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PCB Levels and Accumulation Patterns in Waterbird Eggs and in Their Prey at Lake Kerkini, a North-Eastern Mediterranean Wetland of International Importance

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Abstract. Seven ''target'' PCB levels were determined and compared in waterbird eggs, in their prey, and in water at Lake Kerkini, northern Greece, to investigate PCB bioaccumulation patterns and to define the best bioindicator of target PCBs for this area. PCBs were analysed from eggs of *Phalacrocorax carbo*, *Podiceps cristatus, Ardea cinerea, Egretta garzetta*, and *Nycticorax nycticorax* and from prey types *Alburnus alburnus, Rutilus rutilus*, *Lepomis gibbosus. Carassius auratus*, and *Rana* sp. PCBs analysed were detected in all bird eggs, prey, and water but contamination patterns differed among these sample types. The lipid-corrected geometric means of the congeners analysed were significantly different among most bird species and among some prey species. PCB congeners 118, 138, 153, and 180 accounted for around 80% of the total PCB contamination in bird egg samples. Percent congener concentrations of high-chlorinated PCBs tended to increase from water through prey to most bird egg samples whereas the low chlorinated PCBs (28 and 52) decreased. Bioaccumulation factors (BAFs) also exhibited an increasing trend for higher chlorinated PCBs from prey types to bird eggs. The greatest BAFs of six of the congeners were shared between *Phalacrocorax carbo* and *Ardea cinerea.* Among prey, the BAFs of four PCBs were highest in *Lepomis gibbosus.* Biomagnification factors varied between 1.01 and 39.57. In contrast to low chlorinated PCBs, high chlorinated congeners biomagnified considerably through fish prey. The highest biomagnification took place in *Phalacrocorax carbo*. No relationship was found between the lipid content of samples and BAFs of PCBs probably due to biotransformation differences of the congeners in the biota sampled.

Due to the greatest PCB concentrations especially of the higher chlorinated PCBs in the eggs of *Phalacrocorax carbo* and its considerable bioaccumulation tendencies, it is proposed as the best PCB biomonitor of target PCBs at Lake Kerkini. *Lepomis gibbosus* had the highest concentrations of most congeners and exhibited the greatest bioaccumulative properties among prey and can be used as an alternative biomonitor.

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Polychlorinated biphenyls (PCBs) were extensively used as plasticizers, as additives in hydraulic and dielectric fluids in industry, and as fire retardants. Nevertheless, PCBs have high toxicity and persistence in the environment. Studies have shown that some congeners can negatively affect wildlife reproduction and population levels and/or cause various embryonic deformities and mortality (Yamashita *et al.,* 1993; Custer *et al.,* 1999). Their long ago banning from most of the world seems to have reduced levels (Hebert *et al.,* 1999) but they persist in biota mainly due to their lipophilic structure and bioaccumulative properties thus reaching greater concentrations in higher-level consumers (Tanabe *et al.,* 1987).

In the Mediterranean, these organochlorines have been found in a great variety of biota (Swindlehurst *et al.,* 1995; Fasola *et al.,* 1998; Albanis *et al.,* 2003; Albaigés, 2005). Monitoring and comparative studies using higher trophic level receptors are scarce in this part of the region and those available (such as mentioned above) have been carried out mainly in coastal wetlands and are greatly lacking from lakes. Greek lakes are of particular interest for such studies because they have been used as irrigation water sources, are epicenters where human populations have developed, and consume local resources such as water and fish and also provide refuge to internationally important populations of wildlife, especially birds. PCBs and other organochlorines accumulate in higher concentrations in fish-eating waterbirds such as cormorants and herons, which, living in association with aquatic environments as top consumers, have been proved to be particularly useful bioindicators of organochlorines (Scharenberg, 1991; Weseloh *et al.,* 1995; De Luca-Abbott *et al.* 2001). The accumulation of PCBs through their food chains is being investigated but due to the complexity of the food chains and particularities in the response of each species to these chemicals, pollution studies should continue.

The purposes of this paper are (1) to determine ''target'' PCB levels in waterbird eggs and in their prey at Lake Kerkini, northern Greece, (2) to investigate PCBs bioaccumulation patterns from water through prey to the bird eggs, and (3) to define the best bioindicator(s) of target PCBs at Lake Kerkini.

Materials and Methods

Study Area

Lake Kerkini is situated in northern Greece (41°22′N, 22°13′E). It is an artificial lake constructed in 1932 for irrigation and flood prevention of the transboundary river Strymon originating from Bulgaria and flowing into the north Aegean Sea. Water levels are managed through a dam installed in 1982. Lake depth from September to February is 31.5 m and lake surface extends over 4500 ha whereas its depth from May to June is 35.5 m (7300 ha). About 30% of the river's total length and 35% of its catchment belong to Greece. A riverine forest is situated along the mouth of the river in the lake composed mainly by *Salix alba* and *Salix hybrids* hosting colonies of waterbirds such as pelecaniform and ardeid (herons and relative) species. Lake Kerkini is a wetland of international importance under the Ramsar Convention and also a Special Protected Area and Important Bird Area of Greece (Liordos, 2004).

Field Sampling

Egg samples were collected between the end of March to the end of April 2004, depending on the time of laying of each bird species. The levels of organochlorines in bird eggs reflect the diet of the female prior to egg lying and pollutant levels in body reserves, thus constituting a useful indicator of environmental contamination (Muñoz Cifuentes *et al.,* 2003). Under license, one egg was randomly sampled from different nests of the following waterbird species: *Phalacrocorax carbo* (great cormorant), *Podiceps cristatus* (great-crested grebe), *Ardea cinerea* (grey heron), *Egretta garzetta* (little egret), and *Nycticorax nycticorax* (black-crowned night heron). The eggs were placed in cotton-spread cartons, were opened as soon as possible after collection and their contents poured in chemically cleaned glass containers. The prey types of some of the waterbirds studied were known from previous investigations in the area (Table 1). Most bird species' diet included in considerable proportions *Alburnus alburnus* (bleak), *Rutilus rutilus* (roach), *Lepomis gibbosus* (pumpkinseed), *Carassius auratus* (goldfish), and *Rana* sp. (amphibians, preyed by herons). The preponderance of the above-mentioned four fish species in the birds' diet is probably due to their greater availability in the lake; thus, it is also supposed to constitute prey of the grey heron and great-crested grebe, though further proof is needed. Samples of fish prey were collected during the birds' egg laying period using an electrofishing device and professional nets of appropriate size at the birds' main feeding grounds. Fish of sizes known to be taken by each species were selected for chemical analysis. Frogs were collected by hand net and euthanised in the field by placing in a solution of 3-aminobenzoic acid ethyl ester. All material was carried to the laboratory in a field fridge and was subsequently deep frozen to −20°C. Then the material was transported to the University of Ioannina for chemical analyses.

Water samples were collected in the above-mentioned period by boat, using a Ruttner sampler (1 L) along 15 stations 150 m distant from each other in a straight line starting from the Strymon river mouth across a west direction in the lake. In each station, three water samples were taken (surface, column, bottom), homogenized in a container, and 1.5 L of the mix were placed in chemically cleaned glass bottles and transported to the laboratory where they were preserved in a fridge until analysis.

Chemicals

All solvents used (hexane, acetone, dichloromethane, methanol, ethyl acetate), were pesticide residue analysis grade, purchased from Pestiscan (Labscan Ltd, Dublin, Ireland). Alumina, copper, sodium sulphate (pro-analysis), and concentrated sulphuric acid 98%, were from Merck (Darmstadt, Germany). Octadecyl bonded silica (C18, 500 mg) solid-phase extraction (SPE) disks of 47 mm diameter and 0.5 mm thickness were obtained from Empore^{TM} (Saint Paul, MN, USA). Glassware was soaked, cleaned with chromic solution, thoroughly rinsed with distilled water and acetone, and heated at 150°C for 12 hours. Aluminum foil was rinsed with acetone and dried at ambient temperature prior to use. Cellulose extraction thimbles of 35 mm i.d. and 100 mm long were from Whatman (Maidstone, England). Sodium sulphate and thimbles were pre-cleaned by Soxhlet extraction with hexane:dichloromethane (3:1, v/v) for 3 h before use.

The following seven PCB (in terms of the PCB IUPAC numbers) congeners were analysed: PCB 28, 52, 101, 118, 138, 153, and 180 belonging to the group known as ''target'' or ''indicator'' PCBs (Bachour *et al.,* 1998). PCB-standards (Dr. Ehrensdorfer GmbH Laboratory, Augsburg, Germany) were obtained in concentrations of 10 mg/mL.

Analytical Procedures

Extraction of water samples. SPE and chromatographic techniques were applied to quantify pesticides in lake water samples. Methanol modifier (10 mL) was added to 1-L water samples to allow better extraction (Albanis *et al.,* 1998). Prior to extraction, the C18 disks were washed with 10 mL of acetone under vacuum, followed by 10 mL of methanol. Disks were not allowed to dry, as recommended (Albanis *et al.,* 1998). Samples were mixed well and allowed to percolate through the disks at a flow-rate of 50 mL/min under vacuum. After extraction, the pesticides trapped in the disks were collected by using 2×10 mL of dichloromethane–ethyl acetate (1:1, v/v) as eluting solvent. The fractions were evaporated to 0.5 mL under a gentle stream of nitrogen prior to GC injections.

Extraction of egg samples. Whole egg contents were homogenized in a blender and an aliquot of 5–10 g was homogenized again with anhydrous sodium sulfate in 250-mL glass beakers. The mixture was extracted first with 50 mL of a hexane:dichloromethane (3:1, v/v) mixture, and then twice with 20 mL of the mixture, using a mechanical shaking for 2 min, sonication bath for 10 min, and manual mixing for 1 min with a glass rod. The extracts were collected in polypropylene centrifugation tubes of 50 mL and centrifuged at 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 aliquots of concentrated sulfuric acid under stirring until the organic layer remained colourless.

Extraction of fish and frog tissues. PCB congeners were measured in individual fish and frogs or pooled composites of multiple items depending on their size and availability. The pooled composites containing approximately equal tissue mass for PCB analysis. Whole body homogenates of 10–15 g were dried by blending with anhydrous sodium sulfate in a mortar until a fine powder was obtained. The granular mixtures were introduced into pre-cleaned cellulose thimbles and were Soxhlet extracted with n-hexane:dichloromethane (3:1, v/v) for 16 h. Lipid content was determined gravimetrically using about

Table 1. Information on prey use of nestlings (expressed as percentages of total prey numbers) for some of the waterbirds studied at Lake Kerkini

Prey	*P. carbo*	*P. carbo*	*E. garzetta*	*N. nycticorax*
Alburnus alburnus	83.6	62.5	19.4	71.5
Rutilus rutilus	2.4	28.8	2.6	7.4
Lepomis gibbosus	7.4	0.3	1.5	4.1
Carassius auratus	5.0	8.2	0.4	0.9
Other fish	1.6*	1.0**	5.0***	2.6*
Indetermined fish	0.0	0.0	2.1	0.0
Insects+ other arthropods	0.0	0.0	29.8	1.5
Tadpoles	0.0	0.0	25.8	11.8
Frogs	0.0	0.0	0.0	0.3
Crustaceans	0.0	0.0	9.6	0.0
Annelids	0.0	0.0	3.8	0.0
Source	Nazirides (2004)	Liordos (2004)	Tsachalidis (1989)	Birtsas (2002)
Year of data collection	1989–1990	1999–2002	1988–1989	1995–1996

The number or asterisks (*) indicates the number of species involved in this category.

20% of the extract. The extract was subsequently concentrated by vacuum rotary evaporation and cleaned up with concentrated sulphuric acid aliquots under stirring for a first lipid purification until the organic layer remained colourless. The extracts were dried, reconstituted in hexane, and the cleanup was completed by adsorption chromatography, eluting the colourless layer through short chromatography columns prepared in plastic syringes (10 mL) fitted with glass wool (Singh *et al.,* 1998). Solvent blanks analysed for organochlorines cross contamination showed the absence of co-eluted organochlorines. The syringes were layered with 5 cm alumina (5% water-deactivated) followed by 1 cm acid-activated copper for the removal of any residual elemental sulphur in the extracts (Wainwright *et al.,* 2001) and dried sodium sulphate. The column was washed with 50 mL of the extraction solvent mixture. The purified samples were evaporated in a rotary evaporator to approximately 5 mL and in a gentle N_2 stream at 35°C to approximately 0.5 mL, then samples were stored in silanized vials in a refrigerator (4°C).

Chromatographic Analysis and Quality Assurance

Samples were analyzed with a Shimadzu 2010 gas chromatograph equipped with ^{63}Ni electron capture detector (ECD). Separation was achieved with a DB-5 (30 m × 0.25 mm i.d., J & W Scientific, Folsom, CA) capillary column. The column oven temperature was programmed as follows: 150°C (4 min), 150–260°C (5°C/min), 260°C (15 min), 260–290°C (10°C/min), 290°C (10 min). The temperatures were set at 240°C for the injector and 300°C for the detector. Helium was used as carrier at a flow of 1.5 mL/min and nitrogen was used as make-up gas at a flow of 35 mL/min. Injections (1 μL) were performed using a Shimadzu AOC–20i auto injector in splitless mode with the valve open for 30 sec. Quantitation of PCBs was performed using internal standard (tetrachloro-m-xylene). Congeners were positively identified if the relative retention time (versus the internal standard) differed no more than 0.05 from that of the calibration standards. Procedural blanks were also analysed for every set of 10 samples. Procedures were tested for recovery using PCB 209 as surrogate spike. Recoveries of spiked PCBs into samples and passed through the analytical procedure were between 87 and 106% (RSD <10 %). Reported concentrations were not corrected for the recoveries of surrogate standard. Peaks less than three times the noise level were considered below the detection limit. Detection limits ranged from 0.1 to 0.2 ng/g and from 0.02 to 0.05 ng/g for egg and fish or frog samples, respectively, on a wet weight basis.

Secondary confirmation was performed on representative samples using a GC-MSD, QP 5000 Shimadzu equipped with DB-5 MS capillary column, 30 m × 0.25 mm i.d. contained (5% phenyl) methyl polysiloxane (J & W Scientific, Folsom, CA), following the previous oven temperature program. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector and interface temperatures were 240°C and 290°C, respectively. The spectra were obtained at 70 eV. The splitless mode was used for injection of 1 μL volume, with the valve opened for 30 sec. Two cluster of ions M^+ and $(M+2)^+$ for each biphenyl congener were chosen for screening analysis in selected ion monitoring mode (SIM).

Data Analysis and Statistical Procedures

Data on concentrations of PCB contaminants are presented as geometric mean values accompanied with minimum and maximum values. A value one-half the lowest limit of detection was assigned to samples with undetectable contaminant concentrations if detectable quantities were found in at least half of the samples. All statistical assessments were limited to the chemicals that were detected in more than 50% of all sample sets. Due to the small sample sizes and the non-normal distribution of the congener concentrations (Shapiro-Wilks tests), the statistical evaluation was based on the use of non-parametric tests. Residue differences among bird egg samples and among prey species were tested separately using the Kruskal-Wallis test. Differences between pairs in the same group were tested by the Mann-Whitney test using Bonferroni correction for multiple comparisons. Correlations were tested using non-parametric Spearman rank correlation. All statistical comparisons refer to lipid-corrected geometric means. Cluster analysis (with Euclidean-distances as distance measure and single linkage as a linkage rule) was used to evaluate overall differences in pollution patterns among sample types studied.

Although also calculated on a wet weight basis (Meylan *et al.,* 1999), bioaccumulation factors (BAFs) depend on the lipid content of an organism. Therefore, lipid-normalized BAF values are more useful when comparing across animals, as the variation due to variable lipid content is eliminated (Kelly *et al.,* 2004), and were calculated in the present study according to the equation:

$$\mathrm{BAF} = (\mathrm{PCB\,in\,organism})_{\mathrm{lipid\,adjusted}} / (\mathrm{PCB\,in\,raw\,water})$$

The way to evaluate dietary PCB enrichment in an organism is to calculate biomagnification factors (BMF) according to the equation:

$$BMF = (PCB\ in\ predator)_{\ lipid\ adjusted} / (PCB\ in\ prey)_{\ lipid\ adjusted}$$

BMF values higher or lower than 1 indicate biomagnification and elimination of the pollutant, respectively.

All statistical analyses were completed with STATISTICA 6.0 (Statsoft Inc.).

Results

PCB Levels in Water, Prey, and Waterbird Eggs

All congeners analyzed were detected in the water and prey samples from Lake Kerkini in very low concentrations (Table 2). The ΣPCB levels detected in water samples ranged between 3.22 and 15.1 ng/L with a geometric mean value of 6.64 ng/L. The low chlorinated PCBs dominated over the heavier congeners as shown in Figure 1. Geometric mean concentrations of all congeners except PCB 101 and 118 were significantly different among prey species. Among prey, *L. gibbosus* had the highest geometric mean levels of PCB 52, 138, 153, and 180. *R. rutilus* showed maximum levels of PCB 101 and *Rana* sp. showed maximum levels of PCB 28, 118, and ΣPCBs. The difference between maximum and minimum concentrations for congeners PCB 28, 52, 101, 138, and ΣPCBs was greatest in *Rana* sp., of PCB 153 and 180 in *L. gibbosus*, and of PCB 118 in *C. auratus.*

All seven PCBs analyzed were detected in all bird species' egg samples but geometric mean concentrations were low and varied considerably. The lipid-corrected geometric means of all congeners were significantly different among species (Table 3). However, geometric mean concentrations of PCB 138, 153, and 180 were highest in *P. carbo, A. cinerea, E. garzetta*, and *N. nycticorax* samples while PCB 153 was highest in *P. cristatus* eggs. Except in *P. carbo* eggs, PCB 153 geometric mean concentrations were higher than those of the other congeners.

Among birds, *P. carbo* eggs had maximum levels of PCB 118, 138, 153, and ΣPCBs. Maximum levels of congeners PCB 28, 52, and 180 were found in *A. cinerea* eggs and of PCB 101 in *P. cristatus* eggs. Noticeably, PCB 52 levels were much similar in *P. cristatus, A. cinerea*, and *N. nycticorax* egg samples. The difference between maximum and minimum concentrations was greatest for congeners PCB 101, 118, 153, and ΣPCBs in *P. cristatus* eggs. PCB 28 and 52 concentration ranges had the greatest differences in *A. cinerea* egg samples. Minimum and maximum levels of PCB 138 differed mostly in *P. carbo* and of PCB 180 in *E. garzetta* eggs.

The high chlorinated PCB congeners 138, 153, and 180 dominated in most bird egg samples (Fig. 1). Patterns of percent congener concentrations in all types of samples analyzed indicated the general tendency of PCBs 118, 138, 153, and 180 to increase from water through prey to most bird egg samples whereas an inverse situation held for congeners 28, 52, and 101 (Fig. 1).

A cluster analysis of percent congener levels separated water from all other samples. Furthermore, *Rana* sp. were separated form all other samples, which formed two distinct clusters, one including waterbird species and another fish prey types (Fig. 2).

Table 2. Concentrations of target PCBs in waterbirds' prey (ng/g) and in water samples (ng/L) from Lake Kerkini

	C. auratus (n = 10) *Geometric mean*			*A. alburnus* (n = 10) *Geometric mean*			*R. rutilus* (n = 9) *Geometric mean*			*L gibbosus* (n = 11) *Geometric mean*			*Rana* sp. (n=10) *Geometric mean*			Water (n = 15) *Geometric*			K-Wallis H (n = 50, df = 4)
	wet wt	lipid wt	Min	Max	wet wt	lipid wt	Min	Max	wet wt	lipid wt	Min	Max	wet wt	lipid wt	Min	Max	mean	Min	Max	*P*
PCB 28	1.02	25.0[a]	7.01	117	2.28	27.2	9.07	281	2.08	42.8	8.76	144	1.45	48.9	12.6	154	1.18	**110**[a]	0.65	317	2.99	0.15	7.27	14.7	0.005
PCB 52	0.79	19.5[a]	6.45	65.9	1.14	13.6	3.07	43.1	1.43	29.4	9.01	107	1.90	**63.8**[a]	19.7	133	0.58	53.5	0.65	270	1.27	0.29	5.15	17.5	0.015
PCB 101	1.26	30.8	12.8	101	2.56	30.4	12.1	63.3	2.78	**57.4**	18.9	97.3	1.35	45.5	10.7	106	0.53	49.6	13.8	144	0.29	0.10	0.79	6.87	n.s.
PCB 118	0.43	8.04	0.10	75.3	0.90	10.7	3.32	74.5	0.39	8.07	1.50	37.4	0.74	21.3	1.32	69.8	0.27	**25.0**	5.35	54.7	0.30	0.05	0.70	8.20	n.s.
PCB 138	0.81	20.0	6.63	50.0	2.27	27.0	5.41	95.4	0.92	18.9	3.90	60.4	1.15	**38.9**	0.83	121	0.43	35.3	0.30	314	0.16	0.05	1.39	11.0	0.027
PCB 153	0.52	12.8[a]	4.30	130	1.96	23.4	6.30	178	0.83	17.1	3.85	176	1.80	**60.5**[a]	7.70	274	0.59	55.0	7.61	201	0.36	0.14	0.65	12.0	0.017
PCB 180	0.31	7.67	1.71	55.6	0.84	9.99	4.21	31.9	0.21	4.46[ab]	1.25	22.2	0.66	**22.2**	3.64	94.6	0.18	17.1[b]	9.56	51.0	0.26	0.10	2.46	14.6	0.006
ΣPCBS	6.44	146[a]	61.7	458	20.1	191[b]	100	464	14.5	224[c]	113	414	15.0	374	93.1	727	5.71	**465**[abc]	59.5	866	6.64	3.22	15.1	18.3	0.001

Bold values indicate the highest geometric mean concentration of each compound detected among all prey species. For each compound, values sharing a common letter are significantly different (Mann-Whitney U-test, Bonferroni corrected $P = 0.005$). Geometric means of wet weight are given for comparison with the literature. Statistics did not include water data.

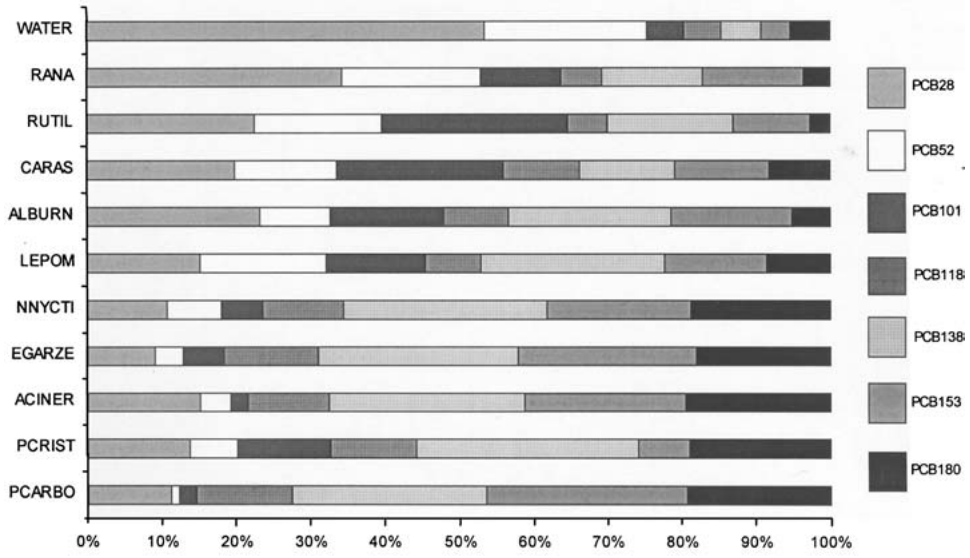

Fig. 1. Relative percentages of PCB congeners in samples from Lake Kerkini. RANA: *Rana* sp.; RUTIL: *Rutilus rutilus*; CARAS: *Carassius auratus*; ALBURN: *Alburnus alburnus*; LEPOM: *Lepomis gibbosus*; NNYCTI: *Nycticorax nycticorax*; EGARZE: *Egretta garzetta*; ACINER: *Ardea cinerea*; PCRIST: *Podiceps cristatus*; PCARBO: *Phalacrocorax carbo*

Bioaccumulation Patterns

Bioaccumulation factors (BAF) for PCBs 28, 52, and 101 were frequently greater in prey whereas most of the PCBs 118, 138, 153, and 180 were generally greater in bird eggs (Table 4). BAF of PCB 118, 138, and 153 were highest in *P. carbo* eggs, BAF of PCB 180 was highest in *A. cinerea* eggs, whereas BAF of PCB 101 was highest in *R. rutilus*, of PCB 52 in *L. gibbosus* and of PCB 28 in *Rana* sp.. Among herons, all BAFs except of PCB 101 were much higher in *A. cinerea* eggs. Among prey, in four out of seven PCBs (52, 118, 153, 180) the highest BAFs were found in *L. gibbosus.* For none of the congeners did the bioaccumulation factors show a significant relationship with the lipid content of samples (Spearman rank correlation). The relationship between the apparent fish/water bioaccumulation factors and n-octanol/water partition coefficients (K_{ow}) for the determined PCBs is shown in Figure 3. BAFs seemed to indicate a deviation from theoretical equilibrium partitioning for some congeners. Log K_{ow} of PCBs analysed indicated statistically significant positive correlation with the relative PCB BAFs in *P. carbo, E. garzetta* (both $r_s = 0.821$, $P = 0.023$), *A. cinerea*, and *N. nycticorax* (both $r_s = 0.857$, $P = 0.014$).

Biomagnification factors (BMF) in bird eggs were much lower than BAFs and varied between 1.0l and 39.57. PCB congeners 28, 52, and 101 (especially in the last two) showed a weak tendency to biomagnify. In contrast, the rest of the congeners (118, 138, 153, and 180) generally biomagnified considerably through fish prey especially in *P. carbo* and *A. cinerea* eggs (Table 5). The highest biomagnification took place in *P. carbo* eggs. Of all bird species, *P. carbo* and *A. cinerea* showed the greater tendency to biomagnify PCBs through all prey types.

Discussion

Pollutant Levels and Patterns

Lake water ΣPCB levels were similar to or lower than those recorded for various surface waters of Greece (n.d.-92 ng/L) and particularly for river Strymon (20 ng/L) whose water supplies Lake Kerkini (Kamarianos *et al.* 2002; Katsoyiannis, 2006). As expected, the PCB pattern in water was skewed towards the low chlorinated or less lipophilic congeners (PCB 28 and PCB 52) reflecting a decreasing water solubility and higher partitioning to sediments with increasing chlorination of the PCB congeners. Similar levels of PCBs (i.e., 9.1 ng/L) were recorded at Marnay site in the River Seine (France) characterizing the site as slightly polluted (Blanchard *et al.* 1997).

A shift was found in the proportions of the low chlorinated biphenyls, which were higher in water and prey than in birds' eggs. This chlorine shift is readily explained by the physical and biological properties of PCB isomers. Fish and frogs are more exposed to waterborne PCBs either in a dissolved form or in a form that is not tightly sorbed to suspended matter and, in both instances, these are low-C1 isomers. In contrast, high-C1 isomers are mainly transferred via invertebrate prey than via water. In addition, low-C1 PCBs are more easily metabolized than high-C1 PCBs and, therefore, more easily eliminated from the body of fish and birds. This typical stepwise increase in concentrations of PCBs with $\log K_{ow} < 6$ as they move up the food chain is also observed in the aquatic food chains of Lake Ontario with tri, tetra, and penta-chloro congeners comprising a higher fraction in water and plankton than the higher chlorinated congeners, which are higher at the upper trophic levels (fish) (Oliver and Niimi, 1988; Metcalfe and Metcalfe, 1997). The observed relative percentages of PCB congeners in the prey species could be partially supported by the feeding habits of the organisms, Species such as *C. auratus* or *R. rutilus* that are omnivorous or a large part of the diet include plants or phytophilous invertebrates, respectively, presented relatively higher percentages of low-chlorinated congeners (PCB 28, 52, and 101). In contrast, species such as *A. alburnus* and *L. gibbosus* that feed mainly with zoobenthos and zooplankton presented relatively higher percentages of high-chlorinated congeners (PCB 138 and 153).

The high chlorinated PCB congeners were found in more elevated levels in birds compared to their prey, though it was not always the case. Evidently, *P. carbo* and *A. cinerea* eggs

Table 3. Concentrations of target PCBs (ng/g) in waterbird eggs from Lake Kerkini

		P. carbo (n = 11) *Geometric mean*			*P. cristatus* (n =13) *Geometric mean*			*A. cinerea* (n = 11) *Geometric mean*			*E. garzetta* (n = 11) *Geometric mean*			*N.nycticorax* (n = 13) *Geometric mean*			K-Wallis H (n = 59, df = 4)
	1997[†]	wetwt this study	lipid wt	Min	Max	wetwt	lipid wt	Max	Min	wet wt	lipid wt	Min	Max	Wet wt	lipid wt	Min	Max	wetwt	lipid wt	Min	Max	P
PCE 28	4.00	5.37	116^{abc}	71.1	216	2.46	33.8^{cf}	10.1	235	6.80	**132**^{def}	58.1	332	1.42	25.4^{ad}	11.6	74.5	2.21	36.4^{be}	14.6	76.7	32.9	< 0.001
PCE 52	1.87***	0.39	6.32	2.50	33.0	1.48	20.3	6.52	56.1	1.25	**21.6**	2.50	85.2	0.44	6.58	2.50	51.4	1.20	19.4	2.50	54.2	13.3	0.010
PCE 101	2.45*	0.90	19.4	4.49	51.8	3.21	**44.2**^{abc}	13.8	121	0.80	14.7^{c}	1.25	45.5	0.73	12.8^{a}	1.25	56.6	1.09	18.0^{b}	4.93	35.0	17.2	0.002
PCE 118	ND	5.80	**125**^{abc}	55.4	265	1.79	20.4^{adef}	3.21	250	4.97	96.8^{dg}	42.2	277	1.95	34.8^{beg}	16.2	110	2.03	35.5^{cf}	11.2	72.5	30.5	< 0.001
PCB 138	ND	12.1	**260**^{abc}	118	488	0.80	110^{cdf}	1.25	222	9.96	194^{eg}	81.6	428	3.19	56.9^{ade}	16.0	273	3.82	63.0^{bfg}	15.7	152	40.0	< 0.001
PCB 153	NA	11.2	**241**^{abc}	77.6	517	4.43	61.0^{cf}	9.37	568	12.0	233^{def}	103	632	4.33	77.4^{ad}	42.5	201	5.61	92.5^{be}	35.4	191	27.9	< 0.001
PCB 180	4.57	7.46	160^{ab}	30.6	404	1.97	27.1^{be}	3.89	486	9.06	**177**^{cde}	79.6	303	2.44	43.5^{ac}	139	909	3.26	53.7^{d}	11.0	196	25.4	< 0.001
ΣPCBs	19.6	60.3**	**959**^{abc}	360	1932	29.5	251^{ad}	99.7	1708	63.9	937^{d}	651	1955	17.9	276^{b}	139	909	26.3	339^{c}	126	714	30.4	< 0.001

[†] Data transformed from Konstantinou *et al.* (2000).

*** $P \leq 0.001$; ** $P = 0.01$; ** $P < 0.05$.

ND: Not detected in any egg sample; NA: Not analyzed.

Bold values indicate the highest geometric mean concentration of each compound reported among all bird species (lipid weight). For each compound, values sharing a common letter are significantly different (Mann-Whitney U-test, Bonferroni corrected $P = 0.005$). Geometric means of wet weight are given for comparison with the literature.

were by far the most polluted with these congeners. It is widely known that elevated concentrations of higher chlorinated PCBs are detected in higher links of the food chain, a fact mainly attributed to the structure and bioaccumulative properties of these congeners (Guruge *et al.,* 2000). In some studies, PCB 118 has been found in elevated levels in fish, birds, and mammals (Zimmermann *et al.,* 1997; Hong *et al.,* 1998). In a previous study at Lake Kerkini, this congener was below detection limits in great cormorant egg samples (Konstantinou *et al.,* 2000) suggesting recent, probably transboundary imputs.

Herons and cormorants exhibited higher PCB egg burdens than grebes. Herons and cormorants feed on bigger, i.e., older fish, which contain higher PCB levels than young fish, which are preferred by the grebes (Zimmermann *et al.,* 1997).

The situation greatly differed with regard to low chlorinated PCBs found in more elevated percentages in water and prey than in birds' eggs. Of them, PCB 28 seems to have more bioaccumulative properties and a greater persistency due to its low metabolic rate (Guruge and Tanabe, 1997). Elevated PCB 28 levels in the frogs may be related to skin absorption due to persistent exposure to this congener being more water soluble (Dannenberger *et al.* 1997). The greater water solubility of low chlorinated congeners favors absorption in biota such as fish and other organisms with filter feeding habits as circulating high volumes of water through their bodies (Pastor *et al.,* 2004). Low chlorinated congeners have also been found to contribute remarkably to the total body burden PCBs in trout (*Salmo trutta*) and eel (*Anguila anguila*) (Bordajandi *et al.,* 2003). Among the herons studied, PCB 153 was present at the highest concentration, followed by the congeners 138, 180, and 118, in a pattern that is similar to that previously reported for most other fish-eating birds (Boumphrey *et al.,* 1993; Jenssen *et al.,* 2001).

The variability of PCB levels in different waterbird species such those studied may be due to different diets and metabolic characteristics of each species (Zimmermann *et al.,* 1997; Hoshi *et al.,* 1998; Connell *et al.,* 2003). Certainly, at Lake Kerkini the differences among birds' prey composition and sizes taken would partly have accounted for the egg PCB burden differences detected. However, evidence supporting the idea that metabolism particularities also accounted for the differences among species is derived by comparing the concentrations of individual PCBs detected in the eggs from Lake Kerkini with those in muscle tissue of the same birds from a study in Switzerland (Zimmermann *et al.,* 1997). We found statistically significant correlations between geometric means congener contents of eggs and lipid-corrected average concentrations of muscle in *P. carbo* ($r_s = 0.75$, $P = 0.052$) and *A. cinerea* ($r_s = 0.79$, $P = 0.036$). This may indicate that metabolic pathways lead to excretion of PCB to eggs by the females dependent on the burdens accumulated in their bodies. Notably, this tendency was not detected in *P. cristatus* ($r_s = 0.71$, $P = 0.071$). The relationship of female body concentrations of organochlorines and those in their eggs is also indicated in other studies (Barron *et al.* 1995; Scharenberg and Ebeling, 1998).

The levels of PCBs detected at Lake Kerkini were much lower than those recently detected in other parts of the Mediterranean region (Fasola *et al.* 1998; Crivelli *et al.* 1999; Albaigés 2005). Levels at Lake Prespa (northern Greece) seem to decline compared to the past (Crivelli *et al.* 1999) but this trend is not obvious at Lake Kerkini. Though levels are still

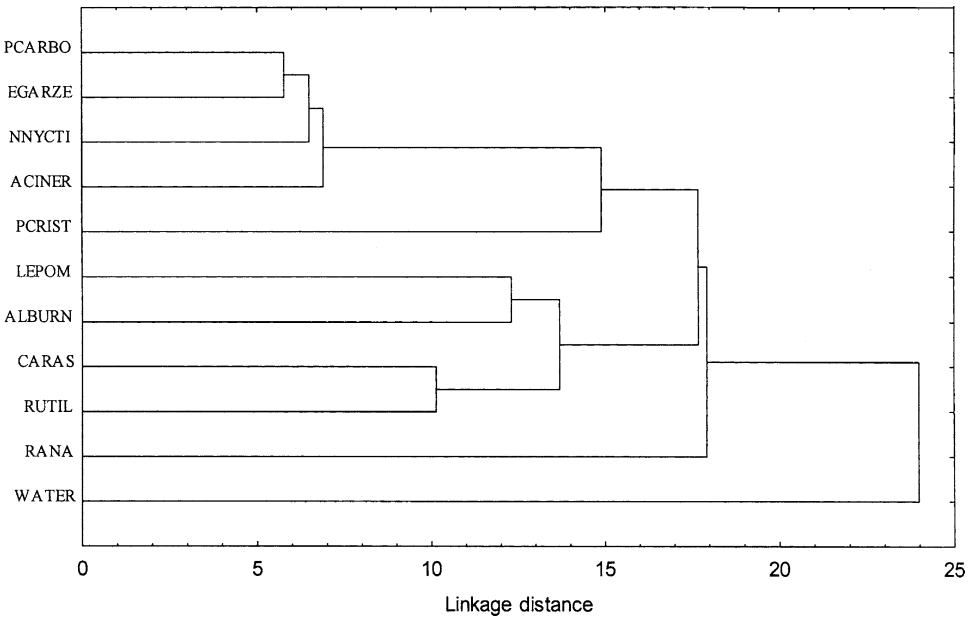

Fig. 2. Cluster analysis of the relative percentages of PCB congeners indicating PCBs contamination patterns of the sample types from Lake Kerkini. Symbols of sample types as in Figure 1

Table 4. Logarithms of bioaccumulation factors (BAF) of target PCBs in waterbird eggs and their prey species

		Birds					Prey				
	Log Kow	*P. carbo*	*P. cristatus*	*A. cinerea*	*E. garzetta*	*N. nycticorax*	*C. auratus*	*A. alburnus*	*R. rutilus*	*L. gibbosus*	*Rana* sp.
PCB 28	5.6	4.59	4.05	**4.65**	3.93	4.09	3.92	3.96	4.16	4.21	5.52
PCB 52	5.8	3.70	4.20	**4.23**	3.71	4.18	4.19	4.03	4.36	4.70	4.62
PCB 101	6.4	4.82	**5.18**	4.70	4.65	4.79	5.03	5.02	5.30	5.19	5.23
PCB 118	6.7	**5.61**	4.83	5.51	5.06	5.07	4.43	4.55	4.43	4.85	4.92
PCB 138	6.8	**6.21**	4.84	6.08	5.55	5.59	5.10	5.23	6.07	5.39	5.35
PCB 153	7.1	**5.83**	5.23	5.81	5.33	5.41	4.80	4.81	4.68	5.23	5.18
PCB 180	7.4	5.79	5.02	**5.83**	5.22	5.32	4.47	4.58	4.23	4.93	4.82
% mean lipid content		4.67	7.30	5.24	5.64	6.10	5.10	8.52	5.15	3.45	1.20
SD		0.54	0.67	0.97	0.74	0.62	3.08	1.57	1.94	2.34	0.72

Bold numbers indicate BAF maximum value of each compound reported among bird species. Values in squares are BAF maximum values among prey species.

low, monitoring and localization of pollution sources are of concern.

Regarding the effects of PCBs on these species, the lowest levels in eggs associated with reproductive failure and hard tissue malformations to cormorants range from 1600 to 7300 ng/g (Yamashita *et al.,* 1993; Dirksen *et al.,* 1995) and those associated with reproductive problems in herons seem to start with concentrations from 4 μg/g (black-crowned night heron, Hoffman *et al.,* 1986) to greater than 10 μg/g (Fitzner *et al.,* 1988; Barren *et al.,* 1995). Geometric mean concentrations of ΣPCBs in bird eggs (either wet or lipid corrected weight), supposed to account for about 50% of the total PCB egg content (Dirksen *et al.,* 1995), even if multiplied by 2, seem too low to have any adverse biological effects.

Bioaccumulation and Biomagnification

In Great Lakes' food chain, salmonids had greater PCBs accumulation than the lower levels of the food chain due to their higher lipid concentrations. The BAFs were correlated to congeners' K_{ow} for the various trophic levels, and chlorine substitution pattern of the isomer played an important role in the bioaccumulation process (Oliver and Niimi, 1988). Lower congeners (i.e., 52) may degrade more extensively than higher congeners (i.e., 138), whereas PCB 153 and 180 may not be metabolised and accumulated compared with the situation in prey (Looser and Ballschmiter 1998). Congeners 118 and 138 (which sometimes exhibit greater BMP values than 153) are interesting from the point of view of the reaction between detoxifying activity and bioaccumulation. They are very persistent because the biphenyl ring does not have adjacent m-p unsubstituted carbons but does have adjacent 1 or 2 o-m unsubstituted carbons (Fossi *et al.,* 1995). Moreover, PCB 153 is more difficult to metabolize than PCB 138 (no vicinal free position) and accumulates in the eggs at a greater proportion (Dietrich *et al.,* 1997).

Accumulation of contaminants in fish lipids can occur by two routes: (1) diffusion from the water across the gills into the body, and (2) transfer from the gut into the body after

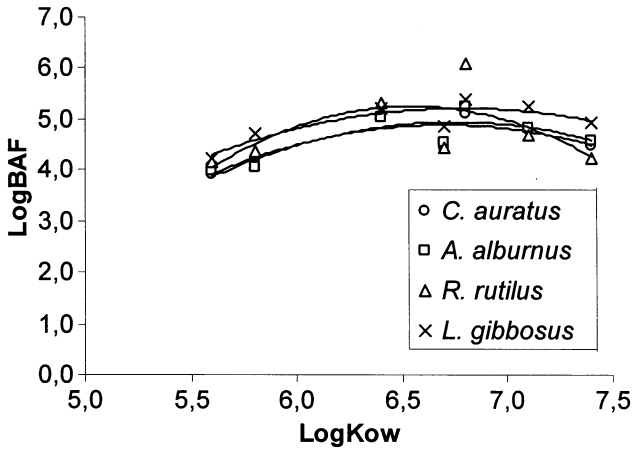

Fig. 3. Relationship between the apparent LogBAFs and LogKow for PCB congeners detected in Lake Kerkini

consumption of contaminated food. The relative importance of these routes is affected by species, locality, food web, and contaminants' physical-chemical properties (Swackhamer and Hites, 1988). Comparison of bioaccumulation and biomagnification data between studies must be done with caution since both biological and chemical variables may differ and, thereby, confound the interpretation. It was, however, important to relate the present results to earlier findings. The calculated BAFs for fish species had generally similar values compared to those reviewed in Table 6. Generally, BAFs decreased with increasing lake PCB concentrations; thus, differences in water PCB measurements most likely explain the variation in BAFs between studies (Borga *et al.* 2005).

Exposure time of organisms to the PCBs may also affect the BAFs. The organism PCB concentrations represent integrated measures of contamination over a period of time and water measures are instantaneous. Therefore, changes in water concentrations may not be reflected in organism concentrations due to a lag period for equilibrium to be reached. Finally, different methods of lipid extraction can vary in efficiency for different lipid classes, which may affect lipid-normalised BAFs (Berglund *et al.* 2000).

The apparent fish/water BAFs seemed to indicate a deviation from theoretical equilibrium partitioning (linear relation) for highly chlorinated PCB congeners and especially PCB 180. In fact, the relation between BAF and K_{ow} was more parabolic (curvilinear) than linear (Fig. 3) with a distinct drop-off in the relation at log K_{ow} above 7. These results are consistent with previously published studies (Scharenberg *et al.* 1994; Blanchard *et al.* 1997). Parabolic profile of correlation between BAF in fish and K_{ow} was also reported for PCB accumulation in pumpkinseed with similar correlation factor and regression equation (Crimmins *et al.* 2002). This effect may be due to: (1) resistance to transport of high molecular weight molecules across membranes (steric effect); (2) exceptionally long times to reach steady-state partitioning or metabolism; (3) the high degree of binding of very non-polar compounds to food in the gastrointestinal tract or to dissolved and particulate organic material in water; (4) overestimation of bioavailable water concentrations or inaccurate K_{ow}; and (5) elimination into feces (Metcalfe and Metcalfe 1997; Crimmins *et al.* 2002; Fisk *et al.* 1998). For low-chlorinated PCBs, the relation between BAF and K_{ow} although linear deviates from 1:1 model which includes the assumptions PCBs are distributed mainly into the neutral lipid pool of the organism and that dietary PCB uptake leading to biomagnification is negligible. In organisms, hydrophobic contaminants may partition into other phases in addition to lipid and also biomagnification frequently occurs thus leading to deviation from 1:1 relationship (Borga *et al.,* 2005). Similarly, deviations were also reported for zooplankton and fish species elsewhere (Borga *et al.* 2005; Crimmins *et al.* 2002). If we assumed only the PCB congeners with $\log K_{ow} < 7$, then the slopes of the BAF function of K_{ow} of the four fish species ranged between 0.72 to 1.06 and were comparable to the slope value of 0.9 reported for small rainbow smelt (Oliver and Niimi, 1988).

The observed BAF range could be explained as the result of trophic level effects in combination also with body size effects (Burreau *et al.* 2004). Increased trophic position of the fish could lead to increased PCB intake while increased body size could lead to less efficient PCB clearance over the gills due to reduced gill area-to-body-volume ratio or increased distance between PCB storage tissues and sites of elimination in the organism (LeBlanc 1995).

The data in Table 5 show the biological magnification of PCB residues in the food web of Lake Kerkini. Though birds studied are fish predators, some PCBs, especially the lower chlorinated congeners, did not seem to biomagnify (as similarly found in Lake Ontario's food chain; Metcalfe and Metcalfe 1997). This probably was a consequence of a greater degree of biotransformation of lower PCBs compared to the higher chlorinated congeners in bird bodies (Guruge and Tanabe 1997) thus appearing in reduced concentrations in the eggs. The heavier congeners are more hydrophobic; they have longer elimination half-lives in biota and are more efficiently transferred from prey to predator through the diet. However, in addition to hydrophobicity, the substitution pattern also has an influence on the metabolism of PCBs and thus on their biomagnification potential. At Lake Kerkini, congeners 118, 138, 153, and 180 biomagnified through most prey types in bird eggs. All these congeners share the recalcitrant 4,4'-substitution or no viscinal H-atoms in both aromatic rings and, therefore, would be biomagnified and not metabolized. PCB accumulation in both fish and birds seems to be associated with their adipose tissue content (Colombo *et al.,* 2000). Considerable bioaccumulation was found in both *L. gibbosus* and *Rana* sp. especially of highly chlorinated congeners. This fact cannot be explained by their lipid content, since it is lower than the other three prey types. In addition, discrepancies have been reported regarding the nature of the associations between PCB residues and lipid contents in aquatic animals and this relation appears to be a function of many factors, such as species, season, reproductive status, and the ecosystem being investigated (Crimmins *et al.* 2002). Nevertheless, as no relationship was found between the lipid content of samples and BAFs of PCBs, it seems that the biotransformation difference of the congeners in the biota sampled probably was a primary factor affecting PCB bioaccumulation at Lake Kerkini. This is supported by the smaller BAFs and BMFs of the lower chlorinated congeners in the eggs of birds.

Table 5. Biomagnification factors (BMF) of target PCBs in waterbird eggs for each prey type used

	C. auratus (n = 10)					*A. alburnus* (n = 10)					*R. rutilus* (n = 9)					*L. gibbosus* (n = 11)					*Rana* sp. (n = 10)		
	PCA	PCR	ACI	EGA	NYN	PCA	PCR	ACI	EGA	NYN	PCA	PCR	ACI	EGA	NYN	PCA	PCR	ACI	EGA	NYN	ACI	EGA	NYN
PCB 28	4.63	136	**5.30**	1.02	1.46	4.25	1.25	4.87	-	1.34	2.70		3.09	–	–	2.36	–	2.71	–	–	1.20	–	–
PCB 52	[a]	1.04	1.11	–	–	–	1.49	**1.59**	–	1.43	–	–	–	–	–	–	–	–	–	–	–	–	–
PCB 101	–	1.43	–	–	–	–	**1.45**	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–
PCB 118	**15.52**	2.53	1.20	4.33	4.42	11.63	1.90	9.02	3.25	3.31	15.47	2.53	11.99	4.32	4.40	5.86	–	4.55	1.64	1.67	3.86	1.39	1.42
PCB 138	13.00	–	9.71	2.85	3.15	9.60	–	7.17	2.10	2.33	**13.73**	–	10.26	3.01	3.33	6,67	–	4.98	1.46	1.62	5.49	1.61	1.78
PCB 153	**18.95**	4.78	18.27	6.07	7.26	10.30	2.60	9.93	3.30	3.94	14.13	3.57	13.62	4.53	5.41	3.99	1.01	3.85	1.28	1.53	4.23	1.41	1.68
PCB 180	20.92	3.53	23.00	5.67	7.00	16.06	2.71	17.66	4.35	5.38	35.99	6.07	**39.57**	9.76	12.05	7.23	1.22	7.95	1.96	2.42	10.30	2.54	3.13

Bold numbers indicate BMF maximum value of each compound reported among all bird species.-: no biomagnification (BMF < 1). PCA: *P. carbo.* PCR: *P. cristatus.* ACI: *A. cinerea.* EGA: *E. garzetta.* NYN: *N. nycticoraxr*

[a] BMFs were not calculated because congeners occurred in < 50% of samples.

Table 6. Physicochemical properties and summary BAFs of PCBs determined in the present study in comparison to previously reported values

Congeners	S[a] (25°C) (mg/L)	$LogK_{ow}^{b}$	LogBAF fish[c]	LogBAF fish[d]	LogBAF fish[e]	LogBAF *Rutilus rutilus*[f]	LogBAF *Rutilus rutilus*[g]
PCB-28	0.65	5.6	4.2	33.9[d]	3.92–4.21	2.7–3.74	4.16
PCB-52	0.26	5.8	4.6	2.66–6.38	4.03–4.70	3.3–42.2	4.36
PCB-101	0.099	6.4	5.0	3.18–8.02	5.02–5.30	3.64–4.68	5.30
PCB-118	0.099	6.7	5.0	–[h]	4.43–4.85	3.36–4.92	4.43
PCB-138	0.038	6.8	5.4	–	5.10–6.07	4.41–4.81	6.07
PCB-153	0.038	7.1	5.4	4.63–4.82 4.48–5.23[i]	4.68–5.23	3.87–4.98	4.68
PCB-180	0.014	7.4	5.8		4.23–4.93	4.06–5.34	4.23

[a] From Serrano *et al.* (2003).
[b] Mean value from Mackay *et al.* (2000).
[c] From Erickson (2001).
[d] Values from Mackay *et al.* (2000).
[e] Determined BAF values for all fish species in the present study.
[f] Reported BAF values for roach for slightly and highly polluted sites, respectively (Blanchard *et al.* 1997).
[g] Reported BAF values for roach in the present study.
[h] Data not available.
[i] Calculated based on solubility.

BMFs of non-metabolizable congeners, e.g., PCB 153, typically range between 20 and 60 in birds (Kelly *et al.* 2004). In addition, BMFs from fish to grey herons ranged from 3 to 15, depending on the individual congener while BMFs of ΣPCBs from cod or polar cod to Brunnich's guillemot or Glaucous gull ranged between 9.4–17 and 638–1144 respectively (Zimmermann *et al.,* 1997; Borga *et al.* 2001). Although some of the BMF values calculated in the present study as well as the maximum value for PCB 153 (18.95) lie within the above-mentioned ranges, many of BMF values were lower or showed no biomagnification. This was probably due to the fact that, in contrast to other studies reporting concentrations in bird tissues, in the present study concentrations in eggs were measured. There is little information on BMFs of PCBs from fish species to bird eggs (Henny *et al.* 2003; Braune and Norstrom, 1989). BMF values for high-chlorinated PCBs (138, 153, 180) similar to our study have also been reported from fish to osprey eggs while BMFs for PCB 101 were considerably lower (Table 7).

The basic assumption in the biomagnification model (that trophic position alone can be used to describe biomagnification of PCBs) is too simplified to accurately describe the fate of PCBs in food chains. However, there is a trophic level increase for the high chlorinated congeners (Burreau *et al.* 2004). The heavier congeners are more persistent, more hydrophobic, and more efficiently transferred from prey to predator through the diet. Many studies have shown that even when lipid is normalized, food web structure and trophic level may affect persistent organochlorine concentrations (Kidd *et al.* 1998; Bentzen *et al.* 1996).

In Search of the Best Bioindicator

Eggs of the same or relative waterbird species analysed in this study have been used extensively as PCB and other organochlorine biomonitors in aquatic systems (e.g., cormorants, Ryckman *et al.,* 1998; Custer *et al.,* 1999); grey heron (De Cruz *et al.,* 1997) and great blue heron (*Ardea herodias*, Fitzner *et al,* 1988); little egret (Fasola *et al.,* 1998; Connell *et al.,* 2003); black-crowned night heron (Hothem *et al,* 1995; Fasola *et al.,* 1998); great-crested grebe (Scharenberg and Ebeling, 1998). In an analogous manner, fish have also been used as bioindicators of PCBs (Colombo *et al.,* 2000; Bordajandi *et al.,* 2003).

At Lake Kerkini, once all congeners analysed were found in detectable concentrations, practically all species examined could be used as biomonitors of PCBs. *P. carbo* eggs indicated the greatest concentration and bioaccumulation especially of

Table 7. Summary BMFs of PCBs determined in the present study in comparison to previously reported values

Congener	BMF (gull egg/alewife)[a]	BMF (osprey egg/fish)[b]	BMF range (Bird's egg/fishes)[c]	BMF (bird egg / prey diet)[d]		
				P. carbo	*N. nycticorax*	*E.garzetta*
PCB-28	–[e]	–	<1–5.30	4.0	1.0	2.5
PCB-52	–	–	<1–1.59	0.4	0.4	0.9
PCB-101	7.9	10	<1–1.45	0.6	0.5	1.0
PCB-118	31	11	<1–15.52	11.2	2.2	15.0
PCB-138	42	16	<1–3.73	9.6	2.1	11.0
PCB-153	48	16	1.01–18.95	9.6	2.0	14.4
PCB-180	53	19	1.22–39.57	15.3	3.2	26.5

[a] From Braune and Norstrom (1989).
[b] From Henny *et al.* (2003) for composite (3 species) fish diet of ospreys based on wet weight concentrations.
[c] Range of BMF values determined in the present study for all bird species and all fish prey.
[d] BMF values determined on the basis of bird's diet (% prey—fish and frogs—consumption) reported in Table 1.
[e] Data not available.

the higher PCBs and seemed to be the most useful as a biomonitor among all biota analysed. In this area, breeding cormorants occur in considerable populations, thus the use of egg samples for organochlorine pollutant monitoring will not affect the survival of the breeding population. In contrast, among birds, both *E. garzetta* and *N. nycticorax* are the least suitable. In addition, the diet of these two species is too diverse, thus the identification of pollution origin becomes complicated. Also, *P. cristatus* eggs, showing considerable variability of four PCB levels probably due to specialized diet of the pairs sampled, do not seem to indicate the PCB pollution in the lake. This fact renders *P. cristatus* unsuitable as biomonitor.

Evidently, among fish, *L. gibbosus* had the highest concentrations of most congeners and exhibited the greatest bioaccumulative properties. Sampling of *L. gibbosus* is much easier and less invasive than egg sampling and larger samples can be obtained. Thus, this species is also proposed as an alternative biomonitor of PCBs at Lake Kerkini.

Some other threatened colonial waterbird species such as *Phalacrocorax pygmeus* (pygmy cormorant), *Platalea leucorodia* (spoonbill), *Ardeola ralloides* (squacco heron), and *Plegadis falcinellus* (glossy ibis) also breed at Lake Kerkini but in relatively low numbers (Nazirides, 2004). Due to their endangered status, no evaluation was made for their use as PCBs biomonitors.

Conclusion

This study provides latter PCB levels in waterbird eggs and in their prey at Lake Kerkini, an investigation of PCBs bioaccumulation patterns, and finally an assessment of the best bioindicator. The results have shown that the PCB pattern changed with trophic position from fish and frogs to waterbirds with a decreasing contribution of low chlorinated congeners and an increasing contribution of the most persistent compounds. The pattern change reflects the different biomagnification degree of congeners, which depends upon the organism's physiology with respect to xenobiotic elimination and the congener's physicochemical properties. The source of biomagnification is considered to be trophic transfer whereby contaminants are concentrated in organisms through the ingestion and absorption of residues accumulated in prey species occupying lower trophic niches. Higher concentrations and bioaccumulations were found in the cormorant, which can be used as an indicator/sentinel species (an integrator of contaminants) by collecting only egg samples, and projecting via BMFs and BAFs an estimated contamination in the lake ecosystem. The determined bioaccumulation and biomagnification factors provide some basic understanding of the relationships between PCB burdens in prey species of fish-eating birds and PCB incorporated into their eggs, and may prove useful in understanding the sources of contaminants in migratory species although additional studies are needed. Compared to previous studies, the composition and concentrations of pollutants vary; thus, collaboration with neighbouring countries is suggested for the identification and elimination of pollution sources.

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References

Albaigés J (2005) Persistent organic pollutants in the Mediterranean Sea. In: The Mediterranean Sea. The handbook of environmental chemistry, Vol 5, part K, Springer, Heidelberg, Germany, pp 899–149

Albanis TA, Hela DG, Sakellarides TM, Konstantinou IK (1998) Monitoring of pesticides residues and their metabolites in surface and underground waters from Imathia (N. Greece) by means of solid phase extraction disks and gas chromatography. J Chromatogr A 823:59–71

Albanis T, Goutner V, Konstantinou I, Frigis K (2003) Organochlorine contaminants in eggs of the yellow-legged gull (*Larus cachinnans michaellis*) in the northeastern Mediterranean: is this gull a suitable biomonitor for the region? Environ Pollut 126:245–255

Bachour G, Failing K, Georgii S, Elmadfa I, Brunn H (1998) Species and organ dependence of PCB contamination in fish, foxes, roe deed and humans. Arch Environ Contam Toxicol 35:666–673

Barren MG, Galbraith H, Beltman D (1995) Comparative reproductive and developmental toxicology of PCBs in birds. Comp Biochem Physiol 112C:1–14

Bentzen E, Lean DRS, Taylor WD, Mackay D (1996) Role of food web structure on lipid and bioaccumulation on organic contaminants by lake trout (*Salvelinus namaycush*). Can J Fish Aquat Sci 53:2397–2407

Berglund O, Larsson P, Ewald G, Okla L (2000) Bioaccumulation and differential partitioning of polychlorinated biphenyls in freshwater planktonic food webs. Can J Fish Aquat Sci 57:1160–l168

Birtsas PK (2002) Ecology and conservation of the black-crowned night heron (*Nycticorax nycticorax* (L., 1758)) at the Kerkini reservoir, Macedonia, Greece. Doctoral Dissertation, Department of Forestry and Natural Environment, Aristotle University of Thessaloniki, p 151

Blanchard M, Teil MJ, Carru AM, Chesterikoff A, Chevreuil M (1997) Organochlorine distribution and mono-orthosubstituted PCB pattern in the roach (*Rutilus rutilus*) from the river Seine. Wat Res 31:1455–1461

Bordajandi LR, Gómez G, Fernández MA, Abad E, Rivera J, González MJ (2003) Study on PCBs PCDD/Fs, organochlorine pesticides, heavy metals and arsenic content in freshwater fish species from the river Turia (Spain). Chemosphere 53:163–171

Borga K, Gabrielsen GW, Skaare JU (2001) Biomagnification of organochlorines along a Barents Sea food chain. Environ Poll 113:187–198

Borga K, Fisk AT, Hargrave B, Hoekstra PF, Swackhamer D, Muir DC (2005) Bioaccumulation factors of PCBs revisited. Environ Sci Teclinol 39:4523–4532

Boumphrey RS, Harrad SJ, Jones KC, Osborn D (1993) Polychlorinated biphenyl congener patterns in tissues from a selection of British birds. Arch Environ Contam Toxicol 25:346–352

Braune BM, Norstrom RJ (1989) Dynamics of organochlorine compounds in Herring Gulls: III. Tissue distribution and bioaccumulation in Lake Ontario gulls. Environ Toxicol Chem 8:957–968

Burreau S, Zebuhr Y, Broman D, Ishaq R (2004) Biomagnification of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) studied in pike (*Esox lucius*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) from the Baltic Sea. Chemosphere 55:1043–1052

Colombo JC, Bilos C, Lenicov MR, Colautti D, Landoni P, Brochu C (2000) Detritivorous fish contamination in the Rio de la Plata estuary: a critical accumulation pathway in the cycle of anthropogenic compounds. Can J Fish Aquat Sci 57:1139–1150

Connell DW, Fung CN, Minh TB, Tanabe S, Lam PKS, Wong BSF, Lam MHW, Wong LC, Richardson BJ (2003) Risk to breeding success of fish-eating ardeids due to persistent organic contaminants in Hong Kong: evidence from organochlorine compounds in eggs. Wat Res 37:459–467

Crimmins BS, Doelling Brown P, Kelso DP, Foster GD (2002) Bioaccumulation of PCBs in aquatic biota from a tidal freshwater marsh ecosystem. Arch Environ Contam Toxicol 42:396–404

Crivelli AJ, Marsili L, Focardi S, Renzoni A (1999) Organochlorine compounds in pelicans (*Pelecanus crispus* and *Pelecanus onocrotalus*) nesting at Lake Mikri Prespa, northwestern Greece. Bull Environ Contam Toxicol 62:383–389

Custer TW, Custer CM, Hines RK, Gutreuter S, Stromborg KL, Allen PD, Melancon MJ (1999) Organochlorine contaminants and reproductive success of double-crested cormorants from Green Bay, Wisconsin, USA. Environ Toxicol Chem 18:1209–1217

Dannenberger D, Andersson R, Rappe C (1997) Levels and patterns of polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls in surface sediments from the Oder River estuarine system. Mar Pollut Bull 34:1016–1024

De Cruz I, Mougin C, Grolleau G (1997) Chlorinated hydrocarbons in eggs of grey heron (*Ardea cinerea L.*) in France. (Lac De Grandieu). Chemosphere 35:1003–1009

De Luca-Abbott SB, Wong BSF, Peakall DB, Lam PKS, Young L, Lam MHW, Richardson BJ (2001) Review of effects of water pollution on the breeding success of waterbirds, with particular reference to ardeids in Hong Kong. Ecotoxicology 10:327–349

Dietrich S, Büthe A, Denker E, Hötker H (1997) Organochlorines in eggs and food organisms of avocets (*Recurvirostra avosetta*). Bull Environ Contam Toxicol 58:219–226

Dirksen S, Boudewijn T, Slager LK, Mes RG, Van Schaick MJM, De Voogt P (1995) Reduce breeding success of cormorants (*Phalacrocorax carbo sinensis*) in relation to persistent organochlorine pollution of aquatic habitats in the Netherlands. Environ Pollut 88:119–l32

Erickson MD (2001) Introduction: PCB properties, uses, occurrence and regulatory history. In: Robertson LW, Hansen LG (eds) PCBs: Recent advances in environmental toxicology and health effects. The University Press of Kentucky, U.S.A., pp xi–xxx

Fasola M, Movalli PA, Gandini C (1998) Heavy metal, organochlorine pesticide, and PCB residues in eggs and feathers of herons breeding in northern Italy. Arch Environ Contam Toxicol 34:87–93

Fisk AT, Norstrom RJ, Cymbalisty CD, Muir DCG (1998) Dietary accumulation and depuration of hydrophobic organochlorines: Bioaccumulation parameters and their relationship with the octanol/water partition coefficient. Environ Toxicol Chem 17:951–961

Fitzner RE, Blus LJ, Henny CJ, Carlile DW (1988) Organochlorine residues in great blue herons from the northwest United States. Colon Watb 11:203–300

Fossi MC, Massi A, Lari L, Marsili L, Focardi S, Leonzio C, Renzoni A (1995) Interspecies differences in mixed function oxidase activity in birds: Relationship between feeding habits, detoxication activities and organochlorine accumulation. Environ Pollut 90:15–24

Guruge KS, Tanabe S (1997) Congener specific accumulation and toxic assessment of polychlorinated biphenyls in common cormorants, *Phalacrocorax carbo*, from Lake Biwa, Japan. Environ Pollut 96:425–433

Guruge KS, Tanabe S, Fukuda M (2000) Toxic assessment of PCBs by the 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalent in common cormorant (*Phalacrocorax carbo*) from Japan. Arch Environ Contam Toxicol 38:509–521

Hebert CE, Nostrom RJ, Zhu J, Macdonald CR (1999) Historical changes in PCB patterns in Lake Ontario and Green Bay, Lake Michigan, 1971 to 1982, from herring gull egg monitoring data. J Gt Lakes Res 25:220–233

Henny CJ, Kaiser JL, Grove RA, Raymond Bentlet V, Elliott JE (2003) Biomagnification factors (fish to osprey eggs from Willamette river, Oregon, USA) for PCDDs, PCDFs, PCBs and OC pesticides. Environ Monit Assess 84:275–315

Hoffman DJ, Rattner BA, Bunck CM, Crynitsky A, Ohlendorf HM, Lowe RW (1986) Association between PCBs and lower embryonic weight in black-crowned night herons in San Francisco Bay. J Toxicol Environ Health 19:383–391

Hong CS, Xiao J, Bush B, Shaw SD (1998) Environmental occurrence and potential toxicity of planar, mono- and di-ortho polychlorinated biphenyls in the biota. Chemosphere 36:1637–1651

Hoshi H, Minamoto N, Iwata H, Shiraki K, Tatsukawa R, Tanabe S, Fujita S, Hirai K, Kinjo T (1998) Organochlorine pesticides and polychlorinated biphenyl congeners in wild terrestrial mammals and birds from Chubu region, Japan: interspecies comparison of the residue levels and compositions. Chemosphere 36:3211–3221

Hothem RL, Roster PL, King KA, Keldsen TJ, Marois KC, Wainwright SE (1995) Spatial and temporal trends of contaminants in

eggs of wading birds from San Francisco Bay, California. Environ Toxicol Chem 14:1319–1331

Jenssen BM, Nilssen VH, Murvoll KM, Skaare JU (2001) PCBs, TEQs and plasma retinol in grey heron (*Ardea cinerea*) hatchlings from two rookeries in Norway. Chemosphere 44:483–489

Kamarianos A, Karamanlis X, Galoupi E (2002) Pollution of coastal areas of N. Creece by organochlorine pesticides and polychlorinated biphenyls (PCBs). In: Proceedings of the 1st Environmental Conference of Macedonia, 1–4 March, Thessaloniki, Greece, pp 116–121

Katsoyiannis A (2006) Occurence of polychlorinated biphenyls (PCBs) in the Soulou stream in the power generation area of Eordea, nortwastern Greece. Chemosphere 65:1551–1561

Kelly BC, Gobas FAPC, McLachan MS (2004) Intestinal absorption and biomagnification of organic contaminants in fish, wildlife and humans. Environ Toxicol Chem 23:2324–2336

Kidd KA, Schindler DW, Hesslein RH, Muir DCG (1998) Effects of trophic position and lipid on organochlorine concentrations in fishes from subarctic lakes in Yukon Territory. Can J Fish Aquat Sci 55:869–881

Konstantinou IK, Goutner V, Albanis TA (2000) The incidence of polychlorinated biphenyl and organochlorine pesticide residues in the eggs of the cormorant (*Phalacrocorax carbo sinensis*): an evaluation of the situation in four Greek wetlands of international importance. Sci Total Environ 257:61–79

LeBlanc GA (1995) Trophic-level diffrences in the bioconcentration of chemicals: implications in assessing environmental biomagnification. Environ Sci Technol 29:154–160

Liordos V (2004) Biology and ecology of great cormorant ((*Phalacrocorax carbo* L., 1758) populations breeding and wintering in Greek wetlands. Doctoral Dissertation, Department of Biology, Aristotle University of Thessaloniki, p 307

Looser R, Ballschmiter K (1998) Biomagnification of polychlorinated biphenyls (PCBs) in freshwater fish. Fres J Anal Chem 360:816–819

Mackay D, Shiu WY, Ma KC (2000) Handbook of physical-chemical properties and environmental fate. CRCnetBase, Chapman & Hall, CRC Press, Boca Raton, FL

Metcalfe TL, Metcalfe CD (1997) The trophodynamics of PCBs, including mono- and non-ortho congeners, in the food web of north-central Lake Ontario. Sci Total Environ 201:245–272

Meylan WM, Howard PH, Boethling RS, Aronson D, Printup H, Gouchie S (1999) Improved method for estimating bioconcentration/bioaccumulation factor from octanol/water partition coefficient. Environ Toxicol Chem 18:664–672

Muñoz Cifuentes J, Becker PH, Sommer U, Pacheco P, Schlatter R (2003) Seabird eggs as bioindicators of chemical contamination in Chile. Environ Pollut 126:123–137

Nazirides T (2004) Comparative biological-ecological study of the cormorant (*Phalacrocorax carbo* (Linnaeus, 1758)) and pygmy cormorant (*Phalacrocorax pygmeus* (Pallas, 1773)) at Lake Kerkini. Doctoral Dissertation, Department of Forestry and Natural Environment, Aristotle University of Thessaloniki, p 118

Oliver BG, Niimi AJ (1988) Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. Environ Sci Technol 22:388–397

Pastor D, Sanpera C, Gonzáles-Solís J, Ruiz X, Albaigés J (2004) Factors affecting the organochlorine pollutant load in biota of a rice field ecosystem (Ebro Delta, NE Spain). Chemosphere 55:567–576

Ryckman DP, Weseloh DV, Hamp P, Fox GA, Collins B, Ewins PJ, Norstrom RJ (1998) Spatial and temporal trends in organochlorine contamination and bill deformities in double-crested cormorants (*Phalacrocorax auritus*) from the Canadian Great Lakes. Environ Monitor Assess 53:169–195

Scharenberg W (1991) Cormorants (*Phalacrocorax carbo sinensis*) as bioindicators for chlorinated biphenyls. Arch Environ Contam Toxicol 21:536–540

Scharenberg W, Gramann P, Pfeiffer WH (1994) Bioaccumulation of heavy metals and organochlorines in a lake ecosystem with special reference to bream (Abramis brama). Sci Total Environ 155:187–197

Scharenberg W, Ebeling E (1998) Organochlorine pesticides in eggs of two waterbird species (*Fulica atra, Podiceps cristatus*) from the same habitat: reference site Lake Belau, Germany. Chemosphere 36:263–270

Serrano R, Simal-Julian A, Pitarch E, Hernandez F, Varo I, Navarro JC (2003) Biomagnification study on organochlorine compounds in marine aquaculture: The sea bass (*Dicentrachus labrax*) as a model. Environ Sci Technol 37:3375–3381

Singh AK, Spassova D, White T (1998) Quantitative analysis of polychlorinated biphenyls, organochlorine insecticides, polycyclic aromatic hydrocarbons, polychlorinated hydrocarbons and polynitrohydrocarbons in spiked samples of soil, water and plasma by selected-ion monitoring gas chromatography-mass spectrometry. J Chromatogr B 706:231–244

Swackhamer DL, Hites RA (1988) Occurrence and bioaccumulation of organochlorine compounds in fishes from Siskiwit Lake, Isle Royale, Lake Superior. Environ Sci Technol 22:543–548

Swindlehurst RJ, Johnston PA, Tröndle S, Stringer RL, Stephenson AD, Stone IM (1995) Regulation of toxic chemicals in the Mediterranean: the need for an adequate strategy. Sci Total Environ 171:243–264

Tanabe S, Kannan N, Subramanian A, Watanabe S, Tatsukawa R (1987) Highly toxic coplanar PCBs: occurrence, source, persistency and toxic implications to wildlife and humans. Environ Pollut 47:147–163

Tsachalidis EP (1989) Biology and behavioral ecology of little egret (*Egretta garzetta*) in the artificial Lake Kerkini, Serres. Doctoral dissertation, Department of Forestry and Natural Environment, Aristotle University of Thessaloniki, p 146

Wainwright SE, Mora MA, Sericano JL, Thomas P (2001) Chlorinated hydrocarbons and biomarkers of exposure in wading birds and fish of the lower Rio Grande Valley, Texas. Arch Environ Contam Toxicol 40:101–111

Weseloh DV, Hamr P, Bishop CA, Norstrom RJ (1995) Organochlorine contaminant levels in waterbird species from Hamilton Harbour, Lake Ontario: an IJC area of concern. J Gt Lakes Res 21:12l–l37

Yamashita N, Tanabe S, Ludwig JP, Kurita H, Ludwig ME, Tatsukawa R (1993) Embryonic abnormalities and organochlorine contamination in double-crested cormorants (*Phalacrocorax auritus*) and Caspian terns (*Hydroprogne caspia*) from the upper Great Lakes in 1988. Environ Pollut 79:163–173

Zimmermann G, Dietrich DR, Schmid P, Schlatter C (1997) Congener-specific bioaccumulation of PCBs in different water bird species. Chemosphere 34:1379–1388