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Spatiotemporal variations of organochlorine pesticides in an apex predator: Influence of government regulations and farming practices

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ABSTRACT

Background: Intensification of agricultural practices has caused several negative effects to the environment. The use of fertilizers and pesticides may alter geochemical cycles or cause direct wildlife intoxication. Detrimental effects of organochlorine pesticides (OCPs) have forced the authorities to ban or restrict its use. This study evaluates the variation in levels of OCPs in a sentinel species in relation to changes in government regulations and the spatial configuration of agricultural practices around the nests.
Methods: Between 2003 and 2007, we analysed OCP levels in 256 blood samples of Eurasian Eagle-owl (*Bubo bubo*) chicks nesting in area of intensive commercial agriculture with historical frequent use of pesticides, in South-eastern Spain. We studied year-to-year variations in OCP concentrations and their relation with land use configuration around raptor nests by Generalized Linear Mixed Models (GLMM).
Results: OCPs were detected in 100% samples surveyed in 2003 and 2004, while dropped to 27% in 2005, 6.8% in 2006 and 6.3% in 2007, coinciding with the ban of OCPs. The presence of the main OCPs was related to agricultural practices. In particular, endosulfan and lindane were related to irrigated crops and urban areas, while DDT-related compounds and dieldrin were associated with dry land farming.
Conclusions: OCP concentrations in blood samples of Eurasian Eagle-owls may respond quickly to the implementations of new regulations about the use of agricultural products. This raptor was confirmed as a good sentinel species allowing rapid detection of changes in pesticides use.

1. Introduction

During the last decades, human population growth has led to an intensification of agricultural practices to cover the increasing food demand (Godfray et al., 2010). This has undergone several negative effects to the environment such as native habitat loss, habitat fragmentation, alteration of hydrologic systems, introduction of exotic species, or loss of biodiversity associated to agro-ecosystems changes (Chamberlain et al., 2000; Donald et al., 2001; Tscharntke et al., 2005; Henle et al., 2008), and an increase of contaminants in the environment, in particular, pesticides and fertilisers (Firbank et al., 2007). These chemicals are not innocuous in the ecosystem because they can alter the proper functioning of geochemical cycles or cause direct intoxication of wildlife. In the case of organochlorine pesticides (OCPs), detrimental effects have been related to their use in several species of different taxa (Facemire et al., 1995; Guillette et al., 1994; Henny and Elliott, 2007). These effects have forced the authorities to ban the use of different substances, which has led to a subsequent decrease of their concentrations both in the environment and animal tissues (García-Fernández et al., 2008). However, due to the high persistence and ability to bioaccumulate and biomagnificate through the trophic chain, environmental concentrations of these compounds may still be

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important and exert potential risks (Van den Steen et al., 2009).

The Mediterranean is one of the areas that have undergone major changes during the last century (Blondel and Aronson, 1999). Large areas of the Mediterranean basin have been occupied for intensive commercial agriculture, specifically in South-eastern Spain, with an intensive use of pesticides (Sánchez-Picón et al., 2011). OCPs were introduced for the first time in the 1930s, and widely used in Spain between the 1950s and 1990s (Sánchez-Gelabert et al., 2008). Although most of them were banned more than 30 years ago in Spain and most countries, some compounds such as lindane or endosulfan were authorised and recommended for a variety of crops grown in our study area and were only relatively recently banned by the EU legislation (Decision 2005/864/EC, Regulation (EC) 850/2004).

The value of birds as biomonitoring organisms of environmental contaminants has been broadly recognized (García-Fernández, 2014) and several governmental established monitoring programmes are the proof for it (Gómez-Ramírez et al., 2014). Among them, birds of prey are considered especially suitable for monitoring contaminants, mainly due to their position at the top of the trophic web and susceptibility to bioaccumulate and integrate contaminants over time (Furness, 1993; García-Fernández, 2014; Espín et al., 2016). In this sense, the suitability of raptor samples to evaluate changes in contaminant levels due to shifts in agricultural practices has been evidenced in previous studies (Martínez-López et al., 2012). Even though birds of prey can be successfully used to evaluate the implementation of regulatory actions and changes in the agricultural practices, these studies are still scarce and more information is needed (Gómez-Ramírez et al., 2014).

The aim of this study was to evaluate variations in levels of OCPs in relation to changes in governmental regulations and the spatial configuration of agricultural practices around raptor nests. To achieve this goal, the Eurasian Eagle-owl (*Bubo bubo*) was chosen as biomonitor species. This raptor can be considered suitable to monitor environmental pollutants in our study area (South-eastern Spain) because it is an abundant species, relatively easy to capture, and exposed to environmental contaminants (García-Fernández, 2014). Moreover, because this is a long-lived, territorial resident owl, with a relatively small home range (León-Ortega et al., 2017a), it is likely to reflect local contamination (Dell'Omo et al., 2008; Gómez-Ramírez et al., 2011). Agricultural practices that require artificial irrigation are usually related to a higher use of pesticides, as the continuous presence of water in warm climate conditions creates a favourable habitat for breeding insects (Ongley, 1996). In this sense, our hypothesis suggests that pesticide levels in blood of Eurasian Eagle-owls are spatially related to the percentage of irrigated crops around nests, and these compounds are expected to decrease along the study period due to the implementation of the European regulations. In order to test this hypothesis, we analysed OCPs levels in blood of Eurasian Eagle-owl nestlings born in an area with a mosaic of natural and intensive agriculture patches and assessed the changes of these levels in relation to the ban of their use.

2. Methods

2.1. Study area

The study area comprises a 1110 km^2 in the South of the province of Alicante and the East of Murcia Region (South-eastern Spain; 37° 57 N, 0° 55 W, Fig. 1). The climate is thermo-Mediterranean semiarid with 275–400 mm of annual rainfall and a high annual average temperature of 19 °C.

The landscape is formed by low hills close to the sea and low rocky mountains covered by a mosaic of intensive irrigated agriculture (citrus crops and vegetables), dry land farming (almond, olive and carob trees), remnants of natural vegetation composed of Mediterranean shrubs *Pistacea lentiscus*, *Rosmarinus officinalis*, *Rhamnus lycioides*, *Chamaerops humilis*, *Thymus* sp. and pines forest (*Pinus halepensis* and *P. pinea*). Urban developments and sparse human settlements are distributed throughout the area, especially around the river valley and near the coast. Besides, several golf courses have been placed within the study area over the past decade.

2.2. Biomonitoring species

The Eurasian Eagle-owl is one of the largest strigiforms in the world and it is widely distributed in the Palaearctic (del Hoyo et al., 1999). In Spain it is sedentary and highly territorial during the whole year, occupying territories whose size and foraging area seem to depend on prey availability (León-Ortega et al., 2017a). In South-eastern Spain, the Eurasian Eagle-owl population shows the highest breeding density described for its distribution range and a high productivity (Pérez-García et al., 2012; León-Ortega et al., 2017b), higher than other populations studied in the whole Palearctic (see review in Marchesi et al., 2002). The breeding population was estimated as 170 pairs in the study area, during the study period (Pérez-García et al., 2012; 2016; León-Ortega, 2016).

2.3. Blood sampling

Between 2003 and 2007 a total of 50 Eurasian Eagle-owl nests were monitored. Reproductive activity was rigorously checked during all breeding period to determine the age of fledglings. Two-hundred and fifty-six samples were obtained from 28 to 30 days-old Eurasian Eagle-owl chicks, by the veterinarian of the Wildlife Rehabilitation Centre "Santa Faz" (Alicante, Spain), who proceeded to evaluate clinically the health status of the chicks prior to blood sampling. In order to avoid stress in the nestlings, careful steps were taken, like covering the head of the nestlings during manipulation. Once sampled, nestlings were returned to the nest. Blood samples from the nestlings (3–5 mL) were taken by puncturing the brachial vein with a 23G needle and a syringe. These samples were taken immediately to the laboratory under refrigerated conditions and frozen to −40 °C until processing. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

2.4. Organochlorine pesticides analyses

Two-hundred and fifty-six blood samples were analysed according to the method developed by María-Mojica et al. (2000), adapted for blood samples by Martínez-López et al. (2009), to detect the traditionally most frequently used OCPs in the study area (hexachlorocyclohexane (HCH) isomers (α-HCH, β-HCH, γ-HCH or lindane and δ-HCH), endosulfan I, endosulfan II, aldrin, endrin, dieldrin, dichlorodiphenyltrichloroethane (*p,p'*-DDT), and heptachlor and their metabolites dichlorodiphenyldichloroethane (*p,p'*-DDD), dichlorodiphenyldichloroethylene (*p,p'*-DDE), endrin aldehyde and heptachlor epoxide. A volume of 200 µL of whole blood was sonicated and homogenized using hexane: acetone (3:1 v/v) as solvent. The samples were filtered using anhydrous sodium sulphate and the solvent collected was evaporated until dryness. After redissolution in 5 mL hexane, samples were cleaned up with Florisil-column chromatography (SepPak, Waters®) using a petroleum ether-diethyl ether mix (21:4) as elution. The extract was evaporated until dryness and re-dissolved in 1 mL of n-hexane. One microliter was injected into a gas chromatograph with electron capture (GC-ECD 17 Shimadzu) for the detection of OCPs. The SPB-608 capillary column (Supelco®) was 30 m long, 0.25 mm i.d. with a 0.25 mm-thick coating. Helium was used as the carrier gas. The injector was set at the splitless mode; the injector temperature was 290 °C. The column program was: 2 min 50 °C, from 50 to 150 °C at 40 °C min^{-1}, 2 min 150 °C, from 150 to 290 °C at 8 °C min^{-1}, 10 min 290 °C. The detector temperature was 330 °C and nitrogen used as gas make up. Quantification was based on an external standard containing 16 organochlorine pesticide compounds from Supelco® which is

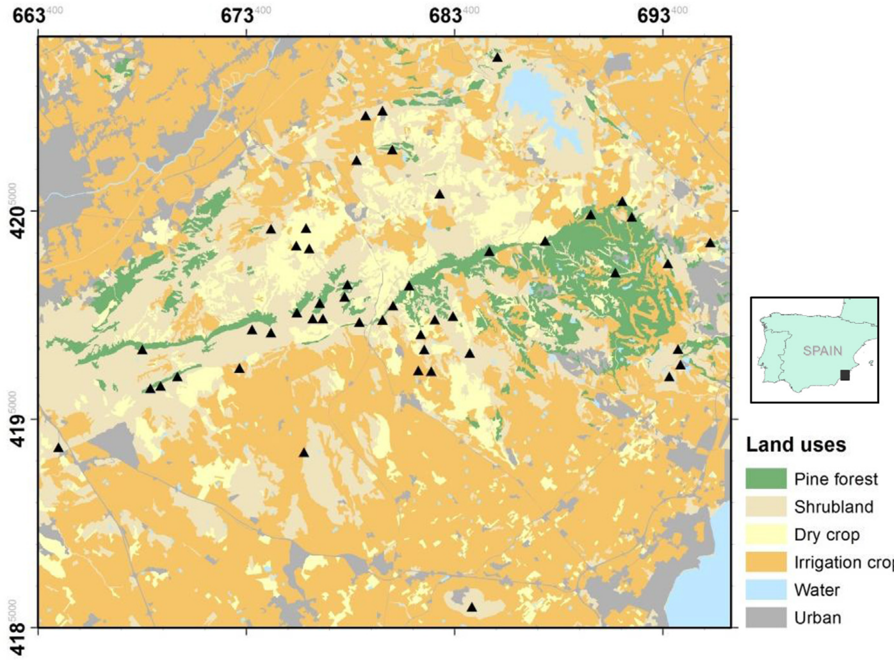

Fig. 1. Location and land use configuration of the study area in south-eastern Spain. Sampled Eurasian Eagle-owl nests are shown as black triangles.

dissolved in n-hexane (1:25), obtaining the following final concentrations: 10 μg mL^{-1} for *α*-HCH, *β*-HCH, γ-HCH, *δ*-HCH, lindane, heptachlor, heptachlor epoxide and aldrin; 20 μg mL^{-1} for endosulfan I, endosulfan II, *p,p'*-DDE, dieldrin and endrin; and 60 μg mL^{-1} for *p,p'*-DDD, *p,p'*-DDT, and endrin aldehyde. Quality assurance criteria were based on the application of quality controls, which included the analysis of blanks and duplicate samples covering the complete analytical procedure. For each compound, the percentage of variability between duplicates varied from 0.8 to 12% and mean recovery (accuracy) in spiked samples ranged from 85.8 to 146.0%. Quality assurance criteria were based on the application of quality controls which included the analysis of blank and duplicate samples covering the complete analytical procedure. Detection limits ranged from 0.20 to 0.50 μg L^{-1}. A dissolution of methoxychlor (PolyScience®) was prepared periodically prepared at 1 mg/mL and used as an internal standard in every sample analysed. A blank with hexane was incorporated in the batch every five samples.

2.5. Land use configuration

To characterize land uses in each Eurasian Eagle-owl territory, we selected an area of 1 km radius buffer around the most frequently used Eurasian Eagle-owl nests throughout the study period. We selected this distance because it is the nearest neighbour distance (NND = 0.9 km; Pérez-García et al., 2012) and encompasses the mean home range estimates for this raptor in the study area (León-Ortega, 2016; León-Ortega et al., 2017a). In each buffer area, we calculated the percentage of the following land uses: natural vegetation (pine forest and scrublands), dry cereals crops, dry fruit trees (almond and olive trees), citric crops (orange and lemon trees), irrigated vegetables (tomatoes, lettuce, broccoli), water (water surfaces and water infrastructures such as ponds or dams) and urban (urban or industrial area). Additionally, we calculated two summary crop covers one for total dry crops (cereals and dry fruits trees) and other for total irrigated crops (citric + vegetables + others fruits trees). Moreover, we calculated the presence of water ponds for irrigation in the 1 km buffer (1/0), and the minimum distance between each nest and the nearest water pond, urban area and golf courses. Land use distribution for the study period was obtained from CORINE land cover 2006 (EEA, 2011). We used QGIS software (Quantum GIS, 2012) to calculate the landscape variables.

2.6. Statistical procedures

Spatial-temporal trends and correlations with OCPs were studied for only five main compounds: lindane, Σ endosulfan (sum of endosulfan I and II), dieldrin, *pp'*-DDE and *p,p'*-DDT. These were chosen based on their higher frequency of detection and concentrations in blood of Eurasian Eagle-owls. To study spatial autocorrelations of these main OCPs levels per territory and year, these were summed (total OCPs), and we used Moran's Index (Sokal and Thomson, 1987). This index is frequently used as a spatial autocorrelation measure because it evaluates the correlation of data and variables collected between nearby locations in space. We studied inter-annual differences in OCPs levels in relation to European regulations by Kruskal-Wallis test and Wilcoxon rank sum test. To study the relationship between the four main OCPs and land uses, we used Linear Mixed Models (LMM) using land use variables as fixed effect and territory as random effects to control for the possible effects of pseudo replication (McCulloch and Searle, 2004). Prior to modelling, we assessed collinearity of land use variables by mean Variance Inflation Factor (VIF) (Belsley et al., 1980). This is an effective and comprehensive approach for multicollinearity assessment showing advantages over other methods such as establishing relationship between several independent variables at a time. Variables with VIF > 5 were excluded from further analyses (Belsley et al., 1980). We constructed models including land use variables and the interaction between land use variables and the factor year (variable * year). All models were compared with null model and these comparisons were carried out by corrected Akaike information criterion (AICc; Burnham and Anderson, 2002). We computed delta AICc to determine the strength of evidence and AICc weights to represent the relative likelihood of each model (Burnham and Anderson, 2002). This process was performed for the five main contaminants studied (lindane, Σ endosulfan, dieldrin, *pp'*-DDE and *p,p'*-DDT). All analyses were conducted using R statistical software (R Development Core Team, 2018) with lme4 packages for LMM analysis (Bates et al., 2015). All tests were two tailed, statistical significance was set at $\alpha \leq 0.05$, and all means are given ± 1 SD. Values below detection limits were given a value of zero.

3. Results

3.1. Organochlorine concentrations

OCPs were quantifiable in all Eurasian Eagle-owl nestlings surveyed in 2003 and 2004 (n = 58), while the frequency dropped to 27% in 2005 (n = 62), 6.8% in 2006 (n = 59) and 6.3% in 2007 (n = 79) (see supplementary material, Table S1). Lindane, *p,p'*-DDE, endosulfan I and α-HCH were the most frequent compounds. However, the highest concentrations were observed in endosulfan I, dieldrin, DDT and lindane (418.08, 246.50, 93.54 and 68.46 $\mu g\,L^{-1}$, respectively) (see supplementary material, Table S1).

Significant differences between years were found in levels of total OCPs (Kruskal-Wallis $X_4^2 = 133.99$, $p < 0.001$) showing a decreasing trend over the study period. Specifically for the five main compounds, we found significant differences comparing the concentrations of 2003 and 2004 with the following years (Wilcoxon test lindane W = 2153.5, $p < 0.001$; endosulfan W = 947.5, $p < 0.001$; dieldrin W = 4372, $p < 0.001$; *p,p'*-DDE W = 2653, $p < 0.001$, *p,p'*-DDT W = 4235, $p < 0.001$). In fact, during 2005 and 2006, only few individuals showed quantifiable lindane (n = 8), dieldrin (n = 5) and *p,p'*-DDE (n = 7). In the last year of monitoring (2007), only one individual was found with quantifiable residues of all of these five main OCPs (Fig. 2).

3.2. Spatial and land uses correlations

The main OCPs did not show spatial auto-correlation in any year of study (all Moran's I > 0.05; Fig. 3). Lindane and endosulfan showed similar response to land use variables (see Table 1). Two best models for lindane included the interaction of distance to urban areas and year and the interaction between year and the percentage of irrigated crops. This last model explained endosulfan variation. However, the model that best explained the levels of diphenylaliphatic compounds (*p,p'*-DDE and *p,p'*-DDT) and dieldrin included the interaction between cereal crops and year. All best models for the five main OCPs included the interaction between a land use and the year indicating the temporal variation in concentrations during the study period (see Table 1).

4. Discussion

Our study shows a sharp decrease of quantifiable OCP concentrations in blood samples of Eurasian Eagle-owl nestlings born in the first

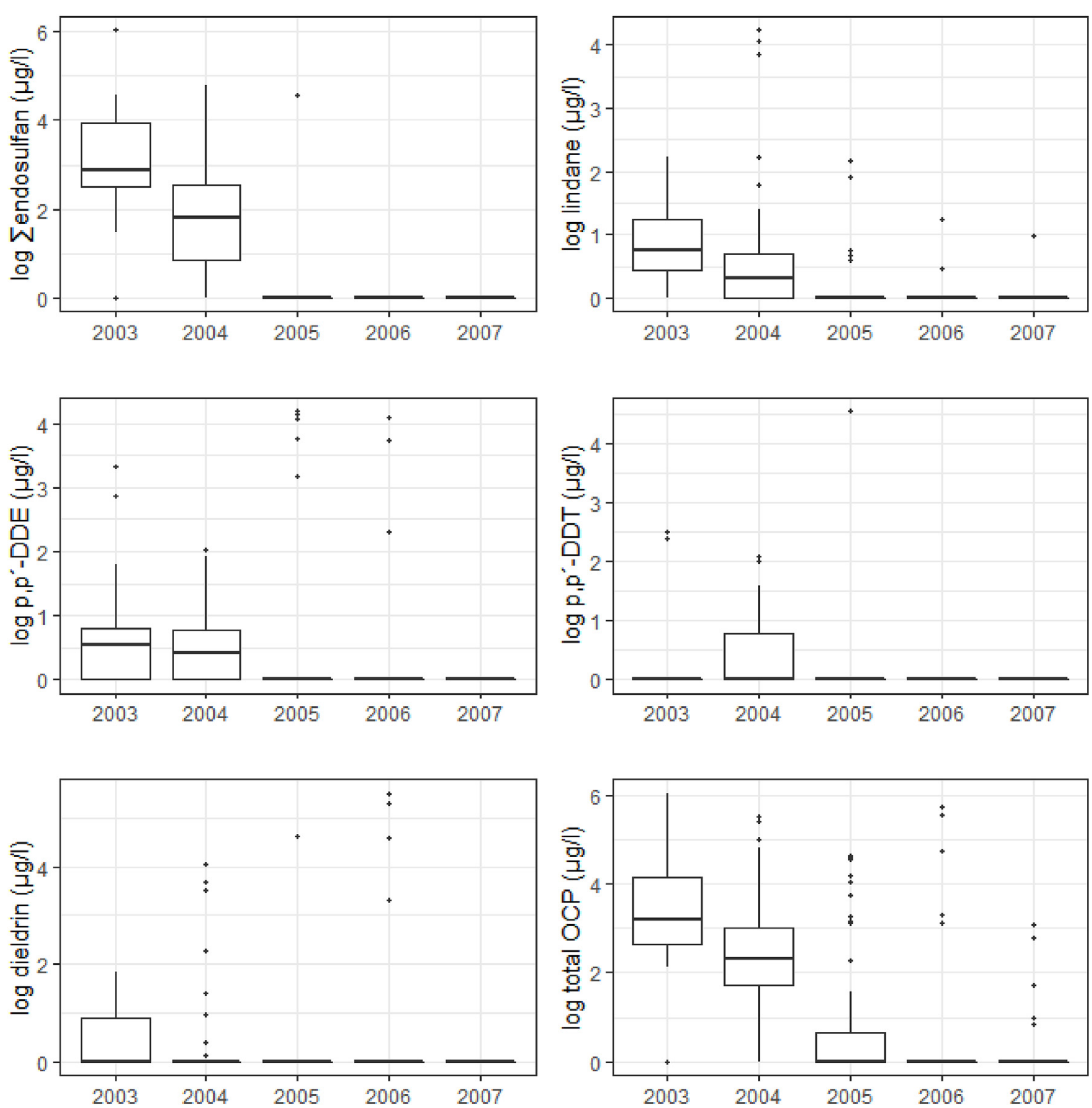

Fig. 2. Annual distribution of the five main OCPs and total OCPs concentrations ($\mu g\,L^{-1}$) in Eurasian Eagle-owl blood samples during the study period. We show mean (midline), standard error (box), standard deviation (whiskers) and outliers (dots). OCP levels have been log-transformed by log (value + 1).

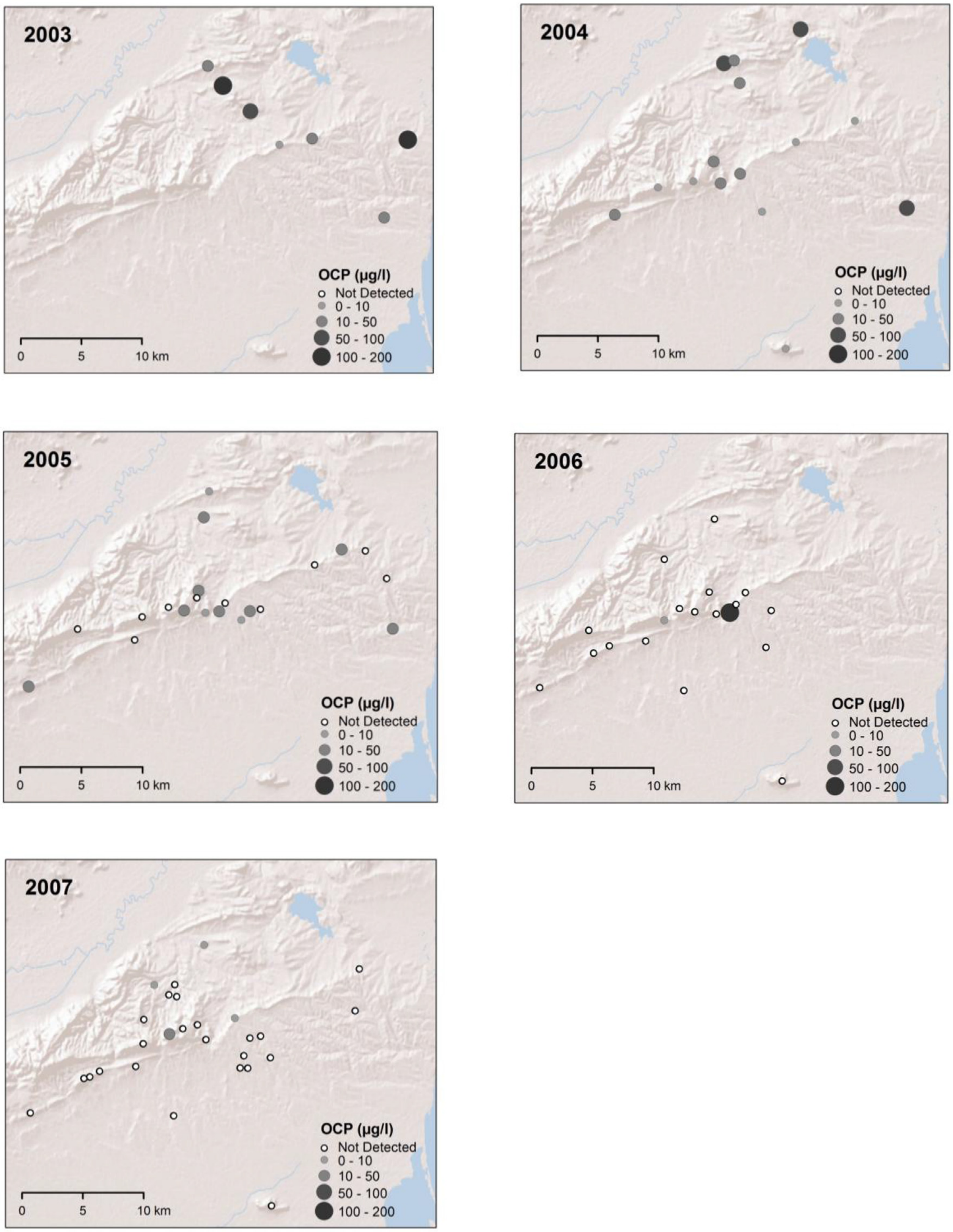

Fig. 3. Annual spatial distribution of total OCPs levels (µg L^{-1}) in blood samples of Eurasian Eagle-owl's fledglings in south-eastern Spain. Each point represents the mean concentration per sampled nest.

two years of monitoring (2003–2004), reaching non-detectable values in the latter years. These declines may be related to the legal restrictions of use of phytosanitary products in agricultural practices (see Table S2). Similar results were found in Booted eagle nestlings also born in Murcia Region, whose concentrations of OCP insecticides varied simultaneously to agricultural practices (Martínez-López et al., 2009). In particular, Σ endosulfan and lindane were related to the presence of irrigated crops and urban areas, while diphenylaliphatic compounds (*p,p'*-DDE and *p,p'*-DDT) and dieldrin were related to the presence of dry herbaceous ones (see Table 1).

4.1. Organochlorine levels in Eurasian Eagle-owl nestlings

Concentrations of OCPs between 2003 and 2004 were generally lower or similar to those in blood or plasma of other nestlings of birds of prey hatched in the same decade but in different parts of the world (see Table 2). However, lindane concentrations in nestlings born in 2004 were higher (mean = 5.11 µg L^{-1}) than in Common Buzzards (*Buteo*

Table 1
Linear Mixed Models of main OCPs levels in Eurasian Eagle-owl fledglings in relation to land uses at 1 km around their nests using as random effect the Eagle-owl territory. k = total number of parameters; AICc = corrected Akaike's Information Criterion; ΔAICc = difference of AICc between models; Wt = Akaike's weights. Only models with a ΔAICc < 3 are presented. Variables included in the models: percentage of irrigated crops (Irrig Crops), dry crops (Dry Crops), water surface (Water), urban area (Urb), citric crops (Citric) and cereal crops (Cereal) and the distance to nearest urban area (Dist Urb).

OCP	Model	k	AICc	Δ AICc	Wt
Lindane	~ Dist Urb*Year	12	371.48	0	0.36
	~ Irrig Crops * Year + Water	13	372.17	0.69	0.25
	~ Water * Year	12	373.4	1.93	0.14
	~ Irrig Crops * Year + Water + Dry Crops	14	374.13	2.66	0.09
	~ Irrig Crops * Year + Water + Urb	14	374.39	2.91	0.08
Σ Endosulfan	~ Irrig Crops * Year + Water	13	519.49	0	0.54
	~ Irrig Crops * Year + Water + Urb	14	520.57	1.08	0.31
	~ Irrig Crops * Year + Water + Urb + Dist Urb	15	522.47	2.99	0.12
P,p'- DDE	~ Cereal * Year	12	563.29	0	0.67
	~ Urb * Year	12	565.87	2.58	0.19
P,p'- DDT	~ Cereal * Year	12	265.07	0	0.97
Dieldrin	~ Cereal * Year	12	569.76	0	0.62
	~ Irrig Crops * Year + Water	13	572.47	2.71	0.16
	~ Citric * Year	12	572.53	2.77	0.16

buteo) and Goshawks born close to our study area, in Murcia Region, between 1999 and 2003 (Martínez-López, 2005). Aldrin, endrin and heptachlor have been rarely analysed in blood samples from raptors, but our levels were lower than in the Goshawk, Common Buzzard, or Booted Eagle nestlings born in south-eastern Spain between 1999 and 2003 (Martínez-López, 2005). Finally, endosulfan I concentrations prior to the ban (2003–2004) were similar to the average in Booted Eagles born between 1999 and 2002 in Murcia Region (Martínez-López et al., 2009).

4.2. *OCP and land use practices*

In agreement to our hypothesis, higher OCPs concentrations were mainly related to a higher percentage of intensive land use as irrigation crops and urban areas. Similarly, the concentrations of diphenylaliphatics and dieldrin were lower in Eagle-owl territories with a higher percentage of dry crops, because the use of pesticides is lower in these crops. The absence of spatial autocorrelation in OCP levels through the study area suggests that sources of contaminants are spatially localized, and therefore closely related to the land use practices that are performed within each Eurasian Eagle-owl territory. This could be explained by the very small home ranges (c.a. 0.7–1.5 ha; Lombardi et al., 2007) of the European rabbit (*Oryctolagus cunniculus*), the main prey of Eurasian Eagle-owls in our study area (71–90% of the total prey consumed; León-Ortega, 2016; Pérez-García et al., 2012). Therefore, both links in the trophic web of bioaccumulation of the OCP from the places of application (e.g., irrigated crops) to the species used as a monitor (Eurasian Eagle-owl) are highly correlated spatially. In fact, previous studies in nestlings of this species and in the same study area, showed that Hg levels in blood were highly correlated to Hg levels in rabbits (Espín et al., 2014a). This reinforces the idea of the spatial representability of the sentinel species used.

The obvious relationship between the presence of irrigated crops and water infrastructure around the nest (e.g. dams or ponds) and compounds as lindane and Σ endosulfan seem to be explained by the use of these compounds in irrigated crops. This is in agreement with the fact that endosulfan was still authorised for this type of crops in the study area during the study period (Decision, 2005/864/EC). Although lindane was only authorised for veterinary and forestry applications

Table 2
Levels of the five main organochlorine pesticides from this study reported as mean (range) compared to other studies in nestlings of birds of prey. Concentrations are reported as μg L^{-1}.

Species; Sampling year	Dieldrin	Lindane	Σ Endosulfan	p,p'-DDT	p,p'-DDE	Reference; Location, sample size
Eurasian Eagle-owl (Bubo bubo); 2003	*0.96 (ND-5.25)*	*1.98 (ND-8.32)*	*51.54 (ND-418.08)*	*1.24 (ND-11.16)*	*3.36 (ND-27.27)*	*This study; Spain, (n = 17)*
Eurasian Eagle-owl (Bubo bubo); 2004	*3.67 (ND-57.17)*	*5.11 (ND-68.46)*	*16.25 (ND-118.75)*	*0.82 (ND-7.20)*	*1.21 (ND-6.50)*	*This study; Spain, (n = 39)*
Eurasian Eagle-owl (Bubo bubo); 2005	*1.65 (ND-102.23)*	*0.27 (ND-7.84)*	*96.34*	*93.54*	*4.01 (ND-64.91)*	*This study; Spain, (n = 62)*
Booted Eagle (*Hieraetus pennatus*); 1999–2003	6.51 (ND-81.15)	10.10; ND-123	29.65 (ND-239.03)	7.5; (ND-63)	5.6 (ND-25)	Martínez-López et al. (2009); Spain (n = 62)
Common buzzard (*Buteo buteo*); 2001	1.85 (1.3–2.4)	0.76 (ND-1.4)	10.58 (3.7–8.6)	5.45 (3.7–7.2)	1.57 (ND-3)	Martínez-López, 2005*; Spain (n = 2)
Common buzzard (*Buteo buteo*); 2003	34.9 (11.61–93.63)	0.66 (ND-3.16)	48.27 (ND-145.05)	11.31 (ND-31.74)	5.53 (ND-13.99)	Martínez-López, 2005*; Spain (n = 11)
Goshawk (*Accipiter gentilis*); 2002–2003	7.88 (ND-18.17)	0.56 (ND-2.85)	28.8 (ND-19.64)	2.4 (0.6–7.9)	5.35 (ND-25)	Martínez-López, 2005*; Spain (n = 11)
Golden eagle (*Aquila chrysaetos*); 2008	–	–	–	–	0.6–4.88	Sonne et al. (2012); Norway (n = 2)
Golden eagle (*Aquila chrysaetos*); 2010	–	–	–	–	8 (0.9–18.2. 9)	Sonne et al. (2012); Norway (n = 9)
Goshawk (*Accipiter gentilis*); 2008	–	–	–	–	7.5 (1.77–24.7)	Sonne et al. (2012); Norway (n = 16)
Goshawk (*Accipiter gentilis*); 2009	–				3.4 (0.85–11)	Sonne et al. (2012); Norway (n = 23)
Goshawk (*Accipiter gentilis*); 2010					4.8(1.8–10)	Sonne et al. (2012); Norway (n = 16)
Black harrier (*Circus maurus*); 2012–2014	–	–	–	–	0.29 (ND-2.21)	García-Heras et al. (2018); South Africa (n = 90)
Montagu's harrier (*Circus pygargus*); 2007–2008	–	–	–	–	0.96 (ND-0.82)	Espín et al. (2018); NE Spain **(n = 14)
Montagu's harrier (*Circus pygargus*); 2007–2008					0.17 (ND-5.3)	Espín et al. (2018), W Spain (n = 17)
Montagu's harrier (*Circus pygargus*); 2007–2008					0.35 (ND-6.5)	Espín et al. (2018); NW Spain (n = 18)
Pallid harrier (*Circus macrourus*); 2008	–	–	–	–	0.12 (ND-3.7)	Espín et al. (2018); Kazakhstan **(n = 21)

ND = not detected *Σendosulfan for this study included endosulfan I, endosulfan II and endosulfan sulfate; **Geometric mean (range).

(Regulation (EC) No 850/2004; Table S2), it could have been used illegally for agriculture. In addition, both OCPs were reported to be indiscriminately used to control insects and parasites (Li, 1999; WHO, 2015) in urban areas and surroundings. This may also explain the relation of both compounds with the presence of human residences and other urban infrastructures around the nest.

4.3. Temporal trends and regulations

Blood can be considered a useful matrix to evaluate recent exposure to contaminants such as OCPs (Martínez-López et al., 2009). Thus, the decreasing concentrations of OCPs in blood of Eurasian Eagle-owl nestlings during the study period could reflect the variations in farming practices due to European OCP regulation. However, the declines of Σ endosulfan and lindane were more evident one or two years before the definitive ban. This could be attributed to the fact that pesticide companies tend to promote the sale of alternative products one or two years before the ban. Thus, farmers would prove the applicability of the new products and will probably continue using them after the definitive prohibition. Unfortunately, no data about the sales and uses of insecticides during the study period are available to test this hypothesis.

Lindane was banned for agricultural use in 2000 (Decision, 2000/801/EC), but was still allowed for professional remedial and industrial treatment of lumber, timber and logs and for indoor industrial and residential applications until 2006. Moreover, until 2007, technical HCH was allowed for use as an intermediate in chemical manufacturing and products with > 99% of lindane for public health and veterinary topical insecticide (Regulation (EC) No 850/2004). Hence, in spite of the rapid metabolism of lindane in birds, traces in the environment could be still found. Ban of aldrin, endrin and heptachlor took part as early as in 1994 in Spain (Orden de 4 de Febrero de 1994), but dieldrin has shown long persistence in soil (Martijn et al., 1993).

Endosulfan was only recently banned in the study area (Decision, 2005/864/EC) which would explain that it could still be detected in our study, in spite of its low persistence in the organisms (Wiemeyer, 1996). According to the ban, authorisations for plant protection products containing endosulfan were withdrawn by 2 June 2006 but some products used for hazelnut, cotton or tomato crops could be authorised until 2007 in Spain. This could explain the detection of quantifiable concentration of this compound in our samples until 2005.

P,p'-DDE is the most stable and toxic metabolite of *p,p'*-DDT and the most frequently found in tissues from living beings and in the environment (Turusov et al., 2002). In Spain, *p,p'*-DDT was widely used in agricultural applications but, similarly to other European countries, it was restricted in the late 1970s and finally banned in 1994 (Orden de 22 de marzo de, 1971 and Orden 4 de Febrero de 1994). Due to this ban, concentrations of diphenylaliphatics are expected to show a decreasing trend. However, stabilizing or even increasing concentrations have been found in other studies (Bustnes et al., 2007; García-Fernández et al., 2008; Gómez-Ramírez et al., 2012). A possible hypothesis for this current input has been related to the use of dicofol (Martínez-López et al., 2007; García-Fernández et al., 2008; García-Heras et al., 2018), an insecticide recommended for citrus farming in our study area during the study period (Orden de 1 de Febrero de 2006; Resolución de 28 de Marzo de 2007) and permitted until 2009 (Decision, 2008/764/EC). Because DDT is an intermediate product in the synthesis of dicofol, a small fraction of DDT-related compounds can be found in the formulations of this insecticide (Council Directive 90/533/EEC), probably justifying its presence in the environment (Qiu et al., 2005). However, the decreasing levels of DDT through the study period may be attributed to an abandonment of agricultural labours, especially on lemon trees (CARM, 2010; Gómez-Ramirez et al., 2012).

Despite all this, we must also assume that, although many of these compounds have been technically banned in a given year, many farmers may have continued using their stocked and stored products, and therefore the reduction of environmental concentrations may be delayed after the ban. This could be represented in outlier concentrations detected in some individuals during the banned period. This suggests that Eagle-owl could be used as an early sentinel of use of restricted OCPs.

4.4. Owls as sentinels

Our results suggest that the Eurasian Eagle-owls can be considered an effective sentinel species to study spatiotemporal differences in pesticide use at local scale. Birds of prey are generally considered suitable sentinel animals for biomonitoring of environmental contaminants (Gómez-Ramírez et al., 2014). Previous studies in the same species and study area found that levels of metals in blood of nestlings were higher in ancient mining sites or close to industrial activities (Espín et al., 2014a, 2014b; García-Fernández et al., 1995; Gómez-Ramírez et al., 2011). In addition, one of the aims of these biomonitoring studies is to assess the efficiency of policies and legislative instruments to address environmental contamination in terms of bioavailability (Gómez-Ramírez et al., 2014). In this sense, several studies have shown decreasing concentrations of contaminants such as *p,p'*-DDT and its metabolites (*p,p*-DDE and *p,p*'-DDD) in eggs or tissues from raptors from South Greenland (Vorkamp et al., 2009), UK (Newton and Wyllie, 1992) or Norway (Bustnes et al., 2007) or lead in livers of Kestrels from rural and city regions of South-eastern Spain and in feathers of Tawny Owls in Norway (Bustnes et al., 2013; García-Fernández et al., 2005).

4.5. Conclusions

The rapid decrease in levels of OCPs in Eurasian Eagle-owl blood related to ban of these compounds and land use may indicate the sensitivity of this species and matrix (blood) to indicate environmental changes, and therefore, its value as a sentinel species to biomonitoring pesticides. This has allowed us to confirm that the implementations of new regulations about the use of phytosanitary products used in agricultural practices can cause a very rapid effect on the ecosystem, as has been shown by the drop in quantifiable OCP concentrations in a sentinel species, which is also found at the top of the trophic web.

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Appendix A. Supplementary data

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