SEASONAL VARIATION OF DDT AND PCB ACCUMULATION IN MUSCLE OF CARP (CYPRINUS CARPIO) AND EELS (ANGUILLA ANGUILLA) FROM THE EBRO DELTA, SPAIN

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DDT
PCB
CARPS
EELS
EBRO DELTA

ABSTRACT – The seasonal variations in PCB and DDT accumulation levels in carp and eels from the Ebro Delta troughout an annual cycle are described. The two types of organochlorine compounds are ubiquitous in the samples analyzed. Variations in accumulation patterns are related to biological events (e.g. reproduction) and environmental changes (water flow, pesticide treatment, etc.). When comparing the levels of pollutant accumulation in carp (detritivore-herbivore) and eels (carnivore), PCB levels reached in the two species are very similar, while DDTs display an important variation, being higher for eels than carp. The total organochlorine load in eels from the Ebro Delta is lower than in eels from a similar ecosystem, the Venice Lagoon. This difference is mainly due to a greater PCB load in Venetian eels. Conversely, DDT concentrations are higher (double) in eels from the Ebro Delta. The PCB and DDT concentrations in carp and eels from the Ebro Delta are lower than the tolerance levels set by the U.S.F.D.A., but controls extended to further species are needed in order to assess risk factors associated with organochlorine pollution in this area.

DDT
PCB
CARPE
ANGUILLE
DELTA DE L'EBRE

RÉSUMÉ – Les variations saisonnières de PCB et du DDT des Carpes et des Anguilles du delta de l'Ebre sont suivies pendant un cycle annuel. Les deux types de composés organochlorés sont omniprésents dans les échantillons analysés. Les niveaux d'accumulation sont mis en relation avec les différentes phases du cycle biologique des deux espèces de Poissons (reproduction) et aussi par les changements d'ordre écologique (flux aquatique, traitements phytosanitaires). Quand on compare les niveaux d'accumulation des Carpes (détritivores-herbivores) et des Anguilles (carnivores), on constate que les niveaux de PCB des deux espèces sont très similaires. Au contraire, les DDT montrent une variation importante, présentant une concentration plus élevée pour les Anguilles que pour les Carpes. La quantité totale de pesticides organochlorés des Anguilles du delta de l'Ebre ne dépasse pas les niveaux de tolérance donnés par l'U.S.F.D.A., mais les contrôles doivent être étendus à d'autres espèces pour pouvoir évaluer les facteurs de risque associés avec la pollution par des organochlorés dans cette zone méditerranéenne.

INTRODUCTION

The Ebro Delta is a natural ecosystem which has been highly stressed by human activities (Ruiz *et al.*, 1981). It is also the wetland environment most highly polluted by organochlorines of all those studied in Spain (Alberto 1979; Ruiz *et al.*, 1979; Alberto & Nadal, 1981; Grimau, 1983; Ruiz, 1982; Llorente, 1984) and the only area on the European Mediterranean coast where p.p'. DDE and PCBs have been claimed to produce a decrease in avian eggshell thickness (Ruiz *et al.*, 1983; Llorente, 1984).

Nevertheless, no detailed study of pollution by organochlorines on fish from the Ebro Delta coastal lagoons has been undertaken up to now.

In this paper we estimate the variations of DDTs and PCBs in fish from the Ebro Delta throughout an annual cycle (see study area). In order to do so, we have chosen two species, both of which are very abundant and consumed by humans : Carp (*Cyprinus carpio*) and eel (*Anguilla*

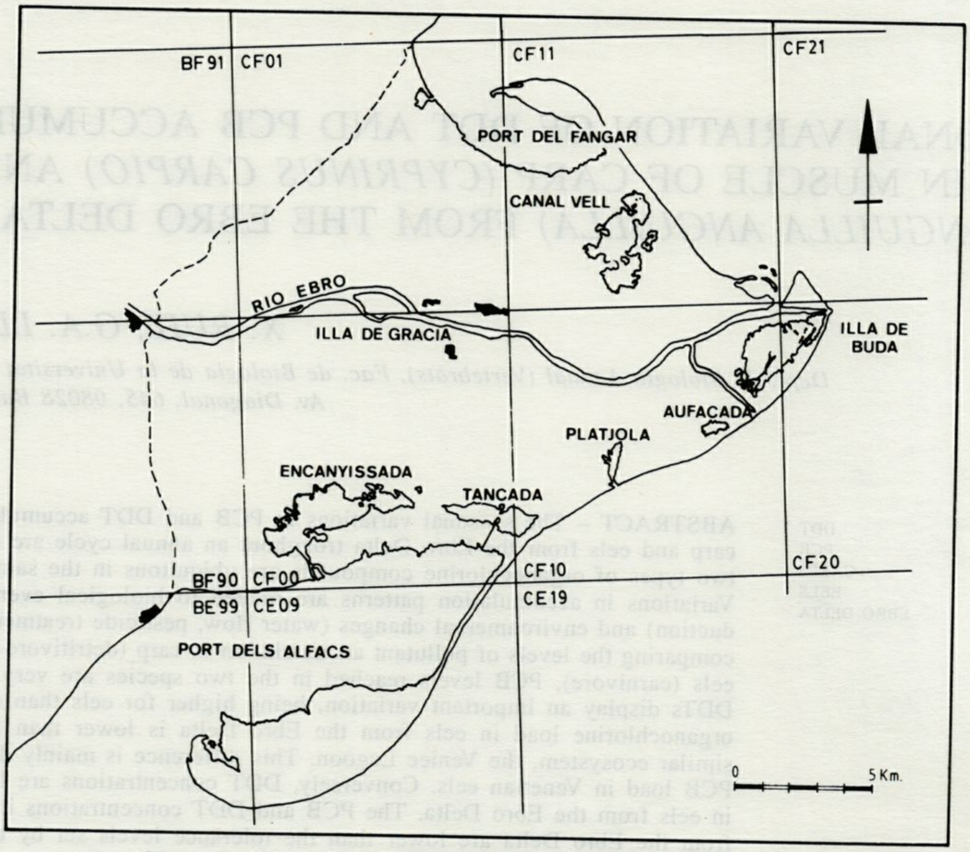

Fig. 1. – Map of the Ebro Delta showing sampling locality.

anguilla). These two species are situaed at different levels in the food-web of the Delta ecosystem. Carp is a detritivore-herbivore and the eel is a carnivore. This provides the basis for discussing the relative importance of biomagnification and of the seasonal variations in the organochlorine accumulation in those fish.

Prospects of creating fish and aquaculture developments in the Ebro Delta add further relevance to the evaluation of the organochlorine pollution impact on fish from this area.

STUDY AREA

The Ebro Delta (Fig. 1) is an alluvial plain which is over 350 km^2 in extension. It is cultivated intensively and rice fields occupy aprox. 40 % of the total surface area.

Rice cultivation needs a constant water supply because rice fields must always be flooded. Water is diverted from the Ebro river into the fields by means of an extensive network of irrigation channels all over the Delta plain. The cultivation cycle begins in April, when the channels are opened and the fields are flooded by water. Thereafter, active circulation of water continues down to the coastal lagoons until the end of rice harvesting in November. Channels are closed in December.

This hydrological cycle has great biological significance because it regulates the dynamics of productivity in the Ebro Delta and determines the timing of reproduction for most species (Ruiz *et al*., 1981; Ruiz, 1985; Llorente & Ruiz, 1985). Moreover, organochlorine pollutants and pesticides in general reach wildlife through this cycle.

MATERIAL AND METHODS

Sampling

Carp and eels were sampled during 1985 at monthly intervals in the Canal Vell lagoon or in the channels near it (Fig. 1). The specimens were measured, weighed, labelled, and preserved frozen at – 20°C until analysis.

From these collections we prepared composite samples of dorsal muscle for both species separately, following Bernhard's methods (1976), in order to obtain direct mean levels for DDT and PCB, as well as for extractable lipid on a fresh weight basis. It has to be noted, however, that data calculated on a fat basis would only be arithmetic

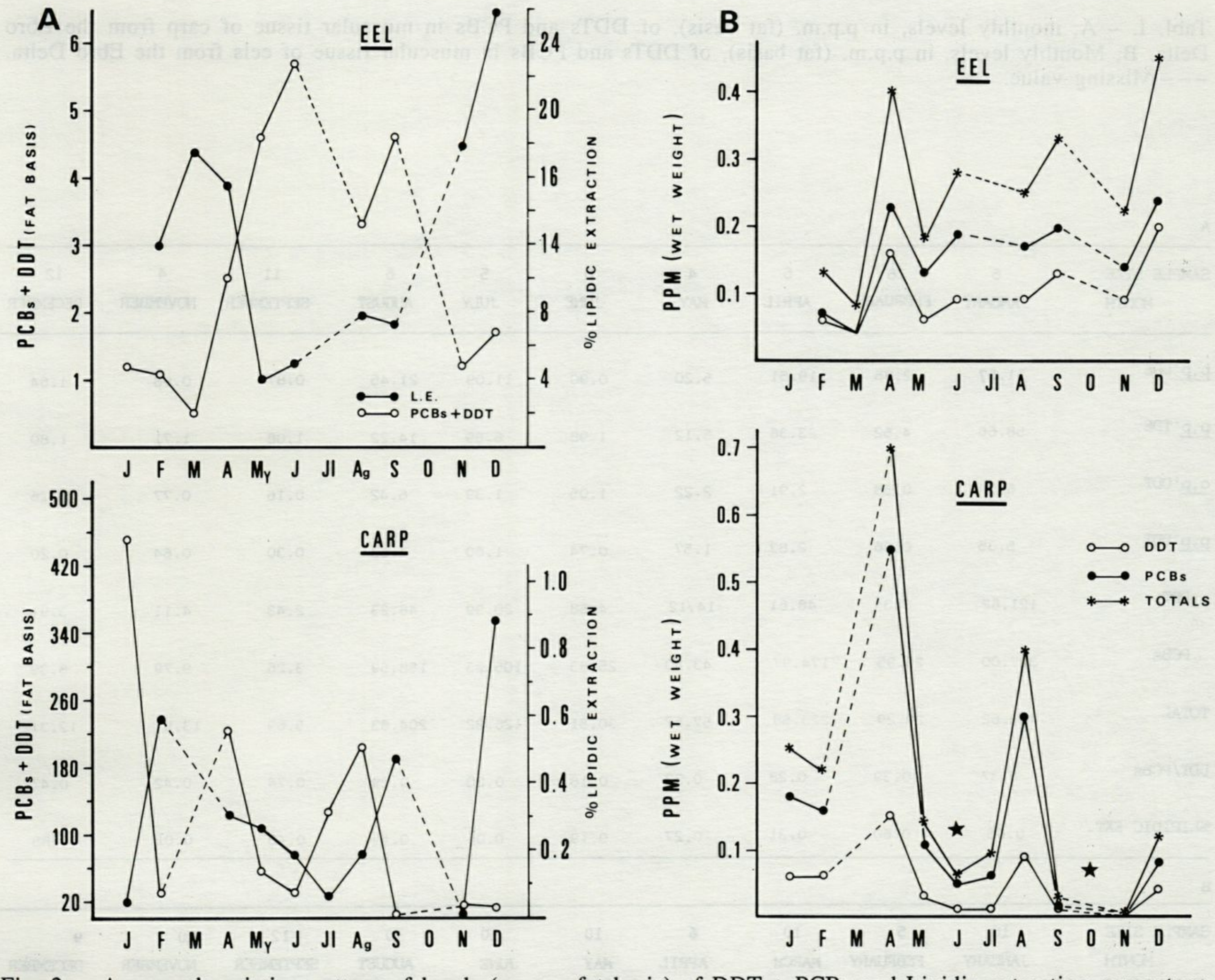

Fig. 2. – A, annual variation pattern of levels (p.p.m., fat basis) of DDT + PCBs and Lipidic extraction percentage for carp and eels from the Ebro Delta. Reproduction periods for carp starred. B, annual variation pattern of mean levels (p.p.m., wet weight) of DDT, PCBs and DDT + PCBs for carp and eels from the Ebro Delta.

mean values if all individual samples of a composite sample contained the same amount of fat.

Because of the intrinsic variability in lipid contents of the axial musculature of finfish (Brandes and Dietrich in Phillips, 1980), special care was given to take always the same portion and weight of muscle.

Analytical procedures

The muscular tissue was ground with anhydrous sodium sulphate and extracted with n-hexane (residue-free quality) in a Soxhlet apparatus for 4 hours. A portion of the extract was used to weigh the quantity of extractable fat per gram of muscle. An aliquot containing 1 g of extractable fat was cleaned-up with sulphuric acid following the method of Murphy (1972).

Chromatographic analysis was carried out on a Perkin-Elmer Sigma 3B model GLC, equipped with a Grob Splitter (250°C), Ni63 E.C.D. (350°C) and a Sigma 15 data station. The chromatography was carried out on a fused silica capillary column (0.25 mm internal diameter, 60 m long) with SPB-1 as stationary phase (0.25 µm film thickness). Splitless technique was used to inject 1 µl of the purified extract (equivalent to 1 mg of extractable fat).

Temperature was programmed as follows: injection at 40°C to 160°C at 25°C min^{-1} rate. Temperature constant for one min., and then increased from 160°C to 250°C at 2°C min^{-1} rate. Temperature constant at 250°C until the end of the analysis. Heptachlor was used as an internal standard to asses the intrinsic variability of the analyses (Aguilar and Borrell, 1985). The DDTs (p.p' DDE, p.p' TDE (=DDD), o.p' DDT, p.p' DDT) were confirmed by an alkaline attack (OHNa) which yielded their olefins, and reinjection of the sample. Polychlorobiphenyls were not altered by this procedure, so it was used to quantify the two groups of compounds as suggested by Watts (1980). A

Tabl. I. – A, monthly levels, in p.p.m. (fat basis), of DDTs and PCBs in muscular tissue of carp from the Ebro Delta. B, Monthly levels, in p.p.m. (fat basis), of DDTs and PCBs in muscular tissue of eels from the Ebro Delta. --- Missing value.

A

SAMPLE SIZE	6	16	6	4	6	5	6	11	4	12
MONTH	JANUARY	FEBRUARY	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	NOVEMBER	DECEMBER
p,p'DDE	51.57	2.85	19.51	5.20	0.90	11.09	21.45	0.87	0.98	1.64
p,p'TDE	58.66	4.52	23.36	5.12	1.98	6.89	14.22	1.08	1.71	1.80
o,p'DDT	6.04	0.58	2.91	2.22	1.05	1.39	6.42	0.16	0.77	0.26
p,p'DDT	5.35	0.36	2.83	1.57	0.74	1.60	4.13	0.30	0.64	0.20
DDT	121.62	8.34	48.61	14.12	4.68	20.99	46.23	2.43	4.11	3.91
PCBs	327.00	20.95	174.97	43.40	25.83	105.23	158.59	3.26	9.79	8.39
TOTAL	448.62	29.29	223.58	57.52	30.51	126.22	204.83	5.69	13.91	12.31
DDT/PCBs	0.37	0.39	0.28	0.32	0.18	0.20	0.29	0.74	0.42	0.47
%LIPIDIC EXT.	0.05	0.60	0.31	0.27	0.19	0.06	0.19	0.48	0.01	0.88

B

SAMPLE SIZE	10	5	10	6	10	10	10	12	10	9
MONTH	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	AUGUST	SEPTEMBER	NOVEMBER	DECEMBER
p,p'DDE	0.18	0.15	0.09	0.34	0.48	0.48	0.32	0.37	0.17	0.43
p,p'TDE	0.20	0.26	0.11	0.45	0.65	0.84	0.56	1.14	0.23	0.32
o,p'DDT	0.01	0.02	0.01	0.03	0.05	0.15	0.03	0.04	0.01	0.02
p,p'DDT	0.01	0.05	0.17	0.24	0.17	0.36	0.17	0.31	0.02	0.02
DDT	0.40	0.50	0.23	1.06	1.41	1.85	1.10	1.87	0.44	0.81
PCBs	0.78	0.58	0.25	1.48	3.16	3.89	2.17	2.74	0.78	0.93
TOTAL	1.19	1.09	0.48	2.55	4.58	5.75	3.27	4.61	1.23	1.74
DDT/PCBs	0.52	0.86	0.89	0.72	0.44	0.47	0.50	0.68	0.57	0.87
%LIPIDIC EXT.	---	11.93	17.51	15.52	4.00	4.96	7.81	7.22	17.88	25.88

mixture of Aroclor^{R} 1254/1260 (50/50) was used to quantify PCBs following the Webb and McCall (1973) method with a computer program. PCB concentration was calculated as the sum of individual peaks.

RESULTS

The levels on an extractable fat basis (see above) of PCB and DDT in the muscle of carp and eels throughout the annual cycle are given in tables I and II. Both DDTs (p.p' DDE, p.p' TDE, o.p' DDT, p.p' DDT) and PCBs are ubiquitous, although there are considerable monthly variations (Fig. 2).

In carp, there is a concentration peak in January, followed by two smaller peaks in April and August. The minimum is attained in September. If the percentage of extractable fat is compared to organochlorine load expressed on fat basis, it can be seen that, in general, there is an inverse relationship between the two parameters (Fig. 2A), except for spring (Apr.-June) and autumn (Sept.-Nov.). In these periods there is a decrease in both organochlorine load and muscle fat contents. If the total amount of organochlorine in muscle (PCBs + DDT) is expressed on a wet weight basis, a different variation profile is obtained (Fig. 2B). The smallest pollutant levels are detected in Nov., the maximum peak for January disappears, April and August peaks continue to be evident, and a new peak appears in Dec., indicating a net intake of organochlorines in muscle. When expressing results on a fat basis, this last peak is masked by an increase in fat reserves which dilutes pollutants.

Tabl. II. – Geometric mean values for April + December subsample levels of organochlorines in carp and eels from the Ebro Delta.

geometric mean	fat basis levels		wet weight levels		total load	
	eel	carp	eel	carp	eel	carp
DDT	0.932	13.799	0.187	0.076	6.782	2.603
PCBs	1.175	38.335	0.235	0.210	8.550	7.233
total	2.109	52.462	0.423	0.288	15.354	9.898
% Lipid ext	20.041	0.523	-	-	-	-
g muscul tissue	36.310	36.075	-	-	-	-

In eels, a clear inverse relationship between the percentage of extractable fat and the organochlorine load (on a fat basis) also occurs, except for December, when a recovery of fat reserves in muscle appears together with an increase in organochlorine load.

Similarly to carp, the variation profile of the PCBs + DDT load is different when expressing values on a wet weight basis (Fig. 2B). In this case the maximum levels are attained in April and Dec., not in June and Sept. The minimum is maintained for March in the two variation profiles.

DISCUSSION

The striking difference in organochlorine concentrations per unit weight of lipid between carp and eels is highly attenuated when expressing results on a wet weight basis. This is explained by the great difference in extractable fat contents between the two species. Therefore, in order to compare them, we used monthly mean levels expressed on a wet weight basis since the applied method of preparing composite samples does not yield mean concentrations on the lipid basis anyhow (see Material and Methods).

In the case of carp, the pollutant concentration peak for April can be related to the opening of channels at the beginning of the rice cycle (see study area). The massive inflow of water stirs up the sediment, thus re-suspending particles and rendering possible the intake by fish of pollutants adsorbed to these particles (Phillips, 1980). Later, when turbidity decreases, the fish partition pollutants back to the environment (Hamelink *et al.*, 1971; Scura & Theilacker, 1977; Shea *et al.*, 1980; Schneider, 1982). This process might explain the decrease in organochlorine levels in May. Thereafter, a decrease in both pollutants and fat contents occurs, which is probably linked to the reproduction period.

In August, a new increase in organochlorine levels is noted. This peak indicates a net intake of pollutants related to pesticide use in the Ebro Delta both for land-farming and in mosquito treatment. In autumn, a new process of de-contamination occurs and a minimum of PCBs and DDT loads is reached in November. This process is similar to that which takes place in spring (May-June) and can be explained by a second period of reproduction, as is known to occur in the carp of the Ebro Delta.

In December there is a new increase in pollution levels which occurs together with an increase in the fat contents of muscle. This is the time when channels are completely closed and water circulation ends. Under such conditions, the water level decreases by evaporation, thus concentrating both food and pollutants. As a result of this process, carp fatten and incorporate more pollutants.

With regard to eels (Fig. 2) there are two clearly delimited periods in which muscle fat contents differ. They are higher from November to April, and lower from May to September. The difference is significant (U = O, p 0.01) and could be attributable to the fact that eels sampled in autumn and winter are migrants, caught just before leaving the lagoon, while Spring and Summer eels are residents which are not yet ready to initiate migration and have low fat contents in muscle tissues.

When expressing levels on a wet weight basis, two clear peaks appear in April and December, as happened with carp. The factors producing these two peaks for eels are probably the same as for carp : the influx of water and stirring up of sediments in April, and the concentration of residual water in December.

When comparing overall residue levels between carp and eels, it is worth noting that from June to December eels display higher mean levels than carp, while between January and April the situation is reversed. Carp are more contaminated when the water supply is cut off and the water concentrates and becomes turbid (Shea *et al.*, 1980; Young *et al.*, 1977; Saiki and Schmitt, 1986).

In eels the process is very similar, but the changes appear to be less clear because eels almost exclusively inhabit the lagoons in which the water mass fluctuates much less and, consequently, the seasonal variation in pollutants is less marked.

Another point to be taken into consideration is the biological cycle of the two species. In the Ebro Delta, carp have two periods of reproduction, whereas eels do not breed there. Consequently, carp release pollutants associated with the lipidic fraction of eggs and sperm during Spring and Autumn, whereas eels do not. This produces higher fluctuations in the pollutant load in carp.

In order to compare PCBs + DDT accumulation related to the trophic level, we have only chosen samples from April and December because these months correspond to coincident peaks of maximum accumulation for the two species.

Thus, we have calculated the geometric means for values given in tables I and II. Total organochlorine pollutant loads expressed on a fat basis are higher in carp than in eels. However, levels expressed on a wet weight basis do adjust to the biomagnification principle. Although apparently contradictory, these results are in agreement with those reached by many authors for several species, when studying bioaccumulation through food webs both in the field and in laboratory experiments (Phillips, 1980).

It is worth noting that on a wet weight basis PCB levels are very similar for the two species, DDT being mainly responsible for the differences. In the case of PCBs, it is well established that fish incorporate them mainly by direct absorption through the gills or skin (Phillips, 1980; Courtney & Langston, 1980; Larsson, 1984) although there is some evidence that they can also be incorporate via food (Sodergren, 1984).

Jensen *et al.* (1975) and Addison & Zinck (1977) have suggested the possibility that the carrying capacity for DDT in fish tissues is related to their lipidic content, in the sense that, at low fat levels, the lipidic turnover is much higher. In this way the DDT may be relocated together with the fat, making its metabolization and/or elimination easier. At first glance, this hypothesis could explain the results from this paper because eels have much higher extractable fat levels in muscle (40 fold) than carp (G.M. eels = 20.0, carps = 0.52). However this hypothesis would require the proportion of DDT metabolites to be higher for carp than for eels, which is not the case (p.p' TDE/DDT U = 22 n.s; p.p' DDE/DDT U = 36 n.s.; p.p' DDT/DDT U = 40.5 n.s.; two tailed Mann-Withney U test, n.s. = not significant).

Another possibility is that concentration obtained from a subsample data are not representative of the whole muscle load as suggested by Moriarty (1984). It is recommendable, therefore, to estimate the absolute amount of pollutants present in the fish muscle. In order to do so, we have calculated the geometric mean for total muscle weight of all individuals in the sample (April and December) for each species, and then used these means as conversion factors for obtaining the μg. of pollutants in muscle per fish. The results obtained show the same trend as the figures on wet weight basis, reconfirming that eels have more DDT in muscle than carp.

A very important factor to be taken into account is the difference in habitat location of sampling for eels and carp. As was mentioned above, carp were caught in channels and rice-fields and eels in lagoons.

There is no data available for sediments, but unpublished data on another fish (*Gambusia affinis*) and bird (*Bubulcus ibis*) inhabiting the area support the idea of different availabilities of PCBs and DDTs in rice fields and channels than lagoons, although this possibility needs to be confirmed.

Pollutant levels in eels from the Ebro Delta can be compared to those present in the species from the Venice lagoon, a similar ecosystem in the Mediterranean. In order to compare pollutant loads, we have calculated geometric means for the monthly values in the Ebro Delta, and the average for the two localities studied by Fossato (1983) in the Venice lagoon. It can be seen that eels from the Ebro Delta present total organochlorine levels of about half those of Venice (G.M. DDT + PCBs E.D. = 0.232; V = 0.485). However, it is worth noting that differences are reversed for PCBs and DDT. The DDT level is higher for the Ebro Delta eels (G.M. = 0.090) than for those of Venice (G.M. = 0.035). Conversely, PCB contamination is three times higher in Venice animals (G.M. E.D. = 0.140, V = 0.450). The greater impact of DDT in the Ebro Delta is consistent with previous data obtained from waterfowl in this area (Ruiz *et al.*, 1979, 1983, 1984; Alberto, 1979; Alberto & Nadal, 1981; Llorente *et al.*, 1983, 1987).

Finally, the mean levels on a wet weight basis are far from exceeding the tolerance levels for fish tissues set by the United States Food and Drug Administration (USFDA). So the impact of PCBs and DDT in these species do not seem to constitute a risk factor for the development of fish cultures in the Ebro Delta, although further studies covering more species are needed to evaluate organochlorine pollution in the area comprehensively.

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