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Growth and condition indices of juvenile turbot, *Scophthalmus maximus*, exposed to contaminated sediments: Effects of metallic and organic compounds

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ABSTRACT

Since sediments have the potential to form associations with several classes of pollutants, they have been recognized as a possible and significant source of contamination for the benthic environment. Flatfish maintain a close association with sediments for food and cover, and are therefore more likely to be exposed to contaminated sediments, especially in coastal areas (e.g. nursery grounds). The assessment of these potential biological effects involves the use of adapted biomonitoring tools. The main objective of this study was to assess and compare the response of several physiological biomarkers measured on juvenile turbot (*Scophthalmus maximus*) exposed to contaminated sediments. Sediments were collected from three stations in a harbour in northern France (Boulogne-sur-Mer), in an anthropogenic French estuary (the Seine), and in a reference site (exposed sandy beach of Wimereux). Unexposed lab-reared juvenile turbots were exposed to sediments for 7 and 21 days in laboratory conditions. Sediments were analysed for metals, PAH and PCB contamination. Several fish growth and condition indices were individually analysed in fish according to the chemical contaminant availability in sediment, the metal concentrations in gills and the estimation of PAH metabolites in their bile. Significant decreases in growth rates, morphometric index, RNA:DNA ratio and the lipid storage index, based on the ratio of the quantity of triacylglycerols on sterols (TAG:ST), were observed with increasing level of chemical contamination. This decrease in the fish's physiological status could be related to the significant increase of several metal concentrations in contaminated fish gills and the significant increase of PAH metabolites in bile. In a field situation, such a reduction in growth and energetic status of juvenile fish could dramatically decrease their over-winter survival in contaminated nursery grounds.

1. Introduction

Contamination of sediment in coastal areas, caused by multiple anthropogenic activities, is considered a major environmental concern for marine ecosystems. Although emissions into water and the atmosphere have been reduced, because of environmental regulations and the ban on the production and use of some substances (e.g., PCBs, DDT, some polybrominated diphenyl ethers), sediment now serves as a source of many contaminants (EPA, 2000; Van der Geest et al., 2010). Indeed, sediments have the potential to form associations with several classes of anthropogenic pollutants, and may not only function as sinks for chemical contaminants, but also as sources of such pollutants through resuspension of particulate matter (Viguri et al., 2007; Otte et al., 2008). In particular, hydrophobic organic compounds and metals are two important groups of contaminants whose chemical constitution makes them prone to adsorption by particles. Although exposure to environmentally relevant concentrations of these contaminants may not be acutely lethal, they have the potential to induce sublethal effects in a variety of marine organisms (Van der Geest et al., 2010). In this context, toxicology methods have been developed to monitor the effects of sediment-associated pollutants on organisms, population and communities (Luoma and Ho, 1992). Sediment toxicity bioassays are instruments of increasing importance for scientists

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to test the toxicity and bioavailability of chemical compounds in sediments to organisms. Indeed, one method for exposing aquatic organisms to contaminated sediment is to place the organisms with sediment layered on the bottom. It appears to be of great importance to select the appropriate type of test organisms, as well as the appropriate life stage, and the relevant sublethal measurements, based on the route of exposure to be assessed.

For several reasons, fish species have attracted considerable interest in studies assessing biological and biochemical responses to environmental contaminants (Amara et al., 2009). Benthic and epibenthic fish are the most heavily exposed to contaminants originating in sediments, and may integrate several routes of exposure by accumulating contaminants dissolved in water (through gills and skin) and through the ingestion of food and sediment. Moreover, fish often resuspend sediment, increasing the availability of contaminants. In particular, flatfish appear good model species to examine the effects of contaminated sediment. Following metamorphosis, flatfish display a predominantly benthic life style and maintain an intimate contact with sediments, where they seek shelter, waylay their prey (Aarnio et al., 1996), and therefore are likely to be exposed to sediment-associated contaminants. Among the different flatfish species, turbot could represent an appropriate study species, as it lives in shallow coastal areas likely to be impacted by nearshore activities. This species is reared in aquaculture and its biology and physiology are well known. It has also a rapid growth, particularly during its juvenile stage, and is able to change its physiology with easily detectable external stressors. Hatchery production of turbot has led to an increased availability of individuals with known exposure history, which is an essential element in any toxicological study (Boisson et al., 1998).

Toxicity tests that involve sublethal measurements have been shown to be sensitive and provide relevant information about the adverse effects associated with chemical contaminants (Jiménez-Tenorio et al., 2007). Among the different levels of responses analysed, physiological responses to chemical contaminants have often been ignored by ecotoxicologists, because they are regarded as being too generalized and too difficult to measure routinely (Depledge et al., 1995). However, evidence is now emerging that there may be some advantages in identifying integrated responses to the sum of the stresses imposed by pollutants and natural environmental factors. Moreover, few laboratory studies have examined the toxicity of contaminated sediment on early life stages, despite evidence that juveniles are more sensitive to contaminants than adults are. Juvenile fish physiological biomarkers, as growth or lipid storage, may provide the key to integrating various molecular and cellular responses in an organism with impaired fitness (Amara et al., 2007); these processes must be functional for juvenile fish to survive and so contribute to the population's renewability (Depledge et al., 1995). Indeed, a commonly observed sublethal response of organisms exposed to chemical contaminants chronically is a change in their energy allocation (Rowe et al., 2001). In such a way, maintenance costs associated with combating chemical toxicants would be expected to ultimately reduce growth, and so a general decrease in juvenile fish condition.

The main objective of this study was to use some fish physiological biomarkers to analyse the potential adverse effects of contaminated sediments. Juvenile turbots were exposed in laboratory conditions to different contaminated sediments from a highly industrialised harbour (Boulogne-sur-Mer) and from an anthropogenic French estuary, the Seine. Several fish growth and condition indices were analysed according to the sediment chemical contamination, the metal concentrations in gills and the estimation of PAH metabolites in their bile. We used growth rates (somatic and estimated from otolith microstructure), RNA:DNA ratios, morphometric (Fulton's K condition index), and lipid (triacylglycerols:sterols ratio, TAG:ST) condition indices to estimate the health status of turbot related to the level of chemical contamination in sediments.

2. Materials and methods

This experiment was conducted in accordance with European Commission recommendation 2007/526/EC, on revised guidelines for the accommodation and care of animals used for experimental and other scientific purposes. The University of Littoral Côte d'Opale is authorised to conduct experimentation on animals in its capacity as a certified establishment; according to the administrative order No. B62-160-2.

2.1. Sediment collection

Sediments were collected from different sites located along the French coast of the Eastern English Channel at the same time in February 2010. These sediments were sampled from three stations in a harbour in northern France (Boulogne-sur-Mer: BSM); from an anthropogenic French estuary (the Seine) and from a reference site (Fig. 1). The BSM harbour is an intensively developed and industrialised harbour impacted on by municipal and industrial discharges, fishing and shipping activities, and marinas. The sediment was collected using a Van Veen grab (250 cm^2 sampling area) in three different locations in the harbour: station A in the front, and stations B and C in the inner part. Estuarine inter-tidal sediment was collected at low tide from the north bank of the Seine estuary. The Seine basin currently accounts for 25% of French agriculture, 25–30% of French industrial activity and 23% of the French

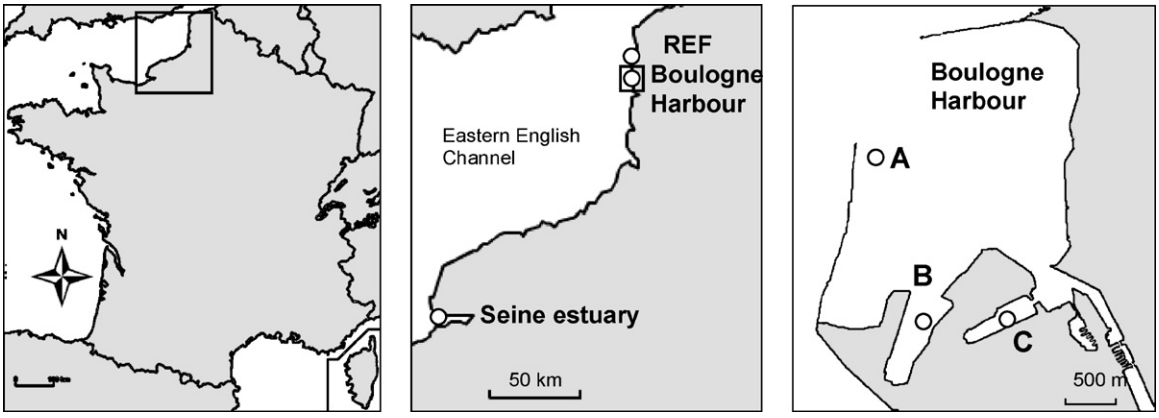

Fig. 1. Locations of the three sediment sampling sites (Reference, the Seine estuary and harbour of Boulogne sur Mer) and the three stations in the harbour (A–C).

population. The last sample was taken at low tide from Wimereux beach, used as the reference site. For each of these five locations, about 12 L of sediment were collected and put into polyethylene bags. Sediments were not sieved; however, large sediment particles and organic debris were removed. About 10 L were stored at 4 °C until the experimental assay and 2 L were frozen at −20 °C for sediment analyses. Sediment exposure was initiated approximately two weeks after sediment collection.

2.2. Sediment exposure conditions

Four-month-old turbots, *Scophthalmus maximus*, (weight: 6.83 ± 0.72 g) were obtained from a hatchery (France Turbot) and acclimatised in two clean tanks (160 L) in semi-static conditions for two weeks. The water was aerated with air pumps and the photoperiod was set at a 10 h light and 14 h dark cycle. During acclimation, the water temperature was 14.3 ± 0.8 °C, and the fish were fed with a commercial fish food once a day. The daily feeding amount was maintained at approximately 1% of the total fish weight. Before the beginning of the experiment, each fish was anaesthetised in a 200 μl L^{-1} 2-phenoxyethanol solution, weighed (0.01 g accuracy), measured for total length (1 mm accuracy) and individually marked (Visual Implant Tag, 1.2 mm × 2.7 mm, Northwest Marine Technology).

The experimental 21-day assay consisted of a static water system of 37 L capacity glass tanks, in which 5 L of sediment and 25 L of clean seawater were allocated. The assay was performed in duplicate for the five different sediments, thus ten tanks were used. Sediments were homogenised and allowed to settle 48 h before the beginning of the assay. The conditions of treatment remained the same as those ones applied during the period of acclimation. Fifteen tagged fish were randomly distributed in each tank (30 per treatment) and fed with a commercial fish food once a day. In order to maintain an acceptable quality of overlying water, a daily water change (1/3 of total water volume) was performed, and water parameters (temperature, salinity, pH, oxygen and turbidity) were monitored every day before the water renewal.

Fifteen fish were sampled before exposure to the sediment to establish t_0 (the background level of the different biological parameters) and after 7 and 21 days of exposure. Another group of fifteen fish (7–8 per duplicate tank) was sampled per treatment group and anaesthetised with 2-phenoxyethanol. The turbots were identified (tagged), weighed and measured. Their bile was sampled, frozen in liquid nitrogen and preserved at −80 °C. Their gills and muscles were sampled and stored at −20 °C and their otoliths (sagittae) were extracted and preserved in ethanol (95%).

2.3. Additional starvation assay

Complementary to the contaminated sediment exposure, a starvation assay was undertaken to analyse the responses of physiological biomarkers under severe stress. Two 37 L glass tanks were used in which 5 L of the reference sediment were allocated. A number of 24 tagged turbots were distributed in each duplicate tank. Experimental conditions were strictly identical to the sediment exposure assay, but these fish were not fed during the experiment. Twelve fish (6 per duplicate tank) were sampled after 2, 7, 10 and 21 days. The fish were anaesthetised, weighed and measured. Muscles and otoliths were sampled as previously.

2.4. Sediment analysis

Sediment samples were analysed to determine granulometry, organic matter contents, metals, PAHs and PCBs contents.

The grain size distribution was analysed using a laser Beckman-Coulter LS 230. A classification was established using the proportion of clay (<4 μm), fine silt (4–20 μm), coarse silt (20–50 μm), fine sand (50–200 μm), medium sand (200–500 μm) and coarse sand (500–2000 μm).

For the measurement of total organic matter (TOM, mg g^{-1}), the sediment samples were dried at 60 °C for 24 h and subsequently baked at 450 °C for 5 h (Luczak et al., 1997). The total organic carbon (TOC) and total organic nitrogen (TON) contents were determined using a CHNS analyser (NA-2100, CE instruments).

In order to determine selected metals (Al, Cd, Cr, Cu, Mn, Ni, Pb, V and Zn) in the total and bioavailable fractions, the sediments were dried in a oven at 40 °C to constant weight and then ground into powder. For the determination of total metals, about 0.250 g of ground sediment were digested with HF (Suprapur, Merck) at 110 °C for 48 h followed by a mixture of concentrated acids HCl:HNO_3 (3:1, v:v, Suprapur Merck) at 120 °C for 24 h. This operation was repeated once. Metals associated with the reactive fractions of sediment, considered as bioavailable fractions, were estimated using the method of Huerta-Diaz and Morse (1990). The reactive fraction shows values comparable to the sum of the first three reactive fractions in the Tessier extraction scheme (Tessier et al., 1979). The reactive fraction, such as metals extracted by HCl 1 M, comprises of metal exchangeable and linked to carbonates, partially to oxy-hydroxides of Fe–Mn, and to acid volatile sulfides (AVS). About 0.5 g of sediment was leached during 24 h with 20 mL of 1 M HCl (Suprapur, Merck). The total and extractable heavy metals were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES, VARIAN Vista Pro, axial view). For quality assurance, reagents blanks, sample replicates and standard reference materials (MESS-3 and PACS-2, National Research Council Canada) were used to assess the accuracy and precision of the analyses. In all cases, the recovery efficiency was better than 85% for the total digestion of standard reference materials.

The extent of sediment contamination was assessed using the enrichment factor (EF) (Salomons and Forstner, 1984; Windom et al., 1989). Commonly, normalization of the metals to a conservative element such as Al is employed as an index (EF) to evaluate anthropogenic influences to the sediments. The EF is defined as $EF = (X/Al)_{sample}/(X/Al)_{background}$, where $(X/Al)_{sample}$ is the metal to Al ratio in the sample of interest and $(X/Al)_{background}$ is the same ratio in the upper crustal material (Taylor and McLennan, 1995). According to previous studies (Birth, 2003; Han et al., 2006), EF < 2 indicates sediment uncontaminated by metals (crustal origin), 2 < EF < 10, moderately contaminated, EF > 10 significantly contaminated (non-crustal source).

The persistent organic pollutants, including PAHs (EPA's 16 priority PAHs) and PCBs (7 congeners: 28, 52, 101, 118, 138, 153, 180) were analysed. Briefly, organic compounds were extracted from 2 g of dried sediment by a microwave oven (120 °C for 15 min, 1200 W), assisted with a 40 mL mixture of acetone and hexane (1:1, v:v). The solvent was evaporated under a stream of nitrogen in a TurboVap, and then concentrated to 1 mL of hexane. Simultaneous determination of PAHs and PCBs was performed on a gas chromatography–mass spectrometer (GC–MS, VARIAN, CP 3800-1200 MS TQ). A ZB-MultiResidue column (30 m, 0.25 mm, 0.25 μm) was used (Phenomenex). Identification of PAH compounds and PCB congeners was based on the comparison of their GC-retention times and their mass spectrum, with appropriate individual standards.

A way to assess sediment toxicity is the use of contamination sediment quality guidelines. Long et al. (1995) identified two guideline values: the effects range-low (ERL) and the effects range-median (ERM). Concentrations above the ERL, represent a possible-effects range within effects would occasionally occur. The concentrations above the ERM values represent a probable-effects range within which effects would frequently occur. In the present study, chemical contaminant contents in sediments were analysed according to theses guidelines.

2.5. Biological analysis

2.5.1. PAH metabolites in bile

Bile samples were diluted 1:3000 times in 95% ethanol and, fixed wavelength fluorescence (FF) was measured at the excitation/emission wavelengths 343/383 nm and 380/430. By $FF_{343/383}$ and $FF_{380/430}$ mainly pyrene and benzo(a)pyrene type metabolites are detected (Aas and Klungsaryr, 1998). The two fluorescence intensities were summed up and the relative PAH metabolite detection was expressed as an arbitrary unit of fluorescence.

2.5.2. Growth index

Turbot specific growth rates in weight (% per day) were estimated as:

$$GW = \frac{100(\ln W_2 - \ln W_1)}{t_2 - t_1},$$

where W_1 and W_2 are fish total body weight at times t_1 (beginning of the experiment) and t_2 (time of collection). Similarly, the specific growth rate in length was estimated as:

$$GL = \frac{100(\ln L_2 - \ln L_1)}{t_2 - t_1},$$

where L_1 and L_2 are fish total length at times t_1 and t_2 respectively.

The recent growth index (RG) was determined by measuring the width of the peripheral daily increments of the otoliths. We used the width of the daily otolith increments from the previous 5 days before the end of the experiment as an indicator of recent growth (mean distance between the margin of the otolith back to the 5th ring). Sagittae were cleaned and photographed to determinate the diameter, the perimeter and the area of each otolith. Then, they were mounted on a glass slide in thermoplastic cement (Crystal Bond). Sections of sagittae were obtained by polishing them on both sides with grinding paper of decreasing grit sizes (5–0.1 μm) until increments at the outer edge were visible. Otoliths were etched for 30 s with 0.1 M EDTA and analysed under transmitted light, using a video system fitted to a compound microscope. All the measurements were done along the same axis (anteroposterior) using an Image Analysis System (TNPC, 5.0, NEOSIS). The increment measurement was repeated three times by the same reader at different interval of time and the mean was calculated.

2.5.3. Condition indices

We estimated three health indices in turbot exposed to sediments at t_7 and t_{21} and in the fish sacrificed at the beginning of the experiment (t_0): RNA:DNA ratio and TAG:ST ratio as indicators of nutritional status, and Fulton's K condition index as an indicator of the general well being of the fish. This latter morphometric index assumes that heavier fish, for a given length, are in better condition. We calculated Fulton's K condition index with the formula: $K = 100(W/L^3)$, where W is the body mass (mg) and L is the total length (mm).

The procedure used to determine RNA and DNA concentrations in individual fish was based on the Clemmesen method (1988). Nucleic acids were measured on muscle fragments (0.05 g) by homogenizing the sample in ice-cold Tris–EDTA buffer (0.05 M Tris, 0.1 M NaCl, 0.01 M EDTA, pH 8.0) with proteinase-K (pro-K) and sodium dodecyl sulfate (SDS). Nucleic acids were extracted by purification step involving phenol:chloroform:isoamylalcohol (Amara et al., 2009). The quantity of RNA and DNA was determined by the fluorescence-photometric technique, using a specific nucleic acid fluorescent dye-ethidium bromide (Sigma–Aldrich Chemicals, France) (Amara et al., 2009).

The third health index was a lipid storage index based on the ratio of the quantity of triacylglycerols (TAG; reserve lipids) to the quantity of sterols (ST; structural lipids) in the fish. The amount of total lipids in each individual was measured on a sample of lyophilised muscle (0.07 g). Lipid extraction was conducted using the method of Bligh and Dyer (1959) slightly modified as described by Amara et al. (2007). Lipids were extracted using a mixture of water:chloroform:methanol (1:1:1, v/v/v). TAGs and sterols were separated from other lipids by performing thin layer chromatography (TLC).

2.5.4. Metal analysis in gills

Because of the low quantity of juvenile fish gills for metal analysis, due to the small size of the juveniles, the gills of five fish were pooled and thus three samples of gills were analysed for each condition. The gills were rinsed with Milli-Q water, mixed and lyophilised for analysis of metal concentrations. Samples were digested with HNO_3 (65%, Suprapur Merck) at an ambient temperature for 24 h, and then at 100 °C for 4 h. Metal concentrations were determined by inductively coupled plasma-mass spectrometry (ICP-MS; VARIAN 820). Standard curves were used to determine both Mn and Zn, whereas a standard addition technique was applied for the resolution of matrix effects to calculate As, Cd, Co, Cr, Cu, Ni, Pb, Se and V. International certified standard (DORM-3, NRC Canada) was used to control the accuracy of the analytical procedure.

2.6. Statistical analysis

Statistics were performed with XLSTAT 2007. Comparison of biological parameters between the five exposure conditions were analysed with one-way ANOVA, followed by post hoc Tukey tests. If biological data did not comply with the parametric assumption of normality (Shapiro–Wilk tests) and homogeneity of variance (Levene tests) after various transformation techniques were tested, the non-parametric Kruskall–Wallis test and Mann–Whitney U test for post hoc pairwise comparisons were used. These non-parametric tests were also used to analyse differences in metal bioaccumulation in gills. Using data from all the individuals collected, a Pearson product moment correlation matrix was computed to test the strength of the relationships between the variables measured. A principal component analysis (PCA) was performed with data means for each condition to evaluate the contributions of chemical and physiological biomarkers in the explanation of variations between exposure conditions.

3. Results

3.1. Environmental parameters

No mortality was observed in any of the exposure tanks. Temperature, salinity, pH and oxygen levels were constant and similar in the different exposure tanks throughout the experimental assay. Sediment grain size distribution was different between the reference sediment, which was sandy, whereas, harbour and estuarine sediments were mainly composed of mud (Table 1). The turbidity was low in reference tanks (6.14 ± 3.74 NTU), varying from 214 ± 96 NTU in the A sediment tanks, to 398 ± 124 NTU in the Seine sediment tanks. This difference of turbidity could be related to the physical properties of the sediment. TOM, TOC and TON were also lower in the reference sediment, compared to the other sediments. These three organic compounds increased from A to C harbour sediments, and the Seine estuary sediment showed values similar to the A sediment.

No measurable PCB congeners were detected above 0.01 mg kg^{-1} in any of the sediments tested. As expected, the reference sediment was the least concentrated in both metal and PAHs compounds (Table 1). Among the three harbour sediments, the A sediment located in front of the harbour, presented the lowest concentrations in metals, (which were however three times

Table 1
Mean (±SD) abiotic parameters, sediment metal, PAHs and PCBs concentrations (mg kg^{-1} dry weight) in the five sediments (Ref, Seine, A, B and C).

	Concentrations in sediment						
	REF	Seine	A	B	C	ERL	ERM
Turbidity (NTU)	6.14 ± 3.74	398 ± 124	214 ± 96	391 ± 196	216 ± 77		
TOM (mg g^{-1})	2.41 ± 0.04	57.7 ± 1.0	41.8 ± 0.7	116 ± 2	155 ± 3		
TOC (mg g^{-1})	1.89 ± 0.11	15.3 ± 0.9	9.42 ± 0.55	27.2 ± 1.6	43.9 ± 2.6		
TON (mg g^{-1})	0	2.38 ± 0.05	1.34 ± 0.03	4.68 ± 0.11	3.95 ± 0.09		
Mud (%)	0	83.8 ± 6.2	83.2 ± 6.0	91 ± 1.8	90.2 ± 2.1		
Sand (%)	100 ± 2.61	16.2 ± 3.5	16.8 ± 4.3	9.04 ± 1.4	9.8 ± 1.9		
Metals							
Cd	<0.1	0.62 ± 0.11	0.26 ± 0.03	1.61 ± 0.10[a]	0.97 ± 0.02	1.20	9.60
Cr	4.98 ± 0.23	40.3 ± 1.4	15.5 ± 0.7	37.3 ± 0.3	41.1 ± 1.0	81	370
Cu	0.67 ± 0.02	15.5 ± 0.1	3.73 ± 0.09	16.9 ± 0.1	94.1 ± 1.8[a]	34	270
Mn	52.1 ± 1.15	396 ± 7	124 ± 1	1758 ± 17	543 ± 12		
Ni	1.36 ± 0.06	10.3 ± 0.3	4.55 ± 0.13	12.5 ± 0.2	14.6 ± 0.3	20.9	51.6
Pb	3.56 ± 0.04	23.4 ± 0.5	7.51 ± 0.02	42.6 ± 0.4	32.8 ± 0.9	46.7	218
V	4.72 ± 0.1	36.0 ± 0.7	17.8 ± 0.2	52.7 ± 3.8	66.4 ± 1.6		
Zn	6.39 ± 0.34	73.9 ± 2.8	22.8 ± 0.5	161 ± 2[a]	273 ± 19[a]	150	410
Al	9810 ± 370	26700 ± 200	14170 ± 160	29240 ± 600	32050 ± 1200		
PAHs							
Naphtalene	<0.05	0.07 ± 0.03	<0.05	<0.05	0.05 ± 0.02	0.16	2.10
Acenaphtylene	<0.05	<0.05	<0.05	<0.05	<0.05	0.04	0.64
Acenaphtene	<0.05	<0.05	<0.05	<0.05	<0.05	0.02	0.50
Fluorene	<0.05	<0.05	<0.05	<0.05	<0.05	0.02	0.54
Phenanthrene	<0.05	0.12 ± 0.02	<0.05	0.10 ± 0.01	0.13 ± 0.02	0.24	1.50
Anthracene	<0.05	0.07 ± 0.02	<0.05	<0.05	0.13 ± 0.04[a]	0.09	1.10
Fluoranthene	<0.05	0.20 ± 0.04	<0.05	0.17 ± 0.03	0.22 ± 0.04	0.60	5.10
Pyrene	<0.05	0.17 ± 0.03	<0.05	0.12 ± 0.02	0.25 ± 0.04	0.67	2.60
Benzo(a)anthracene	<0.05	0.14 ± 0.01	<0.05	0.12 ± 0.01	0.19 ± 0.02	0.26	1.60
Chrysene	<0.05	0.16 ± 0.03	<0.05	0.13 ± 0.03	0.46 ± 0.09[a]	0.38	2.80
Benzo(b)fluoranthene	<0.05	0.22 ± 0.10	<0.05	0.16 ± 0.8	0.31 ± 0.15		
Benzo(k)fluoranthene	<0.05	0.07 ± 0.01	<0.05	0.06 ± 0.01	0.11 ± 0.02		
Benzo(a)pyrene	<0.05	0.09 ± 0.06	<0.05	0.09 ± 0.06	0.12 ± 0.09	0.43	1.60
Indeno(123-cd)pyrene	<0.05	0.15 ± 0.02	<0.05	0.11 ± 0.02	0.21 ± 0.03		
Dibenzo(a.h)anthracene	<0.05	0.07 ± 0.05[a]	<0.05	0.12 ± 0.09[a]	0.12 ± 0.09[a]	0.06	2.60
Benzo(ghi)perylene	<0.05	0.10 ± 0.01	<0.05	0.09 ± 0.01	0.14 ± 0.01		
Total PAHs		1.63 ± 0.64		1.27 ± 0.50	2.44 ± 0.95		

TOM, TOC, TON: total organic matter, carbon or nitrogen concentrations. ERL and ERM guidelines values defined by Long et al. (1995) are also presented.
[a] Concentration that exceeds the ERL.

higher than in reference sediment), with no PAHs being detected. The B and C sediments showed different levels of contamination among metals. The B sediment showed the highest levels of Cd, Mn, and Pb, which were approximately 16, 33 and 12 times higher than reference sediment, respectively. In contrast, Cu and Zn were more abundant in the C sediment, with concentrations respectively 140 and 43 times higher than those measured in the reference site were. All metal concentrations were higher in the Seine sediment compared to the A sediment, but they remained lower than the two other harbour sediments, except for the chromium concentration, which was of the same order of magnitude. EF values confirm that a moderate contamination (EF > 2) is present in the inner harbour area for Cu, Mn, Pb and Zn and the difference of metal contamination between B and C sediments (Table 2). High ratios (EF > 10) were found for Cd for Seine and harbour sediments.

Concerning organic compounds, higher total PAH concentrations were found in the C sediment (2.44 ± 0.95 mg kg^{-1}) compared to the B one (1.27 ± 0.50 mg kg^{-1}). The total PAH concentration measured in the Seine sediment (1.63 ± 0.64 mg kg^{-1}) was between those measured in the B and the C sediments. Among the different aromatic compounds detected in this sediment, PAHs with intermediate–high molecular weight were dominant.

To evaluate sediment toxicity, Table 1 also gives the levels of two contamination sediment quality guidelines for some elements. Among the five sediments, the Seine, B and C sediments presented at least one compound concentration above the ERL values. Dibenzo(a,h)anthracene concentrations exceeded the ERL value for the three sediments, Zn in both harbour sediments, and Cu, anthracene and chrysene only in the C sediment.

The percentage results of bioavailable metals in the sediments collected in the five locations are given in Table 2. The reactive fractions of Cd, Cu and Pb were not detected in the reference sediment due to their low contents in the sediment total fraction. Differences in bioavailability among elements appeared similar among the sediments sampling: Cd, Mn and Pb showed a strong affinity with the acid-soluble fraction, which represented more than 60%, while Cr, Ni and V remained lower than 40%.

Table 2
Percentage (%) of metals potentially reactive towards biota and enrichment factor (EF) values for the five sediments (Ref, Seine, A, B and C).

	REF	Seine	A	B	C
EF					
Cd	6	15	19	45	25
Cr	1	3	3	3	3
Cu	0	1	2	2	9
Mn	1	1	2	8	2
Ni	1	1	2	2	2
Pb	1	2	4	6	4
V	1	2	2	2	3
Zn	1	2	3	6	10
Reactive fraction (%)					
Cd	–	63	83	82	85
Cr	18	25	20	20	18
Cu	–	71	66	76	93
Mn	56	62	65	70	63
Ni	22	38	40	37	40
Pb	–	89	73	95	96
V	31	22	35	30	33
Zn	80	73	72	66	46

Table 3
Mean (±SD) metal concentrations (mg kg^{-1} dry weight) in gills of turbot exposed to the five sediments (Ref, Seine, A, B and C) during 7 days (a) and 21 days (b) (t_0: reference status). $n = 3$. (^{0}), (^{1}), (^{2}), (^{3}), (^{4}), (^{5}) represent significant difference ($p < 0.05$) compared to "t_0", Seine, A, B and C respectively.

Metals	t_0	REF	Seine	A	B	C
(a) Concentrations in gills (t_7)						
As	1.18 ± 0.04	1.24 ± 0.17	1.24 ± 0.15	1.53 ± 0.19	1.39 ± 0.11	1.37 ± 0.12
Cd	0.11 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.02
Co	0.18 ± 0.02	0.22 ± 0.02	0.24 ± 0.01	0.22 ± 0.01	0.27 ± 0.01^{0,1,3}	0.27 ± 0.03^0
Cr	1.26 ± 0.02	1.41 ± 0.06	1.03 ± 0.03	1.04 ± 0.35	0.99 ± 0.01	0.98 ± 0.18
Cu	3.14 ± 0.03	3.73 ± 0.75	3.05 ± 0.17	3.08 ± 0.09	3.04 ± 0.11	3.63 ± 0.21
Mn	158 ± 17	188 ± 8.6	163 ± 15	172 ± 7	193 ± 22	188 ± 3
Ni	2.86 ± 1.17	1.91 ± 0.12	1.73 ± 0.28	1.77 ± 0.08	1.78 ± 0.10	1.87 ± 0.14
Pb	0.13 ± 0.09	0.19 ± 0.02	0.25 ± 0.03	0.64 ± 0.04^{0,1}	1.02 ± 0.20^{0,1}	0.61 ± 0.12^{0,1}
Se	3.09 ± 0.12	2.83 ± 0.23	3.35 ± 0.28	3.49 ± 0.65	3.45 ± 0.33	3.11 ± 0.33
V	0.63 ± 0.04	0.69 ± 0.01	0.75 ± 0.07	2.03 ± 0.05^{0,1,4}	1.80 ± 0.17^0	1.76 ± 0.17^0
Zn	97.8 ± 8.5	101 ± 3	93.4 ± 9.8	92.9 ± 3.49	94.1 ± 6.2	98.0 ± 7.9
(b) Concentrations in gills (t_{21})						
As	1.18 ± 0.04	1.29 ± 0.04	1.37 ± 0.14	1.65 ± 0.19^0	1.75 ± 0.50^0	1.18 ± 0.06
Cd	0.11 ± 0.01	0.08 ± 0.02	0.09 ± 0.01	0.06 ± 0.01	0.09 ± 0.02	0.07 ± 0.01
Co	0.18 ± 0.02	0.19 ± 0.02	0.26 ± 0.01^0	0.21 ± 0.02	0.34 ± 0.12^{0,1}	0.27 ± 0.04^{0,1}
Cr	1.26 ± 0.02	1.16 ± 0.44	1.65 ± 0.41	1.48 ± 0.46	2.26 ± 0.92	2.30 ± 0.97
Cu	3.14 ± 0.03	3.64 ± 0.76	3.63 ± 0.47	3.12 ± 0.83	4.05 ± 1.64	3.76 ± 0.54
Mn	158 ± 17	168 ± 2	170 ± 12	161 ± 14	224 ± 91	174 ± 9
Ni	2.86 ± 1.17	1.75 ± 0.23	2.18 ± 0.27	2.11 ± 0.61	2.69 ± 1.35	1.90 ± 0.24
Pb	0.13 ± 0.09	0.16 ± 0.05	0.42 ± 0.04	0.53 ± 0.16	1.23 ± 0.44^{0,1}	0.58 ± 0.16^{0,1}
Se	3.09 ± 0.12	3.17 ± 0.21	3.34 ± 0.22	3.23 ± 0.55	4.01 ± 1.20	3.00 ± 0.07
V	0.63 ± 0.04	0.68 ± 0.07	0.90 ± 0.05	1.77 ± 0.11^{0,1}	2.27 ± 0.65^{0,1}	1.67 ± 0.19^0
Zn	97.8 ± 8.45	101 ± 6	101 ± 7	101 ± 7	124 ± 47	102 ± 16

3.2. Metal concentrations in gills

The fish exposed for 7 days to sediment collected in the three harbour stations, presented a significant increase in Pb and V concentrations in their gills compared to t_0 (Table 3). Cobalt concentrations were also significantly higher in the gills from fish exposed to both harbour sediments compared to t_0. No major differences in metal concentrations in the gills at t_{21} were observed compared to t_7. A significant increase of Pb, V and Co was observed in the harbour stations. The fish exposed to the Seine sediment for 21 days, presented significantly higher levels of Co concentrations. Arsenic concentrations were significantly higher in turbot exposed to the A and B harbour sediments, as compared to t_0. Cd, Cr, Cu, Mn and Zn concentrations were not higher in the gills from fish exposed to contaminated sediments at either exposure times, even if they presented different levels of concentrations in the five conditions.

3.3. PAHs metabolites in bile

Whereas low fluorescence was detected in the bile from the fish exposed to the reference and the A sediments at both exposure times, a significant increase in fluorescence signals was observed for those fish exposed to the Seine, B and C sediments (Fig. 2). The turbot exposed to the sediment from the innermost station of the harbour (C), displayed significantly higher bile fluorescence signals, compared to the four other sediments.

3.4. Physiological biomarkers

The fish exposed to the reference sediment for 7-days showed a positive GW. Fish from the other treatments lost weight (Fig. 3). This loss of weight was significantly higher in the fish exposed to the C sediment, compared to other contaminated sediments. Similar results were observed following the 21-days exposure, but with global higher GW values compared to t_7, and a weight gain observed in the fish exposed to the A sediment. The specific growth rate in length (GL) was significantly lower in the fish exposed to the four contaminated sediments compared to the reference sediment, following both exposure times. Those fish exposed to the C sediment had generally not grown in length and presented significantly lower GL compared to the Seine and the A sediments. No difference in turbot recent growth, estimated by the five last otolith daily increments, was observed between the fish exposed to the reference, Seine, A and B sediments. On the other hand, a decrease in recent growth was found in those fish exposed to the C sediment, which was significant compared to those exposed to the A sediment.

For both exposure times, the reference fish showed a similar K index to the t_0 fish (Fig. 4). Following 7 days exposure, the fish exposed to the Seine and the three harbour sediments had a significantly lower K index compared to the t_0, reference. At t_{21}, the K index of fish exposed to A sediment was no longer different from the reference fish. A gradual significant decrease of the K index was observed in the t_{21} fish exposed to the Seine, B and C sediments, respectively compared to the reference fish. No significant

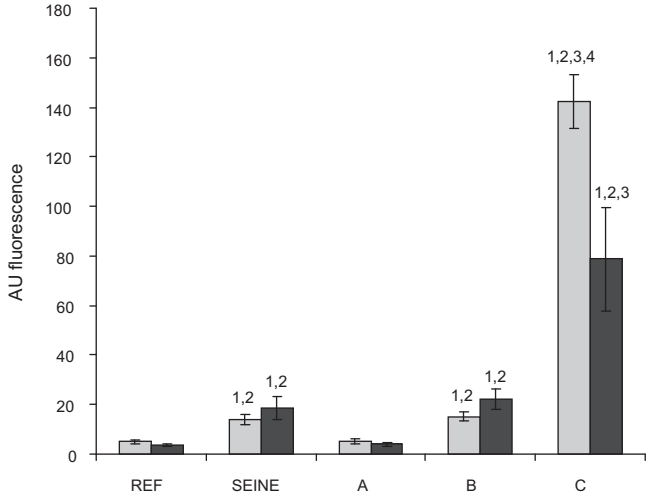

Fig. 2. Differences in relative PAH metabolites in bile from turbot exposed to the five sediments (Ref, Seine, A, B and C) during 7 (□) and 21 (■) days. Data represent mean (±SE) relative PAH metabolites fluorescence in bile. $n = 10$. (^{0}), (^{1}), (^{2}), (^{3}), (^{4}), (^{5}) represent significant difference ($p < 0.05$) compared to "t_0", Seine, A, B and C respectively.

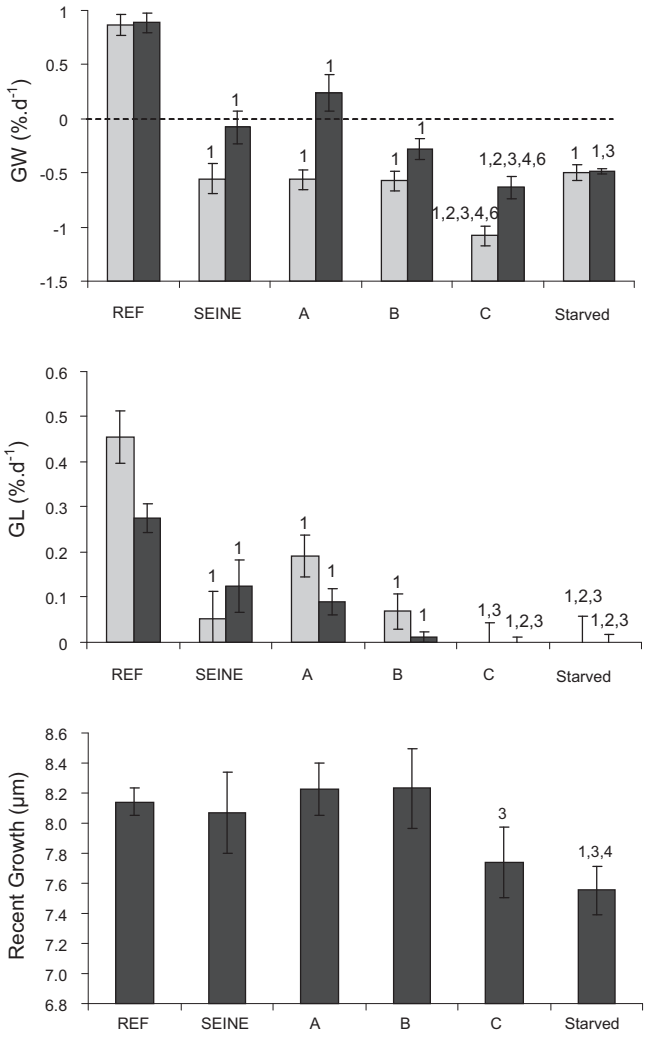

Fig. 3. Differences in the specific growth rate in weight (GW), length (GL) and the recent otolith growth (RG: obtained by the last five otolith increments) of juvenile turbot exposed to the five sediments (Ref, Seine, A, B and C) after 7 (□) and 21 (■) days (mean ± SE). $n = 15$. (^{0}), (^{1}), (^{2}), (^{3}), (^{4}), (^{5}), (^{6}) represent significant difference ($p < 0.05$) compared to "t_0", Seine, A, B, C and starved fish, respectively.

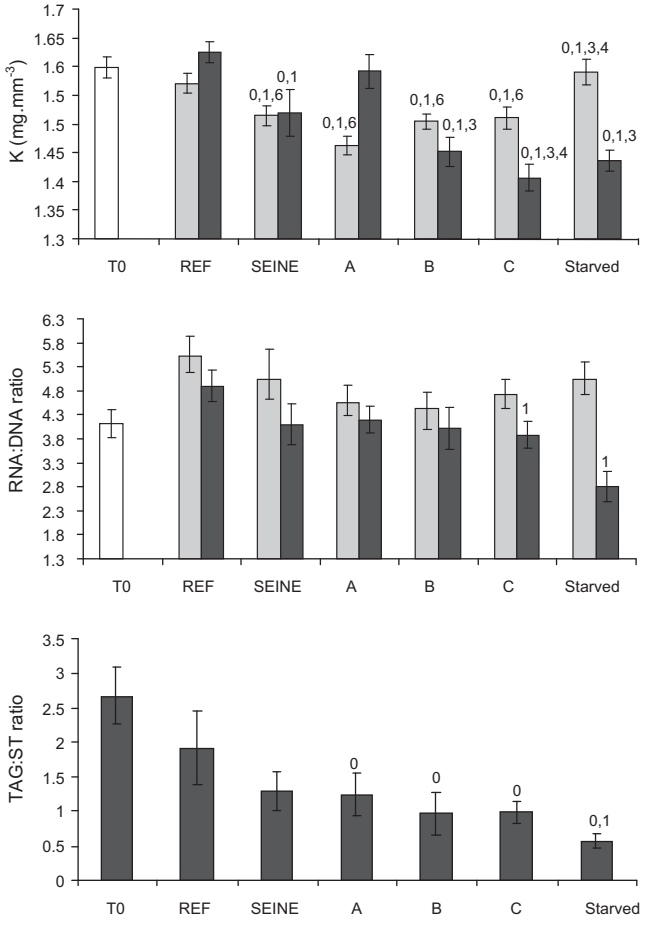

Fig. 4. Differences in the Fulton's K condition index, the RNA:DNA ratio and the lipid index (TAG:ST ratio) from turbot exposed to the five sediments (Ref, Seine, A, B and C) during 7 (□) and 21 (■) days (mean ± SE). $n = 15$. (^{0}), (^{1}), (^{2}), (^{3}), (^{4}), (^{5}), (^{6}) represent significant difference ($p < 0.05$) compared to "t_0", Seine, A, B, C and starved fish, respectively.

difference in RNA:DNA ratio was observed in the fish exposed to the reference, Seine, A and B sediments. On the other hand, those fish exposed to the C sediment presented a significantly lower RNA:DNA ratio compared to the reference fish. A decrease of the TAG:ST ratio was observed in the fish exposed to the four contaminated sediments, and the decrease was significant for those fish exposed to the three harbour stations sediments, compared to t_0 fish.

3.5. Starvation effects on physiological performance

When starved, fish presented a weight loss within 2 days, and stopped growing in length after 7 days. A significant decrease in Fulton's K condition index was observed after 10 days of starvation compared to the t_0 fish (Table 4). RNA:DNA and TAG:ST ratios measured on the starved fish were significantly lower only following 21 days of starvation compared to the fed fishes.

Growth rates, recent growth and Fulton's K condition index of the fish exposed to the C sediment, presented values in the same order of magnitude as the starved fish. The significant decreases of RNA:DNA and TAG:ST observed in the fish exposed to the C

Table 4
Comparison of specific growth rate in length (GL) and in weight (GW), Fulton's K index, RNA:DNA ratio and TAG:ST ratio (mean ± SE) for turbot starved at t_2, t_7, t_{10} and t_{21}. Reference turbot values are also presented at t_0, t_7 and t_{21}. $n = 12$. (^{0}) and (^{1}) represent significant difference ($p < 0.05$) compared to "t_0" and "reference" respectively.

Sample time	Condition	GL	GW	K	RNA:DNA	TAG:ST
t_0				1.60 ± 0.02	4.44 ± 0.28	2.49 ± 0.44
t_2	Fed	–	–	–	–	–
	Starved	0.19 ± 0.31	-0.33 ± 0.10	1.53 ± 0.02	3.74 ± 0.52	1.20 ± 0.42
$t7$	Fed	0.46 ± 0.06	0.87 ± 0.10	1.57 ± 0.02	5.52 ± 0.43	
	Starved	0	-0.50 ± 0.07^1	1.59 ± 0.02	5.03 ± 0.36	2.22 ± 0.73
$t10$	Fed	–	–	–	–	–
	Starved	0	-0.69 ± 0.07	1.49 ± 0.02^0	4.11 ± 0.29	2.36 ± 0.81
$t21$	Fed	0.27 ± 0.03	0.89 ± 0.09	1.62 ± 0.02	4.90 ± 0.32	1.91 ± 0.53
	Starved	0	-0.49 ± 0.02^1	$1.44 \pm 0.02^{0,1}$	2.81 ± 0.31^1	$0.57 \pm 0.11^{0,1}$

Table 5
Pearson's correlation between growth indices (specific growth rate in length (GL) and weight (GW) and recent growth (RG)) and condition indices (Fulton's condition Index (K), RNA:DNA and TAG:ST ratios) measured in turbot exposed to the five sediments (Ref, Seine, A, B and C). $n = 75$. Significant correlation for $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.

	GW	GL	K
(A)t_7			
GL	0.68***	–	
K	0.43***	0.17	–
ARN:ADN	0.26*	0.04	0.02

	GW	GL	RG	K	RNA:DNA
(B) t_{21}					
GL	0.71***	–			
RG	0.09	0.142	–		
K	0.84***	0.51	−0.04	–	
ARN:ADN	0.19	−0.01	−0.02	0.08	–
TAG:ST	0.25*	0.12	−0.10	0.26*	0.03

sediment were slightly lower than those measured on the starved fish.

3.6. Relationships between physiological biomarkers

The two specific growth rates (GW and GL) were significantly correlated with each other and K indices were significantly correlated with GW (Table 5). Theses correlations were stronger at t_{21} compared to t_7. On the contrary, RNA:DNA ratios were significantly correlated with GW only at t_7. TAG:ST ratios were weakly but significantly correlated with GW and K indices.

The two first axes of the PCA allowed the explanation of 93.02% of the global inertia in the data with mean explanations for the first axis (77.69%) (Fig. 5). The first axis differentiates on its right-hand side, the five sediment exposure conditions according to the global chemical contamination, and on its left, the biological parameters. When exposure condition were projected on this axis, physiological biomarkers appeared to decrease gradually with the level of chemical contamination from the reference, A, Seine, B and C exposure conditions, respectively. No major difference in contributions was observed between variables (comprised between 3.29% and 8.12%). Metal and PAH contaminations were the main factors on the second axis, which allowed us to distinguish the B and C sediments. This distinction could be explained by the highest contributions of Mn (23.1%), Cu (13.4%) and Cd (10.2%).

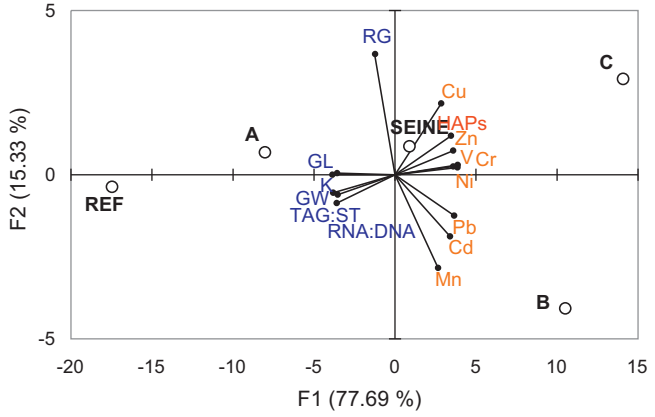

Fig. 5. PCA based on physiological biomarkers (specific growth rate in length (GL) and weight (GW), recent otolith growth (RG), Fulton's condition Index (K), RNA:DNA and TAG:ST ratios) of turbot exposed to the five sediments (Ref, Seine, A, B and C) after 21 days in relation to sediment chemical contamination (metals and total PAHs).

4. Discussion

In the present study, the effects of contaminated sediments from an important harbour in northern France, and from an estuary with human impact, the Seine, were analysed on juvenile turbot growth and condition. According to contamination sediment quality guidelines identified in Long et al. (1995), the reference sediment has good criteria of sediment quality, thus validating its reference status. On the contrary, sediments from harbour stations B, C, and the Seine estuary, corresponded to polluted marine sediments in which some metals and/or PAHs compounds exceeded the ERL (effects range-low) guidelines. The reference turbots showed a Fulton's K condition index similar to turbots of the same size (ou: of similar size) sampled in an estuary in northern France, the Canche, (data not shown); considered as a relatively clean estuary, used as a nursery ground by numerous fish (Selleslagh et al., 2009). This similitude of K index shows the relatively good health of fish exposed to the reference sediment. On the contrary, the exposure of juvenile turbots to the harbour and the Seine sediments, led to a decrease of their biological performance. Indeed, turbot growth and condition indices decreased with the level of chemical contamination. As a decrease of growth and condition indices were observed in relation with the chemical contamination in sediments, a dose-dependent effect of chemical contamination on fish health appeared evident. Fish exposed to the most contaminated sediment showed growth and condition indices values similar to the starved fish. This similarity suggested the severity of the deleterious effect of such contaminated sediment on turbot health.

4.1. Sediment contaminants vs contaminants in fish

Chemical concentrations in sediment were similar to those measured in previous studies in the same harbour (Berthet et al., 2003; Amara et al., 2007) and in the Seine estuary (Cachot et al., 2006; Amara et al., 2009). The absence of detectable PCBs in all the sediment samples suggested that PCB contamination was unlikely to be a plausible factor influencing the response of turbot fish in our experiment. The high contents of metals in the harbour sediment could have been a result of the proximity of metallurgical activities. In particular, the high manganese contents could have been the consequence of the activities of a former ferromanganese factory. The metal contamination of the Seine sediment was intermediate between harbour sediments A and B. In the Seine estuary, the metallic contamination was related to the effluents from the upstream Paris urban area, and local inputs from the heavily industrialised Rouen and Le Havre regions. These comparisons of metal concentrations with the reference sediment could be influenced by the difference of granulometry and mineralogical composition since metal have a preferential affinity with muddy sediment (Förstner, 1989; Loring, 1991). However, the normalization with Al, confirms the metal contamination in those investigated areas. Bioaccumulation patterns of metals in fish tissues can be used as selective indicators of environmental metal contamination (Sultana and Rado, 1998). In particular, gills are the first organ to be in contact with water and resuspended sediment particles, so they can be relevant sites of interaction with metals (Fernandes et al., 2007). On the other hand, fish gills are able to accumulate chemicals that were taken up by other exposure routes, due to their position between the venous and arterial circulation, thus receiving nearly all of the cardiac output (Levine and Oris, 1999). It is documented that fish gills respond earlier than the kidney and liver under field contamination exposure (Ahmad et al., 2004; Santos et al., 2004). In the present study, higher concentrations of As, Co, Pb and V were observed in turbot exposed to most contaminated sediments, with higher significant increases in turbot exposed to the two innermost sediments of the harbour. Gills

have been considered as a more sensitive organ than liver response for arsenic in field tilapia (Liao et al., 2005), and a similarity of Pb and Co with calcium in their deposition and mobilisation from gills has been observed in previous studies in field fish (Masoud et al., 2007) and under water exposure (Richards and Playle, 1998). As and Pb, having no known role in biological systems, are toxic even in trace amounts. Conversely, vanadium is beneficial to normal cell growth (Colina et al., 2005) and cobalt acts as a co-factor for several enzymes (Richards and Playle, 1998). However, theses metals could produce toxic effects at high concentrations. At higher concentrations, vanadium can become toxic to cells, inducing several injury effects on specific target organs, lipid peroxidation and changes in haematological and respiratory systems (Zychlinski et al., 1991; Byczkowski and Kulkarni, 1998). Little is known about cobalt toxicity, but water exposure of carp to Co has been shown to cause an edematous separation of the gill's secondary lamellar epithelium (Richards and Playle, 1998).

The Cu, Mn and Zn concentrations found in the turbot gills did not reflect the different levels of contamination found in the sediments, in spite of their relatively high availability. These results suggested that turbots were able to regulate tissue metal concentrations as previously reported in others species (Kraemer et al., 2005; Fernandes et al., 2007). Indeed, the concentrations of essential metals in organisms tend to be highly regulated compared to non-essential ones (Pereira et al., 2009). Consequently, essential metal concentrations in fish may not reflect environmental exposure to the same extent as those of non-essential metals (Schmitt et al., 1993). In the present study, there was no evident increase of metals in t_{21} compared to t_7 whereas several authors have observed an increase of metal concentrations in fish gills with the duration of water exposure to contaminants (Annune and Iyaniwura, 1993; Gbem et al., 2001). This phenomenon of time-dependence would most likely have been observed in our study if we had increased the duration of exposure.

Harbour activities and maritime traffic, have also lead to the contamination of sediments in PAHs, which exhibit high toxicity in the marine environment. These carcinogenic and mutagenic compounds were found in the Seine sediment in concentrations between harbour stations B and C. Following metabolism by Phase I and Phase II detoxification enzymes, the major route of excretion of molecular weight metabolites is the bile (Van der Oost et al., 2003). That is why PAH metabolites detected in fish bile have been applied as a biomarker of PAH exposure (Aas et al., 1998; Ariese, 1993; Hellou and Payne, 1987; Lin et al., 1996). The results of total biliary fluorescence indicated a relation with PAH concentrations in the sediments since the higher fluorescence was observed in turbot exposed to the C harbour sediment - the most contaminated in PAHs. Although, the biliary metabolite content did not represent an adverse biological effect by itself, these results showed the different levels of PAH exposure of turbot among the different conditions. This corroborated that biliary florescence was a good biomarker for PAH exposure and serves as a tool that is complementary to chemical analysis in biomonitoring programs.

4.2. Fish physiological performance

Several studies have shown that chemical contaminants inhibit growth of fish in a number of species, especially at early life stages, such as larvae and juveniles (Al-Yakoob et al., 1996). Indeed, a decrease in growth and condition has been observed in fish exposed to different contamination levels (Rowe et al., 2001; Alquezar et al., 2006). Differences in fish growth could represent a sum-up of the sublethal responses to chemical contaminants as growth integrates many processes (Morales-Nin et al., 2007). In particular, exposure to chemical contaminants could lead to a change in energy allocation which would be used preferentially for resistance to chemical stress to the detriment of growth (Rowe et al., 2001).

A decrease of the RNA:DNA ratio was observed in the four contaminated conditions compared to the reference one, which confirmed the decrease of turbot growth and energetic status under chemical contamination. However, the decrease was significant only for turbot exposed to the highest contaminated sediment (C sediment). We observed the same results for the recent growth. In the present study, these two parameters were not correlated with specific growth rates in the length of juvenile turbot. Mesocosm and field studies have also revealed considerable variability in RNA:DNA and recent growth of individual fish, even when variability in size and/or age is removed (Buckley et al., 1999). The otolith daily increments are used in many studies showing short term variations in fish growth rates (Geffen, 1982; Morales-Nin and Aldebert, 1997). However, in the fish aged several months, the complex otolith structure and its irregular shape, lead to variability in its measurement, and could explain this lack of correlation. In the same way, high variability in RNA:DNA ratios was observed in the present study. The RNA:DNA ratio is an index commonly used, relating growth and nutritional condition of juvenile fish. Several studies have shown a fast response of this index to feeding and growth fluctuations in the early life stage of fish (Clemmesen, 1988; Buckley et al., 1999). However, individual nucleic acid determinations have revealed a large and unexplained inter-individual variability in the RNA content and in the RNA:DNA ratios of fed fish reared under identical conditions (Clemmesen, 1988; Robinson and Ware, 1988; McGurk et al., 1992; Ueberschar and Clemmesen, 1992; Mathers *et al.*, 1993). In the present study, a significant decrease of RNA:DNA ratios measured on the starved fish were only observed following the 21 days exposure whereas, several studies have shown a decrease in this after only few days of starvation (Buckley, 1980; Tanaka et al., 2007; Bergeron et al., 1994). However, many of these studies were carried out on larval fish. Fish age could have influenced the RNA:DNA variability, since this factor is known to influence this physiological biomarker. For example, a decrease of RNA concentrations has been observed with age in rainbow trout (*Oncorhynchus mykiss*) (Perago et al., 2001).

In the present study, a decrease of the lipid index was observed in the turbot exposed to the three harbour sediments. The TAG:ST ratio was correlated with the specific growth rates and Fulton's K condition index. This nutritional index, based on the ratio between quantities of lipid reserves (TAG) and structural lipids (ST), has been used to investigate the nutritional status of fish (Galois et al., 1990; Amara et al., 2000; Gilliers et al., 2006). A lipid depletion has been identified as a general metabolic response to stress (Lemly and Esch, 1984; Lemly, 1997). The TAG decrease could be the direct consequence of the xenobiotic detoxification and regulation, which involves both passive and active mechanisms and therefore require energy (Alquezar et al., 2006). In a mesocosm study, Claireaux et al. (2004) showed that the value of the TAG:ST ratio of juvenile sole, exposed for 24 h to PAHs, diminished by 75% after a 3 months period in clean sea water relative to the control fish. In the present study, the fish were fed up to 1% of their weight during exposure; a value recommended by Sprague (1969) for fish bioassay. When food was introduced, direct observations showed that the fish fed actively in all tanks. However, a slight tendency of a decrease of consumption rate with the contamination level was observed. Indeed, fish feeding could have been influenced by turbidity or chemical contamination, since exposure of organisms to pollutants could have induced alterations in their feeding behaviour or swimming activities. For instance, turbot exposed to oil have shown reduced feeding rates (Saborido-Rey et al., 2007) and reduced swimming activities (Stephens et al., 2000). Moreover, an increase of turbidity was observed in the four contaminated conditions compared to the reference one. In spite of the preference of turbot for high turbidity

areas (Florin et al., 2009), turbot are visual daylight predators (Gibson, 2005), implying that increased turbidity can reduce feeding efficiency (Moore and Moore, 1976). However, turbidity was similar in the four contaminated conditions, whereas decreases of many physiological biomarkers were found from those fish exposed to sediment A and to sediment C. Thus, the turbot growth and energetic reductions shown in this study could also be the result of a decline in feeding activity combined with a major reduction in the ability to assimilate and/or convert food to energy.

The results of growth and condition indices of the fish exposed to the harbour sediments could be compared to a previous study in which juvenile turbot were caged in the harbour of Boulogne Sur Mer (Kerambrun et al., 2011). In particular, a cage was fixed to the sediment at station B where sediment was sampled in the present study. The growth in length and in weight measured in the turbot exposed to sediment B was similar to those found in the caged fish. However, condition indices (K index, RNA:DNA and TAG:ST ratios) were found lower in the caged fish. This comparative result suggests that environmental factors other than sediment associated contaminants, could have influenced the condition of the fish. In particular, in the present study, a daily water change was performed to avoid a decrease in seawater quality. However, this renewed seawater could have decreased the transfer of chemical contaminant by the water column. Moreover, the fish were fed uncontaminated food, but prey items in contaminated areas are likely to be an additional source of chemical contaminants.

5. Conclusion

Our study has shown the deleterious effects of exposure to contaminated sediments on the health of juvenile turbot, using growth and condition indices. Although all biological parameters used in this study showed variations in the most contaminated condition, growth rates and Fulton's K condition indices appeared to be more sensitive to the level of the chemical contamination. The decrease in biological performance of the turbot exposed to the Seine sediment, corroborates the results observed by Amara et al. (2009), which found a decrease in growth and condition of juvenile flounder (*Platichthys flesus*) sampled in the Seine estuary, compared to relatively clean estuaries. Growth during the initial year following settlement is likely to be critical to subsequent survival and recruitment. Fish experience their most rapid growth during the juvenile phase (Smith et al., 1995), and any reduction during juvenile growth would prolong the length of the juvenile stage. Juvenile fish with reduced growth would be more susceptible to predation and might compete less successfully for food than larger fish. Moreover, the observed changes in nutritional status and condition with the level of sediment chemical contamination may limit the ability of juvenile fish to tolerate difficult conditions such as diseases or starvation. In conclusion, such a reduction in growth and energetic status of juvenile fish could dramatically decrease their over-winter survival in contaminated nursery grounds. However, to extrapolate these results in the field, other abiotic and biotic factors would have to be considered.

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