Ecotoxicity of Sediments Contaminated by the Oil Spill Associated with the Tanker “Prestige” Using Juveniles of the Fish Sparus aurata

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Received: 27 October 2005/Accepted: 4 February 2006

Abstract. In November 2002, the oil spill from the tanker Prestige in the Galician Coast caused an ecological catastrophe in Spain. The adverse effects associated with the contaminants bound to sediments were tested using juveniles of the fish Sparus aurata (seabream). The approach evaluates sediment quality by using an integrated assessment including chemical and ecotoxicological data. Sediment samples were physicochemically characterized, and the concentration of contaminants (polycyclic aromatic hydrocarbons—(PAHs) and metals) was measured. Different biomarkers of exposure (metallothioneins and ethoxyresorufin O-deethylase activity (EROD)) and biomarkers of effect (histopathology) were analyzed along the time. A multivariate analysis approach was used to correlate concentration of contaminants and sublethal effects measured in individuals of fish. Results show that increasing concentrations of PAHs in sediments were related to increased EROD activities and histopathological lesions. This is the first evidence showing adverse effects associated with petroleum contamination of PAHs in sediments after this spill, and it demonstrates the utility of the sublethal toxicity tests for monitoring the impact of petroleum spills.

It has long been demonstrated that sediments can adsorb persistent and toxic chemicals to levels many times higher than water column concentrations (DelValls et al. 2002), whereas the sediment may become sufficiently polluted to disrupt natural biological communities (Adams et al. 1992; Tolun et al. 2001).

For a better assessment of the pollution process in the marine coastal environment, several authors have proposed determinations based on chemical measurements together with laboratory toxicity tests (Chapman 1988; Luoma and Ho 2001).

Sediment toxicity bioassays are instruments used to test the ecotoxicity and bioavailability of chemical compounds in sediments to benthic organisms. In this kind of bioassay, the organisms are exposed to sediment samples collected in situ and after the incubation period, a biological response is measured; this response must be sensitive, ecologically relevant, and easy to standardize (Stebbing et al. 1980). Bioassays provide information on the toxicity of contaminated sediments that can be neither derived from chemical analysis nor from ecological surveys performed alone (Chapman and Long 1983; Long and Chapman 1985). Interest in the effects of environmental stressors on health and disease in fish and other marine organisms has increased in recent years, and in particular, histological and cellular alterations have been observed in marine fish from polluted coastal waters and estuaries (Malins et al. 1984; Stein et al. 1992). These sublethal responses have been found to be a powerful tool to evaluate sediment toxicity effects (DelValls et al. 1998a).

Biomarkers in fishes have been previously studied in the assessments of oil spills such as Exxon Valdez (Varanasi et al. 1995; Jewett et al. 2002), Braer (Ritchie and O'Sullivan 1994), and Sea Empress (Kirby et al. 1998). In the present study, histopathology was conducted as a biomarker of effect in order to measure the damage caused in the target tissues by the presence of chemicals in the sediments. Two biomarkers of exposure were selected to address the biological adverse effects associated with contaminants present in the studied sediments. The toxicity of metals was assessed by metallothionein (MT) induction, whereas ethoxyresorufin O-deethylase activity (EROD) represents a good marker in MFO (mixed-function oxygenase), which is the first mode of detoxification of many organic pollutants (polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls). The EROD measurement in fish is considered a monitor of pollution exposure and an indicator of potential future problems in the health of fish populations (Carballeira 2003). Furthermore, EROD induction can be documented in fish exposed to spilled petroleum despite low tissues of PAHs (George et al. 1995; Whyte et al. 2000); The reason for using metallothionein induction is because we are studying a mixture of contaminants in the environment, and metallothioneins are one of the main biomarkers. In addition, it has been proved that the induction of this biomarker is not always just related to metals (Steigeman et al. 1992; Muto et al. 1999; Van der Oost et al. 2003).

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The composition of the oil spilled by the tanker *Prestige* was a mixture of saturated hydrocarbons, aromatic hydrocarbons, resins, and asphaltenes, with most of the PAHs being of medium to high molecular weight (Albaigés and Bayona 2003). Furthermore, it presents some trace metals such as Ni, V, Cu, Pb, and Zn (CSIC 2003; Albaigés and Bayona 2003; Prego and Cabelo-Garcia 2003; Prego and Cabelo-Garcia 2004). The physicochemical characteristics of this fuel show that the soluble fraction is low and the kinetics of degradation are slow under natural conditions so it is expected to be accumulated in sediments. The biological effects associated with the chemicals from the oil spill will be dependent on the nature of the ecosystem that accepts them and the organisms living in it (DelValls 2003). The first research notes about the early impact support the prediction that the acute toxicity of the weathered fuel (Mariño-Balsa 2003), very rich in high molecular weight compounds, was relatively low for the organisms tested (clams and microalgae). However, although concentrations of individual PAHs in aquatic environments are usually much lower than concentrations that are acutely toxic to aquatic organisms, sublethal effects can be produced (Albers 2003).

The results presented in this work show the status of the sediments 2 years after the accidental spill by linking sublethal responses measured in the fish exposed to oil-contaminated sediments with chemical data determined in sediments.

**Materials and Methods**

**Approach**

The present study was carried out by using sediment samples collected along different littoral areas in the North and the South of Spain. In the North, we chose sampling stations that have been affected by the oil spill in differing degrees and located along the Galician Coast (Ga1, Ga2, Ga3). Another sample was located in the South of Spain, in the Bay of Cádiz (BC) which is considered a pristine area (Riba et al. 2004a) and was used as the negative control reference. An artificial sample (TM) was made by mixing a toxic mud from an accidental mining spill in Spain (Aznalcóllar, April 1998) with the clean sediment and used as positive control (Riba et al. 2003).

Sediment samples from each station were collected with a 0.025-m² Van Veen grab and placed in a cooler until a sufficient amount of sediment was collected from a particular station (about 30 L). The contents of the cooler were homogenized with a Teflon® spoon until no color or textural differences could be detected. The samples were subsampled for physical characterization and chemical quantification. After that, sediment samples were maintained in the cooler at 4°C in the dark until used in sediment toxicity tests. Testing occurred within 2 weeks of collection. Sediment was filtered (0.5 mm) prior to the toxicity test in order to remove means interferences such as shells, predators, and other residues.

**Chemical Analysis**

Sediment aliquots from each station were dried at room temperature prior to chemical analysis and then gently homogenized. Geochemical matrix characteristics were studied analyzing total organic carbon concentration and sediment grain size. Organic carbon content was determined using the method of Gaudette et al. (1974) with El Rayis (1985) modification. For sediment grain size, an aliquot of wet sediment was analyzed using a laser particle size Fristch (model Analysette 22) following the method reported by DelValls and Chapman (1998b).

For trace metal analysis, the sediments were digested as described by Loring and Rantala (1992). Fe, Mn, Zn, and Cu concentrations in the extracts were determined with a Perkin–Elmer 2100 flame atomic absorption spectrophotometer. Concentrations of Hg and As were determined by means of Perkin–Elmer MHS-FIAS coupled with a Perkin–Elmer 4100 ZL spectrophotometer. The other trace metals were measured by graphite furnace atomic absorption spectrophotometry (Perkin–Elmer 4100 ZL). Results are expressed as mg kg⁻¹ dry sediment. The analytical procedures were checked using reference material (MESS-1 NRC and CRM 277 BCR) and showed a 90–110 range.

PAHs were analyzed by using gas chromatography/mass spectrometry (U.S. Environmental Protection Agency SW-846 Method 8270); briefly, dried samples were Soxhlet extracted with n-hexane for 18 h, and the extracts were isolated by column chromatography on Florisil-alumino-silica. PAHs were eluted and their fractions were dried in a rotary evaporator and redissolved in isooctane. Aromatic fractions were analyzed on a Hewlett–Packard (HP) 5890 Series II gas chromatograph coupled with HP 5970 mass spectrometer. Chromatographic resolution was achieved with a 30 m × 0.250 mm DB-5 capillary column, which has a 0.25-µm film thickness, with helium as carrier gas. Quality control was carried out using NRC-CNRC HS-6 sediment reference material. The analytical procedure allows agreement with the certified values in a 90–112 range.

**Sediment Bioassays**

Toxicity tests were carried out using juveniles of *Sparus aurata* obtained from an aquaculture farm and transported to the laboratory where the fish spent 1 month to acclimatize. *S. aurata* was selected because is a common species along the Spanish coast. Its biology is well known, having been used in previous pollution studies (DelValls et al. 1998a), and it is easy to acclimatize to laboratory conditions. The sea water used during the acclimatization period and the bioassay was clean marine water. A baseline of 10 randomly chosen individuals were weighed to provide data for feeding calculations. After the acclimatization period, the fish had a weight that averaged 4 ± 1 g.

Approximately 4 L of sediment from the negative control (BC) and the other stations (Ga1, Ga2, Ga3, TM) were placed in replicate 25-L glass tanks with clean sea water before the beginning of the experiment. After 24 h of particle settling, aeration was provided to maintain adequate oxygen concentrations (greater than 80% saturation). At the beginning of the test, another baseline group of 10 randomly chosen individuals was measured, weighed, anesthetized, and processed for biomarker responses (exposure and effect) to be used as the initial cellular control. Twelve individuals were placed in every tank after checking each tank’s water quality and were fed 2 or 3 times per day with commercial food (approximately 0.2 g per fish per day of “Mar Perla T” 1.4–2.2 mm). The test was conducted over 2 months, during which time no mortality was recorded. After the exposure period, individuals from each station were anesthetized and processed for histopathological, MTs, and EROD analysis. During the experiment natural photoperiod was selected and constant temperature was maintained (19 ± 1°C). The physicochemical parameters pH, temperature, oxygen, and salinity were recorded and controlled when necessary to maintain quality control during the test. Water replacement was performed every day by renewing 33% of the water column using a peristaltic pump.
Histological Procedures

Organisms from the toxicity tests were analyzed to determine the histopathological damages in different target tissues (liver and gills). When the water was renewed, the survival rate for all tanks was determined. Fish were removed from the tanks after 56 days of exposure time and samples were collected. Fish were anesthetized with 0.1% of 99% pure 2-phenoxethanol during 5–10 min, then weighed, measured for length, and externally examined. Target tissues (liver and gills) from all of the organisms were obtained by dissection and then fixed in phosphate-buffered 10% formaldehyde (pH 7.2) for 24 h and embedded in paraffin. The histological sections were stained with hematoxylin–eosin and hematoxylin–VOF (Gutiérrez 1967). Sections were reviewed by light microscopy (Leitz Laborlux S) and photographed (Sony DDC-CM30). Damage to the tissues was semi-quantified by detecting the frequency of the lesions in each detected alteration.

Biochemical Analysis

Fish were sampled for biochemical analysis, and after dissection, the liver was kept at −80°C prior to the homogenization. The samples were homogenized following the procedure developed by Lafontaine et al. (2000).

Metallothionein Concentration (MT)

Samples obtained to determine metallothionein content were centrifuged at 28,000 g for 40 min. The supernatant was added to 0.9 ml of NaCl (0.9%), heated to 95°C for 4 min, and centrifuged at 10,000 g for 15 min at 4°C. Supernatant was stored at −80°C prior to MT concentration determinations by Anodic Stripping Voltammetry (Olafson and Olsson 1987) using purified rabbit metallothionein (Sigma-Aldrich). Total protein determination was carried out using the methodology described by Bradford (1976). Concentrations were expressed as μg MT/mg total protein.

Mixed Function Oxidase Assay (EROD)

After homogenization of the samples, EROD samples were centrifuged at 10,000 g for 30 min, and the supernatant was used for the EROD activity determination and the total protein content described by Bradford (1976). Mixed function oxygenase activity was measured using the adapted EROD assay (Gagné and Blaise 1993). Briefly, 50 μl of supernatant (homogenate 10,000 g for 30 min), 10 mM 7-ethoxyresorufin, and 10 mM reduced NADPH in 100 mM KH₂PO₄ buffer (pH 7.4). The reaction was started by the addition of NADPH, was allowed to proceed for 60 min at 30°C, and stopped by the addition of 100 μl of 0.1 M NaOH. The 7-hydroxyresorufin was determined fluorometrically using 520 nm (excitation) and 590 nm (emission) filters. 7-Hydroxyresorufin concentration in the samples was achieved through a standard calibration curve developed with concentrations of 7-hydroxyresorufin. Results were expressed as pmol/mg total protein.

Statistical Analysis

Analysis of variance was performed in order to determine significant differences (p < 0.05; p < 0.01) among sites in relation to the biomarkers responses; the Tukey test was used as the post-hoc comparison. Also, contamination and toxicity data were linked by factor analysis, and using principal components analysis (PCA) as the extraction procedure, which is a multivariate statistical technique to explore variable distributions (Riba et al. 2003). The original data set used in the analysis included two biomarkers (EROD activity and metallothionein induction), two histopathological indexes (lesions in gills (LIG), and lesions in liver (LIL)), the concentration of different contaminants (PAHs, Cd, Cu, Ni, Pb, Zn, Hg), and the geochemical matrix characteristics (including total organic carbon and grain size distributions). The objective of PCA is to derive a reduced number of new variables as linear combinations of the original variables. This provides a description of the structure of the data with the minimum loss of information.

Results

Sediment Contamination

Summarized results of total organic carbon, grain size (percent of fine grain <63 μm), concentration of metals and PAHs are shown in Table 1. Of all the stations, the negative control (BC) showed the lowest values of most of contaminants. In general, it is observed that the concentration of PAHs in the area of Galicia (Ga3>Ga2>Ga1) was higher than those measured in the toxic mud and the sediments from the Bay of Cádiz (not detected). The toxic mud, used as positive control, showed high levels of metals in comparison to the other sample sites. These chemical data can be compared to international sediment quality guidelines (SQGs) that account for the chemical contaminants levels associated with biological effect (DelValls et al. 2004). In Table 1, the contaminants that exceed any SQG are highlighted. The letter that appears with the number indicates which SQG is surpassed.

Biomarker Responses

The basal level of metallothioneins measured in liver on day 0 of exposure was 20.1 μg⁻¹ mg⁻¹ and was lower than the levels of this biomarker after 56 days of exposure; the measures show that metallothionein levels in liver were significantly different (p < 0.5; p < 0.01) between fishes exposed to control and those exposed to other sediment (Figure 1). These differences were more significant for the station Ga3 and TM (p < 0.01) than for the other two stations located in the area of Galicia, Ga1, Ga2 (p < 0.05). EROD activity determined in the liver of juveniles of S. aurata on day 0 was 0.3 pmol mg⁻¹ min⁻¹. Results after 56 days of exposure are higher than basal levels and showed low values in fish exposed to sediment samples with absence or low levels of total PAHs (TM and BC, respectively) and were significantly different (p < 0.05) from stations affected to a different degree by the oil spill (Ga#) (Figure 1). The fish from these stations present high values of EROD activity and high levels of PAHs in their sediment (Ga1, Ga2, and Ga3). These relationships increase when the concentration of PAHs in sediments increases (Ga3 > Ga2 > Ga1). The EROD activity in liver of fish exposed to sediments from station Ga2 and Ga3 showed a significant difference (p < 0.01) between the control
treatment. Also, those from station Ga1 were significantly different from control treatment but with different value of the statistical $p(0.05)$.

For metallothioneins, Tukey test results set the five stations in three homogeneous groups according to the differences of averages among the sites. The first group includes only BC, which is the negative control; the second group is constituted by Ga1, Ga2, and Ga3; and the third group includes TM, which is the positive control.

For EROD activity, there are three homogeneous groups set by the Tukey test results. The first group includes BC and TM, both negative and positive control, which do not present significant differences; the second group is constituted by Ga1 ($p < 0.05$) and Ga2 ($p < 0.01$); and the third group includes Ga3 ($p < 0.01$).

### Histopathological Approaches

The organisms analyzed on day 0 did not present histopathological damages. Different alterations were observed in target tissues (gills and liver) of fish exposed to sediment collected after 56 days of exposure in the different stations, mainly in gills, which showed shortening of secondary lamellae, hypertrophy, and hyperplasia, necrosis, and loss of epithelial cells in Ga# and TM; fusion of the secondary lamellae above all in Ga3 and TM; and presence of edematous areas in the distal portion of lamellae in Ga# and TM. Also, liver showed lesions: vacuolization of hepatocytes, necrosis, and decrease of the zymogen granules of the exocrine pancreas in Ga# and TM. In general, an increase of cytoplasmic basophilia was detected in the liver and exocrine pancreas of all exposed fish related to the increase of PAHs. An example of some of these lesions is shown in Figure 2.

These lesions have been previously recorded as related to contaminants bound to sediments in $S$. aurata (DelValls et al. 1998a) and in other fish species such as $Solea senegalensis$ (Riba et al. 2004b, 2004c). As previously reported by DelValls et al. (1998a) and based on the damage observed in the different tissues, histopathological alterations were evaluated semiquantitatively in the fishes exposed to the different stations by ranking the frequency of lesions measured in a total number of 6 individuals: – (0 individuals), +/− (1 individual), + (2 individuals), ++/− (3 individuals), ++ (4 individuals), ++++/− (5 individuals), and finally the maximum is associated with the presence of a disease in the total number of individuals, +++ (6 individuals sampled). Gills were shown to be the most damaged tissue, showing different lesions mainly in Ga#.
and TM. An average of this semiquantitative evaluation of the frequency of the lesions measured from the different replicate results is shown in Table 2.

General indexes of lesion (lesion index in gills [LIG] and lesion index in liver [LIL]) were calculated for each tissue as an average value of the fish damage semiquantified (Figure 3).

Fish exposure to sediment samples produced lesion damage related to the increase of the concentrations of contaminants (PAHs in Ga# and high levels of metals in TM) in the sediments selected in the bioassay. The lesions identified in all the tissues analyzed were almost always present in animals exposed to sediments from stations Ga2 and Ga3. Evaluations of histology of gills and liver revealed clear significant differences \( (p < 0.05) \) between the negative control of toxicity and the Ga3 station and the toxic mud. The severity of the lesions detected in the tissues of fish exposed to sediments collected in Bay of Cádiz was lower than those measured in the area of Galicia. Results show the lowest indexes in the Bay of Cádiz (BC), which were significantly different \( (p < 0.05) \) from the values from Galicia (Ga#) and toxic mud (TM). The index of lesions measured for gills (LIG) in Galicia increase with the presence of PAHs in the sediment samples (Ga3 > Ga2 > Ga1). The LIL results show that TM has the highest index related to liver lesions.

**Discussion**

To link the set of data obtained, the original variables from chemical concentration and sublethal responses were analyzed by factor analysis, using PCA as the extraction procedure, which is a multivariate statistical technique (MAA) to explore variable (chemical concentration, \( n = 25 \); toxicity data, \( n = 4 \)) distributions. The factor analysis was performed on the correlation matrix, and the variables were autoscaled (standardized) so as to be treated with equal importance (Riba et al. 2004a). The applications of MAA to the original 29 variables indicate that they can be grouped in two new factors. These factors explain 88.4% of the variance in the original data set. Negative values of sorted rotated factor loadings (negative salience) are as important as positive values (positive salience). In the present study, we selected to interpret a group of variables as those associated with a particular component where loading was 0.40 or higher (Table 3). This approximates Comrey's cutoff of 0.55 (Comrey 1973) for a good association between an original variable and a factor, and also takes into account discontinuities in the magnitudes of loadings approximating the original variables.

The first principal factor, \#1, is predominant and accounts for 62.4% of the variance; it explains the toxicity of individual PAHs and combines the concentrations of PAHs (fluorene, acenaphthene, naphthalene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, dibenzo[a,h]anthracene, indene[123-cd]pyrene, benzo[g,h,i]perylene) in sediment, the total organic carbon, all the indexes of histopathological lesions (gills, LIG; and liver, LIL), and the EROD activity. The second factor, \#2, accounts for 26.1% of the variance; it explains the toxicity associated with the metals in sediment combining, with negative loading, the chemical concentrations of the metals Cd, Cr, Cu, Ni, Pb, Zn, and Hg with the grain size with all the indexes of lesions (gills, LIG; and liver, LIL) and with the induction of metallothioneins (MTs). Figure 4 shows the influence of both factors in the five different stations. Factor 1, with positive loading, is defined as the toxic responses of the fish to PAHs bound to sediments; thus, station Ga2 (0.44) and especially Ga3 (1.48) show the significant prevalence of this factor, whereas this factor does not affect station Ga1, and both controls positive (TM) and negative (BC). The definition of Factor 2 as the toxic responses of the fish to metals bound to sediment only has prevalence in the positive control (TM); the negative loading of the toxic responses and the metals concentration in sediment implies that the prevalence of these factors in the station is associated with negative factor scores. Furthermore, a linear relationship can be observed in the scores of this factor from BC to Ga3, which confirms the increase of toxicity when PAHs increase in sediments.

It is estimated that about 63,000 tons of heavy fuel oil were lost from the single-hull tanker Prestige. Although a large quantity of this fuel was collected and removed from the coast, a large amount likely settled down at the bottom of the sea covered with sediment reaching the littoral area of the Galician coast after the first months of the spill (Albaiges and Bayona 2003). In the present study, we aimed to assess the impact of this enrichment in littoral sediments collected in different affected areas and 2 years after the oil spill using juveniles of
the fish *S. aurata* by means of different sublethal endpoints such as histopathological lesions, metallothionein induction, and EROD activity.

Previous studies have shown how in water the toxicity of individual PAHs increases as molecular weight (MW) increases up to MW 202 and beyond it; solubility reduces and so does lethal toxicity, but sublethal effects can result (Albers 2003). In the present study, it has been shown that the Galician sediments (mainly Ga2 and Ga3) analyzed present levels of PAHs with low MW (fluorene, acenaphthene, naphthalene, phenanthrene, anthracene), medium MW (pyrene), and high MW (benzo[a]anthracene, chrysene, benzo[a]pyrene, dibenz[ah]anthracene), higher than some of the SQGs proposed by international agencies. Furthermore, all individual PAHs seem to have induced hepatic EROD and to produce histopathological damage; it is quite difficult to determine which of the individual PAHs is the main pollutant that has caused the biological effects (explained by factor 1 in the MAA); however, it can be concluded that PAHs are the compounds that are producing the adverse effects to the fishes.

The significant differences of EROD induction between Ga# (Ga1, *p* < 0.05; Ga2 and Ga3, *p* < 0.01) show a strong relationship with the concentration of PAHs in the Galician sediments (Ga1, Ga2, and especially in Ga3)—impacted by the oil spill—and the histopathological lesions in gills and liver, studied in the MAA. Despite differences in the induction of EROD among these Galician samples, the validity of this biomarker of contamination was shown.

Twbn the absence of fish mortality, other research on the impact of the “Sea Empress” oil spill in the UK in 1996 (Edwards and White 1999) showed the possibility of sublethal and chronic effects using a variety of techniques such as EROD activity. In these studies, there was evidence of high levels of EROD activity in the sites exposed to oil constituents in comparison with the control sites. There are other studies, carried out using biomarkers as EROD activity, that

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### Table 2. Frequency of lesions detected in microscopic abnormalities of individuals of juveniles of the fish *Sparus aurata* sampled in the Bay of Cádiz (BC), Galicia coast (Ga#) and toxic mud (TM) treatments on day 56 of exposure

<table>
<thead>
<tr>
<th>Organ</th>
<th>Histopathology</th>
<th>Samples zones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BC</td>
</tr>
<tr>
<td>Gills</td>
<td>Hypertrophy/hyperplasia</td>
<td>+/-</td>
</tr>
<tr>
<td>Fusion of secondary lamellae</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Shortening of secondary lamellae</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Edematous areas or aneurysm in distal portion of lamellae</td>
<td></td>
<td>+/++</td>
</tr>
<tr>
<td>Necrosis and lost of cells epithelial</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Liver</td>
<td>Increase of lipid vacules in the hepatocytes</td>
<td>+/-</td>
</tr>
<tr>
<td>Increase of cytoplasmic basophilia of hepatocytes</td>
<td></td>
<td>+/</td>
</tr>
<tr>
<td>Necrosis and decrease of the zymogen granules of exocrine pancreas</td>
<td></td>
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</table>

(0 individuals), +/- (1 individual), + (2 individuals), ++/+ (3 individuals), ++ (4 individuals), +++/++ (5 individuals) and finally the maximum is associated with the presence of a disease in the total number of individuals, +++ (6 individuals sampled).
because gill filaments and lamellae provide a very large surface area for direct and continuous contact with contaminants in water. Fish gill and liver are highly sensitive to pollutant exposure; however, as previously indicated (Arellano et al. 1999), these pointed histopathological alterations are, in general, nonspecific effects, meaning that they are responsive to a variety of pollutants, and therefore only indicative of the general quality of the environment rather than specific types of pollutants (Au 2004). The increase of lipid vacuoles (small size) present in the hepatocytes can indicate an alteration of lipid metabolism or a partial change in their morphology, or in that of lysosomes (Arellano et al. 1999; Segner and Storch 1985). The cause–effect relationships and detailed mechanisms leading to the development of most pathological symptoms are not generally clear. Nevertheless, certain hepatic lesions in fish have been well correlated with contaminant exposure (Au 2004). Lamellar fusion of gills could be a protective effect for diminishing the amount of vulnerable gill surface area (Mallat 1985).

The comparison between chemical analysis and the different toxic response (biomarkers of exposure and of effect at different levels) is a useful tool to determine the quality of the studied sediments. The importance of the use of chronic bioassays that provide long-term information on the effects of the exposure to a toxic compound has been proved, because a compound cannot reflect a considerable lethal toxicity, but it is able to produce lesions at different levels to the organism exposed.
Despite the repercussion of the spill in the biota, shown as a decrease of the abundance of the microfauna (Junoy et al. 2005), previous studies have shown that there was not an important toxic effect in different marine organisms (clams and microalgae) exposed to samples of the sediments and their elutriates associated with the spill caused by the tanker “Prestige” (Mariño-Balsa et al. 2003). The bioassay using juveniles of the fish S. aurata showed results sensitive enough to determine the hazard associated with this oil-contaminated sediment, displaying good correlation between the toxicity and the contaminant levels using a sublethal set of measurements including both biomarkers of exposure and effect. This study demonstrates the necessity to monitor the impact of the spill on sediment quality in the areas affected. Furthermore, it shows that a subchronic test using a sensitive and sublethal endpoint is a powerful tool to identify the risk associated with the enrichment of PAHs in affected sediments. The higher sensitivity of this bioassay compared to the acute tests previously used indicates the need to incorporate this kind of approach as part of a more complete and integrated study based on a weight-of-evidence approach, as previously recommended by some authors (Carballeira 2003).

Acknowledgments. The described work was supported by a Grant funded by the Ministry of Education and Science VEM2003-20563. Carmen Morales-Caselles thanks the Ministry of Education and Science for funding her research fellowship (FPU). We thank the members of the ICMAN-CSIC for their support and help.

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