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Organochlorine residues in blood of cinereous vultures and Eurasian griffon vultures in a northeastern Mediterranean area of nature conservation

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Abstract In the National Park of Dadia-Lefkimi-Soufli Forest (Dadia NP, Greece), seven "target" PCBs and 16 organochlorine pesticides (OCs) were analysed in blood samples of cinereous vultures (*Aegypius monachus*) and Eurasian griffon vultures (*Gyps fulvus*). PCB congeners 138, 153 and 180 predominated in both species' blood samples. In both species, no differences were detected in congener levels between successive age classes, but in cinereous vulture, there were significant differences between adult and nestling in levels of PCB 28, 52, 101, 118 and between nestling and immature in levels of PCB 101. Regarding pesticides, p,p'-DDE dominated in both vultures followed by β-HCH, lindane and endosulfan sulphate, but ∑OCs were higher in griffon vulture. Significant differences were detected only between nestling and sub-adult cinereous vultures in heptachlor levels and between nestling and adult in p,p'-DDT. The origin of pollutants differs between the two vulture species and pollution patterns may not reflect those at Dadia NP.

Keywords Organochlorines · Vultures · Dadia National Park · Greece

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Introduction

Persistent organic pollutants such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) are distributed in food webs globally and, due to their bioaccumulative properties and persistence, they biomagnify to their greater concentrations in higher-level consumers (Senthil Kumar et al. 2002a). They have been associated with negative effects in humans and wildlife (Longnecker et al. 1997; Senthil Kumar et al. 2002a).

Especially toxic are those PCBs with a structure resembling dioxin (one or no chlorine in the ortho-positions) showing strong effects to the en-

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zymic systems of animals and especially to hepatic function (Senthil Kumar et al. 2002b). Frequently, PCBs and OCs have synergistic effects in birds (Henny 1998). Moreover, since many OCs biomagnify in food chains, birds of prey are subject to the highest exposures of these chemicals which resulted in dramatic population declines of some species: in Europe and America, the effects of dichlorodiphenylotrichloroethane (DDT) on peregrine falcons (*Falco peregrinus*) have been well documented (Henny 1998), whereas high levels of DDE have been detected in the rare subspecies *F. peregrinus tundrius* (Johnstone et al. 1996). Both groups of pollutants can be transferred through aquatic food chains so that some fish-eating raptors such as osprey (*Pandion haliaetus*) and bald eagle (*Haliaeetus leucocephalus*) have been affected and thus are used as bioindicators (Bowerman et al. 2000; Elliott et al. 2000; Senthil Kumar et al. 2002a).

Vultures are large-sized birds of prey occupying a higher position in food chains. They feed on carrion and can be exposed to toxic chemicals accumulated in a variety of prey through the food chain. There are a few studies focusing to the detection of organochlorine residues in tissues of various species of vultures (Van Wyk et al.1993, 2001, Senthil Kumar et al. 2003; Snyder and Meretsky 2003; Gómara et al. 2004; Muralidharan et al. 2008).

Although organochlorine contaminants, being highly lipophilic, are usually measured in fatty tissues, blood analysis has greatly been suggested in wildlife monitoring studies (Olsson et al. 2000; Senthil Kumar et al. 2002b, 2003; Bustnes et al. 2006; Martínez-López et al. 2009). Blood transports lipid-based organochlorines to different tissues and organs and offers several benefits over traditional tissue sampling especially for sensitive species: it can be collected easily and relatively non-destructively from free-ranging populations and facilitates the repeated collection of larger numbers of samples, which improves both the monitoring of organochlorine levels and the assessment of toxicological effects (Keller et al. 2004).

In the National Park of Dadia-Lefkimi-Soufli Forest (hereafter Dadia NP), vultures, and particularly cinereous vulture (*Aegypius monachus*) and Eurasian griffon vulture (*Gyps fulvus*; hereafter griffon vulture), are primary objects of study and conservation (Poirazidis et al. 2002; Skartsi and Poirazidis 2002). During the last 15 years, the cinereous vulture population initially increased (1987–1994) and thereafter rather stabilised whereas although griffon vultures' breeding activity ceased after 1995, population increased due to visitors coming from other areas (Skartsi and Poirazidis 2002; Skartsi et al. 2008).

This research investigated the levels of organochlorines in blood of cinereous vultures and griffon vultures to control for the possibility that the birds captured at Dadia NP could have been affected. In addition, it is the first account of analysis of toxicants present in populations of vultures in northeastern Mediterranean.

Materials and methods

Study area and sampling

Dadia NP is situated in the Evros Province, Northeastern Greece, and extents over 428 km^2. Two cores, totaling 78 km^2, have been declared areas of strict protection. The habitats in Dadia NP include oak and pine forests, cultivations, pastures, streams with aquatic vegetation and stony hills (see also Poirazidis et al. 2004). Since 1987, the vultures' populations have been monitored by guards and scientific personnel and, since 1994, by WWF-Greece in collaboration with the Bureau of Environment of Evros Prefecture.

The study of cinereous vulture was one of the basic actions of a Life-Nature (2002–2005) project implemented by WWF-Greece entitled "Conservation of birds of prey in Dadia Forest" (LIFE02NAT/GR/8497). Cinereous vultures and griffon vultures were caught (between October 2003 and January 2005) within the framework of a monitoring program implemented during the Life-Nature project. Captures, marking and blood sampling (see further) were made with a permission provided by the Department of National Parks of the Ministry of Rural Development and Food. The birds were attracted and caught at a suitable metal cage constructed at a forest opening where food and a decoy vulture were placed (Elorriaga et al. 2004b). Cinereous vulture nestlings were sampled when 65–70 days. They

were carried head-covered down to the ground in a bag and returned just after marking and blood sampling. The heads of all other vultures captured were also covered during sampling and marking. Blood was extracted from the brachial vein by a sterile disposable syringe and immediately transferred in appropriate tubes containing heparin. Samples were soon transported to the laboratory and stored in −20°C until analysis, which was performed in the Department of Chemistry, University of Ioannina. The blood sample size was maximum 4 ml for nestlings and 8 ml for the free-living individuals respectively (to cover the needs of studies on genetics, haematology, plasma biochemistry and haemoparasites). No vultures were injured during the sampling. As all sampled birds were individually marked with alphanumeric rings and patagial tags, all were monitored after sampling either on their nests until post-fledging or at the Dadia NP feeding station.

Both vulture species were aged according to their moulting pattern following De la Puente and Elorriaga (2011). Thus, age classes that were determined (and further used in age–class comparisons for pollutant levels) were: adult (age ≥5 calendar years (CY)), sub-adult (>3–4 CY), immature (2–3 CY), juvenile (1) and nestling (at nest, 0). During the study, recaptured individuals were also sampled to investigate potential changes in organochlorine levels in relation to age.

Reagents and standards

The following seven PCB congeners (in terms of the PCB IUPAC numbers) were analyzed: PCB 28, 52, 101, 118, 138, 153 and 180 belonging to the group known as "target" or "indicator" PCBs (Ishikawa et al. 2007). PCB standards were obtained from Dr. Ehrensdorfer's laboratory (Augsburg, Germany) and the organochlorine pesticides α-hexachlorocyclohexane isomer (α-HCH), β-HCH, lindane (γ-HCH), δ-HCH, heptachlor, endosulfan I, II and endosulfan sulfate, γ-chlordane, heptachlor epoxide, aldrin, dieldrin, endrin, *p*,*p*′-DDT, *p*,*p*′-DDD and *p*,*p*′-DDE were purchased from Supelco (Bellefonte, PA). All solvents used were pesticide residue analysis grade, purchased from Pestiscan (Labscan Ltd, Ireland). Alumina, silica and sodium sulphate (pro analysis) were from Merck (Darmstadt, Germany). Glassware was soaked, cleaned with chromic solution, thoroughly rinsed with distilled water and acetone and heated at 150°C for 12 h.

Analytical procedures

The concentration of OCs in blood wet weight was used as a measure of OC burden, since wet weight is usually considered most relevant for studying potential toxic effects (Henriksen et al. 1998). The solvent mixture used for extraction (hexane/ethyl ether) was based in previous studies (Voltz et al. 2001; Johnstone et al. 1996). An aliquot of homogeneous blood (2 ml) was transferred to a pre-weighed container and the sample weight was determined. The analytes were extracted from the blood with 5 ml hexane: ethyl ether (1:1, *v*/*v*), vortex mixed for 1 min, sonicated for 10 min and mechanical shaken for 2 min. This procedure was repeated three times and, each time, the extract was centrifuged for 5 min at 4,500 rpm, and the supernatant was removed. The combined supernatants were concentrated to 0.5 ml using a rotary evaporator and a gentle nitrogen stream. Subsequently, the extract was cleaned by column chromatography using 1 g of silica (deactivated with 5% *w*/*v* water), 2 g of alumina (deactivated with 5% *w*/*v* water) and 1 g Na_2SO_4 packed into a 1 cm glass column. The column was eluted with 50 ml hexane/ethyl ether (1:1, *v*/*v*), and the entire elute for each sample was finally evaporated in a rotary evaporator to about 5 ml and brought to a final volume of 0.1 ml under a gentle N_2 stream at 35°C. The samples were then capped, vortex mixed and transferred to GC vials with microinserts for quantification of OCs via GC analysis. The procedures were checked for recovery using decachlorobiphenyl (PCB 209) as internal standard. Percent recoveries of OCs spiked into samples and passed through the analytical procedure were between 72–94% (RSD <15%, $n = 3$). Residue levels were not adjusted for percent recovery. The detection limits for PCB congeners and OCs were determined as three times the background noise level and ranged from 0.01 to 0.05 ng/g wet weight depending on the compound. Blank samples were performed with every set of real samples analyzed in order to identify any con-

tamination throughout the analytical procedure. No background interference was found to be introduced by the methodology proposed.

Chromatographic conditions

A Shimadzu 2010 gas chromatograph equipped with ^{63}Ni electron capture detector was used for the organochlorine residue analysis. Separation was achieved with a fused-silica capillary column coated with 5% phenyl–95% dimethylpolysiloxane (ZB-5, Phenomenex, 30 m × 0.25 mm i.d., 0.25 μm film thickness). The column oven temperature was programmed as follows: 150°C (2 min), 150–180°C (2°C/min), 180–184°C (0.5°C/min), 184°C (2 min), 184–200°C (2°C/min), 200°C (20 min), 200–280°C (10°C/min), 280°C (5 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. The splitless mode was used for injection of 1.5 μl volume, with the valve opened for 30 s. Compounds were positively identified if the relative retention time (versus the internal standard) differed no more than 0.01 from that of the calibration standards. Quantification of OCs and PCBs was performed using 2,4,5,6-tetrachloro-*m*-xylene and PCB 209 (decachlorobiphenyl) as internal standards.

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., J and 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 2-μl volume, with the valve opened for 30 s. Two ions from the molecular ion cluster (M^+ and $[M+2]^+$) for each of the OCs were chosen for screening analysis in selected ion monitoring mode. The relative retention times to the nearest internal standard and ratios between the monitored ions were used as identification criteria. A deviation of ion ratios of less than +20% from the theoretical value was considered acceptable for identification.

Table 1 Levels of PCBs in blood samples of cinereous vultures (ng/g wet weight, $n = 30$) and Eurasian griffon vultures ($n = 15$) in Dadia National Park

	Cinereous vulture						Eurasian griffon vulture					
	Mean	Geometric mean	Minimum	Maximum	% of total	%CV	Mean	Geometric mean	Minimum	Maximum	% of total	%CV
PCB 28	0.28	0.18	0.05	0.76	3.30	75	0.34	0.22	0.05	0.78	3.18	71
PCB 52	0.28	0.17	0.05	0.77	3.28	83	0.36	0.23	0.05	0.82	3.38	71
PCB 101	0.38	0.25	0.04	0.83	4.43	64	0.50	0.38	0.04	0.93	4.70	52
PCB 118	0.76	0.61	0.04	2.48	8.96	60	1.21	1.12	0.50	2.12	11.39	40
PCB 138	2.02	1.82	0.87	4.82	23.73	49	2.54	2.27	0.92	5.61	23.85	49
PCB 153	3.42	3.02	1.23	7.68	40.09	49	4.00	3.58	1.42	7.83	37.57	45
PCB 180	1.38	1.21	0.40	4.08	16.22	58	1.70	1.51	0.60	2.96	15.93	45
∑PCBs	8.52	7.56	2.86	21.21		51	10.64	9.50	3.58	20.44		46

CV coefficient of variation

Data analysis and statistical procedures

Data on concentrations of contaminants are presented as geometric mean values accompanied with minimum and maximum values. A value one half the minimum detection limit 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 (Kolmogorov–Smirnov tests), the statistical evaluation was based on the use of non-parametric tests (Kruskal–Wallis test). Residue concentrations were compared among age–classes of birds (as previously defined) by box-plot analyses. Statistically significant differences were further tested by Mann–Whitney tests using Bonferroni correction for multiple comparisons. Comparisons of concentrations in samples of re-captured birds were made by sign test.

Organochlorine pesticides were grouped according to their structure in HCHs, cyclodienes (Cycls) and DDTs. Comparisons in the concentrations of each category were made between the two vulture species using contingency table analyses.

Results

Polychlorinated biphenyls

All seven PCBs analysed were detected in both vultures' blood samples. In cinereous vultures' blood samples, individual congener geometric mean concentrations varied from 0.17 ng/g wet weight (PCB 52) to 3.02 ng/g ww (PCB 153) whereas for the sum of the congeners geometric mean and maximum concentrations were 7.56 and 21.21 ng/g, respectively (Table 1). Similarly, the geometric means of griffon vulture blood samples varied from 0.22 ng/g ww (PCB 28) to 3.58 ng/g ww (PCB 153) whereas the sum of the congeners' geometric mean and maximum concentrations were 9.50 and 20.44 ng/g, respectively (Table 1). Total concentrations of PCBs were approximately estimated at 100–106 ng/g ww, multiplying the sum of the individual congeners by a factor of 5 according to DIN 51527 (DIN 1987). Statistical comparison of geometric mean congener levels between species indicated significant difference only for PCB 118 ($H = 10.258$, $p = 0.0014$, Kruskal–Wallis test) levels being higher in griffon vultures.

Congener fingerprints (percentage levels for each congener in the whole sample) were similar in the two species (Fig. 1). Congeners 138, 153 and

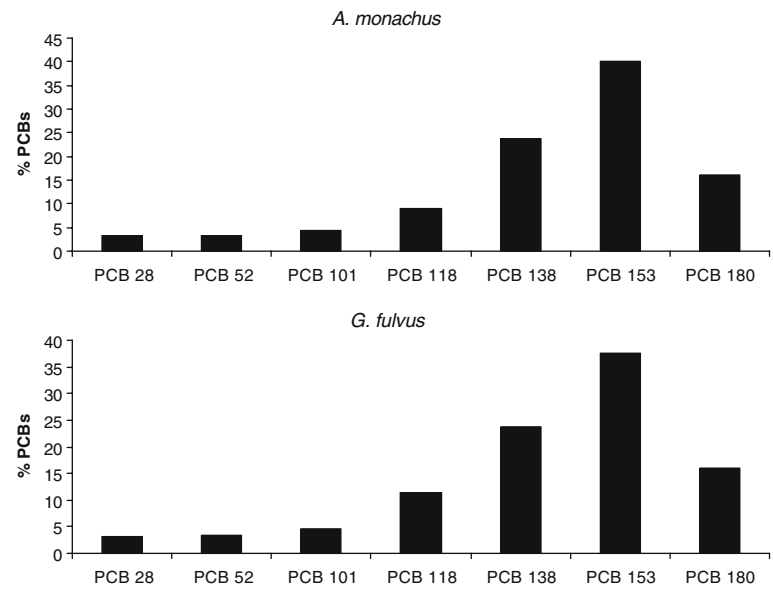

Fig. 1 Percent levels of PCBs in blood of the two vulture species in Dadia National Park

Table 2 Comparisons of PCB levels in recaptured cinereous vultures

Bird code/year	*Z*	*p* Level	Trend
H_{11}/2003 vs. H_{11}/2005	2.268	0.023	2005>2003
H_{17}/2003 vs. H_{17}/2005	0.894	0.371	–
H_{37}-Jul/2004 vs. H_{37}-Oct/2004	2.268	0.023	Oct/2004>Jul/2004
H_{38}/2004 vs. H_{38}/2005	2.041	0.041	2005>2004

A numbered H indicate the code of a bird sampled in a particular year. Trend indicates the year when levels were higher. Comparisons were made using sign test

180 (especially the first two) predominated in both species' samples.

In three out of four recaptured cinereous vultures, PCB levels were significantly different (Table 2) being higher in the date of recapture representing a greater bird age. This comparison was impossible for griffon vultures as only one recapture was made.

In cinereous vulture, there were overall statistically significant differences among age–classes for all PCB congener levels (Fig. 2). Comparisons between pairs of cinereous vulture age–class con-

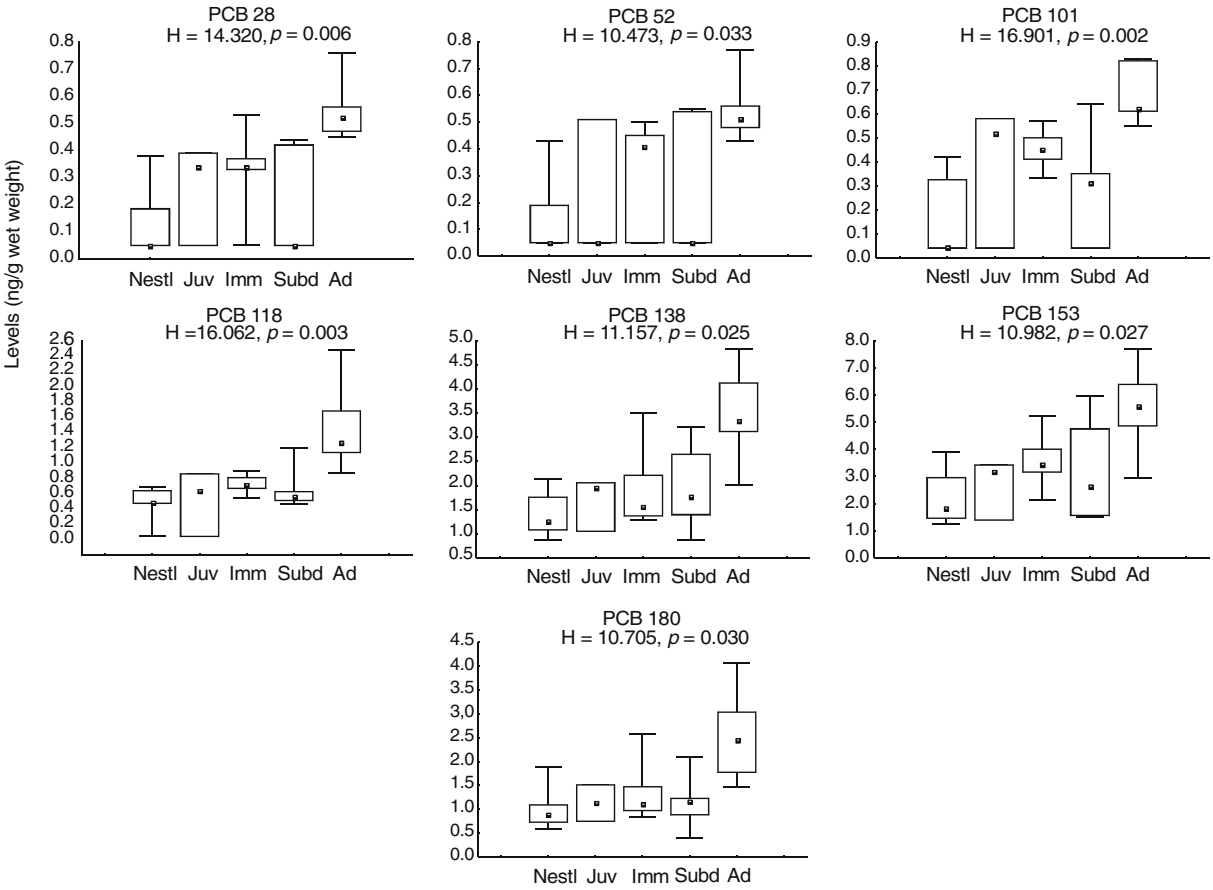

Fig. 2 Box-plot analysis of age class levels of each PCB congener in cinereous vulture. Indicated are: median (*square dot*), 75% range of values (*square box*) and range of values (*vertical lines*). On axis *X*, age abbreviation symbols mean (from left to right): *nestl* nestling ($n = 8$); *juv* juvenile ($n = 3$); *imm* immature ($n = 9$); *subd* subadult ($n = 5$); *ad* adult ($n = 5$)

Table 3 Levels of organochlorine pesticides in blood samples of cinereous vulture (ng/g wet weight, $n = 30$) and Eurasian griffon vulture ($n = 15$)

	Cinereous vulture					Eurasian griffon vulture					Kruskal–Wallis	
	Mean	Geometric mean	Minimum	Maximum	% of total	Mean	Geometric mean	Minimum	Maximum	% of total	H	*p*
α−HCH	0.66	0.60	0.03	0.94	4.49	0.88	0.86	0.69	1.28	4.39	14.291	<0.001
β−HCH	2.03	1.93	0.66	2.88	13.76	2.31	2.25	1.52	3.57	11.58	0.487	n.s.
γ−HCH (lindane)	1.13	1.08	0.41	1.64	7.64	1.69	1.66	1.19	2.37	8.48	18.162	<0.001
δ−HCH	0.41	0.28	0.03	0.66	2.79	0.53	0.45	0.03	0.78	2.64	4.506	0.034
Aldrin	0.55	0.29	0.03	2.19	3.73	0.78	0.26	0.03	3.94	3.89	0.012	n.s.
Dieldrin	0.73	0.32	0.04	2.12	4.93	0.48	0.14	0.04	1.79	2.41	–	–
Endrin	0.26	0.13	0.05	0.90	1.79	0.23	0.11	0.05	0.79	1.14	–	–
Heptachlor	0.88	0.82	0.39	2.09	5.98	1.20	1.14	0.65	2.24	6.03	7.014	0.008
Heptachlor epoxide	0.38	0.24	0.04	1.04	2.59	0.46	0.30	0.04	1.12	2.29	0.115	n.s.
γ−chlordane	0.09	0.05	0.04	0.61	0.58	0.22	0.08	0.04	0.88	1.10	–	–
Endosulfan I	0.79	0.36	0.04	2.14	5.35	1.28	0.48	0.04	3.89	6.38	0.433	n.s.
Endosulfan II	0.31	0.18	0.05	0.81	2.11	0.57	0.21	0.05	1.82	2.87	–	–
Endosulfan sulfate	1.86	1.01	0.06	4.01	12.62	3.76	2.48	0.62	10.42	18.82	1.822	n.s.
p, *p*′-DDT	0.20	0.11	0.06	0.88	1.33	0.25	0.13	0.06	0.98	1.28	–	–
p, *p*′-DDD	0.67	0.52	0.05	1.87	4.56	0.85	0.63	0.05	2.26	4.26	0.377	n.s.
p, *p*′-DDE	3.80	3.34	0.95	9.42	25.75	4.49	3.82	1.52	10.59	22.45	0.557	n.s.
∑OCs	14.76	13.87	5.05	27.65		19.98	18.15	9.42	45.27		3.994	0.046

gener levels (with Mann–Whitney tests and Bonferroni corrected $p = 0.005$) indicated significant differences between adult and nestling in levels of PCB 28 ($Z = 3.079$, $p = 0.002$), PCB 52 ($Z = 3.007$, $p = 0.003$), PCB 101 ($Z = 3.012$, $p = 0.003$) and PCB 118 ($Z = 2.932$, $p = 0.003$) and between nestling and immature in levels of PCB 101 ($Z = -3.118$ $p = 0.002$). No differences were detected between successive age–classes.

A respective analysis in griffon vulture did not reveal an overall significant difference neither among age classes (juvenile, immature, sub-adult) nor between pairs of age classes. Anyway, the power of these tests was low due to small sample sizes in age classes juveniles and sub-adults (both $n = 2$) and immatures ($n = 9$). No samples from adult griffons were available for examination.

Organochlorine pesticides

All 16 OCs analysed were detected from both vulture species' blood samples. Nevertheless, endrin, γ-chlordane and $p,p'-$DDT in cinereous vulture and dieldrin, endrin, γ-chlordane, endosulfan II and $p,p'-$DDT in griffon vulture were found in less than 50% of samples. In both vultures, $p,p'-$DDE dominated among compounds with highest geometric mean and percent levels. β-HCH, lindane and endosulfan sulphate followed in levels, the last being more important in griffon vulture (Table 3). The geometric mean of $\sum$OCs was 13.87 ng/g ww with maximum 27.65 ng/g ww in cinereous vulture and 18.15 ng/g ww with maximum 45.27 ng/g ww in griffon vulture.

Significant differences were detected in geometric mean levels of α-, γ- and δ-HCH, heptachlor and $\sum$OCs, all being higher in griffon vulture (Table 3). Anyway, percent levels of $\sum$Cycls, $\sum$DDTs and $\sum$HCHs were not significantly different between the two vulture species resulting in similar fingerprints ($\chi^2 = 2.130$, $df = 2$, n.s., Fig. 3).

Where possible, a statistical comparison of OC levels in blood samples of cinereous vultures of different age, showed significant difference in three compounds (heptachlor, $p,p'-$DDT and $p,p'-$DDE (Fig. 4). Pairwise comparisons (Mann–Whitney test, Bonferroni probability $p = 0.005$), indicated a significant difference between nestling and sub-adult in heptachlor ($Z = -2.928$, $p = 0.003$) and between nestling and adult in $p,p'-$DDT ($Z = -2.861$, $p = 0.004$). These suggest that in cinereous vultures (as in the case of PCBs) there were not profound age differences in OCs' levels.

Regarding griffon vultures, while in Kruskal–Wallis test overall statistical differences were detected among age-groups in levels of β-HCH ($H = 7.293$, $p = 0.026$), heptachlor epoxide ($H = 7.000$, $p = 0.030$), $p,p'-$DDT ($H = 6.600$, $p = 0.037$) and marginal differences ($p \approx 0.05$) in levels of lindane and dieldrin, a further pairwise analysis (with Mann–Whitney test and Bonferroni corrected $p = 0.0167$) did not reveal statistical differences between age classes probably, due to

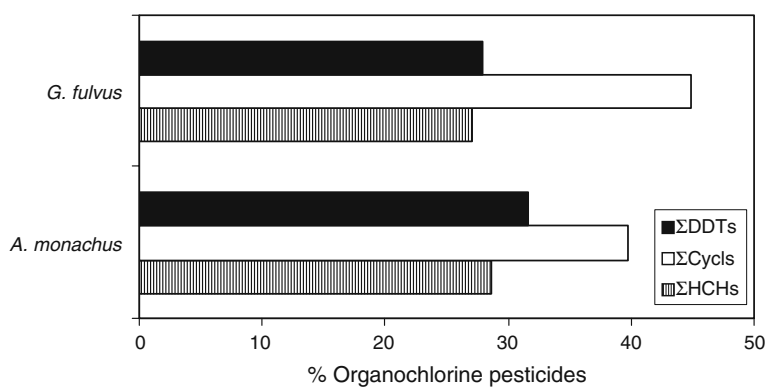

Fig. 3 Percent levels of the three groups of organochlorine pesticides in blood of the two vulture species in Dadia National Park

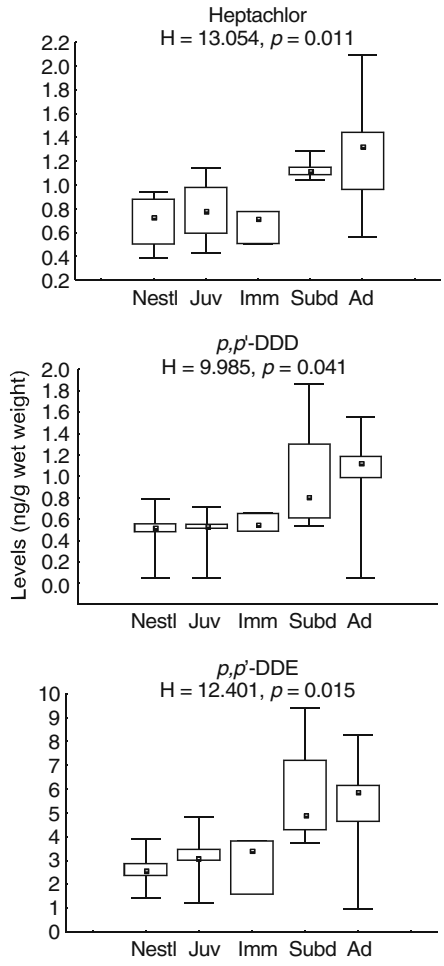

Fig. 4 Box-plot analysis of age class levels in some organochlorine pesticides in cinereous vulture. For age symbols and sample sizes, refer to Fig. 2

small sample sizes in age classes juveniles and subadults (as mentioned above).

In only one of the four cases of cinereous vulture recaptures a statistical difference was found in total OC levels being higher in an older aged bird (Table 4). In griffon vultures no respective test was possible as only one bird was recaptured.

Discussion

Levels of both PCBs and OCs found in the blood samples of both vulture species in Dadia NP were low. Some studies, investigated PCB and OC residues in vulture blood but concentrations have frequently been given as micrograms per millilitre instead as micrograms per gram of this study. Measurements with the former unit provide higher concentrations. Levels of PCBs detected in our samples in Dadia NP were generally in the range of those previously detected in blood serum of Egyptian vulture (*Neophron percnopterus*) in Spain (Gómara et al. 2002, 2004). In addition, levels of DDT were much lower in Dadia NP whereas those of DDE were comparable with some Spanish areas (Gómara et al. 2004).

Mean levels of all OC pesticides analysed in blood samples of the whitebacked vulture (*Pseudogyps africanus*) were considerably higher than these in Dadia NP (Van Wyk et al. 2001). Similar were the results on two South African vultures, cape vulture (*Gyps coprotheres*) and lappet-faced vulture (*Torgos tracheliotos*). Similarly, to our study, levels of endosulfan sulphate were higher than levels of endosulfan I and II (Van Wyk et al. 2001). Blood concentrations of dioxin-like PCBs were in the range of 0.815–4.627 ng/l ww in American black vultures (*Coragyps atratus*) and 0.753–3.611 ng/l ww in Turkey vultures (*Cathartes aura*) from South Carolina, USA (Senthil Kumar et al. 2003). Where detected in carcass tissues of white-backed vulture (*Gyps bengalensis*) in India, levels of DDTs, dieldrin, ∑HCH, ∑endosulfan and heptachlor epoxide were much higher than

Table 4 Comparisons of OC levels in recaptured cinereous vultures

Bird code	*Z*	*p* Level	Trend
H11/03 and H11/05	0.000	n.s.	–
H17/03 and H17/05	2.598	0.009	03>05
H37–7/04 and H37–10/04	0.802	n.s.	–
H38/04 and H38/05	0.516	n.s.	–

A numbered H indicates the code of a bird sampled in a particular year. Trend indicates the year when levels were higher. Comparisons were made using sign test

the respective of our vultures from Dadia NP (Muralidharan et al. 2008). Considering that blood concentration of OCs and PCBs is usually higher than that in tissues (in glaucous gull *Larus hyperboreus* ranged from 1 to 5, Henriksen et al. 1998) it seems that Indian vultures carried much higher levels of contaminants in their blood.

Compared to other raptor species, arithmetic means of ∑PCBs including those analysed in this study (PCB 28, 118, 138, 153, 180), β-HCH and *p*,*p*′-DDE in whole blood samples from white-tailed sea eagles (*Haliaeetus albicilla*; Baltic Coast, Sweden) ranged 54–230, 0.20–2.5 and 10–39 ng/g wet weight, respectively (Olsson et al. 2000). Much higher levels of HCHs and endosulfans were reported by Martínez-López et al. (2009) in blood of booted eagle (*Hieraaetus pennatus*) in Spain. Compared to other raptor species, whose tissues (subcutaneous fat and liver) have been analysed in Greece (Hela et al. 2006), the respective pollutant levels of the two vulture species from Dadia NP were considerably lower. The differences may be due to the manner chlorinated compounds are partitioned in the two types of tissues (Henriksen et al. 1998). In addition, greatly migrative behaviour, and particular feeding habits of those raptors may have also partly accounted for the differences.

The similar accumulation profiles of homologues and congeners between Turkey vulture and American black vulture suggested similar ecology, migratory movements and feeding habits (Senthil Kumar et al. 2003). Where differences were detected in organochlorine levels between cinereous vulture and griffon vulture in Dadia NP, they were higher in the latter species probably partly due to different metabolic capacities.

What is the origin of pollutants in the vultures sampled in Dadia NP? Pesticides such as those reported in the vultures' blood, have been reported in water, sediments and biota in the lower part of the river Evros, crossing the eastern border of the study area (Golfinopoulos et al. 2003; Erkmen and Kolankaya 2006; Vryzas et al. 2008). Additionally, pesticides and PCBs were detected in waterbird eggs in the Evros Delta at the south of Dadia NP (Konstantinou et al. 2000; Albanis et al. 2003; Goutner et al. 2004). These might suggest that pollution reflected in vultures' blood could have partly originated from the river pollution. Nevertheless, the most important food taken by both vulture species in Dadia NP is provided in feeding stations and consists of carcasses of livestock, mainly pigs and bovines, originating from local intensive livestock breeding; also of dogs and wild animals such as foxes, martens, hedgehogs and tortoises (taken mainly by cinereous vultures) (Skartsi and Poirazidis 2002; Skartsi et al. 2003; Elorriaga et al. 2004a, 2005). Cinereous vultures (and a part of griffon vulture population) also forage in a wider area including mountainous and hilly areas of Rhodope and Evros provinces (Greece) and the southeastern mountainous area of Bulgaria (Skartsi et al. 2003; Elorriaga et al. 2004a, 2005; Vasilakis et al. 2006, 2008). The intensive management of livestock farms and the feeding habits of omnivorous animals (constituting vulture prey) foraging in agricultural lands and rubbish dumps probably increase the possibilities of their exposure to organochlorine pollutants such γ-HCH (whose use was prohibited in Greece by June 2002), endosulfan isomers and endosulfan sulphate that are currently used in Greece.

Thus, the river Evros as source of organochlorine pollution may be of secondary importance for Dadia NP vultures due to their dependence on feeding stations and terrestrial sources of food. Regarding cinereous vulture, the low concentrations of PCB and OC residues in blood reflects banning of most of them in Greece in the mid-1970s (Albanis 1997) and the low concentrations expected in their food types. In contrast, griffon vultures present at Dadia NP originated from different breeding populations of countries such as Croatia, Serbia, Bulgaria and Israel (WWF-Greece recovery data) and may have mostly lived far from Dadia NP. Therefore, organochlorine levels may greatly reflect the exposure of griffons to pollutants outside Dadia NP.

A higher proportion of high-chlorinated PCB congeners (138, 153 and 180) have also been found in other studies on vultures (Senthil Kumar et al. 2003; Gómara et al. 2004) and on other species such as bald eagles (Henriksen et al. 1998; Keller et al. 2004; Donalson et al. 1999), attributed to PCBs' bioaccumulative properties

and to the slow rate of biotransformation of these congeners (Senthil Kumar et al. 2001; Van den Brink and Bosveld 2001). The occurrence of the high-chlorinated PCB congeners in Greece have also been verified by their detection in eggs of a variety of waterbird species (Albanis et al. 1996; Albanis et al. 2003; Goutner et al. 1997; Goutner et al. 2001; Goutner et al. 2004; Konstantinou et al. 2000).

Temporal variability of pollutants in blood could be expected for free-living vultures that experience fluctuations in nutritional condition and have a diverse diet. The blood levels of organochlorines can be largely variable in cases of positive energy levels reflecting levels in diet and during fasting periods reflecting stored fat levels of organochlorines. Blood organochlorine concentrations are especially labile as they are highly correlated to recent feeding (Henriksen et al. 1998) and affected by fasting and lipid mobilisation. Changes in body condition and blood lipid content may explain a significant portion of the variation in blood concentrations of the organochlorines. The levels of more easily metabolized congeners demonstrate greater coefficient of variation than the more highly chlorinated PCBs suggesting recent food intake (Olsson et al. 2000). In our samples, in addition to the highest variations found in the lower PCBs, most of the variations of the other PCBs (118 and above) were relatively high (being 49–60% for cinereous vulture and 40–49% for griffon vulture). These suggest that their blood contaminant concentrations may have been affected by recent feeding activities.

Although in the few cases of cinereous vulture recaptures bioaccumulation of PCBs through age classes occurred, it was generally weak and visible only when adult and juvenile levels were compared. The absence of significant changes in blood concentrations between consecutive age classes suggests that the dietary intake of these contaminants is approximately equal to the dilution by growth of contaminants and that intake and elimination are balanced. The interpretation of temporal differences of pollutant levels in relation to age must be considered with caution due to the small sample sizes. Similarly, temporal variability was not pronounced for dioxins, furans and PCBs in small number of blood samples from American black vultures and Turkey vultures from South Carolina (Senthil Kumar et al. 2003).

Levels of OCs and/or PCBs that negatively affect birds of prey (reported in Van Wyk et al. 2001; Gómara et al. 2004; Hela et al. 2006) suggest that residue levels detected in vultures sampled in Dadia NP were too low to affect them.

Conclusion

This study is the first account of analysis of toxicants present in populations of different species of vultures in northeastern Mediterranean. The current data present baseline values for a number of organochlorines, which have not been analyzed in vulture species in the past. The detected organochlorine levels in the blood samples of vultures were low compared to other studies, and the vulture population sampled was under no direct threat from the present contamination levels. However, negative effects cannot be excluded in starvation periods while synergistic effects could also increase the toxicity. The possible confounding factor of recent feeding may be important when sampling from free-living birds.

References

Albanis, T. A. (1997). *Pesticides: applications, effects and regulations*. Ioannina: University of Ioannina.

Albanis, T. A., Hela, D., Papakostas, G., & Goutner, V. (1996). Concentration and bioaccumulation of organochlorine pesticide residues in herons and their prey, in wetlands of Thermaikos Gulf, Macedonia, Greece. *Science of the Total Environment, 182*, 11–19.

Albanis, T. A., Goutner, V., Konstantinou, I., & Frigis, K. (2003). Organochlorine contaminants in eggs of the yellow-legged gull (*Larus cachinnans michahellis*) in the north-eastern Mediterranean: Is this gull a suitable biomonitor for the region? *Environmental Pollution, 126*, 245–255.

Bowerman, W. W., Bestb, D. A., Grubbc, T. G., Sikarskied, J. G., & Giesy, J. P. (2000). Assessment of environmental endocrine disruptors in bald eagles of the Great Lakes. *Chemosphere, 41*, 1569–1574.

Bustnes, J. O., Tveraa, T., Henden, J. A., Varpe, Ø., Janssen, K., & Skaare, J. U. (2006). Organochlorines in Antarctic and Arctic avian top predators: A com-

parison between the south polar skua and two species of northern hemisphere gulls. *Environmental Science and Technology, 40*, 2826–2831.

De la Puente, J., & Elorriaga, N.J. (2011). Ageing of Eurasian black vulture (*Aegypius monachus*) using the moult. In *Proceedings of the international symposium on the black vulture Aegypius monachus*. Córdoba: Junta de Andalucía.

DIN (Deutsches Institut für Normung e.V.) (1987). *Testing of petroleum products, determination of polychlorinated biphenyls (PCB)*. DIN 51527–1, Berlin: DIN.

Donalson, G. M., Shutt, J. L., & Hunter, P. (1999). Organochlorine contamination in bald eagle eggs and nestlings from the Canadian Great Lakes. *Archives of Environmental Contamination and Toxicology, 36*, 70–80.

Elliott, J. E., Machmer, M. M., Wilson, L. K., & Henny, J. (2000). Contaminants in ospreys from the Pacific northwest: II. Organochlorine pesticides, polychlorinated biphenyls, and mercury, 1991–1997. *Archives of Environmental Contamination and Toxicology, 38*, 93–106.

Elorriaga, N. J., Skartsi, T., & Vasilakis, D. (2004a). *Black vulture and griffon vulture monitoring in the National Park of Dadia-Lefkimi-Soufli Forest*. Annual Tech. Rep. 2004. Athens: WWF-Greece.

Elorriaga, N. J., Vasilakis, D., & Skartsi, T. (2004b). Vulture trapping with sliding-door cage trap in the National Park of Dadia-Lefkimi-Soufli Forest. *Vulture News, 51*, 69–72.

Elorriaga, N. J., Skartsi, T., & Vasilakis, D. (2005). *Black vulture and griffon vulture monitoring in the National Park of Dadia-Lefkimi-Soufli Forest*. Annual Tech. Rep. 2005. Athens: WWF-Greece.

Erkmen, B., & Kolankaya, D. (2006). Determination of organochlorine pesticide residues in water, sediment, and fish samples from the Meriç Delta, Turkey. *International Journal of Environmental Analytical Chemistry, 86*, 161–169.

Golfinopoulos, S. K., Nikolaou, A. D., Kostopoulou, M. N., Xilourgidis, N. K., Vagi, M. C., & Lekkas, D. T. (2003). Organochlorine pesticides in the surface waters of northern Greece. *Chemosphere, 50*, 507–516.

Gómara, B., Ramos, L., & González, M. J. (2002). Determination of polychlorinated biphenyls in small-size serum samples by solid-phase extraction followed by gas chromatography with micro-electron-capture detection. *Journal of Chromatography B, 766*, 279–287.

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.

Goutner, V., Charalambidou, I., & Albanis, T. A. (1997). Organochlorine insecticide residues in eggs of the little tern (*Sterna albifrons*) in the Axios Delta, Greece. *Bulletin of Environmental Contamination and Toxicology, 58*, 61–66.

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

Goutner, V., Albanis, T. A., & Konstantinou, I. K. (2004). PCBs and organochlorine pesticide residues in eggs of threatened colonial charadriiform species (Aves, Charadriiformes) from wetlands of international importance in northeastern Greece. *Belgian Journal of Zoology, 134*(Suppl. 1), 39–46.

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. *Archives of Environmental Contamination and Toxicology, 50*, 603–613.

Henny, C. J. (1998). Toxic chemicals and birds of prey in the mid-1990s: A personal prespective. In R. D. Chancellor, B-U. Meyburg, & J. J. Ferrero (Eds.), *Holarctic birds of prey* (pp. 483–500). Berlin: ADENEX-WWGBP.

Henriksen, E. O., Gabrielsen, G. W., & Skaare, J. U. (1998). Validation of the use of blood samples to assess tissue concentrations of organochlorines in glaucous gulls, *Larus hyperboreus*. *Chemosphere, 37*, 2627–2643.

Johnstone, R. M., Court, G. S., Fesser, A. C., Bradley, D. M., Oliphant, L. W., & MacNeil, J. D. (1996). Long-term trends and sources of organochlorine contamination in Canadian tundra peregrine falcons, *Falco peregrinus tundrius*. *Environmental Pollution, 93*, 109–120.

Ishikawa, Y., Noma, Y., Mori, Y., & Sakai, S. (2007). Congener profiles of PCB and a proposed new set of indicator congeners. *Chemosphere, 67*, 1838–1851.

Keller, J. M., Kucklick, J. R., & McClellan-Green, P. D. (2004). Organochlorine contaminants in loggerhead sea turtle blood: extraction techniques and distribution among plasma and red blood cells. *Archives of Environmental Contamination and Toxicology, 46*, 254–264.

Konstantinou, I. K., Goutner, V., & Albanis, T. A. (2000). The incidence of polychlorinated biphenyl and organochlorine pesticide residues in eggs of the cormorant (*Phalacrocorax carbo sinensis*): Evaluation of the situation in four Greek wetlands of international importance. *Science of the Total Environment, 257*, 61–79.

Longnecker, P., Rogan, W. J., & Lucier, G. (1997). The human health effects of DDT (dichlorodiphenyltrichloroethane) and PCBs (polychlorinated biphenyls) and an overview of organochlorines in public health. *Annual Review of Public Health, 18*, 211–244.

Martínez-López, E., Romero, D., María-Mojica, P., Martínez, J. E., Calvo, J. F., &García-Fernández, A. J. (2009). Changes in blood pesticide levels in booted eagle (*Hieraaetus pennatus*) associated with agricultural land practices. *Ecotoxicology and Environmental Safety, 72*, 45–50.

Muralidharan, S., Dhananjayan, V., Risebrough, R., Prakash, V., Jayakumar, Z. R., Peter, H., et al. (2008). Persistent organochlorine pesticide residues in tissues and eggs of white-backed vulture, *Gyps bengalensis*

from different locations in India. *Bulletin of Environmental Contamination and Toxicology, 81*, 561–565.

Olsson, A., Ceder, K., Bergman, Å., & Helander, B. (2000). Nestling blood of the white-tailed sea eagle (*Haliaeetus albicilla*) as an indicator of territorial exposure to organohalogen compounds-An evaluation. *Environmental Science and Technology, 34*, 2733–2740.

Poirazidis, C., Skartsi, T., & Catsadorakis, G. (2002). *Monitoring plan for the protected area of Dadia–Lefkimi–Soufli Forest*. Athens: WWF-Greece.

Poirazidis, C., Goutner, V., Skartsi, T., & Stamou, G. (2004). Modelling nesting habitat as a conservation tool for the Eurasian black vulture (*Aegypius monachus*) in Dadia Nature Reserve, northeastern Greece. *Biological Conservation, 118*, 235–248.

Senthil Kumar, K. S., Bowerman, W. W., DeVault, T. L., Takasuga, T., Rhodes, O. E. Jr., Brisbin, I. L., et al. (2003). Chlorinated hydrocarbon contaminants in blood of black and Turkey vultures from Savannah River Site, South Carolina, USA. *Chemosphere, 53*, 173–182.

Senthil Kumar, K., Kannan, K., Giesy, J. P., & Masunaga, S. (2002a). Distribution of chlorinated hydrocarbons in tissues of bald eagles from the upper peninsula of Michigan, USA. *Environmental Science and Technology, 36*, 2789–2796.

Senthil Kumar, K., Kannan, K., Corsolini, S., Evans, T., Giesy, J. P., Nakanishi, J., et al. (2002b). Polychlorinated dibenzo-p-dioxins, dibenzofurans and polychlorinated biphenyls in polar bear penguin and polar skua. *Environmental Pollution, 119*, 151–161.

Senthil Kumar, K., Kannan, K., Subramanian, A., & Tanabe, S. (2001). Accumulation of organochlorine pesticides and polychlorinated biphenyls in sediments aquatic organisms, birds, bird eggs and bat collected from South India. *Environmental Science and Pollution Research, 8*, 35–47.

Skartsi, T., Elorriaga, N. J., Vasilakis, D., & Poirazidis, C. (2008). Population size, breeding rates and conservation status of Eurasian black vulture in the Dadia National Park, Thrace, NE Greece. *Journal of Natural History, 42*, 345–353.

Skartsi, T., & Poirazidis, C. (2002). *Management plan of black vulture population in the protected area of Dadia-Lefkimi-Soufli Forest*. Athens: WWF-Greece.

Skartsi, T., Vasilakis, D., & Elorriaga, N. J. (2003). *Black vulture and griffon vulture monitoring in National Park of Dadia-Lefkimi-Soufli Forest*. Annual Technical Report. Athens: WWF-Greece.

Snyder, N. F. R., & Meretsky, V. J. (2003). California condors and DDE: A re-evaluation. *Ibis, 145*, 136–151.

Van den Brink, N. W., & Bosveld, B. T. C. (2001). PCB concentrations and metabolism patterns in common terns (*Sterna hirundo*) from different breeding colonies in The Netherlands. *Marine Pollution Bulletin, 42*, 280–285.

Van Wyk, E., Van Der Bank, F. H., Verdoorn, G. H., & Bouwman, H. (1993). Chlorinated hydrocarbon insecticide residues in the Cape griffon vulture (*Gyps coprotheres*). *Comparative Biochemistry and Physiology C, 104*, 209–220.

Van Wyk, E., Bouwman, H., Van Der Bank, F. H., Verdoorn, G. H., & Hofmann, D. (2001). Persistent organochlorine pesticides detected in blood and tissue samples of vultures from different localities in South Africa. *Comparative Biochemistry and Physiology C, 129*, 243–264.

Vasilakis, D., Poirazidis, C., & Elorriaga, N. J. (2006). Breeding season range use of a Eurasian black vulture (*Aegypius monachus*) population in the Dadia National Park and the adjacent areas, Thrace, NE Greece. In S. E. Piper, & D. C. Houston (Eds), *Proceedings of the international conference on the conservation and management of vulture populations* (p. 180). Thessaloniki: NHMC, WWF-Creece.

Vasilakis, P. D., Poirazidis, C., & Elorriaga, N. J. (2008). Range use of a Eurasian black vulture (*Aegypius monachus*) population in the Dadia National Park and the adjacent areas, Thrace, NE Greece. *Journal of Natural History, 45*, 355–373.

Voltz, S. A., Johnston, J. J., & Griffin, D. L. (2001). Solid phase extraction gas chromatography/electron capture detector method for the determination of organochlorine pesticides in wildlife whole blood. *Journal of Agricultural and Food Chemistry, 49*, 2741–2745.

Vryzas, Z., Vassiliou, G., Alexoudis, C., & Papadopoulou-Mourkidou, E. (2008). Spatial and temporal distribution of pesticide residues in surface waters in northeastern Greece. *Water Research, 43*, 1–10.