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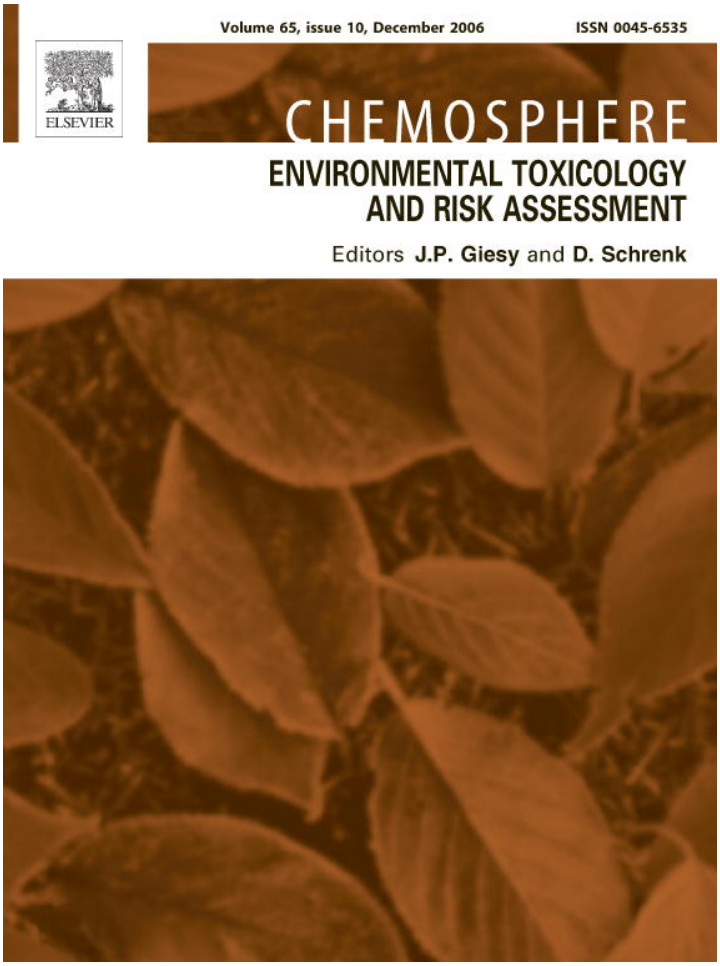


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# Biomarker responses in European eel (*Anguilla anguilla*) exposed to persistent organic pollutants. A field study in the Vaccarès lagoon (Camargue, France)

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**Abstract**

A screening of relevant biomarkers was carried out in order to evaluate metabolic and cellular damages in European eels exposed to a non-point source contamination by persistent organic pollutants (POP) such as polycyclic aromatic hydrocarbons (PAH) and organochlorine compounds (OC) in a protected area, the Nature Reserve of Camargue (France). Investigations were focused on metabolic responses including detoxification mechanisms (biotransformation, antioxidant process), energy requirements and enzymatic membrane markers either involved in neuronal conduction (acetylcholinesterase, AChE) or in osmoregulation and energy metabolism (ATPases). The hepatic and muscular glycogen rates seemed to be suitable biomarkers as well as three hepatic activities involved in the protection against oxyradicals: catalase, glutathione peroxidase (SeGPx) and superoxide dismutases (SOD). The muscle and gill ATPases as well as the muscle and brain AChE showed more significant relevance in terms of biomarkers than the biotransformation enzymes: ethoxyresorufine-*O*-deethylase (EROD) and uridine diphospho-glucuronyl transferase (UDPGT). However, most of these enzymatic activities depend on numerous abiotic factors, which must be taken into account in such a biomarker assessment approach. Our study provides some conclusive elements to approve the use in situ of biomarkers developed from laboratory studies. It also raises a question regarding the location of contaminant impregnation in fish organ, in relation with age, development status or mode of contamination, and its influence on biomarker response. If the relevance of membrane indicators is confirmed, this study provides an original statement of the extent of the ecotoxicological threat for the aquatic species in a protected area, due to the occurrence of POP in the cell membranes.
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*Keywords:* Biomarkers; PAH; Organochlorine pesticides; Eels; Camargue reserve

## 1. Introduction

Most ecotoxicological research on aquatic ecosystems involves the biomonitoring of inland or coastal waters. This concept deals with the detection of early warning responses of aquatic biocoenosis exposed to pollutants or other environmental stressors. Such biomonitoring programs are usually based on the study of the biochemical and physiological responses induced in exposed organisms (Lionetto et al., 2003), that resulted in the development of biomarkers over the last decades (Van der Oost et al., 2003). However, although biomarkers are intended to be useful tools for environmental assessment in the field, most of them were developed under laboratory controlled toxic conditions, generally very different of in natura exposures. Moreover, the most frequent ecotoxicological experiments refer to isolated organisms under controlled conditions. Such approaches are frequently discussed because of the deficiency – or the too great – specificity of the selected

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indicators facing the diversity and the variability of the contaminants and the abiotic factors (Wu et al., 2005; Forbes et al., 2006). The multiplicity of factors have conducted the scientists to develop models taking into account the complexity of the ecosystems and the instability of responses. Currently the efforts of modeling in ecotoxicology are seldom conclusive on the ecosystem scale. Consequently, the relationship between biomarker responses and population-level effects in the field are not still well defined. Therefore the assessment of biomarkers using natural populations in natural ecosystems is necessary to develop efficient tools for in situ use. This has already been achieved in different areas such as the North-West of the Mediterranean Sea (Burgeot et al., 1996), the Gulf of Naples in Italy (Naso et al., 2005), the North Sea (Van der Oost et al., 1996, 1997) or in river Douro estuary (Portugal) (Ferreira et al., 2006).

We had then planned to investigate the ecotoxicological status of the brackish ecosystems of the French national Nature Reserve of Camargue (NRC), registered in the Man and Biosphere Program in 1977.

The NRC is located in a rather remote area, free of neighboring industrial activities. It includes many aquatic ecosystems characterized by a large variety of freshwater bodies. The Camargue ecosystems are exposed to continuous inputs of pesticides used in surrounding agricultural areas (Persic et al., 2004). Pollutants may be transferred to the Camargue waters by means of atmospheric precipitations (dry or wet), leaching, runoff, or irrigation canals. Our previous works confirmed the presence of persistent organic pollutants (POP) as organochlorine pesticides (OC), polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) in fish collected in the Vaccarès lagoon, the main water body of the NRC (Roche et al., 2000, 2002a,b; Oliveira Ribeiro et al., 2005). The assessment of risks related to these pollutants implies the evaluation of the relationships between individual fish tissue contamination and selected biological parameter variations. This evaluation is necessary as the first step of the estimation of in situ long-term effects induced by fluctuating low level pollution.

In the present study, we focused on the biological effects of organic pollutants on a population of European eels (*Anguilla anguilla*). The biomarkers were selected in order to characterize several metabolic processes. They concerned energetic metabolism parameters, (neutral and polar lipids, protein, glycogen contents), and muscle and gills ATPase activities. ATPases play a key role in osmoregulation (gill ATPases) and/or energy metabolism (muscle ATPases). Moreover, due to their location in the membranes, ATPases are considered to be good membrane markers (Comoglio et al., 2005). Other membrane enzyme activities were measured, such as muscle and brain acetylcholinesterase (AChE), known as carbamates and organophosphorus targets (Galgani et al., 1992; Forget et al., 2003). We also studied biotransformation enzyme activities – 7-ethoxyresorufin *O*-deethylase (EROD), uridine-5′-diphospho-glucuronyltransferase (UDPGT) and glutathione-*S*-transferase (GST) – and antioxidant enzymes – superoxide dismutase (SOD), catalase and total and selenium-dependant glutathione peroxidases (GPx and Se-GPx). Their use has been recommended in numerous ecotoxicological studies (Roche and Bogé, 2000; Van der Oost et al., 2003).

## 2. Material and methods

### 2.1. Study area and organisms

The Camargue Biosphere Reserve is located in the Rhône delta in Southern France. It constitutes the largest coastal wetland of Western Europe (13,000 ha). This natural Reserve is limited at the North by the Vaccarès lagoon and at the South by the last undamaged sand dunes on the Mediterranean coast (Fig. 1).

European eels ($n = 176$) were collected from the Vaccarès lagoon during semi-annual campaigns in 1999 and 2000. According to their length, eels were split in two groups ($n_1 = 92$, $n_2 = 84$). Even if eel length and weight are not systematically correlated to its age, the first group ($95.8 \pm 5.1$ g, $47.6 \pm 0.9$ cm) was incorrectly called 'juveniles' and the second one ($257.4 \pm 16.3$ g, $36.8 \pm 0.7$ cm) 'yellows'.

### 2.2. Chemical analysis methods

Livers and muscles were dissected for metabolic and POP analysis. Organochlorine compounds (OC), lindane (γ-HCH), dieldrin, *pp*′-DDE, hexachlorobenzene (HCB), 23 PCB congeners and 16 PAH were extracted from liver and muscle lipid fractions, with a chloroform–methanol solution. PAH were also extracted from bile. Purification procedures were performed by means of solid phase extraction (SPE) on Florisil column according to the EPA method 3620 (Bond Elut Florisil, 1 g, 200 μM particle size – Varian). OC analyses were performed by gas chromatography with AutoSystem XL (Perkin–Elmer), using electron capture detection (ECD $^{63}$Ni Source) and nitrogen as the carrier gas following an adapted procedure of the EPA Method 8081a. The detection limit ranged from 0.05 to 0.20 g kg$^{-1}$ in fish tissues.

The identification and quantification of the 16 priority PAH (naphtalene, acenaphtylene, acenaphtene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(*a*)-anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, indeno(1,2,3-*cd*)pyrene, benzo (*ghi*)perylene, dibenzo(*ah*)anthracene) were carried out by gas chromatography–mass spectrometry (GC/MS) using an electronic impact ionization detector (EI).

### 2.3. Biochemical methods

In liver and muscle, lipid content was estimated using a chloroform–methanol extraction (Folch et al., 1957) and protein content was assessed using the Lowry method

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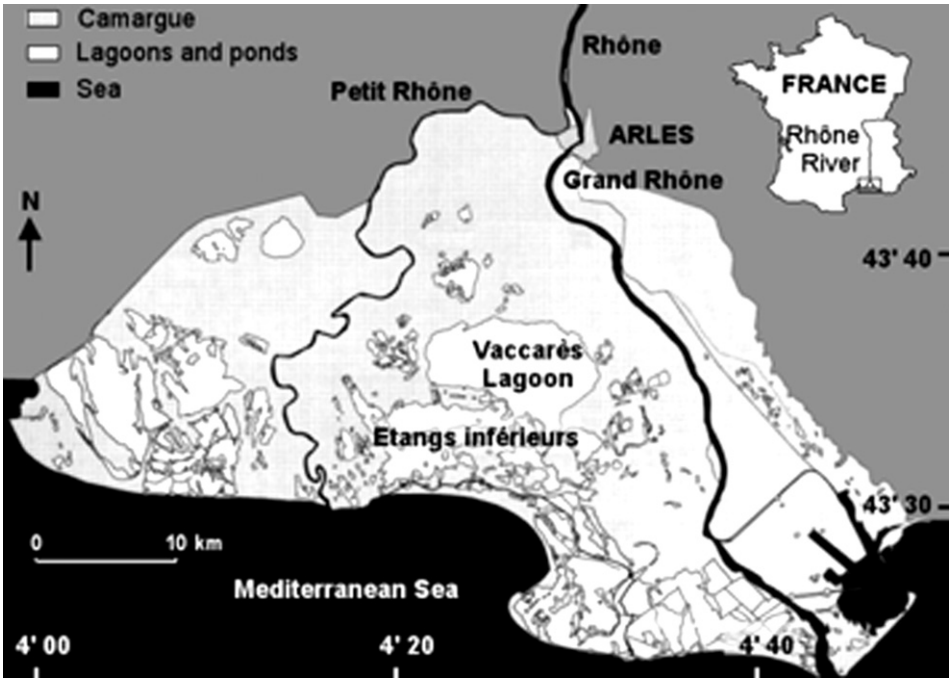


Fig. 1. Study area: the Vaccarès lagoon in the Rhône Delta (Camargue – South of France).

(Lowry et al., 1951). Lipidic phosphorus was estimated by spectrophotometry (Bartlett, 1959). The glucose released after glycogen enzymatic hydrolysis (amyloglucosidase) was measured using glucose oxidase, according to a method adapted from Hugget and Nixon (1957) using ABTS as chromogen. The EROD activity was measured in the microsomal fractions of liver using a spectrofluorometric method of Burke and Mayer (1974). The GST activity in cytosol was assessed by monitoring the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) according to Habig et al. (1974). The total SOD activity was determined according to the modified method of Misra and Fridovich (1972) (Buet, 2002), based on the oxidation of epinephrine to adrenochrome. The catalase activity was evaluated using the hydrogen peroxide breakdown method (Aebi, 1984). The GPx activities were measured as described by Tappel (1978), using cumene hydroperoxide or hydrogen peroxide as the substrate. ATPases activities were measured in gill and muscle by a method described by Schmitz et al. (1973). The Na,K-ATPases were distinguished using the ouabain-resistant activity. The method of Ellman et al. (1961) was used to measure AChE activity in brain and muscle. All enzyme activities were measured in kinetic with at least two replicates (four replicates for SOD).

### 2.4. Data analysis

Inter-age variability was compared using one-way analysis of variance (ANOVA) followed by Scheffé's and Bonferroni/Dunn post hoc tests and paired comparisons were used to check differences between muscular and hepatic concentrations. Pearson correlations between contamination level and biological data were calculated. All tests were regarded as statistically significant when $p < 0.05$.

## 3. Results

### 3.1. Contaminant contents in tissues

All the investigated POP were found in tissue of eels from Vaccarès lagoon. Concentrations showed a high variability (Table 1). The PCB represented the most concentrated OC compounds in liver and muscle, in both yellow and juvenile eels. These pollutants exhibited the single significant inter-age variation of OC: PCB contents in muscle were slightly higher in juvenile eels than in yellow ones ($p < 0.04$). The average concentrations (geometric mean) were upper to $14 \times 10^3$ ng g$^{-1}$ in liver and upper to $4 \times 10^3$ ng g$^{-1}$ in muscle. The maximum PCB content was observed in the liver of a juvenile eel ($691 \times 10^3$ ng g$^{-1}$ dry weight).

The most common OC pesticide found in the hepatic tissue was lindane, nevertheless the concentrations of the other banned substances (dieldrin, HCB and *pp*′-DDE) were often high, notably the *pp*′-DDE in liver of yellows. In France, lindane use was prohibited since July 1998 (the year before our first fishing). However, more recent fish analyses showed that the concentrations of this insecticide, previously used in the bordering rice fields, decreased in liver, but increased in muscle despite the new regulations (Oliveira Ribeiro et al., 2005).

Except for HCB, OC contents were lower in muscle than in liver. The maximum HCB content ($18 \times 10^3$ ng g$^{-1}$) was measured in the muscle of a juvenile eel. Quantifiable levels

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of prohibited pesticides OC were found in more than 80% of the eels. PCB were detected in 94% of the liver samples and in all the muscle samples (Table 2).

The most concentrated PAHs in muscle and liver were phenanthrene, naphtalene and anthracene. The last mentioned was mostly found in juveniles. In bile, only phenanthrene shown contents higher than $100 \times 10^3$ ng $g^{-1}$. A study carried out in 2003 (Oliveira Ribeiro et al., 2005) showed that the PAH contamination profile in bile was approximately the same.

The inter-individual variations were considerable. For example, fluoranthene was undetectable in some samples and up to 5 μg $g^{-1}$ in muscle and bile of some other eels. Tissues concentrations of the heaviest molecules (from benzo(*a*)anthracene to benzo(*ghi*)perylene in Table 1) were generally low and often undetectable. Few inter-age variations were noted, except the muscle concentration of phenanthrene and benzo(*a*)anthracene ($p = 0.040$ and $p = 0.046$ respectively). All the livers were contaminated with phenanthrene and more than 93% with anthracene (Table 2). On the contrary, in the few tested yellows, none of the heaviest PAH molecules (benzo(*b*)fluoranthene, indeno(1,2,3-*cd*) pyrene, dibenzo(*a*)anthracene and benzo(*ghi*)perylene) were detected in hepatic lipids. Even if the contamination

Table 1
Organochlorines (pesticides and PCB) and PAHs concentrations in eels (juveniles and yellows) from the Vaccarès lagoon

| | Liver ng $g^{-1}$ dry weight | | Muscle ng $g^{-1}$ dry weight | | Bile ng $g^{-1}$ | |
|---|---|---|---|---|---|---|
| | Juveniles | Yellows | Juvenile | Yellows | Juveniles | Yellows |
| *Organochlorine compounds* | | | | | | |
| Lindane | 279 (53) [nd–7831] | 234 (36) [9.2–3181] | 67.6 (53) [nd–7841] | 91.9 (45) [1.4–1846] | nm | nm |
| Dieldrin | 70.1 (55) [nd–3579] | 14.4 (37) [nd–1371] | 10.5 (53) [nd–2714] | 6.6 (45) [nd–799] | nm | nm |
| HCB | 12.7 (43) [nd–2749] | 3.3 (39) [nd–2912] | 67.4 (42) [nd–17909] | 38.8 (40) [nd–8317] | nm | nm |
| *pp′*-DDE | 142 (43) [nd–57727] | 303 (36) [0.3–19885] | 21.7 (40) [nd–7755] | 18.9 (39) [nd–3713] | nm | nm |
| ∑PCB | 17012 (41) [459–691696] | 14799 (36) [308–381227] | 5122 (41) [80–195347] | 4249 (40)* [701–69628] | nm | nm |
| *PAH* | | | | | | |
| Naphtalene | 229 (12) [0.20–27467] | 406 (16) [nd–31427] | 726 (9) [8.85–18184] | 167 (9) [6.46–15301] | 6.12 (28) [0.04–437] | 5.98 (29) [0.14–223] |
| Acenaphtylene | 0.64 (12) [nd–3246] | 18.9 (11) [nd–4129] | 196 (11) [23.7–923] | 35.1 (9) [5.03–836] | 4.29 (29) [nd–711] | 6.92 (22) [nd–265] |
| Acenaphtene | 0.25 (11) [nd–8132] | 0.85 (11) [nd–10048] | 4.71 (11) [0.02–2076] | 0.12 (8) [nd–551] | 37.0 (29) [0.25–4054] | 21.6 (22) [nd–1716] |
| Fluorene | 0.34 (12) [nd–1172] | 0.86 (15) [nd–88] | 0.71 (11) [0.01–45] | 0.07 (9) [nd–66] | 15.1 (28) [0.25–762] | 22.0 (28) [nd–861] |
| Phenanthrene | 422 (11) [17.4–2147] | 508 (16) [50.3–3233] | 540.4 (11) [7.55–6169] | 79.3 (9)* [8.31–2035] | 241 (28) [20.9–8730] | 194 (29) [3.99–10002] |
| Anthracene | 227 (11) [33.7–1878] | 85.9 (16) [0.05–705] | 77.7 (10) [5.67–403] | 19.8 (9) [3.16–1031] | 23.9 (29) [1.42–337] | 15.7 (22) [0.61–402] |
| Fluoranthene | 0.12 (12) [nd–1285] | 33.7 (16) [nd–1020] | 297 (11) [5.77–5403] | 8.42 (9) [nd–1659] | 21.4 (28) [0.25–5844] | 31.8 (28) [nd–5365] |
| Pyrene | 26.7 (12) [0.20–939] | 24.4 (15) [0.03–931] | 122 (11) [0.01–3640] | 2.80 (9) [nd–589] | 9.72 (28) [nd–626] | 14.7 (28) [0.25–871] |
| Benzo(*a*)anthracene | 45.7 (12) [0.12–1258] | 21.5 (15) [0.03–3324] | 9.80 (11) [nd–2075] | 0.49 (9) [nd–123] | 22.5 (29) [1.45–800] | 14.4 (22) [1.07–968] |
| Chrysene | 15.7 (12) [nd–989] | 16.4 (15) [0.03–769] | 5.23 (11) [nd–910] | 0.43 (9) [nd–75] | 61.6 (28) [5.16–975] | 32.5 (28) [1.7–974] |
| Benzo(*b*)fluoranthene | nd | 0.03 (7) [nd–129] | 1.41 (11) [nd–1012] | 0.02 (9) [nd–129] | 5.09 (28) [nd–629] | 20.5 (21) [nd–572] |
| Benzo(*k*)fluoranthene | 0.71 (5) [0.12–611] | 0.43 (7) [0.01–107] | 1.29 (11) [nd–684] | 0.02 (9) [nd–95] | 1.86 (29) [nd–322] | 12.7 (22) [nd–919] |
| Benzo(*a*)pyrene | 25.2 (12) [0.12–1727] | 1.31 (16) [nd–1748] | 0.36 (11) [nd–374] | 0.01 (9)* [nd–114] | 1.05 (29) [nd–150] | 5.78 (22) [nd–1716] |
| Indeno(1,2,3*cd*) pyrene | nd | 0.012 (7) [nd–117] | 0.37 (11) [nd–1268] | 0.01 (9) [nd–317] | 0.34 (29) [nd–113] | 1.27 (21) [nd–100] |
| Dibenzo(*a*)anthracene | nd | 0.015 (7) [nd–569] | 0.18 (11) [nd–1670] | 0.05 (9) [nd–322] | 0.0005 (28) [nd–8] | 0.001 (22) [nd–38] |
| Benzo(*ghi*)perylene | nd | 0.010 (7) [nd–43] | 0.63 (10) [nd–2216] | 0.03 (9) [nd–1724] | 0.10 (29) [nd–184] | 0.19 (22) [nd–252] |

Geometric mean; (*n*): number of samples; [min value–max value].
Significant difference juveniles vs yellows $^*p < 0.05$; nd: not detected; nm: not measured.

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Table 2
Percent of individuals contaminated by POP

| Analysed individuals | Total | % Contaminated | Sum juveniles | % Contaminated | Sum yellows | % Contaminated |
|---|---|---|---|---|---|---|
| *Bile impregnation* | | | | | | |
| Naphtalene | 52 | 91.2 | 27 | 93.1 | 25 | 89.3 |
| Acenaphtylene | 51 | 80.4 | 22 | 86.4 | 29 | 75.9 |
| Acenaphtene | 51 | 82.4 | 22 | 86.4 | 29 | 79.3 |
| Fluorene | 56 | 82.1 | 28 | 89.3 | 28 | 75.0 |
| Phenanthrene | 57 | 100.0 | 29 | 100.0 | 28 | 100.0 |
| Anthracene | 51 | 100.0 | 22 | 100.0 | 29 | 100.0 |
| Fluoranthene | 56 | 83.9 | 28 | 89.3 | 28 | 78.6 |
| Pyrene | 56 | 85.7 | 28 | 92.9 | 28 | 78.6 |
| Benzo(*a*)anthracene | 51 | 100.0 | 22 | 100.0 | 29 | 100.0 |
| Chrysene | 56 | 100.0 | 28 | 100.0 | 28 | 100.0 |
| Benzo(*b*)fluoranthene | 49 | 79.6 | 21 | 85.7 | 28 | 75.0 |
| Benzo(*k*)fluoranthene | 51 | 76.5 | 22 | 86.4 | 29 | 69.0 |
| Benzo(*a*)pyrene | 51 | 78.4 | 22 | 86.4 | 29 | 72.4 |
| Indeno(1,2,3*cd*)pyrene | 50 | 68.0 | 21 | 76.2 | 29 | 62.1 |
| Dibenzo(*a*)anthracene | 50 | 10.0 | 22 | 13.6 | 28 | 7.1 |
| Benzo(*ghi*)perylene | 51 | 47.1 | 22 | 50.0 | 29 | 44.8 |
| *Liver impregnation* | | | | | | |
| Lindane | 93 | 93.5 | 56 | 91.1 | 37 | 97.3 |
| Dieldrin | 93 | 89.2 | 56 | 92.9 | 37 | 83.8 |
| HCB | 83 | 66.3 | 44 | 72.7 | 39 | 59.0 |
| *pp′*-DDE | 83 | 94.0 | 44 | 95.5 | 39 | 92.3 |
| ∑PCB | 80 | 96.3 | 42 | 97.6 | 38 | 94.7 |
| Naphtalene | 28 | 92.9 | 16 | 93.8 | 12 | 91.7 |
| Acenaphtylene | 23 | 56.5 | 11 | 72.7 | 12 | 41.7 |
| Acenaphtene | 22 | 27.3 | 11 | 36.4 | 11 | 18.2 |
| Fluorene | 27 | 40.7 | 15 | 53.3 | 12 | 25.0 |
| Phenanthrene | 27 | 100.0 | 16 | 100.0 | 11 | 100.0 |
| Anthracene | 27 | 96.3 | 16 | 93.8 | 11 | 100.0 |
| Fluoranthene | 28 | 67.9 | 16 | 87.5 | 12 | 41.7 |
| Pyrene | 27 | 74.1 | 15 | 80.0 | 12 | 66.7 |
| Benzo(*a*)anthracene | 27 | 74.1 | 15 | 73.3 | 12 | 75.0 |
| Chrysene | 27 | 74.1 | 15 | 73.3 | 12 | 75.0 |
| Benzo(*b*)fluoranthene | 12 | 16.7 | 7 | 28.6 | 5 | 0.0 |
| Benzo(*k*)fluoranthene | 12 | 25.0 | 7 | 28.6 | 5 | 20.0 |
| Benzo(*a*)pyrene | 28 | 53.6 | 16 | 43.8 | 12 | 66.7 |
| Indeno(1,2,3*cd*)pyrene | 12 | 16.7 | 7 | 28.6 | 5 | 0.0 |
| Dibenzo(*a*)anthracene | 12 | 16.7 | 7 | 28.6 | 5 | 0.0 |
| Benzo(*ghi*)perylene | 12 | 16.7 | 7 | 28.6 | 5 | 0.0 |
| *Muscle impregnation* | | | | | | |
| Lindane | 99 | 96.0 | 54 | 92.6 | 45 | 100.0 |
| Dieldrin | 99 | 88.9 | 54 | 88.9 | 45 | 88.9 |
| HCB | 88 | 85.2 | 45 | 88.9 | 43 | 81.4 |
| *pp′*-DDE | 81 | 79.0 | 41 | 78.0 | 40 | 80.0 |
| ∑PCB | 81 | 100.0 | 41 | 100.0 | 40 | 100.0 |
| Naphtalene | 18 | 100.0 | 9 | 100.0 | 9 | 100.0 |
| Acenaphtylene | 20 | 100.0 | 9 | 100.0 | 11 | 100.0 |
| Acenaphtene | 19 | 52.6 | 8 | 25.0 | 11 | 72.7 |
| Fluorene | 20 | 65.0 | 9 | 55.6 | 11 | 72.7 |
| Phenanthrene | 20 | 100.0 | 9 | 100.0 | 11 | 100.0 |
| Anthracene | 19 | 100.0 | 9 | 100.0 | 10 | 100.0 |
| Fluoranthene | 20 | 90.0 | 9 | 77.8 | 11 | 100.0 |
| Pyrene | 20 | 80.0 | 9 | 66.7 | 11 | 90.9 |
| Benzo(*a*)anthracene | 20 | 60.0 | 9 | 55.6 | 11 | 63.6 |
| Chrysene | 20 | 60.0 | 9 | 55.6 | 11 | 63.6 |
| Benzo(*b*)fluoranthene | 20 | 45.0 | 9 | 22.2 | 11 | 63.6 |
| Benzo(*k*)fluoranthene | 20 | 45.0 | 9 | 22.2 | 11 | 63.6 |
| Benzo(*a*)pyrene | 20 | 40.0 | 9 | 22.2 | 11 | 54.5 |
| Indeno(1,2,3*cd*)pyrene | 20 | 35.0 | 9 | 11.1 | 11 | 54.5 |
| Dibenzo(*a*)anthracene | 20 | 35.0 | 9 | 33.3 | 11 | 36.4 |
| Benzo(*ghi*)perylene | 19 | 36.8 | 9 | 22.2 | 10 | 50.0 |

Variation as a function of the age. The eels are considered as 'contaminated' when the concentrations of pollutants are upper to the confidence limit of detection (i.e. 0.05 ng $g^{-1}$ and 0.2 ng $g^{-1}$ for OC and PAH respectively).

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level was lower in muscle, naphthalene and 3-rings PAH, such as acenaphtylene, phenanthrene and anthracene, were detected in all the eels. These two last PAH were also systematically found in bile, as well as benzo(*a*)anthracene and chrysene.

### 3.2. Relationships between contaminant contents and metabolic markers

Few significant correlations were detected between biliary micro-contaminant concentrations (Fig. 2) and metabolic markers (Table 3).

In muscle, glycogen showed a great number of correlations with low molecular weight PAH (less than 230 g $mol^{-1}$). However, most frequent correlations concern the muscular protein rate and PAH and PCB concentrations, especially in muscle. Only three relations were pointed out between the hepatic EROD activity and the contamination levels. They involved the concentrations of fluoranthene and dibenzo(*ah*)anthracene in bile and HCB in liver. On the other hand, hepatic GST seemed to be activated by the presence of naphthalene, anthracene, indeno(1,2,3-*cd*)perylene and dibenzo(*ah*)anthracene in muscle, despite the low abundance of these PAH. Only two significant relationships were observed between UDPGT activity and micro-pollutants, a negative one with hepatic fluoranthene and a positive one with hepatic HCB. Antioxidant responses were numerous but complex and variable in nature and intensity. Indeed, hepatic peroxydases (GPx, Se-GPx and catalase) appeared to be inhibited by anthracene and benzo(*a*)anthracene in bile, and by chrysene, benzo(*a*)pyrene and/or lindane in liver. At the same time, this group of enzymes, notably catalase, was activated by some low molecular weight PAH (naphthalene, phenanthrene, fluoranthene, pyrene) and OC compounds (PCB in liver and muscle, *pp′*-DDE in muscle). Statistical relationships between micro-contaminant concentrations and total hepatic SOD activity were unexpected. These correlations were mainly negative (PAH in bile, acenaphtylene and fluoranthene in liver, lindane in liver and muscle). The positive correlations were uncommon, when noted, they concerned primarily PCB. Among enzymatic activities in erythrocytes, GPx and Se-GPx were correlated with many tissue POP impregnation. Se-GPx activity was significantly correlated with all hepatic and muscular OC (except muscle HCB). These correlations were either negative (*pp′*-DDE and ∑PCB), or positive (lindane, dieldrin, HCB). Blood GPx activity also was correlated with biliary concentrations of phenanthrene, pyrene, chrysene and benzo(*b*)fluoranthene.

The relationships between contaminant contents and lipidic components were investigated. Actually, in liver and muscle, xenobiotic concentrations were seldom correlated with neutral lipid burdens (Table 4), except for dieldrin and benzo(*a*)anthracene. Nevertheless, in muscle, significant correlations were observed between some PAH or OC concentrations and phospholipid rates. These primarily concerned lower molecular weight PAH (3-and 4-aromatic rings plus benzo(*b*)fluoranthene and benzo(*b*)-fluoranthene), that are less likely to undergo biotransformation processes (Sundberg et al., 2005).

Many positive correlations were noted between membrane enzymes activities (ATPases and AChE) and PAH

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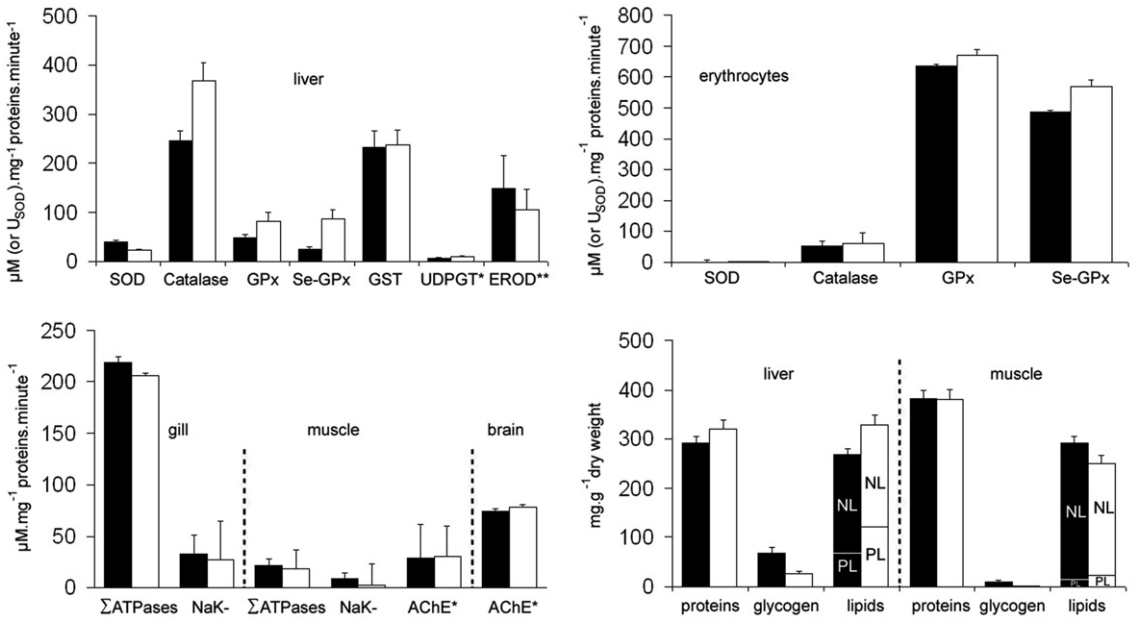


Fig. 2. Enzymes activities in liver, gills, muscles, brain and erythrocytes of eels from the Vaccarès lagoon and metabolic components in liver and muscles. ■ yellows, □ juveniles. Enzymatic activities are expressed in μM $mg^{-1}$ proteins except: $U_{SOD}$ (1U = 50%inhibition of the reaction); ∗ ng $mg^{-1}$ proteins; ∗∗ pg $mg^{-1}$ proteins. Other values are in ng $g^{-1}$ dry weight. PL = phospholipids; LN = neutral lipids. *n* = number of analyses; hepatic enzymes $n > 55$ except UDPGT and EROD $19 > n > 25$; erythrocytes enzymes $44 > n > 48$; ATPases $n > 50$; AChE $29 > n > 49$.

Table 3
Significative Pearson correlations between tissue concentration (in bile, liver and muscle) of PAH and OC, and potential biomarkers, metabolic parameters, tissue composition and activities of detoxification enzymes, in eels from Vaccarès

| Concentrations | Tissue composition | | | | Enzymes activities | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glycogen | | Proteins | | Liver | | | | | | | Erythrocytes | | | |
| | Liver | Muscle | Liver | Muscle | GPx | Se-GPx | Catalase | SODs | EROD | UDPGT | GST | GPx | Se-GPx | Catalase | SODs |
| *Bile (50 < n < 57)* | | | | | | | | | | | | | | | |
| Acenaphtylene | | | | | | | | 0.006(−) | | | | | | | 0.016 |
| Acenaphtene | | 0.0002 | | | | | | 0.017(−) | | | | | | | |
| Phenanthrene | | | | | | | | | | | | 0.043 | | | |
| Anthracene | | 0.003 | | 0.030 | 0.047(−) | | | | | | | | | | |
| Fluoranthene | 0.043 | | | | | | | | 0.0042 | | | | | | |
| Pyrene | | | | | | | | | | | | 0.010 | | | |
| Benzo(*a*)anthracene | | 0.0001 | 0.003 | 0.003 | | | 0.029(−) | | | | | | | | |
| Chrysene | | | 0.037 | | | | | | | | | 0.004 | 0.056 | | |
| Benzo(*b*)fluoranthene | | | | | | | | 0.006(−) | | | | 0.049 | | | |
| Benzo(*k*)fluoranthene | | | | | | | | 0.019(−) | | | | | | | |
| Indeno(1,2,3*cd*)pyrene | | | | | | | | 0.031(−) | | | | | | | |
| Dibenzo(*ah*)anthracene | | | | | | | | | 0.0007 | | | | | | |
| Benzo(*ghi*)perylene | | | | | | | | | | | 0.030 | | | | |
| *Liver (n = 28)* | | | | | | | | | | | | | | | |
| Naphtalene | | | 0.011 | 0.035 | | | 0.033 | | nm | | | nm | nm | nm | nm |
| Acenaphtylene | 0.016 | | | | 0.015 | | | 0.001(−) | nm | | | nm | nm | nm | nm |
| Phenanthrene | | | 0.025 | 0.046 | | | 0.005 | | nm | | | nm | nm | nm | nm |
| Anthracene | | | | | 0.0005 | | | | nm | | | nm | nm | nm | nm |
| Fluoranthene | | 0.016 | | | | | 0.0001 | 0.037(−) | nm | 0.015(−) | | nm | nm | nm | nm |
| Pyrene | | | | | | | 0.019 | | nm | | | nm | nm | nm | nm |
| Chrysene | | | | | 0.013(−) | | | | nm | | | nm | nm | nm | nm |
| Benzo(*b*)fluoranthene | | | 0.002 | 0.003 | | | 0.037 | 0.002 | nm | | <0.0001 | nm | nm | nm | nm |
| Benzo(*k*)fluoranthene | | | | | | | | | nm | | | nm | nm | nm | nm |
| Benzo(*a*)pyrene | | | | 0.040 | 0.040(−) | | | | nm | | | nm | nm | nm | nm |
| Indeno(1,2,3*cd*)pyrene | | | 0.001 | 0.046 | | | | | nm | | | nm | nm | nm | nm |
| Dibenzo(*ah*)anthracene | | | 0.001 | 0.002 | | | | | nm | | | nm | nm | nm | nm |
| Benzo(*ghi*)perylene | | | | | 0.033 | | | | nm | | | nm | nm | nm | nm |
| HCB | | | | 0.006 | 0.017 | | | | 0.0007 | | <0.0001 | | 0.006 | | |
| Lindane | | | 0.031 | | | 0.022 | 0.030(−) | 0.002(−) | | 0.0012 | 0.033 | | 0.009 | | 0.032(−) |
| Dieldrin | | | 0.024 | | | <0.0001 | | | | | | | 0.0005 | | |
| *pp*′-DDE | | | 0.0005(−) | | | | | | | | | | 0.036(−) | | |
| ∑PCB | | | 0.0004(−) | 0.019 | | | <0.0001 | 0.009 | | | | | 0.028(−) | | |
| *Muscle (n = 20)* | | | | | | | | | | | | | | | |
| Naphthalene | | <0.0001 | | <0.0001 | 0.048 | 0.039 | 0.006 | 0.026 | nm | | 0.0031 | nm | nm | nm | nm |
| Acenaphtylene | | 0.0001 | | 0.0006 | | | | | nm | | | nm | nm | nm | nm |
| Phenanthrene | | 0.014 | 0.008 | <0.0001 | 0.011 | 0.032 | 0.037 | 0.016 | nm | | | nm | nm | nm | nm |
| Anthracene | | <0.0001 | | | | | 0.021 | | nm | | <0.0001 | nm | nm | nm | nm |
| Fluoranthene | | <0.0001 | | <0.0001 | | | 0.040 | | nm | | | nm | nm | nm | nm |

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| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pyrene | <0.0001 | | <0.0001 | | 0.042 | | nm | | | nm | nm | nm | nm |
| Benzo(*a*)anthracene | <0.0001 | | <0.0001 | | | | nm | | | nm | nm | nm | nm |
| Chrysene | <0.0001 | | 0.001 | | 0.030 | | nm | | | nm | nm | nm | nm |
| Benzo(*b*)fluoranthene | | 0.0002 | 0.002 | | | | nm | | | nm | nm | nm | nm |
| Benzo(*k*)fluoranthene | | 0.005 | 0.001 | | | | nm | | | nm | nm | nm | nm |
| Benzo(*a*)pyrene | | | 0.008 | | | | nm | | | nm | nm | nm | nm |
| Indeno(1,2,3*cd*)pyrene | | | 0.036 | <0.0001 | | 0.032 | nm | | 0.022 | nm | nm | nm | nm |
| Dibenzo(*ah*)anthracene | | | | <0.0001 | | | nm | | 0.004 | nm | nm | nm | nm |
| Benzo(*ghi*)perylene | 0.048 | 0.001 | 0.007 | | 0.0002 | 0.006 | nm | | | nm | nm | nm | nm |
| HCB | | | | | | | | | 0.019 | <0.0001 | 0.022 | | |
| Lindane | 0.0020 | | 0.046 | | 0.0224 | 0.027 | 0.009(−) | | | 0.005 | <0.0001 | | |
| Dieldrin | | | 0.017(−) | | <0.0001 | | | | | | | | |
| *pp*′-DDE | | 0.001(−) | 0.007(−) | | | <0.0001 | | | | | 0.036(−) | | |
| ∑PCB | | 0.007(−) | 0.005(−) | | | <0.0001 | 0.0015 | | | | 0.010(−) | | <0.0001 |

*p* Value; (−) negative coefficient; nm: not measured.

concentrations, indicating a possible unspecific enzymatic activation. Nevertheless, the response of these enzymes depended on the location of the contamination in eel tissues (Table 5). In the present study, the AChE activity of muscle appeared to be a good marker for PAH contamination in bile and in muscle (for example pyrene and fluoranthene $p < 0.0005$), when muscle ATPase activities and gill Na,K-ATPase were more significant for heavier PAH in liver. Therefore, bile concentrations of PAH containing 5 or less aromatic rings, except benzo(*a*)anthracene and benzo(*a*)pyrene showed strong positive relations with muscular AChE activity. Many correlations were found between muscle AChE activity and muscle PAH concentrations, although few correlations were found with hepatic contaminants. The links between the brain AChE and PAH contents in muscle and liver were not tested, but no significant correlations were found with bile concentrations. Conversely, brain AChE was negatively correlated with lindane and dieldrin in the liver and positively correlated with muscle PCB concentrations.

In gill, the total ATPases activity increased with the level of pyrene, benzo(*k*)fluoranthene and benzo(*a*)pyrene in the bile and with naphthalene, phenanthrene and fluoranthene in liver. In addition, interesting correlations concerned the gill Na,K-ATPases and the three heaviest PAH molecules (dibenzo(*ah*)anthracene, indeno(*cd*)pyrene and benzo(*ghi*)perylene), the HCB and the PCB in muscle. On the other hand, the activity of gill ouabain-resistant ATPases (Na,K-ATPases) increased with the increasing concentrations of fluorene, pyrene, benzo(*k*)fluoranthene and benzo(*a*)pyrene in bile; HCB and PCB in muscle and, in a very significant manner, the three heaviest PAH molecules in liver. The muscle ATPases (total and Na,K-) showed significant positive correlations with hepatic contaminants as chrysene and hydrocarbons containing 5 or 6 rings. Furthermore, pyrene, chrysene dibenzo(ah)anthracene in bile, dibenzo(ah)anthracene and indeno(1,2,3-*cd*)pyrene in muscle, seemed to stimulate only the muscle $Na^+$,$K^+$-ATPases activity. Surprisingly, correlations of muscle ATPases with OC concentrations were missing.

## 4. Discussion

The various aquatic biotopes of the NRC are exposed to xenobiotics coming from atmospheric transfer, runoff, leaching and drainage of surrounding agricultural fields. Fish populations living in this protected area are contaminated by two distinct ways: a direct transfer from the water column via the gills and the skin (bioconcentration) and a trophic transfer by ingestion of contaminated food (biomagnification). Bioaccumulation of xenobiotics, resulting of the addition of these two previous processes, depends on the ability of organisms to metabolize and eliminate these exogenous compounds.

In a previous work (Oliveira Ribeiro et al., 2005), eels sampled in various sites in the Vaccarès lagoon exhibited high levels of POP and metal contaminations, and

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Table 4
Relationship between tissue concentration of POP and lipid constituents in liver and muscle of eels from Vaccarès

| | Hepatic concentration ($n = 28$) | | | Muscular concentration ($n = 20$) | | |
|---|---|---|---|---|---|---|
| | Total lipids | Neutral lipids | Phospholipids | Total lipids | Neutral lipids | Phospholipids |
| Naphtalene | | | | | | |
| Acenaphtylene | | | | | | 0.0045 |
| Acenaphtene | | | | | | |
| Fluorene | | | | | | |
| Phenanthrene | | | | | | 0.012 |
| Anthracene | | | | | | |
| Fluoranthene | | | | | | 0.015 |
| Pyrene | | | | | | 0.014 |
| Benzo(*a*)anthracene | 0.003 | 0.012 | | | 0.012 | 0.008 |
| Chrysene | | | | | | 0.043 |
| Benzo(*b*)fluoranthene | | | | | | 0.030 |
| Benzo(*k*)fluoranthene | | | | | | 0.034 |
| Benzo(*a*)pyrene | | | | | | |
| Dibenzo(*ah*)anthracene | | | | | | |
| Indeno(*cd*)pyrene | | | | | | |
| Benzo(*ghi*)perylene | | | 0.049 | | | |
| | Hepatic concentration ($77 < n < 91$) | | | Muscular concentration ($78 < n < 97$) | | |
| HCB | | | | | | |
| ∑PCB | | | | | | |
| Lindane | | | | | | <0.0001 |
| Dieldrin | <0.0001 | 0.003 | 0.0003 | | | <0.0001 |
| *pp*′-DDE | | | | | | |

Pearson $p$ value ($n$): number of pairs.

histopathological lesions in liver, spleen and gills, indicating chronic effects of the pollutants. Some of these damages were characterized by the presence of tumors in both liver and spleen.

The main objective of the experimental approach developed in the present study, was to measure suitable biomarkers, which are useful in situ. This includes the evaluation of defense mechanisms (physiological or compensatory biochemical responses), biodegradation or biotransformation processes and chemical analysis of contaminants in eel tissues. This biomonitoring program required the assessment of numerous eco-physiological parameters involved in various metabolic processes (energy, oxidative stress, biotransformation, neuronal conduction, ...). The contamination of the Vaccarès fish community was confirmed by the detection of 16 priority PAH and organochlorine residues in eel tissues. The acceptable PAH concentrations in marine organisms were estimated by the AFSSA (French Agency of Medical Security of Food) after the wreck of the Erika tanker off the French coasts. As part of this evaluation, Narbonne et al. (2000). recommend threshold values of 0.5 mg $kg^{-1}$ of dry weight for the 16 prioritary PAHs in bivalves. In 90–100% of tested eels, the selected micro-pollutants were detected in the liver, the muscle and/or the gall bladder, except the heavy PAH (more than four aromatic rings). The large intraspecific variation found was analogous to that from other European regions and may be explained by uptake, age, sex and individual metabolic response to detoxification (Van der Oost et al., 2003; Vives et al., 2005).

In addition to the ecotoxicological impact related to an exposure to these xenobiotics, eel metabolism depends on temporal variations of biotic (age, maturity, nutritional state, reproduction, migration, ...) and abiotic factors (temperature, salinity, oxygen rate, ...). This implies a great diversity of responses. Moreover, the hepatic enzymatic activities involved in detoxification processes (biotransformation and antioxydant defense) depend on season, site of capture and abiotic factors (Förlin et al., 1995).

Ecotoxicological studies carried out in the lentic ecosystems of NRC imply the absence of a 'control fish population'. Indeed, in this protected area, no biotope can be classified as non-exposed because of the permanent input of pollutants. That is why the use of comparative tests is limited. Consequently, the data interpretation was achieved by statistical analysis. Various relationships between biological responses and the intensity of the contamination have been exhibited, as well as specific biomarkers. However, these biomarkers do not provide information about the amplitude of the global contamination. Several correlations were noted. Their significance seems to be related to the molecular weight of the contaminants (especially for PAH) and to their physico-chemical characteristics (i.e. $\log K_{ow}$) as described by de Maagd et al. (1997). The heaviest molecules are the most easily and quickly metabolized.

Glycogen is a significant marker of energetic reserves and numerous positive correlations were observed with organochlorine compounds and volatile PAH. Thus, the increase in the glycogen contents should be used as a bio-

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Table 5
Significative Pearson correlations between membrane enzyme activities and organic micro-pollutants in bile, liver and muscle of eels from the Vaccarès lagoon

| | Gill ATPases | | Muscle ATPases | | Muscle | Brain |
|---|---|---|---|---|---|---|
| | Total | NaK | Total | NaK | AChE | AChE |
| *Bile impregnation ($29 < n < 36$)* | | | | | | |
| Naphtalene | | | | | 0.043 | |
| Acenaphtylene | | | | | 0.033 | |
| Acenaphtene | | | | | 0.034 | |
| Fluorene | | 0.013 | | | 0.008 | |
| Phenanthrene | | | | | 0.007 | |
| Anthracene | | | | | 0.012 | |
| Fluoranthene | | | | | 0.005 | |
| Pyrene | 0.039 | 0.0003 | | 0.004 | 0.0003 | |
| Benzo(*a*)anthracene | | | | | | |
| Chrysene | | | | 0.002 | 0.001 | |
| Benzo(*b*)fluoranthene | | | | | 0.001 | |
| Benzo(*k*)fluoranthene | 0.0004 | <0.0001 | | | 0.008 | |
| Benzo(*a*)pyrene | <0.0001 | <0.0001 | | 0.047 | | |
| Dibenzo(*ah*)anthracene | | | | | | |
| Indeno(*cd*)pyrene | | | | | | |
| Benzo(*ghi*)perylene | | | | | | |
| *Liver impregnation (PAH $n = 8$; OC $n > 70$)* | | | | | | |
| Naphtalene | 0.026 | | | | | nm |
| Acenaphtene | | | | | | nm |
| Acenaphtylene | | | | | | nm |
| Fluorene | | | | | | nm |
| Phenanthrene | 0.036 | | | | | nm |
| Anthracene | | | | | | nm |
| Fluoranthene | <0.0001 | | | | | nm |
| Pyrene | | | | | | nm |
| Benzo(*a*)anthracene | | | | | | nm |
| Chrysene | | | 0.003 | 0.007 | | nm |
| Benzo(*b*)fluoranthene | | | | | | nm |
| Benzo(*k*)fluoranthene | | | | | | nm |
| Benzo(*a*)pyrene | | | 0.017 | 0.010 | | nm |
| Dibenzo(*ah*)anthracene | | <0.0001 | 0.0003 | 0.0003 | | nm |
| Indeno(*cd*)pyrene | | <0.0001 | 0.0003 | 0.0003 | | nm |
| Benzo(*ghi*)perylene | | <0.0001 | 0.0003 | 0.0003 | | nm |
| HCB | | | | | | |
| Lindane | | | | | 0.001 | 0.016(−) |
| Dieldrin | | | | | | 0.008(−) |
| *pp′*-DDE | | | | | 0.024(−) | |
| ∑PCB | | | | | | |
| *Muscle impregnation (PAH $n = 15$; OC $n > 70$)* | | | | | | |
| Naphtalene | 0.002 | | | | | nm |
| Acenaphtene | | | | | 0.007 | nm |
| Acenaphtylene | | | | | | nm |
| Fluorene | | | | | | nm |
| Phenanthrene | | | | | 0.028 | nm |
| Anthracene | 0.013 | | | | 0.006 | nm |
| Fluoranthene | | | | | 0.0002 | nm |
| Pyrene | | | | | <0.0001 | nm |
| Benzo(*a*)anthracene | | | | | 0.001 | nm |
| Chrysene | | | | | 0.002 | nm |
| Benzo(*b*)fluoranthene | | | | | | nm |
| Benzo(*k*)fluoranthene | | | | | | nm |
| Benzo(*a*)pyrene | | | | | | nm |
| Dibenzo(*ah*)anthracene | 0.013 | | | 0.0002 | 0.026 | nm |
| Indeno(*cd*)pyrene | 0.043 | | | 0.001 | 0.043 | nm |
| Benzo(*ghi*)perylene | <0.0001 | | | | | nm |
| HCB | 0.0003 | 0.002 | | | | |
| Lindane | | | | | | |
| Dieldrin | | | | | | |
| *pp′*-DDE | | | | | 0.004(−) | |
| ∑PCB | 0.006 | 0.005 | | | 0.001(−) | 0.012 |

*p* Value; $n$ = number of pairs; nm: not measured.

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marker of organic contamination in eel (Roche et al., 2002b). Nevertheless, these results contradict the observations generally described. In most ecotoxicological studies, acute contamination leads to glycogen depletion (Gimeno et al., 1995; Sancho et al., 1998; Strmac and Braunbeck, 1999; Walter et al., 2000). This depletion is probably due to a food aversion and a decrease, often dose-dependent, of the gluconeogenesis induced by the inhibition of key gluconeogenic enzymes (Feeley, 1995; Viluksela et al., 1999). In addition to glycogen and lipid depletion, Braunbeck and Appelbaum (1999) described gut and liver ultrastructural changes at extremely low doses of endosulfan in carp (*Cyprinus carpio*). On the contrary, Thomas et al. (1999) showed a lack of such physiological response in PAH exposed mussels. They suggest that chronic exposure to such contaminants may involve a physiological tolerance in aquatic organisms. In the same way, Oruc and Uner (1998) concluded that, in carp, chemical stress induced an elevation of glycogenolysis while chronic exposure induced compensatory mechanisms. The positive correlations between glycogen content and micro-pollutant concentrations in the NRC eel population tend to prove both a pathological alteration and a general stimulation of the hepatic metabolism.

The muscle proteins content, as glycogen one, increased with the concentrations of contaminants in fish tissue. This may be due to a metabolic effect in relation with the energy state or to a protein–enzyme synthesis in relation with the induction process. Results seem to prove that protein action in the contamination response is mainly due to their role as a tissue component.

The induction of enzymatic defense systems by xenobiotics in the bile is seldom exhibited. These observations are in disagreement with most biomonitoring studies that recommend the use of the EROD activity as a biomarker of chemical exposure in aquatic ecosystems (Livingstone et al., 1993; Van der Oost et al., 1996, 2003). However, the responses of hepatic antioxidant activities are numerous but very different. GPx and catalase are inhibited by some compounds but stimulated, often strongly, by a majority of POP (notably 2–3 rings PAH). Moreover, catalase was very dependent on PCB accumulation and answered by hyperactivity. On the other hand, the hepatic SOD activity seems to be inhibited by liver and gall bladder contamination (except PCB) and activated when pollutants are present in muscle (except lindane). This opposition may be explained by the fact that we analyzed not only the effects on oxidative metabolisms but also the effects on protein synthesis and maturation. In erythrocytes, the responses of antioxidant enzymes did not follow those of hepatic enzymes. Indeed, biliary contaminants affected only GPx activity, and total GPx and Se-GPx were activated by the more polar OC pesticides and inhibited by *pp′*-DDE and PCB.

Due to the lipophilic nature of these xenobiotics, it is accepted that their uptake and bioaccumulation are related to the composition of the storage tissues (Stange and Klungsoyr, 1997). The inclusion of lipophilic compounds in lipid fractions of fish tissue was investigated and described in a previous paper (Roche et al., 2002a). We have demonstrated that non-polar (or lesser polar) PAH and PCB, especially easily degradable PAH (with a high molecular weight), were incorporated in neutral lipid fractions. On the other hand, significant xenobiotic concentrations were detected in polar lipid fractions containing membrane phospholipids. The inclusion of such contaminants in membrane lipid structures induced the selection of enzymatic membrane markers (ATPases and AChE) as potential biomarkers. In the present paper, our results show that the concentrations of POP are seldom correlated with lipidic fraction contents, except the 'medium' molecular weight PAH (3,4,5 rings PAH) with muscle phospholipids. That is why membrane markers, such as the ATPase activity in gill and muscle or AChE in muscle have been measured. The correlation analysis has confirmed the relevance of the use of these enzymatic systems as biomarkers of PAH exposure. Actually, it is well known that the interaction of xenobiotics with biological membranes may lead to an alteration of cell metabolism, due to an action on membrane-bound enzymes or systems (permeability, transport, receptors, ...). The determination of cellular location of xenobiotic accumulation would be a new approach that would provide information on the chronicity of the contamination or on the amplitude of the detoxification processes. The response of membrane enzymes depends on the localization of the contamination in fish tissues. The activity of AChE in muscle appears to be a good marker of the presence of PAH in bile and in muscle. Although OC contaminants often inhibited AChE. ATPases respond to PAH contamination, notably with an high $\log K_{ow}$ as well as naphthalene (the most volatile), anthracene and pyrene. Curiously, few correlations with OC have been observed and none with lindane. However, lindane is structurally similar to inositol-triphosphate and its incorporation in membrane lipid bilayers and the induced disturbance of membrane-bound enzyme activities are now well documented (Antunes-Madeira and Madeira, 1989; Rodriguez-Fuentes and Gold-Bouchot, 2000; Forget et al., 2003).

## 5. Conclusion

In aquatic organisms, the mechanisms involved in detoxification processes are the first defense against xenobiotic exposure effects. However, in the Vaccarès lagoon, the level of eel contamination by organic pollutants does not seem sufficient to induce a response from biotransformation enzymes. Nevertheless, these enzymatic systems are often used in programs monitoring polluted areas. In the present study, gill and muscle ATPases as well as muscle AChE activities appear as potential biomarkers of this chronic contamination. Indeed, their response is related to the incorporation of lipophilic substances in the phospholipidic membrane matrix, whereas the EROD activity

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would be more related to acute chemical stress. On the other hand, the glycogen content is a significant indicator of the state of energy reserves. The significant link observed between energy reserves and the defense enzyme activities would support the use of the glycogen burden as a biomarker. Catalase and glutathione-dependent activities are sensitive to lipophilic contaminants. That is why they should also be considered as potential biomarkers of eel contamination in such protected areas. Moreover, the variations of these parameters depend on both the nature of the storage tissue and the potential of degradation of the xenobiotics. In addition, the markers of membrane integrity seem to be useful as biological indicators of long-term effects. These concern ATPase activities, involved in energy metabolism and osmoregulation process, and AChE, involved in neuronal conduction and known as an indicator of organophosphate and carbamate intoxication.

The evaluation of the eel population response using exposure and effect biomarkers indicates that the persistent exposure of fish populations and communities to organic pollutants in NRC certainly leads to eco-physiological and toxicological deleterious effects.

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