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Multi-organ histopathology in gobies for estuarine environmental risk assessment: A case study in the Ibaizabal estuary (SE Bay of Biscay)

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A B S T R A C T

Multi-organ (liver, gills, kidney and spleen) histopathology in gobies (*Pomatoschistus* spp.) together with metal bioaccumulation and sediment contamination levels were studied during 2011–2013 for estuarine environmental risk assessment in the Ibaizabal estuary (SE Bay of Biscay). Results indicate that sediments were moderately-strongly impacted by metals and organic compounds, suggesting that adverse biological effects could be likely. Similar metal bioaccumulation levels and multi-organ histopathological indices were detected in gobies collected along the estuary, indicating a similar affection degree. Accordingly, both metal bioaccumulation levels and histopathological indices decreased in gobies collected in 2013 reflecting a lower impact on fish health status. Liver, gills and kidney presented higher histopathological damage than spleen. Fat vacuolation of hepatocytes, lamellar fusion and melanomacrophage centers were the most prevalent hepatic, branchial and renal alterations, respectively. These histopathological changes may indicate exposure to non-specific toxicants. Nevertheless, the influence of other environmental variables should not be excluded as causative factors. No severe pathological traits were registered in gonads, suggesting undisturbed reproductive status. In conclusion, the use of multi-organ histopathology in gobies in combination with metal bioaccumulation and sediment contamination levels, contribute to a better understanding of sub-lethal effects and a more accurate environmental risk assessment in the Ibaizabal estuary.

1. Introduction

Estuaries are major sinks of potentially hazardous chemicals derived from human activities. Most of these substances are highly persistent and may provoke adverse effects on estuarine organisms, with severe consequences for ecosystem health, local economies and human health (Borja et al., 2008; Chapman et al., 2013; Gredilla et al., 2013). Hence, the monitoring and protection of estuarine ecosystems has long been regarded as a priority for environmental protection agencies and stakeholders worldwide (Stentiford et al., 2003; Monteiro et al., 2006). Within the estuarine environment, sediments constitute a dynamic and essential component, at least due to their provision of habitat and food resources to benthic and demersal species (Apitz, 2012). However, aquatic sediments, especially those of confined coastal waterbodies, also tend to accumulate many pollutants, since many substances tend to be adsorbed to particular matter or to become trapped in pore water (Martínez-Lladó et al., 2007). Those pollutants may be remobilized to the water column through the resuspension of sediments (Eggleton and Thomas, 2004).

Traditionally, sediment contamination risk has been determined by assessing the bulk chemical content of each toxicant with the subsequent comparison with background or reference values (Belzunce et al., 2004; Rodríguez et al., 2006). Due to the little insight on the true ecological impact retrieved from this approach, more integrated assessments are recommended, implying the analysis of different lines-of-evidence (LOEs) in order to link sediment contamination to adverse biological effects (Montero et al., 2013). In this respect, Chapman et al. (1997) proposed the Sediment Quality Triad, which integrates contamination levels, toxicity testing and benthos analysis for sediment risk assessment. Later on, this integrated approach has been updated integrating other LOEs

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(Chapman and Hollert, 2006; Chapman et al., 2013). Among these LOEs, bottom fish histopathology has been recommended and effectively employed in many biomonitoring programmes (*e.g.* Stentiford et al., 2003; Lang et al., 2006; Fricke et al., 2012; Costa et al., 2013; Schultz et al., 2013), due to its ability to disclose the true health status of animals (Chapman and Hollert, 2006). Liver, gills, kidney and spleen, together with gonad as supporting organ, have already been assessed histopathologically as target organs in field or laboratory works (Monteiro et al., 2006; Costa et al., 2010; Schultz et al., 2013).

Teleosts have been widely used in ecotoxicological studies due to their ecological relevance, availability and ability to act as surrogates for higher vertebrates (Costa et al., 2013). The gobies, *Pomatoschistus* spp., are ubiquitous and abundant benthic species in most European estuaries and near shore waters, and as such, they are susceptible to sustain contamination induced injury (Stentiford et al., 2003; Martinho et al., 2006; Dolbeth et al., 2007; Fonseca et al., 2014). Gobies are among the most abundant fish species inhabiting the Ibaizabal estuary (SE Bay of Biscay) (Revilla et al., 2014), which makes them suitable sentinel fishes for estuarine sediment monitoring.

The Ibaizabal estuary has been highly impacted for many decades by several human activities such as discharges of urban effluents, mineral sluicing and industrial wastes (Cearreta et al., 2000; Belzunce et al., 2004). Consequently, this estuarine system suffered a progressive deterioration along the 20th century, showing low oxygen levels, high contaminant contents and disappearance of fauna in several areas (Borja et al., 2006). However, the decline of the industrial activity in mid-90's together with the implementation of modern sewerage systems in 2001 have led to a gradual improvement of the environmental health status of the system (Borja et al., 2010). Nevertheless, although recent studies on sediment toxicity tests using sea urchins and amphipods indicate lack of acute toxicity, sediment contaminants levels are still relevant (Montero et al., 2013). In order to contribute to a better assessment of estuarine environmental health status, evaluation of sub-lethal effects (measured in terms of multi-organ histopathology) in gobies together with metal bioaccumulation and sediment contamination levels were studied in a three year survey carried out along the Ibaizabal estuary. Hence, the specific aims of the present study are (1) to provide a multi-organ (liver, gills, kidney and spleen) histopathological assessment in gobies and (2) to evaluate the use of the multi-organ histopathology in combination with metal bioaccumulation and sediment contamination levels for environmental risk assessment of the Ibaizabal estuary.

2. Material and methods

2.1. Sampling

Sampling campaigns were carried out in the Ibaizabal estuary in autumn with the "Ortze" oceanographic vessel. Sediments were collected from 2009 to 2013 by a Day grab at four sampling sites (obtaining in total 20 sediment samples) for sediment characterisation and contaminant determination (Fig. 1). Similarly, gobies (*Pomatoschistus* spp.) were collected from 2011 to 2013 by trawling at four transects situated upstream (Olabeaga and Rontegi) and downstream (Lamiako and Inner Abra) the sewage treatment plant (Fig. 1). Water depth in these areas was between 8 and 15 m. The sampling was carried out by using a 2.5 m wide beam trawl with a tickler chain. Three hauls of 10 min at 2.5 knots were carried out at each sampling transect and survey. No fish were obtained at Lamiako on the 2011 campaign. Approximately 30 entire individuals per haul were fixed in 10% neutral buffered formalin for 24 h at 4 °C for histological examination. Another set of 30 individuals per haul was pooled and stored at −20 °C for metal bioaccumulation analyses.

2.2. Sediment characterisation and contamination levels

The granulometric characterisation of sediments [% gravel (>2 mm), sand (2 mm–63 μm) and mud (<63 μm)] was estimated by two procedures depending on the percentage of fine fraction. Samples with low percentage of fine sediment (<10%) were determined by dry sieving according to Folk (1974); while samples with high content of fine sediments (>10%) were determined by Laser Diffraction Particle Size Analyser (LDPSA); due to the underestimation of the finest fraction, mud content was transformed following the method proposed by Rodríguez and Uriarte (2009). Total organic matter (TOM) was determined by loss of weight on ignition (450 °C, 6 h) according to Dean (1974).

Metal (Cd, Cr, Cu, Hg, Ni, Pb and Zn) contents were measured in triplicate in acid extracts from the fine fraction of the sediments (<63 μm). In brief: dried sediment was digested in an acid mixture ($2HCl:1HNO_3$) using microwave system (MARS 5 Xpress CEM Corporation Instrument). Afterwards, metal levels were determined by Atomic Absorption Spectrometry, AAS (AAS800 Perkin Elmer): Cd was analysed by THGA graphite furnace, using Zeeman background correction; Cr, Cu, Ni, Pb and Zn were determined in an air-acetylene flame; finally, total Hg was measured by quartz furnace AAS following cold vapor method. Analytical accuracy was checked by the PACS-2 reference material (National Research Council of Canada, NRC) and the measured values were found to be within the certified range.

Organic compounds such as polychlorinated biphenyls (PCBs), other organochloride pesticides and polycyclic aromatic hydrocarbons (PAHs) were also determined (see Table 1). Sediment samples (5–10 g) were pre-concentrated with a mixture of solvents (pentane:diclorомethane; 50:50) by Accelerated Solvent Extraction, ASE (200 system DIONEX). Organic extract was purified by Gel Permeation Chromatography (GPC) and different extracts were collected, evaporated and reconstituted by isooctane for PCBs or by ethyl acetate for organochlorides. 8 ml of sulphuric acid were added to PAHs extract and then it was centrifuged. Organic phases were collected and determined by Gas Chromatography-Mass Spectrometry, GC–MS (Agilent 6890 GC coupled with an Agilent 5973 MSD instrument).

Contaminant levels were contrasted with the published values of the Threshold Effects Level (TEL) and Probable Effects Level (PEL) proposed for metals (Menchaca et al., 2012); PCBs and PAHs (Menchaca et al., 2014); plus DDTs (Macdonald et al., 1996). The contaminants potential to cause adverse biological effects was assessed through the estimation of sediment quality guideline quotients (SQG-Qs). The SQG-Qs were calculated as the ratio between the concentration of individual chemicals and their respective PEL value (Long and MacDonald, 1998). The sediments were then ranked as proposed by MacDonald et al. (2004) according to their toxicological risk for each class of contaminants (metals, PAHs and DDTs) and for total contamination: SQG-Q < 0.1 as non-impacted sediments; 0.1–1 as moderately impacted and >1 as strongly impacted. In this attempt, the mean of individual SQG-Qs of each chemical group was calculated.

2.3. Metal bioaccumulation

Pools (n ≈ 30 individuals per sampling transect and campaign) of whole gobies were homogenised and approximately 0.5 g of freeze-dried samples were digested with 10 ml of concentrated nitric acid by a microwave oven (MARS 5 Xpress CEM Corporation Instrument). After digestion, Cd, Cu, Hg, Pb and Zn were analysed by