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Organochlorine and mercury residues in eggs of the lesser kestrel (*Falco naumanni*) from a long term study in the eastern Mediterranean

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ABSTRACT

Organochlorine and mercury residues were analyzed in unhatched eggs of the lesser kestrel (*Falco naumanni*) (2002–2012) in central Greece. Concentrations graded as ∑DDTs > ∑PCBs > HCB > ∑HCHs > ∑Chlordanes. Temporal declines were found in the concentrations of ∑DDTs, ∑HCHs and ∑Chlordanes but not in Hg, HCB and ∑PCBs. TEQs of PCBs and their degree of metabolisation showed no time trend. The reproductive parameters showed neither a temporal trend nor a significant year effect. No relationships occurred between the reproductive parameters per year and nest type (natural, artificial) with any of the contaminants analyzed except HCB influenced by year and clutch size. Low pollutant concentrations suggest that either lesser kestrels ranged across the year in unpolluted areas or may be caused by their short food chain. The low concentrations seem improbable to have affected the reproduction of these birds, although critical levels are still to be defined.

1. Introduction

Contaminants such as anthropogenic mercury (Hg) and organochlorines (OCs) are widespread in the environment. Hg is a very toxic metal, widely occurring in the Mediterranean environment due to natural emissions from volcanoes, other geological procedures (Ferrara et al., 2000) and anthropogenic activities (Pirrone et al., 2003; Pacyna et al., 2010). Hg becomes bioavailable mainly in its methylated form. Among OCs, organochlorine pesticides and the industrial polychlorinated biphenyls (PCBs) are two groups well known for their short and long term effects on biota and humans and are widely detected in the Mediterranean region (Gómez-Gutiérrez et al., 2007). Despite bans, OC residues are still detected in environmental samples and find their way in the food chains due to their high persistence and ability to bioaccumulate and biomagnificate (Metcalfe and Metcalfe, 1997; Gómez-Ramirez et al., 2014).

Birds of prey are particularly vulnerable to bioaccumulated contaminants because they are at the top of the terrestrial food chain and have high rates of food consumption; PCBs and DDTs have been of the OCs the most commonly detected (Gómara and González, 2006; Fernie and Letcher, 2010; Luzardo et al., 2014). PCBs, particularly the most toxic congeners, have been associated with reduced reproductive success, embryonic deformities, edema and induction of cytochrome P450 (Hoffmann et al., 1998) and disrupted reproductive behavior (Fisher et al., 2006). Effects usually attributed to DDE, the most toxic DDT metabolite, are reduced eggshell thickness and hatchability, both observed on the peregrine (*Falco peregrinus*) and other falcons (Fox and Donald, 1980; Elliott et al., 2005). Therefore, both a thorough understanding of the trends and present levels of contaminants along with the conservation needs of birds of prey make their use as bioindicators necessary and prominent within the frame of monitoring programs (NRC, 1991; Gómez-Ramírez et al., 2014). Although there have been several monitoring schemes in Europe, they are lacking in eastern European countries (Gómez-Ramírez et al., 2014).

Although Greece is a region hosting a rich diversity of birds of prey, studies on OC pollutants accumulation on this group have been scarce (Clark and Peakall, 1976; Hela et al., 2006; Goutner et al., 2011a) while Hg has not been studied at all. All those pollutants are listed in Annexes A and B of the Stockholm Convention,

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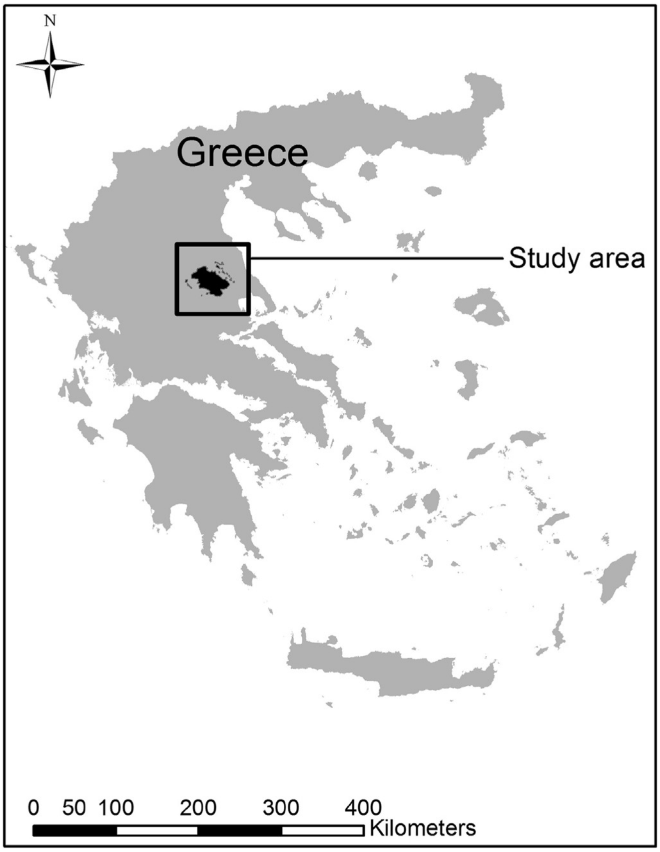

Fig. 1. The location of the study area.

and countries should take appropriate measures to ensure that their use do not potentially harm both, human health and wildlife.

The lesser kestrel (*Falco naumanni*) is a small raptor with an estimated global population of 25,100–52,000 pairs, whilst the European breeding population lies between 25,000 and 42,000 breeding pairs (BirdLife International, 2015). The western European population suffered a dramatic decline of 95% since 1950, however, recently it has been stabilized and thus the species qualified as "least concern" (BirdLife International, 2015) although considerable declines have locally occurred. Its population in Greece, comprising c. 15% of the European total, has also been declining and it is mostly concentrated in Thessaly, central Greece, in 98 colonies totaling c. 2900 breeding pairs (Vlachos et al., 2004). Lesser kestrels are associated with intensively cultivated fields as foraging places (Vlachos et al., 2003), and this behavior was considered a possible reason for the population decline throughout its breeding geographical range in Europe (Donázar et al., 1993). The current study is conducted within the framework of a long term study for the conservation of lesser kestrel and its habitats in Greece (Bakaloudis, 2012; Vlachos et al., 2015), and its aim is twofold: a) to investigate the long term trends of Hg and some environmentally important OCs in lesser kestrel eggs, and b) to evaluate possible effects of these contaminants to the studied population, in relation to its breeding parameters.

2. Materials and methods

2.1. Study area

The study area is "Periochi Thessalikou Kampou", Special Protected Area, SPA: (GR-1420011), situated at the eastern part of the Larissa plain, Thessaly, central Greece (Fig. 1). It is near the village of Armenio (39°29′07″N, 22°41′39″E) and is surrounded by intensively cultivated agricultural land. The climate is thermo-Mediterranean, with mild rainy winter, dry and hot summer and a mean annual precipitation of about 465 mm. The habitat types are mainly cultivations dominated by cotton, corn, cereals and other of minor importance, whereas natural habitats are grasslands and marginal habitats, such as the edges of cultivations including the dykes among them (Vlachos et al., 2015).

2.2. Site, egg sampling and preparation

The study was carried out from 2002 to 2012 in a colony of approximately 120 breeding pairs. The colony has been developed mainly supported by nest boxes set up on pine trees but, until 2006, some other natural nest sites such as on old houses, barns and similar constructions have been monitored. Each nest was monitored during the breeding season from the start of egg laying (mid-April) to nestlings' fledging period (mid-July) to accurately record breeding chronology and success. Data were collected on number of eggs laid, hatched and nestlings fledged. Only unhatched eggs were collected. From 2002 to 2006 54 eggs from 54 clutches (Table 1) were collected from both natural sites and nest boxes nearly in the same amount per year whereas from 2007 to 2012 70 eggs originated only from 20 clutches from nest boxes. The contents of one or more unhatched eggs from the same clutch were homogenized and stored deep frozen in chemically clean containers until analysis. So, the clutch is the sample unit reflecting the contamination of the egg-laying female. Compound groups chosen for analysis are persistent so that their original composition would not change due to 'post-hatching' microbiological degradation (Herzke et al., 2002; Custer et al., 2014).

2.3. Reproductive parameters

The reproductive parameters analyzed were (a) the clutch size (number of eggs laid) of the nests from where eggs were sampled, (b) the number of eggs hatched per clutch, and (c) the hatching success (proportion of eggs hatched from eggs laid per clutch) as a measure of breeding success.

2.4. Chemical analysis

All chemical analyses were made by the Institute of Avian Research at ICBM-Terramare, Wilhelmshaven, Germany. Egg contents were thawed and then homogenized using an Ultra Turrax and freeze-dried.

We analyzed organochlorines (OCs) and the heavy metal mercury. Among the OCs we focused on the industrial chemicals PCBs (62 congeners) and HCB (hexachlorobenzene), and on the pesticides DDT and metabolites, HCH (hexacholocyclohexane) isomers, chlordane and nonachlor compounds. For the determination of the OCs each sample of 0.5 g egg-homogenate was spiked with the internal standard solution, dried with sodium sulfate, cleaned by silica gel column, eluted with n-hexane:dichloromethane (8:2), evaporated and redissolvedin 250 μl isooctane. For detection a GC–MS Agilent 6890 was used, coupled to a mass-selective detector Agilent 5973 and CIS, with helium as the carrier gas. The electron impact ionization was used and measured in the SIM mode. For separation, a HAT-5-column with a length of 25 m was used. The internal standard added before extraction allows correct losses during extraction and clean-up process (automatically done by the calibrated GC–MS software). The qualification and the quantification of the pesticides and PCBs were performed according to Büthe and Denker (1995). Forty one of 62 PCB congeners could be determined separately (PCB 28, PCB 52, PCB 64, PCB 66, PCB 70, PCB 74, PCB 85, PCB 95, PCB 99, PCB 105, PCB

Table 1
Mean concentrations (ng g^{-1} dry weight ± 1 SD; TEQs: pg g^{-1}) of pollutants detected in lesser kestrel eggs from Greece. Range (min–max) values are in parentheses.

Year (n[a])	Contaminants								Breeding output		
	Hg	∑DDTs	∑PCBs	Degree of chlorination of the PCBs	TEQs of PCBs (pg g^{-1})	∑HCHs	HCB	∑Chlordanes	Mean no. eggs laid	Mean no. eggs hatched	Mean no. eggs hatched/laid
2002 (5)	401.7 ± 89.7 (310.8–529.8)	317.3 ± 229.9 (115.6–650.8)	84.3 ± 32.8 (31.6–121.2)	6.1 ± 0.2 (5.8–6.4)	0.9 ± 0.3 (0.5–1.4)	21.0 ± 13.0 (11.4–35.3)	14.1 ± 2.1 (12.4–17.7)	1.2 ± 1.6 (0.0–4.0)	3.8 ± 1.3 (2–5)	2.2 ± 1.3 (0–3)	0.52 ± 0.30 (0.00–0.75)
2003 (8)	105.9 ± 23.1 (64.1–135.8)	160.1 ± 90.2 (47.5–324.7)	59.5 ± 22.5 (27.5–92.0)	5.9 ± 0.4 (5.1–6.3)	0.9 ± 0.4 (0.5–1.6)	26.6 ± 11.3 (13.7–43.3)	28.0 ± 16.8 (8.3–53.2)	2.3 ± 2.9 (0.0–8.2)	4.3 ± 0.7 (3–5)	2.5 ± 1.2 (0–4)	0.57 ± 0.27 (0.00–0.80)
2004 (15)	232.9 ± 147.6 (50.2–715.2)	411.4 ± 533.7 (53.3–1711.3)	87.4 ± 29.8 (55.1–165.8)	5.9 ± 0.3 (5.1–6.3)	281.7 ± 228.7 (0.6–831.1)	20.0 ± 10.4 (2.9–43.0)	17.9 ± 8.2 (4.7–30.9)	3.2 ± 4.0 (0.0–14.2)	3.6 ± 1.1 (1–5)	1.5 ± 1.6 (0–4)	0.35 ± 0.37 (0.00–0.80)
2005 (13)	152.3 ± 64.0 (87.0–337.4)	263.7 ± 326.8 (31.0–1266.1)	97.6 ± 29.6 (56.1–152.2)	6.0 ± 0.3 (5.5–6.5)	317.3 ± 245.3 (0.9–690.6)	17.0 ± 8.6 (4.0–41.1)	22.5 ± 10.9 (8.2–37.7)	1.9 ± 1.5 (0.0–5.4)	4.1 ± 0.9 (3–6)	2.0 ± 1.4 (0–3)	0.48 ± 0.34 (0.00–0.75)
2006 (13)	326.1 ± 212.0 (108.8–783.0)	452.7 ± 310.5 (26.1–1060.4)	94.7 ± 46.0 (23.7–205.1)	6.1 ± 0.2 (5.8–6.4)	365.5 ± 97.0 (110.6–501.9)	11.9 ± 3.6 (7.0–20.4)	24.0 ± 13.7 (2.6–41.4)	4.2 ± 3.8 (0.0–11.9)	3.8 ± 1.4 (1–6)	2.0 ± 1.5 (0–4)	0.46 ± 0.35 (0.00–0.75)
2007 (14)	142.3 ± 98.7 (29.8–416.9)	191.5 ± 315.3 (17.6–1229.4)	84.7 ± 33.8 (42.6–174.2)	6.1 ± 0.2 (5.9–6.5)	165.5 ± 147.6 (1.0–431.9)	20.2 ± 14.8 (7.1–54.6)	10.3 ± 5.8 (3.6–26.2)	0.3 ± 0.4 (0.0–1.2)	4.1 ± 1.1 (1–5)	2.6 ± 1.4 (0–4)	0.56 ± 0.29 (0.00–0.80)
2008 (7)	211.5 ± 97.0 (101.8–369.9)	63.3 ± 40.7 (14.0–138.7)	73.8 ± 32.7 (44.6–113.0)	6.3 ± 0.1 (6.1–6.5)	369.9 ± 236.9 (151.1–872.4)	8.8 ± 3.2 (5.8–15.6)	13.0 ± 11.8 (2.6–35.8)	0.5 ± 0.7 (0.0–1.8)	3.7 ± 0.5 (3–4)	1.7 ± 1.1 (0–3)	0.45 ± 0.29 (0.00–0.75)
2009 (14)	119.3 ± 71.8 (33.3–294.5)	231.2 ± 246.8 (65.6–936.4)	92.0 ± 37.8 (40.0–169.8)	6.2 ± 0.2 (5.9–6.6)	362.7 ± 165.1 (130.9–601.6)	11.6 ± 6.8 (5.7–32.0)	38.6 ± 27.3 (8.1–106.5)	2.0 ± 3.0 (0.0–11.6)	3.7 ± 1.4 (1–5)	2.2 ± 1.5 (0–4)	0.51 ± 0.31 (0.00–0.80)
2010 (10)	126.5 ± 78.2 (43.4–319.0)	83.1 ± 51.7 (28.5–174.0)	51.7 ± 13.0 (26.1–70.7)	6.4 ± 0.1 (6.3–6.6)	357.7 ± 136.6 (150.7–671.0)	8.4 ± 2.0 (5.7–11.6)	27.3 ± 12.6 (7.0–46.1)	0.6 ± 1.0 (0.0–2.9)	4.0 ± 1.2 (1–5)	2.6 ± 1.1 (0–4)	0.60 ± 0.23 (0.00–0.80)
2011 (13)	262.0 ± 113.4 (107.0–465.8)	107.9 ± 58.7 (28.6–211.7)	84.4 ± 36.2 (37.2–153.6)	5.9 ± 0.2 (5.6–6.3)	209.6 ± 205.8 (0.9–591.4)	4.4 ± 4.1 (0.0–15.3)	13.7 ± 7.0 (5.6–35.2)	0.6 ± 1.2 (0.0–4.4)	3.8 ± 1.1 (1–5)	2.2 ± 1.5 (0–4)	0.51 ± 0.33 (0.00–0.80)
2012 (12)	160.3 ± 87.2 (45.8–312.9)	144.6 ± 65.1 (23.5–245.1)	78.4 ± 23.8 (31.8–116.3)	5.8 ± 0.3 (5.3–6.1)	302.8 ± 90.4 (150.7–451.1)	10.7 ± 14.2 (1.4–53.2)	10.1 ± 4.0 (5.0–18.8)	0.9 ± 1.3 (0.0–4.4)	3.4 ± 1.7 (1–6)	1.6 ± 1.5 (0–4)	0.37 ± 0.34 (0.00–0.80)

[a] Sample sizes per year.

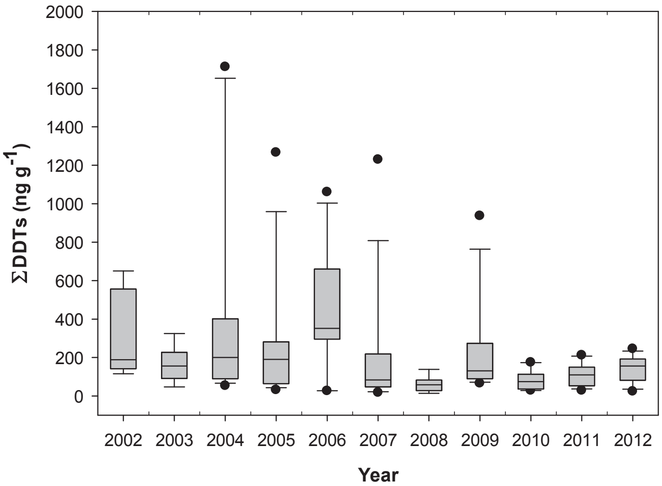

Fig. 2. Trend of ∑DDT concentrations (ng g^{-1} dry weight) in lesser kestrel eggs, 2002–2012. Indicated are: Box plots with 25%–75% percentiles; median is drawn as horizontal line in each box; percentiles 10%–90% are shown as a vertical line and dots outside represent outliers. See Table 1 for means and sample size.

107, PCB 110, PCB 114, PCB 118, PCB 123, PCB 126, PCB 128, PCB 130, PCB 138, PCB 141, PCB 149, PCB 153, PCB 155, PCB 156, PCB 157, PCB 166, PCB 167, PCB 169, PCB 170, PCB 171 PCB 172, PCB 174, PCB 177, PCB 178, PCB 183, PCB 189, PCB 190, PCB 194, PCB 195, PCB 199, PCB 202). The remaining 21 congeners were detected and determined in peaks with two or three, respectively nine peaks with two PCBs (PCB 47/48, PCB 84/92, PCB 87/115, PCB 101/90, PCB 132/146, PCB 158/129, PCB 175/187, PCB 180/193, PCB 196/203), and one peak with three PCBs (PCB160/163/164; see Table A1 for further details on the congeners and its structure). Hereafter, the concentrations of OCs analyzed are referred as: ∑PCB: the sum of 62 congeners. The toxic equivalents (TEQs) of ten dioxin-like PCB congeners were also calculated, namely the non-ortho PCB congeners PCB 126 and 169 and mono-ortho congeners PCB 105, 114, 118, 123, 156, 157, 167 and 189 (e.g. Muñoz, 2004; Becker and Dittmann, 2009, 2010; Table A1), using bird-specific 2,3,7,8-TCDD toxic equivalency factors (TEF) proposed by the WHO (Van den Berg et al., 1998). As measure for the composition of the PCB-mixture by congeners of different degree of chlorination (3 and 8 Cl-atoms; Table A1; see Beyerbach et al., 1993; Denker et al., 1994; Becker et al., 2001 for list of PCB congeners) we calculated an average degree of chlorination of the mixture in the egg (by adding different amounts of congeners with 3, 4, 5, 6, 7 or 8 chlorine atoms according to their proportion of ∑PCB; Table A1). The concentration sum of hexacholocyclohexanes, namely α-HCH, β-HCH and γ-HCH is hereafter mentioned as ∑HCHs; the sum of DDT metabolites *p,p*′-DDE, *p,p*′-DDT and *p,p*′-DDD as ∑DDTs; the ratio of *p,p*′-DDT*100/*p,p*′-DDE; the sum of chlordane and nonachlor compounds namely trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor as ∑Chlordanes; and hexachlorobenzene as HCB. Detection limits are within the range 0.1–0.9 ng g^{-1} (*p,p*′-DDT 0.3 ng g^{-1}; chlordanes, nonachlors 0.2 ng g^{-1}; other organochlorines incl. PCB congeners 0.1 ng g^{-1}). The concentrations are given as average of two determinations in ng g^{-1} dry weight (dw), for TEQs in pg g^{-1}. See Becker et al. (2001) for more details.

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

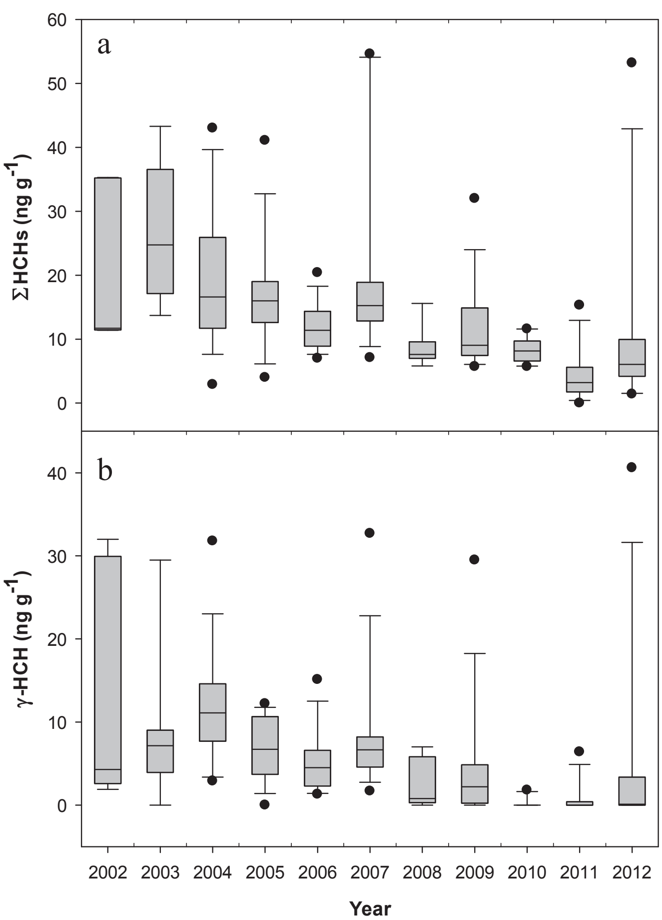

Fig. 3. Trend of ∑HCHs (a) and γ-HCH (lindane) (b) concentrations (ng g^{-1} dry weight) in lesser kestrel eggs, 2002–2012. For symbols and statistics refer to Fig. 2.

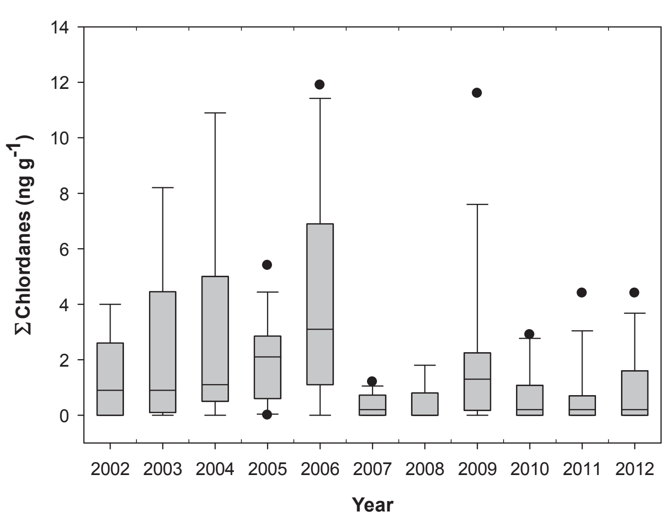

Fig. 4. ∑Chlordanes concentrations (ng g^{-1} dry weight) in lesser kestrel eggs, 2002–2012. For symbols and statistics refer to Fig. 2.

ready-to-use analytical method to analyze a large number of samples in a short time frame (Roy and Bose, 2008; Maggi et al., 2009). Detection limit was 0.003 ng g^{-1}, and concentrations are given in ng g^{-1} dw.

The conversion factor for dry-weight/fresh weight based concentrations is about 0.2 in raptors (Baum, 1981).

2.5. Statistical analysis

Annual levels of the chemicals' concentrations and of reproductive parameters were calculated and presented as means ± SD. Temporal trends for the years 2002–2012 were calculated using Spearman Rank correlations ($r_{s,}$ two-tailed test). The results are presented as box plots with medians, 25%–75% and their 10%–90% confidence intervals. The reproductive parameters were used as covariates in General Linear Models (GLM) using year and nest type (2002–2006) and their interaction as factors.

3. Results

Chemicals' concentrations detected in the eggs are presented in Table 1.

3.1. Organochlorine pesticides

The mean concentrations of ∑DDTs ranged from 63.3 ± 40.7 (SD) ng g^{-1} (2008) to 452.7 ± 310.5 ng g^{-1} (2006). The main part of ∑DDTs resulted from *p,p*′-DDE detected in all eggs analyzed. *p,p*′-DDD was detected in 76.2% of all eggs whereas *p,p*′-DDT in 66.7% of them. DDT/DDE ratio was on average 5.8 ± 7.7. A decrease between 2002 and 2012 was found in the mean concentrations of ∑DDTs ($r_s = -0.282$, $p < 0.01$, Fig. 2), with a tendency of a less variation through the years. The DDT/DDE ratio did not change temporally ($r_s = -0.025$, $p = 0.786$).

The mean concentrations of ∑HCHs ranged from 4.4 ± 4.1 ng g^{-1} (2011) to 26.6 ± 11.3 ng g^{-1} (2003). ∑HCHs concentrations also decreased between 2002 and 2012 ($r_s = -0.629$, $p < 0.01$, Fig. 3a). Like ∑HCHs, also β-HCH ($r_s = -0.297$, $p < 0.01$) and γ-HCH ($r_s = -0.617$, $p < 0.01$, Fig. 3b) concentrations decreased during this period.

Also the γ-HCH proportion of the ∑HCHs level decreased temporally ($r_s = -0.510$, $p < 0.01$). ∑Chlordanes were found in concentrations ranging from 0.3 ± 0.4 ng g^{-1} (2007) to 4.2 ± 3.8 (2006) ng g^{-1} with a decreasing trend during the study period ($r_s = -0.277$, $p < 0.01$, Fig. 4).

3.2. Polychlorinated biphenyls (PCBs)

The mean concentrations of ∑PCBs varied from 51.7 ± 13.0 ng g^{-1} (2010) to 97.6 ± 29.6 ng g^{-1} (2005) (Table 1). ∑PCBs did not show statistically significant temporal trends. The TEQ means varied from 0.9 (2002/3) to 369.9 (2008, Table 1), also without obvious temporal trends. Furthermore, the chemical composition of ∑PCBs was rather stable with means between 5.5 and 6.5 chlorine atoms per congener in the mixture over the years.

3.3. Mercury

The mean Hg concentrations ranged from 105.9 ± 23.1 ng g^{-1} (2003) to 401.7 ± 89.7 ng g^{-1} (2002), and the mean HCB concentrations from 10.1 ± 4.0 ng g^{-1} (2012) to 38.6 ± 27.3 ng g^{-1} (2009). Hg and HCB did not show statistically significant temporal trends.

3.4. Breeding success and contaminant concentrations

In the total study period, the mean number of the eggs laid per nest ($n = 124$) was 3.84 ± 1.18 (SD) (range 1–6) with a mean number of hatchlings 2.07 ± 1.41 (0–4). The hatching success was 48.3 ± 31.5% (0–80). The three reproductive parameters showed neither temporal trends (r_s ranged from −0.024 to 0.010, n.s., respectively) nor a significant year effect (GLM). In the data subset from 2002 to 2006 no influence of year and nest type was detected on clutch size, number of eggs hatched nor on hatching success, but there was an effect of the interaction between year and nest type on the number of eggs laid ($F = 3.403$, d.f. = 4, $p = 0.016$; clutch size was lower in nest boxes in 2002 and 2006). There was no significant relationship between the mean numbers of eggs laid, hatched and hatching success per year and nest type (2002–2006) with any of the contaminants analyzed except HCB whose concentrations were influenced by year ($F = 5.776$, d.f. = 10, $p < 0.0001$) and increased significantly in relation to clutch size ($F = 7.136$, d.f. = 1, $p = 0.009$, Fig. 5).

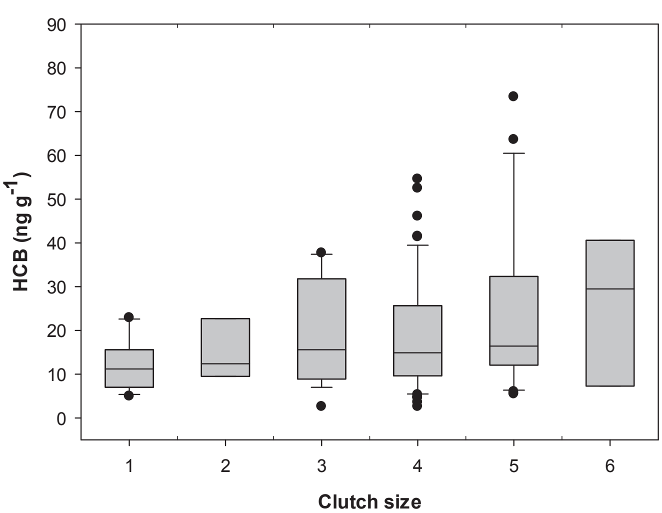

Fig. 5. HCB concentrations (ng g^{-1} dry weight) in lesser kestrel eggs from nests with different clutch sizes ($n = 124$, data from 2002 to 2012). For symbols and statistics refer to Fig. 2.

4. Discussion

4.1. Pollutant concentrations and temporal trends

Egg-laying is a major route of excretion of OCs and Hg in birds (Bogan and Newton, 1983; Lewis et al., 1993; Custer et al., 2014). The concentrations of all chemicals detected in the lesser kestrel eggs were comparably low. The concentrations of OCs analyzed graded as ∑DDTs > ∑PCBs > HCB > ∑HCHs > ∑Chlordanes. DDTs have also been found to predominate in other birds' egg samples from Greece; and PCBs and HCHs also followed the same pattern for some species (i.e. Goutner et al., 2001, 2011b) whereas in others the patterns were as HCHs > PCBs (Goutner et al., 2004) denoting different foraging and other habits of the species studied, and agrochemical or industrial pollution, respectively. Chlordanes (those analyzed in this study) and HCB when analyzed in birds' eggs in Greece have been detected in low concentrations (Sakellarides et al., 2006; Goutner et al., 2011b). The preponderance of DDT metabolites in ∑DDTs, beyond a declining temporal trend, denoted that a recent contamination of the birds with DDT appears less likely. Also, the decreasing variation of DDTs concentration through the years suggests a declining usage of DDT in the wintering areas of the lesser kestrels and probably is an evidence of measures taken.

Migratory raptors have been found to uptake DDTs, PCBs, HCHs and other OCs along their wintering and stopover sites (Becker and Sieg, 1987; Gómara et al., 2004; Kunisue et al., 2003; Martinez-Lopez et al., 2007; Chen et al., 2009), and accumulation patterns seem to reflect the birds' dietary habits (i.e. Elliott and Martin, 1994; Glaser and Connolly, 2002; Martinez-Lopez et al., 2007; Gómez-Ramírez et al., 2014). Similarly to other parts of its distribution, in our area, lesser kestrels are mainly insectivorous (BirdLife International, 2015; Vlachos et al., 2015) and thus involved in a relatively short food chain explaining the low concentrations of pollutants in their eggs. It is also likely that the studied population of lesser kestrels ranged year-round in habitats with limited pesticide applications and low industrialization. The degree of chlorination of the PCB mixture in the eggs did not change significantly over time, indicating that the lesser kestrels faced the same industrial PCB mixture in their environments, and that there was no major change in the metabolisation of the mixture (Beyerbach et al., 1993; Denker et al., 1994; Becker et al., 2001).

Despite the extensive ringing of nestlings and adults in our study colony since 2007, there are no recoveries to suggest the wintering or migration ways of this population. It has been suggested that the European lesser kestrel populations winter in southern Turkey, Malta and across much of Africa, particularly South Africa (BirdLife International, 2015). If, as expected, Africa is involved in the migration and wintering range of the Greek population, OCs and DDTs in particular might have been uptaken within this range, since in our study area OC pesticides have long ago been banned. DDT was produced and used in the Mediterranean region as long as other OCs had already been banned in the EU (UNEP-Chemicals, 2002). DDT seems to be still in use in African countries, as suggested by sediment analyses, and in some areas it may have biological effects (Bouwman et al., 2008; Adu-Kumi et al., 2010; Deribe et al., 2011; Barakat et al., 2012a; Polder et al., 2014; Yohannes et al., 2014); while in some areas levels suggested declines and/old inputs (Barakat et al., 2012a, 2012b). Declining trends of DDT as a result of use restrictions have also been found in long-term studies using eggs of other birds of prey in Spain whereas PCBs trends varied (González et al., 1984; Hernández et al., 1986). PCBs and other OCs like those analyzed in this study have also been detected in the aforementioned studies.

The decrease of $\sum$HCHs and particularly γ-HCH in the egg samples also seems to concur with the history of its ban in Greece: it was used in some cultivations and seed treatment until June 2002, when it was phased out according to EC Directive 2000/801. Lindane is a commonly detected pesticide in surface waters of Greece due to its higher application rates and widespread use and/or higher run-off hazard probably due to transboundary pollution (Konstantinou et al., 2006). Nevertheless the presence of lindane (and other OCs) in our study area may have been due to wet and dry deposition as they undergo atmospheric long-range transport (Cleemann et al., 1995; van Pul et al., 1998).

The preponderance of DDTs and PCBs but in much higher levels has also been detected in livers and fat of other raptors in Greece from birds that died in a bird hospital. Although the origin of the contaminants also remained unknown, a downward trend in OC contamination was also confirmed (Hela et al., 2006). HCB undergoes a rather fast clearance rate in birds (Donaldson et al., 1997) and might therefore not indicate concrete accumulation trends.

Mercury has also originated from terrestrial feeding of the birds. As reported in other studies in Greece, Hg concentrations in several breeding bird species where terrestrial prey prevailed was low (Goutner and Furness, 1998; Goutner et al., 2013). Also, Hg concentrations from 11 terrestrial (agricultural) areas ranged from 25 to 98 ng g^{-1}, mostly denoting uncontaminated soils (Haidouti et al., 1985). There have been no cues of Hg charges in the study area, explaining the relatively stable concentrations in the egg samples over the years.

4.2. Pollutant levels and potential biological effects

Hg can cause mortality and reproductive impairment in wild birds with possible adverse effects starting at about 4000 ng g^{-1} dw (Eisler 1987). Levels of Hg concentrations that affect breeding performance vary among falcons: In American kestrels (*Falco sparverius*) that were experimentally fed with methyl-Hg, major reproductive decline was found in concentrations between 7.7 and 13.5 μg g^{-1} (wet weight hereafter ww) for eggs laid and c. 2 μg g^{-1} ww for fledging (Albers et al., 2007), whereas in another study reproductive impairment was found with egg concentrations of 18.1 μg g^{-1} (ww) (Bennet et al., 2009). Nevertheless the median lethal concentration was less than 0.25 μg g^{-1}Hg (ww) in methyl-Hg injections in this bird's eggs (Heinz et al., 2009). In merlins (*Falco columbarius*) productivity fell markedly in clutches where mercury exceeded 3 μg g^{-1} dw (Newton and Haas, 1988) whereas females with eggs with mean concentrations of 1.10 μg g^{-1} Hg (considered “contaminated”) did not differ in breeding performance from those with “uncontaminated” eggs with 0.04 μg g^{-1} (Fox and Donald, 1980). An overall mean of 0.22 μg g^{-1} ww in peregrine falcon eggs from seven eastern American locations (range of means 0.05–0.43) was not considered to have significant effects (Clark et al., 2009) whereas >1 μg g^{-1} ww in eggs is considered critical (Peakall et al., 1990). The critical levels for the lesser kestrel are not known although in a Spanish population mean concentration of 379 ng g^{-1} (ww) in eggs had no effect on breeding (Negro et al., 1993). Nevertheless, embryo health and survival declines at egg-Hg concentrations ranging from 200 to 5200 ng g^{-1} dw across a broad range of bird taxa (as summarized by Hargreaves et al., 2010). In five of our study years Hg levels were >200 ng g^{-1}, higher than the lowest reported for the most sensitive species. Thus, although judging from the above mentioned information it seems unlikely that the Hg concentrations observed in the current study could cause adverse effects, the accurate concentrations causing impairment to the lesser kestrel are still to be defined.

Regarding DDE, concentrations of 15–20 μg g^{-1} DDE were reported to result in eggshell thinning in Canadian peregrines (Peakall et al., 1990). It seems that lowest DDE levels at which productivity is affected in falcons and other raptors generally range between 1.2 and 10 μg g^{-1} ww (Noble and Elliott, 1990). Gervais et al. (2000) summarised a number of studies where impaired reproduction in some raptors is caused by DDE concentrations of 2 μg g^{-1} (prairie falcon) to 20 μg g^{-1} (peregrine falcon) ww in eggs. DDE between 0.7 and 1.2 μg g^{-1} was not considered toxic to egg development of ospreys (Clark et al., 2001). Spanish lesser kestrels with $\sum$DDTs means of 2.1 μg g^{-1} ww showed no differences in the hatchability of clutches grouped by levels below and above 5 μg g^{-1} DDE (Negro et al., 1993). Compared to the above mentioned, concentrations in our population seem to be very low to have reproductive effects on the lesser kestrels.

PCBs cause endocrine disruption, immunotoxicity and teratogenesis whereas sublethal effects in birds occur at dietary PCB concentrations of 2–10 μg g^{-1} in several bird species such as kestrels (Barron et al., 1995). PCB concentrations of 1.8–3.2 μg g^{-1} were not considered harmful toxic to egg development of ospreys (Clark et al., 2001) and 4.0 μg g^{-1} was a no observable adverse effect concentration established for bald eagles (*Haliaeetus leucocephalus*) (Wiemeyer et al. 1984). The highest concentrations of $\sum$PCBs in prairie falcons (*Falco mexicanus*) eggs from California was 600 ng g^{-1} ww, probably not toxicologically important (Jarman et al., 1996), whereas there was no evidence of reproductive impairment of prairie falcons at PCB concentrations of 580 ng g^{-1} ww (Fyfe et al., 1976). Therefore the concentrations found in lesser kestrel eggs in Thessaly seem too low to affect their reproduction.

Although all HCH isomers are toxic and persisting for years in the environment (Badea et al., 2009) lindane is practically nontoxic to birds (Karmin, 1997). Concentrations of 10–30 ng g^{-1} ww in peregrine falcon eggs (similar to our study) had not any apparent effects on breeding (Augspurger and Boynton, 1998).

HCB, a pesticide and industrial by-product, has been detected in many wild bird populations and affects the breeding performance of birds both in the laboratory and in the wild (Jarman et al., 1996). Biological effects are generally associated with egg concentrations >4 μg g^{-1} (Peakall et al., 1990). Effects have been described in diet concentrations of 1000 ng g^{-1} ww for Japanese quail (*Coturnix japonica*), 7670 ng g^{-1} ww in common tern (*Sterna hirundo*) eggs (Courtney, 1979). For prairie falcon, a sensitive species, it is considered that mean concentrations of 800 ng g^{-1} ww may have deleterious effect on the hatching success (Jarman et al., 1996). Nevertheless the concentrations found in our study are much lower to lead to biological effects.

Components and metabolites of chlordane have been reported in a wide range of environmental samples (Jarman et al., 1993). Concentrations from 1 to 16 μg g^{-1} in the brain was associated with mortality (Peakall et al., 1990). The highest levels of summed chlordanes in the East Coast peregrine-falcon eggs at 230 and 530 ng g^{-1} ww seem to lower than those associated with acute toxicity in adults (Jarman et al., 1993). The concentrations found in this study are negligible to have biological effects.

According to the results reported here, individually, each pollutant or pollutant group analyzed in this study did not seem to affect directly the breeding performance of the lesser kestrel. However, the lowest possible concentrations that might affect reproduction are not known for the species.

In addition to the aforementioned, the egg laying and hatching parameters of lesser kestrels in our area were constant and relatively high over the years. Analyses did not detect relationships between pollutants' concentrations and the reproductive success of the species. These suggest that the concentrations of the pollutants analyzed do not pose a direct threat to our population of lesser kestrels.

Interestingly, concentrations of HCB were increasing significantly with clutch size, and in years characterized by high clutch size also HCB-levels were high (Fig. 5, Table 1). A probable explanation would be that robust females (having higher body condition and more fat content) and/or under favorable environmental conditions, might produce more eggs and deposit higher concentrations of HCB in the bigger clutches and higher quality eggs, usually produced by older females and/or those in better condition (Furness, 1984; Meathrel and Ryder, 1987; Meathrel et al., 1987; Wendeln, 1997; Wendeln and Becker, 1999). Alternatively findings may be associated to a potential increase of HCB concentrations with laying order (Nisbet, 1982). Hence, unhatched lesser kestrel eggs such as the ones sampled were probably either the last laid or the smallest egg in the clutch. Hatching failure is higher to smaller eggs depending on the ambient temperature whose effect on hatching success is evident for females in poor body condition (Serrano et al., 2005). These considerations suggest that the type of sample may have affected the results taken for some pollutants but in this case the drawbacks are counterbalanced by the avoidance of reducing the breeding success of a conservation-needing species using viable eggs. The use of unhatched eggs for monitoring contaminants is still an extensively used practice (i.e. Clark and Peakal, 1976; Froslie et al., 1986; Negro et al., 1993; Jarman et al., 1996).

5. Conclusions

The non-viable eggs of lesser kestrels analyzed from Greece contained Hg, PCBs and organochlorine pesticides indicating once more the presence of these pollutants in the environment and urging the need of continuing their monitoring. This study beyond establishing a raptor-based monitoring system in this part of the region, also verified that pollutant levels have been either reduced or remained stable, depending on the contaminant, with no detectable and obvious effect on the breeding performance of this species. Further investigation should define the critical levels for the lesser kestrel, locate the range within which these birds take up contaminants and the potential usefulness of the lesser kestrel as a sentinel species for environmental contamination.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2015.09.021.

References

Adu-Kumi, S., Kawano, M., Shiki, Y., Yeboah, P.O., Carboo, D., Pwamang, J., Morita, M., Suzuki, N., 2010. Organochlorine pesticides (OCPs), dioxin-like polychlorinated biphenyls (dl-PCBs), polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo furans (PCDD/Fs) in edible fish from Lake Volta, LakeBosumtwi and WeijaLake in Ghana. Chemosphere 81, 675–684.

Albers, P.H., Koterba, M.T., Rossmann, R., Link, W.A., French, J.B., Bennett, R.S., Bauer, W.C., 2007. Effects of methylmercury on reproduction in American kestrels. Environ. Toxicol. Chem. 26, 1856–1866.

ASTM D6722-01, 2006. Standard test methode für total quecksilber in kohle und kohleverbrennungsrückständen durch direkte verbrennungsanalyse. MLS MikrowellenLaborsysteme, Leutkirch.

Augspurger, T., Boynton, A., 1998. Organochlorines and mercury in peregrine falcon eggs from western north Carolina. J. Raptor Res. 32, 251–254.

Badea, S.L., Vogt, C., Weber, S., Danet, A.F., Richnow, H.H., 2009. Stable isotope fractionation of gamma-hexachlorocyclohexane (lindane) during reductive dechlorination by two strains of sulfate-reducing bacteria. Environ. Sci. Technol. 43, 3155–3161.

Bakaloudis, D.E., 2012. A preliminary overview of monitoring for raptors in Greece. Acrocephalus 33, 293–295.

Barakat, A.O., Mostafa, A., Wade, T.L., Sweet, S.T., El Sayed, N.B., 2012a. Spatial distribution and temporal trends of persistent organochlorine pollutants in sediments from Lake Maryut, Alexandria. Egypt. Mar. Pollut. Bull. 64, 395–404.

Barakat, A.O., Mostafa, A., Wade, T.L., Sweet, S.T., El Sayed, N.B., 2012b. Assessment of persistent organochlorine pollutants in sediments from Lake Manzala, Egypt. Mar. Pollut. Bull. 64, 1713–1720.

Barron, M.G., Galbraith, H., Beltman, D., 1995. Comparative reproductive and developmental toxicology of PCBs in birds. Comp. Biochem. Physiol. C 112, 1–14.

Baum, F., 1981. Chlorierte Kohlenwasserstoffe in wildlebenden Tieren und Nahrungsnetzen: Vorkommen, Bedeutung und Nachweis. Ecol. Birds 3, 65–71.

Becker, D.M., Sieg, C.H., 1987. Eggshell quality and organochlorine residues in eggs of merlins, *Falco columbarius*, in southeastern Montana. Can. Field Nat. 101 (3), 369–372.

Becker, P.H., Muñoz, C.J., Behrends, B., Schmieder, K.R., 2001. Contaminants in Bird Eggs in the Wadden Sea. Temporal and Spatial Trends 1991 – 2000. Wadden Sea Ecosystem No. 11. Common Wadden Sea Secretariat, Trilateral Monitoring and Assessment Group, Wilhelmshaven, Germany.

Becker, P.H., Dittmann, T., 2009. Contaminants in bird eggs. Thematic report No. 5.2. In: Marencic, H., Vlas, J de (Eds.), Quality Status Report 2009. Wadden Sea Ecosystem No. 25.Common Wadden Sea Secretariat. Trilateral Monitoring and Assessment Group, Wilhelmshaven, Germany.

Becker, P.H., Dittmann, T., 2010. Contaminants in bird eggs in the Wadden Sea: trends and perspectives. In: Marencic, H., Eskildsen, K., Farke, H., Hedtkamp, S. (Eds.), Science for Nature Conservation and Management: the Wadden Sea Ecosystem and EU Directives. Proc 12th Int Scientific Wadden Sea Symp Wilhelmshaven, Germany, 30 March – 3 April 2009. Wadden Sea Ecosystem No. 26. Common Wadden Sea Secretariat, Wilhelmshaven, Germany, pp. 205–209.

Bennett, R.S., French Jr., J.B., Rossmann, R., Haebler, R., 2009. Dietary toxicity and tissue accumulation of methylmercury in American kestrels. Arch. Environ. Contam. Toxicol. 56, 149–156.

Beyerbach, M., Becker, P.H., Büthe, A., Denker, E., Heidmann, W.A., Staats de Yanés, G., 1993. Variation von PCB-Gemischen in Eiern und Vögeln des Wattenmeeres. J. Ornithol. 134, 325–334.

BirdLife International, 2015. Species Factsheet: Falco Naumanni. Downloaded from. http://www.birdlife.org. on 11/03/2015.

Bogan, J.A., Newton, I., 1983. Eff. Organochlor. Compd. Br. Birds Prey. Vet Res Commun. 7, 119–124.

Bouwman, H., Polder, A., Venter, B., Skaare, J.U., 2008. Organochlorine contaminants in cormorant, darter, egret, and ibis eggs from South Africa. Chemosphere 71, 227–241.

Büthe, A., Denker, E., 1995. Qualitative and quantitative determination of PCB congeners by using a HAT-5 column and an efficient quadruple MS. Chemosphere 30, 753–777.

Chen, D., Zhang, X., Mai, B., Sun, Q., Song, J., Luo, X., Zeng, E.Y., Hale, R.C., 2009. Polychlorinated biphenyls and organochlorine pesticides in various bird species from northern China. Environ. Pollut. 157, 2023–2029.

Clark, A.L., Peakall, D.B., 1976. Organochlorine residues in Eleonora's falcon *Falco eleonorae*, its eggs and its prey. Ibis 119, 353–358.

Clark, K.E., Zhao, Y., Kane, C.M., 2009. Organochlorine pesticides, PCBs, dioxins, and metals in postterm peregrine falcon (*Falco peregrinus*) eggs from the mid-Atlantic states, 1993–1999. Arch. Environ. Contam. Toxicol. 57, 174–184.

Clark, K.E., Stansley, W., Niles, L.J., 2001. Changes in contaminant levels in New Jersey osprey eggs and prey, 1989 to 1998. Arch. Environ. Contam. Toxicol. 40, 277–284.

Cleemann, M., Poulsen, M.E., Hilbert, G., 1995. Deposition of lindane in Denmark. Chemosphere 30, 2039–2049.

Courtney, D.C., 1979. Hexachlorobenzene (HCB): a review. Environ. Res. 20, 225–266.

Custer, C.M., Custer, T.W., Thyen, S., Becker, P.H., 2014. Incubation stage and polychlorinated biphenyl (PCB) congener patterns in an altricial and precocial bird species. Environ. Pollut. 195, 109–114.

Denker, E., Becker, P.H., Beyerbach, M., Büthe, A., Heidmann, W.A., Staates de Yanes, G., 1994. Concentrations and metabolism of PCBs in eggs of waterbirds on the German North Sea coast. Bull. Environ. Contam. Toxicol. 52, 220–225.

Deribe, E., Rosseland, B.O., Borgstrøm, R., Salbu, B., Gebremariam, Z., Dadebo, E., Norli, H.R., Eklo, O.M., 2011. Bioaccumulation of persistent organic pollutants (POPs) in fish species from Lake Koka, Ethiopia: the influence of lipid content and trophic position. Sci. Total Environ. 410–411, 136–145.

Donaldson, G.M., Braune, B.M., Gaston, A.J., Noble, D.G., 1997. Organochlorine and heavy metal residues in breast muscle of known-age thick-billed murres (*Uria lomvia*) from the Canadian Arctic. Arch. Environ. Contam. Toxicol. 33, 430–435.

Donázar, J.A., Negro, J.J., Hiraldo, F., 1993. Foraging habitat selection, land-use changes and population decline in the lesser kestrel *Falco naumanni*. J. Appl. Ecol. 30, 515–522.

Eisler, R., 1987. Mercury Hazards to Fish, Wildlife, and Invertebrates: a Synoptic Review. U.S. Fish and Wildlife Service Biological Report 85(1.10) (Laurel, MD, USA).

Elliott, J.E., Martin, P.A., 1994. Chlorinated hydrocarbons and shell thinning in eggs of (*Accipiter*) hawks in Ontario, 1986–1989. Environ. Pollut. 86, 189–200.

Elliott, J.E., Miller, M.J., Wilson, L.K., 2005. Assessing breeding potential of peregrine falcons based on chlorinated hydrocarbon concentrations in prey. Environ. Pollut. 134, 353–361.

Fernie, K.J., Letcher, R.J., 2010. Historical contaminants, flame retardants, and halogenated phenolic compounds in peregrine falcon (*Falco peregrinus*) nestlings in the Canadian Great Lakes Basin. Environ. Sci. Technol. 44, 3520–3526.

Ferrara, R., Mazzolai, B., Lanzillotta, E., Nucaro, E., Pirrone, N., 2000. Volcanoes as emission sources of atmospheric mercury in the Mediterranean basin. Sci. Total Environ. 259, 115–121.

Fisher, S.A., Bortolotti, G.R., Fernie, K.J., Bird, D.M., Smits, J.E., 2006. Behavioral variation and its consequences during incubation for American kestrels exposed to polychlorinated biphenyls. Ecotox Environ. Safe 63, 226–235.

Fox, G.A., Donald, T., 1980. Organochlorine pollutants, nest-defense behavior and reproductive success in merlins. Condor 82, 81–84.

Froslie, A., Holt, G., Norheim, G., 1986. Mercury and persistent chlorinated hydrocarbons in owls Strigiformes and birds of prey Falconiformes collected in Norway during the period 1965–1983. Environ. Pollut. 11, 91–108.

Furness, R.W., 1984. Influences of adult age and experience, nest location, clutch size and laying sequence on the breeding success of the Great skua *Catharacta skua*. J. Zool. Lond 202, 565–576.

Fyfe, R.W., Risebrough, R.W., Walker, W., 1976. Pollutant effects on the reproduction of the prairie falcons and merlins of the Canadian prairies. Can. Field Nat. 90, 346–355.

Gervais, J.A., Rosenberg, D.K., Fry, D.M., Trulio, L., Sturm, K.K., 2000. Burrowing owls and agricultural pesticides: evaluation of residues and risks for three populations in California. USA. Environ. Toxicol. Chem. 19, 337–343.

Glaser, D., Connolly, J.P., 2002. A model of *p,p*-DDE and total PCB bioaccumulation in birds from the Southern California Bight. Cont. Shelf Res. 22, 1079–1100.

Gómara, B., Ramos, L., Gangoso, L., Donázar, J.A., González, M.J., 2004. Levels of polychlorinated biphenyls and organochlorine pesticides in serum samples of Egyptian vulture (*Neophron percnopterus*) from Spain. Chemosphere 55, 577–583.

Gómara, B., González, M.J., 2006. Enantiomeric fractions and congener specific determination of polychlorinated biphenyls in eggs of predatory birds from Doñana National Park (Spain). Chemosphere 63, 662–669.

Gómez-Gutiérrez, A., Garnacho, E., Bayona, J.M., Albaigés, J., 2007. Assessment of the Mediterranean sediments contamination by persistent organic pollutants. Environ. Pollut. 148, 396–408.

Gómez-Ramírez, P., Shore, R.F., van den Brink, N.W., van Hattum, B., Bustnes, J.O., Duke, G., Fritsch, C., García-Fernández, A.J., Helander, B.O., Jaspers, V., Krone, O., Martínez-López, E., Mateo, R., Movalli, P., Sonne, C., 2014. An overview of existing raptor contaminant monitoring activities in Europe. Environ. Int. 67, 12–21.

González, M.J., Hernández, L.M., Rico, M.C., Baluja, G., 1984. Residues of organochlorine pesticides, polychlorinated biphenyls and heavy metals in the eggs of predatory birds from Doñana National Park (Spain), 1980–1983. J. Environ. Sci. Health B 19, 759–772.

Goutner, V., Furness, R.W., 1998. Feathers of white stork *Ciconia ciconia* chicks in north-eastern Greece, as indicators of geographical variation in mercury contamination. Toxicol. Environ. Chem. 67, 379–390.

Goutner, V., Albanis, T., Konstantinou, I., Papakonstantinou, K., 2001. PCBs and organochlorine pesticide residues in eggs of Audouin's gull (*Larus audouinii*) in the north-eastern Mediterranean. Mar. Pollut. Bull. 42, 377–388.

Goutner, V., Albanis, T., Konstantinou, I., 2004. PCBs and organochlorine pesticide residues in eggs of threatened colonial charadriiform species (Aves, Charadriiformes) from wetlands of international importance in northeastern Greece. Belg J. Zool. 135, 157–163.

Goutner, V., Skartsi, T., Konstantinou, I.K., Sakellarides, T.M., Albanis, T.A., Vasilakis, D., Elorriaga, J., Poirazidis, K., 2011a. Organochlorine residues in blood of cinereous vultures and Eurasian griffon vultures in a northeastern Mediterranean area of nature conservation. Environ. Monit. Assess. 183, 259–271.

Goutner, V., Becker, P.H., Liordos, V., 2011b. Organochlorines and mercury in livers of Great Cormorants (*Phalacrocorax carbo sinensis*) wintering in northeastern Mediterranean wetlands in relation to area, bird age, and gender. Sci. Total Environ. 409, 710–718.

Goutner, V., Becker, P.H., Liordos, V., 2013. Low mercury contamination in Mediterranean gull *Larus melanocephalus* chicks in Greece. Chem. Ecol. 29, 1–10.

Haidouti, C., Skarlou, V., Tsouloucha, F., 1985. Mercury contents of some Greek soils. Geoderma 35, 251–256.

Hargreaves, A.L., Whiteside, D.P., Gilchrist, G., 2010. Egg concentrations of 17 elements, including mercury, and their relationship to fitness measures in arctic shorebirds and their eggs. Sci. Total Environ. 408, 3153–3161.

Heinz, G.H., Hoffman, D.J., Klimstra, Z.D., Stebbins, K.R., Kondrad, S.L., Erwin, C.A., 2009. Species differences in the sensitivity of avian embryos to methylmercury. Arch. Environ. Contam. Toxicol. 56, 129–138.

Hela, D.G., Konstantinou, I.K., Sakellarides, T.M., Lambropoulou, D.A., Akriotis, T., Albanis, T.A., 2006. Persistent organochlorine contaminants in liver and fat of birds of prey from Greece. Arch. Environ. Contam. Toxicol. 50, 603–613.

Hernandez, L.M., Rico, M.C., Gonzalez, M.J., Hernan, M.A., Fernandez, M.A., 1986. Presence and time trends of organochlorine pollutants and heavy metals in eggs of predatory birds of Spain. J. Field Ornithol. 57, 270–282.

Herzke, D., Kallenborn, R., Nygård, T., 2002. Organochlorines in egg samples from Norwegian birds of prey: congener-, isomer- and enantiomer specific considerations. Sci. Total Environ. 291, 59–71.

Hoffman, D.J., Melancon, M.J., Klein, P.N., Eiseman, J.D., Spann, J.W., 1998. Comparative developmental toxicity of planar polychlorinated biphenyl congeners in chickens, American kestrels and common terns. Environ. Toxicol. Chem. 17, 747–757.

Jarman, W.M., Norstrom, R.J., Simon, M., Bums, S.A., Bacon, C.A., Simoneit, B.R.T., 1993. Organochlorines, including chlordane compounds and their metabolites, in peregrine falcon, prairie falcon, and clapper rail eggs from the USA. Environ. Pollut. 81, 127–136.

Jarman, W.M., Burns, S.A., Bacon, C.E., Rechtin, J., DeBenedetti, S., Linthicum, J.L., Walton, B.J., 1996. High levels of HCB and DDE associated with reproductive failure in prairie falcons (*Falco mexicanus*) from California. Bull. Environ. Contam. Toxicol. 57, 8–15.

Karmin, M.A., 1997. Pesticide Profiles: Toxicity Environmental Impact, and Fate. CRC Press, LLC. Florida.

Konstantinou, I.K., Hela, D.G., Albanis, T.A., 2006. The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part I. Review on occurrence and levels. Environ. Pollut. 141, 555–570.

Kunisue, T., Watanabea, M., Subramanian, A., Sethuraman, A., Titenko, A.M., Qui, V., Prudente, M., Tanabe, S., 2003. Accumulation features of persistent organochlorines in resident and migratory birds from Asia. Environ. Pollut. 125, 157–172.

Lewis, S.A., Becker, P.H., Furness, R.W., 1993. Mercury levels in eggs, tissues, and feathers of herring gulls *Larus argentatus* from the German Wadden Sea coast. Environ. Pollut. 80, 293–299.

Luzardo, O.P., Ruiz-Suárez, N., Henríquez-Hernández, L.A., Valerón, P.F., Camacho, M., Zumbado, M., Boada, L.D., 2014. Assessment of the exposure to organochlorine pesticides, PCBs and PAHs in six species of predatory birds of the Canary Islands, Spain. Sci. Total Environ. 472, 146–153.

Maggi, C., Berducci, M.T., Bianchi, J., Giani, M., Campanella, L., 2009. Methylmercury determination in marine sediment and organisms by Direct Mercury Analyser. Anal. Chim. Acta 641, 32–36.

Martinez-Lopez, E., Maria-Mojica, P., Martinez, J.E., Calvo, J.F., Wright, J., Shore, R.F., Romero, D., Garcia-Fernandez, A.J., 2007. Organochlorine residues in booted eagle (*Hieraaetus pennatus*) and goshawk (*Accipiter gentilis*) eggs from south-eastern Spain. Environ. Toxicol. Chem. 26, 2373–2378.

Meathrel, C.E., Ryder, J.P., 1987. Intraclutch variation in the size, mass and composition of ring-billed gull eggs. Condor 89, 364–368.
Meathrel, C.E., Ryder, J.P., Termaat, B.M., 1987. Size and composition of Herring Gull eggs: relationship to position in the laying sequence and the body condition of females. Colon Waterbirds 10, 55–63.
Metcalfe, T.L., Metcalfe, C.D., 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.
Muñoz, C.J., 2004. Seabirds at Risk? Effects of Environmental Chemicals on Reproductive Success and Mass Growth of Seabirds Breeding at the Wadden Sea in the Mid-1990s. Wadden Sea Ecosystem No. 18. Common Wadden Sea Secretariat, Wilhelmshaven, Germany, pp. 27–51.
Negro, J.J., Donázar, J.A., Hiraldo, F., Hernández, L.M., Fernández, M.A., 1993. Organochlorine and heavy metal contamination in non-viable eggs and its relation to breeding success in a Spanish population of Lesser Kestrels (*Falco naumanni*). Environ. Pollut. 82, 201–205.
Newton, I., Haas, M.B., 1988. Pollutants in Merlin eggs and their effects on breeding. Brit Birds 81, 258–269.
Nisbet, I.C.T., 1982. Eggshell characteristics and organochlorine residues in common Terns: Variation with egg sequence. Colon Waterbirds 5, 139–143.
Noble, D.G., Elliott, J.E., 1990. Levels of contaminants in Canadian raptors, 1966–1988: effects and temporal trends. Can. Field Nat. 104, 222–243.
NRC (US National Research Council), 1991. Animals as Sentinels of Environmental Health Hazards. National Academy Press, Washington.
Pacyna, E.G., Pacyna, J.M., Sundseth, K., Munthe, J., Kindbom, K., Wilson, S., Steenhuisen, F., Maxson, P., 2010. Global emission of mercury to the atmosphere from anthropogenic sources in 2005 and projections to 2020. Atmosph. Environ. 44, 2487–2499.
Peakall, D.B., Noble, D.G., Elliott, J.E., Somers, J.D., Erickson, G., 1990. Environmental contaminants in Canadian peregrine falcons, *Falco peregrinus*: a toxicological assessment. Can. Field Nat. 104, 244–254.
Pirrone, N., Ferrara, R., Hedgecock, I.M., Kallos, G., Mamane, Y., Munthe, J., Pacyna, J.M., Pytharoulis, I., Sprovieri, F., Voudouri, A., Wangberg, I., 2003. Dynamic processes of mercury over the Mediterranean region: results from the Mediterranean Atmospheric Mercury Cycle System (MAMCS) project. Atmos. Environ. 37 (Suppl. 1), 21–39.
Polder, A., Müller, M.B., Lyche, J.L., Mdegela, R.H., Nonga, H.E., Mabiki, F.P., Mbise, T.J., Skaare, J.U., Sandvik, M., Skjerve, E., Lie, E., 2014. Levels and patterns of persistent organic pollutants (POPs) in tilapia (*Oreochromis* sp.) from four different lakes in Tanzania: geographical differences and implications for human health. Sci. Total Environ. 488–489, 252–260.
Roy, N.K., Bose, S.S., 2008. Determination of mercury in thirty-three international stream sediment and soil reference samples by Direct Mercury Analyser. Geostand. Anal. Res. 32, 331–335.
Sakellarides, T.M., Konstantinou, I.K., Hela, D.G., Lambropoulou, D., Dimou, A., Albanis, T.A., 2006. Accumulation profiles of persistent organochlorines in liver and fat tissues of various waterbird species from Greece. Chemosphere 63, 1392–1409.
Serrano, D., Tella, J.L., Ursúa, E., 2005. Proximate causes and fitness consequences of hatching failure in lesser kestrels *Falco naumanni*. J. Avian Biol. 36, 242–250.
UNEP-Chemicals, 2002. Regionally Based Assessment of Persistent Toxic Substances. Mediterranean Regional Report. Global Environment Facility, Geneva, Switzerland.
Van den Berg, M., Birnbaum, L., Bosveld, A.T.C., Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., Rolaf van Leeuwen, F.X., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillit, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect. 106, 775–792.
Van Pul, W.A.J., de Leeuw, F.A.A.M., van Jaarsveld, J.A., van der Gaag, M.A., Sliggers, C.J., 1998. The potential for long-range transboundary atmospheric transport. Chemosphere 37, 113–141.
Vlachos, C., Bakaloudis, D., Chatznikos, E., Papadopoulos, T., Tsalagas, D., 2003. Aerial hunting behavior of the Lesser Kestrel *Falco naumanni* during the breeding season in Thessaly (Greece). Acta Ornithol. 38, 47–52.
Vlachos, C., Bakaloudis, D., Chatznikos, E., 2004. Status of the lesser kestrel *Falco naumanni* in Thessaly, central Greece. In: Chancellor, R.D., Meyburg, B.-U. (Eds.), Raptors Worldwide. Hungary, Berlin and Budapest: World Working Group on Birds of Prey/MME-Birdlife, pp. 731–736.
Vlachos, C.G., Bakaloudis, D.E., Kitikidou, K., Goutner, V., Bontzorlos, V., Papakosta, M.A., Chatzinikos, E., 2015. Home range and foraging habitat selection by breeding lesser kestrels (*Falco naumanni*) in Greece. J. Nat. Hist. 49, 371–381.
Wendeln, H., 1997. Body mass of female common terns (*Sterna hirundo*) during courtship: relationships to male quality, egg mass, diet, laying date and age. Colon Waterbirds 20, 235–243.
Wendeln, H., Becker, P.H., 1999. Effects of parental quality and effort on the reproduction of common terns. J. Anim. Ecol. 68, 205–214.
Wiemeyer, S.N., Lamont, T.J., Bunck, C.N., Sindelar, C.R., Gramlich, F.J., Fraser, J.D., Byrd, M.A., 1984. Organochlorine pesticide, polychlorobiphenyl, and mercury residues in bald eagle eggs —1969–1979— and their relationships to shell thinning and reproduction. Arch. Environ. Contam. Toxicol. 13, 529–549.
Yohannes, Y.B., Ikenaka, Y., Nakayama, S.M.M., Ishizuka, M., 2014. Organochlorine pesticides in bird species and their prey (fish) from the Ethiopian Rift Valley region, Ethiopia. Environ. Pollut. 192, 121–128.