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Blood concentrations of *p,p'*-DDE and PCBs in harriers breeding in Spain and Kazakhstan

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Abstract

Organochlorine compounds (OC) are of interest in current biomonitoring studies because of their well-known persistence, accumulation capacity and the adverse effects they caused in the past. *P,p'*-DDE has been shown to cause severe reproductive failures and population declines in birds of prey. However, there are knowledge gaps regarding OC exposure for some species (e.g. harriers) and to the historical record and the broader picture. The main goal was to evaluate exposure to *p,p'*-DDE and PCBs in two raptor species: Montagu's and pallid harriers (*Circus pygargus* and *Circus macrourus*), and to investigate if birds from different breeding areas and wintering grounds differ in pollutant levels. For this purpose, we collected blood of adult and nestling Montagu's and pallid harriers breeding in the natural steppes of Kazakhstan, and adult and nestling Montagu's harriers breeding in agricultural and natural habitats of Spain, in 2007-2008. We determined the blood concentrations of *p,p'*-DDE and PCBs. Adult harriers generally showed higher concentrations of *p,p'*-DDE and PCBs than nestlings, probably because they had more time for a progressive accumulation of these compounds due to a higher intake than excretion rate. The *p,p'*-DDE concentrations in adults were equivalent in all the studied areas. The ratio *p,p'*-DDE/PCB 153 was higher in adults than in nestlings, suggesting that a portion of the *p,p'*-DDE in adult harriers may have come from *p,p'*-DDT applied in the past in the wintering areas. Overall, the concentrations of *p,p'*-DDE and ∑PCBs reported were generally low and below any demonstrated threshold of harm.

Keywords: DDE; PCBs; migratory birds; raptor; Montagu's harrier; pallid harrier

1. Introduction

Current biomonitoring programs using raptors to evaluate organochlorine compounds (OC) exposure and related effects are frequent in many areas, including Europe (Gómez-Ramírez et al., 2014), where results suggest that the priority compounds of interest remain persistent organic pollutants (POPs), including pesticides such as dichlorodiphenyltrichloroethanes (DDTs) and pollutants such as polychlorinated biphenyls (PCBs) (Espín et al., 2016). This is mainly due to their well-known persistence, their accumulation capacity and the adverse effects they cause on the immune, endocrine, nervous and reproductive systems in birds (e.g. Walker, 2003; Letcher et al., 2010; Milton et al., 2011; Tanabe, 2002; Fry, 1995). Although the use of OCs is restricted or banned in most countries, they are frequently detected in wild birds, particularly predators (Luzardo et al., 2014; Ortiz-Santaliestra et al., 2015; Sonne et al., 2010) because persistent residues can be transferred, bioaccumulated and biomagnified along food chains (Furness, 1993). Nowadays, *p,p'*-DDT is the only OC still recommended by the WHO for disease vector control because of the absence of equally effective and efficient alternatives (WHO, 2011), and there is ongoing legal use of *p,p'*-DDT in countries such as India (82% of the global use of DDT in the period 2000-2009) and some African countries (Ethiopia contributing 11.3%; van den Berg et al., 2012; Bouwman et al., 2013). In addition, despite decades of regulation, the levels reported in the environment and in wild birds could suggest an illegal use of OCs in different countries or a contamination from the legal use of dicofol formulations that may contain up to 0.1% of DDTs (Abbasi et al., 2016; Ali et al., 2014; Gómara et al., 2008). Furthermore, even when declining *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) concentrations are expected from the ban on *p,p'*-DDT use, dietary shifts in raptor species (e.g. a reduction of rabbit population may produce an increase in the biomass consumption of Passeriformes, which are known to accumulate higher levels of OC) could have noticeable consequences on *p,p'*-DDE exposure (Mañosa et al., 2003).

Almost all the biomonitoring programs using raptors are restricted to specific areas (e.g. western countries in Europe, USA and Canada) and a huge data gap exist for countries in Asia, Africa and South America (Abbasi et al., 2016; Gómez-Ramírez et al., 2014). One of these areas is Kazakhstan, where, to the best of our knowledge, there is only a single study reporting OC concentrations in a bird species, the saker falcon (*Falco cherrug*) (Kenward et al., 1998). Furthermore, pollutant concentrations found in tissues of migratory species may reflect exposure in migration or wintering areas (Elliott and Shutt, 1993; Henny et al., 1982; Kunisue et al., 2002), so the study of contaminant accumulation in birds of prey wintering in potentially polluted environments may also help provide a more comprehensive overview of the OCs exposure worldwide.

The Montagu's harrier (*Circus pygargus*) and the pallid harrier (*Circus macrourus*) are two closely related ground-nesting migratory raptors of conservation concern. Both species breed in sympatry in northern Kazakhstan, where they use natural vegetation to breed (Terraube et al., 2009, 2010). The Montagu's harrier breeding range extends to western Europe, where the species commonly breed in farmlands (Arroyo et al., 2002). Therefore, harriers in Europe could be more exposed to legacy OC pesticides used for agriculture in the past. Montagu's and pallid harriers winter in either India or the Sahel/Ethiopia fringe in Africa (Terraube et al., 2012; Trierweiler et al., 2014), where they use agricultural areas for foraging, which also makes them vulnerable to farmland toxic compounds that were used there, including DDTs (Dhananjayan et al., 2011; Kunisue et al., 2003; Yohannes et al., 2014, 2017). Additionally, these species may be also exposed to PCBs, depending on the level of industrialization in their breeding and wintering grounds. Contaminant levels in harriers are not well-documented, but according to the International Action Plan for the pallid harrier (Galushin et al., 2003), the use of harmful pesticides is one of the threats for this Near-Threatened species. Further research evaluating the exposure to OC is required to assess spatial and temporal trends in concentrations of bioaccumulative chemicals and fill the knowledge gap for some species and geographical areas improving the historical record and the broader picture.

The main aim of this study was to evaluate exposure to *p,p'*-DDE and PCBs in breeding Montagu's and pallid harriers, and to investigate the role of the breeding habitat and wintering grounds as a source of variation in pollutant levels for these migratory birds. For this purpose, we carried out an extensive sampling to collect blood of adult and nestling Montagu's and pallid harriers breeding in the natural steppes of Kazakhstan, and Montagu's harriers breeding in agricultural and natural habitats of Spain, and determined the concentrations of *p,p'*-DDE and PCBs. Using this information, we tested for differences in levels of *p,p'*-DDE and PCBs between species and age-groups, considering the different wintering areas (Eastern Africa, India or Western Africa) and breeding habitat (agricultural and natural vegetation areas). Based on previous findings, we expect to find higher *p,p'*-DDE levels in populations using agricultural habitats as main breeding habitats (Spain) than in birds breeding in Kazakh steppes. We also hypothesize that the concentration of *p,p'*-DDE and PCBs will be higher in adults than nestlings. Due to the lack of information on concentrations of *p,p'*-DDE and PCBs in the wintering grounds, we cannot make clear predictions at this point, although differences in wintering areas and diet of each species during the non-breeding season will probably affect blood concentrations of *p,p'*-DDE and PCBs.

2. *Material and methods*

2.1. Species and study areas

The Montagu's harrier breeds from western Europe to central Europe across Russia and central Asia, wintering in either sub-Saharan Africa (European breeding populations) or the Indian subcontinent (Asian breeding populations) (del Hoyo et al., 1994; Trierweiler et al., 2014). This species is categorized as vulnerable or conservation-dependent in most countries of western Europe (Arroyo and García, 2007; Kitowski, 2002; Koks and Visser, 2002; Millon et al., 2004) and has a generalist diet, with birds dominating in all the monitored study areas, and small mammals, reptiles and insects being other prey (Terraube and Arroyo, 2011). In the wintering grounds, however, its diet mainly consists of insects (grasshoppers; Augiron et al., 2015).

The pallid harrier breeds mainly in the steppes of Asiatic Russia, Kazakhstan and north-west China (BirdLife International, 2015). Pallid harriers breeding in Kazakhstan can spend the winter either in Africa or in the Indian subcontinent, although satellite tracking showed that most pallid harriers from our study area wintered in Eastern Africa (Terraube et al., 2012). The pallid harrier is categorized as near threatened on the IUCN Red List (BirdLife International, 2015) and is a vole-specialist predator with a narrower trophic niche than the Montagu's harrier in their sympatric Kazakh breeding grounds (Terraube et al., 2011).

The study area in north-central Kazakhstan was in and around the Naurzum National Nature Reserve (Kostanay Oblast, 51° N, 64° E) located at the southern limit of Siberian forests and northern limit of Eurasian steppes. It is characterised by a mosaic of dry steppes, riverbeds, bushy areas and woodland patches

(Terraube et al., 2009). The two study species breed there in sympatry, using slightly different vegetation types for nesting (Terraube et al., 2009, 2010), but the same habitats (mainly steppes dominated with *Stipa lessingiana*) for foraging. All individuals from Kazakhstan (breeding adults and nestlings from the two species) included in this study were sampled from nests located in natural steppe areas.

The three study areas for Montagu's harrier in Spain were in Castellón, Extremadura and Galicia. The Montagu's harriers sampled in Castellón province (Eastern Spain, 40° N, 0° E) used Mediterranean scrub dominated by Kermes oak (*Quercus coccifera*) as nesting habitat. This habitat is dominant in the study area, but other vegetation types include mainly non-irrigated orchards and, secondarily, herbaceous crops (including cereal fields) and pine plantations (Limiñana et al., 2011). The Montagu's harriers sampled in Extremadura (Southwestern Spain, 38°30' N, 6°30' W) bred within cereal fields. Land-use of the study area consisted of a mosaic of non-irrigated herbaceous crops (dominated by winter cereal), vineyards, olive groves, and extensive pastures (Arroyo et al., 2009). Finally, the harriers sampled in Galicia (Northwestern Spain, 42°N, 7°40' W) bred in Atlantic-heathland shrubs dominated by *Ulex* sp., mixed with heather *Erica* sp., and pastureland. Traditional extensive pastoralism is the main land-use in this mountainous area but there is a sharp increase in recent years in the number of afforested parcels or maize crops to the detriment of shrubland (Tapia et al., 2016).

2.2. Sampling and measurements

A total of 153 blood samples of nestling and adult harriers were collected: 119 from Montagu's harriers (90 from the three areas of Spain and 29 from Kazakhstan) and 34 from pallid harriers in Kazakhstan. Most samples were collected in 2008, except 20 from Montagu's harriers (9 from Extremadura and 11 from Kazakhstan) that were collected in 2007.

Adult birds were caught using dho-gaza nets and lured using a decoy of a fox or owl placed at the proximity of active nests during the brood-rearing period (nestlings older than 10 days), which elicited the birds to attack the predator decoy and fall into the net. Samples from nestlings were collected during nest visits performed as part of population monitoring programs for both species. We measured harriers' body mass (to the nearest 1g), wing length (nearest 1mm) and tarsus length (nearest 0.1 mm) when possible (not all birds were measured due to logistical constrains). Nestlings of Montagu's harriers were aged based on body measurements, and sexed based on iris colour (Arroyo, 1995). Nestlings of pallid harriers were aged using wing-length growth curves for a similar sized species (hen harrier *Circus cyaneus*; Terraube et al., 2009), and were genetically sexed using the primers 0057F and 002R (Round et al., 2007).

Blood samples were collected by venipuncture of the brachial vein with a 0.5 mm-needle, and then placed directly into heparinized microhematocrit capillary tubes. Blood was then transferred into glass tubes filled with hexane for sample conservation at room temperature. The conservation of the blood sample directly into analytical grade hexane was selected because it was not possible to keep samples refrigerated nor frozen in Kazakhstan. Samples were transported to the laboratory within 1 to 2 months after collection and then were frozen at −20°C until organochlorine analysis.

When available, morphometric measurements were used for estimating individual body condition. For adult individuals, we used the scaled mass index (M) proposed by Peig and Green (2009), which has been used before in other raptor species (Ortiz-Santaliestra et al., 2015). This index is calculated as follows: $M = M_i (L_0/L_i)^\wedge b_{SMA}$, where Mi and Li are the body mass and tarsus length of individual i respectively, L_0 is the arithmetic mean tarsus length considering all individuals, and b_{SMA} is the quotient between the slope of the regression of the natural log-transformed body mass on the natural-log transformed tarsus length of each individual and the Pearson's correlation coefficient of such regression. For nestlings, we used the residuals from the linear regression of body mass against age as a condition index (this relationship was linear in the range of ages of our sampled nestlings, from 9 to 32 days old).

2.3. Organochlorine analyses in blood

Concentrations of PCBs and *p,p'*-DDE were determined in blood samples (40-80 µl) following the method previously described and validated in Mateo et al. (2012) that permits working with small blood volumes. This method is based on the extraction of blood samples with n-hexane and the clean-up of the extract with sulfuric acid. Organochlorine concentrations were measured by gas chromatography coupled to an electron capture detector (GC-ECD) equipped with a column HP-5 30m, 0.32mm, 0.25µm purchased both from Agilent Technologies. Pesticide-Mix 13, PCB-Mix 20 and PCB 209 purchased from Dr. Ehrenstorfer

(Augsburg, Germany) were used for calibration purposes and PCB 209 as an internal standard. Recoveries of the analysed compounds were calculated with blood samples of farm reared red-legged partridges (*Alectoris rufa*) spiked with 0.25, 0.5, 1 and 2 ng/ml (N=3 for each level). Cyclodienes and methoxychlor are completely lost in the clean-up step, but this method gives acceptable recoveries for DDTs and PCBs detected in the blood of harriers. The observed recoveries were (mean ± RSD) 95.5 ± 5.4% for *p,p'*-DDE, 112.2 ± 9.8% for PCB 153, 102.5 ± 7.3% for PCB 138 and 112.9 ± 13.6% for PCB 180. Limits of quantification of the detected OC were 0.04 ng/ml for *p,p'*-DDE and PCB 153, 0.03 ng/ml for PCB 138 and 0.01 ng/ml PCB 180.

2.4. Statistical analyses

All analyses were carried out using R (version 3.2.3). Average levels of *p,p'*-DDE and PCBs are given as geometric mean (GM), with the observed range (min-max) being also reported. We studied the combined effects of age (nestlings vs. adults), sex and zone (i.e. study area: Kostanay province in Kazakhstan, and Galicia, Extremadura and Castellón in Spain) on the blood concentrations of *p,p'*-DDE and PCBs of each species using Generalized Linear Mixed Models (GLMMs; package nlme; normal distribution and identity link function). The concentrations of *p,p'*-DDE and PCBs were log-transformed (x+1) using the natural logarithm to approximate a normal distribution. For Montagu's harriers, GLMMs were built including the concentration of *p,p'*-DDE and PCBs as the response variables and age, sex, zone and the two-way interactions between them as explanatory variables. Nest identity was included as a random factor in the models in order to account for the non-independence of data from nestlings sampled in the same nest. For pallid harriers, GLMMs were done including age, sex and the interaction between age and sex as fixed variables, and nest identity as a random factor. We used the Akaike's information criterion-approach (AIC) for selecting the best supported models. AIC-values corrected for sample sizes (AICc) and Akaike weights (w_i) were used for model comparisons. Model averaging was used to report the results from the best supported models (those with ΔAICc < 4). For statistical analyses, concentrations below the detection limit were substituted with a value equal to half the limit of quantitation (LOQ/2). When testing for differences between species in the concentration of *p,p'*-DDE and PCBs, GLMMs were done selecting the samples of Montagu's and pallid harriers breeding in Kazakhstan. The concentrations of *p,p'*-DDE and PCBs were the response variables, and the species, age, sex and the interactions between these three explanatory variables were selected as fixed factors, including nest as a random factor. One-way ANOVA and Tukey's test for post hoc pairwise comparisons were used to test for differences in PCBs and *p,p'*-DDE concentrations among zones and species for adults and nestlings separately.

To evaluate the effect of age (in days), body condition and zone on nestling *p,p'*-DDE and PCBs concentrations, GLMMs were built including *p,p'*-DDE or PCBs as the response variables and age (in days), body condition, zone and their interactions as explanatory variables for each species (zone was not included for pallid harriers). Nest identity was included as a random factor. An effect of the age on concentrations of *p,p'*-DDE and PCBs may not be found when most nestlings have reached more than half of their adult weight (Olsson et al., 2000). We thus conducted this analysis using only nestling Montagu's harriers from Kazakhstan for which data were available for a wider range of ages (9 to 32 days), and excluding zone from the models. Similar GLMMs were done in Kazakh adult individuals for each species, including the concentrations of *p,p'*-DDE and PCBs as the response variables and body condition, sex and their interaction as fixed factors, with nest identity as a random factor in the models.

The relative contribution of PCB-congeners was also compared among age groups (nestlings vs. adults), zones, and species with one-way ANOVA and Tukey's test. Pearson (r_p) or Spearman's (r_s) correlation coefficient was used to explore correlations between different OCs and between *p,p'*-DDE and PCBs and body condition depending on the normality of data (checked using Kolmogorov-Smirnov test). The level of significance for these tests was set at α=0.05.

3. *Results*

Overall, the OC found at the highest concentrations was *p,p'*-DDE, followed by PCB 153, 180 and 138 (Table 1). In terms of occurrence, *p,p'*-DDE was the most frequently detected (71.9%), followed by PCB 180 (40.5%), PCB 153 (37.9%) and PCB 138 (12.4%).

[Table 1 near here]

The best model explaining the concentration of ∑PCBs in pallid harriers included only the variable “age group” (adults had higher concentrations than nestlings; Table 2 and Table S1, Figure 1). For Montagu’s harriers, the best model included the interaction age x zone: nestlings had lower levels of ∑PCBs than adults, but the difference was statistically significant only in individuals from Extremadura (Table 2 and Table S1, Figure 1). The best model explaining *p,p’*-DDE levels in both species only included the variable age group: adults had higher levels than nestlings (Table 2 and Table S1, Figure 1). The ratio of *p,p’*-DDE to the more persistent PCB 153 was higher in adults (Table 1).

[Table 2 near here]

[Figure 1 near here]

One-way ANOVA tests for levels of ∑PCBs in Montagu’s harriers showed significant differences among zones for nestlings ($p < 0.001$, one-way ANOVA tests; nestlings from Kazakhstan had higher levels of ∑PCBs than those from Spain, see Tukey’s test in Figure 1) and for adults ($p < 0.001$, one-way ANOVA tests; adults from Extremadura and Kazakhstan had higher levels of ∑PCBs than those from Galicia, Figure 1). For *p,p’*-DDE, there were no marked differences among zones in adult Montagu’s harriers ($p = 0.114$, one-way ANOVA tests, Figure 1), while significant differences were found in nestlings ($p < 0.01$, one-way ANOVA tests; nestlings from Castellón had higher *p,p’*-DDE levels than those from Extremadura and Kazakhstan, Figure 1). We observed a negative relationship between body condition and *p,p’*-DDE concentrations in adult Montagu’s harriers from Kazakhstan ($r_P = -0.70$, $p = 0.007$, Figure 2).

[Figure 2 near here]

When comparing both species in the same zone (Kazakhstan), GLMMs showed that the best model explaining the concentration of ∑PCBs included age and species (Table 3 and Table S2); nestling pallid harriers had lower concentration of ∑PCBs than nestling Montagu’s harriers ($p = 0.019$, one-way ANOVA; Table 1, Figure 1). These differences in the concentrations of ∑PCBs resulted in a different DDE/PCB 153 ratio in nestlings of the two species in Kazakhstan (0.7 in Montagu’s harrier vs. 3.7 in pallid harrier, Table 1). For *p,p’*-DDE concentrations, only the age group was kept in the best model (Table 3 and Table S2), and levels were very similar for Montagu's and pallid harrier nestlings ($p = 0.57$, one-way ANOVA) or adults ($p = 0.34$, one-way ANOVA; Figure 1). No effect of sex was found in any of the previous models.

[Table 3 near here]

Considering all data together, significant correlations were found between the levels of *p,p'*-DDE and ∑PCBs ($r = 0.44$, $p = <0.001$, $n = 153$) and among the concentrations of PCB congeners ($r_S = 0.31\text{-}0.67$, $p < 0.001$, $n = 153$): individuals with more contaminants of one type were also more contaminated with the other ones.

4. *Discussion*

The observed concentrations of *p,p'*-DDE and ∑PCBs in blood of Montagu's and pallid harriers were relatively low, but differed in some compounds between study areas, species and age classes. Here we discuss how differences in diet or migratory routes can help in the interpretation of these results.

4.1. PCBs and p,p'-DDE: effect of age, differences between study areas and species

Adult harriers generally showed higher concentrations of *p,p'*-DDE and ∑PCBs in blood than nestlings. The most likely explanation for this result is that they have had more time, being older, for a progressive accumulation of these persistent compounds due to a higher intake than excretion rate (Goutner et al., 2011; Ortiz-Santaliestra et al., 2015). The data showed that the ratio of *p,p'*-DDE to PCB 153 is three to eight times higher in adults than in nestlings. PCB 153 and *p,p'*-DDE are very similar in both their physical-chemical properties and in their environmental stability (PubChem, 2017). Thus, a plausible interpretation is that a portion of the *p,p'*-DDE had come from the wintering areas, whether in Africa or India. Although there is ongoing legal use of *p,p'*-DDT in India and some African countries (van den Berg et al., 2012; Bouwman et al., 2013), the parent compound *p,p'*-DDT was not detected in the present study, suggesting that most of the *p,p'*-DDE therefore is derived from *p,p'*-DDT applied in the past. In addition, although it was not possible to measure the blood lipid content due to a very limited amount of blood, age-related differences in blood lipids could also explain the differences in the concentrations of PCBs between age groups. Moreover, diet composition bears significant influence on the concentration of contaminants in top predators (Espín et al., 2014; Mañosa et al., 2003; Palma et al., 2005), particularly when there are no ongoing points or important regional sources of contamination that may mask the effect of the trophic level (Elliott et al., 2009). In this sense, nestling Montagu's harriers in Kazakhstan had higher concentrations of ∑PCBs than nestling pallid harriers and Montagu's harriers in Spain. These differences resulted in a different DDE/PCB 153 ratio in nestlings from Kazakhstan (0.7 in Montagu's harrier vs. 3.7 in pallid harrier). This could be related to a recent ingestion of contaminated prey by Montagu's harrier nestlings in Kazakhstan, since PCB levels in the blood of raptors are known to vary because of recent food intake (Olsson et al., 2000). Furthermore, in Kazakh study area a very low vole abundance was reported in 2007, and Montagu's harriers targeted mainly lizards, passerine birds and insects (Terraube et al., 2011). Adults can discriminate between the prey they feed themselves and those they feed their nestlings (Catry et al., 2016, Rey et al., 2012). Thus, adults could have brought to the nest a higher proportion of the most energetically valuable prey, i.e. birds, to feed the nestlings. Most harrier's bird prey in Kazakhstan are long-distance migrants wintering probably in India where they are more susceptible to PCB contamination: a higher atmospheric contamination by PCBs in India than in Western and Eastern African countries has been reported (Chakraborty et al., 2013; Gioia et al., 2011; Klánová et al., 2009).

Additionally, adult harriers from Extremadura and Kazakhstan had higher levels of ∑PCBs than those from Galicia. This might be also related to different diet habits, although Galicia may have a lower level of environmental PCB contamination. In the breeding grounds, adult Montagu's and pallid harriers in Kazakhstan feed on the same prey types (Terraube et al., 2011) and are thus probably exposed to similar levels of PCBs. Montagu's harriers breeding in Kazakhstan winter in India, as do at least part of the Kazak breeding population of pallid harriers, while Montagu's harriers breeding in Spain winter in sub-Saharan Africa (del Hoyo et al., 1994; Trierweiler et al., 2014). India has higher atmospheric levels of PCBs than most African countries (Chakraborty et al., 2013; Gioia et al., 2011; Klánová et al., 2009). This could explain the higher levels of ∑PCBs detected in harriers wintering in the Indian subcontinent. Regarding adult harriers breeding in Extremadura, a variation in the diet composition in that area in the study year or a recent ingestion of contaminated items could explain the higher concentration of ∑PCBs.

Unlike with PCBs, adult harriers showed very similar *p,p'*-DDE concentrations throughout the different sampling areas in Spain and Kazakhstan. The legal use of the pesticide DDT was banned in Spain in 1977 (BOE, 1975), but its main metabolite *p,p'*-DDE may persist during years in the environment and is frequently detected. The lack of an obvious geographical trend may reflect a decreasing use of this compound in agricultural environments and its degradation over time, or else that contamination occurs mainly in the wintering grounds, and that DDE contamination levels in Africa and India are similar. Although we collected samples from both 2007 and 2008, due to the sampling design the inter-annual differences in *p,p'*-DDE and PCBs concentrations cannot be addressed in this study.

We also found a negative relationship between *p,p'*-DDE concentrations and body condition in adult Montagu's harriers from Kazakhstan, although not from other areas. The sampling of adult Montagu's harriers from Kazakhstan was done in years of low and intermediate vole abundance (Terraube et al., 2011) and abnormally cold weather in spring 2007. Food restrictions and adverse conditions at the arrival on the breeding grounds may have consequences in body weight and condition and associated mobilization of fat reserves, the major storage depot of OCs of the animal (Drouillard et al., 2001), with the subsequent remobilisation of lipophilic *p,p'*-DDE and its distribution via the bloodstream to highly metabolically active organs (Hela et al., 2006). This could explain that, in adults, a worse body condition was associated with higher *p,p'*-DDE concentrations in blood.

After completing a long migration journey to the breeding grounds, birds may be in a comparatively lean condition and lipophilic substances in females may be readily excreted from body tissues into egg yolk (Espín et al., 2016). Thus, several studies have suggested that adult females have lower OC concentrations than males, probably due to the transference of these compounds from mother to egg (e.g. Bustnes et al., 2008). However, no effect of gender on the concentration of *p,p'*-DDE and PCBs was found in this study, which may indicate a lower investment in the egg from body reserves (and higher from recently ingested food) in comparison with other bird species. Raptors are often classified as income breeders as they are largely thought to rely on food intake rather than reserves (i.e. capital breeders) to service the energetic costs of breeding (Durant et al., 2000), and so egg concentrations in raptor eggs most likely reflect exposure within breeding territories (Espín et al., 2016). The influence of egg residues on blood concentrations in chicks has been reported to be most pronounced in small chicks and decrease with age and weight due to growth dilution (Charnetski, 1976; Lemmetyinen et al., 1982). In the present study, *p,p'*-DDE and PCBs in blood did not decrease with age in nestlings, probably because most of the nestlings had reached more than half of their adult weight when the samples were collected, because contaminants in the egg were relatively small, or because dietary intake of these contaminants compensate the dilution by growth of maternally transferred contaminants (Olsson et al., 2000).

4.2. Comparison with other studies and toxicological significance

We are unaware of any other studies reporting blood OCs levels in Montagu's or pallid harriers, but similar works have been conducted on other raptors (Table 4). The concentrations of *p,p'*-DDE and PCBs reported in this study are generally lower than levels found in nestling and adult individuals of other *Accipitridae* species from different regions (see Table 4 for an overview). Studied species differ in their feeding habits, migratory behaviours and geographical locations. Moreover, most data are from studies that were conducted ca. 2-3 decades ago (1979-1998), when these legacy OCs were present in considerable concentrations in the environment, while a drop in their levels can be expected in more recent studies. The similar patterns of blood *p,p'*-DDE concentrations found in adult harriers throughout the different sampling sites and the positive correlation found between *p,p'*-DDE and ∑PCB concentrations suggest that the body burden in birds responds to the global distribution of these OC and the studied areas are not impacted by major local point sources. Therefore, this reduces local spatial variability and other parameters such as age, trophic level and diet sources become important factors influencing concentrations of the legacy *p,p'*-DDE and ∑PCBs (Elliott et al., 2009). In general, the concentrations of *p,p'*-DDE and PCBs reported for harriers in this paper were comparable to those reported in particular zones by different authors that inferred that such low exposure levels and concentrations may not be necessarily associated with deleterious effects on survival or reproduction (Goutner et al., 2011; Martínez López, 2005; Ortiz-Santaliestra et al., 2015; Smith and Bouwman, 2000). However, a recent study on nestling black harrier (*Circus maurus*) found that plasma ΣDDTs (*p,p'*-DDE + *p,p'*-DDT) levels averaged 1.9 ± 2.0 ng/ml (min-max: nd-9.8 ng/ml), and DDT concentrations were negatively associated with circulating carotenoid levels and coloration (García-Heras et al., 2017a). In addition, the white blood cell count increased with higher DDT concentrations, indicative of a compensatory response of the immune system in exposed nestlings (García-Heras et al., 2017b). In sampled nestlings in the present study, *p,p'*-DDE concentrations averaged 1.3 ± 2.0 ng/ml (min-max: nd-9.8 ng/ml). Therefore, a *p,p'*-DDE-related disruption on carotenoid-based signalling cannot be discarded, although diet is also an important factor affecting carotenoid levels (García-Heras et al., 2017a).

[Table 4 near here]

It is well known that *p,p'*-DDE is strongly associated with eggshell thinning and decreased productivity in different bird species (Blus, 2011). Henny and Meeker (1981) proposed a method to adjust plasma residues from laying females of four species of birds of prey (American kestrels, *Falco sparverius,* and accipiters) to the estimated residues in eggs for the purpose of residue interpretation [$\sum$DDT in egg contents (µg/g ww) = 6.243 ($\sum$DDT in plasma, µg/g ww)$^{1.033}$]. The application of this predictive equation should be interpreted with caution because of species differences, but may give some insights for species without any prior information, such as harriers. Assuming that the concentration in blood is equivalent to the concentration in plasma, the observed *p,p'*-DDE concentrations in adult Montagu's and pallid harriers (geometric means ranging from 2.6 to 6.6 ng/ml depending on the area) would result in an estimated 0.013-0.035 µg/g in eggs. These concentrations are lower than those reported in Montagu's harrier eggs from Voronezh, Russia (0.45 µg/g; Henny et al., 1998). It has been suggested that the lowest-observable-adverse-effect level (LOAEL) for fertility is 3.5 µg/g (ww) in Spanish Imperial eagle (*Aquila adalberti*) eggs (Hernández et al., 2008), and the non-observable-adverse-effect level (NOAEL) falls between 1 and 2 µg/g (ww) in goshawk (*Accipiter gentilis*) eggs (Mañosa et al., 2003). In general, DDE residues in eggs from 1 µg/g (ww) upwards are associated with eggshell thinning in different wild bird species (Blus, 2011). Although we do not know if Montagu's and pallid harriers respond at the same level of DDE as other raptor species do, in general the estimated egg concentrations are much lower than 1 µg/g, suggesting that DDE concentrations may not be impairing eggshell thickness in the study populations. Only three adult Montagu's harriers from Extremadura presented high blood *p,p'*-DDE concentrations (63-94 ng/ml) that would correspond to DDE levels in eggs (0.36-0.54 µg/g) still below 1 µg/g (although this should be interpreted with caution due to the inter-specific differences in tolerance to OCs).

5. Conclusions

The present study reports for the first time baseline data on blood concentrations of *p,p'*-DDE and PCBs in harriers from Spain and Kazakhstan that will be useful for comparative purposes in future monitoring studies. The *p,p'*-DDE concentrations in adults were equivalent in all the studied areas. The ratio *p,p'*-DDE/PCB 153 was higher in adults than in nestlings, suggesting that a portion of the *p,p'*-DDE in adult harriers comes from *p,p'*-DDT applied in the past in the wintering areas, whether in Africa or India, with no evidence of recent *p,p'*-DDT use in those areas. Overall, the concentrations of *p,p'*-DDE and ∑PCBs reported are generally low, and *p,p'*-DDE levels are below any demonstrated threshold of harm. While resident birds directly reflect the specific local pollution, interpreting blood concentrations in migratory species may pose some difficulties when faced to limited literature.

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Table captions

Table 1. Geometric means and range (min-max) of PCBs and *p,p'*-DDE levels (ng/ml, ww) in the blood of Montagu's harriers and pallid harriers from Spain and Kazakhstan.

Table 2. Conditional model-averaged parameter estimates (considering only the set of models with ΔAICc<4) describing the variables affecting variation in PCBs and DDE concentrations in Montagu's and pallid harriers.

Table 3. Conditional model-averaged parameter estimates (considering only the set of models with ΔAICc<4) describing the variables affecting variation in PCBs and DDE concentrations in harriers from Kazakhstan.

Table 4. Overview of the PCBs and *p,p'*-DDE concentrations reported in blood samples from Accipitridae species from different areas, at time periods and of different age groups (nestlings versus adults).

Table S1. Model selection results (ΔAICc<4) for variables explaining variation in PCBs and DDE concentrations in Montagu's and pallid harriers.

Table S2. Model selection results (ΔAICc<4) for variables explaining variation in PCBs and DDE concentrations in harriers from Kazakhstan.

Figure captions

Figure 1. Mean **(**± SE) blood concentrations of *p,p'*-DDE and ∑PCBs (ng/ml, wet weight, natural log(x+1)-transformed values) in Montagu's harriers (CP) from Spain and Kazakhstan and in pallid harriers (CM) from Kazakhstan according to age group (nestlings: white dots; adults: black dots). Different letters above the error bars denote significant differences between areas for Montagu's harrier, and different letters below error bars indicate significant differences between species (in Kazakhstan only). Uppercase and lowercase letters are used to highlight differences in adults and nestlings, respectively. Tukey's test was used for post hoc pairwise comparison ($p < 0.05$).

Figure 2. Relationship between body condition and *p,p'*-DDE blood concentrations (ng/ml, wet weight; natural log(x+1)-transformed) in adult Montagu's harriers (*C. pygargus*) from Kazakhstan.