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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

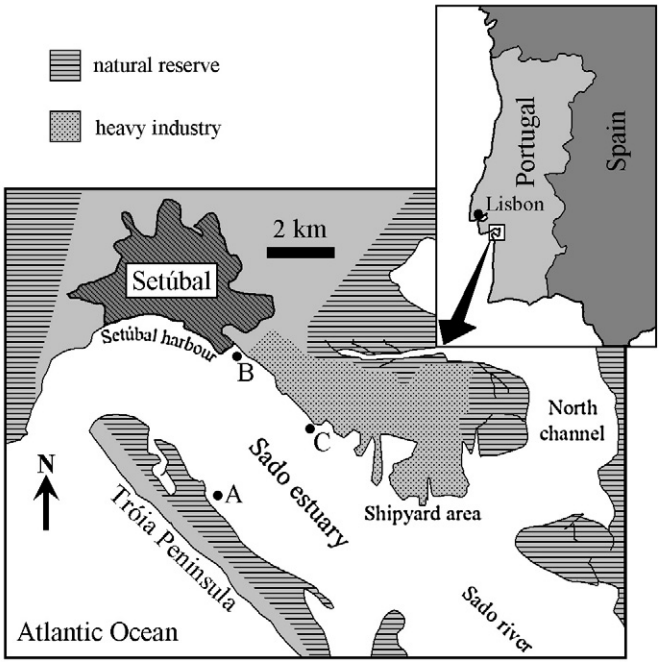

Fig. 1. Map of the Sado estuary showing the selected study sites A, B and C (•).

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 ± 0.2, salinity = 33 ± 1 $g.L^{-1}$, total ammonia = 3 ± 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.

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.

Sediment contaminants (metallic, polycyclic aromatic hydrocarbons and organochlorines) were selected according to previous findings for the same proximate areas, where levels of concern have been found (see Caeiro et al., 2005; Neuparth et al., 2005; Costa et al., 2008). Metallic sediment contaminants were determined by inductively coupled plasma mass spectrometry (ICP-MS) using a Thermo Elemental X-Series spectrometer after total digestion of sediment samples with a mixture of acids (HF, HCl, and $HN0_3$) in closed TFE vials, according to the procedure described by Caetano et al. (2007). The procedure was validated by analysis of the reference sediments MESS-2 (NRC, Canada), PACS-2 (National Research Council, Canada) and MAG-1 (USGS, USA), having found the obtained concentrations within the certified ranges. Polycyclic aromatic hydrocarbons (PAHs) were determined using a GCQ Trace Finnigan gas chromatography–mass spectrometry (GC–MS) apparatus in selected ion mode (SIM) after Soxhlet-extraction with an acetone–hexane (1:1) mixture, as described by Martins et al. (2008). Polychlorinated biphenyl (PCBs) congeners and the pesticide residues *pp′*DDD (i,i-dichloro-2,2-bis[*p*-chlorophenyl]ethane), *pp′*DDE (l,l-dichloro-2,2-bis[*p*-chlorophenyl] ethylene) and *pp′*DDT (l,l,l-trichloro-2,2-bis[*p*-chlorophenyl]ethane) were analyzed by GC–MS using a Hewlett–Packard 6890 gas chromatograph with an electron capture detector (ECD) after Soxhlet-extraction with *n*-hexane (Ferreira et al., 2003). Quality control for organic contaminant determination was obtained from an analysis of the reference sediment SRM 1941b (NIST, USA) and the measured values were found within the certified ranges.

The impact potential for observing adverse biological effects of the tested sediments was evaluated by calculating the *PEL* quotient (*PELQ*) based on the published guideline values for coastal waters, namely the Threshold Effects Level (*TEL*) and the Probable Effects Level (*PEL*), according to MacDonald et al. (1996). These guidelines were originally developed for coastal waters and have been largely used in estuarine sediment ecological risk assessment studies. The *PELQ* indices were calculated for each contaminant according to the formula described by Long and MacDonald (1998):

$$PELQ_i = \frac{C_i}{PEL} \quad (1)$$

where *PEL* is the guideline value for the contaminant *i* and C_i the measured concentration of the contaminant in the surveyed sediment. The Sediment Quality Guideline Quotient indices (*SQGQ*), developed to compare sites impacted by mixtures of contaminants, were calculated for each sediment according to Long and MacDonald (1998) as:

$$SQGQ = \frac{\sum_{i=1}^{n} PELQ_i}{n} \quad (2)$$

where $PELQ_i$ is the indice deriving from Eq. (1) for the contaminant *i* and *n* the number of contaminants under analysis. Stations were scored according to their overall potential of observing adverse biological effects, as proposed by MacDonald et al. (2004): $SQGQ < 0.1$ — unimpacted; $0.1 \leq SQGQ < 1$ — moderately impacted; and $SQGQ \geq 1$ — highly impacted.

2.3. Histological analyses

Animals were anaesthetized on ice and euthanized by cervical sectioning. Organ samples (first and second gills arches from the eyed side and body kidney) were immediately excised and fixed in Bouin–Hollande's solution (10% v/v formaldehyde and 7% v/v acetic acid to which picric acid was added to saturation) for 48 h. Samples were then washed for 24 h in distilled water (kidney samples) or in a 6% v/v formic acid solution to promote decalcification (gills). Samples were afterwards dehydrated in a progressive series of ethanol and embedded in paraffin (xylene was used for intermediate impregnation). The procedure from fixation to embedding was adapted from Martoja and Martoja (1967). For both organs, 2–3 μm thick sections were obtained. Slides were prepared in duplicate for each organ and staining procedure, with 6–8 sequential sections per slide. Kidney and gill were stained with haematoxylin and eosin (H&E) for structural analyses as described by Martoja and Martoja (1967). Gill sections were additionally stained through the alcian blue histochemical method using nuclear fast red as counterstain (AB&NFR) for identification of goblet (mucous secreting) cells (Kiernan, 2008) and with the acridine orange fluorochrome stain (AO) for nucleic acids to identify mitochondria in chloride cells (Costa and Costa, 2008). Slides were cleared with xylene and mounted in DPX resinous media (BDH). A DMLB model microscope adapted for epifluorescence with an EL6000 light source for mercury short-arc reflector lamps and an I3 filter was used for analyses. All equipments were supplied by Leica Microsystems. Identification of normal and pathological features on gills and kidneys was primarily based on Hibyia (1982) and Arellano et al. (1999, 2004).

2.4. Statistical analyses

Cell measurements and counts were analyzed by the non-parametric Kruskall–Wallis ANOVA by ranks *H* ($\alpha = 0.05$). Statistics were computed with the software Statistica 6.0 (Statsoft inc., Tulsa, OK, USA), according to Zar (1998).

3. Results

Overall mortality at the end of the assays was much differentiated between tests: 2% as result of exposure to sediment A, 13% for test B and 48% for C.

3.1. Sediment characterization

The tested sediments revealed distinct characteristics and patterns of contamination (Table 1). Sediment A, collected from the reference site, was the least contaminated and the least anoxic. Sediment C was essentially contaminated by organic compounds and was found to have the lowest redox potential, whereas sediment B was contaminated by metallic and organic toxicants and was the sediment with highest OM and FF percentages. Inference on *SQGQ* indices (Eq. (2)) indicate that site A is unimpacted while sites B and C are moderately impacted, with the sediments from site B presenting the highest potential ecological hazard, according to the classification proposed by MacDonald et al. (2004). Contamination of sediments B and C can, however, be considered low to moderate, since *PEL* thresholds are only reached for the elements copper (Cu) and zinc (Zn) in sediment B.

3.2. Gill and chloride cell histopathology

Fish collected at T_0 essentially exhibited normal gill morphology of filaments and lamellae (Fig. 2A). No considerable amounts of sediment particles were found in the gills and fish did not exhibit evidence for gill parasites. Normal gill epithelium on filament and lamella is typically formed by a single layer of cells. Chloride cells stained with H&E have a densely-stained granulous cytoplasm. Discrimination of chloride cells from goblet (mucous secreting) and regular epithelial cells could be aided by the observation of the crypt opening to the exterior and by the vesicular aspect of goblet cells' cytoplasm, which differentially retains the haematoxylin pigment according to the mucosubstances' pH. Staining with the nuclei-acid specific fluorochrome allows good discrimination of chloride cells due to the fluorescence of mitochondrial DNA (Fig. 2B). Chloride cells are mostly located in the interlamellar epithelium of the filament ($\approx 2 \pm 1$ cells between lamellae) but smaller, flattened, chloride cells can

Table 1
General characterization and contaminant ranges of tested sediments. *PEL* and *TEL* guidelines were obtained from MacDonald et al. (1996).

			Site						
			A			B		C	
Organic matter (%)			3.2			11.8		7.7	
Fine fraction (%)			37.3			97.9		76.8	
Redox potential (mV)			−233			−290		−316	
Contaminant class			*TEL*[a]	*PEL*[b]	*PELQ*[c]		*PELQ*[c]		*PELQ*[c]	
Metallic (mg kg^{-1} sediment dw)									
As			7.24	41.6	7.25 ± 0.15*	0.17	27.43 ± 0.55*	0.66	12.38 ± 0.25*	0.30
Cd			0.68	4.21	0.04 ± 0.00	0.01	0.22 ± 0.00	0.05	0.15 ± 0.00	0.04
Cr			52.3	160	24.20 ± 0.48	0.15	76.33 ± 1.53*	0.48	21.85 ± 0.44	0.14
Cu			18.7	108	22.57 ± 0.45*	0.21	167.32 ± 3.35**	1.55	41.18 ± 0.82*	0.38
Ni			15.9	42.8	12.97 ± 0.26	0.30	33.67 ± 0.67*	0.79	9.03 ± 0.18	0.21
Pb			30.2	112	23.70 ± 0.47	0.21	66.49 ± 1.33*	0.59	45.17 ± 0.90*	0.40
Zn			124	271	147.48 ± 2.95*	0.54	312.23 ± 6.24**	1.15	87.75 ± 1.76	0.32
Organic (μg kg^{-1} sediment dw)										
	3-ring	Acenaphthene	6.71	88.9	1.41 ± 0.24	0.02	9.42 ± 1.60*	0.11	4.19 ± 0.71	0.05
		Acenaphthylene	5.87	128	0.24 ± 0.04	0.00	1.83 ± 0.31	0.01	1.95 ± 0.33	0.02
		Anthracene	46.9	245	1.03 ± 0.17	0.00	10.60 ± 1.	0.04	15.34 ± 2.61	0.06
		Fluorene	21.2	144	1.32 ± 0.22	0.01	8.70 ± 1.48	0.06	8.03 ± 1.37	0.06
		Phenanthrene	86.7	544	7.96 ± 1.35	0.01	50.77 ± 8.63	0.09	54.09 ± 9.20	0.10
PAHs	4-ring	Benz(a)anthracene	74.8	693	4.53 ± 0.77	0.01	64.60 ± 10.98	0.09	86.52 ± 14.71*	0.12
		Chrysene	108	846	2.20 ± 0.37	0.00	28.31 ± 4.81	0.03	37.19 ± 6.32	0.04
		Fluoranthene	113	1494	18.05 ± 3.07	0.01	170.80 ± 29.04*	0.11	184.30 ± 31.30*	0.12
		Pyrene	153	1398	14.66 ± 2.49	0.01	131.74 ± 22.40	0.09	171.39 ± 29.14*	0.12
	5-ring	Benzo(a)pyrene	88.8	793	7.56 ± 1.28	0.01	69.81 ± 11.87	0.09	85.88 ± 14.60	0.11
		Dibenzo(a,h)anthracene	6.22	135	0.74 ± 0.13	0.01	7.45 ± 1.27*	0.06	6.99 ± 1.19*	0.05
PCBs		ΣPCB	21.6	189	1.87 ± 0.32	0.01	5.64 ± 0.96	0.03	15.34 ± 2.61	0.08
		pp′DDD	1.22	7.81	0.10 ± 0.02	0.01	0.28 ± 0.05	0.04	0.60 ± 0.10	0.08
DDTs		pp′DDE	2.07	374	0.05 ± 0.01	0.00	0.27 ± 0.05	0.00	0.65 ± 0.11	0.00
		pp′DDT	1.19	4.77	0.70 ± 0.12	0.15	4.39 ± 0.75*	0.92	1.18 ± 0.20	0.25
		SQGQ[d]				0.08		0.32		0.14

* Concentrations above *TEL*.
** Concentration above *PEL*.
[a] Threshold Effects Level — concentration below which contamination effects rarely occur.
[b] Probable Effects Level — concentration above which contamination effects occur frequently.
[c] *PEL* quotient (Eq. (1)).
[d] Sediment Quality Guideline quotient (Eq. (2)).

occasionally be observed between lamellar pavement cells. Structural lesions in gills of exposed fish were in general moderate and more frequent in individuals exposed to sediment B and moreover to sediment C. Gill damage was observed, however, to increase from T_0 to T_{28} for all tests. Some of the most recurrent alterations consisted of moderate interlamellar hyperplasia (proliferation) and hypertrophy of lamellar pavement cells (although no rod-shaped filaments were observed). In fish exposed to sediments B and C, however, hypertrophy of chloride cells was a frequent alteration (Fig. 3). Chloride cell hypertrophy was uncommon on A-tested fish or restricted to small localized foci, whereas in B and C-tested individuals this alteration was typically observed throughout the gill arches (one or two hypertrophied cell were commonly observed in basal interlamellar epithelia, and could also occasionally be found in lamellar epithelia). Hypertrophied chloride cells appeared as empty, vacuole-like structures (loosing their typical granular cytoplasm) that could be positively discriminated from other epithelial cells by differential staining (Fig. 4A–C). Nuclei and remaining cytoplasmatic structures were observed compressed against the cell's membrane, depending on the section under observation. These cells were found to be ellipsoidal in shape. The average largest axle of the hypertrophied chloride cells measured 17 ± 2 μm and no statistical differences were found between tests and sampling times (Kruskall–Wallis $H = 9.57$, $p = 0.09$). Due to cell enlargement, the surrounding tissue was often compressed. No significant changes were observed in the total number of chloride cells (normal/hypertrophied) in the interlamellar basal epithelium between all tests and sampling times (Kruskall–Wallis $H = 1.79$, $p = 0.77$).

3.3. Body kidney histopathology

Fish exposed to sediments B and C sustained strong renal damage when compared to A-tested individuals, which depicted normal kidneys, similar to those observed in T_0-collected fish (Fig. 5A). Individuals collected at T_0 and those exposed to sediment A for 14 and 28 days exhibited normal kidney tubules (renal parenchyma) and a normal hematopoietic interstitial (intertubular) tissue surrounding the tubules (mostly containing erythrocytes and blast cells). As typical in saltwater fish, glomeruli were found to be rare or even absent and reduced in size when present. A prominent regression of tubules was observed in fish exposed to sediments B and C at the assays' midterm (T_{14}). Cloudy swelling (also termed albuminous degeneration) became an evident alteration of tubular epithelial cells, resulting in cell hypertrophy (and loss of shape) with a granular cytoplasm. Altered cells did not appear to have nuclear alterations. With respect to tubular structure, a very evident regression was observed, typically associated to degenerating or necrotic cloudy-swollen cells. This lesion implied the loss of tubule shape, reduction of lumen diameter and, frequently, tubule disappearance. Necrosis appears to be preceded by cloudy swelling and followed by tubule disorganization and disappearance. However, whereas in the kidneys of B-tested fish collected at this sampling time some intact tubules could be found (Fig. 5B), in fish exposed to sediment C the regression of tubules was more severe (Fig. 5C). The most severe damage was observed in fish exposed to sediment C for 28 days, with some individuals exhibiting entirely necrotic sections, affecting both tubular and hematopoietic tissue. Many melanomacrophages and haemosiderin deposits

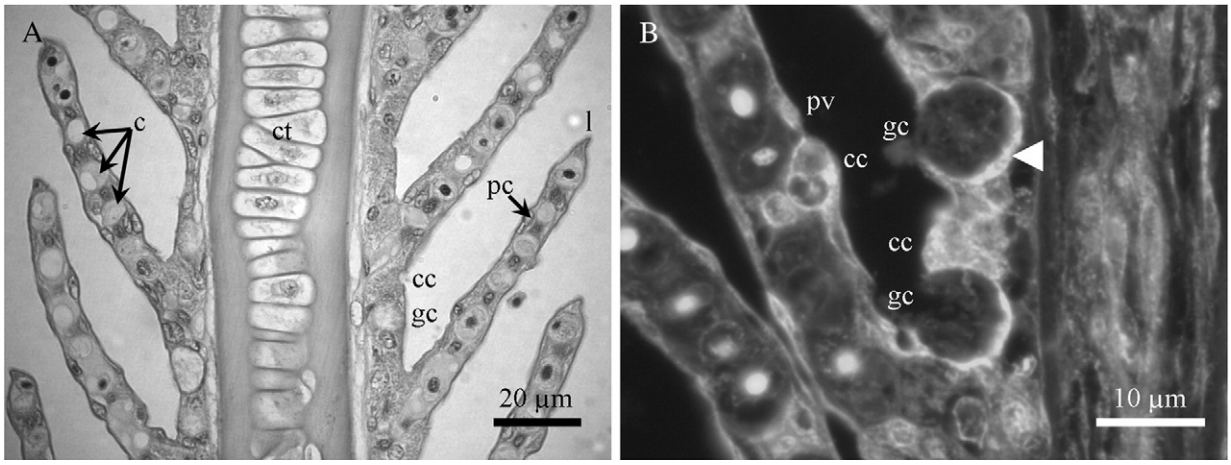

Fig. 2. Normal morphology of gills of a fish collected at the beginning of the assay (T_0). A) Bright-field micrograph (H&E). c) Lamellar capillaries; cc) chloride cell where the crypt opening to the exterior is clearly visible; ct) gill filament supporting cartilage; gc) goblet (mucous secreting) cell; l) gill lamella; pc) pillar cell. B) Epifluorescence micrograph (AO) of a normal gill, exhibiting chloride cells in lamella and filament epithelia (cc). Chloride cells are strongly fluorescent and have a granular cytoplasm due to their high concentration of mitochondria. Goblet cells (gc) also possess a highly fluorescent cytoplasm typically compressed against the cell's basal surface (arrowhead) but most of the cell's intraplasmatic space is occupied by mucous vesicles that do not retain the dye. Non-secreting epithelial cells such as pavement cells (pv) do not exhibit a densely fluorescent cytoplasm.

(appearing as reddish granules) were observed on necrotic tissue. Kidneys of B-tested fish did not exhibit significant changes in kidney damage from T_{14} to T_{28}. No clear evidence for mitosing cells was in the renal parenchyma of fish exposed to sediments B and C, at both sampling times. Due to the reduced number of Malpighian corpuscles, glomerular lesions could not be scored.

4. Discussion

The present work demonstrated that exposure to sediments that might be considered low to moderately contaminated may cause severe damage to the kidneys and gill chloride cells in juvenile Senegalese soles. The severity of lesions and overall mortality were not, however, directly related to the cumulative sediment contamination since exposure to sediment C, to which was obtained an intermediate *SQGQ* contamination indice (valuing 0.14, whereas sediments A and B *SQGQ*s were 0.08 and 0.32, respectively), caused greater mortality and more pronounced kidney alterations. Still, exposure to both most contaminated sediments (B and C) caused high damage to kidneys and gill chloride cells, probably compromising the animals' capability to regulate their internal osmotic balance. The similarity between chloride cell alterations in fish exposed to both sediments and the higher severity of renal lesions observed in fish exposed to sediments essentially contaminated by organic compounds (namely, sediment C) may indicate that organic xenobiotics, especially PAHs and PCBs were the primary xenobiotics accountable for toxicity, even though the action of well represented metals such as Cu and Zn should not be excluded.

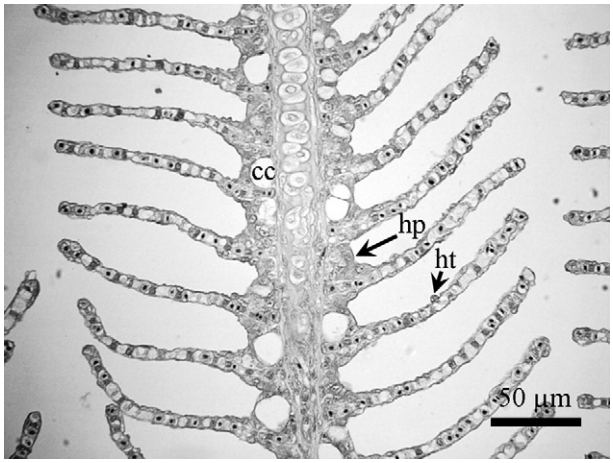

Fig. 3. Overview of the gills of a fish exposed to sediment C (mostly contaminated by organic compounds) for 28 days (H&E), exhibiting many hypertrophied chloride cells (cc). Structural damage to the gills is low but foci of moderate pavement cell hyperplasia (hp) can be observed in the interlamellar spaces, as well as hypertrophied pavement cells (ht).

The observed chloride cell alterations imply a change in the cellular structure by fluid retention (causing cell enlargement) and loss of mitochondria and other nucleic acid-bearing cytoplasmatic structures like the rough endoplasmatic reticulum. These findings are in accordance to the chloride cell dystrophies observed by Arellano et al. (1999) in *S. senegalensis* exposed to waterborne copper. The presence of intact nuclei and plasmatic membranes indicate that hypertrophy of chloride cells is not directly related to necrosis. This alteration is, nevertheless, likely to impair active transport of ions due to a reduction in the number of mitochondria and consequently in cellular energy production. Chloride cell hypertrophy and hyperplasia in fish gills have been found to be an adaptation to hypertonic media (Karnaky et al., 1976b; Foskett et al., 1981). These changes imply an increase in cell activity and microstructure density (Karnaky et al., 1976a; Foskett et al., 1981). However, the alterations observed in the present work indicate loss of subcellular structures and fluid retention in chloride cells of fish exposed to contaminated sediments. Due to the constancy of the assays' water parameters, it can be reasoned that the observed hypertrophy is putatively caused by xenobiotics released from the sediments and not by osmotic-driven chloride cell hypertrophy. The hypertrophied cells observed in the gills of fish exposed to sediments B and C, rather than being a contaminant-triggered overresponse to environment salinity should actually result from atrophy/regression of cellular constituents. In fact, chloride cell hypertrophy and impairment of gill active transporter enzymes have already been found to be linked to exposure to different classes of xenobiotics, from metal to pesticides (Arellano et al., 1999; Fanta et al., 2003; Monette et al., 2008), which substantiates the present findings and indicates that this alteration is an unspecific effect of contamination. It is noteworthy that no traces of sediment particles or organisms were found in the digestive tracts of exposed fish, what may indicate that fish were feeding essentially on pellets. It can therefore be argued that gill epithelium was the principal entry point of contamination.

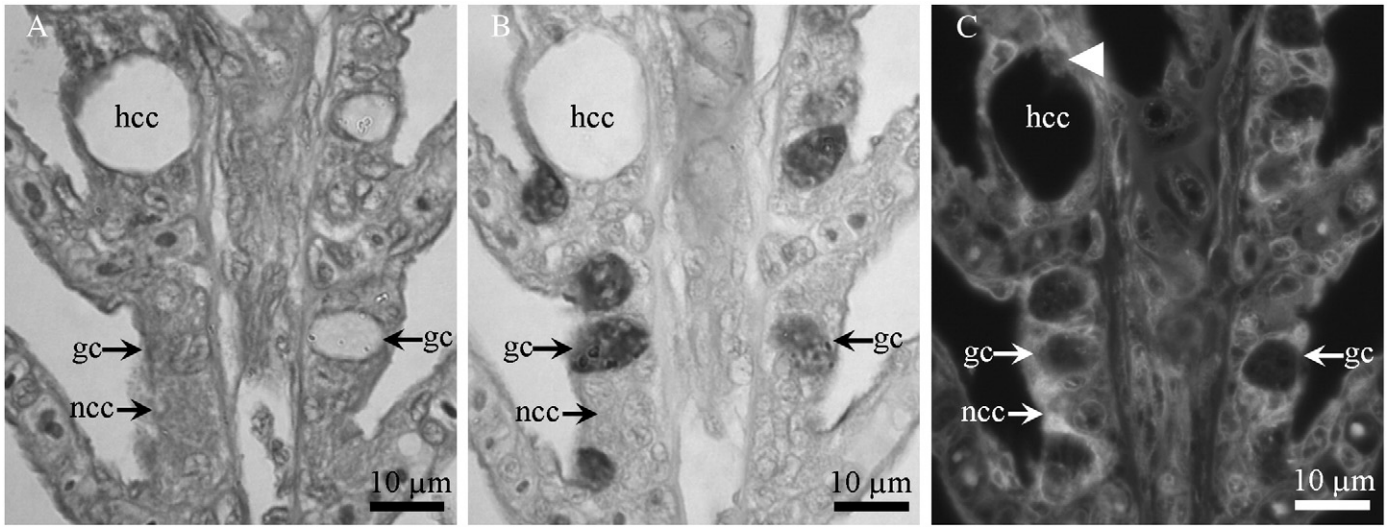

Fig. 4. Sequential sections of the gills of a fish exposed to sediment B (contaminated by metallic and organic xenobiotics) for 14 days where hypertrophied (hcc) and normal (ncc) chloride cells can be observed, among many goblet cells. Sections are stained by different techniques: A) H&E; B) AB&NFR for mucosubstances; C) AO for DNA (epifluorescence micrograph). Normal chloride cells retain the typical granulous, dense, cytoplasm, whereas hypertrophied cells appear as empty-like, unstained structures, indicating probable fluid retention. Goblet cells appear strongly or weakly stained by haematoxylin depending on mucous pH (basic or acid, respectively), but are always stained by the alcian blue reaction and are thus well distinguishable from other epithelial cells. Hypertrophied chloride cells do not exhibit the strong AO fluorescence of mitochondria-rich cytoplasm in normal cells but the nucleus (arrowhead) is retained.

Many of chronic histopathological traits described by toxicological studies in fish and other vertebrates, such as tubule cell vacuolation and peptide hyaline inclusions (e.g. Triebskorn et al., 2004; Camargo and Martinez, 2007; McCoy et al., 2008), were not observed in the present study. Instead, the major alterations observed, such as cloudy swelling, necrosis and tubule regression, combined with the absence of tubular regeneration, should indicate severe, acute damage to the body kidney of fish exposed to the most contaminated sediments. Cloudy swelling of tubule cells has nevertheless been found as one of the most recurrent alterations in the kidneys of fish exposed to complex mixtures of contaminants (Triebskorn et al., 2004; Camargo and Martinez, 2007; Giari et al., 2007). According to our findings, it is possible to suggest a progressive series of tubule lesions according to the time of exposure: cloudy swelling of tubule epithelial cells; tubule cell necrosis; tubule disorganization leading to tubule disappearance. This pattern is consistent with the proliferative kidney disease (PKD) described in feral or laboratory-tested fish exposed to undiscriminated contaminant mixtures (e.g. Triebskorn et al., 2002). The relation between the observed renal lesions and the sediment contaminants may indicate that the damage should be linked to uptaken sediment organic contaminants (especially PAHs and PCBs) rather than being a response to overall contamination. The severe lesions observed in the tubules of body kidney (the secretory portion of fish kidneys) might be linked to the excretion of the highly reactive and toxic organic contaminant metabolites. Some of these metabolites (such as PAH *o*-quinones and diol epoxides) are formed by cytochrome monooxygenase systems to transform the hydrophobic compounds into more water soluble, thus more excretable, forms (Flowers-Geary et al., 1996; Burchiel et al., 2007). Flatfish have already been found, for instance, to rapidly catabolise PAHs (Varanasi and Gmur, 1981), which may contribute to a fast detoxification but also diffuse the highly toxic metabolites through the organism via the blood stream, eventually reaching the kidneys. Although the liver is considered to be the most important organ involved in contaminant metabolism, accumulation and excretion of xenobiotics and their metabolites are important renal functions (see Pritchard and Miller, 1997 for a review), a premise that is supported by the current findings. The severe PKD sustained by the body kidney of fish exposed to sediment C might contribute to explain the high mortality that occurred during this test. Nevertheless, much information is still lacking regarding the specific effects of xenobiotics, especially of organic compounds, in fish kidney.

In spite of its lower contaminant levels than sediment B, exposure to sediment C caused more severe damage to renal tubules than B. Still, both tests were responsible for more severe lesions in the chloride cells and body kidney that would be expected from the comparison between the measured sediment contaminants and the sediment quality guidelines. The current findings confirm that these guidelines are in essence a primary screening tool and should not make toxicity bioassays expendable, although they have been shown capable of predicting sediment toxicity in laboratorial studies (Long et al., 1998). It's noteworthy, however, that the concentrations of some metallic contaminants in sediment B reach up to 3- and 4-fold the levels in sediment C, such as copper and zinc (both above the PEL thresholds). The results thus suggest that organic contaminants appear to have higher toxic effects on osmotic balance mechanisms than metals. The toxicity effects observed, and more specifically the differences between tests B and C renal tubule lesions, might also be explained by the prevalence of some organic compounds in sediment C, such as total PCBs (reaching a 3-fold level in comparison to sediment B) and some PAHs, especially the higher molecular weight (more toxic) 4- and 5-ringed compounds. On the other hand, sediment handling prior to bioassay preparation and sediment resuspension caused by the animals' scavenging activities, may have promoted bioavailability of contaminants. In fact, bioturbation has been found responsible for increased availability of contaminants in the water column (Atkinson et al., 2007). Also, high levels of sediment fine particle fraction and organic matter favour contaminant retention in sediments but, combined with anoxia, enhance the release of contaminants into the water column during sediment disturbance events (Eggleton and Thomas, 2004), which is likely to have occurred in all tests, especially during exposures to sediments B and C. This toxicity magnification effect should not, however, explain on its own the differences between the kidney lesions observed in fish exposed to these sediments.

Exposure to moderate sediment-based contamination may cause severe lesions in organs involved in the maintenance of internal osmotic balance of juvenile *S. senegalensis*. Even though laboratorial bioassays may promote the release of xenobiotics adsorped to sediment particles

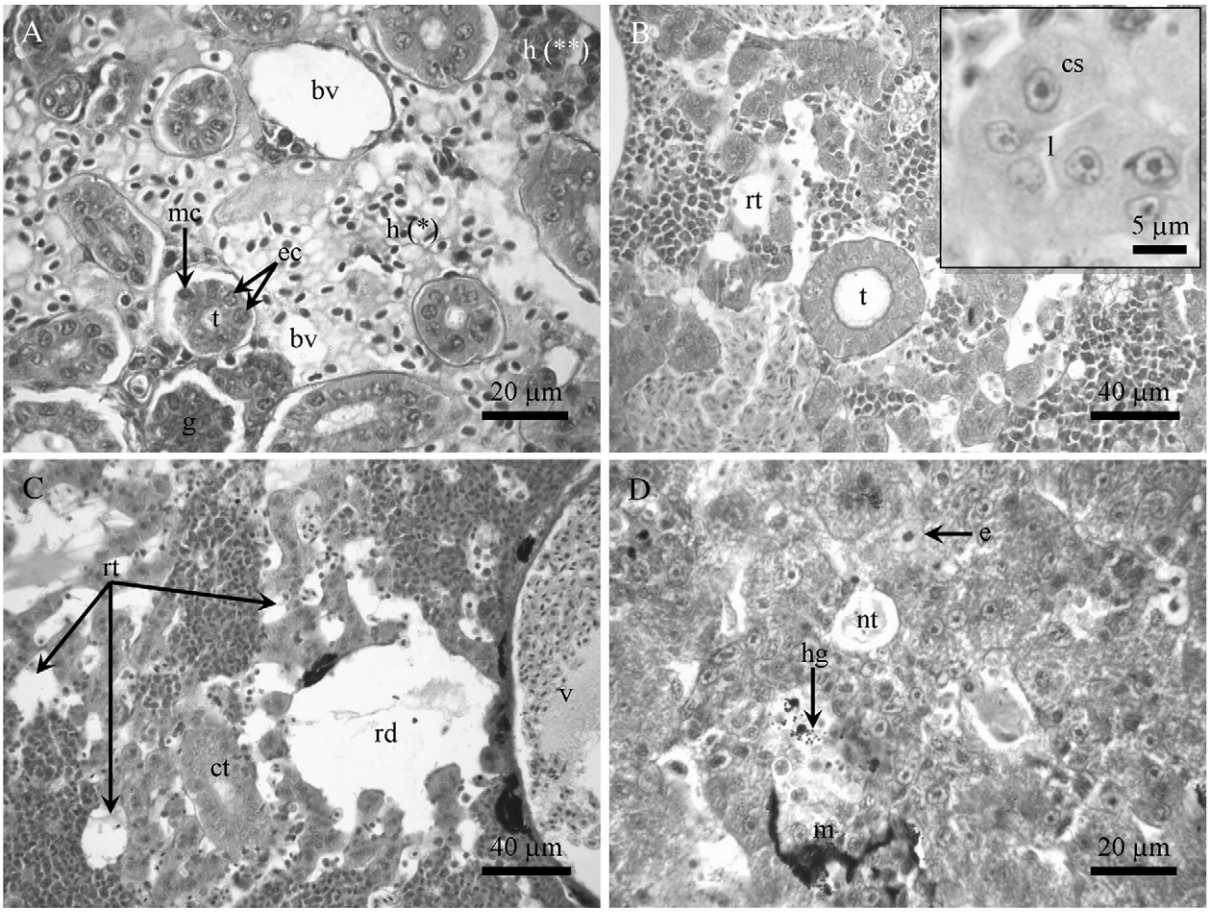

Fig. 5. Body kidney (proximal convoluted segment) morphology of exposed individuals (H&E). A) Normal kidney of an individual collected at T_0. bv) blood vessels; g) glomerulus inside a Malpighian corpuscle; h) haematopoietic tissue (with *erythrocyes and **blast cells); t) renal tubule lined by normal cuboidal epithelial cells (ec), internally lined by a microvili layer. Mitosing cells (mc) could frequently be observed, indicating parenchyma regeneration. B) Overview of the kidney of a fish exposed to sediment B (contaminated with metallic and organic substances) for 14 days, exhibiting normal (t) among many regressing (rt) tubules formed by cloudy-swollen and necrotic cells. Side-panel: detail of a tubule lined by cloudy-swollen epithelial cells (cs), from the same sample, an alteration that typically causes a pronounced reduction of the lumen (l) diameter. C) Overview of the renal parenchyma of a fish after 14 days of exposure to sediment C (essentially contaminated by organic toxicants), exhibiting tubules completely lined by cloudy-swollen epithelial cells (ct) and regressing tubules (rt). A regressing urinary duct is also evident (rd). v) renal vein with erythrocytes inside. D) Detail of the heavily-damaged kidney of a fish exposed to sediment C for 28 days, exhibiting acute necrosis of parenchymal and interstitial tissue with melanomacrophages (m) and haemosiderin granules (hg). The few persisting tubules (nt) are clearly lined by necrotic epithelial cells. The haematopoietic (intertubular) tissue is also suffering from advanced necrosis, with few, sparse, intact erythrocytes (e) or blast cells.

and organic fraction, it can be concluded that low to moderate sediment contamination, at least when associated to sediment disturbance events, may cause severe lesions in osmotic regulation structures in juvenile *S. senegalensis*. On the other hand, organic contaminants such as PAHs and PCBs appeared to have a more pronounced toxic effect on the mechanisms of osmotic balance than metallic elements and DDTs on tested fish. The results also confirm that, although a useful tool as a primary indicator of toxicity to biota, sediment quality guidelines should be complemented by the actual analysis of effects of contaminants to organisms.

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