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Relationships between persistent organic chemicals residues and biochemical constituents in fish from a protected area: the French National Nature Reserve of Camargue

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Abstract

The Reserve of Biosphere of Camargue [French National Nature Reserve of Camargue (NNRC)] is a protected area frequently exposed to natural and anthropogenic environmental alterations. To evaluate potential contamination of fish with lipophilic chemicals—organochlorines (OCs) and polycyclic aromatic hydrocarbons (PAHs)—a biological monitoring survey was carried out. Metabolic reserve levels were evaluated to select appropriate biological indicators able to be significant biomarkers. In addition, the incorporation of xenobiotic molecules in the lipid compartments was investigated. The contents of glycogen, total lipids, proteins and lipidic phosphorus were analyzed in liver and skeletal muscles of three teleostean: the European eel (*Anguilla anguilla*); the crucian carp (*Carassius auratus*); and the catfish (*Ictalurus melas*). The atmospheric origin of the PAH detected in any season in the biomass and the OCs compounds contamination by derive from agricultural treatments are established. In contradiction with some laboratory acute intoxication studies, we observe a positive correlation between tissue concentrations of contaminants and the muscular glycogen amount, a sensitive energy reserve marker. Moreover, it seems likely that the incorporation of these xenobiotics is located preferentially in the membrane structures.

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Keywords: Protected area; Ecotoxicology; Fish; PAH; Organochlorines; Metabolism; Glycogen; Lipids

1. Introduction

The French National Nature Reserve of Camargue (NNRC) (Fig. 1) is an UNESCO Biosphere Reserve. It is located in the southeastern part of France. It ranks among the most famous coastal Mediterranean reserves due to its outstanding ecological characteristics and especially to its biodiversity (Ramade, 1997, 1999). It currently preserves a complex mosaic of both terrestrial and aquatic habitats: sand dunes; halophytic grass-

lands; mudflats; and various coastal wetlands. A number of shallow paralic ecosystems (lagoons) occurs in the lowest central and southern parts of this protected area where a wide range of salinity exists. In spite of the absence of industry in the Rhône delta and low human density at European scale in the vicinity of this Reserve, these ecosystems are under various stresses due to anthropic environmental modifications (Ramade, 1993). They are especially exposed to tourism development. During the last decade, a significant area of natural habitats around the NNRC has been converted to agriculture, mostly in rice-fields. Consequently, the areas covered by salted steppes

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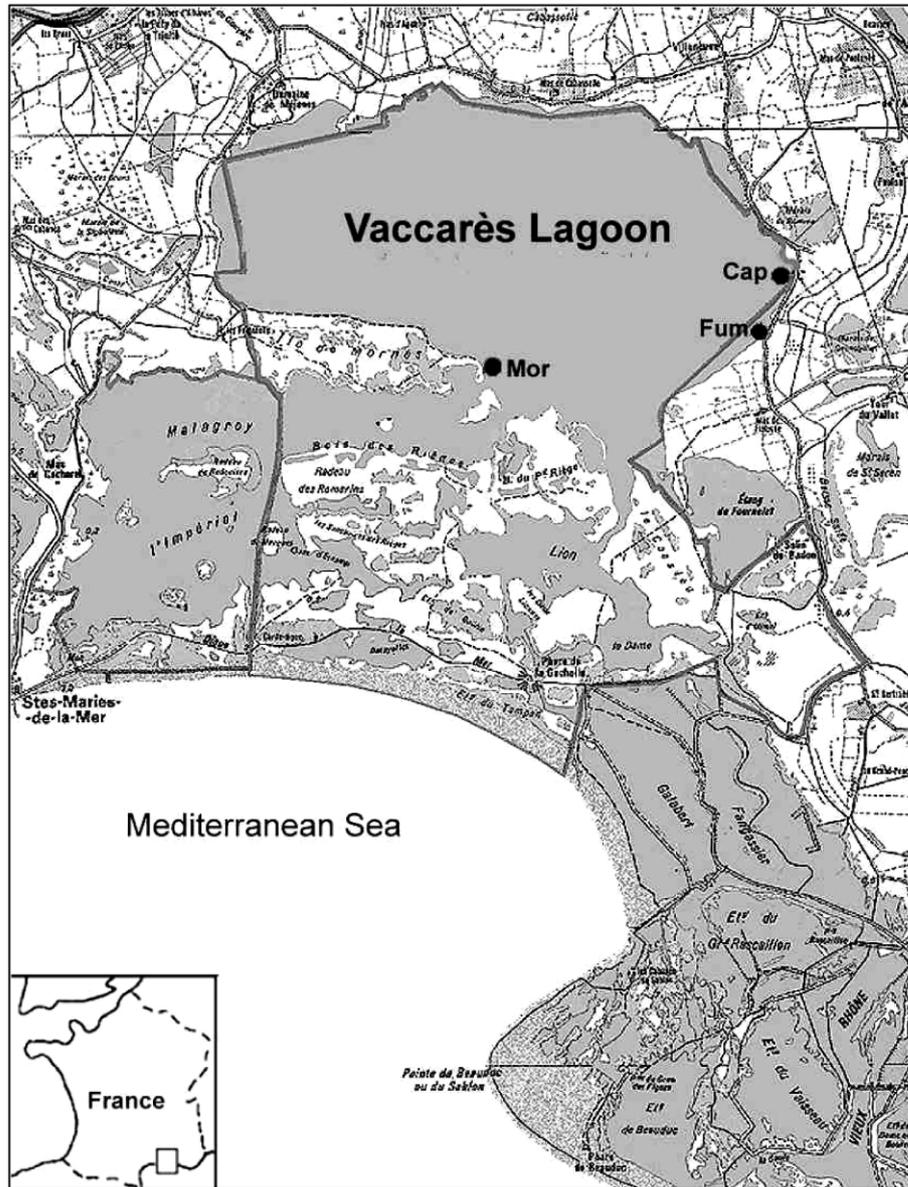


Fig. 1. The Camargue Regional Nature Park (France). Reference sites: Fum, Fumemorte; Cap, La Capelière; and Mor, Mornès. Scale: 1/20000.

(locally named 'sansouïres') have been more especially concerned by this conversion (Tamisier and Grillas, 1994). The 'sansouïres' are classified by the EU as vulnerable habitats prioritized for conservation. The draining ditches from the rice fields currently bring a number of persistent organic pollutants into the major water body of this natural reserve, the Vaccarès lagoon. Intensive and frequent pesticide spraying is carried out, especially organochlorine (OC) insecticides such as lindane.

In addition, irrigation ditches contribute to the high input of fresh water coming from the industrialized Rhône river valley, associated with a low water exchange with the Mediterranean Sea. These artificial water inputs are the major source of perturbation for these coastal wetlands, as their hydrological seasonal balance depends on evaporation in Summer and precipitations mainly equinoctial. They also influence the salinity balance in the lagoons. Presently, the average salinity of

brackish wetlands is 17 g l^{-1} . However, in this site, it dropped down to approximately 4.5 g l^{-1} as a consequence of unusual rainfalls. This variation led to a decline in native plant and animal species and subsequently, to an invasion by freshwater fishes such as catfishes and crucian carps. These species were formerly limited to the northernmost part of the Reserve and to the irrigation and drainage ditches.

Another potential source of pollution is related to the occurrence eastward of a petrochemical complex (Fos-sur-mer) and a large city (Marseille) at a distance of approximately 40 km. Persistent organic pollutants (POP) such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), are brought via the atmospheric transfer to these aquatic habitats.

Fish biomarkers for monitoring the anthropogenic chemicals are frequently used (Stein et al., 1993; Strandberg et al., 1998). The Camargue Nature Reserve provides an opportunity to test the suitability of biological responses as biomarkers in fishes from sites exposed to periodical xenobiotics spills. In a previous work we have shown that because of the vicinity of agricultural areas, the pesticides burden in fish tissues is permanent though at a rather low level (Roche et al., 2000), and it depends on the sedimentary load of persistent chemicals and on the water supply. The effects of organic xenobiotics on fish have been extensively documented. A number of reports have brought evidence that contamination with lipophilic pollutants triggers many biochemical responses, like disturbance of endocrine functions, activation of metabolization systems or more generally, disorder of the normal physiology (Stein et al., 1993; Burgeot et al., 1996; van der Oost et al., 1996, 1997). However, the difficulty to select appropriate biological indicators is related to the chemical complexity of the potential contaminants and the nature of target organisms. Organic xenobiotic toxicity involves interactions among several metabolites operating by distinct mechanisms translating into a vast array of biological disorders.

Due to the nature of the contamination, previous investigations about validation of biomarkers in eels from the Vaccarès lagoon were focused on numerous biological indicators including the detoxification mechanisms (biotransformation, antioxidant process), energy requirements, but also some unspecific metabolic processes (Roche et al., in press). We have then showed that three hepatic

activities involved in the protection against oxyradicals: catalase; glutathion peroxidase and superoxide dismutases; muscle and gill ATPases as well as muscle and brain acetylcholinesterase are more significant in term of biomarkers than the biotransformation enzymes ethoxyresorufine-*O*-deethylase and uridine diphosphate glucosyl transferase. Moreover, we have presumed that glycogen and protein tissue rates could also be sensitive biomarkers. Consequently, we propose in the present study, to assess the responses of energetic markers (glycogen, protein and lipids) to fluctuating POP contaminations in three fish species from the NNRC lagoons.

These investigations are part of a research program, intended to estimate the health of the Vaccarès lagoon ecosystem. Then, we have measured the metabolic reserve levels and the xenobiotics tissue content—lindane (γ BHC), PCBs, naphthalene, phenanthrene, anthracene, fluoranthene and benzo(*a*)pyrene [B(*a*)P]—in eel (*Anguilla anguilla*), crucian carp (*Carassius auratus*) and catfish, the black bullhead (*Ictalurus melas*).

2. Materials and methods

Fishes were collected from three riparian locations in the Vaccarès lagoon (Fig. 1). The sampling sites were selected in correspondence with previous studies performed by IFREMER (the French Institute of Research and Exploitation of the Sea) as part of its monitoring network (RNO, 1998). They were: (1) the mouth of the Fumemorte canal (FUM), which drains rice fields irrigation wastewaters, in the southeast of the Vaccarès lagoon, located at the NNRC border; (2) the site named 'la Capelière' (CAP) in the eastern part of the lagoon, approximately 300 m northward from the canal mouth; (3) riparian waters from the Mornès peninsula (MOR), the reference site, to the furthest end of the fresh waters inputs in the most remote area of the reserve. During the experiment, the average salinity of the water of this lagoon varied from 3.4 to 8 g l^{-1} . Fishes were caught in Autumn (November 1996); Winter (January–February 1997) and at the end of Spring (June 1996 and June 1997).

Fifty-three eels ($225.1 \pm 22.1 \text{ g}$), 31 crucian carps ($335.3 \pm 20.3 \text{ g}$) and 32 black bullheads ($129.5 \pm 12.8 \text{ g}$) were analyzed. The European eel (*Anguilla anguilla*) stands as an eurhythaline and migratory species, however, sedentary for a long

time (approx. 9–15 years) in Vaccarès lagoon. This teleostean is a predator and carnivorous fish living both in contact with sediments and in free waters. In this study, all the sampled eels were immature. The crucian carp (*Carassius auratus*) was recently (approx. 18 years) and accidentally imported into Camargue. Due to a sudden decrease of salinity, its population had increased in 1993 and 1994. It is a littoral stationary cyprinidae, preferably herbivorous, which lives in stagnant waters and marshes. In Camargue, this species is gynogenetical, therefore all the examined crucian carps were mature females with full gonads. Catfish (*Ictalurus melas*) is a sedimentary fresh water siluriform, coming from North America. Its natural feeding diet is manifold; indeed, this omnivorous fish is highly predatory towards eggs, alevins and yearlings of other species. The experimental population was composed of 8 males and 24 females.

Livers and muscles were dissected and cut into small parts for chemical and biochemical analysis. The gall bladders were removed and stored at -30°C for subsequent PAHs analysis. Water content of liver and muscle samples was determined by oven drying at 105°C for 24 h. Total lipids were extracted with a chloroform–methanol solution (Folch et al., 1957) and weighed. Lipidic phosphorus was estimated by a spectrophotometric determination (Fiske and Subbarow, 1925). After lipid extraction, the lipid-free residues were filtered and then digested in a NaOH solution (1 N) for protein and glycogen determinations. Protein content was assessed by the Lowry method (Lowry et al., 1951). The glycogen, precipitated by ethanol, was hydrolyzed by amyloglucamylase and glucose was measured by the glucose oxidase technique adapted of Hugget and Nixon (1957) using ABTS as chromogen.

The OCs were extracted from lipids, after previous isolation and concentration from liver and muscle samples, and purified on a column of Florisil 60/100 Mesh. PCBs or γBHC were eluted with hexane or hexane/diethyl ether (95/5), respectively. Analysis of OC extracts were performed by gas chromatography with a Girdel 3000 using electron capture detection (^{63}Ni Source) with argon/methane (90/10) as carrier gas. Recovery of lindane and PCBs from the extraction and clean-up procedures were $84 \pm 8\%$ and $69.5 \pm 8.3\%$, respectively. For PCBs analysis the external standards were the Aroclor 1242, 1254 and 1260, including 22 congeners: 18; 31; 44; 47; 49; 52;

66; 101; 105; 110; 118; 119; 138; 151; 153; 156; 170; 180; 194; 195; 199; and 209. PAHs were extracted from liver and bile using a soxhlet device with dichloromethane as solvent. After solvent evaporation, lipid removal and PAHs purification were performed on florisil column with hexane/dichloromethane (90:10) as eluant. Five PAH have been selected according to their number of aromatic cycles, their abundance in this area and their carcinogenicity: naphthalene (two cycles), phenanthrene and anthracene (three cycles), fluoranthene (four cycles) and B(a)P (five cycles). Analysis and detection of PAH were carried out by means of gas chromatography/mass spectrometry (GC/MS) with a mass spectrometer HP 5972, coupled to a chromatograph HP5890 (Hewlett–Packard) with electron impact (EI) ionization detector and helium as carrier gas.

In these field studies, POP concentrations are expressed with regard to dry weight (or in default, to the body weight) to point up the exact pollutant burden of an organism located at a given level of the trophic web or by extension, of the food chain, and to allow comparison. This choice is comforted by the lack of relation between the trophic level and the global content in lipids (Russell et al., 1999) and the infrequency of correlation between contents of lipophilic contaminants and the lipid tissue concentration (this work; Roche et al., 2000, 2002). In addition, bile PAH concentrations are related to total fish body weight because gall bladder volume fluctuates with the nutritional state and the extraction procedure does not allow to evaluate the percentage of water in samples. All analyses were performed with individual samples.

Hexane and other solvents (analytical grade) were purchased from SVIP company (F75012 Paris, France) and OC standards from Alltech France SARL (F59242 Templeve, France); florisil was purchased from Touzart et Matignon (F94403 Vitry sur Seine, France). Other chemical products were obtained from Sigma Chemical Company Europe (38297 St Quentin Fallavier, France).

Statistical analyses were achieved with the Statview program (version 4.02, Abacus Concepts Inc., 1992–1993). When the normality and the variance homogeneity of data were demonstrated, statistical differences were checked using parametric Student's *t*-test. Correlations were calculated using Pearson's coefficient. When normality could not be demonstrated, Mann–Whitney's test was used for comparison.

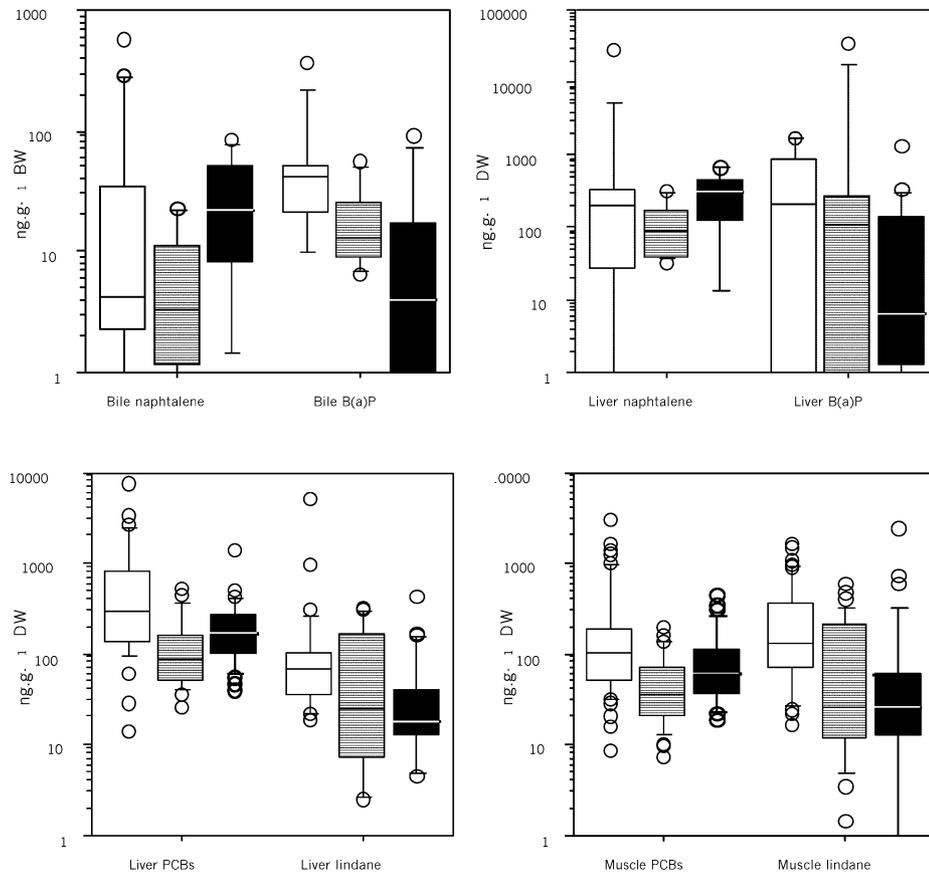


Fig. 2. Box-plots representation of individual bile and liver concentrations of naphtalene and B(a)P, and liver and muscle concentrations of PCBs and lindane in the □ eels (*Anguilla anguilla*), ■ crucian carps (*Carassius carassius*) and ▨ black bullheads (*Ictalurus nebulosus*) from the French Nature Reserve of Camargue. Values are expressed in Log (concentration). Geometric mean and 95% of values are in the box.

3. Results

3.1. Analysis of organic microcontaminants

3.1.1. Inter-specific comparisons

Inter-species variation for global contamination by the more frequent PAH and organochlorine molecules is shown in Fig. 2, in which box-plots indicate the repartition of individual values around the geometric mean.

In the three species, the bile naphtalene contamination was low. In eels, many values exceeded the geometric mean signifying that a number of eels were strongly contaminated. The contaminant burden appeared lower in crucian carps and black bullheads displayed the highest naphtalene accumulation. The distribution scheme of the contamination was similar for the liver naphtalene concentration, except in eels where the interval

was reduced and most of the values remained below the geometric mean.

B(a)P occurred in all samples of bile and liver from the three species. Eels were the most contaminated and black bullheads the least ones. Liver B(a)P concentrations showed a large variability, thus many eels and crucian carps were less contaminated whereas very high values (saturation of detection capacity) were found in crucian carps.

In the studied fishes, PCBs reached the highest concentrations in liver and the highest levels were found in eels. In eel muscle, γ BHC concentration ranges were higher than in black bullheads and in crucian carps. In the latter, values showed a larger variability.

3.1.2. Inter-sites and inter-seasons comparisons

Concentration ranges of PAH (geometric mean and lower/upper values), as a function of location

and season, are listed in Table 1. Individual PAH concentrations were very fluctuating in bile and in liver; they ranged from $<0.05 \text{ ng g}^{-1}$ to $>300 \mu\text{g g}^{-1}$ dry wt. The highest PAH concentrations were found in eels and crucian carps caught in winter (as naphthalene in bile and liver of eels from Fumemorte and La Capelière; B(a)P in liver of crucian carps from Fumemorte). Bile phenanthrene concentrations were regular—geometric means ranged from 143 in eels to 240 ng g^{-1} body wt. in black bullhead. The highest phenanthrene concentrations were found in the livers of eels and black bullheads caught in Autumn (geometric means were approx. 450 ng g^{-1} dry wt.), and the lowest in the liver of crucian carps captured in Autumn and Winter ($<200 \text{ ng g}^{-1}$ dry wt.). In the three species phenanthrene concentrations were correlated to anthracene concentrations, Pearson's coefficients (R^2) ranged from 0.792 ($P=0.031$) in bile to 0.896 ($P=0.0001$) in liver with $n=7-15$. Nevertheless, anthracene concentrations were lower than phenanthrene; in bile, geometric means were scattered between 61 ng g^{-1} in crucian carps and more than 200 ng g^{-1} body wt. in black bullhead, and hepatic concentrations varied from 54 in crucian carps liver to 264 ng g^{-1} dry wt. in eels from Fumemorte in Winter. Fluoranthene was detected in each sample but concentrations were low, except in bile of black bullhead and in liver of eels from Fumemorte in Autumn.

A number of variations in OC burden regarding season or sampling sites are significant. Geometric mean and lower/upper values are summarized in Table 2. When OCs contamination levels were elevated, γ BHC was dominant in muscle and PCBs in liver. For example, in Spring 1996, fishes from Fumemorte were characterized by a high OCs muscle contamination in which γ BHC figured between 40 and 60% of total OCs. In the same way crucian carps and black bullheads from La Capelière showed a high spring γ BHC contamination. Samples of Spring 1997 make an exception for the three species, indeed, in fish caught in La Capelière or Mornès, PCBs stood as the major OC muscular contaminant. In eels, this observation was connected with a high hepatic level of γ BHC. For those from Mornès, γ BHC concentrations were low and PCBs concentrations were equivalent (or higher) to fish from other locations. Finally, in Autumn all the fishes showed low rates of γ BHC and in Winter, hepatic and muscular OCs concen-

trations were especially reduced in crucian carps and black bullheads.

Biochemical constituent concentration showed notable interspecies differences. The global repartition in three species from different trophic levels indicates great disparities in the three metabolic compartments of hepatic and muscular tissues. The hepatic glycogen concentration was higher in crucian carps and black bullheads than in eels, and the white muscle contents of glycogen were lower in black bullheads than in crucian carps and eels. *Anguilla anguilla* is a fat fish for which tissue lipids were preferentially neutral lipids (up to 70% of total lipids in liver and approx. 90% in muscle). On the contrary, muscles of *Carassius auratus* and *Ictalurus melas* are less fatty and phospholipids were dominant (approx. 60%). The liver of black bullheads was more lipid-rich than hepatopancreas of the crucian carp, the major discrepancy being for phospholipids which represented approximately 60% of total lipids for the first and 40% for the second.

Statistical analyses were carried out in order to show seasonal or inter-site variations. Tissue components measured in the three species are presented in Table 3 (geometric mean and lower/upper values). Results of Student's *t*-tests are exposed in Table 4, values are considered statistically significant at $P<0.05$. The diversity of tissue metabolite content suggested a continuous adjustment in each species.

3.1.3. The eels

In eels from La Capelière and from Mornès, a number of variations were observed between Spring 1996 and Spring 1997. In Table 4 (eels, inter-site variation) are observed significant differences in liver structural constituents—proteins increase and phospholipids decrease—and a severe reduction in Spring 1997 of muscular glycogen and lipids concentrations. Therefore, the description of seasonal variations becomes complex.

In a simplified way, during the annual cycle (Spring 1996 to Winter 1996–1997), hepatic glycogen levels were influenced by both season and capture-sites [Table 4, eels (seasonal variations)], whereas proteins levels were stable. Eels from Fumemorte were characterized by their hepatic glycogen burden in spring. Lipid contents were of course, depending upon the season but fluctuated, otherwise, as a function of the capture-site and phospholipids followed such variations. In Spring,

Table 1

PAHs concentrations (geometric mean, minimum–maximum) in bile referred to the body weight (body wt.) and in liver expressed with regard to dry weight (dry wt.) of fish from the Vaccarès Lagoon

	<i>n</i>	Bile (PAH ng g ⁻¹ body wt.)					Liver (PAH ng g ⁻¹ dry wt.)				
		Naphtalene	Phenanthrene	Anthracene	Fluoranthene	B(a)P	Naphtalene	Phenanthrene	Anthracene	Fluoranthene	B(a)P
<i>Anguilla anguilla</i>											
Autumn 1996											
FUM	5						190.1 87–340	433.2 258–828	93.4 46–154	147.6 71–326	0.12 0–3.5
Winter 1996–1997											
CAP	5	25.5 2–2862	225.6 37–1551	135.5 17–680	0.08 <0.05–1.8	570.3 195–3562	169.8 <0.05–27468	300.3 17–953	109.1 34–468	0.002 0.0–177	352.7 86–1217
FUM	5	304.3 3–56690	142.8 89–267	145.2 70–525	0.92 0.1–21	68.0 <0.05–664	29.1 0.2–742	303.6 78–2148	264.2 34–1878	0.005 0.0–0.2	664.1 208–1749
<i>Carassius carassius</i>											
Autumn 1996											
FUM	3						53.3 33–84	62.0 19–131	53.5 38–84	5.0 0.4–19	0.009 <0.05–0.7
Winter 1996–1997											
FUM	7	5.6 0.1–231	190.7 79–2430	61.34 28–219	0.02 <0.05–15	158.6 65–563	109.3 38–320	173.9 58–688	62.0 21–172	2.7 <0.05–96	467.0 29–347 319
<i>Ictalurus melas</i>											
Spring 1996											
CAP	4–6						304.1 63–663	348.5 90–624	70.4 28–132	0.3 <0.05–59	0.2 <0.05–12
Autumn 1996											
FUM	5–7	379.6 108–839	239.6 121–573	209.9 109–483	138.6 52–428	1.18 0.11–54	240.4 112–688	472.0 132–947	145.7 51–410	19.9 0.2–92	5.8 0.2–39
Winter 1996–1997											
FUM	6	25.1 0.2–268	235.6 151–375	85.8 50–148	3.92 0.2–131	294.6 130–927	26.7 0.2–609	239.8 62–785	100.1 29–217	0.3 <0.05–30	259.5 127–1340

Table 2

PCB and lindane concentrations in muscle and liver of fish from the Vaccarès Lagoon; values (geometric mean, minimum–maximum) are expressed with regard to dry weight

	<i>n</i>	Muscle (OC ng g ⁻¹ dry wt.)		Liver (OC ng g ⁻¹ dry wt.)	
		PCB	Lindane	PCB	Lindane
<i>Anguilla anguilla</i>					
Spring 1996					
CAP	8	82.5 47–278	132.7 80–439		
FUM	6	50.2 9–201	1133.4 870–1716		
MOR	2	96.1 77–120	103.8 84–128		
Autumn 1996					
FUM	5	75.4 47–172	80.4 0.5–448	148.5 110–207	68.6 40–104
Winter 1996–1997					
CAP	5	77.1 32–389	216.4 112–632	1124.0 138–2691	13.9 0.05–129
FUM	5	56.5 29–124	32.7 0.02–396	250.7 62–472	37.7 23–70
MOR	5	114.3 42–187	125.3 91–178	1155.0 141–7547	31.7 19–47
Spring 1997					
CAP	5	627.0 111–2991	72.2 32–614	157.7 14–873	602.3 200–5169
MOR	7	414.8 43–1664	39.0 17–339	236.1 29–1111	85.8 38–181
<i>Carassius carassius</i>					
Spring 1996					
CAP	8	56.0 29–166	301.6 170–595		
FUM	4	38.1 14–132	145.8 105–230		
Autumn 1996					
FUM	3	39.5 21–71	23.3 21–27	82.4 26–447	4.61 0.5–68
Winter 1996–1997					
FUM	7	13.0 8–21	7.53 0.9–17	79.8 36–161	7.48 2.5–12
Spring 1997					
CAP	9	69.9 27–197	14.2 1.5–49	123.9 46–532	140.7 24–334
<i>Ictalurus melas</i>					
Spring 1996					
CAP	6	23.8 0–446	221.8 14–2399	138.9 56–244	19.6 0.05–170
Autumn 1996					
FUM	10	52.1 28–86	10.8 0.1–40	142.8 67–392	10.1 4.6–33
Winter 1996–1997					
FUM	6	37.5 18–77	3.49 0.1–588	75.4 40–169	16.2 10–23
Spring 1997					
CAP	10	146.4 59–344	21.3 4.4–137	334.2 128–1448	44.2 13–429

Table 3

Liver and muscle constitution of fish from Vaccarès Lagoon; values are expressed as geometric mean, minimum–maximum

Tissue constituents (mg g ⁻¹ dry wt.)	<i>n</i>	Weight (g)	Liver proteins	Liver glycogen	Liver lipids	Liver phospholipids	Muscle proteins	Muscle glycogen	Muscle lipids	Muscle phospholipids
<i>Anguilla anguilla</i>										
Spring 1996										
CAP	8	133.5 61–374	277.6 222–364	5.99 2.07–14.0	220.4 152–249	100.7 78.4–125.3	460.0 305–646	44.0 25.4–99.7	325.4 244–479	16.8 12.6–24.7
FUM	6	260.2 151–458	296.3 210–459	43.9 19.6–72.9	163.5 133–260	65.7 57.1–70.9	472.1 237–607	45.5 19.3–61.8	261.5 134–459	13.5 6.9–23.7
MOR	2	527.6 458–607	356.6 334–381	2.31 1.72–3.1	271.3 244–301	46.1 45.0–47.2	363.6 319–415	27.2 20.0–37.0	386.9 372–402	20.0 19.2–20.8
Autumn 1996										
FUM	5	369.5 301–420	320.1 255–424	7.68 3.98–13.2	445.9 328–711	43.3 25.3–73.8	248.5 212–325	4.29 1.56–7.29	405.9 213–545	18.3 10–36.8
Winter 1996–1997										
CAP	5	174.6 71–561	287.7 83–454	0.38 0.23–0.69	333.6 195–887	109.4 57.7–153.7	281.1 240–357	1.74 0.55–2.92	384.2 293–656	16.9 11.2–24.6
FUM	5	82.7 70–115	338.9 268–408	0.3 0.17–0.59	244.2 188–335	139.7 85.9–195.8	574.7 467–752	1.47 0.43–7.6	317.8 170–724	35.3 23.4–55.2
MOR	10	149.0 40–589	329.7 233–490	1.28 0.4–18.4	247.8 181–352	82.7 52.7–104.5	271.4 180–378	0.86 0.1–2.5	219.2 102–407	14.6 8.8–21.9
Spring 1997										
CAP	5	71.5 38–253	535.5 312–936	4.03 0.87–27.5	231.3 168–314	31.2 13.4–52.5	492.9 404–598	0.95 0.52–2	163.3 113–249	9.15 2.8–16.5
MOR	7	200.2 120–301	355.5 234–491	0.49 0.002–6.3	224.9 136–490	29.6 14.6–51.0	583.2 321–722	0.17 0.005–0.94	190.5 147–254	5.78 3.8–9.1
<i>Carassius carassius</i>										
Spring 1996										
CAP	8	264.4 176–468	244.5 151–310	4.22 0.64–23.9	117.0 95–173	38.6 31.5–57.0	370.7 323–514	41.9 28.4–89.8	47.0 35–92	27.1 20.2–52.8
FUM	4	329.4 265–378	213.3 149–264	13.5 3.7–25.0	98.9 87–109	32.7 28.6–35.8	364.2 339–426	44.4 38.5–66.8	40.3 38–45	23.3 22.1–26.0
Autumn 1996										
FUM	3	323.2 116–685	207.8 140–363	60.1 50.5–76.8	106.9 55–270	57.9 27.5–197.2	316.8 266–394	14.5 7.06–23.9	5 9.0 46–67	13.7 10.9–20.0
Winter 1996–1997										
FUM	7	303.9 254–361	93.6 70–158	143.0 97–192	50.6 23–190	14.0 6.7–46.6	244.6 220–275	5.7 0.68–9.51	42.5 35–55	26.0 23.1–28.6
Spring 1997										
CAP	9	377.4 300–591	177.6 122–282	61.0 1.5–718	67.3 44–109	21.5 9.1–35.7	326.9 255–367	8.0 2.9–32.7	25.4 20–40	14.5 9.9–22.1

Table 3 (Continued)

Tissue constituents (mg g ⁻¹ dry wt.)	<i>n</i>	Weight (g)	Liver proteins	Liver glycogen	Liver lipids	Liver phospholipids	Muscle proteins	Muscle glycogen	Muscle lipids	Muscle phospholipids
<i>Ictalurus melas</i>										
Spring 1996 CAP	6	155.4 82–324	421.1 297–530	22.2 5.4–113	134.2 95–248	67.8 51.6–85.7	417.3 274–532	2.17 0.23–7.40	28.2 24–31	21.0 19.8–22.6
Autumn 1996 FUM	10	111.7 38–256	397.1 231–472	85.4 22.0–138	82.2 31–164	48.0 19.6–81.6	450.4 367–498	6.80 3.04–11.72	27.6 23–38	18.0 12.8–22.6
Winter 1996–1997 FUM	6	114.8 55–259	203.3 159–251	256.6 72.6–419	116.1 84–141	58.5 22.3–116	271.0 224–398	1.34 0.70–2.38	53.9 45–88	32.1 27.3–44.4
Spring 1997 CAP	10	93.7 62–169	201.9 148–280	7.3 0.7–62	115.2 79–217	67.5 42.7–94.2	282.7 224–322	0.11 0.07–0.19	43.4 22–98	21.6 12.2–32.4

Table 4
Inter-site and seasonal variations of biological parameters from the French NNRC; Student's *t*-test conclusions

	Sites	Comparisons	d.f.	<i>P</i>
<i>Eels (intersite variation)</i>				
Liver proteins	La Capelière	Spring 1996 vs. Spring 1997	11	0.0058
Liver glycogen	Fumemorte	Spring 1996 vs. Autumn 1996	9	0.0051
	La Capelière	Spring 1996 vs. Winter 1996–1997	10	0.0111
Liver lipids	Fumemorte	Spring 1996 vs. Winter 1996–1997	9	0.0016
	Fumemorte	Spring 1996 vs. Autumn 1996	9	0.0015
	Fumemorte	Spring 1996 vs. Winter 1996–1997	9	0.0305
Liver phospholipids	Fumemorte	Autumn 1996 vs. Winter 1996–1997	8	0.0203
	La Capelière	Spring 1996 vs. Spring 1997	11	<0.0001
	Fumemorte	Spring 1996 vs. Autumn 1996	9	0.0305
	Fumemorte	Spring 1996 vs. Winter 1996–1997	9	0.0013
	Mornès	Spring 1996 vs. Winter 1996–1997	10	0.0110
	La Capelière	Spring 1997 vs. Winter 1996–1997	8	0.0035
Muscle proteins	Mornès	Spring 1997 vs. Winter 1996–1997	15	<0.0001
	Fumemorte	Autumn 1996 vs. Winter 1996–1997	8	0.0013
	Fumemorte	Spring 1996 vs. Autumn 1996	9	0.0045
	La Capelière	Spring 1996 vs. Winter 1996–1997	11	0.0024
	La Capelière	Spring 1997 vs. Winter 1996–1997	8	0.0010
Muscle glycogen	Mornès	Spring 1997 vs. Winter 1996–1997	15	<0.0001
	Fumemorte	Autumn 1996 vs. Winter 1996–1997	8	0.0003
	La Capelière	Spring 1996 vs. Spring 1997	11	0.0009
	Mornès	Spring 1996 vs. Spring 1997	7	0.0001
	Fumemorte	Spring 1996 vs. Autumn 1996	9	0.0002
	La Capelière	Spring 1996 vs. Winter 1996–1997	11	0.0011
	Fumemorte	Spring 1996 vs. Winter 1996–1997	9	0.0001
Muscle lipids	Mornès	Spring 1996 vs. Winter 1996–1997	10	<0.0001
	Mornès	Spring 1997 vs. Winter 1996–1997	15	0.0370
	La Capelière	Spring 1996 vs. Spring 1997	11	0.0026
Muscle phospholipids	Mornès	Spring 1996 vs. Spring 1997	7	0.0004
	La Capelière	Spring 1997 vs. Winter 1996–1997	8	0.0115
	La Capelière	Spring 1996 vs. Spring 1997	11	0.0257
<i>Eels (seasonal variation)</i>	Mornès	Spring 1996 vs. Spring 1997	7	<0.0001
	Fumemorte	Spring 1996 vs. Winter 1996–1997	9	0.0033
	Mornès	Spring 1997 vs. Winter 1996–1997	15	0.0001
	Spring 1996	La Capelière vs. Fumemorte	12	0.0004
Liver glycogen	Spring 1996	Fumemorte vs. Mornès	6	0.0413
	Spring 1996	La Capelière vs. Fumemorte	12	0.0222
Liver lipids	Spring 1996	Fumemorte vs. Mornès	6	0.0302
	Spring 1996	La Capelière vs. Fumemorte	12	0.0002
Liver phospholipids	Spring 1996	La Capelière vs. Mornès	8	0.0014
	Spring 1996	Fumemorte vs. Mornès	6	0.0022
	Winter 1996–1997	Fumemorte vs. Mornès	13	0.0013
	Winter 1996–1997	La Capelière vs. Fumemorte	8	0.0005
Muscle proteins	Winter 1996–1997	Fumemorte vs. Mornès	13	<0.0001
	Winter 1996–1997	La Capelière vs. Mornès	13	0.0187
Muscle lipids	Winter 1996–1997	La Capelière vs. Fumemorte	8	0.0111
	Spring 1997	La Capelière vs. Mornès	10	0.0466
	Winter 1996–1997	Fumemorte vs. Mornès	13	0.0002
<i>Crucian carps (seasonal variation)</i>	Winter 1996–1997	La Capelière vs. Fumemorte	8	0.0009
	Spring 1997	La Capelière vs. Mornès	10	0.0466
Liver proteins	Winter 1996–1997	Fumemorte vs. Mornès	13	0.0002
	Spring 1997	La Capelière vs. Fumemorte	8	0.0009
Liver phospholipids	Spring 1996	Spring 1996 vs. Winter 1996–1997	9	0.0009
	La Capelière	Spring 1996 vs. Spring 1997	15	0.0255

Table 4 (Continued)

	Sites	Comparisons	d.f.	P
	Fumemorte	Autumn 1996 vs. Winter 1996–1997	8	0.0232
Liver glycogen	Fumemorte	Spring 1996 vs. Autumn 1996	5	0.0036
	Fumemorte	Spring 1996 vs. Winter 1996–1997	9	<0.0001
	Fumemorte	Autumn 1996 vs. Winter 1996–1997	8	0.0017
Liver lipids	La Capelière	Spring 1996 vs. Spring 1997	15	0.0007
Liver phospholipids	La Capelière	Spring 1996 vs. Spring 1997	15	0.0022
Muscle proteins	Fumemorte	Spring 1996 vs. Winter 1996–1997	9	0.0001
	Fumemorte	Autumn 1996 vs. Winter 1996–1997	8	0.0205
Muscle glycogen	Fumemorte	Spring 1996 vs. Autumn 1996	5	0.0246
	Fumemorte	Spring 1996 vs. Winter 1996–1997	9	<0.0001
	La Capelière	Spring 1996 vs. Spring 1997	15	0.0003
	Fumemorte	Autumn 1996 vs. Winter 1996–1997	8	0.0300
Muscle lipids	Fumemorte	Spring 1996 vs. Autumn 1996	5	0.0223
	La Capelière	Spring 1996 vs. Spring 1997	15	0.0025
	Fumemorte	Autumn 1996 vs. Winter 1996–1997	8	0.0187
Muscle phospholipids	Fumemorte	Spring 1996 vs. Autumn 1996	5	0.0186
	La Capelière	Spring 1996 vs. Spring 1997	15	0.0025
	Fumemorte	Autumn 1996 vs. Winter 1996–1997	8	0.0006
<i>Black bullheads (seasonal variation)</i>				
Liver proteins	La Capelière	Spring 1996 vs. Spring 1997	14	<0.0001
	Fumemorte	Autumn 1996 vs. Winter 1996–1997	14	<0.0001
Liver glycogen	Fumemorte	Autumn 1996 vs. Winter 1996–1997	14	0.0003
Muscle proteins	La Capelière	Spring 1996 vs. Spring 1997	14	0.0005
	Fumemorte	Autumn 1996 vs. Winter 1996–1997	14	<0.0001
Muscle glycogen	La Capelière	Spring 1996 vs. Spring 1997	14	0.0010
	Fumemorte	Autumn 1996 vs. Winter 1996–1997	14	0.0001
Muscle lipids	Fumemorte	Autumn 1996 vs. Winter 1996–1997	14	0.0002
Muscle phospholipids	Fumemorte	Autumn 1996 vs. Winter 1996–1997	14	<0.0001

eels were less rich in hepatic lipids but glycogen content was relatively higher than in other seasons. In Autumn, their tissues were heavily loaded in lipids and use of such overcharge is indicated by the Winter decrease, except in eels caught in the waters from La Capelière.

The prevalent variations of muscular constitution affected the glycogen content, which displayed seasonal variations, like a large decrease in Winter and an increase in Spring except for the eels from Mornès. Lipids muscular contents were generally stable during the experimental annual cycle, nevertheless, in winter the fishes from la Capelière were fatter than fishes caught in Mornès. Usually, the sampling site had no effect on protein muscular concentrations except in eels from Fumemorte, which exhibited high values in winter. Another point in inter-site diversity deals with the high

glycogen level in muscle of eels from Fumemorte, particularly in Spring.

3.1.4. The crucian carps

In crucian carps, the inter-site analysis is restricted. The fishes were sampled at Fumemorte and La Capelière in Spring and in Autumn and at the Fumemorte site exclusively in Winter. As for eels, differences were significant between fish caught in Spring 1996 and in Spring 1997. They are related to an increase of liver glycogen and a hepatic decrease of proteins, lipids and phospholipids in Spring 1997. In the meantime, muscle glycogen, lipids and phospholipids contents decreased [Table 4, crucian carps (seasonal variation)].

During an annual cycle, crucian carps from Fumemorte showed extensive seasonal fluctuations

Table 5

Pearson's significant correlation between tissue contaminant levels and biological parameters in fish from the French National Nature of Camargue

Biological parameters	Contaminant	Species	Pearson coefficient	<i>P</i>	<i>n</i>
Liver lipids	Liver PCBs	Crucian carps	0.442	0.0576	19
	Liver γ HCH	Black bullheads	0.485	0.0051	31
Liver phospholipids	Liver B(a)P	Black bullheads	0.536	0.0168	19
	Liver PCBs	Crucian carps	0.505	0.0261	19
Muscle lipids	Muscle PCBs	Black bullheads	0.392	0.0257	32
	Muscle γ HCH	Crucian carps	0.460	0.0084	31
Muscle phospholipids	Muscle γ HCH	Crucian carps	0.610	0.0002	31
Liver glycogen	Bile fluoranthene	Crucian carps	-0.673	0.0144	12
	Bile fluoranthene	Black bullheads	-0.600	0.0376	12
	Liver fluoranthene	Eels	0.852	<0.0001	14
	Liver B(a)P	Black bullheads	0.666	0.0013	19
	Liver PCBs	Black bullheads	-0.355	0.0492	31
	Liver PCBs	Crucian carps	0.535	0.0170	19
Muscle glycogen	Bile naphtalene	Black bullheads	0.570	0.0522	12
	Bile anthracene	Black bullheads	0.838	0.0013	10
	Bile fluoranthene	Eels	0.565	0.0209	16
	Bile fluoranthene	Crucian carps	0.621	0.0291	12
	Bile fluoranthene	Black bullheads	0.763	0.0026	12
	Liver phenanthrene	Eels	0.522	0.0447	15
	Liver fluoranthene	Black bullheads	0.708	0.0006	18
	Muscle γ HCH	Eels	0.373	0.0086	48
	Muscle γ HCH	Crucian carps	0.732	<0.0001	31

of hepatic and muscular metabolic constituents. Glycogen burden increased in the hepatopancreas and decreased in the muscle from Spring to Winter. Consequently, in Winter, glycogen was dominant in the hepatic tissue. In addition, in Autumn, the lipid reserves were preferentially hepatic. Crucian carps possessed lean muscles and proteins were largely prevalent but showed a reduction from spring to Winter. Lipid concentration was more important in Winter, and phospholipids decreased from Autumn to Winter.

3.1.5. The black bullheads

The experimental population of black bullheads does not allow statistical analyses about seasonal variations in each caught site, as a consequence only few observations are noticed in Table 4 (black bullheads, seasonal variation). Differences between fish caught in Spring 1996 and Spring 1997 were characterized by a fall in hepatic and muscular protein contents and muscle glycogen.

Catfish livers were less lipid-rich than hepatic tissues of eel and crucian carps, but this lipid reserve did not fluctuate during the experimental

year (Spring 1996–Winter 1996–1997). The glycogen content was variable, nevertheless it represented the more abundant constituent in the liver in Winter. On the contrary, the concentration in muscle glycogen was low (10 times less than eels), as the lipids content. Such energetic reserves varied during the year, but constitutive parameters, proteins and phospholipids were more sensitive to seasonal variations. For instance in Autumn, relative muscular protein concentration was elevated.

3.2. Correlations analysis

We have investigated possible relationships between accumulation of pesticides in fish tissue and biochemical responses (Table 5). Correlation analyses between constitutive parameters of liver and muscle and pesticides concentrations consists of: firstly, in the assessment of (neutral or polar) lipid accumulation; secondly, in the determination of a relationship between contamination and glycogen content, a metabolic parameter considered as a potential biomarker of effect (Schramm et al., 1998; Au et al., 1999).

In eels, no statistical relationship was observed between lipid contents and lipophilic pesticide concentrations, neither in liver nor in muscle. In crucian carps liver the PCB concentration was significantly correlated to the amount of lipid and notably to phospholipid content; whereas in muscle, γ BHC concentration showed an evolution analogous to that of total lipid and phospholipid concentrations. In catfish liver, γ BHC was related to total lipids and PCBs to phospholipids only. In muscle, a significant correlation was found between PCBs concentration and total lipid contents.

In the three species, fluoranthene concentrations were correlated with glycogen contents. Concerning this PAH, the more significant positive correlation appeared in liver eels, whereas the positive relationship between bile fluoranthene concentration and muscle glycogen was similar in all the species. Surprisingly, in crucian carps and black bullheads, an opposite correlation between this molecule and liver glycogen was detected. Yet usually, other correlations were positive, except in black bullheads between liver glycogen and liver PCBs. In addition, the two positive relations between γ BHC and glycogen concentrations must be noticed in muscle eels and in crucian carps.

4. Discussion

The occurrence of chemical contaminants in agricultural environments and the atmospheric transport of industrial emissions lead to exposure and accumulation of pollutant residues in animal tissues from exposed communities. But contamination can be a subsequent effect of previous use of chemicals which are now banned (Fries, 1995). An evaluation of pollutant impacts in fishes from the French National Nature Reserve of Camargue has been carried out from Spring 1996 to Spring 1998. The aim of this study was to assess the extent of major POP contamination, and to achieve the identification and validation of biomarkers in a protected area. Tissue micropollutant (OCs and PAHs) concentrations and their impact on biological parameters have been appraised in three dominant species: eel; crucian carp; and black bullheads.

These three species display significant demoeological and ecophysiological differences such as habitat, means of reproduction and biotic potential, trophic relationships as well as tissue constitution

and metabolic activity. Moreover, aquatic species are sensitive to seasonal variations of biotic and abiotic factors regulating metabolic mechanisms and pesticides bioaccumulation. In addition, rates of persistent lipophilic pollutants in fish depend on the diet and on the pace of transfer across the gills. Therefore, such uptake has a significant effect on toxicant concentration in the body (Randall et al., 1998).

Higher trophic organisms accumulate these contaminants to a raised level (Stange and Klungsoyr, 1997; Dewailly et al., 1999). In a recent study about different food chain levels in the North Pacific Ocean, Miao et al. (2000) stated that high trophic species such as eels highly bioaccumulate PCBs. The reality of inter-species variations involves a potential diversity in responses in relation to persistent chemical exposition. In the present state of our investigations we may conclude that all the investigated micro-contaminants occur in every fish sample. Nevertheless, the magnitude and localization of accumulation in tissue compartments and metabolic responses vary as a function of trophic level. In our work this fact translates into a high variability of pesticides accumulation due to a significant discrepancy between extreme values.

The PAHs were detected in the bile and liver of fish at quite variable rates. They were more abundant in the bile of eels and black bullheads and in the liver of crucian carps. It seems difficult, at this stage of our research, to sort out the most representative molecule of this contamination, although hydrocarbons which are the most ubiquitous in the fishes investigated are naphthalene, phenanthrene and B(a)P.

The hepatic concentrations fluctuated all year long depending on the sampling season: whatever the species, high levels of PAHs contaminants were found in Winter, in particular B(a)P in fish from Fumemorte. Thus, our results show an annual cyclic variation, which seems chronologically related to the seasonal metabolism requirement in eels. However, up to now we are unable to identify a significant variation between the samples coming from different sites. Such homogeneity of geographical repartition suggests an atmospheric transfer of these pollutants. Additionally, in this study, demersal fishes such as catfish, living in the bottom waters with recurrent contact with the sediments, a famous pollution storage compartment, were sometimes less contaminated than eels

or crucian carps, for example by the fluoranthene and the B(a)P in Spring or by the naphthalene in Winter (see Table 1). However, all the PAHs investigated were present though at a variable extent.

The OC contamination was characterized by the invariant presence of lindane and PCB in tissues; lindane was prevalent in muscle (mainly in eels), whereas PCBs were abundant in the liver (mainly in crucian carps). Taking apart these two compounds, considerable tissue concentrations of *pp'*-DDE and dieldrin were found in eels from Fumemorte or in crucian carps from La Capelière (Roche et al., 2000). Seasonal variations were obvious in fish coming from the Fumemorte channel for which contamination was much lower in Autumn than in Spring. However, this site is located at the east of the Vaccarès lagoon near the outlet of irrigation waters of the rice plantations (and various cultures). Logically, in Spring, the insecticide treatment period, the most lindane-contaminated eels were found.

The total OCs burden was less in the crucian carps than in eels. These observations are related to the diet of such preferentially herbivorous fishes, which are located at a lower level in the aquatic trophic web. One might as well quote the rather low bioaccumulation factor observed related to a poor-lipid muscular tissue. However, the hepatic accumulation of PCBs is preferentially located in membrane structures (lipid bilayer), according to the high biomagnification potential hypothesis for such chemicals. The lipid composition influences largely the incorporation of OC and probably their toxicity (Antunes-Madeira and Madeira, 1989; Bremle and Ewald, 1995), in addition they are metabolized slowly (Kutz et al., 1991).

The levels of energy metabolic reserves differed largely from one species to another, moreover, they varied according to season and capture site. No statistical correlation was detected between the neutral lipid contents and contaminant concentrations among the eels investigated, in spite of their muscle being particularly fatty. On the other hand, the suggested hypothesis (Roche et al., 2002), about a preferential accumulation in hepatic membrane structures is supported by the correlation analysis results for lindane and PCBs in the crucian carps and black bullheads. Nevertheless, in the latter non-fatty fishes, the rates of some lipophilic substances (liver γ BHC and muscle PCBs) were also strongly correlated with the

contents of neutral lipids. This observation suggests the role of lipid quality and composition (i.e. relative amount of fatty acids saturation) in the tendency of OC to bioaccumulate (Ewald and Larsson, 1994).

Several significant correlations were shown between hepatic or muscular glycogen and pesticides concentrations in the three fish species. They were generally positive with muscle glycogen and can be negative with liver glycogen, in particular in the crucian carps (bile fluoranthene) and the black bullheads (liver PCBs and bile fluoranthene). The variability of such energy parameters is largely demonstrated. For example, the fishes from the Fumemorte channel displayed in Spring a high content of hepatic glycogen, in the same way the muscle glycogen rate was higher in Spring for the majority of fish except for eels from Mornès. Thus, the increase of the glycogen contents proves to be a significant marker of the organic contamination in the three species. Nevertheless, these observations are contradicting those classically described. Regarding glycogen concentration, a number of contradictory results have been described in ecotoxicological studies. Acute intoxication may lead to a glycogen depletion (Gimeno et al., 1995; Sancho et al., 1998; Strmac and Braunbeck, 1999; Walter et al., 2000) as a consequence of feed refusal and a decrease in gluconeogenesis, often dose-dependent, due to a diminished activity of key gluconeogenic enzymes (Feeley, 1995; Viluksela et al., 1999). Moreover, Braunbeck and Appelbaum (1999) described glycogen and lipid depletion and observed gut and liver ultrastructural changes at extremely low doses of endosulfan in carp. On the contrary, Thomas et al. (1999) have shown a lack of such physiological response in PAHs exposed mussels. They suggest that chronically exposed organisms may develop a physiological tolerance to these pollutants. In the same way, Oruc and Uner (1998) concluded that in *Cyprinus carpio*, an elevation of glycogenolysis occurs following an acute chemical stress and compensatory mechanisms are developed during chronic exposure.

The positive correlation between glycogen content and concentration of micropollutants in fishes of the Camargue Reserve, tends to show a coexistence of a pathological alteration and a general stimulation of hepatic metabolism. This result brings elements for discussion between ecotoxicological data collected in controlled or in situ

conditions and on the other hand, in polluted or protected areas.

5. Conclusion

This paper relates the first study on organic contamination in fish from the protected area of Camargue (France), and more broadly ranks among the few ones ever achieved which concerns such a problem in a coastal wetland reserve from the whole Mediterranean coast.

The absence of a relation between tissue PAH amounts and the sites of capture leads to think of an atmospheric origin of these pollutants found at any season in the biomass. On the other hand, OCs compounds concentrations are unquestionably the consequence of the OC pesticides employment, especially lindane-insecticide allowed in France until August 1998. Moreover, our results suggest a prolonged use, if not persisting, of prohibited substances, such as dieldrin in the rice fields. It is now well documented that the contaminants do not have the same level of metabolization or storage in various tissues. Chronologically, concerning PAHs, the bile accumulation is an indication of a recent intoxication and the liver, location of detoxification metabolism, would give a longer-term image of contamination. The muscular tissue may represent storage if accumulation was related to the use of the energy reserves. Repartition of xenobiotic molecules in the lipid compartments is not systematic in fish from Camargue. We have proved that the incorporation by some of them is located preferentially in the membrane structures (Suwalsky et al., 1998; Donato et al., 2000; Roche et al., 2002).

The glycogen is a sensitive marker of the state of energy reserves. Nevertheless, we updated in eels and crucian carps, relations between this metabolic constituent and enzymatic activities of organism defense (results not yet published). However, in contradiction with some laboratory acute intoxication studies (Oruc and Uner, 1998; Sancho et al., 1998), we have shown a positive correlation between tissue concentrations of contaminants and muscular glycogen amount. If we turn now to the implication of our results regarding the conservation of the aquatic habitats of the reserve, some major conclusions have to be drawn. First of all, one must emphasize that the level of OCs contamination found in the fishes from the NNRC are lower than those documented elsewhere. For

instance in eels, from Tern Island (North Pacific Ocean), the total average PCB concentrations were as high as $96 \mu\text{g g}^{-1}$ dry wt. (Miao et al., 2000), against 70 ng g^{-1} dry wt. in eels from Camargue. In fishes from Michigan waters (including the Great Lakes), Nan et al. (2000) found polychlorinated naphthalenes concentrations up to $31\,400 \text{ pg g}^{-1}$ wet wt. (i.e. approx. 125.6 ng g^{-1} dry wt.). Moreover, PAH biliary concentrations are higher in fish from Camargue lagoon (average $\approx 4700 \text{ ng ml}^{-1}$, work in progress) than in fish from European high-altitude mountain lakes. Indeed, Escartin and Porte (1999) have shown that biliary levels of hydroxylated PAHs ranging from 69 ng ml^{-1} bile in trouts from Redo Lake (Spanish Pyrenees) to 990 ng ml^{-1} bile in those sampled in Bedoichov Lake (Czech Jizera Mountains).

The levels of contaminants in fishes from the NNRC investigated in the present study are less a matter of concern than the case studies thereupon quoted. Nevertheless, the levels of dieldrin—a banned but still used OC insecticide—and even those of lindane and PCBs, if sustained in the future, could really jeopardize the health of the wetland ecosystems from this Reserve and threaten the populations of piscivorous birds, a major component of its protected biodiversity.

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