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A POLLUTION-MONITORING PILOT STUDY INVOLVING CONTAMINANT AND BIOMARKER MEASUREMENTS IN THE SEINE ESTUARY, FRANCE, USING ZEBRA MUSSELS (*DREISSENA POLYMORPHA*)

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Abstract—Zebra mussel (*Dreissena polymorpha*) is an invasive species that has proliferated in European and North American rivers and lakes during the last century. In this study, *D. polymorpha* has been used to provide information on contamination levels and biological effects in the Seine Estuary (France). The bivalves accumulated polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) to a high degree with values reaching 800 ng/g dry weight for PCBs (sum of 20 congeners), and 1,000 ng/g dry weight of PAHs (sum of 14 compounds) in the whole body. These values are among the highest reported of PCBs and, to a lesser extent, of PAHs in other contaminated areas in the world. Toxic equivalent quantities of PCBs and PAHs detected in zebra mussels varied from 20 to 40 pg dioxin equivalents/g dry weight for PCBs and up to 120 ng benzo[*a*]pyrene equivalents/g dry weight for PAHs, indicating a high potential risk for animals feeding on them. Biological impacts, such as altered condition index, decreased lysosomal stability, and high levels of multixenobiotic resistance (MXR) proteins also were detected in mussels living downstream of Rouen, the main city of the Seine Estuary. Taken together, these results indicate that the Seine Estuary is a heavily polluted area with the potential to cause deleterious health effects in some endogenous living organisms. This study also shows that chemical and biological measurements bring different but complementary results that can help diagnose environmental health.

Keywords—*Dreissena polymorpha* Polychlorinated biphenyls Polycyclic aromatic hydrocarbons Multixenobiotic resistance Biomarkers

INTRODUCTION

Ecotoxicology is a multidisciplinary research area. It is now evident that any reliable environmental risk assessment of exposure to a mixture of contaminants should involve several disciplines such as environmental chemistry, toxicology, and ecology. Nevertheless, in many studies, integrating all the existing data and concepts still is an unachieved goal. Environmental chemists and biologists both extensively have used mussels as living organisms for their studies. Indeed, mussels are very useful as biomonitors to overcome difficulties in measuring chemical contamination directly from water, i.e., dealing with very low concentrations and fluctuations over time. Most importantly, they also allow discrimination between the bioavailable fraction and the unavailable forms of potentially toxic compounds and, thus, help to consider the dose that might affect biological activities. The rationale for using mussels as bioindicators is that they can (to a certain extent) accumulate contaminants proportionally to the exposure over a large range of concentrations. They provide valuable information on the associated potential chemical risk and, because these organisms are widespread and easy to collect, mussel watch programs have been conducted for more than three decades using marine bivalves [1,2].

The zebra mussel, *Dreissena polymorpha*, more recently has been used as an indicator organism in various countries [3,4], and its physiological and demographic properties make it an interesting alternative for freshwater environments. This species has spread over European rivers (and in North America) during the 20th century and now can be found in many countries [5]. Zebra mussels have high filtering capacities (1 to 2 L/individual/d) [6,7] and can tolerate high concentrations of many types of pollutants. Although they are smaller than the blue mussels, they can be numerous in some places and thus provide enough samples for environmental analysis.

In this study, as part of the multidisciplinary research program Seine-Aval [8], we used zebra mussels as sentinel organisms in order to provide information on both the levels of contamination and their associated biological effects in the Seine Estuary. Polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) are well-known and widespread contaminants. Concentrations of these classes of pollutants in *Mytilus edulis* tissues collected near the mouth of the estuary are among the highest in the European coasts (600 ng PCB153/g dry wt) [9]. Because these contaminants might arise from inland waters, *D. polymorpha* could be exposed to high doses of these compounds, which represent a potential threat to living organisms. Both PCBs and PAHs usually exist as complex mixtures of numerous congeners in environmental samples. To assess the toxicological risks of these mixtures, it has been proposed to use the toxic equivalency approach [10,11]. The toxic potency of each congener is compared with that of a reference compound, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin or benzo[*a*]pyrene (B*a*P), given a

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value of 1. The amount of each congener in the mixture then is multiplied by its relative potency factor, and, assuming purely additive effects, the products are added together.

Many contaminants, such as dichlorodiphenyltrichloroethane, lindane, mercury, and cadmium, as well as PAHs and PCBs, have been detected in the Seine Estuary [12]. Because environmental contaminants are too numerous and chemical analysis is expensive, it is an unachievable goal to monitor all the compounds that are present in continental or marine waters. Biological analysis has the potential to complement this chemical approach because measurements are performed directly on organisms living in the environment. Organisms such as zebra mussels have to cope with numerous structurally and functionally diverse hazardous compounds. Although these organisms may be considered as adapted or resistant to contaminated environments, they can provide information on sublethal effects of the actual mixtures to which they are exposed [13,14]. Two types of biological responses have been studied. One is the expression of the multixenobitic resistance (MXR) system, which corresponds to a first line of defense against various structurally and functionally unrelated compounds [15]. The resistant cells and organs express high molecular–weight membrane proteins that can transport several chemicals out of the cell. This response has the advantage of being the result of an exposure to numerous compounds and may help to diagnose the environmental contamination [16]. The second type of response corresponds to biological activities or structures whose impairment may lead to dramatic effects on the organism health. In this respect, acetylcholinesterase (AChE) activity and lysosome membrane stability have gained recognition as important mechanisms and biomarkers [17,18]. Acetylcholinesterase activity is involved in the deactivation of acetylcholine at nerve endings, preventing continuous nerve firings, which are vital for normal functioning of sensory and neuromuscular systems. Lysosomes accumulate a diverse range of chemical contaminants that can lead to membrane damage, resulting in leakage of their contents into the cytosol and subsequent damage to cells [19].

This paper presents the results of a two-year monitoring study involving chemical analysis and biomarker measurements. It shows that the Seine Estuary is a highly contaminated area based on the measured concentrations of two major classes of persistent contaminants and on the levels of biomarkers of pollution detected in the resident zebra mussel population.

MATERIALS AND METHODS

Study area

The Seine River, which flows into the English Channel, drains a large industrialized and urbanized basin in France (78,000 km^2) hosting 40% of the French industries and 16 million people. Mean river flow is 410 m^3/s with low waters of less than 200 m^3/s in summer and high waters in winter of more than 1,500 m^3/s. It is an estuary of the macrotidal type with tidal amplitude varying from 3 to 8 m at its mouth and a tidal penetration of 170 km up to the dam located at Poses (pk 202; pk are distances in km from Paris). Salinity varies according to tides and river flows from pk 325 onward and a maximal turbidity zone is located between pk 330 and 350 (Fig. 1). The mean annual suspended matter flow at Poses is 6.5×10^5 tons and the main sewage treatment work located in Rouen (pk 247) discharges 1 m^3/s of secondary treated effluents (capacity: 4.5×10^5 population equivalent).

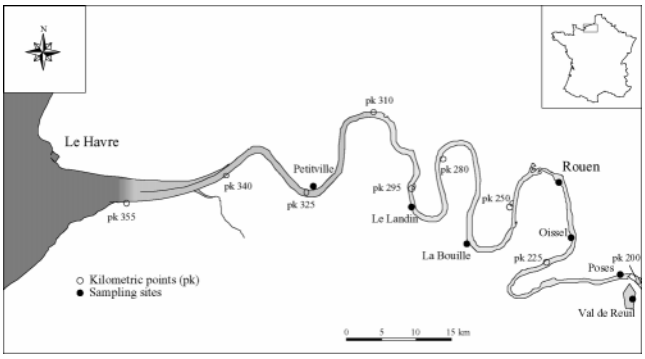

Fig. 1. Location of sampling sites in France; pk are distances (in km) from Paris.

Sample collection and biometric analysis

Zebra mussels, 20- to 27-mm long, were sampled by scuba diving at 1- to 2-m depth at low tide. Six sites (Poses, Oissel, Rouen, La Bouille, Le Landin, and Petitville) from the freshwater zone of the estuary and one control site located at Val de Reuil in a nearby lake (Fig. 1) were studied in July and September in 1997 and 1998, and a temporal study was conducted at Rouen by sampling every month (in 1997) or every two months (in 1998). Forty-two mussels were sized, dissected, drained, and weighed. A condition index (CI), derived from these data, was calculated as follows: CI (in mg/mm) = wet weight/length. Surface water temperature, salinity, pH, suspended organic matter, chlorophyll, and pheopigments were recorded and are part of a database of the Seine-Aval program [20].

Chemical analysis

Fifteen mussels were depurated for 24 h in clean freshwater (from a local source) then shucked and kept at −20°C until further analysis. Analytical procedure for both PCBs and PAHs was performed as described previously [21]. Briefly, it included a solid-liquid (Soxhlet, hexane:acetone 80:20) extraction step on freeze-dried samples, followed by an adsorption chromatography on open alumina-silica columns to remove lipids and other unwanted organic material. Two fractions were obtained. The first fraction, *F*1, eluted with *n*-pentane, contained PCBs that were separated further using a Cosmosil 5-PYE high-performance liquid chromatography to isolate non-*ortho*-chloro–substituted and coplanar PCBs from *ortho*-chloro–substituted compounds. Both subfractions then were analyzed by gas chromatography (with capillary columns: CPSil5-C18 and DB1701; J & W Scientific, Folsom, CA, USA) coupled to an electron capture detector. The second fraction, *F*2, eluted with methylene chloride/*n*-pentane (10/90), contained PAHs that were analyzed by capillary gas chromatography (DB5) and mass spectrometry. Ionization of samples was performed in the electron impact mode and detection was achieved by selected ion monitoring, recording the molecular ion for each PAH. The limit of quantification was 0.1 ng/g dry weight for individual PCBs including the coplanar congeners, and between 0.02 and 0.6 ng/g dry weight for individual PAH components. Both for PCBs and PAHs, the relative uncertainty was less than 15% except for the lower PAHs (fluorene, phenanthrene, anthracene), which partially were lost during the evaporation-concentration steps. One procedural blank and one certified reference material (either cod liver oil Bureau Communautaire de Référence–certified reference ma-

Table 1. Average concentration of polychlorinated biphenyls (PCBs) in zebra mussels from different rivers and lakes worldwide

Sampling sites	PCB congeners (ng/g wet wt) 52	101	118	153	180	% Body lipid	References
Seine (La Bouille, France)	6.5	15.2	9.7	23.5	8.9	1.2	This study
Rhine (Lobith, The Netherlands)	2.9	5.5	2.1	8.4	2.7	2	Hendriks et al. [39]
Meuse (Eijsden, The Netherlands)	4.8	7.2	3.1	9.1	3.8	2	Hendriks et al. [39]
Lake St Clair (ON, Canada)	1.1	1.7	1.3	1.7	0.7	1.8	Morrison et al. [40]
Lake Erie (ON, Canada)	1.7	4.9	—	8.9	7.3	1.7	Morrison et al. [40]
Mississippi (Lynxville, WI, USA)	2.4	8.0	4.1	4.7	1.4	1	Cope et al. [4]
Lake Iseo (Sarnico, Italy)	—	14.5	6.6	18.3	6.0	1	Binelli et al. [13]

terial 349 for PCBs or marine sediment National Institute of Standards and Technology standard reference material 1941a for PAHs) were analyzed in each 10-sample batch. Measured PCB congeners were PCB 31, 28, 52, 101, 110, 149, 118, 153, 132, 105, 138, 187, 128, 156, 180, 170, 194, 77, 126, 169, and PAH compounds were fluorene (F), phenanthrene (P), anthracene (A), fluoranthene (Fluor), pyrene (Pyr), benz[*a*] anthracene (B*a*A), chrysene (Chrys), benzo[*b*]fluoranthene (B*b*F), benzo[*k*]fluoranthene (B*k*F), benzo[*e*]pyrene (B*e*P), B*a*P, indeno[1,2,3-*cd*]pyrene (Ipyr), dibenz[*a*,*h*]anthracene (D*ah*A), and benzo[*a*]perylene (Bper). The tissue lipid content was performed gravimetrically by weighing the hexane-acetone extract.

Biochemical analysis

MXR protein analysis. Gills from 42 fresh-collected mussels (per sampling site) were dissected, transferred in six tubes (pools of 7 gills), and frozen immediately at −80°C. Quantification of MXR protein was performed as described in Minier et al. [22]. Briefly, membrane-bound proteins were extracted in 0.1% sodium dodecyl-sulfate buffer and 10 μg of protein samples were loaded onto nitrocellulose membranes. The C219 monoclonal antibody (Abcys, Paris, France) was used as primary antibody (1 μg/ml) and revealed using alkaline-phosphatase–conjugated secondary antibodies. Semiquantitation of the resulting staining was carried out by image analysis (ImageMasterTotalLab, Amersham, Piscataway, NJ, USA) and intercalibration of the different membranes was performed using positive controls corresponding to extracts (4 replicates) of a pool of mussels sampled in Rouen and of a human cancer cell line (MCF7 cells).

AChE activity. The AChE activity was assessed as described by [23]. Briefly, six pools of seven gills (42 mussels per site) were homogenized and centrifuged for 20 min at 10,000 *g*. The AChE activity of 100-μl supernatant in the presence of 0.01 M dithionitrobenzoic acid and 2.8 mM acetylthiocholine then was measured at 412 nm using a microplate reader. Both MXR proteins and AChE activity assessments were standardized to the protein content of the extracts. Protein concentrations were measured using the Bradford method [24] with bovine serum albumin as a standard.

Lysosomal stability. Hemocytes were withdrawn from the posterior adductor muscle of six mussels using a hypodermic syringe and transferred to a physiological saline solution (20 mM *N*-2-hydroxyethyl piperazine-*N*′-2-ethane sulfonic acid, 150 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, pH 7.5). In a humidity chamber, 40 μl of cell suspensions were allowed to attach to poly-lysine–treated microscope slides for 15 min in the dark at 15°C. Excess suspension was removed from the slide and the cell monolayers were incubated in physiological saline buffer containing 40 μg/ml neutral red (Sigma, Lyon, France) in the humidity chamber for 60 min. Slides then were removed and cells were examined under light microscope for abnormal morphology of haemocytic lysosomes and lysosomal integrity. Thirty granular haemocytes were observed and those with marked increased lysosomal volume or cytosolic staining (indicative of lysosomal membrane breakdown) were recorded.

Statistical analysis

Statistical analysis was performed using the SYSTAT program (Systat Software, Richmond, CA, USA). Dependence of chemical and biochemical data on sampling site or sampling time was tested for statistical significance by one-way analysis of variance. Correlation analysis was performed using the Pearson product moment correlation coefficient.

RESULTS

PCB and PAH body burdens

High PCB and PAH concentrations were measured in *D. polymorpha* in the Seine Estuary. The sum of all the measured PAHs (14 compounds) was above 1,000 ng/g dry weight of mussel tissues. The concentrations of chrysene, the PAH component found in highest concentrations in this study, were in the range of 300 to 700 ng/g dry weight of tissues. Total PCB concentrations (sum of 20 congeners) generally exceeded 800 ng/g dry weight. The two main PCBs congeners measured within the whole organism were PCB 153 and PCB 138, and their body burdens varied between 100 and 180 ng/g dry weight for PCB 153 and between 70 and 140 ng/g dry weight for PCB 138. These values always were significantly higher than those found in tissues of zebra mussels collected in the nearby lake taken as reference site (located at Val de Reuil) for both PAHs and PCBs. Comparison with other sites worldwide shows that zebra mussels from the Seine Estuary are more contaminated by PCBs than those from other investigated areas (Table 1). In contrast to PCBs, the PAH body burden of zebra mussels from the Meuse River is four times that found in the Seine Estuary mussels (Table 2).

Only small and nonsignificant variations of PCB body burden, as exemplified by PCB 153 concentrations, were observed between sites of collection within the Seine Estuary (Fig. 2). Nevertheless, concentrations tended to be higher in Poses and La Bouille. This could indicate that those compounds originated from the upper part of the river (allowing a greater amount of PCBs in organisms living upstream, i.e., at Poses) and that another source possibly was located in the industrial area of Rouen (resulting in the small increased PCBs body burden measured in La Bouille). The temporal study of PCB body burden performed in zebra mussels collected at Rouen

Table 2. Average concentration of individual and total polycyclic aromatic hydrocarbons (PAHs) in zebra mussels from different rivers

River	PAH concn. (ng/g wet wt)[a]								
	A	B*a*A	B*a*P	B*e*P	Chrys	Fluor	Pyr	Total PAHs	References
Seine (France)	2	28	21	30	54	29	36	290	This study
Rhine (The Netherlands)	1	20	6	13	17	1	12	137	Hendriks et al. [39]
Meuse (The Netherlands)	21	250	15	55	65	250	120	1,189	Hendriks et al. [39]

[a] A = anthracene; B*a*A = benz[*a*]anthracene; B*a*P = benzo[*a*]pyrene; B*e*P = benzo[*e*]pyrene; Chrys = chrysene; Fluor = fluoranthene; Pyr = pyrene.

during two years (1997–1998) did not reveal any significant trend during the first year, whereas in 1998, a bell-shape curve was observed with a rise in tissue concentrations during the spring and a subsequent decrease during the autumn of the second year (Fig. 3). These PCB levels were not correlated with lipid content ($r = 0.41$, $p > 0.1$, $n = 11$) but with pheophytin ($r = 0.65$, $p = 0.03$, $n = 11$) and suspended particulate matter ($r = 0.54$, $p = 0.08$, $n = 11$) concentrations in water samples, indicating that PCB body burden might be influenced significantly by filtration rate and algae consumption.

Significant variations of PAH body burdens were observed along the six sampling sites from the Seine Estuary ($p < 0.001$, $n = 6$). The PAH concentrations were 50% lower in Le Landin and Petitville when compared to the other sites located upstream (Fig. 2). Even if the difference was not statistically significant, La Bouille always was recorded as the site where the highest tissue concentrations were determined in zebra mussels, thus indicating the existence of a source of contamination immediately downstream of Rouen. Monthly measurements performed at Rouen showed that highest concentrations were found between March and June for each for the two years of study (Fig. 3), but there also was evidence that high variations (25–50% increase or decrease) in PAH body burdens could arise in a relatively short period of time, i.e., from one month to the other. Noticeably, PAH and PCB concentrations varied in a similar way during the second year of the study ($r = 0.76$, $p = 0.01$, $n = 7$).

Fingerprint analysis

The fingerprint of the 16 PCB congeners measured in the mussel body and normalized against the main congener, PCB 153, is given in Figure 4. The data indicated that, whatever the site or time of sampling, the contaminant pattern was similar and characterized by the presence of high concentrations of the PCB congeners 153, 138, 101, and 149. These fingerprints were nearly identical to those observed in the blue mussel, *M. edulis*, at the mouth of the Seine Estuary, but different from the one obtained in zebra mussel from the reference site, which showed a lack of lower chlorinated PCBs (Fig. 5) and a different ratio of specific congeners such as PCB 101/PCB 118.

For PAHs, the fingerprint was normalized against total PAH body burdens (sum of 14 measured compounds). Patterns were very similar in the four upstream sites with chrysene, pyrene, benzo[*a*]anthracene, fluorine, and benzo[*e*]pyrene, respectively, the compounds found at the highest level. The pattern differed in Le Landin and Petitville and was dominated by benzo[*e*]pyrene, chrysene, benzo[*b*]fluoranthene, phenanthrene, and pyrene. The distribution between aromatic classes was site-dependent (Fig. 6). A decrease in the four-ring PAHs at La Bouille became more pronounced at sites further downstream. The four-ring PAHs gradually were replaced by others, mainly five-ring PAHs.

Calculation of specific compounds ratio [25,26] did not provide a clear-cut partition as to whether or not the PAHs were of pyrogenic or petrogenic origin. Nevertheless, pyrene/anthracene, chrysene/benzo[*a*]anthracene, and benzo[*e*]pyrene/benzo[a]pyrene ratios were greater in Le Landin and Petitville, suggesting a predominance of petrogenic compounds in these sites (Table 3).

Quantification of PAH- and PCB-associated toxicity

In order to assess potential effects of both PCBs and PAHs, toxic equivalents (TEQ) were estimated for these mixtures. With toxic equivalent factors taken from [10,27], dioxin TEQ (2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity equivalent) were evaluated for two di-*ortho*–substituted (PCB congeners 170 and 180), three mono-*ortho*–substituted (PCB congeners 105, 118, and 156), and three non-*ortho*–substituted congeners (PCB congeners 77, 126, and 169). Results showed that TEQ varied between 0.02 and 0.03 ng/g dry weight according to

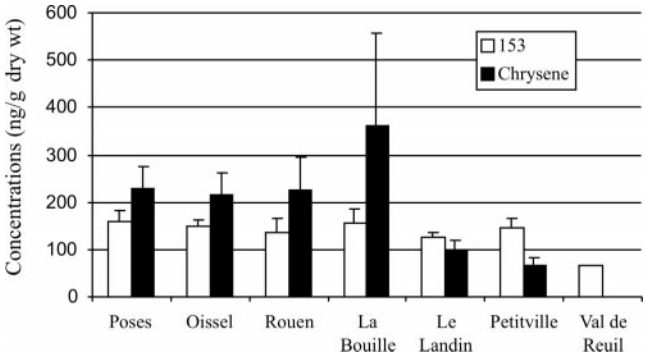

Fig. 2. Zebra mussel tissue concentrations of the polychlorinated biphenyl congener 153 and chrysene in the Seine Estuary (Rouen, France; mean of values obtained in June and September 1997 and 1998). Error bars represent confidence intervals ($\alpha = 0.05$, $n = 4$).

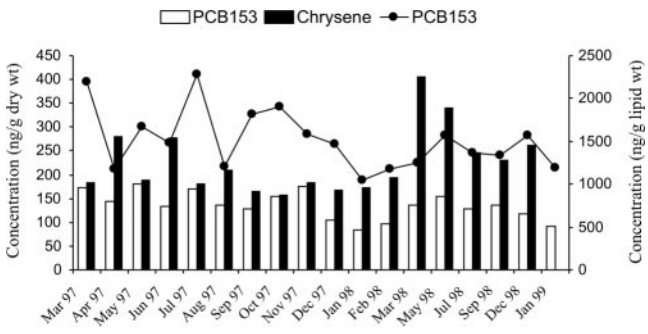

Fig. 3. Seasonal variations of the polychlorinated biphenyl congener 153 (solid bar: In ng/g dry wt; solid line: In ng/g lipid wt) and chrysene body burden (in ng/g dry wt) in *Dreissena polymorpha* sampled in Rouen, France (pools of 15 mussels).

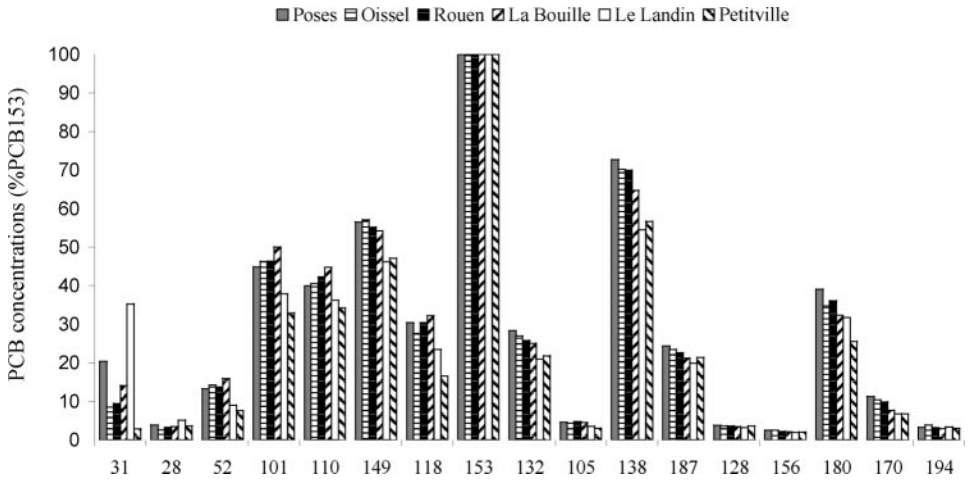

Fig. 4. *Dreissena polymorpha* polychlorinated biphenyl (PCB) fingerprints in sampling sites along the Seine Estuary (Rouen, France).

site or time of sampling (Table 4). Noticeably, PCB congeners 118 and 126 were by far the main contributors to the calculated dioxin equivalent (15–30% and 40–60%, respectively).

For PAHs, TEQ were expressed as B*a*P equivalent [11]. The assessed toxicity was highest at La Bouille with values exceeding 120 ng/g wet weight. Benzo[*a*]pyrene itself was contributing to more than 60% of the calculated toxicity.

Finally, using the approach described by Willett et al. [28], induction equivalents were calculated to compare PAH and PCB's potency to induce 7-ethoxyresorufin-*O*-deethylase activity. Results showed that PAHs contributed to more than 95% of the obtained values that varied between 0.3 and 0.8 ng dioxin equivalent/g dry weight.

Biological responses

The MXR protein content in mussels from the Seine Estuary was double that of the reference site, indicating a higher exposure to pollutants in the river sites (Fig. 7a). In mussels sampled at Rouen, these proteins reached their highest concentrations in summer and decreased to low concentrations in winter ($F = 8.42$, $p < 0.001$, $n = 6$; Fig. 8). These seasonal variations, similar to the ones previously reported for the blue mussel [29], correlated with water temperature ($r = 0.72$, $p = 0.01$, $n = 16$) and chlorophyll *a* content ($r = 0.46$, $p = 0.07$, $n = 16$), suggesting that the MXR protein concentration might be related to filtering rates (which are temperature-dependent) and food supply. When comparing sampling sites along the estuary, values tended to be higher in downstream sites (from Rouen to Petitville), with La Bouille and Petitville having significantly higher amounts of MXR proteins than any other site ($p < 0.05$, $n = 6$).

Similar discrepancies were observed between mussels living upstream or downstream Rouen considering the condition index ($F = 58.8$, $p < 0.001$, $n = 42$; Fig. 7b). Mussels from La Bouille, Le Landin, and Petitville had lower relative weights than those from the other sites within the estuary, although values were reduced significantly only in La Bouille and Petitville. Furthermore, CI of animals leaving in the Seine also were reduced dramatically (by 50%) when compared to CI of mussels living in the reference site.

Measurements of AChE activity in gills were performed the first year of the study. However, results showed that this activity was very low regardless of the site or date of sampling. The values were below 1 nmole/min/mg protein and close to detection limits of the assay. The second year, assessment of lysosome stability then was performed. Results showed that membrane stability was affected significantly in haemocytes of mussels from La Bouille and Petitville compared to mussels from the reference site and river sites upstream Rouen ($F = 6.7$, $p < 0.001$, $n = 6$; Fig. 7c).

DISCUSSION

This study reports a first attempt to combine chemical and biological measurements using zebra mussel, *D. polymorpha*, as indicator organism in the Seine Estuary. The study showed that, like other bivalves such as the marine mussel (*M. edulis*), *D. polymorpha* do concentrate lipophilic compounds such as

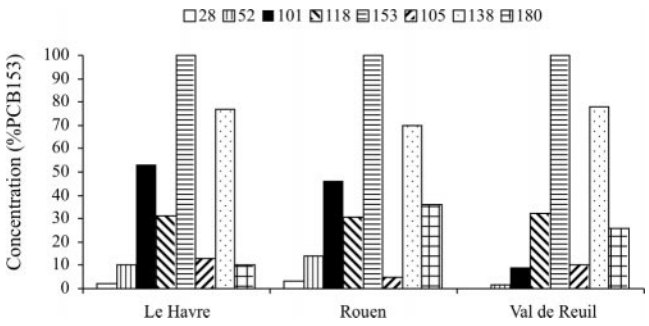

Fig. 5. Polychlorinated biphenyl (PCB) relative concentrations in zebra mussels from the Seine Estuary (Rouen, France) and a nearby lake (Val de Reuil, France) and from blue mussels collected at the mouth of the Seine Estuary (Le Havre, France, from Abarnou et al. [9]).

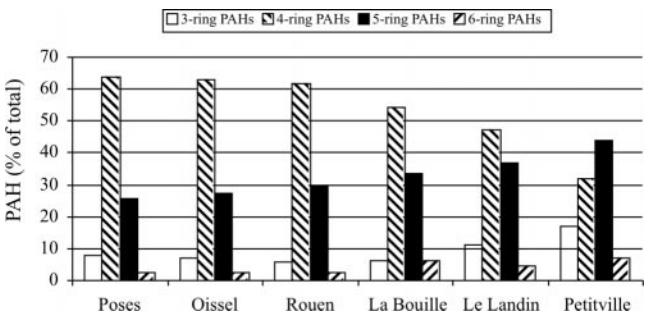

Fig. 6. Polycyclic aromatic hydrocarbon (PAH) relative concentrations in zebra mussels collected in the Seine Estuary (Rouen, France).

Table 3. Ratio between structural isomers (mean of ratios obtained in June, July, and September 1997 and 1998) of polycyclic aromatic hydrocarbons (PAHs) in zebra mussels from the Seine Estuary (France) with confidence interval in brackets ($\alpha = 0.05$)

Station[a]	P/A[b]	Fluor/Pyr[c]	Chrys/B*a*A[d]	B*e*P/B*a*P[e]
Poses	7.33 (2.98)	0.67 (0.06)	2.57 (0.20)	3.47 (1.73)
Oissel	9.875 (4.38)	0.65 (0.06)	2.775 (0.17)	3.1 (1.13)
Rouen	10.025 (3.33)	0.675 (0.05)	2.475 (0.15)	2.65 (0.72)
La Bouille	14.65 (9.71)	0.675 (0.28)	2.35 (0.63)	2.375 (1.14)
Le Landin	14.25 (0.07)	0.45 (0.07)	6.2 (0.42)	3.5 (1.25)
Vieux Port	19.8 (10.27)	0.625 (0.12)	4.425 (0.51)	3.6 (1.31)

[a] Station location sites given in Table 1.
[b] P/A = ratio of phenanthrene concentration to anthracene concentration.
[c] Fluor/Pyr = ratio of fluoranthene concentration to pyrene concentration.
[d] Chrys/B*a*A = ratio of chrysene concentration to benz[*a*]anthracene concentration.
[e] B*e*P/B*a*P = ratio of benz[*e*]pyrene concentration to benz[*a*]pyrene concentration.

PCBs and PAHs to a high extent with values reaching 800 ng/g dry weight for PCBs (sum of 20 congeners) and 1,000 ng/g dry weight of PAHs (sum of 14 compounds) in the whole body. These values are among the highest reported in other contaminated areas in the world for PCBs and, to a lesser extent, for PAHs (Tables 1 and 2). Although comparison between two species should be taken with caution, it can be observed that similar PCB concentrations have been reported in *M. edulis* tissues living near the mouth of the Seine Estuary [9]. Furthermore, relative PCB concentrations were nearly identical in the two bivalve species collected either in the Seine Estuary (zebra mussels) or at the mouth of the Seine Estuary (blue mussels) [21], thus indicating common sources of PCB contaminants. On the contrary, PAH body burdens were 25 times higher in zebra mussels than in *M. edulis* [2]. This difference might be explained by the animals' physiology. *Dreissena polymorpha* generally show higher bioaccumulation capacities than *M. edulis* [30]. Another explanation would be that the two species were exposed to different sources and concentrations of PAHs. Our results suggested that large amounts of PAHs could be released from industries just downstream the city of Rouen, thus contaminating the endogenous zebra mussels.

Seasonal variations of total PCBs and PAHs were recorded. Values generally tended to be higher during spring and summer. Interestingly, similar variations of MXR protein concentrations in gill tissues were measured. It generally is accepted that bioaccumulation in zebra mussels is related to the octanol/water partition coefficient and also to the contamination of sediments rather than water [31,32]. Thus, sediment, particulate organic matter, and algae might be potential sources of contamination for zebra mussels. This indeed was observed in this study, as analysis of seasonal variations of PCB content in zebra mussels were correlated to water pheopigments and suspended matter concentration. High filtering rates increase the probability that populations of zebra mussels will be exposed to a wide range of pollutants, including hydrophobic contaminants such as PCBs and PAHs. As this filtering activity is affected by temperature and food availability [30,31], zebra mussels might accumulate more contaminants during the period of elevated temperature and algal growth. This increase in contaminant exposure, in turn, might have led to induction of the MRX system. The expression of MXR protein is induced by many lipophilic compounds [15] and, in this study, their seasonal variations were correlated to water chlorophyll *a* concentrations and temperature. Taken together, these results indicate that suspended matter and food might be major contamination sources for mussels in the Seine Estuary.

Calculation of toxic equivalent quantities associated with PCBs and PAHs detected in zebra mussels might indicate potential effects mediated by the aryl hydrocarbon receptor. Our results showed that TEQ associated with the PCBs varied from 20 to 30 pg dioxin equivalents/g dry weight, which is more than the concentration limit recommended in any human food in Europe. The PCB congeners 118 and 126 were the main contributors to the calculated dioxin equivalent despite their low concentrations in mussel tissue. The TEQ associated with PAHs were up to 120 ng B*a*P equivalents/g dry weight for mussels living immediately downstream Rouen at La Bouille, corroborating previous results from Munschy et al. [33], who reported that the sediment B*a*P concentrations per g of dried material were high at La Bouille and exceeded 2 mg/g. These

Table 4. Toxic equivalents quantities (TEQs) for, and induction equivalents (IEs) of polychlorinated biphenyl (PCB) and polycyclic aromatic hydrocarbon (PAH) concentrations assessed in zebra mussel tissues collected in various sites along the Seine Estuary (France) in September 1998. Toxic equivalent factors were taken from [11,27,28]

	Sampling sites[a]					
	Poses	Oissel	Rouen	La Bouille	Le Landin	Petitville
TEQ PCBs (pg dioxin equivalent/g dry wt)	27.7	30.6	23.8	28.6	21.8	26.4
TEQ PAHs (ng benzo[*a*]pyrene equivalent/g dry wt)	81.8	75.0	80.5	126.8	40.2	48.4
IE (pg dioxin equivalent/g dry wt)	597	568	560	802	306	350

[a] Sampling site locations given in Table 1.

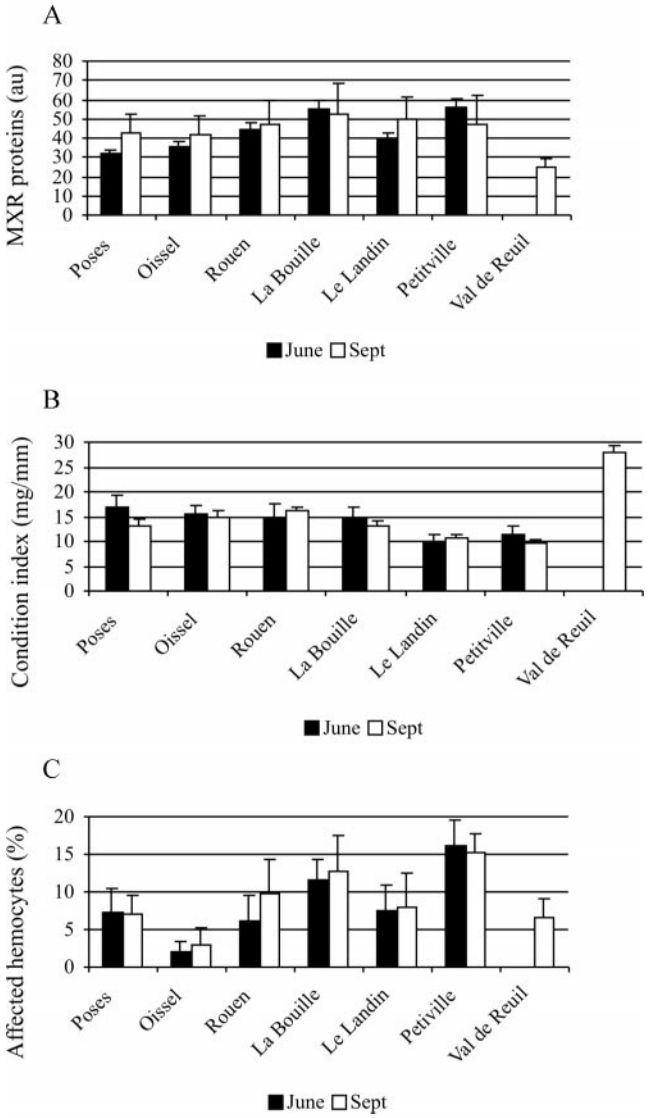

Fig. 7. Multixenobiotic (MXR) protein expression levels (**A**), condition index (**B**), and lysosomal stability (**C**) in zebra mussels collected in the Seine Estuary (Rouen, France). Error bars represent confidence intervals ($\alpha = 0.05$, $n = 6$ for MXR proteins; $n = 42$ for condition index; $n = 6$ for lysosomal stability).

results also are in accordance with previous works that showed, in suspended matter, concentrations of PCBs within the whole estuary and of PAHs at sites downstream Rouen were higher than the acceptable limit effect defined by the Ospar convention [34]. The potential mutagenicity of PAHs is evidenced further by the work of Boillot et al. [35] who reported that flounder (*Platichthys flesus*) sampled in La Bouille had high amounts of DNA adducts in the liver.

The biological responses of *D. polymorpha* living in the estuary are consistent with the potential (calculated) effects of their body burdens of contaminants. Our results showed that the health and development of mussel collected in the river sites, as assessed by their condition index, were poor compared to those of mussels living in a nearby lake taken as reference site. Other differences in markers related to mussel health also were evident between sites within the estuary. They showed that, not only the site of La Bouille, but also the other sites downstream of the city of Rouen, had reduced growth leading to decreased relative weight, higher MXR protein content, and

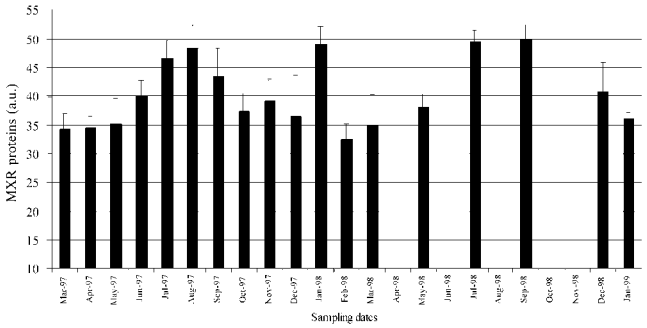

Fig. 8. Seasonal variations of multixenobiotic (MXR) protein expression in zebra mussels sampled in Rouen, France (March 1997–January 1999). Error bars represent confidence intervals ($\alpha = 0.05$, $n = 6$).

altered lysosomal membrane stability. Because mussels have few biotransformation capacities, these results indicate that adverse effects could arise from other mechanisms of toxicity and probably from other compounds than PAHs and PCBs. In this study, no specific mode of action was searched for (except for the AChE activity, which did not provide any significant result). Measurements of lysosomal stability and MXR protein expression allow monitoring of biological effects that can be due to a variety of compounds. They provide results complementary to the chemical analyses as they integrate potential multiple mechanisms of action of compounds that are present as complex mixtures. Zebra mussels also have been used to give more specific information on effects of contaminants, such as endocrine-disrupting effects in other areas, including European and American rivers and lakes [13,14]. Similar analyses should be performed in the Seine, because intersex fish (flounder, roach, and gudgeon) have been observed in this area [36,37].

CONCLUSION

By combining chemical and biological measurements, this study provides concordant information on the presence of contaminants and biological consequences. It shows that the Seine Estuary is highly contaminated by lipophilic compounds such as PAHs and PCBs that bioaccumulate in zebra mussel tissues and might have measurable and significant biological effects on living organisms feeding on mussels. The calculation of TEQ indicated that effects might arise, especially for vertebrates that have high metabolizing capacities. The decrease in fish-population density, which has led to the disappearance of any professional fishing activity in the Seine Estuary since the 1970s [38], at least partly might be related to such toxic effects. Even zebra mussels that might be considered as resistant organisms showed altered condition index, decreased lysosomal stability, and high levels of resistance proteins in the Seine Estuary.

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