary is a large confined coastal area where the effort to preserve environmental quality and sustain human development has dictated an attempt to monitor environmental contamination and its effects on organisms. The estuary is subjected to different sources of contamination: urban from the city of Setúbal, industrial from the city's heavy-industry belt (one of the largest in Portugal) and agricultural from the grounds upstream. The estuary is also an important harbour area, with several shipyards and port facilities, for such reason is being subjected to regular dredging. The estuary is also very important for local fisheries, aquaculture and tourism, each representing an important fraction of the local economy. A large part of the estuary is classified as a natural reserve and the only Portuguese underwater reserve on the mainland territory is located just off the estuary. Current environmental monitoring procedures to assess sediment contamination are being performed on three representative stations of the Sado estuary, selected according to previous information (Caeiro et al., 2005; Neuparth et al., 2005; Costa et al., 2008). These stations (Fig. 1) have different levels of contamination and different sediment and hydrodynamical characteristics: site A is the station farthest from contamination sources and the site with highest hydrodynamics and lower water residence time. Sites B and C (located off the city of Setúbal and the industrial belt, respectively) are the most contaminated, although with distinct patterns of contamination by metallic and organic xenobiotics.

The present work aimed at the identification and description of histological lesions and alterations in kidneys and gill chloride cells in juvenile *S. senegalensis* through a wide set of histological procedures and to relate sediment contamination to toxicity, using a sediment quality indices approach to determine the potential impact on organisms.

2. Methods and materials

2.1. Experimental procedure

The sediments from the selected sites, A, B and C (Fig. 1), were collected with a grab on November 2006. Sediments were subdivided and frozen for analyses (see Section 2.2) while fresh portions were used to prepare the assays after having been stored at 4 °C for a period not extending 5 days. Sediments were homogenized after collection with a spatula to ensure similarity between the samples to be used in the assays and those analyzed for contaminants. The 28-day bioassays were performed with two replicates per treatment, in a closed system tank arrangement with constant aeration and water recirculation (regulated to prevent any hydrodynamic-driven sediment resuspension). Two litres of sediment was allocated in 15 L-capacity polyvinyl tanks with blunt edges (providing approximately a sediment surface of 525 cm^2 and a depth of 3–4 cm) and was added 12 L of clean seawater. Sediments were let to settle for 48 h before the beginning of the assay. Twenty-four randomly selected hatchery-brood and laboratory-reared juvenile *S. senegalensis*, all from the same cohort (69 ± 6 mm standard length), were placed in each tank. A partial weekly water change was made (25% of total water volume) to maintain constant the water parameters: pH $= 7.9 \pm 0.2$, salinity $= 33 \pm 1$ $g.L^{-1}$, total ammonia $= 3 \pm 1$ $mg.L^{-1}$. Water temperature was set at 18 ± 1 °C, O_2 saturation ranged between 40 and 45% and the photoperiod was set at 12:12 h light:dark. The water parameters were monitored weekly to ensure constancy and were found to be equal to the rearing systems. Animals were fed daily with commercial pelleted food for aquaculture fish (Aquasoja M2 grade, from Sorgal, Portugal). For simplification purposes, exposure to the three sediments will hereon be referred to as tests A, B and C. Twelve fish per test (six per replica) were collected for analysis at each sampling time, i.e., at days 14 (T_{14}) and 28 (T_{28}). Fish collected at day 0 (T_0) consisted of twelve animals from the rearing tanks.

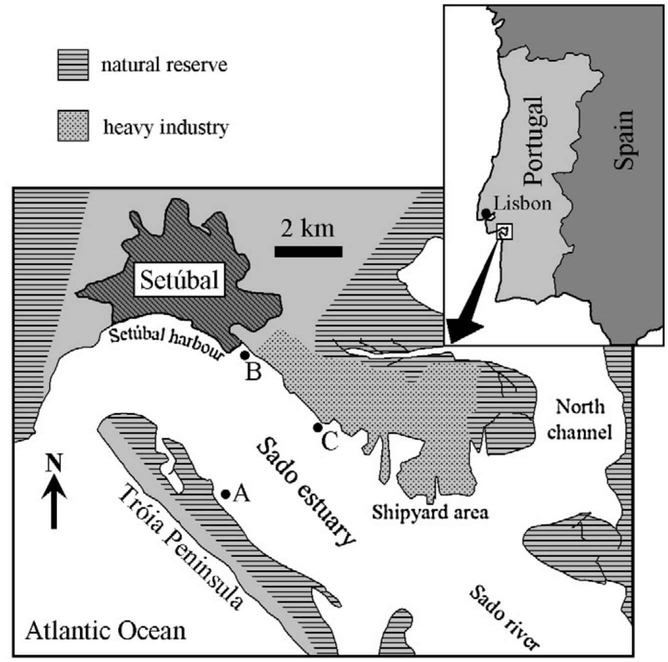

Fig. 1. Map of the Sado estuary showing the selected study sites A, B and C (•).

2.2. Sediment characterization

Sediment redox potential (Eh) was measured immediately after collection and homogenization, using an Orion model 20A apparatus equipped with a H3131 Ag/AgCl reference electrode. In addition, sediments were analyzed for organic matter (OM) and fine fraction (particle size < 63 μm) contents by organic carbon loss-on-ignition (LOI) at 500 ± 50 °C and hydraulic sieving, respectively. Both results are expressed as percentage relatively to sediment dry weight.