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Reintroduction of the Otter (*Lutra lutra*) into Catalan Rivers, Spain: Assessing Organochlorine Residue Exposure Through Diet

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Otter (*Lutra lutra*) populations have been declining in Europe for the last four decades. Several causes, such as hunting, habitat destruction or human disturbances have been involved, but pollution from organochlorine compounds (OCs), and especially polychlorinated biphenyls (PCBs) seemed to play an important role in this decline. Over the last years, PCB concentrations in otter tissues have declined annually some 7-8% and otter populations are recovering in some parts of Europe (Mason 1998).

In Spain, thriving otter populations are present in the West, but have become extinct in many Mediterranean rivers. In Catalonia (NE Spain), otters were present in only 3.1% of 392 transects of rivers in 1984 (Delibes 1990). In the last four decades, a marked decline in otter population has taken place in this region. This is also the case in the Muga and Fluvià rivers, where otters were common in the 1950s but became extinct in the 1980s. However, since the 1980s habitat conservation has been improved as a result of the establishment of four Natural Parks created in the surroundings of these rivers and by the regeneration of the old wetlands in the Empordà marshes, which created a more suitable habitat for the otter. Moreover, the preservation of the riparian forest represents an opportunity to maintain a corridor for animals from different reserves.

All these facts encouraged otter reintroduction programs in these Catalan basins. However, an assessment of the levels of known pollutants responsible for otter declines were necessary. The levels of OCs in freshwater fish from the two studied basins were scarce (López-Martín et al. 1995), and further analyses were needed. This paper describes the results of the analysis of OCs in the prey of otters in the Muga and Fluvià basins in 1994 and discusses the feasibility of the otter reintroduction project.

MATERIALS AND METHODS

Along the rivers Muga and Fluvià, and the Empordà marshes (Aiguamolls de l'Empordà Natural Park) at the mouths of both rivers (Figure 1), six, six and five sampling points, respectively, were analyzed. The Muga river irrigates a farming area,

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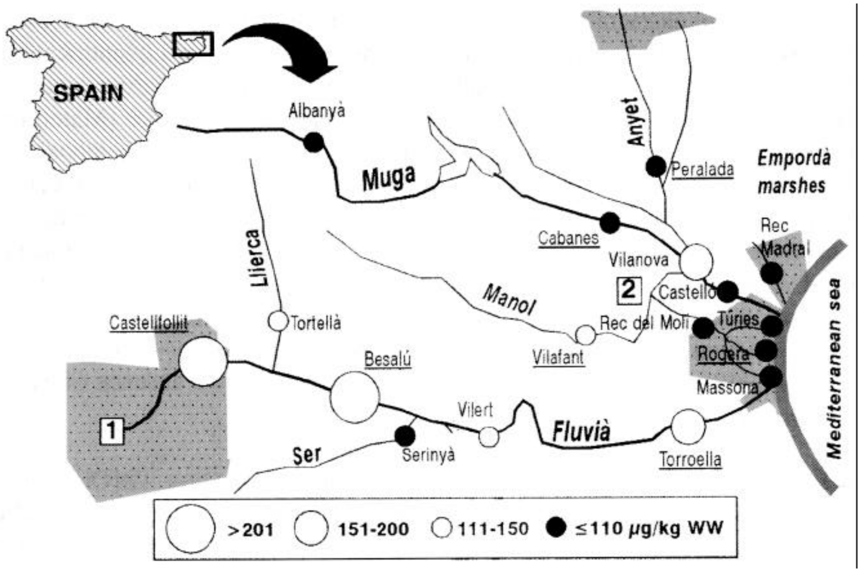

Figure 1. Study area with indication of concentrations of ΣPCBs in µg kg^{-1} WW of fish in the sampling sites. Dark circles correspond to sites with ≤ 10 µg kg^{-1} WW and where otter reintroduction could be carried out. Those sites sampled several times during the year are shown underlined. Shaded areas correspond to Natural Parks and numbers 1 and 2 correspond to the towns of Olot and Figueres, respectively.

and only in the middle and low stretches receives significant urban sewage from the town of Figueres; the Fluvià river crosses an industrial area at Olot near its source and farming lands downstream. The lagoons of the Empordà marshes are flooded from some channels and marine water in the coastal salty lagoons. To study fluctuations in OC levels during 1994, four samples (one per season) were collected at seven points, while the rest were sampled only in winter.

Sampling was carried out by electro-fishing and the first 10 fish were captured, although in some cases this number was not reached. Anguillidae (*Anguilla anguilla* (n, mean weight±s.d. = 105, 73±76 g)), Mugilidae (*Mugil cephalus* (25, 225±259 g), *Chelon labrosus* (3, 404±383 g), *Liza aurata* (9,464±450 g), *Liza ramada* (14, 238±268 g)) and Ciprinidae (*Barbus meridionalis* (126, 57±49 g), *Leuciscus cephalus* (15, 257±147 g), *Rutilus rutilus* (19, 55±35 g), *Scardinius erythrophthalmus* (8,79±101 g), *Cyprinus carpio* (39, 712±618 g)) were analyzed and taken as representative of the potential diet of otters, because prey selection in Spain was assumed to depend mainly on their abundance (Ruiz-Olmo, 1995). Fish from each sample were weighed, ground whole, pooled and an aliquot of 50 g was stored at -20°C until analysis.

Preparation of samples and analyses were done following the method described elsewhere (Mateo *et al.* 1998). Briefly, 2 g of the pooled samples were extracted and cleaned-up with sulfuric acid (Murphy 1972), using part of each sample to calculate the percentage of extractable fat by gravimetry . PCBs #l and #209 were used as internal standards. High resolution chromatographic analyses and quantification of OC residues, using the corrected Ballschmiter and Zell nomenclature system for PCBs (Guitart *et al.* 1993) have been published and detailed before (Mateo *et al.* 1998). A mixture (1:1) of Aroclor 1254 and Aroclor 1260, with the composition of PCB congeners reported by Schulz *et al.* (1989), was used to quantify PCBs cited in Table 1. These are the congeners of PCBs reported in previous studies in freshwater fish from this area (López-Martín *et al.* 1995). Recoveries with fortified samples showed values that ranged from 70 to 100%, except for endrin and dieldrin (0%). Corrections based on recovery data were not introduced in quantification. Variability, as estimated from coefficients of variation of repeated analysis (n=4) was between 1 and 8%. Blanks were processed between samples to check the absence of external contamination.

Concentrations were normalized by logarithm transformation (ln[OC]+l) and parametric tests were used. Differences in OC concentrations between areas (Muga, Fluvià and Empordà Marshes) and seasonality were studied with a two-way ANOVA test without interactions. Ratio ΣDDTs/ΣPCBs did not show a log-normal distribution and were analyzed with a Kruskal-Wallis test. Linear correlations between OC levels and characteristics of the sample (% of weight of cyprinidae, mugilidae and anguillidae, and % of extractable lipid) were studied.

RESULTS AND DISCUSSION

Organochlorine residues in fish samples (Table 1) were mainly ΣPCBs, followed by ΣDDTs (sum of p,p'-DDE, p,p'-DDD and p,p'-DDT) and ΣHCHs (sum of hexachlorocyclohexane isomers). These levels found in the analyzed samples are in the order of those described in fish captured between 1990 and 1992 in Catalan rivers (López-Martín *et al.* 1995). The highest correlations were obtained between ΣPCBs and hexachlorobenzene (HCB) levels (r=0.67, p<0.001).

Differences in OC levels were found between areas (Table 2). Level of p,p'-DDT was significantly higher in the Muga and Fluvià river samples than in the Empordà marshes (p<0.01). The area with highest ΣPCBs concentration was the Fluvià river (p<0.001), that also showed the lowest ratio ΣDDTs/ΣPCBs (p<0.01). The highest levels of ΣPCBs were located just downstream of the two most populated towns, Olot in the Fluvià basin and Figueres in the Muga basin. Seasonality was assessed for γ-HCH (lindane) level: it was higher in spring (geometric mean=6.6 µg kg^{-1} WW; 95% C.I.=2.0-18.2) than in winter (1.7; 1.0-2.6;p<0.01). This finding has also been described analyzing otter faeces from SW England (Mason and Macdonald 1994), and probably is related to the widest use of this pesticide in spring.

The OC levels were also related to fish species pooled in each sample (Figure 2). Two factors, lipid content and migratory behavior, could explain it. In relation to the lipid contents, Ruiz and Llorente (1991) have detected higher levels of ΣDDTs and ΣPCBs in eels than in carps from the Ebro delta (SE Catalonia), and it was mainly due to differences in extractable lipids (20% in eels and 0.5% in carps). On the other hand, the migration of mugilidae to the less polluted sea waters (Albaigés *et al.* 1987) could explain the lower OC levels detected in the Empordà marshes or in the low stretches of Fluvià and Muga rivers.

Table 1. Organochlorine residue levels (μg kg^{-1} WW) in pooled fish samples (n=37).

Compound	Geometric mean	95% C.I.	Range
HCB	2.0	1.6-2.4	0.6-8.6
α-HCH	0.9	0.7-1.1	0.2-3.1
β-HCH	3.0	2.7-3.4	0.6-6.5
γ-HCH	2.5	1.8-3.5	0.5-20.0
δ-HCH	0.4	0.3-0.5	n.d.-1.0
Heptachlor	0.6	0.4-0.8	n.d.-2.1
Heptachlor epoxide	0.2	0.0-0.3	n.d.-6.5
Aldrin	0.5	0.4-0.6	n.d.-1.5
α-Endosulfan	0.7	0.4-1.1	n.d.-8.4
Endosulfan sulphate	1.0	0.8-1.3	n.d.-4.2
p,p'-DDE	16.6	12.3-22.5	1.2-80.8
p,p'-DDD	4.0	2.9-5.3	n.d.-16.9
p,p'-DDT	4.7	3.5-6.2	0.7-34.8
PCB #66+95	12.6	8.7-18.0	n.d.-92.3
#90+101	12.0	8.9-15.9	1.9-70.4
#82+151	2.9	2.0-4.1	n.d.-14.8
#135	5.3	3.9-7.1	0.4-45.8
#123+149	1.5	0.7-2.8	n.d.-20.0
#118	4.1	2.7-6.2	n.d.-39.9
#153	20.0	15.0-26.6	3.1-86.7
#141	2.1	1.5-2.9	n.d.-8.8
#179	0.5	0.3-0.7	n.d.-2.9
#160+138+158	11.1	8.4-14.7	2.4-50.1
#187	5.1	3.9-6.6	0.5-17.2
#128	3.1	2.4-3.9	1.0-13.6
#174	2.1	1.6-2.7	n.d.-8.0
#177	1.8	1.4-2.4	n.d.-6.5
#202+171+156	4.2	3.2-5.5	n.d.-18.4
#180+193	7.0	5.2-9.3	0.9-32.7
#203+196	1.1	0.9-1.4	0.2-3.4
#208+196	0.5	0.5-0.6	0.2-1.3
#194	0.8	0.6-1.0	n.d.-2.4
ΣPCBs	107.2	81.0-136.4	18.6-426.5

The presence of anguillidae in the pooled fish samples was correlated positively with the OC contents, mainly with p,p'-DDT (r=0.44, p<0.01) and p,p'-DDD (r=0.35,

$p<0.05$) levels, but this effect could be explained by the high lipid content of anguillidae, because the presence of anguillidae in the samples was correlated positively with lipid content ($r=0.53$, $p<0.001$). If concentrations were referred to lipid weight, the OC levels, in particular ΣPCBs, were correlated positively with the percentage in weight of cyprinidae in the pooled sample ($r=0.44$, $p<0.01$). One explanation could be that cyprinidae are the predominant species in the samples taken from the most polluted areas of the Fluvià River.

Table 2. Geometric mean (95% C.I.) of organochlorine levels (µg kg^{-1} WW) and arithmetic mean of % of lipid weight (SE) and ratio ΣDDTs/ΣPCBs (range) in fish samples from the studied zones.

Zone/Site	n	% Lipid	HCB	ΣHCHs	ΣDDTs	ΣPCBs	ΣDDTs/ ΣPCBs
Fluvià River							
Castellfollit	4	1.97 a*	4.0 a	12.0 a	52.8 a	281.8 a	0.19 a
		(0.18)	(2.5-6.1)	(4.8-28.1)	(18.7-146.0)	(136.3-581.7)	(0.15-0.22)
Tortellà	1	0.60	2.2	5.1	21.3	137.1	0.16
Besalú	4	2.01 a	3.4 ab	12.0 a	34.1 a	253.1 a	0.15 a
		(0.39)	(1.8-5.8)	(4.8-27.8)	(19.8-58.1)	(101.4-629.8)	(0.09-0.25)
Serinyà	2	1.04	1.0	5.4	3.9	58.3	0.07
Vilert	1	0.89	1.1	4.5	14.1	140.8	1.0
Torroella	4	2.26 a	2.1 b	5.6 a	37.8 a	158.8 a	0.24 a
		(0.43)	(1.9-2.2)	(3.6-8.5)	(26.0-54.8)	(117.4-217.4)	(0.20-0.27)
Total	16	1.79 A	2.5 A	8.0 A	27.9 A	179.1 A	0.17 B
		(0.20)	(1.9-3.3)	(5.9-10.9)	(17.3-44.6)	(123.0-260.6)	(0.05-0.27)
Muga River							
Albanyà	1	0.50	1.0	4.3	3.9	18.6	0.21
Cabanes	4	1.75 a	1.2 a	9.4 a	19.4 b	82.4 a	0.26 b
			(0.7-2.0)	(3.0-25.8)	(6.5-54.7)	(31.9-210.7)	(0.16-0.51)
Peralada	4	2.16 a	1.2 a	4.7 a	71.0 a	64.1 a	1.15 a
		(0.38)	(0.6-1.9)	(2.5-8.3)	(38.7-129.3)	(57.8-71.1)	(0.78-1.55)
Vilafant	3	1.90 a	2.0 a	6.6 a	29.4 ab	124.4 a	0.24 b
		(1.00)	(0.5-5.0)	(4.6-9.2)	(12.4-67.6)	(94.0-164.5)	(0.18-0.29)
Vilanova	1	2.46	8.6	8.6	60.9	179.3	0.34
Castelló	1	1.88	3.2	9.7	56.5	110.8	0.51
Total	14	1.87 A	1.7 A	6.8 A	32.5 A	81.5 B	0.53 A
		(0.27)	(1.1-2.5)	(5.1-9.0)	(19.4-54)	(57.5-115.3)	(0.16-1.55)
Empordà M.							
Rec Madral	1	0.57	1.4	4.3	25.4	41.3	0.61
Turies	1	0.89	1.3	10.2	12.7	50.9	0.25
Rogera	3	1.07	1.6	9.9	14.8	56.1	0.27
		(0.34)	(1.1-2.2)	(4.4-21.0)	(6.7-31.7)	(20.4-150.8)	(0.22-0.31)
La Massona	1	0.39	1.1	5.6	4.1	31.9	0.13
Rec del Molí	1	0.32	1.2	4.9	9.4	81.1	0.12
Total	7	0.77 B	1.4 A	7.4 A	12.4 A	51.5 B	0.27 AB
		(0.18)	(1.2-1.6)	(4.9-10.9)	(7.2-20.9)	(36.5-72.6)	(0.12-0.61)

*Differences among zones or sites into a zone are represented with capital and lower-case letters, respectively. Means with the same letter do not differ significantly (Tukey and Kruskal-Wallis tests).

Otters in Catalonia are present in rivers where prey contain less than 110 µg kg^{-1} WW of ΣPCBs (Ruiz-Olmo 1995). In our study area, this critical level was not reached in

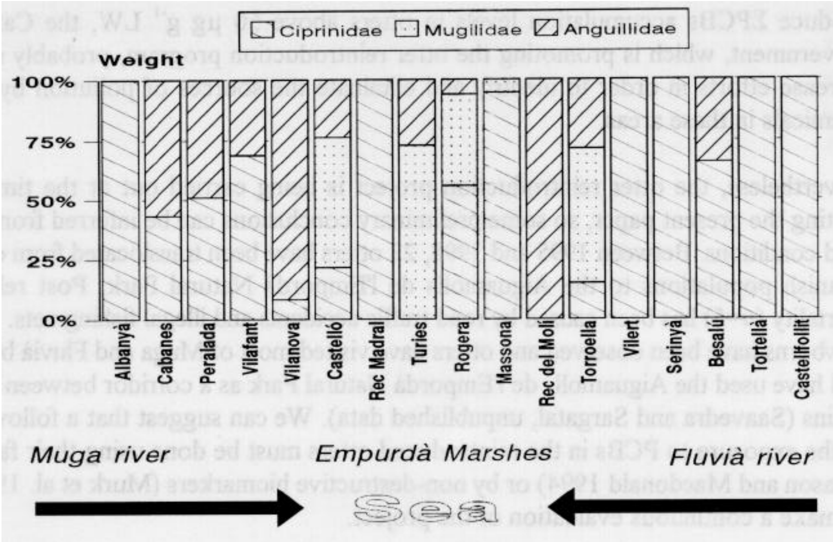

Figure 2. Composition of the samples analyzed throughout the study area (% of a family by weight).

67% (4/6) of the sampling points in the Muga basin, 17% (1/6) in the Fluvià basin and 100% (5/5) in the Empordà marshes (Figure 1). Considering the number of samples, instead of the sampling points, 57% (8/14), 6.25% (1/16) and 100% (7/7) had less than 110 µg kg^{-1} WW ΣPCBs, respectively. Although the most toxic coplanar PCB congeners (non-ortho) were not determined with the method employed here, the risk assessment has been done with the same PCB congeners studied by Ruiz-Olmo (1995). Some mono-ortho (#118) and di-ortho (#128, #135 and #194) congeners were determined individually and their concentrations appear in Table 1. The concentrations of these mono-ortho and di-ortho substituted congeners were significantly correlated with the concentration of ΣPCBs (r=0.841 and r= 0.978, respectively, both p<0.001).

Although the reintroduction could be undertaken in the Empordà Marshes (Aiguamolls de 1'Empordà Natural Park), the movement of otters between these marshes and both rivers could increase the exposure to PCBs above the critical level. If we assume the bioconcentration factor x2.9 of PCBs from fish to otter tissues obtained for Catalan rivers (López-Martín and Ruiz-Olmo 1996), then a mean (95% C.I.) level of ΣPCBs in the tissue of reintroduced otters of 22.6 (17.4-29.0) µg g^{-1} LW (lipid weight) can be predicted. Leonards *et al.* (1997) have found a higher biomagnification factor (x14), on the basis of different PCB congeners. The estimated accumulation in otters with the first factor is also below the critical level of 50 (40-60) µg g^{-1} LW. This level is related to reproductive failures in other mustelid species such as mink (Leonards *et al.*, 1995) and to pathologic changes in otters (Smit *et al.* 1996; Murk *et al.* 1998), but its implications on otter populations have been discussed (Kruuk and Conroy 1996). Although only 18.9% of collected samples can produce ΣPCBs accumulation levels in otters above 50 μg g^{-1} LW, the Catalan Government, which is promoting the otter reintroduction program, probably must increase efforts in order to identity and eliminate the sources of pollution by OC chemicals in these areas.

Nevertheless, the otter reintroduction project is being carried out at the time of writing the present paper, so some preliminary conclusions can be inferred from the field conditions. Between 1995 and 1998, 23 otters have been translocated from other Spanish populations to the Aiguamolls de 1'Empordà Natural Park. Post release mortality (n=5) has been caused by road traffic accidents and illegal fishing nets. Two newborns have been observed and otters have visited most of Muga and Fluvià basins and have used the Aiguamolls de I'Empordà Natural Park as a corridor between both basins (Saavedra and Sargatal, unpublished data). We can suggest that a follow-up of the exposure to PCBs in the reintroduced otters must be done using their faeces (Mason and Macdonald 1994) or by non-destructive biomarkers (Murk et al. 1998) to make a continuous evaluation of the project.

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