Multimarker Approach in Transplanted Mussels for Evaluating Water Quality in Charentes, France, Coast Areas Exposed to Different Anthropogenic Conditions

M. Roméo,[1] Y. Mourgaud,[2] A. Geffard,[2] M. Gnassia-Barelli,[1] J. C. Amiard,[2] H. Budzinski[3]

[1]UMR INRA UNSA 1112 R.O.S.E., Faculté des Sciences, BP 71, 06108 Nice Cedex 2, France

[2]ISOMer, SMAB, Faculté des Sciences, 2 rue de la Houssinière, BP 92208, 44322 Nantes Cedex 3, France

[3]LPTC, UPRESA 5472, Université de Bordeaux I, 33405 Talence, France

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ABSTRACT: An active biomonitoring experiment was performed using mussels collected at a clean site, Fier d'Ars, and transplanted to two locations, outside the harbor of La Rochelle and in the Baie de L'Aiguillon along the coast of Charentes (French Atlantic coast) beginning in April for several months. Mussels were collected in June and October. The cadmium, copper, and zinc concentrations of all resident and transplanted mussel samples and the polycyclic aromatic hydrocarbon and polychlorinated biphenyl concentrations in some mussel samples and in the sediment samples were determined. Mussel response was evaluated for several biochemical biomarkers: concentrations of metallothionein, activities of glutathione S-transferase and acetylcholinesterase (AChE) and levels of thiobarbituric reactive substance (TBARS). The physiological status of the animals was assessed using the condition index. A principal component analysis performed with the chemical and biochemical results of the evaluations of the resident and transplanted mussels collected in June allowed them to be separated into three groups: resident mussels from la Rochelle with high metal and TBARS levels, resident mussels from Baie de L'Aiguillon with a very high condition index, and resident mussels from Fier d'Ars and transplanted mussels at La Rochelle and Baie de L'Aiguillon with low TBARS and AChE activities. Strong seasonal variation from June to October of all parameters was noted. Mussels transplanted to La Rochelle appeared to be the most "polluted" in their pollutant concentrations and biochemical responses; moreover, the La Rochelle site had the highest concentration of organics in sediments of all the sites. The choice of Fier d'Ars as a reference site may be questionable because some of the biomarker responses of the mussels were higher than expected there, although these pollutants in mussels and sediment were present at the lowest concentrations measured. Environ Toxicol 18: 295–305, 2003.

Keywords: mussels; biomarkers; transplantation; heavy metals; PAHs; PCBs; Charentes coasts; northwest Atlantic

Correspondence to: M. Roméo; e-mail: romeo@unice.fr.

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INTRODUCTION

Recent articles have shown that transplanting mollusks from a reference site to a polluted area can be a feasible strategy for biomonitoring the effects of environmental changes in coastal or estuarine zones (Amiard-Triquet et al., 1998; Da Ros et al., 2000; Geffard, 2001). In western France along the Atlantic Ocean, the Charentes coast includes distinct zones, allowing the design of field experiments or the conducting of observations according to the gradients of various pollutants: metals, polycyclic aromatic hydrocarbons (PAHs), and pesticides. Contaminated (Baie de L'Aiguillon, Baie de La Rochelle) and comparatively clean (Fier d'Ars) sites were considered in an active "biomonitoring" experiment (de Kock and Kramer, 1994; Herve et al., 2001) that was performed at these three sites using mussels, as these animals are of great value for biomonitoring because they are sedentary and filter feeders for nutrition. Active biomonitoring is based on comparing chemical and/or biological properties of samples collected from one population that, after randomization and translocation, have been exposed to different environmental conditions at monitoring sites (de Kock and Kramer, 1994).

In the framework of this article, the sample mussel population was constituted from *Mytilus galloprovincialis* mussels resident in the "clean" area. In fact, Fier d'Ars is a zone devoted to oyster culture and located far from agricultural, urban, and industrial influence. The Baie de L'Aiguillon is a highly productive farming zone for edible mussels. The environmental quality in terms of pesticide contamination of this mussel-farming area is a major concern because it previously experienced was contaminated by lindane, a organochlorine insecticide (Radenac et al., 1998). The transplantation site in the Baie de La Rochelle is outside the old harbor (where urban influence is strong) and the Minimes Marina, considered one of the greatest pleasure-boat harbors in Europe. The metal-rich Gironde estuary is located to the south of the transplantation area considered. Miramand et al. (2000) reported a gradient of cadmium concentration in wild oysters (*Crassostrea gigas*), ranging from 15 to 28 μg/g d.w. in the Gironde estuary to the North, where concentration reached 1.5 μg/g d.w. near the island of Ré and 2.4 μg/g d.w. at La Rochelle. In this study the mussels from Fier d'Ars were transplanted to Baie de L'Aiguillon and Baie de La Rochelle for 9 months, from March to November 1999. Heavy metal levels (of cadmium, copper, and zinc) were determined in both resident and transplanted mussels, and PAH and polychlorinated biphenyl (PCB) levels were determined in some mussel samples and in the sediment samples from the three sites. Biochemical biomarkers were used to evaluate the responses of mussels to the pollution gradient. Lagadic et al. (1997) underlined the importance of measuring several biomarkers at the same time in the same animals, which enables a pertinent approach to evaluating the effects of pollutants on individuals. Several biomarkers were thus measured in transplanted and resident mussels: metallothionein (MT) concentrations, glutathione S-transferase (GST) and acetylcholinesterase (AChE) activities, and thiobarbituric reactive substance (TBARS) levels. Determining metallothionein concentration can be a suitable monitoring procedure for assessing metal contamination in the marine environment (Langston et al., 1998; Cosson, 2000; Cosson and Amiard, 2000). GSTs are involved in the metabolism of organochlorine pesticides (Fitzpatrick et al., 1997; EC 2.5.1.18) have begun to be used as biomarkers of these substances and of PCBs in mollusks (Fitzpatrick et al., 1997; Hoarau et al., 2001) because ethoxyresorufin *O*-deethylase (EROD) activity as an exposure biomarker of organic compounds does not give satisfactory responses in these animals (Cajaraville et al., 2000). Moreover, a marker of oxidative stress, thiobarbituric acid reactive substance (TBARS), was also measured as reflecting the state of lipid peroxidation of the membranes (Knight et al., 1988; Pellerin-Massicotte, 1997). Acetylcholinesterase activity, inhibited by the presence of organophosphorus compounds and carbamates, is considered an exposure biomarker for these substances (Galgani and Bocquené, 1989). It has been used in coastal biomonitoring programs (Escartin and Porté, 1997; Stien et al., 1998; Mora et al., 1999, Roméo et al., 2003). In addition to these data on chemical bioaccumulation and biochemical effects, the impact on organisms was also assessed using a general index, namely, the condition index, which reflects the physiological status of the transplanted animals.

MATERIALS AND METHODS

Sampling and translocation sites are shown in Figure 1. Mussels, selected according to their size, were collected from Fier d'Ars in the intertidal zone; about 200 individuals were put into plastic bags used for oyster culture and translocated to the chosen sites [Fier d'Ars (ARS), Baie de L'Aiguillon (BA), and Baie de La Rochelle (LR)] and placed at the same level of the strand line. Until November 1999 about 20 specimens were collected each month at the three sites along with resident mussels collected from BA and LR. The current article focuses on two of the study's sampling dates: June and October, when a multiparametric approach including both chemical and biochemical analyses was attempted.

To do heavy metal and metallothionein determination, the mussels, once collected, were kept in clean seawater for 24 h (Fier d'Ars). They were dissected into digestive glands, gills and remaining tissues. Samples were then deep-frozen at -20°C until analysis. As for the biochemical biomarkers, as soon as the collection was performed, the samples were dissected (digestive gland and gills) and sent in dry ice from Charentes to Nice by special delivery and then kept at

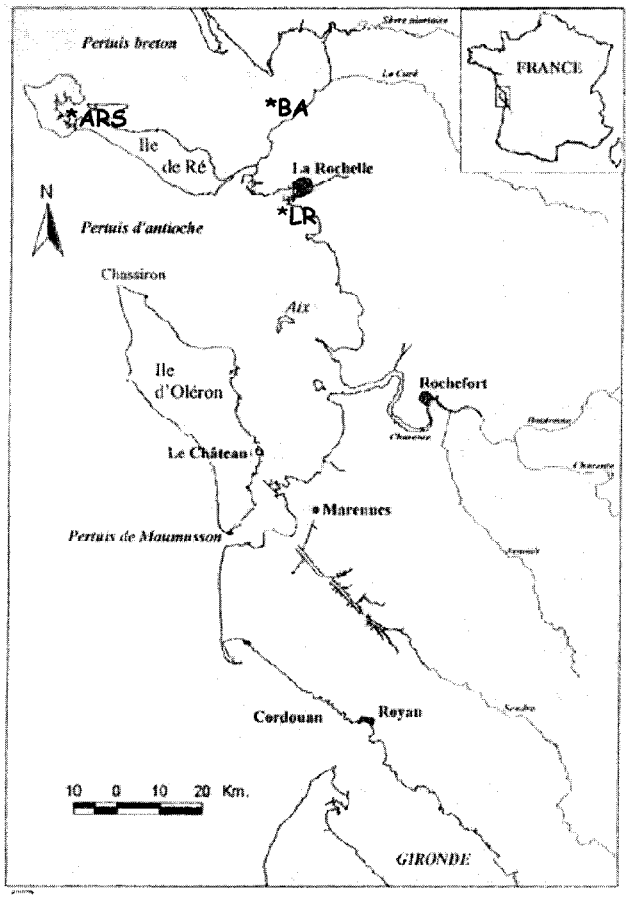

Fig. 1. Location of the transplantation sites—LR: harbor of La Rochelle; BA: Baie de L'Aiguillon. All mussels originated from Fier d'Ars (ARS), a bay inside the island of Ré. The geographical coordinates of La Rochelle are 16°9′ N, 1°10′ W.

−80°C before analysis. The organics were analyzed in mussels and sediments that were freeze-dried.

The condition index was measured on five animals in each bag, using the ratio of the weight of the soft tissue to the total weight (shell + soft tissues + palleal liquid) of the mussel, multiplied by 100 (Amiard et al., 1998).

Metal and Metallothionein Determinations

After acid digestion metals were analyzed by atomic absorption spectrophotometry according to methods validated by controls of internal quality (standard reference materials of oyster and mussel tissues, BCR/278R No. 188) and external quality (Campbell et al., 2000). Metal concentrations in the gills and the digestive gland were determined.

For metallothionein analyses (MT), individual digestive glands were homogenized in a buffer solution (composed of 20 m*M* TRIS, 10^{-5} m*M* β-mercaptoethanol, and 150 m*M* NaCl adjusted to pH 8.6). The cytosolic fraction was recovered by centrifugation (25 000 *g* for 55 min). The heat-stable MTs were isolated by centrifugation of the cytosolic fraction (15 000 *g* for 10 min) after heat treatment (75°C for 10 min). The amount of MT was determined in the heat-denaturated cytosol by differential pulse polarography, a technique based on —SH compound determination according to the Brdicka reaction (Brdicka, 1933), as described by Thompson and Cosson (1984). Although MT was probably not the only heat-stable sulfhydryl-containing compound remaining in the solution to be analyzed, other species potentially present (glutathione, free cysteine, β-mercaptoethanol) had no effect on the polarographic response compared to that of MT (Olafson and Olsson, 1991). A PAR Model 174 analyzer, a PAR/EG&G Model 303 static mercury drop electrode (SMDE) and an X-Y recorder (RE 0089) were used. The temperature of the cell was maintained at 5°C. The standard addition method was used for calibration with rabbit liver MT (Sigma Chemical Co., St Louis, MO) in the absence of purified bivalve MT. The institute ISOMer in which the MT analysis was carried out is involved in BEQUALM (Biological Effects Quality Assurance in Monitoring Programmes; Mathiessen, 2000).

PAH and PCB Concentrations in Mussels and Sediments

The sediments sampled at Fier d'Ars, Baie de L'Aiguillon, and Baie de La Rochelle were freeze-dried and then sieved at 2 mm. For PAH determinations, 2–5 g of dry sediment was extracted using microwave-assisted extraction as described elsewhere (Budzinski et al., 1995; Letellier et al., 1996), using the dichromate as the solvent of extraction. The organic extract was desulfurized using activated copper. Then, according to a procedure adapted from Behar et al. (1989), it was purified in a microcolumn containing alumina and fractionated in a microcolumn containing silica in order to collect separately the saturated and aromatic compounds. Five of the collected mussels were used for the determination of aromatic compounds. Soft tissues were separated from the shell, freeze-dried, and homogenized. Assay samples (1–2 g dry weight) were extracted according to a procedure described by Baumard et al. (1997). The aromatic fraction of sediments and mussels was analyzed by gas chromatography/mass spectrometry (GC/MS) using a HP 5890 series II gas chromatograph coupled to a HP MSD 5972 mass spectrometer. The accuracy of the quantification method was described in Amiard-Triquet et al. (1998).

The details of the procedure for PCB determinations was described in Thompson et al. (1999). The matrix was extracted by microwave-assisted extraction (1–2 g dry weight of mussel tissue, 2–5 g of sediment, time = 10 min at 30 W, Maxidigest 350 PROLABO, Paris) with dichloromethane. Analyses were performed on an HP 5890 series II gas chromatograph (Hewlett-Packard, Avondale, MA) coupled to an ^{63}Ni electron capture detector (ECD) equipped with an

automatic injector. The PCBs were analyzed as individual congeners, with 20 congeners determined (among them, the results for the PCBs with the highest concentrations are given here, i.e., PCB 52, PCB 101, + PCB 90 and PCB 153). The relative response factors of the different compounds were determined by injecting a standard solution of PCBs (Promochem, Molsheim, France; in solution at 99%+ purity) spiked with the same solution of internal standards PCB 30, PCB 103, PCB 155, and PCB 198 as that used for spiking the samples. Blank injections of isooctane were performed between each injection of a sample to ensure the cleanliness of the injector. Procedural blanks were regularly performed, and all results presented have been corrected for blank levels. All glassware was rigorously cleaned with detergent followed by pyrolysis at 450°C. The sodium sulfate and silica gel were preextracted with dichloromethane in an ultrasonic bath, dried, and then rinsed with dichloromethane just before utilization.

GST, AChE, and TBARS Measurements

The GST, AChE, and TBARS measurements were carried out on the gills and digestive glands of individual mussels ($n = 6$). All procedures were carried out at a temperature of 0°C–4°C. Tissues were homogenized in a TRIS buffer (50 m*M* TRIS, 150 m*M* NaCl, pH 7.4), 1 m*M* PMSF (phenylmethylsulfonylfluoride), and 1 m*M* dithiothreitol (DTT) at a 1:4 ratio (w/v) using an Ultra-Turrax. The homogenates were then centrifuged for 30 min at 9000 *g*. Aliquots of the supernatant (called S9 fraction) were frozen at −80°C until use. All determinations were performed on S9 fractions. Total protein was determined for all samples according to Bradford (1976).

GST activity was measured spectrophotometrically at 340 nm by following conjugation of the acceptor substrate 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione (Habig et al., 1974). Acetylcholinesterase activity was determined using the method of Ellman et al. (1961) adapted to a microplate reader by Galgani and Bocquené (1991). Lipid peroxidation was estimated by the formation of thiobarbituric reactive substances (TBARS). Considered to be malonedialdehyde (MDA)-like peroxide products, TBARS, were quantified by reference to MDA absorbance ($\varepsilon = 156.10^3\ M^{-1}\ cm^{-1}$). Results were not expressed as MDA concentrations, as TBA can react with a range of chemical compounds (Csallany et al., 1984), but as TBARS levels.

Statistical Analyses

The variations of each biomarker were tested by one-way analysis of variance considering site or season as a variable. When an ANOVA was significant, post hoc pair-wise comparisons between locations (or seasons) were done using the Scheffé test to determine which values differed significantly.

Principal component analysis (PCA) was used to discriminate the different transplantation sites. This statistical analysis previously has been used in the same type of field experiment (Herve et al., 2002). Data were normalized using the log $(1 + x)$ transformation. Two PCAs were performed with the digestive glands. Variables taken into consideration were: cadmium, copper, and zinc concentrations; GST and AChE activities; TBARS levels; and MT concentrations, as well as the condition index of the animals.

RESULTS

Condition Index of Mussels

The condition index (CI) values of the resident and transplanted mussels are shown in Table I. The index values appeared to be greatly homogeneous. In June the highest CI was found in resident mussels of Baie de L'Aiguillon, followed by Fier d'Ars; the lowest was observed in La Rochelle. In transplanted mussels in June, the CI did not vary, 14.16 (Baie de L'Aiguillon) and 15.25 (La Rochelle), whereas in October the transplanted mussels collected at Baie de L'Aiguillon showed a high condition index compared to those transplanted at la Rochelle. The resident mussels of Fier d'Ars presented a relatively high condition index in October (17.28).

Biomarker Levels in Transplanted and Resident Mussels

The results for the biomarker levels in the transplanted and resident mussels also are shown in Table I. The biomarker levels measured in the gills were significantly higher than those in the digestive gland. They followed the same pattern as that observed for the digestive glands.

As for the biomarker levels in resident mussels measured in June, GST activity in the digestive gland from Baie de L'Aiguillon was significantly lower (Scheffé test significant at $P < 0.05$), whereas of the three stations the highest TBARS content and the highest AChE activity were observed in La Rochelle (Scheffé test significant at $P < 0.05$). MT concentrations in the digestive gland were significantly lower in Fier d'Ars than in Baie de L'Aiguillon (Scheffé test significant at $P < 0.05$) and in La Rochelle (Scheffé test significant at $P < 0.05$). In October only resident mussels from Fier d'Ars could be analyzed. TBARS levels in the digestive gland were significantly lower than in June, whereas MT concentrations increased slightly.

In June the transplanted mussel samples from Baie de L'Aiguillon showed a tendency for lower TBARS levels in the digestive gland and the gills than those from La Roch-

TABLE 1. Mean values (±1 standard deviation, $n = 6$ in each case) of biomarkers: glutathione *S*-transferase (GST) activity, TBARS levels, acetylcholinesterase (AChE) activity and metallothionein concentrations (MT μg/g fresh wt) in digestive gland (DG) and gills of resident and transplanted mussels from La Rochelle, Baie de L'Aiguillon, and Fier d'Ars

		Resident Mussels				Transplanted Mussels			
		June			October	June		October	
		La Rochelle	Baie de L'Aiguillon	Fier d'Ars	Fier d'Ars	La Rochelle	Baie de L'Aiguillon	La Rochelle	Baie de L'Aiguillon
Condition Index		11.01	20.76	16.58	17.28	15.25	14.16	14.2	19.44
Biomarker	Organ								
GST	DG	271.8 ± 113.9[a]	154.0 ± 19.4[b]	331.8 ± 53.1[a]	301.8 ± 19.2	289.3 ± 35.3	310.6 ± 55.3	303.8 ± 12.3[a]	224.7 ± 8.0[b]
(nmol/min/mg)	Gills	436.5 ± 117.1	552.2 ± 77.5	706.2 ± 110.4	397.3 ± 122.5	671.1 ± 134.2	523.2 ± 76.2	219.8 ± 65.0	336.8 ± 22.7
TBARS	DG	8.5 ± 1.4[a]	4.0 ± 0.5[b]	2.8 ± 1.9[b]	0.5 ± 0.2	2.9 ± 1.8	0.5 ± 0.2	1.9 ± 1.0[a]	0.5 ± 0.4[b]
(nmol/g)	Gills	10.2 ± 1.6	6.3 ± 0.6	3.6 ± 1.6	2.4 ± 0.5	7.5 ± 4.2	2.6 ± 0.2	3.4 ± 2.1	2.0 ± 0.8
AChE	DG	6.2 ± 2.2[a]	3.1 ± 0.5[b]	3.0 ± 0.3[b]	3.9 ± 0.1	2.5 ± 0.2	2.6 ± 0.4	3.5 ± 0.5[a]	2.5 ± 0.4[b]
(nmol/min/mg)	Gills	25.6 ± 5.0	20.9 ± 3.4	17.6 ± 3.0	16.3 ± 2.4	14.8 ± 1.6	10.6 ± 2.4	7.2 ± 2.0	10.4 ± 2.1
MT (μg/g fresh weight)	DG	3.1 ± 0.6[a]	2.9 ± 0.4[a]	2.1 ± 0.3[b]	3.6 ± 0.6	3.8 ± 0.5[a]	2.6 ± 0.6[b]	2.7 ± 0.7	2.7 ± 0.4

In June resident mussels could be obtained from the three sites, whereas in October only resident mussels from Fier d'Ars were obtained. Mussels from Fier d'Ars were transplanted to La Rochelle and Baie de L'Aiguillon and collected in June and October, i.e., 4 and 8 months after their transplantation. Data with the same superscript indicates they did not differ significantly at the 95% level (Scheffé test done after a significant ANOVA performed for mussels at each site during the same period).

elle (but the Scheffé test was not significant). MT concentrations were higher in the digestive gland of mussels transplanted at La Rochelle (Scheffé test significant at $P < 0.05$). In October TBARS levels in the digestive gland were higher in la Rochelle than in Baie de L'Aiguillon (Scheffé test significant at $P < 0.05$), and AChE activity was lower (Scheffé test significant at $P < 0.05$) in the digestive gland of the mussels transplanted at Baie de L'Aiguillon compared to that of those from La Rochelle. In contrast, GST activity was significantly higher in the mussels of La Rochelle. MT concentrations were not different between the two sites.

Chemical Analyses of Transplanted and Resident Mussels

The results of chemical analyses in mussels are shown in Table II. In June copper concentrations in the resident mussels were not significantly different in the digestive glands of the resident mussels from the three sites, whereas cadmium concentrations were lower in the mussels from Fier d'Ars than in those from Baie de L'Aiguillon and La Rochelle (Scheffé test significant at $P < 0.05$ in each case), and zinc concentrations were lower in the mussels from Baie de L'Aiguillon than in those from Fier d'Ars and La Rochelle (Scheffé test significant at $P < 0.05$ in each case). Metal concentrations were also significantly lower in October.

For the transplanted animals in June, metal concentrations were not different in the digestive glands in the mussels from both transplantation sites, whereas in October cadmium, copper, and zinc were all significantly higher in transplanted mussels at La Rochelle than in those transplanted at Baie de L'Aiguillon (Scheffé test significant at $P < 0.05$ for each metal).

In the resident mussels collected in June (Table II), ΣPAH, ΣMP (methylphenanthrene),ΣMN (methylnaphthalene), and ΣPCB concentrations (and among them the congeners presenting the highest concentrations such as PCB 52, PCB 101+90 and PCB 153) in mussels (one measurement per pool of 5 mussels) demonstrated that the lowest values were generally found in the animals from Fier d'Ars and the highest in those from La Rochelle. ΣPAH and ΣPCB concentrations in the mussels resident in Fier d'Ars collected in October slightly increased over the corresponding concentrations in June.

In the transplanted mussels organics generally were lower in Baie de L'Aiguillon than in La Rochelle. When comparing both seasons, organics generally showed higher concentrations in mussels from both sites in October than in June.

Metal, PAH, and PCB Concentrations in Sediments

Results of chemical analyses in sediments are presented in Table III. Heavy metal concentrations were lower in the

TABLE 2. Mean values (±1 standard deviation, $n = 6$ in each case) of metal and metallothionein (MT) concentrations (μg/g fresh wt) in digestive gland (DG) and gills of resident and transplanted mussels from La Rochelle, Baie de L'Aiguillon, and Fier d'Ars

		Resident Mussels				Transplanted Mussels			
		June		October		June		October	
		La Rochelle	Baie de L'Aiguillon	Fier d'Ars	Fier d'Ars	La Rochelle	Baie de L'Aiguillon	La Rochelle	Baie de L'Aiguillon
Cd (μg/g)	DG	0.248 ± 0.062^{a}	0.306 ± 0.085^{a}	0.172 ± 0.028^{b}	0.082 ± 0.009	0.145 ± 0.037	0.148 ± 0.029	0.102 ± 0.022^{a}	0.071 ± 0.022^{b}
Cu (μg/g)	DG	5.12 ± 2.07	4.98 ± 0.64	5.08 ± 1.24	2.15 ± 0.49	4.54 ± 1.18	3.42 ± 0.77	2.74 ± 0.67^{a}	2.04 ± 0.27^{b}
Zn (μg/g)	DG	27.07 ± 4.76^{a}	19.60 ± 4.07^{b}	28.87 ± 7.06^{a}	10.54 ± 1.91	20.85 ± 3.18	17.29 ± 4.13	13.92 ± 4.45^{a}	9.99 ± 2.67^{b}
ΣPAH (ng/g)	Mussels	209.14	147.66	105.27	154.92	215.85	180.64	420.30	325.33
ΣMP (ng/g)	Mussels	25.00	9.20	4.48	8.00	11.28	8.20	39.87	9.10
ΣMN (ng/g)	Mussels	9.82	3.01	6.66	5.70	7.24	8.65	5.02	8.37
ΣPCB (ng/g)	Mussels	292.25	106.28	71.15	86.56	192.01	123.67	302.63	135.01
PCB 52 (ng/g)	Mussels	11.65	5.38	<0.2	<0.2	7.56	6.29	10.24	3.58
PCB101 + 90 (ng/g)	Mussels	24.83	6.73	4.02	4.11	16.87	9.38	23.75	8.18
PCB 153 (ng/g)	Mussels	56.32	20.58	11.34	14.99	33.79	25.28	54.86	28.17

Concentrations of organics are expressed as ng/g d.w.; they include ΣPAH, ΣMP (methylphenanthrene), ΣMN (methylnaphtalene), and ΣPCB (among them, PCBs with the highest concentrations are shown, i.e., PCB 52, PCB 101 + PCB 90, and PCB 153) in a pool of mussels from each site. Data with the same superscript indicates they did not differ significantly at the 95% level (Scheffé test done after a significant ANOVA performed for mussels at each site during the same period).

TABLE 3. Mean values (±1 standard deviation) of cadmium, copper, and zinc concentrations (μg/g d.w.) in the sediments from LR (La Rochelle), BA (Baie de L'Aiguillon), and ARS (Fier d'Ars)

	June			October		
	LR	BA	ARS	LR	BA	ARS
Cd (μg/g)	0.18 ± 0.01^a	0.27 ± 0.02^b	0.23 ± 0.01^b	0.30 ± 0.05	0.27 ± 0.02	0.26 ± 0.01
Cu (μg/g)	21.1 ± 2.0^a	16.3 ± 0.5^a	7.5 ± 1.0^b	20.0 ± 1.0^a	16.0 ± 1.5^a	6.0 ± 0.5^b
Zn (μg/g)	160 ± 10^a	140 ± 5^a	70 ± 1^b	150 ± 10^a	145 ± 5^a	60 ± 1^b
ΣPAH (ng/g)	656.55	562.97	381.09	989.11	713.06	504.17
ΣMP (ng/g)	29.35	30.81	17.43	29.88	30.63	33.63
ΣMN (ng/g)	13.08	11.70	5.04	12.64	12.16	5.71
ΣPCB (ng/g)	20.66	9.87	1.63	35.25	13.58	1.40
PCB 52 (ng/g)	1.28	0.56	0.09	1.47	0.82	0.09
PCB101 + 90 (ng/g)	1.61	0.93	0.01	2.92	1.39	0.01
PCB 153 (ng/g)	2.39	1.32	0.27	4.43	1.50	0.20

Concentrations of organics are expressed as ng/g d.w.; they include ΣPAH, ΣMP (methylphenanthrene), ΣMN (methylnaphtalene), and ΣPCB (among them, PCBs with the highest concentrations are shown, i.e., PCB 52, PCB 101 + PCB 90, and PCB 153) in a pool of sediments from each site. Data with the same superscript indicates they did not differ significantly at the 95% level (Scheffé test after a significant ANOVA performed for sediments at each site during the same period).

sediments from Fier d'Ars than in those from Baie de L'Aiguillon and La Rochelle, except for cadmium in June in La Rochelle. Seasonal variation of metal concentrations was not strong. Levels of ΣPAH, ΣMP, and ΣMN in La Rochelle sediments nearly reached twice those of Fier d'Ars, whereas those from Baie de L'Aiguillon presented intermediate concentrations. In the same way, ΣPCB, including the most important congeners, that is, those presenting the highest concentrations, generally showed much higher concentrations in the sediments from La Rochelle than in those from Fier d'Ars, whereas intermediate concentrations were found in the sediments from Baie de L'Aiguillon. Moreover, levels of organics were higher in October than in June.

Principal Component Analyses

Two types of principal component analysis (PCA) were performed: one to compare metal and biomarker levels with condition index values measured in resident and transplanted mussels in June, the second to compare the same parameters in transplanted mussels (and resident mussels of Fier d'Ars) in June and October. The concentrations of PAHs and PCBs in mussels were not taken into consideration in the statistical analyses as these corresponded to one measurement of a pool of mussels.

The PCA that compared resident and transplanted mussels sampled in June is shown in Figure 2; the parameters taken into consideration were: GST and AChE activities, TBARS, copper, cadmium, zinc, and MT concentrations measured in the digestive gland, and the condition index of mussels (240 data items). The first axis shown in Figure 2 represents 40.60% of the total variance, the second axis 23.75%, and the third axis 16.42%. Figure 2 also displays the F1 (horizontal) and F2 (vertical) axes. TBARS and copper levels and AChE activities contribute mainly to the first axis, whereas GST activities, on the one side, and condition index, on the other, are correlated with the second axis. This principal component analysis allowed grouping resident mussels from Fier d'Ars together with transplanted mussels from La Rochelle and Baie de L'Aiguillon. These mussels were characterized by low TBARS and AChE activities in their digestive gland. Resident mussels from La Rochelle, with high TBARS and high AChE activities in their digestive gland, are in the negative part of the first axis. Resident mussels from Baie de L'Aiguillon sampled in June, with high condition index and low GST activity, are clearly separate from the other mussels.

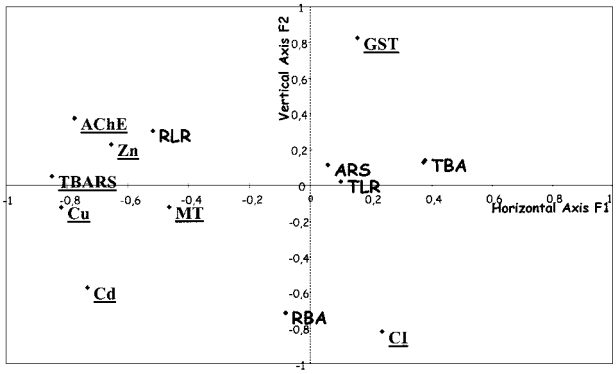

Fig. 2. Principal component analysis including all parameters studied in June in resident mussels, designated as ARS, RLR (resident mussels of La Rochelle), and RBA (resident mussels of Baie de L'Aiguillon); and in transplanted mussels, designated as TLR (transplanted at La Rochelle) and TBA (transplanted at Baie de L'Aiguillon). On the F1 and F2 axes both variables [Cd, Cu, Zn, and MT concentrations; TBARS levels; GST and AChE activities; and condition index (CI)] and the mean scores of the stations are represented.

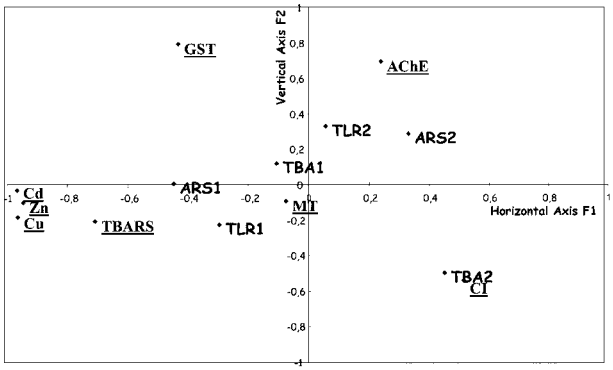

Fig. 3. Principal component analysis including all parameters studied in resident mussels, designated as ARS1, ARS measured in June, and ARS2, ARS measured in October; and transplanted mussels, designated as TLR1 (transplanted at La Rochelle in June), TLR2 (transplanted at La Rochelle in October), TBA1 (transplanted at Baie de L'Aiguillon in June), and TBA2 (transplanted at Baie de L'Aiguillon in October). On the F1 and F2 axes both variables [Cd, Cu, Zn, and MT concentrations; TBARS levels; GST and AChE activities; and condition index (CI)] and the mean scores of the stations are represented.

A comparison of the transplanted mussels sampled in June and October with resident mussels from the Fier d'Ars site was studied by a principal component analysis taking into consideration the same parameters as above (288 data items). The first axis (F1) represents 47.15% of the total variance, the second and third axes 18.63% and 14.49%, respectively. Shown in Figure 3 is a synthesis of the results, in which heavy metal concentrations can be seen to highly correlate with the first horizontal axis and to a lesser extent TBARS levels. The second vertical axis represents GST activity and the third axis (figure not shown) metallothionein concentrations. In Figure 3 mussels from June and October are separated, with those from June, in the negative part of F1, showing higher heavy metal concentrations and TBARS levels than those from October, in the positive part of F1.

DISCUSSION

Wild mussels from a site considered "clean," Fier d'Ars, were transplanted for several months to two other places: the harbor of La Rochelle and Baie de L'Aiguillon. Transplanted mussels were collected in June and October, 4 and 8 months after transplantation, respectively. The transplanted and resident animals (when possible) were analyzed for contaminant concentrations and for biomarker levels.

The data overall demonstrated differences between resident and transplanted mussels as well as seasonal variation in the different parameters studied. Animals did not present high metal or organic concentrations in their tissues compared to the levels published by the Réseau National d'Observation (1995). Metal concentrations in the sediments from the three sites were always lower than the first reference level of sediment quality established by the French regulation (Arrêté Inter-ministériel, June 14, 2000). Below this level the impact of sediment is considered negligible (Circulaire Inter-ministérielle No. 2000-62, June 14, 2000). Metal concentrations in sediments may be affected by the level of organic matter and by the granulometry. In the current study, both these parameters differed little between La Rochelle and Baie de L'Aiguillon. However , the concentration of organic matter was shown to be lower and the granulometry higher in the sediments from Fier d'Ars; these phenomena could lessen the differences observed with the other sites when considering global concentrations of metals.

A comparison was able to be performed in June between mussels originating from all three sites. The most striking feature of the parameters measured in this comparison was that the resident mussels from Fier d'Ars seemed to be less contaminated than the resident mussels from the two other sites. Nevertheless, the high level of acetylcholinesterase activity in June in La Rochelle compared to that in Fier d'Ars or even in Baie de L'Aiguillon seemed unusual because La Rochelle's site is characterized by higher organic levels both in the animals and in the sediments than the two other sites. Moreover, the condition index of the resident animals was the lowest at this site. AChE activity has been reported to be inhibited not only by pesticides such as organophosphorous compounds and carbamates but also by heavy metals (Bocquené et al., 1997). We could hypothesize that mussels from Fier d'Ars, and to a lesser degree from Baie de L'Aiguillon, which appeared not strongly contaminated by metals or organics, could have been exposed to some other substances inhibiting acetylcholinesterase activity. Bocquené et al. (1997) emphasized that AChE may be inhibited by phytotoxins released into the medium during a period of phytoplankton bloom such as the end of spring. Nevertheless, these authors underlined that potential inhibitors of acetylcholinesterase activities are very numerous.

The principal component analyses (Fig. 2) performed on both resident and transplanted animals sampled in June allowed for a clear separation of the resident mussels from the three sites [Fier d'Ars (ARS), resident La Rochelle (RLR), and resident Baie de L'Aiguillon (RBA)]; consequently, the choice of these three sites for the study seems to be fully justified. Grouping transplanted mussels of La Rochelle and Baie de L'Aiguillon and resident mussels from Fier d'Ars was obvious, as can be seen in Figure 2. An important feature is underlined: the mussels that originated from Fier d'Ars and were transplanted for 4 months to La Rochelle and Baie de L'Aiguillon still had the same characteristics as the mussels from Fier d'Ars. Transplanted mussels appeared not to have yet reached equilibrium with their environment after 4 months' experience.

Figure 3 shows there was strong seasonal variation in all parameters from June to October. After 8 months of transplantation, the transplanted mussels (in the case of Fier d'Ars) were separated from mussels transplanted for 4 months and those that were resident and collected in June from Fier d'Ars. The physiological status of transplanted and resident mussels of Fier d'Ars increased from June to October. In October the mussels presenting the most different characteristics were those transplanted at Baie de L'Aiguillon (Fig. 3). At this location the condition index of mussels was indeed very high, possibly because of a higher quantity of available food at this location. Moreover, nearly all other parameters were lower than those in La Rochelle; this phenomenon was present but less marked in June. If all parameters of transplanted mussels in Baie de L'Aiguillon measured in October (last column in Table II) and resident mussels from Fier d'Ars in October (last column in Table I) are compared, their characteristics are on the same order of magnitude except for the concentrations of PAHs and PCBs, which were lower in resident mussels of Fier d'Ars. AChE activity appeared to be inhibited compared to mussels from La Rochelle or even to resident mussels. This apparent inhibition could not influence the physiological status of mussels represented by the condition index, which was high in those mussels transplanted at Baie de L'Aiguillon (CI = 19.44).

The parameters considered in the PCA analyses allowed for either discriminating grouping transplanted or resident mussels according to site. Some biomarkers appeared to be especially "discriminating" compared to others. One of these was AChE activity, whose importance has already been underlined.

In contrast, metallothionein levels did not appear to be a strong "discriminating" factor. These levels were apparently not highly related to heavy metal content in the digestive gland of either transplanted or resident mussels. Mourgaud et al. (2002) found a relationship between concentrations of cadmium, zinc, and, to a less degree, copper and metallothionein concentrations in the whole soft tissues of mussels collected along the Mediterranean coast. In the present work only the digestive gland was studied as this organ has been reported to be the best tissue for considering metallothionein concentrations as biomarkers of heavy metal exposure (Amiard et al., 1998; Geffard, 2001). The relationship reported by Mourgaud et al. (2002) was not found in the case of metallothionein concentrations measured in the digestive gland of transplanted mussels. In the future MT analyses should be performed both in whole soft tissues and in the digestive glands of mussels.

In both PCA analyses TBARS levels were strongly correlated with the first axis. They were linked with heavy metal concentrations in the digestive gland of mussels. Lipid peroxidation as represented by TBARS levels has been shown to increase in mussels and clams exposed to heavy metals, in particular to copper (Viarengo et al., 1990; Roméo and Gnassia-Barelli, 1997).

Also in both PCAs the second (vertical) axis represented GST activity as opposed to the condition index. The opposition between these two parameters may be explained by the low GST activity of animals in good physiological condition. The reported increase in the GST activity of mollusks in the presence of organics such as PCBs (Fitzpatrick et al., 1997; Hoarau et al., 2001) was not observed in this work. The GST activity of the mussels of La Rochelle, which have a relatively high PCB concentration and which live near sediment with a higher level of organics, was not high compared to that of the mussels at the reference site, namely, Fier d'Ars. In this study GST activity was assayed using 1-chloro-2,4-dinitrobenzene (CDNB), a relatively nonspecific GST reference substrate. Thus, the GST-CDNB activity measured reflects the integration of all GST isoenzyme activities. Studies carried out on the blue mussel *Mytilus edulis* have shown that different GST isoforms are induced in this animal as a function of the nature of the pollutants (Fitzpatrick et al., 1997). Hoarau et al. (2001, 2002) reported finding the presence of several GST isoforms more or less expressed in the clams in their studies. Future studies should measur a particular GST isoform that responds to a specific pollutant, instead of investigating the entire range of GST activity. Nevertheless, the striking feature of what is shown in Figure 3 is the opposition between the GST activity and condition index values of the mussels. Some authors have reported that GSTs are biomarkers of toxicity; GST activity was even shown to be elevated in animals in which carcinogenesis had already occurred (Satoh et al., 1985). According to Foureman (1989), induction could be caused by diseases rather than as a direct consequence of exposure to pollutants. In contrast, when mussels have a good condition index, their GST activity seems to be low. For instance, in June the resident mussels of Baie de L'Aiguillon showed low GST activity (154.03 nmol/min/mg), a high condition index (30.76), and relatively high AChE activity (3.96 nmol/min/mg) compared to the resident mussels of Fier d'Ars (GST, 331.9 nmol/min/mg; CI, 16.58; AChE, 2.8 nmol/min/mg). These results tend to demonstrate an unexpected difference between the sites: resident mussels from Fier d'Ars seemed to have been more exposed to pollutants than were resident mussels from Baie de L'Aiguillon, as reflected in the biomarker response measured; the pollutants apparently were not heavy metals, PAHs, or PCBs, but were another type, for instance, toxins. The choice of Fier d'Ars as the reference site because it is devoid of chemical pollution therefore may be discussed as, in fact, another type of pollution may exist there.

And, in conclusion, in the course of the study some interesting variations were determined both in resident and transplanted mussels in the area of the Atlantic Ocean under consideration. In a previous work (Roméo et al., 2003), transplantation experiments were performed with mussels

from an aquaculture farm at three periods in the northwestern Mediterranean, with each experiment lasting 1 month. In the present work mussels were transplanted for much longer periods (the results reported here focused on the 4- and 8-month periods), with equilibrium between transplanted animals and their medium being reached after 3 months, according to Andral and Stanisière (1999). The studied areas were submitted to multiple sources of pollution (metals, PAHs, and PCBs), and chemical determination demonstrated that their levels in both animals and sediment were below acceptable levels set by the French and European legislations. Our experiments allowed the characterization of the response of mussels from different transplantation sites that were not very far geographically from one another.

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