Blood concentrations of PCBs and DDTs in an avian predator endemic to southern Africa: associations with habitat, electrical transformers and diet

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Abstract

Persistent pollutants such as organochlorine compounds (OCs) have been highlighted as a cause of population decline in avian predators. Understanding patterns of OCs contamination can be crucial for the conservation of affected species, yet little is known on these threats to African raptors. Here we report on OC concentrations in an endangered predator endemic to southern Africa, the Black Harrier *Circus maurus*. Blood samples were collected in 2012-2014 from wild nestlings (n= 90) and adults (n= 23) in south-western South Africa, where agriculture and urbanization have rapidly developed since the 1950s. Polychlorinated biphenyl (ΣPCB) and dichlorodiphenyltrichloroethane (ΣDDT, for p,p'-DDT + p,p'-DDE) were detected in 79% and 84% of sampled individuals, respectively, with varying concentrations among demographic groups: nestlings had significantly higher ΣPCB and p,p'-DDT concentrations than adults, while adults had higher levels of p,p'-DDE than nestlings. Levels of ΣPCB significantly increased with an index of electric transformer density, which combines the number and power of electric transformers around active nests. We propose this index as a useful tool for assessing ΣPCB exposure risk in wildlife. Levels of p,p'-DDE significantly increased with the proportion of wetlands within the breeding territory, and also with the proportion of bird biomass in the diet. No association was found between OC levels and the protected area status of nesting sites. Physiological effects of contaminants were also manifest. Higher white blood cell counts were found with higher p,p'-DDT levels. Heterophil to lymphocyte ratio also increased with higher ΣPCB levels, suggesting increased physiological stress and reduced immunity in contaminated individuals. Our results suggest that OCs are still be a current cause of concern for endangered Black Harriers, as well as other sympatric predators.

CAPSULE: Organochlorine levels in the plasma of Black Harriers from South Africa increased with the proportion of wetlands, density of electric transformers and ingested bird biomass, and were associated with sub-lethal physiological effects.

Introduction

Persistent organic pollutants (POPs), such as organochlorine pesticides (DDT and its metabolite DDE) and industrial products such as polychlorinated biphenyls (PCBs), have been detected in all ecosystems (Hoffman et al., 2003). These organochlorine compounds (OCs) are highly persistent, degrade slowly in the environment, and can affect areas far distant from their source of emission through Long Range Atmospheric Transport (LRAT) (Meijer et al., 2003; Roscales et al., 2016). This makes them highly toxic and prone to cause a number of adverse effects on wildlife and humans, even several decades after their withdrawal (Ortiz-Santaliestra et al., 2015). For instance, DDT, was widely used in agricultural areas and wetlands since its invention in the late 1940s, and has been well documented to be responsible for the decline of many raptor populations (e.g. Cade and Burnham, 2003). Despite its ban in the early 1970s, residues from its former use are still found in the wild, with evident effects on wildlife in North America, Europe and Africa (Davies and Randall, 1989; Ortiz-Santaliestra et al., 2015; Gitahi et al., 2002; Bettinetti et al., 2011). Similarly, PCBs have been extensively and widely used since the 1930s for a broad range of applications, particularly in electrical equipment (Hoffman et al., 2003), and have also been found to negatively affect wildlife (Mateo et al., 2016). PCBs were banned in most of the world at the end of the previous century (the Stockholm Convention on POPs was adopted in 2001). However, PCB contamination still persists in African countries, notably because of leakage from, or inadequate disposal of, electric transformers, continued imports of electronic waste from northern countries, shipwreck or biomass burning (Gioia et al., 2014). PCBs may therefore still represent a threat to wildlife and humans in Africa.

Avian predators have been frequently used as bio-indicators of pollutant contamination in the environment (Fox, 2001; Olsson et al., 2000; Gómez-Ramírez et al., 2014) because they are more likely to accumulate them when ingesting contaminated prey (Furness, 1993; Newton et al., 1993; Ortiz-Santaliestra et al., 2015). POPs have been associated with a range of physiological effects in birds, e.g. they affect blood clinical-chemical parameters that disrupt endocrine functions (Sonne et al., 2012). POPs also decrease haemoglobin levels and produce anaemia (Rivera-Rodríguez and Rodríguez-Estrella, 2011), impair immune functions (Grasman et al., 1996; Bustnes et al., 2004) or increase oxidative stress (Wayland et al., 2010; Ortiz-Santaliestra et al., 2015). In terms of immune function, a relative increase in WBC number may be indicative of a response to infection by the immune system, while an increase of the heterophil to lymphocyte ratio (H:L ratio) may be indicative of increased physiological stress and reduced immunity (Siegel, 1985; Ots et al., 1998; Norris and Evans, 2000; Mougeot et al., 2005; Suri et al., 2016). In this context, relating OC levels to WBC or H:L ratios in blood may give an indication of sublethal effects of contaminant exposure.

To identify the possible sources of contamination one must explore associations between OC levels and diet composition or environmental variables. For example, relationships between OC levels and farmland or wetland area may arise if pesticides are sprayed against invertebrate pests in agricultural crops or against mosquitoes in wetlands, where OCs are known to bio-accumulate for years (e.g. Hoffman et al., 2003). Furthermore, relationships between PCB levels and urbanization or industrialization may be found given their use in electrical transformers (Gioia et al., 2014).

In southern Africa, most studies on OCs have been conducted either using unhatched eggs (Davies and Randall, 1989; Bouwman et al., 2008, 2013, 2015) or tissues and organs collected from dead animals (van Wyk et al., 2001). Relatively little work has been published using samples collected from live individuals (van Wyk et al., 2001), which may give less biased information about levels in wild populations. Additionally, most studies investigating the relation between OC exposure and physiological condition in raptor species have been based on experimental work with captive individuals (Bortolotti et al., 2003), often due to the difficulties of accessing nests and capturing adults in natural habitats. However, a growing number of studies highlight the importance of addressing these questions in the wild. This will allow a better understanding of the complexity of the entire system, including the relationships between OC exposure, contamination by bio-accumulation and bio-magnification, the potential sub-lethal effects on individuals, and the implications for the conservation of target species (Rivera-Rodríguez and Rodríguez-Estrella, 2011; Ortiz-Santaliestra et al., 2015).

The Black Harrier *Circus maurus* is a ground-nesting medium-sized bird of prey, endemic to southern Africa. Its population size has been estimated at less than 1000 breeding individuals and the species is currently considered as Endangered in South Africa, Namibia and Lesotho (Simmons et al., 2015; Taylor, 2015). This scarce raptor breeds in indigenous vegetation of south western South Africa, essentially along the coast within the Fynbos biome, and inland within the Karoo biome (Curtis et al., 2004; Simmons et al. 2005; García-

Heras et al., 2016). Due to anthropogenic modification of land use during the second half of the last century, Black Harriers' natural breeding habitats have been reduced by 50%, and many nesting areas are now surrounded by agricultural or urbanized lands. Breeding Black Harriers may, therefore, be currently exposed to OCs from different sources. From 1945 until its withdrawal in the early 1980s, DDT was intensively used in South African agricultural lands, notably in maize and cotton crops. Evidence suggests that it was still used in agricultural crops after 1985 in south-western South Africa (Davies and Randall, 1989; Wells and Leonard, 2006), thereby overlapping the Black Harrier's core breeding range (Curtis et al., 2004; García-Heras et al., 2016). Furthermore, PCBs were still used in electrical transformers as cooling and isolating products at least until 2010 in South Africa (Ministry of Water and Environmental Affairs, 2011). Additionally, given that Black Harriers are known to regularly consume birds, despite being small mammal specialists (García-Heras et al. 2017a,b), they may be exposed to higher OC levels as birds bio-accumulate more OCs than mammals (Fossi et al., 1995). Because a recent study of mitochondrial DNA in adult Black Harrier feathers indicated very low genetic diversity (Fuchs et al., 2014), Black Harriers may be particularly vulnerable to negative effects of pollutants or pathogens. To date, however, there is a lack of knowledge about exposure to pollutants (specifically PCBs and DDTs) and whether these may be affecting Black Harriers.

The main goals of this study were: 1) to assess the occurrence and patterns of OCs contamination in the Endangered Black Harrier population, 2) to identify correlates of OC concentrations (i.e. habitat types within the territories, electric transformer density, and diet composition) to assess sources of contaminations for Black Harriers and 3) to assess whether the detected OCs may affect the physical (i.e. body condition index) or physiological condition (i.e. WBC number and H:L ratio) of individuals. To address these goals, we collected blood samples from wild nestling and adult Black Harriers. We discuss the conservation implications for this endangered raptor species.

Material and methods

Study area

Fieldwork was conducted in South Africa between July and December 2012-2014, in two main regions: along the coast of the Western Cape Province in an area north of the city of Cape Town (-33.700° S, 18.45° E; -33.133° S, 18.083° E), and inland in the Northern Cape Province in the Nieuwoudtville area (-31.316° S, 19.083° E). Nests were located in and around National Parks (South African National Parks), Provincial Protected Reserves (Cape Nature) or on private lands (see García-Heras et al., 2016 for details of nest locations). The mild and temperate climate of the coastal region (Mucina and Rutherford, 2006; Manning, 2007; García-Heras et al., 2016) has contributed to a rapid development of cereal agriculture, viticulture and urbanization in this region. This includes human population expansion to 4 million inhabitants and the presence of the only nuclear power station in Africa since the 1950s. Outside this urbanized environment, large tracts of natural vegetation remain, mainly in protected areas (Curtis et al., 2004; García-Heras et al., 2016). By contrast, the inland region is more rural, sparsely populated (i.e. < 15000 inhabitants) with an old and widespread tradition of agricultural lands and sheep farming, where natural vegetation is highly fragmented (Reyers et al., 2009).

Habitat parameters

OC levels were monitored in adults and nestlings from a total of 49 nests during the study period. Coordinates for these 49 nests were incorporated in a geographical information system (QGIS Valmiera 2.2.0), projected onto WGS84-UTM-34S coordinate reference system. Using the GIS, we first created a 5 km radius buffer around each nest, hereafter the "breeding territory". This corresponds to the average home range of an individual, as estimated from data from 12 GPS-tagged adult Black Harriers (Authors, unpublished data). Within this buffer we identified and calculated for each nest the following three variables as potential sources of OC exposure for Black Harriers: i) Proportion of agricultural land cover, as the potential source of pesticide contamination; ii) Proportion of wetland cover as a potential source of OCs contamination by bioaccumulation in the sediments; iii) An index of the density of electrical transformers (hereafter "Transformer Density Index") as a measure of potential source of PCBs in the environment. Our index was based on the number and power of electrical transformers within the 5 km buffer area. It was calculated as the sum of the kilovolt-ampere or kVA-rating of all the transformers within the 5 km area, divided by the land surface area (kVA/ km^2). The Transformer Density Index ranged from 0 to 227.9 kVA/km^2 depending on nesting sites and averaged 18.7 kVA/km^2. The land use data were obtained from the South African National Land Cover Map (NLC) 2014. The electric transformer data were obtained from the Electricity Supply Commission of South Africa (Eskom's) 2014 shapefiles in GIS.

Finally, each nest was attributed a "protected area status" to test for possible differences in OC levels in and outside protected areas. Nests located inside national parks or natural reserves were considered as "protected" (n= 21), whereas all others were considered "not protected" (n= 28).

Diet assessment

We estimated diet composition at each of the 49 monitored nests through the analysis of regurgitated pellets collected at the nest during the breeding season. Pellet contents including prey remains such as bones, scales, feathers or fur were analysed and identified following the methods described in García-Heras et al. (2017a). Additionally, in 2014, cameras were set at 18 active nests during the nestling period, i.e. when chicks were 7-41 days old. Cameras were programmed to take a picture every 5 seconds, or to record a 60-second video sequence (1 second between two videos), and were set from sunrise until sunset, e.g. 06h00 – 19h59. We obtained a total of 1488.3 hours of recording time (82.7 ± 35.9 hours per nest, range 15.5–142.1 hours). Images and video footage were analysed to identify the type of prey delivered, categorized as small mammal, bird, reptile or unidentified prey item. Data on prey types from pellets and cameras were combined to identify the percentage of each prey type among all identified prey items for each monitored nest. The number of identified prey per nest varied from 1 to 107; a bootstrapping analysis (see Supplementary Material) indicated that a minimum of 10 identified prey was needed to obtain an unbiased estimate of diet composition: both the average values of the proportion of each prey type and their standard deviations converged from 10 identified prey. We used this as our minimum sample. This reduced our sample to 30 nests with at least 10 identified prey to estimate diet composition. Among those nests, the average number of identified prey was 48.4 ± 0.18. Thereafter, the proportion of biomass of each category of prey was estimated as described in García-Heras et al. (2017a). Because the contribution of reptile biomass among the three prey types was so small (~5%), we found that the proportion of bird biomass was strongly negatively related to the proportion of small mammal biomass (Pearson correlation: $r= -0.98$, $p< 0.0001$, $n= 30$).

Therefore, to simplify the analyses, we only used the proportion of bird biomass as indicator of diet composition (García-Heras et al., 2017b).

As with other raptor species, female Black Harriers take care of nestlings at the nest and perform all brooding while the male captures and provides the food in the early nestling period (Simmons, 2000; Redpath et al., 2002). Therefore, females and nestlings feed on the same prey that is provided by males, and we assume that males' diet is likely to be similar to that provided to the nest. We therefore attributed the same diet composition to all members of a given sampled nest.

Field procedures and sample collection

When nestlings were 15-39 days old, we attempted capturing breeding adults using a Dho Gaza net and a mounted Spotted Eagle Owl *Buteo capensis* placed 20-30 m from the nest to simulate a predator intrusion. This elicited mobbing by the adults defending the nestlings. Adults captured in the net, and nestlings at the nest were weighed and measured and individually marked with a metal ring. We measured tarsus length (to the nearest 0.1 mm, using an electronic calliper), and body mass (to the nearest 5-10 g, using a Pesola spring balance). We calculated a body condition index using: i) for nestlings, the residuals from the relation between the body mass and the age, calculated for each sex separately; ii) for adults, the residuals from the relation between weight and tarsus length, as an indicator of size.

Blood samples were taken to determine (i) Organochlorine Compounds (OCs) contamination, (ii) white blood cell counts and (iii) sex using DNA analysis. Each blood sample of 0.7-1 ml was collected from the brachial vein using a heparinized syringe. A drop of fresh blood was deposited and smeared on a glass slide, before being fixed in methanol and dried. The rest of the blood was kept in a heparinazed Eppendorf vial in a polystyrene cool box filled with ice blocks. Within 30-40 min after collection, the samples were centrifuged for 15 min using a Ministar portable centrifuge (VWR, Radnor, Pennsylvania) to separate the plasma from the red cells (i.e. hereafter "blood pellet"). Both set of samples were immediately placed in a portable freezer, and frozen at -80°C on arrival at the lab from the field (< 3 hours after collection) until analyses. Plasma samples were used to quantify the concentrations of OCs. While adult harriers were sexed morphometrically (Simmons et al., 2005), nestlings were sexed genetically using DNA analysis of the blood pellet (see below).

After sampling, nestlings were replaced at the nest, and adults were freed at their place of capture within approximately 20 min. A total of 90 nestlings (n= 40 males, n= 50 females), and 23 adults (15 females and 9 males, of which 7 were breeding pairs) were sampled. The fieldwork protocols were approved by the University of Cape Town's science faculty animal ethics committee, Permit number: A1/2014/2013/V21/GC.

DNA-extraction protocol and molecular sexing method

For the molecular sexing, we lysed blood cells at 55°C for 10 h in 250 µl extraction buffer (0.01 mM Tris-HCl pH= 8,5; 0.01 mM NaCl; 0.05 mM EDTA pH= 8.0, 2 µl of SDS (20%), 8 µl Proteinase K (10 mg/ml). We used differential precipitation with NH_4Ac (4M, pH= 7.5) and 99% ethanol to separate DNA from proteins. Samples were finally diluted in ddH_2O to a working DNA concentration of 25 ng/ml.

DNA from the sex chromosomes (Z and W) was amplified by PCR using the primers 0057F and 002R (Round et al., 2007). Each reaction included approximately 50 ng of genomic DNA. All reactions were performed in 10-µl volumes containing 0.25 U of Taq DNA polymerase (Biotools), 0.125 mM of dNTP's, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 3.0 mM $MgCl_2$ and 4 pmol of each primer. The thermal profile consisted of an initial denaturing step at 94˚C for 3 min, following by 30 cycles of (30 s at 94˚C, 45 s at 50˚C and 45 s at 72˚C), and a final step at 72˚C for 10 min. We evaluated 2.5 µl of each reaction on a 2% agarose gel and using 0.5 x TAE buffer, using 100 bp DNA ladder as reference (Biotools). We routinely used negative controls (samples with ddH_2O instead of genomic DNA as template), and positive controls (genomic DNA from adult male and female Montagu's Harrier), to ascertain that the outcome of each PCR run was not affected by contamination.

Organochlorine compounds analyses

Concentrations of PCBs and organochlorine pesticides were determined in plasma samples following the method previously described and validated in Mateo et al. (2012). This method is based on the extraction of plasma 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 30 m, 0.32

mm, 0.25 µm both purchased from Agilent Technologies. Pesticide-Mix 13 (Dr. Ehrenstorfer standard) containing *cis*-chlordane, *trans*-chlordane, o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'DDT, p,p'-DDT, α-endosulfan, β-endosulfan, HCB, α-HCH, β-HCH, γ-HCH, δ-HCH, ε-HCH, heptachlor, heptachlor-exo-epoxide, methoxychlor, and PCBs 28, 52, 101, 138, 153 and 180 was used for calibration purposes. Recoveries of the analysed compounds were calculated with plasma samples of farm reared Red-Legged Partridges *Alectoris rufa* spiked with 2.5, 5 or 10 ng/ml (n= 5 for each level). Except for some cyclodienes and methoxychlor that are completely lost in the clean-up step, most of the recoveries of the analysed compounds were above 70% and those detected in the Black Harriers all showed recoveries around 100% (Supplementary material, Table S1). OC levels are expressed in ng/ml and we express our results as the mean value ± standard deviation (SD). Overall, OC levels were determined for 23 adults and 90 nestlings.

Blood smear analyses

After fixing with ethanol, blood smears were stained with the May-Grünewald-Giemsa method. To determine the white blood cell (WBC) count, all smears were inspected under a microscope at 1000x magnification with an oil immersion by the same experienced person, who counted the number of leucocytes found in 10000 blood cells from a randomly chosen, central area on the smear. A high WBC count (number of leucocytes / 10000 cells) may be indicative of increased circulation of leucocytes because of an infection (Bustnes et al., 2004; Norris and Evans, 2000). In addition, a total of 100 leucocytes were classified as lymphocytes, monocytes, eosinophils, heterophils and basophils. We calculated the ratio of heterophil to lymphocyte (H:L ratio) for each individual, which may indicate increased physiological stress and reduced immunity (Siegel, 1985; Ots et al., 1998; Mougeot et al., 2005). Overall, WBC and H:L ratio were determined for 23 adults and 88 nestlings.

Statistical analyses

All statistical analyses were conducted using R 3.2.3 (the R Foundation for statistical computing, 2015).

We first looked for general patterns in variation of OC levels. For this, we conducted General Linear Mixed Models (GLMMs), where the log transformed response variables (ΣPCB, ΣDDT, p,p'-DDT and p,p'-DDE) were fitted to models using a normal distribution (package lme4, function lmer and a logit function; Bates et al., 2012). Nest was included as a random effect in all models to take into account the non-independence of samples coming from the same nest.

We then checked for differences among demographic groups (3-level factor: adult females, adult males and nestlings) and years (3-level factor: 2012, 2013, 2014) on OC levels. Pairwise comparisons using Tukey tests were also conducted to compare the significance among demographic groups, two by two. For this model, analyses were conducted on 90 nestlings, 15 adult females and 9 adult males.

We subsequently investigated the relation between OC levels and habitat variables. Specifically, for the ΣPCB we included the following explanatory variables: proportion of wetland area in the breeding territory, protected area status (2-level factors: protected vs. not protected), transformer density index, demographic group (2-level factor: adults vs. nestlings), and the interactions between demographic group and transformer density index, and between demographic group and wetlands. For the ΣDDT, the p,p'-DDT and the p,p'-DDE levels, we included the following as explanatory variables: proportion of wetland, proportion of agricultural cover, protected areas, demographic group, and the interactions between demographic groups and habitat variables.

We also tested for an additional effect of diet composition on OC levels after controlling for significant habitat variables. For this, we included the proportion of bird biomass in the diet as a further explanatory variable in the models. These models were performed using 56 nestlings, 11 adult females and 9 adult males, for which diet data were available.

Finally, to investigate whether contaminants influenced Black Harrier physical or physiological condition we fitted the body condition index, the H:L ratio (square root transformed), and the WBC ratio (log transformed) as response variables to models, using a normal distribution (package lme4, function lmer and a logit function; Bates et al., 2012). As nestlings and adults have different physiological metabolisms, we analysed each group separately. Specifically, for nestlings we first looked at the effects of year, age and sex; subsequently, after accounting for significant variables, we looked at the additional effect of the OC variables (ΣPCB, ΣDDT, p,p'-DDT, p,p'-DDE). Nest was kept as a random term in these models. For adults, models were performed fitting only the OC variables and sex as explanatory variables.

Type III results are presented. A stepwise backward selection process (using the function drop 1 in R) was followed. Non-significant interactions and factors were excluded from final models.

Results

Occurrence and levels of organochlorine compounds in adults and nestlings

A summary of the detected OCs and their levels in the blood plasma of sampled Black Harriers is given in Table 1.

Overall, 79% of sampled individuals (n= 114) had detectable plasmatic PCBs levels (PCB congeners #52, 101, 153, 138 and 180) (Table 1). The sum of the concentrations of all detected PCBs (ΣPCB) did not differ between years (χ^2 = 1.60, d.f. = 2, p = 0.45), but differed significantly among demographic groups (χ^2 = 9.81, d.f.= 2, p = 0.007; Table 1). Pairwise comparisons using Tukey tests indicated that nestlings had significantly more ΣPCB than adult females, while adult males had intermediate levels (Table 1).

We detected DDTs in the plasma of 84% of sampled individuals (n= 114), including one DDT isoform (p,p'-DDT in 53% of sampled individuals) and two metabolites (o,p'-DDE in 0.9% of individuals and p,p'-DDE in 49% of sampled individuals; Table 1). However, as o,p'-DDE was only found in one sampled individual, we only considered p,p'-DDE levels for subsequent analyses. Considering the overall sum of p,p'-DDT and p,p'-DDE (ΣDDT) levels, we found that concentrations did not differ between years (χ^2 = 2.36, d.f. = 2, p = 0.31), but differed significantly among demographic groups (χ^2 = 7.40, d.f. = 2, p = 0.025). Pairwise comparisons showed that adult males had significantly more ΣDDT than nestlings, and adult females had intermediate levels (Table 1). We further investigated variation in p,p'-DDT and p,p'-DDE levels separately. The p,p'-DDT levels did not differ between years (χ^2 = 0.12, d.f. = 2, p = 0.94), but again differed among demographic groups (χ^2 = 18.29, d.f. = 12, p < 0.001). Nestlings had the highest p,p'-DDT levels followed by adult males, whereas adult females had significantly lower values than nestlings (Table 1). We found no significant variation in p,p'-DDE levels between years (χ^2 = 3.78, d.f. = 2, p = 0.15), but marked differences among demographic groups (χ^2 = 134.32, d.f. = 2, p < 0.001). For this metabolite, the lowest levels were found in nestlings. In addition, adult males had significantly higher p,p'-DDE levels than adult females (Table 1).

Territory characteristics and organochlorine compound levels

After considering differences in ΣPCB levels between adults and nestlings (χ^2 = 7.04, d.f. = 1, p = 0.008), we found no evidence for ΣPCB levels to vary with the proportion of wetlands in the breeding territory (χ^2 = 0.70, d.f. = 1, p = 0.40) or the protected area status (χ^2 = 0.04, d.f. = 1, p = 0.84). However, we found a significant positive relationship with the Transformer Density Index (χ^2 = 5.20, d.f. = 1, p = 0.023; Fig. 1) in both adults and nestlings. There was no significant interaction between demographic group and Transformer Density Index (χ^2 = 0.17, d.f. = 1, p = 0.68; Fig. 1).

After accounting for differences in ΣDDT levels between demographic groups (χ^2 = 7.91, d.f. =1 , p = 0.019), we found no significant effect of agriculture cover (χ^2 = 0.791, d.f. = 1, p = 0.37) or protected area status (χ^2 = 0.01, d.f. = 1, p = 0.99), but we found a significant and positive effect of the proportion of wetlands on ΣDDT levels (χ^2 = 6.18, d.f. = 1, p = 0.013; slope ± SE: 0.21 ± 0.085). This effect was similar for adult males, adult females and nestlings: the interaction between demographic group and wetland cover was not significant (χ^2 = 0.38, d.f. = 2, p = 0.83). Considering p,p'-DDT and p,p'-DDE levels separately, we found that the effect of the proportion of wetland was only significant for p,p'-DDE levels (χ^2= 11.62, d.f.= 1, p< 0.001), not for p,p'-DDT (χ^2= 0.304, d.f.= 1, p= 0.58).

Diet composition and organochlorine compound levels

For the sub-sample of 29 nests for which we had detailed information on prey consumed, and after controlling for differences among demographic groups and the effect of transformer density, we found no significant association between ΣPCB levels and diet composition (% birds: χ^2= 0.77, d.f.= 1, p= 0.38). For ΣDDT levels, however, we found that, controlling for differences among demographic groups and the effect of wetland cover, ΣDDT levels increased significantly with the percentage of bird biomass consumed (χ^2= 5.84, d.f.= 1, p= 0.016; slope ± SE: 0.996 ± 0.413).

Considering p,p'-DDT and p,p'-DDE levels separately, we found that the effect of diet composition was only significant for p,p'-DDE levels (χ^2 = 12.41, d.f. = 1, p < 0.001; slope: 0.979 ± 0.278; Fig. 2), but not for p,p'-DDT levels (χ^2 = 0.16, d.f. = 1, p = 0.69).

Physical condition and organochlorine compound levels

We found no significant associations between nestling condition index and ΣPCB or ΣDDT levels (ΣPCB: χ^2 = 0.024, d.f. = 1, p = 0.877; ΣDDT: χ^2 = 1.01, d.f. = 1, p = 0.314), or between adult condition index and OC levels (ΣPCB: χ^2 = 0.70, d.f. = 1, p = 0.40; ΣDDT: χ^2 = 0.20, d.f. = 1, p = 0.65).

WBC count, H:L ratio and organochlorine compound levels

Among nestlings, the WBC count did not vary with ΣPCB levels (χ^2 = 2.3589, d.f. = 1, p = 0.12), but tended to increase with ΣDDT levels (χ^2 = 3.795, d.f. = 1, p = 0.051; slope: 0.13 ± 0.065). The positive association was between WBC count and p,p'-DDT levels (χ^2 = 3.08, d.f. = 1, p = 0.080; slope: 0.11 ± 0.063) rather than between WBC count and p,p'-DDE levels (χ^2 = 0.58, d.f. = 1, p = 0.445). In adult birds, we found that WBC count was greater in females than in males (χ^2 = 4.25, d.f. = 1, p = 0.039; LS means ± SE: 4.35 ± 0.11 and 4.03 ± 0.14, respectively), but did not vary with either ΣPCB levels (χ^2 = 0.10, d.f. = 1, p = 0.75) or with ΣDDT levels (χ^2 = 0.03, d.f. = 1, p = 0.85).

Considering the H:L ratio, we found a positive association with ΣPCB levels in both nestlings (χ^2 = 3.48, d.f. = 1, p = 0.06; slope: 0.066 ± 0.036) and adults (χ^2 = 6.25, d.f. = 1, p = 0.012; slope: 0.050 ± 0.021; Fig. 3). We found no significant association between H:L ratios and ΣDDT levels in either nestling (χ^2 = 0.82, d.f. = 1, p = 0.36) or adult birds (χ^2 = 2.20, d.f. = 1, p = 0.14).

Discussion

This study represents one of the few assessing the presence of organochlorine compounds (OCs) in a southern African raptor using blood samples collected from live (i.e. not dead or moribund) individuals (van Wyk et al., 2001). Furthermore, it highlights associations between OC levels, habitat types, diet composition and indicators of physiological condition that help understand exposure routes and potential sub-lethal effects. ΣPCB and ΣDDT (p,p'-

DDT + p,p’-DDE) levels were detected in 79% and 84% of sampled individuals, respectively, suggesting that environmental contamination is relatively widespread within our study regions. Detected levels were relatively low, but within the range or slightly higher than those found in recent northern-hemisphere studies of other raptors (e.g. Sonne et al., 2012; Bustnes et al., 2013; Eulaers et al., 2014; Gómez-Ramírez et al., 2014; Ortiz-Santaliestra et al., 2015), for which adverse physiological effects have also been reported (e.g. Sonne et al., 2012; Ortiz-Santaliestra et al., 2015). Therefore, the detected levels in Black Harriers seem biologically relevant and may have conservation implications for this scarce and endangered species, as well as other sympatric predators.

PCBs are not produced in South Africa, but they have been imported in large quantities since the 1930s mainly to be used in electricity generating equipment (Ministry of Water and Environmental Affairs, 2011). In South Africa, PCB oils were used in electric transformers and capacitors until at least 2010. According to the Ministry of Water and Environmental Affairs (2011), 32% of all transformers had a PCB content of 1-19 ppm, 2% had 20-49 ppm, 62% had 50-499 ppm, and 4% had a PCB content greater than 500 ppm. In our study, we found a significant association between ΣPCB levels in adult and nestling Black Harriers and the Transformer Density Index (Fig. 1), indicating that those individuals that had the highest levels of ΣPCB in their blood were the ones that had the greater kVA-rating per km^2 within their territory. Electrical transformers are generally considered as a potential source of PCB contamination (Mateo et al. 2016), but this is, to the best of our knowledge, the first time that such an association has been found in a wild animal. PCB leakages from transformers may contaminate the surrounding grounds, sediments, water bodies, and the biota in general by bio-accumulation and bio-magnification. Invertebrates, plants and seeds may then be contaminated, which may consequently contaminate the raptor prey types (e.g. Hoffman et al. 2003) and Black Harriers feeding on them. Remarkably, no association was found between ΣPCB levels and the protected area status, indicating that such areas do not reduce the risk of Black Harriers becoming contaminated by PCBs. In fact, the highest ΣPCB levels (10.0 to 13.7 ng/ml) were found for five nestlings from the West Coast National Park and the Jakkaslfontein Private Nature Reserve and Koeberg Nature Reserve. All are located within the vicinity of the Koeberg nuclear power station, the only one of its type in Africa. Our Transformer Density Index, which combines transformer density and kVA, could be a useful tool to evaluate the relevance of this ΣPCB exposure route in terrestrial wildlife elsewhere.

Other results related to PCBs were less clearly interpretable. We found higher ΣPCB levels in nestling than in adult Black Harriers. Given that the accumulation of OCs in bird tissues occurs over time, i.e. as intake rate exceeds excretion rate (including blood tissue; Goutner et al., 2011), it is usually expected that adults will have higher blood levels of OCs than nestlings, as described in several vulture species (Goutner et al., 2011) and in Bonnelli's Eagle *Aquila fasciata* (Ortiz-Santaliestra et al., 2015). This may indicate that Black Harrier nestlings have a lower P450-enzyme activity (the system responsible for the bio-transformation of PCBs) than adults, as found in other species (Rattner et al., 1997; Jenssen et al., 2001; Frank et al., 2001; Naso et al., 2003), but this would need to be tested. The lack of association between ΣPCB levels and the proportion of avian prey biomass suggests that the ΣPCB contamination in Black Harriers comes from all types of prey similarly. The high levels of ΣPCB came essentially from higher levels of the congener PCB 52, which contributes 4.04 - 5.18% of mass in mixtures with lower chlorination such as Aroclor 1016, 1242 and 1254 (Schulz et al. 1989). This may indicate that, as in some European countries (Georgii et al., 1994) and in accordance with the Stockholm convention, the use of less toxic, lower-chlorinated congeners has been prioritized in South Africa over the use of the more toxic higher-chlorinated congeners such as PCBs 138, 153 and 180 (Ministry of Water and Environmental Affairs, 2011).

Results regarding ΣDDT (p,p'-DDT + p,p'-DDE) levels also provide indications about current risk to wildlife in the study area. Although the use of DDT as an agricultural pesticide has been banned in South Africa since 1983, it remains today an important chemical in the country's fight against malaria. In the north-eastern part of the country, indoor spraying is used as part of the Integrated Vector Control Management Programme, within the malaria control program (Quinn et al., 2011; Ministry of Water and Environmental Affairs, 2011). DDT's main metabolite, p,p'-DDE, is known to persist in the environment for many years (Hoffmann et al., 2003). The presence of p,p'-DDE in Black Harrier blood may thus reflect a former use of the DDT within the species' breeding range, either for agriculture, where there is evidence that DDT continued to be used illegally into the 1990s despite its ban (Wells and Leonard, 2006), or for mosquito control in wetlands. In adult Black Harriers, the exposure to DDTs could also occur during the non-breeding season in areas where DDT is legally used (ca. 2,000 km away from our study sites). GPS and satellite tracking of adults has revealed a migration eastwards to Lesotho, the Eastern Cape and Kwazulu Natal Provinces (see http://blackharrierspace.blogspot.co.za/2015/01/the-season-for-migration-east.html), in areas that are geographically closer to those under malaria control. However, none of the tagged Black Harriers penetrated these zones.

Additionally, the presence of p,p'-DDT in blood (particularly in nestlings) suggest a recent acquisition of the contaminant, and a current and illegal use of this pesticide, or of one of its substitutes, in the region. For example, dicofol is a pesticide registered for use in South Africa and currently sprayed for the control of mites on crops and orchards, and is known to often contain p,p'-DDT (Clark, 1990; Quinn et al., 2011) and to negatively impact raptor species (Clark et al., 1990; Schwarzbach et al., 1991). Thus, the legal use of dicofol may be responsible for the concentrations of p,p'-DDT found in Black Harriers, acquired via contaminated prey in their diet.

Levels of p,p'-DDT were highest in nestlings, and lowest in adult females. Such a pattern could be indicative of maternal transfer (Drouillard and Nostrom, 2001). In females, OCs stored in lipids can be mobilized and transferred to the egg and the embryo, with concentrations that can vary substantially depending on the contaminants (Hoffman et al., 2003; Bourgeon et al., 2013). By contrast, adult males would be continuously bio-accumulating these OCs until they biologically degrade in their bodies (Schnellmann et al., 1985; Hoffman et al., 2003). However, this needs confirmation by evaluating levels of contaminants in unhatched eggs. The differences in concentrations between the p,p'-DDT and p,p'-DDE between adults and nestlings may also be explained by the degradation time needed to metabolize the p,p'-DDT in p,p'-DDE (Wedemeyer, 1968; Hoffman et al., 2003): adults are known to have higher metabolic rates than nestlings, hence a faster capacity to degrade the accumulated p,p'-DDT into p,p'-DDE, than nestlings (Hoffman et al., 2003). This could explain why adults have higher levels of p,p'-DDE relative to nestlings, and nestlings higher levels of p,p'-DDT compared to adults.

Correlates of DDT levels also hint to sources of exposure. We found that ΣDDT concentration in blood significantly increased with the proportion of bird prey biomass taken by Black Harriers, as observed in other birds of prey (Mañosa et al., 2003; van Drooge et al. 2008). This association was significant only with p,p'-DDE levels, not p,p'-DDT. These results may indicate that p,p'-DDE concentrations reflect a contamination of bird prey in other areas. Indeed, Common Quail *Coturnix coturnix* represent 25.2% of the consumed bird species by Black Harriers (García-Heras et al., 2017a), and are known to migrate to areas where the DDT is still widely sprayed (e.g. Namibia, Zambia: Taylor, 2005). As such they offer a potential pathway by which DDE finds its way into harriers. For habitat, while no association between ΣDDT concentrations and agricultural cover was found, we found a significant increase in p,p'-DDE concentrations in Black Harrier blood with an increasing proportion of wetland cover within the breeding territory. This suggests that p,p'-DDE concentrations also reflect former (or current) DDT use for mosquito control in wetlands outside malaria-risk areas, although confirmation is required. This contamination may also be due to the volatilization and condensation properties of POPs from warm source/usage areas to colder regions (Meijer et al., 2003; Ryan et al., 2012; Roscales et al., 2016), but again this requires confirmation. What our results suggest is that wetlands are a source of contamination by OCs in south-western South Africa and these areas need to be considered when assessing OC exposure and implementing conservation measures, as highlighted by Ryan et al. (2012).

Finally, relatively few studies have looked at the effects of the OC concentrations on the physical and physiological condition of wild raptors, which makes comparisons challenging (Rivera-Rodríguez and Rodríguez-Estrella, 2011; Ortiz-Santaliestra et al., 2015). In our study, no association was found between ΣPCB and ΣDDT concentrations and the commonly used body condition index of mass corrected for age or size. This suggests that OC concentrations were unlikely explained by fat mobilization due to poorer nutritional status. On the other hand, and despite the relatively low OC levels detected in Black Harriers, our results suggested an influence on physiological condition: the number of white blood cells (WBC ratio) tended to increase with higher p,p'-DDT levels, and H:L ratio increased with higher ΣPCB levels in both nestlings and adults. The former may be indicative of a compensatory response of the immune system in DDT-contaminated birds; and the latter suggest an increased physiological stress and reduced immunity for PCB-contaminated birds. A decrease of the number of lymphocytes compared to the number of heterophils may indicate an immuno-suppressive action of PCBs, as found in other studies (Bustnes et al., 2004). Similar results were also found by Ortiz-Santaliestra et al. (2015) in a study on Bonelli's Eagle *Aquila faciata*: adults and nestlings contaminated by PCBs, with levels similar to those of Black Harriers, exhibited a reduction of dietary antioxidant (i.e. vitamins and circulating carotenoids), and a reduced concentration of alkaline phosphatase (ALP), an indicator of osteoblastic activity. Furthermore, the levels of PCBs and p,p'-DDE found in Golden Eagles *Aquila chrysaetos*, Northern Goshawks *Accipiter gentilis* and White-Tailed Eagles *Haliaeetus albicilla* by Sonne et al. (2012) were similar to those of Black Harriers, and were also found to affect several blood clinical-chemical parameters and to be of concern for endocrine disruption and thyroid hormones.

Conclusions

This study contributes new knowledge on the exposure and transference of organochlorine compounds to wildlife in South Africa, and on how these contaminants may be affecting top-predators, especially raptors. Using as a study model the endangered and endemic Black Harrier, our results indicate the presence of ΣPCB, p,p'-DDT and p,p'-DDE in a large proportion of the monitored population, indicating current extensive sources of contamination. Furthermore, our results linking contaminant levels and environmental variables revealed the potential sources of contamination, specifically electrical transformers for PCB exposure, and farmland birds and wetlands for p,p'-DDE exposure. Our result also showed an intra-specific relation between diet composition and OC contamination, which is usually described at the inter-specific level, and indicates that individual variation in foraging activity and prey preferences modulate the risk of exposure to these contaminants. Finally, although OCs were detected at relatively low concentrations, we could link these levels with indicators of physiological condition, suggesting that current levels of contaminant exposure represent a risk. In this context, our results on Black Harriers are relevant for other sympatric predators, especially bird-eating raptors, breeding in the same region that may equally be affected by OC contaminants. We encourage more studies on this topic in Africa where little is known on the impacts of the OC exposure on wildlife and where high light levels and temperatures are assumed to break down OCs more rapidly than in temperate areas (Hoffman et al. 2003). Our results are important to raise awareness about the potential sources of contamination and to implement future efficient conservation measures for threatened species.

Acknowledgements

We thank SANParks and Cape Nature for access to study sites. We also thank A. Jenkins, O. Curtis, A. Williams, B. Bester, B. Mackenzie, J.J. Luque-Larena, and all the students from the Percy FitzPatrick Institute of African Ornithology who helped with fieldwork and data collection, especially D. Gaglio. We also thank S. Cunningham for suggestions on sources of transformer information, ESKOM for providing these data, P. Sumasgutner for advice on the blood slide analyses, M. Maritz for conducting the blood slide analyses, Txuso Garcia for the molecular sexing of nestlings and John Jairo López Perea for helping with the laboratory analyses. We are also grateful to G. Cumming for helping MSGH with financial support in 2014.

Funding sources

This study was supported by the National Research Foundation of South Africa (NRF) (Grant no. 90582 to RES), the DST-NRF Centre of Excellence at the Percy FitzPatrick Institute, University of Cape Town, the CSIC (Consejo Superior de Investigaciones Cientificas- PIE 201330E106), a Leslie Brown Award from the Raptor Research Foundation (RRF) and by private landowners and organizations. FM and BA thank UCT for financial support (2015 Science Faculty Distinguished Visitor award to FM; and Foreign Research Fellowship to BA).

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Table 1. Mean (± SD) blood plasma concentrations (ng/ml) of Organochlorine Compounds (OCs) in the Black Harriers from South Africa, 2012-2014. Data ranges [min-max] are given in brackets. Sample size refers to number of individuals. The detection limit was 0.01 ng/ml. ΣPCB is sum of the concentration of all PCB congeners. ΣDDT is the sum of the concentration of p,p'-DDT and p,p'-DDE.

	Adult Females	Adult Males	Nestlings*
PCB 52	1.14 ± 1.57 [0 – 4.40]	0.64 ± 0.83 [0 – 1.92]	2.65 ± 2.71 [0 – 11.44]
PCB 101	0.40 ± 0.93 [0 – 3.61]	0.39 ± 0.50 [0 – 1.34]	0
PCB 138	0.02 ± 0.07 [0 – 0.25]	0	0.01 ± 0.09 [0 – 0.59]
PCB 153	0.11 ± 0.27 [0 – 0.98]	0.13 ± 0.28 [0 – 0.82]	0.37 ± 0.65 [0 – 4.03]
PCB 180	0.21 ± 0.28 [0 – 0.72]	0.25 ± 0.30 [0 – 0.64]	0.52 ± 1.19 [0 – 5.10]
ΣPCB	1.87 ± 2.32 [0 – 6.93]	1.41 ± 1.16 [0 – 3.23]	3.55 ± 3.06 [0 – 13.74]
p,p'-DDT	0.05 ± 0.18 [0 – 0.71]	0.51 ± 0.69 [0 – 2.14]	1.57 ± 1.88 [0 – 9.84]
p,p'-DDE	1.41 ± 0.97 [0 – 3.38]	2.55 ± 1.51 [1.05 – 4.89]	0.29 ± 0.48 [0 – 2.21]
ΣDDT	1.46 ± 0.99 [0 – 3.38]	3.06 ± 1.50 [1.34 – 5.89]	1.86 ± 1.96 [0 – 9.84]
Sample size	15	9	90

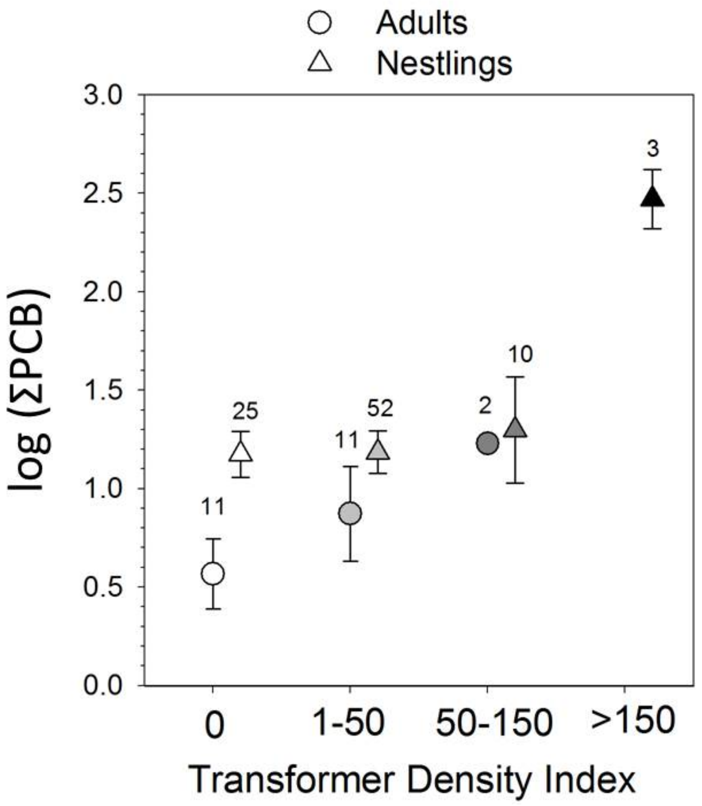
* OC levels did not vary significantly according nestling sex (see results) so data have been pooled for all nestlings.

Figure 1. Mean (± SD) blood plasma concentrations of PCB (ng/ml, log-transformed) according to an index of transformer density within the breeding territory (overall kVA-rating per km2). For illustration purpose, the transformer index has been categorized (four classes: white: 0, light grey: 1-50, dark grey: 50-150, black: >150). Data are shown by demographic group (adults: circles; nestlings: triangles) separately. Numbers above the error bars refer to number of individuals.

Figure 2. Relationship between plasma ΣDDT concentration (log transformed; ng/ml) and the proportion of bird biomass consumed by a) nestling and b) adult Black Harriers.

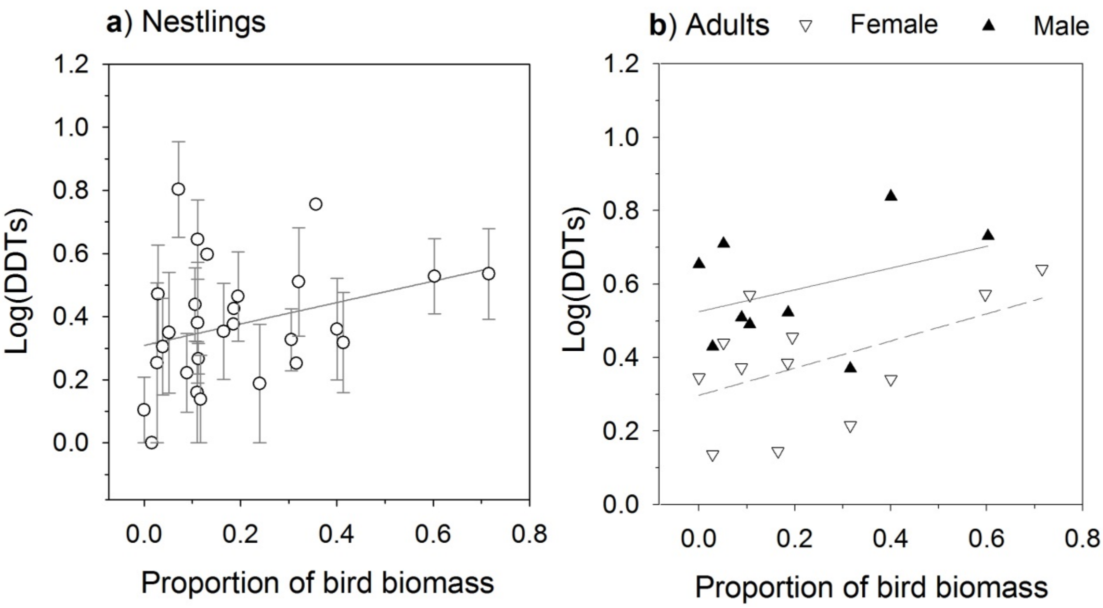
Figure 3. Relationship between the Heterophil:Lymphocyte ratio (squared root transformed) and plasma levels of ΣPCB (ng/ml, log-transformed values) in a) nestling and b) adult Black Harriers. Linear fit (solid line) and ± 95% confidence intervals (dashed line) are showed in dark grey for nestlings and in black for adults.

FIGURE 1.



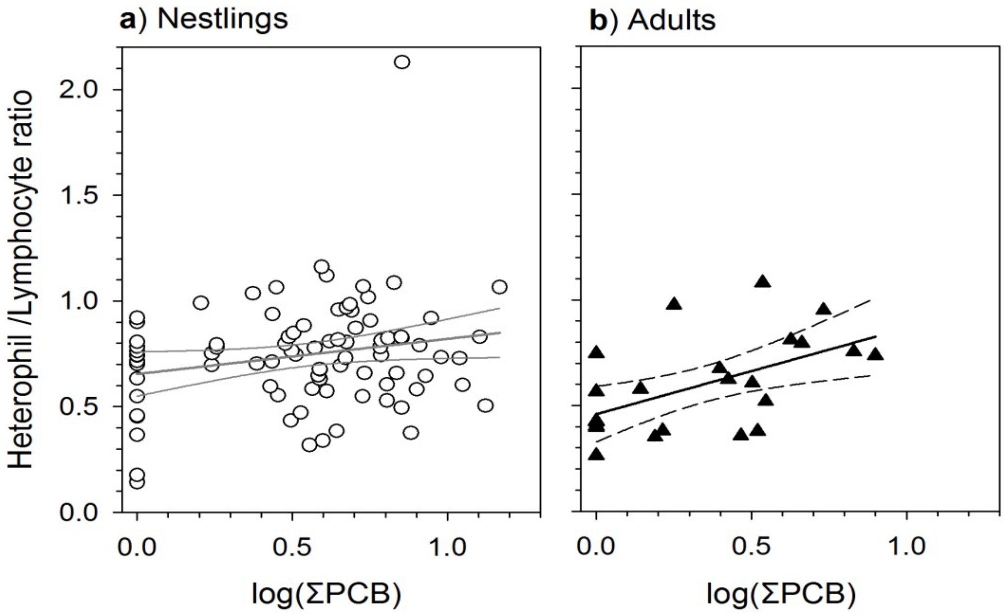
Adults
Nestlings
log (ΣPCB)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
3
10
25
52
2
11
11
0
1-50
50-150
>150
Transformer Density Index

FIGURE 2.


a) Nestlings
b) Adults
Female
Male
Log(DDTs)
Proportion of bird biomass
1.2
1.0
0.8
0.6
0.4
0.2
0.0

886 FIGURE 3.

887



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