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Accumulation of Lipophilic Microcontaminants and Biochemical Responses in Eels from the Camargue Biosphere Reserve

HÉLÈNE ROCHE,* ASTRID BUET AND FRANÇOIS RAMADE
University of Paris-Sud XI, Ecology Systematic and Evolution, ESE UPRESA 8079, Ecology and Zoology, Bldg 442, F91405 Orsay Cedex, France

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Abstract. We assessed the impacts of persistent organic micropollutants on aquatic trophic webs in brackish and freshwater communities in the Camargue National Nature Reserve (NNR). We found that organochlorine compounds affect fish communities, particularly those of the common eel (*Anguilla anguilla*). The aims of this study were (1) to determine the amount of lipophilic xenobiotics such as polycyclic aromatic hydrocarbons (PAHs), which are probably of atmospheric origin, and two organochlorines (OC), lindane and dieldrin, which are from irrigation waters, in liver and muscle (2) to define *in situ* biomarkers and (3) to identify the mode by which fish from 'unpolluted' areas become contaminated.

All of the species were contaminated with low, but fluctuating quantities of PAHs and OCs, regardless of the sampling season. Lindane and dieldrin were always detected and naphtalene was the most abundant hydrocarbon. The OC and PAH content was rarely correlated with the lipid content in storage tissues and their concentrations in the lipidic fractions (neutral and polar) varied greatly. We found a number of correlations between persistent organic pollutant (POP) tissue concentrations and the activities of enzymatic membrane markers. For example, there is a relationship between the concentrations of the most volatile PAHs and the activity of muscle acetylcholinesterase (AChE) and between the concentration of benzo-PAH and the activities of ATPases in the gills and/or muscle.

Keywords: coastal wetland; contamination; fish; lipophilic organic micropollutants; protected area

Introduction

Complex situations occur in aquatic habitats, especially wetlands, due to the combination of natural and anthropic influences (Ramade, 1997). One of the aims of our laboratory is to assess the ecotoxicological threat faced by a protected aquatic area, the NNR of Camargue. This protected area, which is also a biosphere reserve, is used to monitor habitats and communities.

Biomonitoring programs have been used to study the effects of xenobiotics on aquatic organisms in several well-known polluted zones (Burgeot et al., 1996; Van der Oost et al., 1996; Baumard et al., 1999). The aim of this program was to assess the concentrations of organochlorine residues and polycyclic aromatic hydrocarbons in the aquatic trophic web of the Camargue Nature Reserve in Southern France (NNRC, La Capelière F-13200 Arles). Irrigation waters from adjacent rice fields currently

*To whom correspondence should be addressed:
Tel.: (+33)1 69 15 73 12; Fax: (+33)1 69 15 79 47.
E-mail: helene.roche@ibaic.u-psud.fr

contaminate the brackish waters of the nature reserve with a number of OC compounds. Although PAHs can be introduced by atmosphere fall out, they can be transferred from agricultural spray drift, petroleum products, combustion processes and/or natural sources. However, in the Camargue, the major sources of PAHs are a neighboring refinery and petrochemical complex. In addition, natural variations also occur, such as decreases in salinity due to levee collapse and/or excessive rain, variations in temperature, salinity balance and oxygen ratio. All of these factors have major influences on the Camargue ecosystem (Ramade, 1999).

Most lipophilic xenobiotics in aquatic organisms are accumulated in fat-rich tissues. However, a study on fish species from different trophic levels showed that OC and PAH concentrations are not always correlated with the lipid concentration of tissues and a relationship with the rates of membrane phospholipids rates could be revealed (Roche et al., 2000). Therefore, we measured the concentration of two OC molecules (lindane and dieldrin) and 16 PAH compounds in hepatic and muscular lipid fractions from the European common eel (*Anguilla anguilla*).

Knowledge of the cellular localization of these pollutants would make of give more insight into the process of bioaccumulation, thus we also sought membrane-specific biomarkers. This raises the questions about the long-term effects of contamination and subsequent biomagnification (Nendza et al., 1997; Menone et al., 2000). It is probable that the dissolution of organic micropollutants into the membrane bilayer brings about biological changes (changes in fluidity, viscosity, transport, absorption, enzymatic activities etc.). The evaluation of these changes could enable us to identify new biomarkers, specific to the unexposed areas.

Material and methods

Field collection

The Camargue Nature Reserve is the largest protected coastal wetland area in Western Europe (Fig. 1) It is located in the Rhône delta and occupies 13,000 ha between the northern Vaccarès lagoon and the last undamaged sand dunes of the Mediterranean coast (Ramade, 1999). A number of water bodies are located in this protected area. The chosen sampling sites were in the Vaccarès lagoon, the largest body of water in Camargue.

Biological material

The species studied was a catadromous euryhaline fish, the European eel (*Anguilla anguilla*). This migratory species is the most common predator fish in the Camargue, preying on aquatic fauna at the top of the trophic chain. However, this species lives in the bottom waters just above the surface of the sediments and often remains in the Mediterranean coastal wetlands for between 9 and 15 years before returning to the Sargasso Sea for spawning.

We analyzed 31 eels (immatures or males) (weight = 145 ± 22 g), caught in March, June and November 1998. Their nutritional status, as assessed by the contents of their digestive tract, showed that 70% of the eels caught in March were starving and that the eels caught in June were less well-fed than those caught in November.

After an overall external examination, fishes were weighed and measured then their organs were excised. Total lipids were extracted from samples of liver and muscle with a chloroform–methanol solution (Folch et al., 1957) and weighed. Phospholipids were estimated by a standardized colorimetric assay (Fiske and Subbarow, 1925). All analyses were performed on individual samples.

Gills were removed and gill filaments (primary lamellae) were used to measure total and NaK-dependent ATPase activities. Muscle samples were used to evaluate ATPase and acetylcholinesterase AChE activities. Total and ouabain-sensitive ATPase and AChE activities were measured in clarified tissue homogenates (supernatant after centrifugation at $1000 \times g$ for 10 min). Na^+, K^+-ATPase, total ATPase and AChE activities were determined according to the methods described by Schmitz et al. (1973) and by Ellman et al. (1961).

Microcontaminants

Analyzed micropollutants are stable and persistent; their liposolubility means that they can undergo bioaccumulation and biomagnification (Bremle and Ewald, 1995). The concentrations of two organochlorine residues formerly used in agriculture were evaluated in liver and muscle: lindane (γ-HCH) and dieldrin. We also estimated the concentrations of

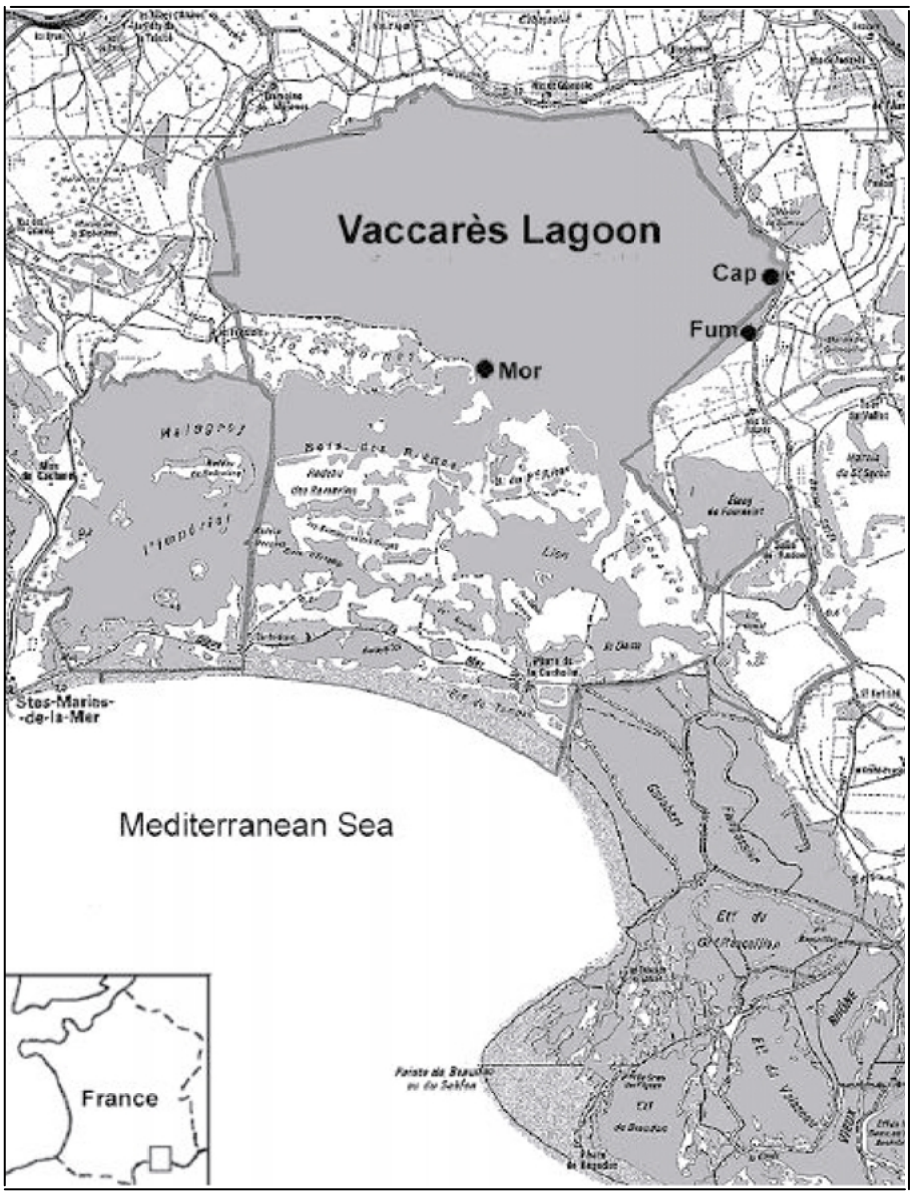

Figure 1. The Camargue (France). Sampling sites in the Vaccarès lagoon, Fum: Fumemorte; Cap: La Capelière; Mor: Mornès.

16 PAHs considered to be priority pollutants by the US Environment Protection Agency (US-EPA) in hepatic and muscular tissues (Table 1).

Extraction and separation

Previously analyses on extracts taken directly from homogenates or the total lipid fraction gave an approximate, but realistic, idea of the amount of accumulated contaminants (Roche et al., 2000). As these results were only approximate for complex compounds, we used a more efficient analytical procedure: lipid groups extracted according to their polarity were analyzed. Lipids and lipophilic substances were separated into three fractions by solid phase extraction (SPE) on a polar silica column ($Si\text{-}CH_2CH_2CH_2NH_2$, Bond Elut NH_2, 1 g 40 µM acid-washed silica 60 Å mean porosity—Varian S.A.,

Les Ulis, France). The first fraction contained natural hydrocarbons and esterified cholesterol, the second one contained the mono-, di- and tri-glycerides, free cholesterol and free fatty acids, and the third one contained the more polar lipids. Separation quality was checked by thin layer chromatography (TLC). Florisil (MgO3Si), an extremely polar, magnesia-loaded silica gel, was used to separate chlorinated micropollutants by an EPA-regulated method (Bond Elut Florisil, 1 g, 200 µM particle size—Varian S.A., Les Ulis, France). Lindane and dieldrin were eluted with hexane/diethyl ether (95 : 5 v/v). PAHs were isolated from muscular and hepatic lipid fractions with hexane/dichloromethane (9 : 1 v/v).

Quantification

OC were identified and quantified by gas chromatography (GC) with electron-capture detection (ECD). PAH were quantified by gas chromatography–mass spectrometry (GC/MS).

Table 1. Molecular characteristics of studied PAH

Name	Abbreviation	MW (g)	Log Kow
Naphtalene	Naph	128	3.3
Acenaphtylene	Acy	152	3.94
Acenaphtene	Ace	154	4.33
Fluorene	Fluo	166	4.18
Anthracene	Anthr	178	4.45
Phenanthrene	Phen	178	5.52
Fluoranthene	Fluora	202	5.2
Pyrene	Pyr	202	5
Benzo(*a*)anthracene	B(a)a	228	5.6
Chrysene	Chrys	228	5.86
Benzo(*b*)fluoranthene	B(b)fluora	252	5.78
Benzo(*k*)fluoranthene	B(k)fluora	252	6.11
Benzo(*a*)pyrene	B(a)pyr	252	6.35
Indeno(1,2,3-*cd*)pyrene	Ind(cd)pyr	276	6.7
Benzo(*ghi*)perylene	B(ghi)pery	276	6.63
Dibenzo(*ah*)anthracene	DiB(ah)anthr	278	6.75

Data analysis

Statistical analyses were achieved with the Statview program (version 4.02, Abacus Concepts Inc. 1992–1993). When the normality and the variance homogeneity of the data were demonstrated, statistical differences were checked by use of the parametric Student's *t*-test. Correlations were calculated by use of Pearson's coefficient.

Results

The eel is a fatty fish in which the liver and muscles are the major reserve tissues (Table 2). In the sample population, the lipid content of fishes caught in the winter (from January to March) was higher than that of fishes caught in the spring or fall despite the relative emptiness of the digestive tract. Contamination and the bioaccumulation of lipophilic xenobiotics were dependent on both the quantity and constitution of tissue lipids. The distribution of lipid classes in analyzed fishes showed that lipids were primarily stored in neutral form ($74.8 \pm 3.3\%$ and $91.7 \pm 0.9\%$ in liver and muscle, respectively).

We have shown previously that the predator fishes in the Camargue, such as the eel and the pikeperch (*Stizostedion lucioperca*), were more contaminated than other species (article in course). Although dieldrin was banned in 1972 and lindane was banned in July 1998, we found residues of these pesticides in all of the samples analyzed (Table 3). This organochlorine was found in all individuals study but at quite

Table 2. Seasonal variations of lipid concentrations in hepatic and muscular tissues of eels from Camargue. Fishes used for ecotoxicological experiments were issuing from this population. Values are expressed in mg g^{-1} of dry weight

		January–March	May–June	October–November
	n	42	82	30
Liver	Total lipids	408.3 ± 32.2	240.6 ± 8.5^{a}	288.3 ± 27.2^{a}
	Neutral lipids	269.8 ± 27.9	154.9 ± 8.8^{b}	221.1 ± 28.9
	Polar lipids	138.5 ± 18.7	81.8 ± 4.7	66.9 ± 4.7
Muscle	Total lipids	345.6 ± 25.1	236.5 ± 10.7^{a}	264.5 ± 24.0^{a}
	Neutral lipids	318.0 ± 24.5	220.2 ± 10.6^{a}	249.3 ± 23.7
	Polar lipids	27.6 ± 3.5	16.3 ± 0.7^{a}	15.2 ± 1.2^{a}

Results are shown as mean ± SE. The value with superscript symbol were significantly different ($p < 0.005$) of [a]Winter samples and [b]both Winter and Autumn samples.

Table 3. Concentration of lindane, dieldrin and total PAH ($\sum$ PAH) in hepatic and muscular tissue in eels from Vaccarès lagoon. Values are expressed in $\mu g\,g^{-1}$ of dry weight

		January–March	May–June	October–November
Liver	Lindane	1.01 ± 0.49 (30)	0.63 ± 0.33 (15)	2.47 ± 1.85 (9)
	Dieldrin	0.88 ± 0.34 (30)	0.16 ± 0.11^{a} (15)	0.038 ± 0.013^{a} (9)
	$\sum$PAH	12.00 ± 3.79 (20)	36.0 ± 10.8^{b} (4)	1.47 ± 0.30^{b} (5)
Muscle	Lindane	0.24 ± 0.08 (31)	0.34 ± 0.08 (31)	0.22 ± 0.05 (9)
	Dieldrin	0.14 ± 0.05 (31)	0.006 ± 0.002^{a} (31)	0.018 ± 0.012^{a} (9)
	$\sum$PAH	2.37 ± 1.25 (11)	67.1 ± 40.3^{a} (5)	90.5 ± 45.9^{a} (4)

Results are shown as mean $\pm$ SE and the value in parentheses indicate number of samples. The value with superscript symbol were significantly different ($p < 0.005$) of [a]Winter samples and [b]both Winter and Spring samples.

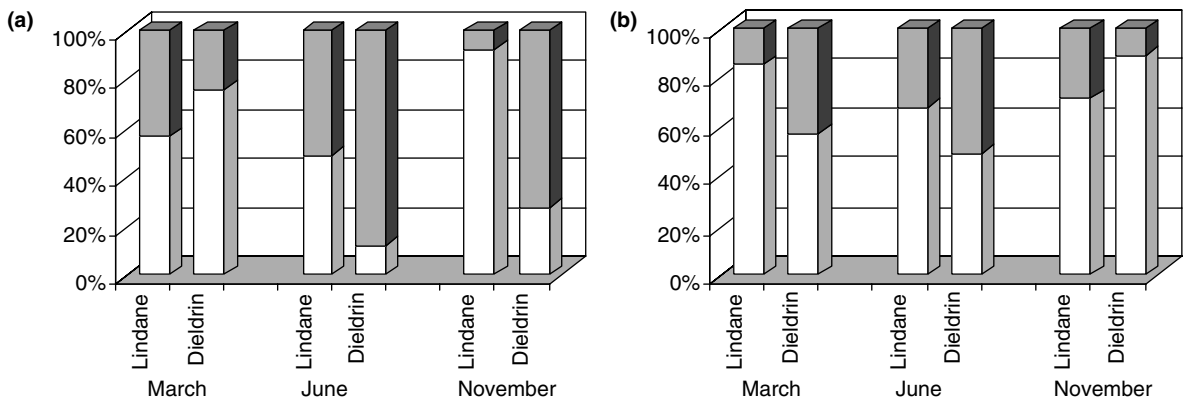

Figure 2. Lindane and dieldrin incorporation in lipid fractions of liver (a) and muscle (b) in March, June and November. Percent of molecule distribution □ in neutral lipids; ■ in phospholipids.

low concentrations. The temporal pattern of contamination was quite different for the two OCs. The levels of lindane in the liver were highest in the autumn, whereas those for dieldrin were highest in the winter.

PAHs concentrations in liver and muscle were higher that those of lindane or dieldrin. An overall analysis of PAH contamination showed strong seasonal variations (Table 3). In spring (May–June) PAH concentrations were elevated both in liver and in muscle. In November, this contamination was localized in muscle tissue. The most abundant and volatile PAH in this eel population was naphtalene, which probably comes from the petrochemical complex at Fos-sur-Mer (about 40 km east). Others PAHs were obviously less concentrated. The contamination was characterized by a large variability.

The xenobiotics studied are non-polar substances, normally eluted in chromatography on silica gel with non-polar solvents, such as pentane, hexane or ethyl ether, which also elute all the neutral lipids. Thus, we analyzed the contaminants in each eluted phase. Figures 2 and 3 show the distribution of lipophilic contaminants in neutral and polar lipids.

Lindane and dieldrin were common in both neutral and polar tissue lipids. (Fig. 2) More than 50% of lindane was found in neutral lipids, except in the less contaminated fishes (caught in June). In November, when the concentration of lindane was higher, about 95% of lindane was extracted from hepatic neutral lipids. Dieldrin concentrations were low in June and November, nevertheless, this organochlorine was preferentially incorporated into hepatic polar lipids (more than 50%) in June.

The most volatile PAHs (naphtalene, acenaphtylene and acenaphtene, when present) were extracted from neutral lipids, both in the liver and in the muscle (Fig. 3). In June (Fig. 3b), most of the other PAHs were found in the hepatic neutral phases. This was particularly of the heaviest PAH, which can potentially induce hepatic biotransformation enzymes such

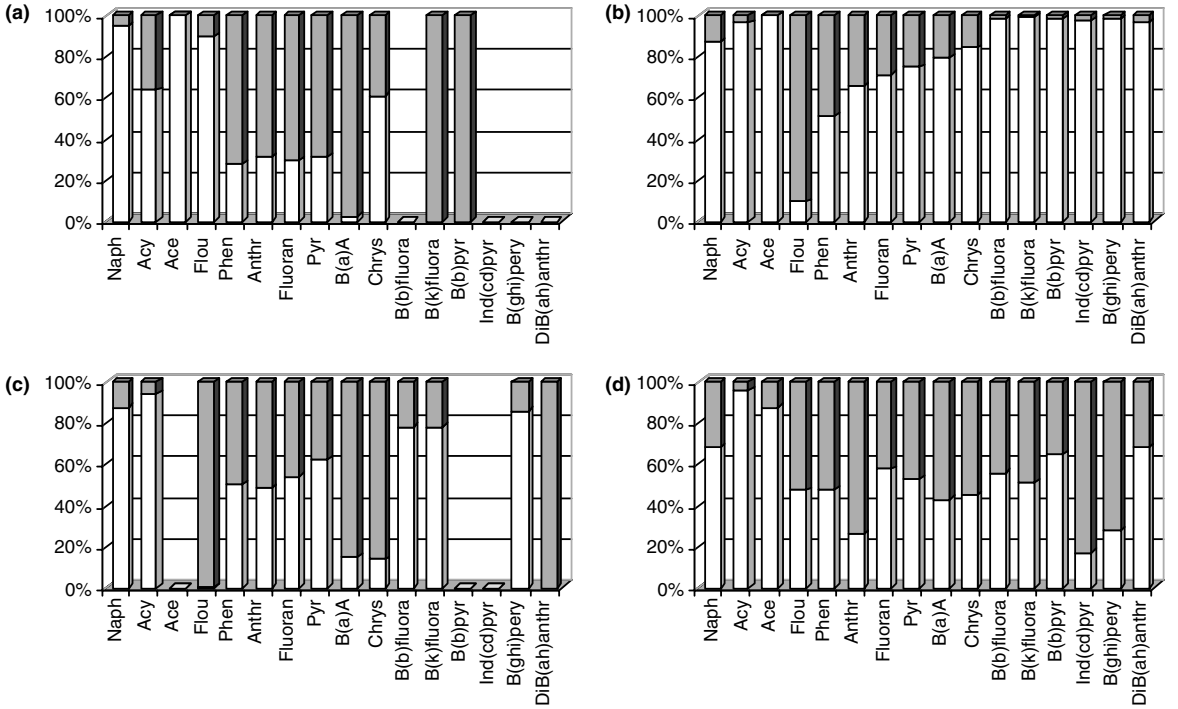

Figure 3. PAH incorporation in lipid fractions of liver—March (a); June (b)—or muscle—March (c); June (d). Percent of molecule distribution □ in neutral lipids; ■ in phospholipids.

as the cytochrome P-450 linked mixed function oxygenase (MFO) system.

In March (Fig. 3a), when total general contamination is low, a significant proportion of the PAHs were detected in the hepatic polar lipids, which are essentially constituted of membrane phospholipids. Some of benzo-PAHs were undetectable at this time. A similar, but less obvious phenomenon was observed in muscle. Indeed, in June (Fig. 3d) all of the 16 PAHs were detected and half of them were retrieved in polar phases, whereas in March the ratios of lipid-polar inclusion for fluorene, benzo(*a*)anthracene, chrysene and dibenzo(*ah*)anthracene were increased and three molecules, including benzo(*a*)pyrene, were not quantifiable.

As xenobiotics might be dissolved in structure lipids we sought ecotoxicological biomarkers in the form of enzymatic membrane markers. A correlation analysis (Pearson's test) was performed to determine whether there are statistical relationships between the tissue concentrations of PAHs and gill and muscle ATPases and/or AChE activities. Total gill ATPases showed numerous positive relations with neutral lipid concentrations of PAHs in the liver (Table 4). When naphtalene and benzo-PAH inclusions in phospholipids increased the activity of gill ATPases also increased. No correlation was detected between the incorporation of the seven lightest PAH molecules (from naphtalene to pyrene) in hepatic and muscular lipids and muscle ATPases activities (except naphtalene). Conversely, other PAHs included in neutral and polar hepatic lipids were often positively associated with muscle ATPases (both total and Na, K-dependent), especially the heavier molecules. Muscle concentrations of the more volatile PAHs (from naphtalene to chrysene) were strongly correlated with muscle AChE when xenobiotics were included or in structural lipids. Surprisingly, correlations between lindane or dieldrin concentrations and enzyme activities were infrequent.

Discussion

The atmospheric transport of industrial emissions and the use of chemicals for agricultural treatments lead to the accumulation of pollutant residues in animal tissues in exposed communities. We analyzed organic

Table 4. Significant Pearson correlations between some membrane enzymatic activity and organic micro-pollutant concentrations in lipid fractions of liver and muscle in eels: p values. $n > 19$

Membrane enzymatic markers		Hepatic contamination			Muscular contamination			
Incorporation of	In	Gill ATPases	Muscle ATPases	Muscle NaK-ATPases	Gill ATPases	Muscle ATPases	Muscle NaK-ATPases	Muscle AChE
∑PAH	Total lipids	0.0022						0.0019
	Neutral lipids	0.0011						0.0099
	Phospholipids							<0.0001
Naphtalene	Neutral lipids	<0.0001						0.0115
	Phospholipids	<0.0001	0.0082	0.0137				0.0002
Acenaphtylene	Neutral lipids							0.0045
	Phospholipids						0.0247	0.0449
Fluorene	Neutral lipids							0.0112
	Phospholipids							
Phenanthrene	Neutral lipids	0.0017						0.0014
	Phospholipids							0.0001
Anthracene	Neutral lipids							
	Phospholipids							0.0019
Fluoranthene	Neutral lipids	0.0309						0.0008
	Phospholipids							<0.0001
Pyrene	Neutral lipids	0.0496						0.0006
	Phospholipids							<0.0001
Benzo(*a*) anthracene	Neutral lipids	0.0084	0.0026	0.0066				0.0026
	Phospholipids					0.042		0.0003
Chrysene	Neutral lipids		0.0083	0.0174				0.0052
	Phospholipids		<0.0001	<0.0001				0.0002
Benzo(*b*) fluoranthene	Neutral lipids	0.026	0.0013	0.0033				
	Phospholipids	0.0528		0.0035				
Benzo(*k*)fluoranthene	Neutral lipids	0.0323	0.0015	0.0036				
	Phospholipids		0.0016					
Benzo(*a*)pyrene	Neutral lipids	0.0418	0.0007	0.0018		0.0001		
	Phospholipids		0.0016			0.0257		
Indeno(*cd*)pyrene	Neutral lipids	0.0468	0.0011	0.0025		<0.0001		
	Phospholipids	0.0528	0.0016	0.0035	0.0425		0.0002	
Benzo(*ghi*)perylene	Neutral lipids	0.0238	<0.0001	0.0002	0.0397	0.0043		
	Phospholipids	0.0528	0.0016	0.0035	0.0426		0.0003	
DiBenzo(*ah*)anthracene	Neutral lipids	0.0501	0.0014	0.0031	0.0018			
	Phospholipids	0.0528		0.0035				
Lindane	Neutral lipids							0.0213
	Phospholipids							
Dieldrin	Neutral lipids							
	Phospholipids			0.0029				

micropollutants (PAHs and organochlorine insecticides) in the tissues of eels collected from the Vaccarès lagoon, the largest body of water in the Camargue Nature Reserve (France). The level of contamination was variable and sometimes worrying. All of the micropollutants sought were detected in all of tissue samples regardless of the sampling period (Roche et al., 2001). Nevertheless, the amplitude and the localization of the pollutants in tissue compartments depended on the season. Fish sampled at the end of the spring (May–June) were more contaminated than fish captured in the winter.

Due to the lipophilic nature of xenobiotics, it is commonly believed that their uptake and

bioaccumulation are related to the composition of the storage tissue (Stange and Klungsyr, 1997). However, we have previously shown that, in fishes from this 'unpolluted' area, the concentrations of these residues in hepatic and muscular tissues are seldom correlated with lipid concentrations (Bremle and Ewald, 1995). Conversely, the contaminant content was significantly correlated with phospholipid concentrations, suggesting that they accumulate in membrane structures. We assessed the level of contamination in the lipid fractions of the liver and muscle to determine the membrane localization of the contaminant. The polarity and the chemical structure of the lipophilic substances are determining factors for the bioaccumulation process in neutral (reserve) and polar (structure) lipids. The cellular localization of contaminants is seldom considered in relation to the timing and duration of the contamination, the amplitude of the detoxification metabolic activity, the relationship between the cellular site of storage and biomarkers responses, etc. Therefore, we measured the micropollutants in the two main lipid components after chromatography on silica gel columns and elution with organic solvents with increasing polarity.

Our results show that at the end of the winter, when the nutritional status has not yet fully recovered and the level of contamination is low, some of the heaviest PAHs (MW $>$ 200) were undetectable. The other PAHs such as benzo(*a*)anthracene, benzo(*k*)-fluoranthene and benzo(*a*)pyrene were found predominantly or only in the most polar phase, and the latter also disappeared from muscle tissue. Although the trend was the same in the muscle, it was less obvious. It appeared that the PAHs with highest Log Kow ($>$5) were incorporated more easily into the membrane lipidic bilayer than those with smaller Log Kow. It is widely thought that the toxicity of lipophilic xenobiotics is related to their interactions with biological membranes, which increases their permeability and disturbs their organization, enzymatic functions and associated systems (Donato et al., 2000).

The binding of PAH with the aryl hydrocarbon receptor (AhR) activates transcription factors that regulate the expression of cytochrome P450 forms and provides indirect support for their biotransformation (Safa et al., 1997; Villeneuve et al., 1998; Willett et al., 2000). This is one of the arguments that confirmed that these enzymes are biomarkers (van der Oost et al., 1997; Collier et al., 1998). The fact that they become part of the membrane suggests that eels in the Vaccarès lagoon have been contaminated for many years and that this contamination is related to a biomagnification phenomenon. This is well documented for lindane (Antunes-Madeira and Madeira, 1989). This delta isomer of hexachlorocyclohexane is structurally similar to inositol-trisphosphate. It is known to alter lipid bilayers, potentially leading to membrane disruption (Verma and Singhal, 1991). Furthermore, it is involved in the metabolism of a number of phosphatidylinositols (Pulido et al., 1992). Thus, the molecular incorporation of POP into the phospholipid matrix may disturb the activities of membrane-bound enzymes, growth and membrane uptake.

Until July 1998 rice fields surrounding the Vaccarès lagoon were extensively sprayed with lindane, which was used to control the rice-borer caterpillar. Therefore, it was not surprising that lindane was detected in the polar phases of hepatic and muscle lipids in eels from Vaccarès. It is noteworthy that our most recent experiments have shown that total tissue impregnation tends to decrease (unpublished results). Dieldrin is an insecticide that has been banned in France since 1973. It was not very concentrated in eel tissues; however, in June and November more than 80% of this molecule was found in the most polar hepatic lipids. It was less concentrated in the muscle. It is possible that dieldrin is still found in the Camargue due to its unusual remanence, but it is more probable that the eels were contaminated via the food chain because it is believed that dieldrin might still be used illegally for rice farming.

The fishes from a protected wetland provide an opportunity to test the suitability of ecotoxicological biomarkers in sites that are relatively free from industrial contaminants but exposed to chronic or periodical transfer of contaminants via water or air and potentially submitted to the biomagnification process. The fish biomarkers that can be used to monitor anthropogenic chemicals are mainly the liver biotransformation enzymes. However, the sensitivity of these hepatic parameters and the intensity of their response are modulated as a function of the biological characteristics of the animal and the amplitude of the chemical stress (Depledge, 1994). For example, the activity of the hepatic enzyme ethoxyresorufin *O*-deethylase (EROD) is affected by a number of seasonal and environmental factors (Rotchell et al., 1999) and is either correlated with reproductive parameters (Kirby

et al., 1999) or influenced by nutritional status (Jørgensen et al., 1999). Due to the characteristics of the contamination, our previous results have not allowed us to show that the activity of EROD can be used as a biomarker in fishes from Vaccarès.

We carried out additional investigations on other metabolic processes that involve membrane enzymatic markers, some of which also have a role in neuronal conduction (AChE), in osmoregulation (gill ATPases) and/or energy metabolism (muscle ATPases). The inhibition of AChE is classically used as a biomarker to monitor contamination with organophosphate insecticides and carbamate (Kang and Fang, 1997; Fernandez-Vega et al., 1999). However, it has been shown that the activity of this enzyme is also sensitive to PAH and PCB (Bocquené et al., 1995). ATPases are responsible for converting electrical potential energy into ATP, their activities are dependent on abiotic factors. The Na, K-ATPase plays a crucial role in the functioning of osmoregulatory cells. Ecotoxicological studies have suggested that heavy metal ions modify the membrane permeability of cells by altering the activity of ATPases, which in turn disturbs active transport mechanisms (Jagoe et al., 1996; Thaker et al., 1996). Sancho et al. (1997) have showed that fenitrothion can inhibit the gill Na,K-ATPase in the eel. In this study, we found a number of weak but significant positive Pearson's correlations between enzymatic activity and of the concentrations of the organic compounds in lipid fractions, which confirm the potential effect on membrane integrity. Apart from the widespread statistical relationships between gill ATPase activities and most of the hepatic contaminants, all of the relationships appeared to be dependent on structural molecular parameters such as molecular weight or log Kow. This suggests a structure–activity relationship, which is hard to assess because of the field working conditions. Nevertheless, our results tended to suggest that gill and muscle ATPases and muscle AChE are better biomarkers than biotransformation enzymes in fish in areas of low contamination.

Conclusion

The Camargue National Nature Reserve is a protected wetland, which is thought to be largely unpolluted and which represents the presumed pollution 'background noise'. In aquatic ecosystems, fishes are located at the highest level of the biomagnification process for organic xenobiotics. Some chemicals are accumulated and others are metabolized (Da Costa and Curtis, 1995). PAH and organochlorine compounds have been considered to be xenobiotic models for monitoring in this protected area because of their ubiquity in the environment, their persistence, their bioaccumulative properties and their potential toxicity to aquatic organisms (Law, 2000). In eels sampled in this reserve, most of the studied non-polar contaminants were found in liver and muscle phospholipids and their concentrations were statistically related to the activities of membrane-bound enzymes.

This study has assessed the ecotoxicological threat that endangers the aquatic community in a protected area in an original manner. Moreover we may conceive a novel aspect of research on the long-term effects of an accidental or chronic pollution including the incorporation of xenobiotic substances in cellular membranes and its consecutive effects on cell functions.

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