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Organochlorines and mercury in livers of great cormorants (*Phalacrocorax carbo sinensis*) wintering in northeastern Mediterranean wetlands in relation to area, bird age, and gender

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ABSTRACT

Wild birds are exposed to pollutants in their habitats. Top consumers of aquatic environments such as the fish-eating great cormorant (*Phalacrocorax carbo sinensis*) are especially affected due to the bioaccumulation of toxic substances in their tissues. This study analysed the livers of 80 great cormorants from Greece to estimate the concentration of organochlorines and mercury and to examine their possible toxic effects and origin. The results showed that mercury (geometric mean 8089 ng g^{-1} dw), *p,p′*-DDE (2628 ng g^{-1} dw), $\sum$HCHs (47 ng g^{-1} dw) and HCB (116 ng g^{-1} dw) concentrations can be considered high compared with those found in great cormorant livers elsewhere except in highly polluted areas, whereas $\sum$PCBs occurred in relatively low concentrations (1091 ng g^{-1} dw). β-HCH was the dominant HCH isomer. Pollutant levels were generally unrelated to area, age and gender. However, *p,p′*-DDE and *p,p′*-DDD showed intersite differences, whilst the proportion of PCBs with 8 chlorine atoms were significantly higher in adult than 1st year great cormorants. Pollution did not reflect local patterns but rather these along the Baltic and Black Seas, whilst differences in *p,p′*-DDE concentration and $\sum$DDTs/$\sum$PCBs ratios between Evros, Axios or Amvrakikos, found on common migration route, suggested different bird origins. Most birds had toxic mercury concentrations; 83.7% above 4000 ng g^{-1} dw and 16% above 17,000 ng g^{-1} dw. Other pollutant levels were too low to have adverse effects.

1. Introduction

Organochlorines (OCs) is a major and diverse group of man-made chemicals. Of the most widespread among the numerous organochlorine pesticides (OCPs), that have been produced and used worldwide, are dichloro-diphenyl-trichloroethane (DDT), hexachlorocyclohexanes (HCHs) and hexachlorobenzene (HCB). Polychlorinated biphenyls (PCBs) have been used as plasticisers, as additives in hydraulic and dielectric fluids in industry, and as fire retardants. In parallel with their applicable properties in agriculture and industry, OCs were proved to have detrimental effects on humans and wildlife. They are persistent in the environment and toxic for organisms, lipophilic and enter food chains accumulating in considerable levels in top predators such as birds of prey and fish-eating birds (Metcalfe and Metcalfe, 1997; Walker, 1990). Their well known harmful effects, particularly of DDT (and its metabolite DDE) and PCBs, on birds are associated with eggshell thinning, resulting in reproductive failure and/or in offspring mortality and deformities (Becker et al., 1993; Custer et al., 1999; Giesy et al., 1994; Ludwig et al., 1995; Yamashita et al., 1993). As a result, the use of the most dangerous OCs was banned in most countries in the 1970s and 1980s, although they are still used in some developing countries (Kunisue et al., 2003).

Mercury (Hg) is a toxic heavy metal that is naturally widespread in the environment but levels in food webs have increased during the last 100 years due to human activities (Monteiro and Furness, 1995). It accumulates in seabirds (Elliott et al., 1992; Furness and Hutton, 1979) and its effect on humans is widely known as Minamata disease (Eisler, 1987).

OCs and Hg accumulate in higher concentrations in top consumers such as fish-eating waterbirds, which are considered suitable bioindicators of these chemicals (Fossi et al., 1995; Scharenberg, 1991; Weseloh et al., 1995). Cormorants are particularly inclined in bioaccumulation due to their fishing habits and incapability of metabolising pollutants (Fossi et al., 1995; Walker, 1990), which impair reproduction (Dirksen et al., 1995) and populations. The severity of OCs and Hg pollution triggered long-term studies using birds as bioindicators (Becker and Muñoz Cifuentes, 2004; Becker et al., 2001; Pekarik and Weseloh, 1998; Ryckman et al., 1998).

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In Greece, OCs have been detected in eggs of great cormorants (*Phalacrocorax carbo sinensis*) (Konstantinou et al., 2000) and other waterbirds (e.g., Albanis et al., 2003; Crivelli et al., 1999; Goutner et al., 2001a) and Hg in feathers (e.g., Goutner and Furness, 1997, 1998; Goutner et al., 2001b). Two more studies reported OCs in livers of raptors and waterbirds (Hela et al., 2006; Sakellarides et al., 2006). Great cormorants are birds breeding and wintering in Greek wetlands (Handrinos, 1993; Liordos and Goutner, 2007). The investigation of OCs and Hg in livers was within the framework of an extensive study on the biology and ecology of the great cormorant in Greek wetlands of international importance between 1999 and 2002 (Liordos and Goutner, 2007, 2008, 2009).

In this paper we address the following topics: (a) we estimate the OC and Hg concentrations in livers of great cormorants wintering in Greek wetlands and potential effects of area, bird age and gender on body loads; (b) we investigate whether pollution levels in birds and in their wintering habitats are correlated; and (c) we examine whether pollutant levels could affect the health of the great cormorant populations.

2. Materials and methods

2.1. Study areas

Great cormorants were studied in the Axios and Evros Deltas, Messolonghi Lagoon, and Amvrakikos Gulf (Fig. 1). These areas have been designated as Wetlands of International Importance under the Ramsar Convention. The Axios Delta (40°27′–40°38′N, 22°33′–22°52′E) belongs to a large wetland complex of 68.7 km^2, situated near the city of Thessaloniki, formed by the rivers Axios, Loudias and Aliakmon. The Evros Delta at the Greek–Turkish border (40°44′–40°51′N, 25°53′–26°8′E) is an extensive area (190 km^2) including diverse habitats. The Messolonghi Lagoon (37°40′–39°40′N, 20°10′–21°30′E) belongs to a large wetland complex, situated in southwestern mainland Greece, totalling 258 km^2. The rivers Acheloos and Evinos discharge at the west and east sides of the area, respectively. The Amvrakikos Gulf, in western Greece (38°59′–39°11′N, 20°44′–21°07′E), is the largest closed gulf in Greece and covers a total of 405 km^2. The rivers Louros and Arachthos flow into the northern part of the Gulf.

2.2. Sampling

A total of 80 birds were sacrificed under license by the Ministry of Rural Development and Food during the wintering season in the years 1999–2002 (see Table 2 for sample size by area and age class). Collected birds were used for the study of several aspects of great cormorant biology and ecology, including diet and morphological sex determination (references cited in Section 1). Birds were aged by examination of plumage characteristics (Cramp and Simmons, 1977) and classified as adults, 2nd winter birds and juveniles (1st winter) and sexed at dissection by gonadal inspection. Details on morphometric measurements are given in Liordos and Goutner (2008). Livers were removed, stored in Hg- and OCs-free bags and preserved in a freezer at −20 °C until analysis.

2.3. Chemical analyses

All chemical analyses were made by the Institute of Avian Research at ICBM-Terramare, Wilhelmshaven, Germany. Livers were thawed and then homogenised using an Ultra Turrax and were freeze-dried. For the determination of the OC pesticides and 62 PCB congeners, each sample of 2 g liver-homogenate was spiked with the internal standard solution, dried with sodium sulfate, clined by silica gel column, eluted with n-hexane:dichloromethane (8:2), evaporated and re-dissolved in 250 µl isooctane. For detection a GC-MS Agilent 6890 was used, coupled to a mass-selective detector Agilent 5973 KAS, with helium as the carrier gas. The electron impact ionization was used and measured in the SIM mode. For separation, a HAT-5-column with a length of

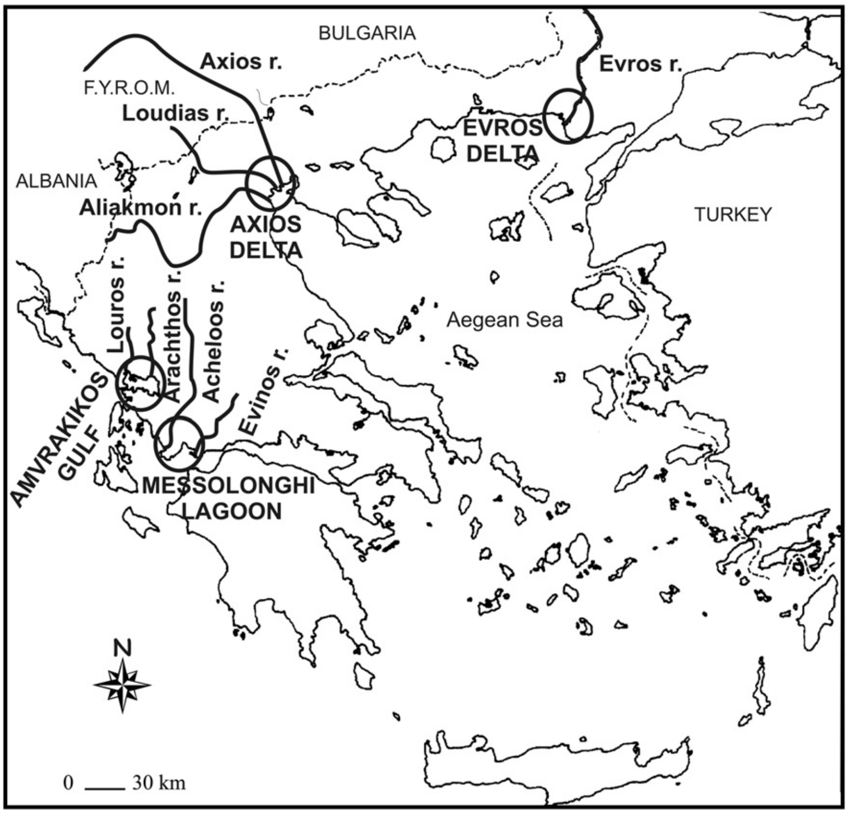

Fig. 1. The study areas with their associated river systems.

25 m was used. The internal standard added before extraction allows correct losses during extraction and clean-up process (automatically done by the calibrated GC-MS software). The qualification and the quantification of the pesticides and PCBs were performed according to Büthe and Denker (1995). Forty one of 62 PCB congeners could be determined separately. The remaining 21 congeners were detected together (nine peaks with two PCBs and one peak with three PCBs). The detection limits are within the range 0.1–0.9 ng g^{-1}. The concentration is given as average of two determinations in ng g^{-1} dry weight (dw). See Becker et al. (2001) for more details.

The Hg measurement was accomplished with the DMA-80 Direct Mercury Analyser by MLS GmbH. This analyser has the potential for sample analysis in a short time without sample preparation and no waste disposal. The dried sample is introduced into the sample boat and burned at 750 °C in oxygen current. The high temperature decomposes Hg compounds and elementary Hg is set free. Hg vapours are collected on a gold amalgamation trap and subsequently desorbed for quantification. Hg content is determined using atomic absorption spectrometry at 254 nm. All samples were analysed in duplicate. The system is based on two measuring methods: EPA Method 7473 and ASTM D6722-01 (2006). The analytical performance of this method has been evaluated by analysis of certified reference materials and has been proposed as ready-to-use analytical method to analyse a large number of samples in a short time frame (Maggi et al., 2009; Roy and Bose, 2008).

2.4. Statistical analysis

The information on great cormorant samples involved area, year, date (used on month level), gender, age (1st year, 2nd year and adult). If not otherwise mentioned, values are presented as geometric means with 95% confidence limits (see later discussions). p,p'-$\sum$DDTs = p,p'-DDE + p,p'-DDD + p,p'-DDT; $\sum$HCHs = α-HCH + β-HCH + γ-HCH; $\sum$PCBs = sum of 62 PCB congeners. For statistical comparisons we performed GLMs based on the logarithmic values to achieve homogeneity of variances. 95% confidence limits from the models are presented as antilog values. The full model including area, age, gender, month of sampling as fixed effects and year as random factor was reduced by the non-significant fixed effects and their interactions to get the final model. Month of sampling and gender never had a significant influence on contaminant levels. If a factor had a significant effect on the contaminant levels, the differences between the factor's categories were tested by post-hoc Scheffé test. Pearson's correlation coefficients between contaminants were calculated using the log values, too. All analyses were made with SPSS17, and level of significance was set at $p \leq 0.05$.

3. Results

Geometric mean Hg concentrations were highest at Messolonghi Lagoon (9975 ng g^{-1} dw) and lowest in the Axios Delta (6147 ng g^{-1}) but without significant differences related to area, bird age, gender or month (Table 1). Two extreme values in the Evros Delta were 215,101 and 106,534 ng g^{-1} Hg.

p,p'-DDE far dominated all other OCs, followed by $\sum$PCBs whereas HCB and HCH isomers were detected in much lower concentrations. Also $\sum$Chlordane-nonachlor was present in negligible concentrations (Table 1). Regarding p,p'-DDE, in relation to the factors examined (area, age, gender, month of sampling and year as random factor), significant effects of area, age and their interaction were found: great cormorants wintering in the Evros Delta showed significantly higher liver concentrations than birds from the Axios Delta (Table 1). Whereas p,p'-DDT was detected in very low levels and insignificantly varied due to area and age, a highly significant p,p'-DDD variation with area was found even on a low level of contamination: livers of great cormorants from Axios Delta were found more contaminated than those originating from the other areas.

Among HCH isomers, β-HCH was detected in much higher levels than the other isomers, whereas γ-HCH occurred in the lowest levels (Table 1). Regarding $\sum$PCBs, the GLM only revealed a significant

Table 1
Variation of concentrations of mercury and organochlorines (ng g^{-1} dry weight) in great cormorant livers from Greece, 1999–2002. Geometric mean values (and 95% confidence limits) are presented. Reduced models are indicated for factors or interactions significant in the first model including area, age, gender, month of sampling and year as random factor. Significant effects from GLMs (*F* values) are given bold. See Table 2 for effects of area vs. age in p,p'-DDE and $\sum$PCBs.

	Amvrakikos Gulf ($n=23$)	Messolonghi Lagoon ($n=16$)	Axios Delta ($n=13$)	Evros Delta ($n=28$)	Totals ($n=80$)	Area ($df=3,65$)	Age ($df=2,65$)	Area*age ($df=6,65$)
Hg	9147.4 (6322.2–13,234.9)	9975 (7065.3–14,082.9)	6147.4 (4012.9–9416.8)	7369.8 (4815.2–11,279.3)	8089.6 (6630.4–9869.9)	0.148	0.3222	0.624[d]
HCB	178.3 (108.5–292.5)	96.5 (45.7–202.6)	36 (23.7–54.4)	155.7 (93.0–260.1)	116.2 (86.7–155.5)	2.372	0.502	0.87
α-HCH	2.5 (1.3–4.2)	4.5 (1.8–9.6)	3.4 (2.4–4.6)	5.8 (3.4–9.7)	4 (3.0–5.3)	0.532	0.501	0.37
β-HCH	33.3 (15.8–68.8)	29.7 (8.7–95.6)	29.1 (20.3–41.6)	72.5 (41.1–127.5)	41.9 (29.0–60.2)	0.377	1.406	0.52
γ-HCH	n.d. (0.0–0.0)	0.2 (0.0–0.8)	n.d. (0.0–0.0)	0.3 (0.0–1.0)	0.1 (0.0–0.3)	0.522	0.157	0.55
p,p′-DDE	2090.4 (1480.9–2950.6)	2712.3 (1382.6–5320.0)	1575.0[a] (1148.0–2160.7)	3949.8[a] (2596.9–6007.2)	2627.9 (2094.5–3297.0)	**3.482***	**3.981***[e]	**3.871****[d]
p,p′-DDD	0.7[**a**] (0.5–1.0)	1.0[**b**] (0.5–1.7)	3.9[**a,b,c**] (1.4–8.9)	1.2[**c**] (0.6–2.1)	1.3 (0.9–1.7)	**8.517*****[d]	1.186	2.200[d]
p,p′-DDT	0.1 (0.0–0.3)	n.d. (0.0–0.0)	n.d. (0.0–0.0)	0.3 (0.0–0.8)	0.1 (0.0–0.3)	1.359	0.261	1.002
$\sum$PCBs	1350.2 (823.5–2213.3)	1099.6 (626.7–1928.6)	859.7 (568.3–1300.2)	1018.6 (657.7–1577.1)	1091.1 (863.5–1378.7)	0.813	2.009	**2.979***[d]
$\sum$Chlordane-nonachlor	n.d. (0.0–0.0)	0 (0.0–0.1)	0.1 (0.0–0.2)	0.1 (0.0–0.2)	0.1 (0.0–0.1)	1.295	0.887	**2.692***[d]
$\sum$DDTs/$\sum$PCBs	0.6[**a**] (0.0–1.3)	1.5 (0.4–3.3)	0.8 (0.3–1.5)	2.9[**a**] (2.0–4.0)	1.4 (1.0–1.9)	**4.149****	0.42	1.528

[a,b,c]Values with a letter in common are significantly different at the $p=0.011$ level (letters in bold $p \leq 0.01$; post-hoc Scheffé tests). [d]Year (random), $p \leq 0.05$. [e]Post-hoc Scheffé test n. s. n.d.: not detected.
* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.001$.

interaction of area and age but no significant effects of the factors separately (Table 1). The ratio ∑DDTs/∑PCBs was significantly higher at Evros Delta than at Amvrakikos Gulf (Table 1).

As we found significant interactions of area and age in case of *p,p′*-DDE and ∑PCBs (Table 1), we split the liver concentrations by area and age (Table 2). Whereas in most areas 2nd year birds had a tendency of elevated concentrations of *p,p′*-DDE, in the Evros Delta adults showed highest concentrations causing the significant interaction. Indeed, a GLM run on *p,p′*-DDE concentrations in relation to area and bird age revealed significant intersite differences only among the adult birds ($F_{3,16}=10.037$, $p=0.001$): differences were significant between Axios and Evros ($p=0.006$, being higher in Evros, Scheffé test) and between Amvrakikos and Evros ($p=0.001$, also higher in Evros; Fig. 2).

Regarding ∑PCBs, the significant interaction of area and age was due to the tendency of adult birds to be more contaminated at Axios Delta, whereas at Amvrakikos Gulf and Messolonghi Lagoon second year birds showed elevated levels compared with Evros Delta (Table 2). Penta-, hexa- and hepta-chlorobiphenyls dominated in the samples. No significant area- or age-related differences were found in the levels of the PCBs of different degree of chlorination. However, the liver proportions of PCBs consisting of 8 chlorine atoms in their molecule were age-related (GLM, $F_{2,65}=3.616$, $p=0.032$, Fig. 3; area $F_{3,65}=1.680$, $p=0.180$; area*age $F_{6,65}=1.487$, $p=0.197$) and highly significant between adult and 1st year old great cormorants ($p<0.001$, Scheffé test).

Significantly positive correlations were detected among most of the pollutants only in the Evros and Amvrakikos areas, being generally stronger in the Evros Delta (Table 3).

4. Discussion

4.1. Hg concentrations

Liver Hg concentrations are the outcome of some complicated physiological processes that contribute to Hg detoxification. These include a species- (and probably age-) dependent demethylation of organic dietary Hg (Thompson and Furness, 1989), diminishing body burdens by placing it into growing feathers (Lewis et al., 1993) and binding it with Se (Furness and Hutton, 1979; Mendes et al., 2008). However, no significant Hg accumulation with age was found in this study, and also for great cormorants in Japan (Nam et al., 2005; Saeki et al., 2000) and razorbills (*Alca torda*, Ribeiro et al., 2009). In contrast, Platteeuw et al. (1995) reported higher Hg concentrations in adult compared to young great cormorants in The Netherlands. Various seabird studies have also reported elevated Hg concentrations in adults (e.g., in Atlantic gannets *Morus bassanus*, Mendes et al., 2008; common guillemots *Uria aalge*, Stewart et al., 1994). In this study, the small sample size and the considerable range of Hg concentrations in each area may have distorted possible age-related differences.

Gender differences in hepatic Hg concentrations were not found in great cormorants from Greece despite differences in body size and diet (Liordos and Goutner, 2008, 2009). Different habitats and prey species may differ in their Hg burdens (Goutner and Furness, 1997; Thompson, 1990) and therefore the extent to which Greek wintering grounds have contributed to great cormorant pollution cannot be evaluated. Gender differences in Hg concentrations have been found

Table 2
Area and age dependent concentrations (ng g^{-1} dry weight; geometric mean, 95% confidence interval in brackets for n≥5) of *p,p′*-DDE and ∑PCBs in great cormorant livers from Greece. See Table 1 for *F* and *p* values. For intersite variation of *p,p′*-DDE concentrations in adults see Fig. 2.

Area	Age	*n*	*p,p′-DDE*	∑PCBs
Amvrakikos Gulf	1st year	9	2098 (1235–3565)	880 (322–2404)
	2nd year	7	3365 (1512–7488)	2943 (1311–6607)
	Adult	7	1292 (714–2340)	1073 (493–2331)
Messolonghi Lagoon	1st year	12	1891 (1099–3252)	938 (519–1694)
	2nd year	2	24,493	4882
	Adult	2	2615	641
Axios Delta	1st year	6	1185 (971–1445)	675 (398–1147)
	2nd year	1	4607	1667
	Adult	6	1751 (1005–3050)	9807 (4097–23,467)
Evros Delta	1st year	18	3547 (1960–6418)	1089 (585–2028)
	2nd year	5	2609 (1536–4425)	564 (305–1042)
	Adult	5	8818 (3391–22,927)	1445 (397–5257)

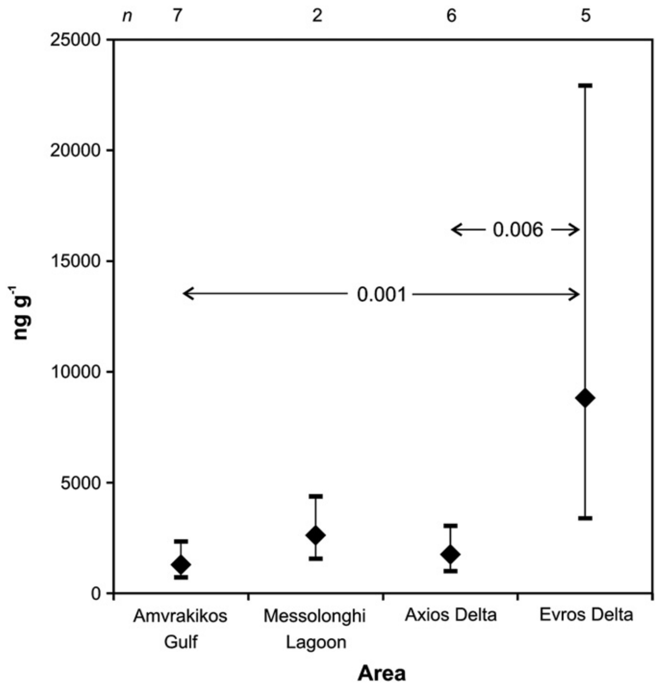

Fig 2. Differences in *p,p′*-DDE levels in adult great cormorant livers among areas studied. Geometric means (diamonds), 95% confidence limits (vertical lines), sample size (*n*) and significant differences (arrows with *p* values) are shown.

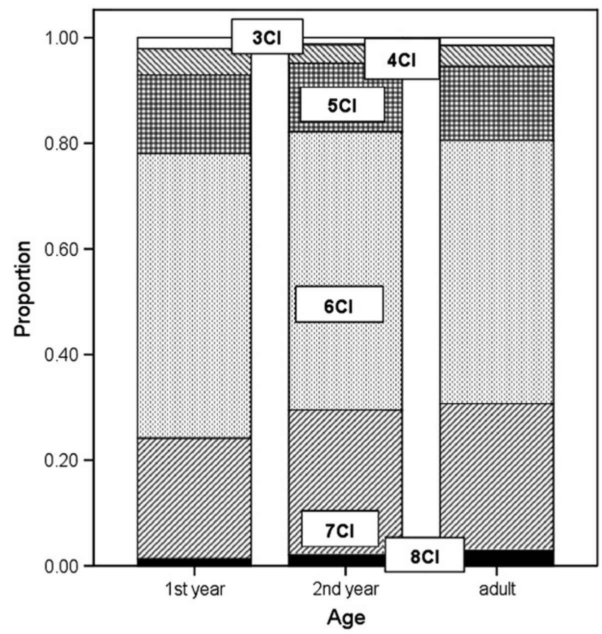

Fig. 3. Composition of PCBs according to their molecule chlorination in livers of different-aged great cormorants from all Greek wetlands. 1st year $n=45$; 2nd year $n=15$; adult $n=20$.

Table 3
Pearson correlations (log values) between pairs of pollutants in the Evros Delta ($n=28$) and Amvrakikos Gulf ($n=23$, numbers in italics). Significant correlations are shown in bold.

	Hg	HCB	∑HCHs	∑DDTs	∑PCBs
Hg		**0.649*** **	**0.809*** **	0.299	**0.530** **
HCB	***0.509****		**0.713*** **	0.076	**0.440** *
∑HCHs	***0.702***** **	***0.565**** *		**0.479** **	**0.706*** **
∑DDTs	*0.378*	*0.174*	***0.533**** *		**0.836*** **
∑PCBs	*0.380*	*0.138*	*0.146*	***0.570**** *	

* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

in American white pelicans (*Pelecanus erythrorhynchos*, Donaldson and Braune, 1999), and in 8 species of ducks in Canada (Braune and Malone, 2006), but neither in great cormorants in Japan (Nam et al., 2005) nor in other seabirds such as common guillemots (despite verified sexual differences in diet, Stewart et al., 1994) or herring gulls (*Larus argentatus*, Lewis et al., 1993). In such studies sample size, being generally small, must seriously be taken into consideration as a great overlap in ranges detected may obscure the biological significance of the findings.

Hg concentrations in great cormorant livers were much lower than those in polluted areas in The Netherlands and Greenland (Nielsen and Dietz, 1989; Platteeuw et al., 1995) and in double-crested cormorants (*Phalacrocorax auritus*) in Canada (Braune, 1987; Elliott et al., 1992), similar in Japanese cormorants (*Phalacrocorax capillatus*, Honda et al., 1990), and generally higher compared to great cormorants from Japan (Honda et al., 1990; Nam et al., 2005; Saeki et al., 2000).

4.2. Organochlorines concentrations

p,p′-DDE dominated over all other OCs in Greek great cormorants' livers. The preponderance of *p,p′*-DDE compared to the other DDT compounds in cormorant livers and eggs is known from other studies (Covaci et al., 2006; Hoshi et al., 1998; Kellner and Lage, 2009; Platteeuw et al., 1995; Senthil Kumar et al., 2005). Also, *p,p′*-DDE dominates in the livers and eggs of other seabirds (Becker et al., 2001; Borgå et al., 2007; Cleemann et al., 2000; Knudsen et al., 2007), raptors (Chen et al., 2009; Hela et al., 2006; Hoshi et al., 1998; Naso et al., 2003) and other bird species (Alleva et al., 2006; Hoshi et al., 1998; Kunisue et al., 2002; Naso et al., 2003). The very low levels of *p,p′*-DDT and its low ratio to *p,p′*-DDE (see Table 1) indicate the reduced use of DDT in recent years (Gabrielsen et al., 1995), photochemical conversion of DDTs in the environment (Brown et al., 1986) and, particularly for the great cormorant, slow rates of metabolisation and/or a high dietary input of chlorinated hydrocarbons leading to bioaccumulation (Fossi et al., 1995; Walker, 1990). Predominance of *p,p′*-DDE seems to be due to its high chemical stability and persistence in the environment and living organisms (Hela et al., 2006).

Regarding the age-related concentrations, elsewhere adult great cormorants' livers have been found with higher levels of *p,p′*-DDE compared to young birds (Guruge et al., 1997; Platteeuw et al., 1995; Scharenberg and Schultz, 1991). Similarly to this study no age-related differences in levels were found in albatrosses (Guruge et al., 2001, partly attributable to limited sample size), black guillemots (*Cepphus grylle*, Òlafsdóttir et al., 2005) and goshawk (*Accipiter gentilis*, Kenntner et al., 2003).

The relationship of *p,p′*-DDE levels with the gender of birds have also been found weak in great cormorants from The Netherlands (Platteeuw et al., 1995), lacking in black guillemots (Òlafsdóttir et al., 2005), northern fulmars (*Fulmarus glacialis*, Knudsen et al., 2007), glaucous gulls (*Larus hyperboreus*, Cleemann et al., 2000), goshawk (Kenntner et al., 2003) and 16 among 18 species of birds of prey in Spain; where existed, the gender difference was attributable to net transfer of OCs to the eggs by females (Van Drooge et al., 2008). In this study, the lower number of adult females (32% of the total), extended ranges in values and generally low sample sizes possibly obscured any gender-related pollution patterns. The differences in *p,p′*-DDE concentrations among the adults probably have geographical origin and are discussed in Section 4.4.

Mean concentrations of *p,p′*-DDE in Greek great cormorant livers in the early 2000s were higher than those reported in most other areas, although were found twice as high in Japan (see comparison of arithmetic means; Table 4) and were higher or within the range of concentrations of other bird species' livers from Greece (Hela et al., 2006; Sakellarides et al., 2006). Mean levels were lower or similar to those in livers of 12 bird species with various feeding habits from Italy (Naso et al., 2003) and similar or higher than those measured in various seabird species' livers around the world (Borgå et al., 2007; Cleemann et al., 2000; Guruge et al., 2001; Knudsen et al., 2007). As discussed in these studies, the great variation in *p,p′*-DDE levels, depending on species' feeding and migratory habits, reflects the pollution patterns in the environment of wide parts of the world where birds live. Thus, the high levels of *p,p′*-DDE in this study seem to be of geographical concern, and this subject is discussed in Section 4.4.

HCH levels were within the range of those detected in great cormorants in other areas but those of the β-isomer were higher (Table 4). β-HCH was far the dominant HCH isomer in the birds' livers from all Greek wetlands studied. This is in agreement with most relevant studies (Becker et al., 2001; Covaci et al., 2006; Guruge et al., 1997). β-HCH is the most lipophilic among the HCH isomers and predominant in animal tissues (Walker 1990). β-HCH concentrations could indicate technical HCH contamination at earlier time because the α- and γ-HCH isomers can be more readily metabolised and would be detected in low concentrations or not at all (Willett et al., 1998).

Mean ∑PCBs concentrations in great cormorant livers can be evaluated as low, compared to those of great cormorants from Mediterranean or other areas (Table 4); levels were found unrelated to age, similarly to cormorants in Japan (Guruge and Tanabe, 1997) and black guillemots (Òlafsdóttir et al., 2005). Higher ∑PCBs concentrations in all tissues of male glaucous gulls were attributed to females' capability of excreting contaminants to eggs (Gabrielsen et al., 1995).

The observed pattern with penta-, hexa-, and hepta-chlorinated PCB congeners predominating in the sample (Fig. 3) is in agreement with other investigations on great cormorants (Falandysz et al., 2002; Guruge and Tanabe, 1997; Guruge et al., 1997, 2000) and other bird species (Becker et al., 2001; Naso et al., 2003). The higher proportions of congeners with eight chlorine atoms detected in adult great cormorants, compared to immature ones, may be explained by the birds' low mixed function oxidase (MFO) activities and consequent slow rates of metabolisation resulting in bioaccumulation through diet, especially of the congeners most resistant to metabolisation (Beyerbach et al., 1993; Fossi et al., 1995; Walker, 1990). Enrichment of higher PCBs such as octachlorobiphenyls in great cormorants via their fish diet suggests a greater capacity to depurate lower chlorinated PCBs (Guruge and Tanabe, 1997). Thus, adult birds accumulated higher PCBs concentrations, probably over the course of several years, in contrast to immature ones that were exposed for shorter periods.

The HCB concentrations detected in this study can be considered high compared with those found in cormorant livers elsewhere except in highly polluted areas (Table 4). Lack of age-related differences in HCB concentrations could be explained by the relatively fast clearance rate of these chemicals in birds (Donaldson et al., 1997).

4.3. Pollutant correlations

Positive correlations of lipophilic pollutants in tissues have also been found in other studies in pelecaniforms particularly between *p,p′*-DDE and PCBs (e.g., Crivelli et al., 1999; Custer et al., 1999, 2001; Furness

Table 4
Levels of organochlorines detected in the liver of great cormorants from various geographical areas. All values have been transformed to ng g^{-1} dry weight. At the lower part of the table, arithmetic mean values of this study have been transformed to lipid weight and wet weight for comparisons given in the text. Transformation was made using as dry weight percentage of livers 27.43% (Platteeuw et al., 1995) and as average fat proportion 13.8% dw (from $n = 6$ of our samples).

Area	Mean or range of means[a]						
	p,p′-DDE	∑DDTs	β-HCH	∑HCHs	∑PCBs	HCB	
Lake Biwa, Japan					7656[h]–26,249[i]		Guruge and Tanabe (1997)
Lake Biwa, Japan[e]		10,572		401	24,790	62	Guruge et al. (1997)
Shinobazu, Tokyo[e]		47,393		583	127,598	547	Guruge et al. (1997)
Chubu region, Japan	252		13		838	9.5	Hoshi et al. (1998)
Sagami River, Japan	11,040	11,040	92	103		179	Senthil Kumar et al. (2005)
Caspian Sea, Iran	58		9			13	Rajaei et al. (2009)
Danube, Papadia canal	215	225		29		10	Covaci et al. (2006)
Danube, japsa Marcova	494	512		69		27	Covaci et al. (2006)
Orbetello, Italy[b]	1640				3150	140	Fossi et al. (1995)
Sardinia, Italy	605				5177		Kannan et al. (2002)
Baltic Sea, Poland					1604–15,312		Falandysz et al. (2002)
Schleswig-Holstein, Germany[c]					8859		Scharenberg (1991)
Schleswig-Holstein, Germany	729[f]–3609[e]	911[f]–401[e,g]	26	77	2771[f]–13,817[e]	109[f]–83[e]	Scharenberg and Schultz (1991)
Lake Ijsselmeer, The Netherlands[d]	2856	2897	94	150	17,892	271	Platteeuw et al. (1995)
Greek wetlands	5744	5747	219	238	1965	353	This study
Equivalent lipid weight ng g^{-1}	41,620	41,647	1585	1726	14,240	2561	This study
Equivalent ww ng g^{-1}	1575	1577	60	65	539	97	This study

[a] Arithmetic means.
[b] Breast muscle.
[c] 6 target PCB means added.
[d] Sums of congener or compound means.
[e] Adult.
[f] Young.
[g] Sum of congener means.
[h] Female.
[i] Male.

and Hutton, 1979; Konstantinou et al., 2000). Correlation coefficients between pollutants' concentrations are increasing with the contamination level and indicate that the birds have accumulated the correlating chemicals from the same sources (Becker et al., 2001). Due to such relationships some effects ascribed to PCBs may have been due to DDE (Custer et al., 1999). Also, PCBs are known to increase the effect of *p, p′*-DDE (Provini and Galassi, 1999) thus, although concentrations of separated OC compounds can range below threshold values for poisoning, their combined effects may affect a bird (Sakellarides et al., 2006). Although other studies did not reveal a clear correlation between Hg and OCs in seabird livers (Furness and Hutton, 1979), a strong correlation of this element with HCB, ∑HCHs and ∑PCBs was detected in this study.

4.4. Explanation of spatial trends

Pollutant concentrations detected in the wintering areas of birds can be considerably affected by pollution in staging and origin areas (Falandysz and Szefer, 1984; Kunisue et al., 2002; Tanabe et al., 1998). We found clear geographic trends in DDT and metabolites (owing to *p, p′*-DDE and *p,p′*-DDD) with great cormorant livers' concentration pattern as Evros > Messolonghi > Amvrakikos > Axios. This pollution pattern seems to contrast these of DDT compounds for the 1990s, based on water analyses of rivers in some of our study areas, being Axios > Evros > Amvrakikos rivers (Louros and Arachthos) (Konstantinou et al., 2006). In Messolonghi area, there is evidence that Acheloos River has a low pollution profile (Dassenakis et al., 1997). The pollution pattern Axios > Evros > Amvrakikos for DDTs, and some other OCs, is also supported from the analysis of biota, particularly waterbird eggs, from their breeding areas (Table 5) whereas for other species (Mediterranean gull *Larus melanocephalus*) the differences found were not significant (Goutner et al., 2005). Consequently, the pattern of pollution in great cormorants, particularly by DDTs, does not seem to reflect that of the environment of their wintering wetlands sampled, being an indication of origin and accumulation from other areas.

The available information from ringing recoveries suggests that there are two major areas from where great cormorants arriving to Greece originate: (a) the Baltic part of Sweden, Germany and Denmark [19 (40%) of all ($n = 47$) recoveries considered — 65.5% of

Table 5
Mean levels (ng g^{-1} wet weight) of organochlorines detected in waterbird eggs and some fish species from the wetland areas studied.

	Species	∑DDTs	∑HCHs	∑PCBs	References
Amvrakikos	Dalmatian pelican	11.36	31.97		Albanis et al. (1995)
	Anguilla anguilla	3.23	20.87		Albanis et al. (1995)
Axios	Little tern	9.61	8.8		Goutner et al. (1997)
	Great cormorant	154.2	56.04	47.99	Konstantinou et al. (2000)
	Mediterranean gull	189	88	35	Goutner et al. (2005)
	Common tern	351	124	95	Goutner et al. (2005)
Evros	Great cormorant	115.16	27.19	16.24	Konstantinou et al. (2000)
	Mediterranean gull	117	205	57	Goutner et al. (2005)
	Avocet	262	77	26	Goutner et al. (2005)
	Yellow-legged gull	214.99	64.45	41.16	Albanis et al. (2003)
	Cyprinus carpio	6.14–62.25[a]			Erkmen and Kolankaya (2006)

[a] Range of means from a number of stations (μg g^{-1} lipid weight).

the recoveries from western Greece] and (b) Ukraine region, Crimean peninsula [11 (23%) of recoveries — 61% of the eastern and central Greece recoveries] (Table 6). Levels high such as in Evros might originate from Ukraine, according to this pattern. Despite the drop in use after the 1970s in western Europe, between 1946 and 1990 Ukraine was the 3rd consumer of DDT among 15 countries of the former Soviet Union, and in the 1990s some quantities were probably still used (Li et al., 2006). The high total levels of DDTs (mostly composed of *p,p′*-DDE) in that region are also reflected in great cormorant eggs from the Danube Delta which, despite a drop compared to that in the 1980s, were still high in the late 1990s (from 58,840 ng g^{-1} to 14,900 ng g^{-1} dw, Aurigi et al., 2000; Fossi et al., 1984).

Nevertheless, if birds recovered in the central and eastern Greece originate from the Black Sea region, how the considerable differences in concentrations between Axios and Evros birds can be explained? Firstly, the birds in Axios/Amvrakikos and Evros may originate from areas with a different pollution regime, although at present this is not supported by evidence. This assertion is supported by $\sum$DDTs/$\sum$PCBs ratios in great cormorant livers, being significantly lower in Amvrakikos Gulf, tended to be lower in Axios Delta than in Evros Delta (Table 1). Secondly, if the birds arriving in these wetlands originate from the same area and are similarly polluted, probably in Evros the birds arrive earlier and feed on more polluted food. There were considerable differences in the diet of the great cormorants analysed in this study between these areas (Liordos and Goutner, 2007, 2009). Data on DDTs concentrations of prey are generally lacking, but they were very low in common carp (*Cyprinus carpio*) from Evros in 2002–2003 (Table 5).

Chemicals other than DDE were found in low levels in great cormorants wintering in Greece and the lack of significant differences among areas does not permit speculation about their origin. Anyway, $\sum$DDTs/$\sum$PCBs ratios in great cormorant livers (Table 1) in Amvrakikos and Axios areas were lowest (<1), probably suggesting that these birds originated from areas with higher industrial pollution. The pattern of $\sum$DDTs/$\sum$PCBs>1 in this study is in agreement with other studies based on waterbird eggs that also suggested a dominance of agrochemical over industrial pollution at least in the north-eastern Mediterranean (Goutner et al., 2001a; Konstantinou et al., 2000; Sakellarides et al., 2006) in contrast to the situation in western Europe (e.g., Becker et al., 2001).

Uniform but high Hg concentrations were detected in great cormorant livers. However, as owing to our knowledge, the Greek areas are not heavily polluted by Hg and most of the Hg burden must had been accumulated in the birds' origin and migration areas. Many of the sampled great cormorants originated from the Baltic and Black Seas (Table 6), heavily polluted by Hg due to domestic and industrial discharge and atmospheric deposition (Burgess et al., 2009; Kullenberg, 1981; Pacyna et al., 2006; Shalovenkov, 2000).

Table 6
Geographical origin of great cormorants' ringing recoveries from Greece. Data from Akriotis and Handrinos (2004).

	Western Greece			Central Greece	Eastern Greece	
	Messolonghi	Amvrakikos	Other		Evros	Other
Sweden	3	3	1	–	1	–
Germany	2	2	2	1	–	–
Denmark	2	–	4	1	–	–
Ukraine	1	–	–	4	2	5
Hungary	1	1	1	–	–	–
Estonia	2	–	–	–	–	–
Serbia-Mont.	1	1	–	–	–	–
Moldova	–	–	–	1	–	1
Croatia	1	–	–	–	–	–
Czechia	–	–	1	–	–	–
Poland	–	–	–	1	–	–
Romania	–	–	–	–	1	–

4.5. Toxicological effects

Sublethal effects of Hg on birds include adverse effects on growth, development, reproduction, blood and tissue chemistry, metabolism, and behaviour; histopathology and bioaccumulation were also noted (Eisler, 1987). Possible adverse effects are between 4000 and 40,000 ng g^{-1} dw (dietary intake) whereas lethal liver residues in birds experimentally treated with methylmercury ranged from 17,000 to 70,000 ng g^{-1} dw (Eisler, 1987). If effects are associated with Hg concentrations of ≥4000 ng g^{-1} dw, totally 67 (83.7%) of great cormorants studied here could have been affected. Birds with levels above 17,000 ng g^{-1} dw ($n = 13$, 16.2%), and particularly two birds with 106,534 and 215,101 ng g^{-1} dw from the Evros Delta, could have suffered acute poisoning.

DDE has been associated with eggshell thinning, reduced hatching success (Blus et al., 1974; Custer et al., 1999) and reduction in the survival of young birds (Connell et al., 2003). In great cormorant eggs, 5% thinning or more occurred at a concentration of approximately 4000 ng g^{-1} ww *p,p′*-DDE and higher (Dirksen et al., 1995), whereas similar levels (3900 ng g^{-1} ww) were associated to decreased reproduction in double-crested cormorants (Custer et al., 1999) but the range of 2200–6300 ng g^{-1} ww was not related with eggshell thinning (Yamashita et al., 1993). DDE concentrations associated with decreased reproduction in various waterbird species' eggs range from >2500 ng g^{-1} ww to >8000 ng g^{-1} ww (summarised in Custer et al., 1999). After transforming the great cormorant DDE liver concentrations to a wet weight basis, concentrations were >2500 ng g^{-1} ww (median 7371 ng g^{-1} ww) in only 7 birds (8.75%). Assuming a 20% maternal transfer rate (Barron et al., 1995) concentrations could be associated with adverse effects only for one female bird from Messolonghi Lagoon (6230 ng g^{-1} ww). Therefore it seems that liver *p,p′*-DDE concentrations would have a limited effect on the reproduction of the great cormorant population wintering in Greece.

Mean total PCBs concentrations in great cormorant eggs associated with adverse effects to hatching and breeding success were 16,000–21,000 ng g^{-1} ww in The Netherlands (Dirksen et al., 1995). Mean levels of 23,800 ng g^{-1} ww in Great Lakes' double-crested cormorants similarly resulted in reproductive failure (Weseloh et al., 1983). In the same region and in the same species, total PCBs means of 3600–7300 ng g^{-1} ww were associated with live deformities (hard tissue malformations, Yamashita et al., 1993); whereas in Green Bay, USA, total PCBs means of 13,600 ng g^{-1} ww were not associated to reduced hatching success and the frequency of deformities (Custer et al., 1999). Great cormorants' total PCBs concentrations in Greece ranged from 39 to 3483 ng g^{-1} (transformed to wet weight). Assuming a transfer rate of 20% (an average value of maternal PCBs, Barron et al., 1995) to the eggs, these levels are too low to have any observable effect on the birds' reproduction.

HCB effects have been described in diet concentrations of 1000 ng g^{-1} ww for Japanese quail (*Coturnix japonica*), 7670 ng g^{-1} ww in common tern (*Sterna hirundo*) eggs (Courtney, 1979) and 10,900 ng g^{-1} ww in great cormorant livers (Koeman et al., 1973). The HCB (wet weight transformed) concentrations in great cormorant livers ranged 3.9 to 2671 ng g^{-1} ww, that is very low to have direct biological effects to the birds.

5. Conclusions

This is the first study carried out on the occurrence of OCs and Hg in livers of great cormorants in Greece. Based on published concentration limits, more than 80% of the sampled birds could have been adversely affected by mercurial pollution. Also, *p,p′*-DDE, β-HCH and HCB concentrations were elevated. These show that parts of the great cormorant population wintering in the European Community may be threatened by pollution. Therefore, further monitoring and control of OCs and Hg levels in their environment are needed. Future research needs to clarify the factors affecting the accumulation of these pollutants in the different sexes. Although the information presented suggests that the Baltic and Black Seas are the most likely regions of origin and accumulation of Hg and DDTs for great cormorants wintering in Greece, more information is needed about the role of the possible local pollution sources, particularly prey.

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