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PCBs and Organochlorine Pesticide Residues in Eggs of Audouin's Gull (*Larus audouinii*) in the North-Eastern Mediterranean

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Levels of eight PCB congeners (IUPAC 8, 20, 28, 52, 101, 118, 138, 180) and 13 organochlorine pesticides (α-BHC, β-BHC, lindane (γ-BHC), heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, 2,4′-DDT, 2,4′-DDD, 4,4′-DDT, 4,4′-DDD, 4,4′-DDE) were measured in unhatched Audouin gull eggs from Aegean Sea (northeastern Mediterranean) colonies in 1997 and 1998. Levels of more persistent congeners 138, 180 and in some colonies 118, predominated among PCBs. Between years significant differences were found in the levels of a variety of contaminants in the same regional colonies (Lipsos, Agathonisi, and Fourni) attributable to temporal changes in diet. Within years differences were limited (1997: PCB congeners 52, 118, 180, heptachlor and 4,4′-DDD; 1998: PCB congener 8 and heptachlor epoxide) which in combination with cluster analysis suggest a temporal rather than spatial pattern of pollution in the Aegean Sea. Maximum median levels of five PCB congeners, total PCBs and eight organochlorine pesticides were found in the Agathonisi and Fourni colonies suggesting an elevated nearby pollution probably from the polluted Menderez River. Levels of all contaminants were too low to have any adverse reproductive effects on the Audouin's gull of the Aegean colonies.

Keywords: polychlorobiphenyls; organochlorines; *Larus audouinii*; Aegean Sea; Mediterranean.

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Introduction

Toxicity coupled with environmental persistence of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) led in their long ago banning in Europe, nonetheless they are still being detected in Mediterranean aquatic habitats and biota (Focardi *et al.*, 1988; Georgakopoulos-Gregoriades *et al.*, 1991; Lopez-Martin *et al.*, 1995; Swindlehurst *et al.*, 1995; Albanis *et al.*, 1994, 1996; Bazzanti *et al.*, 1997; Fasola *et al.*, 1998). Mediterranean sea is a polluted region, receiving considerable discharges of industrial and municipal inputs and, simultaneously, subjected to pollutants' accumulation and dispersion greatly due to its closed formation (Fowler, 1986). The Mediterranean Pollution Monitoring and Research Programme (MED POL), set up in 1983, attempted to co-ordinate pollution monitoring but, on a regional basis, could not provide adequate description of the state of contamination of the marine environment in the Mediterranean (Jeftic, 1993; Swindlehurst *et al.*, 1995). Undoubtedly, a large gap exists in pollution monitoring in the eastern Mediterranean.

Due to PCBs' and OCs' lipophilic properties and persistence, they tend to bioaccumulate and biomagnify in the food chains, thus aquatic birds and their eggs have been proved suitable tools for monitoring organochlorine contamination with the most outstanding case that of the herring gull (*Larus argentatus*) in the Canadian Great Lakes (Weseloh *et al.*, 1979; Mineau *et al.*, 1984; Struger *et al.*, 1985; Fox and Weseloh, 1987; Stow, 1995; Hebert *et al.*, 1997, 1999) and elsewhere (Oxynos *et al.*, 1993). In the Mediterranean, monitoring of PCBs and OCs acquired a particularly interesting dimension through studies on the Audouin's gull (*Larus audouinii*) an endangered gull species breeding only within the Mediterranean (Oro, 1998). Most (70%) of its world population (*ca* 18 600 pairs), are concentrated at the

Ebro Delta colony, Spain, and a generally low number of colonies are scattered mainly on Mediterranean islands (Oro, 1998). Recent surveys carried out by the Hellenic Ornithological Society (HOS) discovered that in 1997, the Hellenic seas, hosted at least 530 pairs in 20 colonies (Goutner *et al.*, 2000). High levels of PCBs and OCs have been detected in Audouin's gull eggs in Italy (Leonzio *et al.*, 1989) and Spain (Gonzalez *et al.*, 1991; Pastor *et al.*, 1995a,b, 1996). Pastor *et al.* (1995b) found that levels of PCBs could potentially cause embryonic diseases. Most of these studies suggested long-term monitoring of organochlorines in Audouin's gull and investigation of their relationship to the breeding performance of the gull.

Considering the conservation importance of the Audouin's gull in the Mediterranean as a whole and in Greece in particular, the absence of pollution monitoring studies in the eastern Mediterranean especially on high trophic-level receptors, and, acting within the framework of a project aiming on the conservation of Audouin's gull in Greece, we investigated the incidence, levels and potential effects of organochlorines on the this gull in Aegean Sea colonies.

Study areas

The general location of the colony areas sampled are shown in Fig. 1. The Audouin's gull colonies were situated on small isolated rocky islands with generally rough terrain. In Dodecanese archipelagos, five colony areas were studied. In both study years (1997 and 1998), Lipsos colony was situated on the same island (22.5 ha). Agathonisi colony, at the north-east, was situated on an almost level island (12.7 ha) in 1997, whereas in 1998 on a smaller island (9.0 ha) with rocky cliffs 8 km distant.

Fig. 1 Map indicating the Audouin's gull colony areas studied within the context of Greece.

During the study years, Fourni colony, at north Dodecanese area, was also situated on different islands (extending over 30 ha in 1997 and 35 ha in 1998) 4 km distant from each other. Astypalea colony (1998), was made on an island extending over 47 ha. Halki colony, at the south-east Dodecanese region, was made on a small (0.8 ha) rocky island and was occasionally sampled in 1997. In the Cyclades archipelago, situated east of Dodecanese, Amorgos colony, at south-eastern part of this area, was made on an island c. 25 ha only studied in 1998. In the Kythera region, south of Peloponnesus, we studied Kythera colony (1997), located on a small (c. 3 ha) rocky island situated near the island of Kythera. Detailed description of 1997 Dodecanese and Kythera colony sites is given in Goutner *et al.* (2000).

Materials and Methods

Field sampling

For reasons of the species protection, eggs which did not hatch were only collected, under license. Eggs collected were only those with uncracked shells. These were placed in cotton-spread cartons (of those used to transport hen's eggs) and were then brought to the HOS field laboratory on Lipsos where they were opened, their contents poured in chemically cleaned glass containers and were subsequently deep frozen to −20°C. After the end of each field season material was transported to the University of Ioannina for chemical analyses.

Reagents

In total, the following eight PCB congeners were analysed: PCB 8, 20, 28, 52, 101, 118, 138, 180. Of these congeners five (in terms of the PCB IUPAC Nos 28, 52, 101, 138, 180) belong to the group known as 'target' or 'indicator' PCBs (Scrimshaw and Lester, 1995; Bachour *et al.*, 1998). PCB 153 is also included in this list but we did not analyse it due to lack of standard. In addition, we measured the levels of PCB IUPAC Nos 8 and 20. Although di-, tri- and tetra-chlorobiphenyls exhibit low accumulation in bird body tissues in comparison to highly chlorinated congeners (Gagnon *et al.*, 1990; Metcalfe and Metcalfe, 1997), their bioaccumulation in fish and marine birds is largely mediated by food and particulate ingestion (Stranberg *et al.*, 1998) and therefore we were stimulated to examine this in the Audouin's gull. The concentrations of several di- and tri-PCBs have also been reported in various waterbird species (Henriksen *et al.*, 1996; Zimmermann *et al.*, 1997; Stranberg *et al.*, 1998). The organochlorine pesticides analysed in this study were α-BHC, β-BHC, lindane (γ-BHC), heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, 2,4′-DDT, 2,4′-DDD, 4,4′-DDT 4,4′-DDD 4,4′-DDE.

PCB-standards were obtained from Dr. Ehrensdorfer GmbH laboratory in concentrations of 10 mg/ml. Supelco No. 4-9151 organochlorine pesticides mixture standard in iso-octane was used in concentrations of mg/ml for the chromatographic analysis. All solvents used (hexane, acetone, petroleum ether), were pesticide residue analysis grade, purchased from Pestiscan (Labscan, Dublin, Ireland). Florisil (50–100 mesh) and sodium sulphate (pro-analysis) were from Merck (Darmstadt, Germany). Glassware was soaked, cleaned with chromic solution, thoroughly rinsed with distilled water and acetone and heated at 150°C for 12 h.

Analytical procedures

Egg contents were homogenized in a blender and a part from each sample (1–2 g) was transferred to a watch glass and then to an oven for drying to constant weight in 60°C for estimating moisture content. A mean egg moisture content of 74.7% (SD: 2.5) was found. Mean lipid content, determined with the Bligh and Dyer method, was 26.2% of dry weight. An aliquot of 5–10 g was homogenized again with 20–30 g of sodium sulphate in glass tubes of 100 ml. The mixture was extracted firstly with 20 ml, and followed by two more times with 10 ml with hexane: petroleum ether (1:1) mixture using a vortex (2 min), sonication bath (5 min) and manual mixing (5 min) with a glass rod. The extracts were collected in polypropylene centrifugation tubes of 50 ml, centrifuged in 4000 rpm for 5 min. The supernatant was evaporated in a rotary evaporator to 10 ml and lipids were then removed by treating the extracts with 0.5–0.8 ml aliquots of concentrated sulphuric acid. The procedure was repeated until the acid layer remained colourless (Pavoni *et al.*, 1991). The clean-up was completed by adsorption chromatographic, eluting the colourless layer through a chromatography glass column 1 cm i.d., 20 cm length, provided with o teflon stopcock. The column was packed as follows: 5 cm Florisil slurry was added first under gentle tapping of the column and keeping stopcock open, to avoid bubbles, and then 2 cm dried sodium sulphate was added. The column was washed with 20 ml *n*-hexane. All solvents used for packing the column were degassed in sonication bath (Fytianos *et al.*, 1997). The purified sample was evaporated in a rotary evaporator to a *ca* 5 ml and in gentle N_2 stream at 35°C to a *ca* 0.5 ml, then samples were stored in silanized vials in a refrigerator (−20°C). Mean recoveries and method detection limits for each congener and compound are given in Table 1.

Chromatographic conditions

GC–ECD. A Shimadzu 14B gas chromatograph equipped with Ni 63 electron capture detector (ECD) was used for the organochlorine residue analysis. The capillary column used was a DB-5, 30 m × 0.32 mm i.d., contained (5% phenyl) methyl polysiloxane (J&W Scientific, Folsom, CA) followed the temperature programme: 150°C (2 min), 150–200°C (5°C/min), 200°C (45 min), 210–270°C (10°C/min), 270°C (3 min). The temperatures were set at 250°C for the injector and 300°C for the detector. Helium was used as the carrier and nitrogen as the make-up gas. Pure reference standard solutions were used for instrument calibration, recovery, quantification and confirmation. The splitless mode was used for injection of 1 μl volume, with the valve opened for 30 s.

TABLE 1

Mean recoveries (%) and method detection limits (MDL) of PCBs and OCs from spiked eggs.

	Mean recovery % ($n = 3$)	MDL (ng/g)
PCBs		
PCB 8	107.2	0.5
PCB 20	106.5	0.5
PCB 28	107.2	0.4
PCB 52	107.3	0.4
PCB 101	106.4	0.3
PCB 118	110.2	0.3
PCB 138	110.5	0.2
PCB 180	111.6	0.2
Organochlorine pesticides		
α-BHC	91.3	0.4
β-BHC	82.2	0.5
Lindane	92.4	0.3
Aldrin	95.2	0.4
Dieldrin	95.7	0.3
Endrin	96.1	0.3
Heptachlor	94.2	0.3
Heptachlor epoxide	86.3	0.6
4,4′-DDE	81.8	0.6
2,4′-DDD	88.9	0.5
2,4′-DDT	97.5	0.3
4,4′-DDD	91.3	0.4
4,4′-DDT	96.3	0.3

GC–MS. The confirmation of organochlorine residues was performed by using a GC-MSD, QP 5000 Shimadzu equipped with DB-5 capillary column, 30 m × 0.32 mm i.d. contained (5% phenyl) methyl polysiloxane (J&W Scientific, Folsom, CA) was used in the following chromatographic conditions: Injector temperature 220°C, column programme of temperatures 55°C (2 min), 55–210°C (5°C/min), 210°C (20 min), 210–271°C (20°C/min), 270°C (4 min). Helium was used as the carrier gas at 14 psi. The interface was kept at 270°C. The spectra were obtained at 70 eV. The splitless mode was used for injection of 1 μl volume, with the valve opened for 30 s. Two ions (M^+, M^{+2}) for each pesticide and biphenyl were chosen for screening analysis in selected ion monitoring mode (SIM). Ions were selected after injecting a concentrated solution of compounds and recording the 'total ion chromatogram'. The ions' traces were divided into five groups that were recorded sequentially during the injection, on the basis of the retention times of the single substances. For the PCBs identification, the selected ions for each degree of chlorination were confirmed by checking the ratios of intensities of ions belonging to the same cluster (Raccanelli *et al.*, 1994; Singh *et al.*, 1998).

Statistical procedures

Concentrations of pollutants (transformed on ng/g dry weight) were checked by the Kolmogorov–Smyrnov χ^2 test, but were not normally distributed, thus median levels of each pollutant and total median concentrations of PCBs (hereafter PCBs) were compared by Mann–Whitney *U* tests (between years in each colony) and Kruskal–Wallis χ^2 tests (among colonies in each year separately). Correlations between contaminants were tested using Spearman rank correlations. We calculated the ratio of total OC concentrations ($\sum$OCs) and $\sum$PCBs ($\sum$OCs/$\sum$PCBs) in the samples of each study area as a measure of agrochemical or industrial pollution (Fossi *et al.*, 1984; Pastor *et al.*, 1995a) and we then compared the medians between and among areas (as previously specified). Cluster analyses (with Euclidean distances as distance measure and single linkage as a linkage rule), separately for PCBs and OCs, were used to evaluate overall differences in pollution patterns among areas studied.

Results

Polychlorinated biphenyls

All eight congeners analysed were detected within the limits of our study area. Levels of low-chlorinated congeners were too low. Levels of congeners 138 and 180 were predominant in all colonies and in both study years (Table 2, Fig. 2). Congener 118 was absent from Lipsos, Fourni and Astypalea colonies in 1998 but in the other colonies (excepting Amorgos) was found in concentrations higher than the other congeners, excepting 138 and 180. The median concentrations of congeners 101 and 180 and $\sum$PCBs differed significantly between 1997 and 1998 at Lipsos colony, of congeners 8 and 28 Agathonisi colonies, and of all congeners excepting 101 and 138 at Fourni colonies. Comparing contaminant levels among all colonies studied in each year, significant differences were only found between median concentrations of congeners 52 and 180 (different in 1997) and 8 (different in both years) (Table 2). Total median PCBs were highest at Agathonisi and lowest at Lipsos, both in 1998. The maximum median concentrations of five out of the eight congeners analysed (8, 20, 28, 52 and 138) were also detected at Agathonisi colonies. Of highest detected total PCBs and maximum levels of congeners 118 and 180 were detected in Kythera, the highest levels of congener 101 and second highest median total PCBs and PCB 138 were detected in Astypalea.

Statistically significant Spearman rank correlations between PCB congeners were generally limited (Table 3) and most (9 out of 17, 53%) were between highly chlorinated congeners (penta- to hepta-PCBs) being mainly positive.

A cluster analysis of percent congener levels, separated Lipsos 1998 from all other colonies. Another two distinct clusters separated colony contaminant levels of 1997 and 1998 (Fig. 3).

Organochlorine pesticides

All 13 organochlorine compounds in the Audouin's gull eggs were analysed were detected within study area. In 1998 endrin was below detection limits in all samples. Significant differences between 1997 and 1998 were found in median concentrations of heptachlor epoxide in samples from Lipsos, Agathonisi and Fourni; also in median concentrations of endrin in Agathonisi and Fourni, heptachlor in Agathonisi and 2,4′-DDD and 2,4′-DDT in Fourni samples. Within years significant differences in median OC concentrations were only found in heptachlor and 4,4′-DDD (1997) and heptachlor epoxide (1998). In all egg samples β-BHC was found in far highest concentrations of other HCHs, reaching a maximum median of 258 ng/g in Agathonisi colony in 1997. Maximum median concentrations of lindane did not exceed 8 ng/g (Astypalea 1998) and of α-BHC 9 ng/g (Fourni 1997). Dieldrin was only found in the one egg sample from Halki colony (1997) in concentration much higher than the highest concentration of others drins in our samples (maximum of aldrin: 5 ng/g, Fourni 1998; maximum of endrin 13 ng/g, Agathonisi 1997). Heptachlor epoxide was found in higher concentrations than heptachlor in all areas with maximum levels found in the area of Fourni (55 ng/g and 3 ng/g, respectively). Of DDT metabolites, 2,4′-DDD was found in highest levels in all areas with a maximum median of 1300 ng/g in Kythera samples. 2,4′-DDT was below detection limits in samples from Halki, Lipsos and Fourni in 1998 and 4,4′-DDD from Lipsos (both years), Fourni and Kythera in 1997. In all areas (excepting Halki) concentrations of 4,4′-DDT (with a maximum median of 48 at Agathonisi in 1998) were below these of 2,4′-DDD. Concentrations of DDE were very low with a maximum median of 7 ng/g found at Agathonisi in 1998. In 1997, significant differences in median concentrations found among colonies were of heptachlor and 4,4′-DDD whereas in 1998 only of heptachlor epoxide (Table 2). The dominance of β-BHC and 2,4′-DDD in the samples from all areas provided a characteristic fingerprint of OCs (Fig. 4). The most elevated median levels of four OC compounds (β-BHC, endrin, 4,4′-DDE, 4,4′-DDT) were detected at Agathonisi colonies whereas the maximum median concentrations of another four OCs (α-BHC, aldrin, heptachlor, heptachlor epoxide) were found at Fourni colonies (situated relatively close to Agathonisi, Fig. 1). Maximum median levels of lindane, 2,4′-DDT, 4,4′-DDD were detected at Astypalea.

A cluster analysis of percent compound levels, as in the case of PCBs, separated Lipsos 1998 from all other colonies. Grouping of colonies did not reveal any geographical pattern in contamination with the exception of Agathonisi and Fourni colonies (1997) that were grouped together. A temporal grouping was also found, with all 1997 colonies grouped on the right-hand side of the cluster (Fig. 5).

Correlations, either positive or negative, were found between BCH compounds and also between BCHs, cyclodiens and DDTs whereas within the last group they were limited (Table 4).

TABLE 2

Concentration of PCB congeners and organochlorine pesticide compounds (ng/g dry weight) in eggs of Audounin's gull from Aegean colonies.[a]

	Lipsos 1997 ($N = 9$)				Lipsos 1998 ($N = 2$)					Agathonisi 1997 ($N = 7$)				Agathonisi 1998 ($N = 7$)					Fourini 1997 ($N = 4$)			
	Mean	Median	Min	Max	Mean	Median	Min	Max	p	Mean	Median	Min	Max	Mean	Median	Min	Max	p	Mean	Median	Min	Max
PCBs																						
PCB 8	10	8	<d. l	23	<d. l.	<d. l.	<d. l.	<d. l.	0.059	26	11	6	77	<d. l.	<d. l.	<d. l.	<d. l.	0.003	17	18	7	23
PCB 20	4	3	<d. l.	9	2	2	2	2	0.814	6	4	<d. l.	17	10	9	5	19	0.116	2	2	<d. l.	5
PCB 28	6	2	<d. l.	19	17	17	14	20	0.099	8	8	<d. l.	20	25	25	<d. l.	42	0.046	7	8	<d. l.	14
PCB 52	5	4	<d. l.	16	5	5	4	6	0.480	18	5	<d. l.	64	18	19	12	24	0.199	3	3	2	5
PCB 101	8	5	2	34	<d. l.	<d. l.	<d. l.	<d. l.	0.034	9	8	3	17	16	14	< d.l.	35	1.000	2	2	<d. l.	3
PCB 118	100	100	<d. l.	213	<d. l.	<d. l.	<d. l.	<d. l.	0.059	68	53	<d. l.	163	36	<d. l.	<d. l.	133	0.317	75	73	60	97
PCB 138	189	184	<d. l.	397	52	52	29	76	0.099	267	159	80	866	404	414	194	545	0.063	136	149	87	160
PCB 180	139	118	49	338	25	25	19	31	0.034	114	91	43	249	107	95	66	176	0.568	94	92	82	110
ΣPCBs	461	444	151	952	102	102	69	135	0.034	516	434	255	996	617	645	354	796	0.253	338	349	275	378
Organochlorine pesticides																						
α-BHC	1	<d. l.	<d. l.	6	<d. l.	<d. l.	<d. l.	1	1.000	<d. l.	<d. l.	<d. l.	2	1	1	<d. l.	2	0.116	11	9	2	24
β-BHC	174	186	68	279	155	155	146	164	0.637	309	258	167	524	200	163	77	347	0.153	216	207	157	295
Lindane	2	1	<d. l.	7	<d. l.	<d. l.	<d. l.	<d. l.	0.239	2	2	<d. l.	5	4	3	<d. l.	8	0.520	<d. l.	<d. l.	<d. l.	1
Aldrin	1	<d. l.	<d. l.	3	1	1	1	1	0.346	1	<d. l.	<d. l.	2	2	1	<d. l.	5	0.830	<d. l.	<d. l.	<d. l.	<d. l.
Dieldrin	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	–	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	–	<d. l.	<d. l.	<d. l.	<d. l.
Endrin	5	5	<d. l.	7	<d. l.	<d. l.	<d. l.	<d. l.	0.059	7	7	<d. l.	13	<d. l.	<d. l.	<d. l.	<d. l.	0.010	4	4	<d. l.	8
Heptachlor	<d. l.	<d. l.	<d. l.	2	1	1	1	1	0.099	<d. l.	<d. l.	<d. l.	1	2	2	1	3	0.003	2	2	<d. l.	3
Heptachlor epoxide	64	55	15	188	1	1	1	1	0.034	51	32	5	140	2	3	1	3	0.003	57	55	20	98
4,4′-DDE	1	<d. l.	<d. l.	3	1	1	1	2	0.637	4	3	1	8	11	7	3	36	0.063	2	2	1	2
2,4′-DDD	1022	549	77	2833	101	101	64	139	0.059	1134	687	331	2639	449	440	204	609	0.153	663	582	455	1035
2,4′-DDT	3	3	<d. l.	6	<d. l.	<d. l.	<d. l.	<d. l.	0.099	1	<d. l.	<d. l.	3	2	<d. l.	<d. l.	15	0.830	3	2	1	8
4,4′-DDD	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	–	17	<d. l.	<d. l.	61	4	3	<d. l.	9	0.830	<d. l.	<d. l.	<d. l.	<d. l.
4,4′-DDT	27	32	<d. l.	49	2	2	1	4	0.099	29	33	11	39	37	48	<d. l.	59	0.253	15	16	<d. l.	27
ΣOCL/ΣPCBs	2.82	3.16	0.60	4.24	2.78	2.78	2.18	3.38	0.637	3.23	3.48	0.70	5.11	1.15	1.10	0.86	1.51	0.032	2.86	2.76	2.11	3.80

	Fourini 1998 ($N = 6$)					Kythera 1997 ($N = 15$)				Amorgos 1998 ($N = 6$)				Astypalea 1998 ($N = 2$)				Halki 1997 ($N = 1$)[b]	Kruskal–Wallis χ^2/p (1997 colonies)[c]	Kruskal–Wallis χ^2/p (1998 colonies)[d]
	Mean	Median	Min	Max	p	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max			
PCB 8	<d. l.[e]	<d. l.	<d. l.	<d. l.	0.011	4	1	<d. l.	17	8	7	<d. l.	21	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	10.062/0.0181	9.263/0.055
PCB 20		13	11	6	22	0.011	6	5	<d. l.	21	7	7	3	10	8	8	6	1	0.292/0.961	8.000/0.092
PCB 28	41	37	26	59	0.011	13	10	5	34	29	35	<d. l.	43	33	33	18	48	<d. l.	1.827/0.609	4.000/0.406
PCB 52	21	22	8	29	0.011	29	11	4	93	16	17	7	25	18	18	12	24	1	12.302/0.006	2.667/0.615
PCB 101	8	<d. l.	<d. l.	44	0.670	7	7	<d. l.	17	3	<d. l.	<d. l.	17	42	42	21	64	16	4.829/0.185	6.155/0.188
PCB 118	<d. l.	<d. l.	<d. l.	<d. l.	0.011	138	121	62	256	21	<d. l.	<d. l.	110	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	7.0401/0.071	4.074/0.396

TABLE 2 (CONTINUED)

PCB 138	314	365	64	408	0.088	238	215	90	500	297	330	100	418	407	407	244	570	13	5.897/ 0.117	8.000/0.092
PCB 180	62	65	34	76	0.011	191	151	50	478	69	62	40	120	82	82	53	110	11	8.642/ 0.035	5.333/0.255
ΣPCBs	458	510	226	571	0.088	624	555	299	1255	449	458	189	593	590	590	354	827	41	5.897/ 0.117	5.333/0.255
Organochlorine pesticides																				
α-BHC	3	2	1	8	0.088	<d. l.	<d. l.	<d. l.	1	2	1	<d. l.	8	3	3	1	4	9	5.897/ 0.117	5.333/0.255
β-BHC	194	175	105	307	0.522	226	213	166	311	124	107	59	220	240	240	205	275	<d. l.	1.182/ 0.757	2.667/0.615
Lindane	8	4	<d. l.	23	0.337	1	<d. l.	<d. l.	6	19	6	<d. l.	51	8	8	<d. l.	16	<d. l.	1.293/ 0.731	2.667/0.615
Aldrin	3	5	<d. l.	5	0.136	1	1	<d. l.	3	2	1	<d. l.	4	3	3	2	3	1	4.296/ 0.231	5.333/0.255
Dieldrin	<d. l.	<d. l.	<d. l.	<d. l.	–	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	101	–	–
Endrin	<d. l.	<d. l.	<d. l.	<d. l.	0.055	4	4	<d. l.	8	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	<d. l.	3.037/ 0.386	–
Heptachlor	3	3	<d. l.	8	0.915	<d. l.	<d. l.	<d. l.	1	2	2	1	3	2	2	1	3	<d. l.	7.773/ 0.051	5.333/0.255
Heptachlor epoxide	5	4	3	10	0.011	46	48	<d. l.	81	3	3	2	5	4	4	2	6	<d. l.	0.292/ 0.961	10.667/0.031
4,4′-DDE	6	<d. l.	<d. l.	27	0.394	22	3	<d. l.	291	3	2	1	9	5	5	4	6	<d. l.	3.326/ 0.344	8.000/0.092
2,4′-DDD	253	248	171	327	0.011	1265	1300	339	2503	323	329	148	445	300	300	180	421	<d. l.	1.827/ 0.609	8.000/0.092
2,4′-DDT	<d. l.	<d. l.	<d. l.	<d. l.	0.011	4	3	<d. l.	16	2	<d. l.	<d. l.	11	7	7	<d. l.	15	<d. l.	4.257/ 0.235	3.602/0.462
4,4′-DDD	6	9	<d. l.	12	0.088	<d. l.	<d. l.	<d. l.	<d. l.	2	<d. l.	<d. l.	7	15	15	<d. l.	29	3	8.485/ 0.037	3.178/0.528
4,4′-DDT	27	34	<d. l.	49	0.394	35	31	16	75	30	33	2	53	16	16	<d. l.	31	<d. l.	4.296/ 0.231	2.667/0.651
ΣOCL/ ΣPCBs	1.21	1.04	0.78	1.94	0.010	2.73	3.07	1.45	4.39	1.19	1.22	0.82	1.53	1.07	1.07	0.94	1.20	1.40	1.293/ 0.731	5.333/0.255

[a] Means values are given for comparisons with other studies.
[b] Halki data were not used in statistical comparisons.
[c] df = 3.
[d] df = 4.
[e] <d. l. :below detection limit.

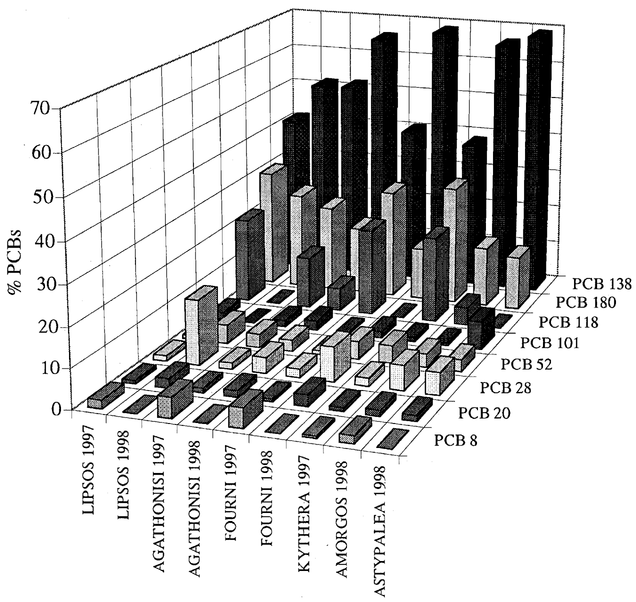

Fig. 2 Fingerprints of PCB congeners (% of total concentration in egg samples of each colony) detected in Audouin's gull colonies studied in the Aegean Sea.

Spearman rank correlations between ∑PCBs and ∑OCs for all Aegean samples in 1997 and 1998 were both positive and highly significant (1997: $r_s = 0.658$, $p < 0.0001$, $N = 35$; 1998: $r_s = 0.789$, $p < 0.0001$, $N = 22$). The medians of the ratio ∑OCs/∑PCBs varied from 1.04 (Fourni 1998) to 3.48 (Agathonisi 1997) but in each year the difference among areas was not significant (Table 2). Median ratios were significantly different between 1997 and 1998 both at Agathonisi and Fourni colonies, being higher in 1998.

TABLE 3

Spearman rank correlations coefficients (r_s) and significance levels (p) between PCB congener concentrations in Audouin's gull eggs from the Aegean Sea. Only significant correlation are given.

Area[a]	PCB congeners tested	r_s	p
Agathonisi 1997	101–138	0.750	0.052
	138–180	0.786	0.036
Fourni 1997	8–20	0.949	0.051
	20–180	−0.949	0.051
Lipsos 1997	20–101	0.661	0.053
	118–138	0.883	0.002
	118–180	0.717	0.030
	138–180	0.883	0.002
Kythera 1997	20–38	−0.511	0.052
	20–118	−0.614	0.015
	20–180	−0.589	0.021
	118–138	0.943	<0.0001
	118–180	0.800	<0.0001
	138–180	0.818	<0.0001
Fourni 1998	101–138	−0.845	0.034
Amorgos 1998	20–138	0.829	0.042
	52–138	0.829	0.042

[a] Lipsos 1998, Astypalea and Halki data were not tested due to small sample sizes.

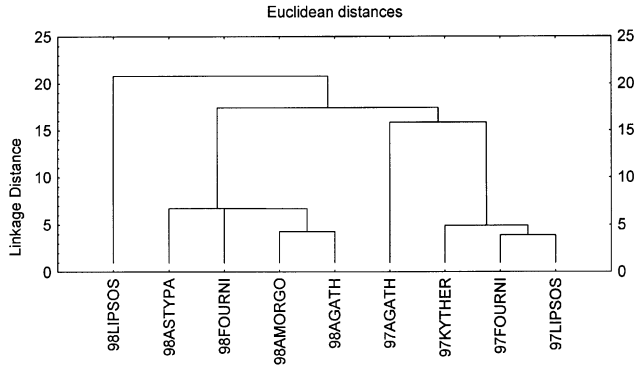

Fig. 3 Relationship of areas studied with regard to levels of PCB congeners measured in Audouin's gull eggs (% of total concentration in egg samples of each colony). Euclidean distances as distance measure and single linkage as a linkage rule were used.

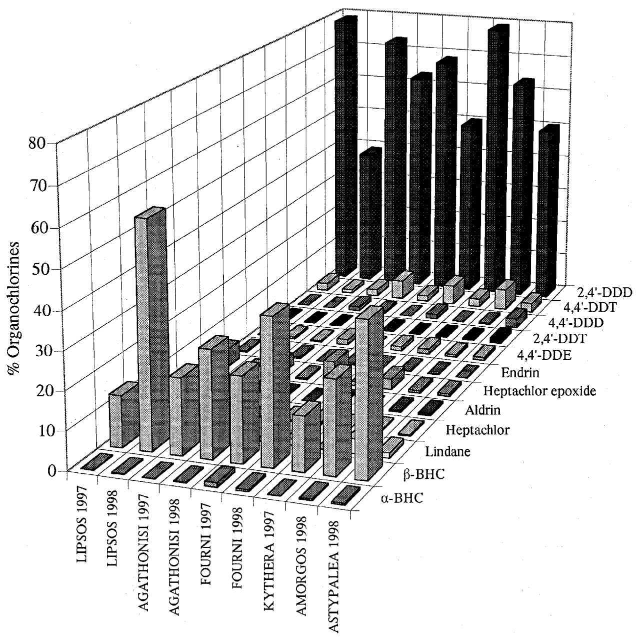

Fig. 4 Fingerprints of organochlorine pesticides (% of total concentration in egg samples of each colony) detected in Audouin's gull eggs in colonies studied in the Aegean Sea.

Discussion

In pollution studies carried out on the Audouin's gull, congeners 138 and 180 were among those found in highest levels (Gonzalez *et al.*, 1991; Pastor *et al.*, 1995a,b). These congeners have also been found among those predominating in the Yellow-legged Gull (*Larus cachinnans michahellis*) in the Mediterranean (Focardi *et al.*, 1988) and in other gulls, waterbirds and biota worldwide (Braune and Norstrom, 1989; Gagnon *et al.*, 1990; Gabrielsen *et al.*, 1995; Gurgue and Tanabe, 1997; Metcalfe and Metcalfe, 1997; Hebert *et al.*, 1999). This fact seems to be due to the persistency and bioaccumulative properties of these congeners which have been attributed to their particular structure characterized by the absence of chlorine unsubstituted adjacent *meta* and *para* positions on the biphenyl ring (Walker, 1990;

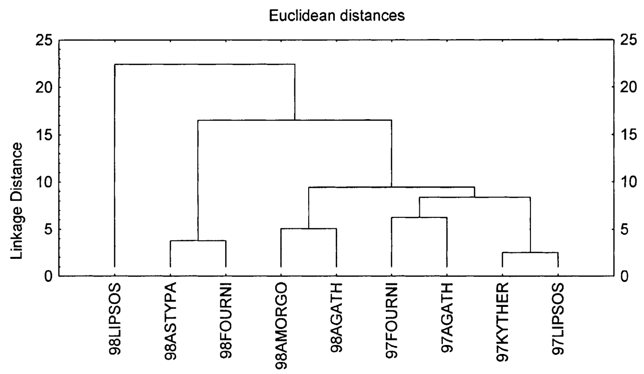

Fig. 5 Relationship of areas studied with regard to levels of organochlorine pesticides in Audouin's gull eggs (% of total concentration in egg samples of each colony). Euclidean distances as distance measure and single linkage as a linkage rule were used.

Gurgue and Tanabe, 1997). As expected, levels of low-chlorinated congeners were low due to their low bioaccumulation. In a variety of studies, concentrations of congeners such as 28 and 52 in other waterbirds' eggs were generally higher than those reported in this study (Bosveld *et al.*, 1995; Dirksen *et al.*, 1995; Haffner *et al.*, 1997).

The source of both PCBs and OCs in waterbirds' eggs is diet and body resources of the female prior to laying (Mineau *et al.*, 1984; Braune and Norstrom, 1989; Furness, 1993). The high correlation found between the concentration of both groups has also been found in other studies (Blus *et al.*, 1974; Gilbertson, 1974; Gabrielsen *et al.*, 1995; Mason *et al.*, 1997; Custer *et al.*, 1999) revealing the common lipophilic properties of organochlorines and probably their common source or common pathways through the food chain. The diet of the Audouin's gull in the study area primarily constituted of fish most of which are actively captured from the epipelagic zone. Highest median concentrations of congeners 8, 20, 28 and 52 were found in samples from Fourni where in both years the chick diet mainly composed by the surface-living *Boops boops* (64.8% and 50% of the totally identified fish, HOS unpub. data). Low PCB congeners (di-, tri- and tetra-chlorinated) have been found to predominate in surface water, plankton and fish in non-point source areas (Tanabe *et al.*, 1984; Hoshi *et al.*, 1998). We have no direct information on the Audouin's gull diet during the pre-laying period, but the most important fish prey fed to chicks in the colonies of Lipsos, Fourni and Agathonisi, presented differences in composition between 1997 and 1998 (HOS unpub. data). Thus, temporal dietary differences probably accounted for the differences in organochlorine levels in each colony. Intercolony differences in contaminant

TABLE 4

Spearman rank correlaions (r_s) and significance levels (p) between organochlorine pesticide compounds in Audouin's gull eggs from the Aegean Sea.[a]

Areas[b]	Compounds tested	r_s	p
Agathonisi 1997	α-BHC – Lindane	0.941	0.002
	Hept. epox. – 4,4′-DDD	−0.757	0.049
	4,4′-DDE – 4,4′-DDT	0.821	0.023
	2,4′-DDD – 4,4′-DDT	0.821	0.023
Fourni 1997	α-BHC – Aldrin	−0.949	0.051
	Heptachlor – Aldrin	−0.949	0.051
Lipsos 1997	α-BHC – Lindane	−0.659	0.053
	Hept. epox. – 4,4′-DDE	−0.730	0.025
	β-BHC – Endrin	0.717	0.030
	Lindane – Endrin	0.783	0.013
	β-BHC – 4,4′-DDT	0.733	0.025
	Lindane – 4,4′-DDT	0.714	0.031
Kythera 1997	Heptachlor – Hept. epox.	−0.586	0.022
	β-BHC – 4,4′-DDE	0.509	0.053
	Aldrin – 2,4′-DDT	0.810	<0.0001
	2,4′-DDD – 4,4′-DDT	0.607	0.016
Agathonisi 1998	β-BHC – Lindane	−0.880	0.021
	β-BHC – 2,4′-DDD	0.829	0.042
	Aldrin – 4,4′-DDD	0.935	0.006
Fourni 1998	β-BHC – Lindane	−0.880	0.021
	α-BHC – 2,4′-DDD	0.829	0.042
	α-BHC – 4,4′-DDT	−0.928	0.008
	Lindane – 4,4′-DDT	0.832	0.040
Amorgos 1998	α-BHC – Lindane	0.812	0.050
	β-BHC – Aldrin	0.928	0.008
	Hept. hepox. – 2,4′-DDD	0.943	0.005
	Heptachlor – 4,4′-DDT	0.829	0.042
	2,4′-DDD – 4,4′-DDT	0.829	0.042

[a] Only significant correlations are given.
[b] Lipsos 1998, Astypalea and Halki data were not tested due to low sample sizes.

levels and composition are probably due to differences in the foraging grounds of each colony. In the western Mediterranean, Audouin's gulls may forage long distances far from their colonies (X. Ruiz, L. Serra pers. comm.) and therefore pollutants in their eggs may reflect pollutant levels over a wide range around their colonies. Nevertheless, elevated concentrations of five PCB congeners, $\sum$PCB levels and of four OC compounds at Agathonisi, the occurrence of maximum levels of another four OCs at Fourni colonies and, in addition, the overall similarity between Agathonisi and Fourni in regard to OCs in 1997 (as revealed by the cluster analysis), may be associated to a nearby pollution source. Indeed, eastern to this area (30–90 km away from the colonies), on the Turkish coast, there is the tributary of the Büyük (Great) Menderez River. This river occupies a drainage area of 24,976 km^2, including tributaries of some other rivers and streams, many populated towns within its limits and is also polluted by an increasing number of geothermal energy production plants (Environmental Foundation of Turkey, 1995). Thus, Menderez River is a much probable source of pollution reflected in the Audouin's gull eggs at nearby colonies.Maximum levels of some organochlorines, especially PCBs, at Kythera and Astypalea may be associated with the pollution by washouts of tankers due to the vicinity of international traffics (T. Kominos, pers. comm.). The cluster analysis of PCBs, separated Lipsos 1998 from all other colonies a fact which, coupled with lowest total organochlorine levels found denotes differences in foraging areas or prey composition than colonies of Fourni and Agathonisi. Separation of 1997 and 1998 data probably suggests that organochlorine uptake by Audouin's gull in the Aegean is differentiated temporally rather than spatially. The source of the pollution of each colony will remain unclear unless detailed studies reveal the foraging grounds of each colony.

Pastor *et al.* (1995a) summarizing studies carried out on the Audouin's gull in the western Mediterranean, indicated much higher levels of PCBs found, ranging from a mean of 2 μg/g dw in the Ebro Delta, Spain, to a mean of 41 μg/g dw in Elba, Italy. In Tuscan Archipelago (Italy), levels of total PCBs found in Audouin's gull eggs between 1981–1986 ranged from 29 μg/g dw to 45 μg/g (Leonzio *et al.*, 1989). Total PCB means in eggs from Ebro Delta and Medes Islands were 13 166 ng/g dw being much higher than the sympatric yellow-legged gulls (897 ng/g) greatly due to higher concentrations of the most toxic non-ortho and mono-ortho compounds in the Audouin's gull (Pastor *et al.*, 1995b). These authors consider levels found are a potential source of embryonic diseases. In the Great Lakes' herring gulls, concentrations that have been associated with reduced hatching success are generally higher than 70 μg/g (Gilman *et al.*, 1977; Weseloh *et al.*, 1979). In Great Lakes' double-crested cormorants total PCB means of c. 4 to 7 μg/g were associated to live-deformities (hard tissue malformations, Yamashita *et al.*, 1993), whereas in the Green Bay, USA, total PCB means of 14 μg/g were not associated to reduced hatching success and the frequency of deformities (Custer *et al.*, 1999). Consequently, the contaminant levels found in this study seem to pose no threat to the populations of Audouin's Gulls in the Aegean.

Regarding OCs, the compound which has been given special attention is DDE due to the well-established inverse relationship between DDE content and eggshell thickness having consequences on the breeding productivity of waterbird populations (Gilbertson, 1974; Blus, 1984; Dirksen *et al.*, 1995; Ludwig *et al.*, 1995; Custer *et al.*, 1999). Mean levels of DDE in the Audouin's gull in the Tuscan Archipelago, Italy, measured in unhatched eggs, therefore comparable to this study, ranged from 6 μg/g to 10 μg/g dw (Leonzio *et al.*, 1989), that is levels affecting eggshell thickness to other waterbirds whereas in Chafarinas Islands and Ebro Delta, Spain levels of 2–3 μg/g (not specified if dw or ww) had no effects on eggshell thickness (Gonzalez *et al.*, 1991). Thus, it seems that levels found in the eggs in the Aegean are too low to affect eggshell thickness. In the above-mentioned studies in the Mediterranean the composition of DDT metabolites, estimated as $\sum$DDTs, could not be compared with our results. In earlier studies in the Mediterranean-Black Sea where a variety of DDT metabolites were analysed in waterbird eggs, 4,4′-DDE was found to predominate (Fossi *et al.*, 1984; Fasola *et al.*, 1987). The increased levels of 2,4′-DDD we detected could be due to that unhatched eggs may have the normal proportions of their constituents altered (Pastor *et al.*, 1995a). Nevertheless, fresh eggs of cormorants (*Phalacrocorax carbo*) from four Greek wetlands recently analysed for the same pollutants, indicated fingerprints much similar to Audouin's gull, with β-BHC and 2-4′-DDD dominating over other residues (Konstantinou *et al.*, in press). This suggests that the proportions of contaminants found in Audouin's gull in the Aegean may not underestimate DDE but could reflect a normal follow up of the ban of these substances in the mid-seventies. Elevated 2-4′-DDD levels could also been explained by that this compound probably was a major constituent in a technical mixture that had been used in our region. Higher concentrations of 4,4′-DDD in the harbour porpoise (*Phocoena phocoena*) from the Black Sea suggested the deductive condition of this marine environment resulting from organic waste pollution (Tanabe *et al.*, 1997).

Regarding other contaminants, drins, commonly, though in low concentrations, found in larids' and ardeids' eggs in Italy (Fasola *et al.*, 1987, 1998) were absent in water, sediments, frogs and waterbirds sampled in coastal Greek wetlands except in eggs of distant south-migrant birds such as the squacco heron (*Ardeola ralloides*) and the little tern (*Sterna albifrons*) (Albanis *et al.*, 1994; Albanis *et al.*, 1995; Albanis *et al.*, 1996; Goutner *et al.*, 1997). Two of Audouin's gull chicks ringed at Fourni colonies during the study periods were recently recovered from Lebanon and Malta indicating that Audouin's gulls from Aegean colonies migrate through and probably overwinter in more contaminated areas from where they may accumulate drins. Dieldrin and endrin levels detected in our samples are too low to cause adverse reproductive effects (Blus, 1982).

Lindane metabolites instead of lindane itself were found in all samples probably due to its short life in the environment (Blus *et al*., 1985) and the elevated amounts of β-BHC may indicate that lindane is still in use in this region. Lindane is not harmful to birds, in contrast to heptachlor and especially its metabolite heptachlor epoxide, being lethal for birds in concentrations ≤ 9 μg/g (Blus *et al*., 1985). Concentrations found in Audouin's gulls did not seem to pose any threat on them.

Most of significant correlations found between organochlorines compounds (15 our of 24, 62.5%, Table 4) were positive. Positive correlations between organochlorines compounds in waterbird eggs are widely known (Blus *et al*., 1974; Ohlendorf *et al*., 1985, 1988; Becker *et al*., 1993). High compound concentrations posing biological threats, result in obscuring the understanding of the effect of each particular contaminant (Blus, 1982; Custer *et al*., 1999). The reasons for which some correlations between the same compounds (i.e. α-BHC-Lindane) were positive in the Audouin's gull eggs in an area and negative in another are at present unknown to us.

Statistically insignificant ratios of $\sum$OCs/$\sum$PCBs among areas in each year indicate a rather uniform mode of pollution depending on the incidence of pollutants over the wider Aegean region. The median ratio of $\sum$OCs/$\sum$PCBs was >1 in all colonies denoting a dominance of agrochemical over industrial pollution in the north-eastern part of the Mediterranean. Agreement with the latter results show other studies in the eastern Mediterranean and Black Sea regions (Fossi *et al*., 1984; Focardi *et al*., 1988; Crivelli *et al*., 1989; Pastor *et al*., 1995a).

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