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Organochlorine Pesticides in the Ferruginous Pygmy Owl (*Glaucidium brasilianum*) in Chiapas, Mexico

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Abstract Concentrations of organochlorine pesticides were quantified in samples of feathers (n = 17) and blood (n = 15) of the ferruginous pygmy owl (*Glaucidium brasilianum*). The individuals were captured near the Protected Natural Area Cerro Sonsonate, Chiapas, Mexico, between February and June 2014. In both tissues, pesticides belonging to seven organochlorine chemical families were detected. However, the organochlorine pesticide concentrations differed between feathers and blood. The highest concentrations of hexachlorocyclohexanes were found in feathers (0.63 ± 0.89 µg/g), whereas the highest concentrations of ΣDrines were found in blood (0.31 ± 0.47 µg/mL). By using the summed concentrations for each of the seven families of pesticides found in feathers, we did not find any significant correlation between the pesticides and pectoral muscle or body weight ($p > 0.15$). The ΣDDT group was the only pesticide family that showed a positive correlation with owl body weight ($r = 0.60$, $p = 0.05$); the concentrations of these pesticides were also high in feather and blood tissues ($r = 0.87$, $p = 0.02$). Our results confirm that ferruginous pygmy owls in the study area are exposed to these pesticides.

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Organochlorine pesticides (OCs) affect all ecosystems (van Wyk et al. 2001), because they are persistent organic pollutants (POPs) that are resistant to degradation, bioaccumulate in both the environment and organisms through the trophic chain, and are highly toxic (Fernández et al. 2004). In Mexico, especially in the region of Soconusco and Frailesca in Chiapas State, pesticides—mainly DDT—have been extensively used in recent decades to control malaria vectors and for intensive agriculture. In 2000, Chiapas State had the second highest pesticide use in Mexico and was the last state in which DDT was used legally (Alegría et al. 2006; Morales 2013). Recently, these organochlorine compounds have been replaced with less-persistent substances (Morales 2013). However, organochlorine contaminants continue to be detected in environmental samples (Kunisue et al. 2003) and still need to be monitored in wildlife populations (Dhananjayaj and Muralidharan 2010).

Birds are recognized as a valuable bioindicator for different types of pollutants (Furness 1993). In particular, because of their trophic position, birds of prey (raptors) bioaccumulate and incorporate pollutants in their bodies that influence their reproduction, which makes this group especially vulnerable (Turusov et al. 2002; Gómez 2011). Studying raptors should help expose any health risks for both humans and the ecosystem. However, organochlorine concentrations vary among tissues; for example, the concentration in the blood indicates recent exposure, while analysis of feathers is useful for studying various physiological, ecological, and toxicological processes inherent in individuals and populations (Smith et al. 2003).

Furthermore, the concentrations of pesticides in adult raptor feathers can be related to their concentrations in blood (Jaspers et al. 2006); therefore, this relationship is useful for biomonitoring POP exposure (Jaspers et al. 2007).

To our knowledge, there have been no previous studies in Mexico to detect POPs in feathers or blood of nocturnal raptors. Mexico has 34 species of Neotropical owls and is one of the countries with the greatest diversity of these raptors; however, their abundance and distribution may be declining in many Neotropical regions because of both habitat fragmentation and pesticide use (Enríquez et al. 2006). The ferruginous pygmy owl (*Glaucidium brasilianum*) is one of the most widely distributed species of owls in Mexico, living in semi-open wooded habitats such as second-growth forests, coffee plantations, and semi-urban areas (Enríquez-Rocha and Rangel-Salazar 2008). These owls feed mostly on insects, such as grasshoppers, crickets, caterpillars, and other large insects, but their diet also can include birds, small mammals, amphibians, and reptiles (often lizards) (König and Weick 2008). Pygmy owls are neither endangered nor require conservation. At present, relatively little information is available on this species; however, because of its wide distribution, it is a good candidate for use to study and monitor OCs in the environment.

In this study, we quantified the concentrations of OCs in two tissue types—feathers and blood—of the ferruginous pygmy owl. We hypothesized that pesticide concentrations would vary between the tissues because pesticides detected in feathers indicate concentrations that were acquired during the period of feather growth (Eulaers et al. 2011b), whereas their presence in blood indicates recent exposure. This study will increase our understanding of one of the major threats to raptor populations (i.e., OCs), particularly for the ferruginous pygmy owl.

Materials and Methods

Bird sampling was conducted between February and June 2014 near the Protected Natural Area Cerro Sonsonate Frailesca. The study area is located between 16°11′18.51″N and 93°19′38.33″W on the Sierra Madre de Chiapas slope in Francisco Villa Ejido (1260 ha) between the Villaflores and Villa Corzo municipalities in Chiapas, Mexico (Fig. 1). These municipalities are ranked third and fourth, respectively, in terms of agricultural area in Chiapas. Livestock activity is also important here—between 40 and 60 ha are used for these activities.

The search, capture, and sampling of owls were carried out from 0430 to 1000 and from 1630 to 2200. We used pre-recorded vocalizations of the species to detect and attract owls to the nets (Bloom et al. 2007). We performed a physical examination of each captured individual that included morphometric and weight measurements. After this, we evaluated the condition of the pectoral muscle (PM) mass, following Ritchie et al. (1994), because the PM is a partial indicator of health or body condition. To assess PM condition, we used the following classification: PM1 = good PM, solid and firm muscle; PM2 = moderate muscle, prominent sternum; and PM3 = small PM, very prominent sternum. Feather samples were obtained using flat dissecting scissors: 15 breast feathers, 15 back feathers, and the third right rectrix were collected. The feathers were wrapped in aluminum foil, placed in sealed plastic bags, and kept at room temperature (~20°C) until analysis. After collecting a rectrix, each individual was temporarily marked by painting its middle toenail (Varland et al. 2007). Blood samples were taken by puncturing the radial vein with a hypodermic needle (G23 Blue, 0.6 mm × 25 mm). Blood was collected in heparinized capillary tubes, deposited in sealed glass tubes, and stored at −4°C for no longer than 1 week until analysis in the laboratory. Storage of the samples at this temperature is recommended in many protocols as POPs are stable under these conditions (EPA 1995).OCs are considered to be POPs because of their half-life in the environment; for example, in areas where the temperature exceeds 20°C, the POP half-life is more than 5 years, whereas that of DDT is close to 10 years.

Feathers were analyzed according the technique described by Dauwe et al. (2005). Feathers were washed with 10 % Triton, rinsed with distilled water, and then cut into small pieces. Each sample was weighed and an internal standard (chlorpyrifos) was added; the sample was incubated overnight at 40°C in 4 mL HCl (4 M) in a mixture of 3 mL hexane:dichloromethane 4:1 (v/v), and then placed in an ultrasonic bath. Extraction of the compounds was carried out twice by liquid–liquid extraction with 4 mL hexane:dichloromethane (4:1, v/v). Extracts were purified using a column chromatograph packed with 0.5 g of silica gel and a layer of anhydrous Na_2SO_4, and were eluted with 4 mL hexane:dichloromethane (1:1, v/v).

The blood samples were analyzed using a technique modified from that of Keller et al. (2004). A mixture of 0.5 mL formic acid and 1 mL hexane:dichloromethane (4:1, v/v) was added to 0.5 mL of sample. The tube was vortexed for 2 min and then left to stand for an hour and a half. The tube was then centrifuged for 5 min at 2500 rpm. The supernatant was vortexed with the same volume of hexane:dichloromethane and then centrifuged for 5 min at 2500 rpm; this process was repeated twice. The extract was purified using a column chromatograph packed with 2 g silica gel, 2 g Florisil, and a layer of anhydrous Na_2SO_4, and eluted with 20 mL hexane, 15 mL dichloromethane, and 15 mL hexane:dichloromethane (4:1, v/v).

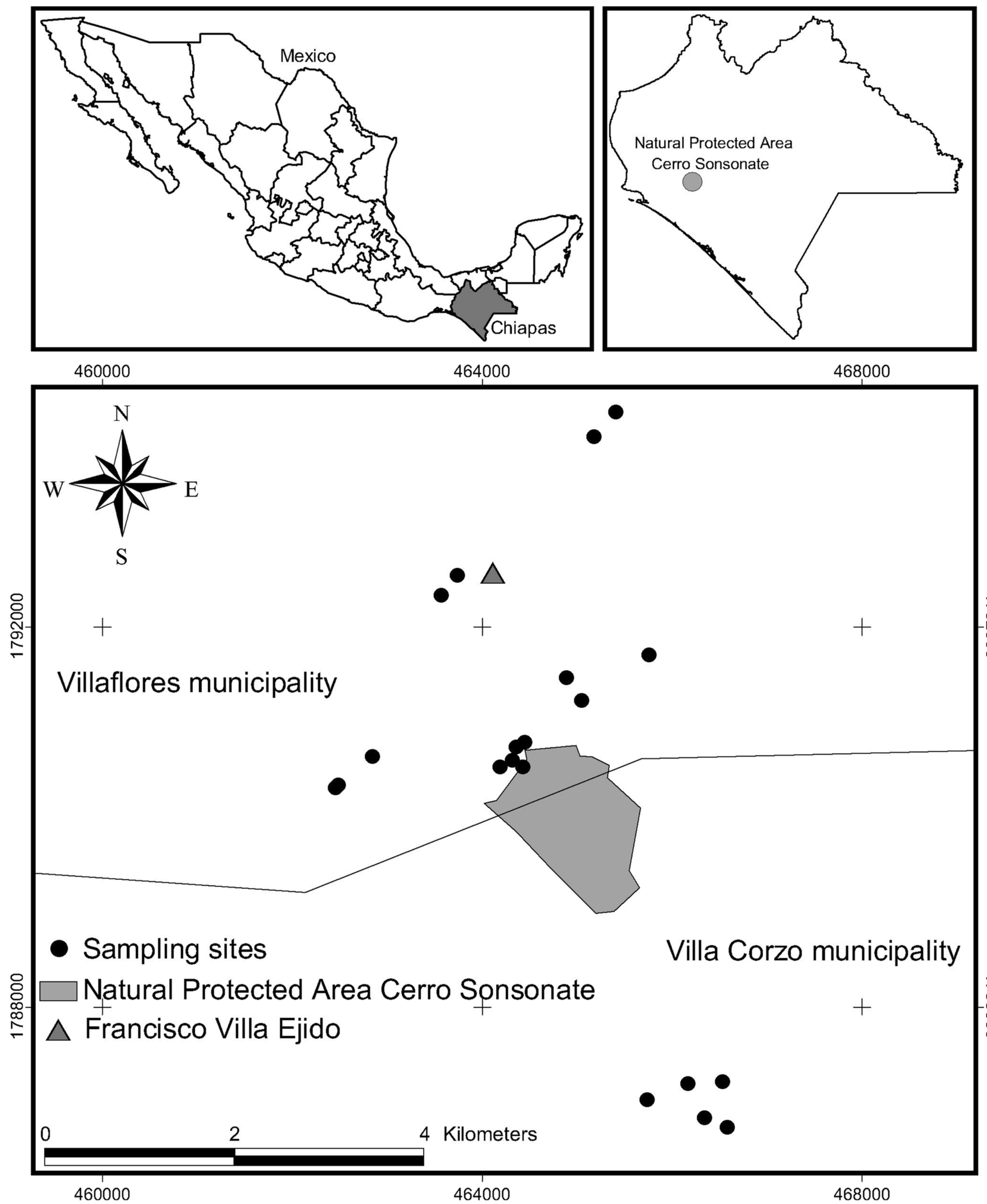

Fig. 1 Sampling sites for ferruginous pygmy owls (*Glaucidium brasilianum*) near the Natural Protected Area Cerro Sonsonate, Chiapas, Mexico. The bisecting line divides the two municipalities

For blood and feather samples, two drops of toluene were added as a keeper to the final elution. This extract was concentrated to a final volume of 50 µL. All samples were analyzed in a Thermo Scientific™ TRACE GC Ultra™ gas chromatograph coupled to a Thermo Scientific™ ITQ™ 900 ion trap mass spectrometer. As an analytical control, a blank of reagents was determined per series (feathers and blood) and an analysis of recovery was carried out (resulting in 85 %–105 %). Samples were injected once. A calibration curve was constructed as an analytical quality control using five different concentrations. Each concentration was run or injected seven times. In total, 20 OCs were analyzed and quantified using the certified reference material SUPELCO-47426-U CLP Organochlorine

Pesticide Mix (99 % purity). The standard comprised the parent compound p,p′-DDT and its degradation products p,p′-DDE and p,p′-DDD, grouped as ΣDDT; endosulfan I and II and their metabolite endosulfan sulfate, grouped as ΣEndosulfan; hexachlorocyclohexane and their four isomers (α-HCH, β-HCH, γ-HCH, and δ-HCH), grouped as ΣHCH; the insecticides aldrin, dieldrin, endrin, and the metabolites endrin aldehyde and endrin ketone, grouped as ΣDrines; the isomers trans-chlordane and cis-chlordane, grouped as ΣChlordane; and the insecticide methoxychlor.

The limits of detection (LOD) for feathers were between 1.19 and 3.21 ng g^{-1}, and the limits of quantification (LOQ) were between 2.62 and 9.19 ng g^{-1} of all of the compounds used as standards. For blood samples, the LOD were between 2.55 and 4.7 ng g^{-1}, and the LOQ were between 6.25 and 13.64 ng g^{-1}. Data were grouped per chemical family per sample for statistical analysis. Spearman's correlation analysis was performed to assess the relationship between the weight of the owl weight and the OC concentrations in feathers and blood. The relationship between the weight and OC concentrations in feathers and blood were also evaluated using Pearson's correlation test. Statistical analyses were performed using Stata version 12.0 (StataCorp 2011) at a significance level of $p < 0.05$.

Results and Discussion

A total of 20 ferruginous pygmy owls were captured. From this pool, feather (n = 20) and blood (n = 15) samples were collected. Seven families of pesticides were detected in 17 of the feather samples and in all the 15 blood samples (Table 1). We found variations in OC concentrations between the tissues. The most frequently occurring families of compounds in feathers were ΣEndosulfan (82 %) and ΣHeptachlor (82 %), followed by ΣDDT (65 %). By contrast, in the blood samples, ΣDrines (100 %) were the most frequent, followed by ΣDDT (73 %) and ΣHCH (73 %).

The ΣHCH family of compounds showed the highest concentration in feathers and the second highest in blood (Table 1). This compound is metabolized and excreted relatively quickly, and it has a shorter life span in the atmosphere than other OCs (Fernández et al. 2004). In Mexico, lindane is currently used in agriculture and is included on the public list of medicines issued by health campaigns for the treatment of head lice and scabies, as well as for various veterinary and industrial purposes (Fernández et al. 2004). In the study area, lindane is used for the treatment of ectoparasite infections in cattle. Therefore, the presence of ΣHCH in ferruginous pygmy owl samples in both tissues may be due to their continued use and recent exposure (Behrooz et al. 2009). Among raptors, this compound has been reported in the feathers of several species, such as barn owls *(Tyto alba)* and long-eared owls *(Asio otus)* (Jaspers et al. 2007), and also in blood samples from the American kestrel *(Falco sparverius)* and osprey chicks (*Pandion haliaetus*) (Rivera-Rodríguez et al. 2007; Rivera-Rodríguez and Rodríguez-Estrella 2011; Table 2). In addition, γ-HCH (lindane) is one of the isomers occurring at a high frequency and concentration in the eagle owl (*Bubo bubo*) and booted eagle (*Hieraaetus pennatus*) (Gómez 2011).

The insecticides aldrin, dieldrin, and endrin showed the highest concentrations in the blood samples and low concentrations in feathers (Table 1). Those pesticides are very hazardous and toxic to wildlife, and they can persist in aquatic environments for several years after their application. In raptors, high concentrations of dieldrin have been reported in populations of eagle owls and great-horned owls (*Bubo virginianus*) and are associated with adult mortality (Frank and Lutz 1999). Dieldrin released into the soil can persist for more than 7 years, although it is rapidly degraded in hot or tropical areas (Scheunert 1989). Other insecticides of this family have been found in osprey chicks and the American kestrel (Rivera-Rodríguez et al. 2007; Rivera-Rodríguez and Rodríguez-Estrella 2011, Table 2).

Table 1 Mean concentrations of organochlorine pesticide residues in the feather and blood samples of the ferruginous pygmy owl (*Glaucidium brasilianum*) in Mexico

Pesticides	n	Feathers		n	Blood	
		Mean ± SD (μg/g)	min–max		Mean ± SD (μg/mL)	min–max
ΣHCH	6	0.63 ± 0.89**	0.01–2.01	11	0.08 ± 0.15	0.001–0.40
ΣHeptachlor	14	0.03 ± 0.03	0.0004–0.14	4	0.06 ± 0.06	0.0002–0.12
ΣDrines	8	0.03 ± 0.05	0.002–0.14	15	0.31 ± 0.47**	0.02–1.77
ΣDDT	11	0.04 ± 0.04	0.0003–0.14	11	0.05 ± 0.07	0.002–0.23
ΣEndosulfan	14	0.34 ± 0.64	0.0007–1.86	9	0.06 ± 0.10	0.002–0.32
ΣChlordane	5	0.01 ± 0.02	0.0004–0.05	2	0.05 ± 0.03	0.02–0.07
Methoxychlor	2	0.03 ± 0.02	0.01–0.05	8	0.05 ± 0.10	0.002–0.29

n = number of individuals; mean ± standard deviation; μg/g dry base; μg/mL wet base; ** highest concentration

Table 2 Concentrations of organochlorine pesticides in feather and blood samples of raptors reported from various studies

Species	Sample		OC Concentration				Results	Site	Reference
	Feathers	Blood	ΣHCH	ΣEndo	ΣDDT	ΣDrines			
Great-horned owl (*Bubo virginianus*) (n = 6) juveniles (n = 2) adults		X	–	–	–	Dieldrin juveniles 220 ppb; adults 340 ppb	Concentrations for survival and mortality assessment	United States	Frank and Lutz (1999)
Burrowing owl (*Athene cunicularia*) (n = 17)	X		–	–	pp-DDE = 214 ppb	–	Concentrations report	United States	Gervais et al. (2000)
Barn owl (*Tyto alba*) (n = 9)	X		5300 ppb	–	48000 ppb	–	Concentrations report	Belgium	Jaspers et al. (2007)
Long-eared owl (*Asio otus*) (n = 10)			2500 ppb	–	110000 ppb	–			
American kestrel (*Falco sparverius*)		X	γ-HCH = 0.4 ppb β-HCH = 0.7 ppb δ-HCH = 0.7 ppb	Endo I = 0.5 ppb, Endo II = 0.7 ppb Endo S = 0.4 ppb	pp-DDE = 1.2 ppb	Dieldrin = 24 ppb	Concentrations report with 50 µl of serum	Mexico	Rivera-Rodríguez et al. (2007)
Great-horned owl (*Bubo virginianus*) (n = 3)		X	–	–	47 ppb	–	Risk assessment	United States	Strausse et al. (2007)
Little owl (*Athene noctua*) (n = 3)	X		46 ppb	–	73 ppb	–			
European scops owl (*Otus scops*) (n = 1)	X		212 ppb	–	295 ppb	–	Concentrations report	India	Behrooz et al. (2009)
Short-eared owl (*Asio flammeus*) (n = 1)	X		28 ppb	–	62 ppb	–			
Barn owl (*Tyto alba*) (n = 1)		X	–	58.3 ppb	70.3 ppb	Dieldrin = 6.3 ppb	Concentrations report	India	Dhananjayaj and Muralidharan (2010)
(*Bubo bubo*) (n = 316)		X	1.20 ppb	Endo I y II = 5.33 ppb	2.65 ppb	Dieldrin = 3.47 ppb	Concentrations report	Spain	Gómez (2011)

Table 2 continued

Species	Sample		OC Concentration				Results	Site	Reference
	Feathers	Blood	ΣHCH	ΣEndo	ΣDDT	ΣDrines			
Osprey (*Pandion haliaetus*) chicks (n = 28)		X	α-HCH = 0.118 ppb β-HCH = 0.177 ppb δ-HCH = 0.064 ppb γ-HCH (lindane) = 0.050 ppb	Endo I = 0.050 ppb Endo II = 0.015 ppb Endo S = 0.027 ppb	pp-DDE = 0.922 ppb pp-DDD = 0.065 ppb	Aldrin = 0.031 ppb Dieldrin = 0.969 ppb Endrin = 0.101 ppb Endrin K = 0.363 ppb Endrin A = 0.868 ppb	Concentrations report Alterations in biochemical parameters	Mexico	Rivera-Rodríguez and Rodríguez-Estrella (2011)

OC = Organochlorines, Endo I = Endosulfan I, Endo II = Endosulfan II, Endo S = Endosulfan Sulfate, Endrin K = Endrin Ketone, Endrin A = Endrin Aldehyde. All units are in parts per billion (ppb)

In Mexico, aldrin was used to control ants and termites, while endrin was used against Lepidoptera in cotton, maize, and sugar cane crops; these compounds were used for 20 and 30 years, respectively (OMS 1996). Endrin appears to be resistant to biodegradation in natural waters and most soils, and it will persist for long periods of time (4–14 years) (FAO 2000). These two pesticides have been banned in Mexico since 1991 (Fernández et al. 2004). However, the presence of ΣDrines, especially in owl blood, indicates that they are still being used in this area in agriculture or that they may have moved to the area through the air from other locations, such as Guatemala or other Central American countries (Alegría et al. 2006).

Endosulfan showed the second highest concentration in feathers and the third highest in blood (Table 1). This pesticide is one of the most toxic on the market today, as it is acutely neurotoxic for mammals and insects (Silva and Gammon 2009). Its presence has not been reported in raptor feathers, but it has appeared in blood samples from eagle owls, osprey chicks, and American kestrels (Rivera-Rodríguez et al. 2007; Gómez 2011; Rivera-Rodríguez and Rodríguez-Estrella 2011; Table 2). Information on the effects of endosulfan on wildlife is rather limited (Espín 2010). This compound does not persist in warm-blooded organisms and exhibits rapid metabolization and excretion; therefore, its presence in *Glaucidium* might indicate recent exposure. In the study area, this product is still used as an insecticide and acaricide in agricultural and industrial settings, even though in May 2011, the Stockholm Convention Committee approved a recommendation to globally eliminate the production and use of endosulfan and its isomers (United Nations 2011).

The concentration of DDT was the third highest of the tested compounds in feathers but was lower in blood samples. This compound shows an increased concentration (biomagnification) in tissues of organisms at successively higher levels in a food chain, with a known half-life of 2–15 + years; it has previously been detected in several species of raptors. For example, it has been reported in feathers of the burrowing owl *(Athene cunicularia)*, little owl *(Athene noctua)*, European scops owl *(Otus scops)*, and short-eared owl (*Asio flammeus*) (Gervais et al. 2000; Behrooz et al. 2009; Table 2). The presence of DDT has been reported in blood samples from the eagle owl, great-horned owl, and the barn owl (Strause et al. 2007; Dhananjayaj and Muralidharan 2010; Table 2). In Mexico, DDT compounds were reported in diurnal raptors, such as osprey chicks and the American kestrel (Rivera-Rodríguez et al. 2007; Rivera-Rodríguez and Rodríguez-Estrella 2011; Table 2). This pesticide was once used throughout the world, and although it has not been used in Mexico for agricultural purposes since 1991 and was banned in 2001, it continues to be used in public health maintenance, as a

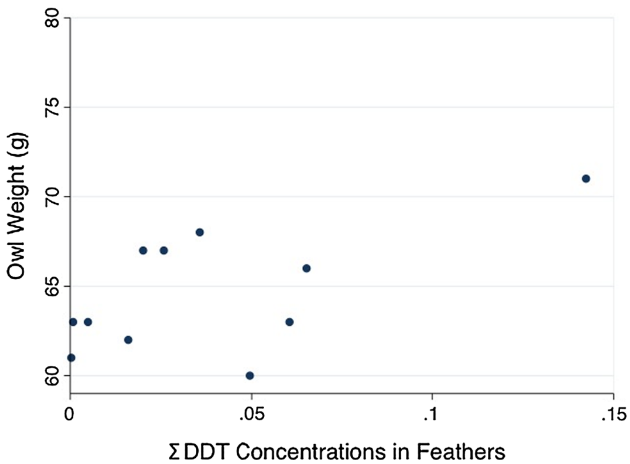

Fig. 2 Correlation between body weights and the DDT concentrations in feathers of the ferruginous pygmy owl (*Glaucidium brasilianum*) in Mexico ($r = 0.60$, $p = 0.05$)

disease vector control in tropical areas (Fernández et al. 2004). The presence of DDT in feathers of owls is likely to be due to its high chemical stability and persistence in both the environment (>30 years) and in organisms (Turusov et al. 2002).

The total concentrations in the feathers of each of the seven families of pesticides showed no significant correlations with either PM (body condition) or body weight ($p > 0.15$). However, ΣDDT did show a marginally positive correlation ($r = 0.60$, $p = 0.05$), indicating that as body weight increases, the concentrations of ΣDDT in feathers also tended to increase (Fig. 2). Exposure to DDT/DDE/DDD has been linked to poor body condition and has been associated with loss, gain, or no effect on body weight. In particular, DDT has been associated with disruption of endocrine systems, hyperthyroidism (loss weight) or hypothyroidism (gain weight) (Jefferies 1969; US Department of Health 2002). More information is necessary, however, to establish a link between DDT in tissues and its effects on the health of owls.

No significant correlations were identified between OCs in feathers and blood tissues ($p > 0.41$) for six of the families of compounds; the exception was ΣDDT ($r = 0.87$, $p = 0.02$, Table 3). Goshawk chicks (*Accipiter gentilis*) also showed a correlation between pp-DDE concentrations in their feathers and blood ($r = 0.86$, $p = 0.01$) (Eulaers et al. 2011a). This species lives in areas with intense agricultural activity. The lack of pesticide concentrations in feathers and blood could be due to several factors. In feathers, OCs accumulate during phases of growth, since birds replace their feathers once a year, and birds can take a few months to molt completely. However, owls molt much more slowly than other birds; they replace a few flight feathers during 1 year, a few more the following year, and only finish molting in the third year (Berger 2005). Thus, it is difficult to know when an individual was contaminated, since a resident owl could have been exposed at any time over a 3-year period. By contrast, OCs in blood indicate a much more recent exposure. However, it is also important to bear in mind that the persistence of different OCs varies and that its half-life is influenced by both environmental temperature and humidity (Scheunert 1989). Other factors can also affect the persistence of OCs, e.g. dose concentrations, duration and frequency of exposure, mode of contact, and the organismal condition (health, sex, and age). The influences of all of these factors on each of the OCs and on physiological pathways are important considerations.

Table 3 Pearson's correlations of organochlorine pesticide (OC) concentrations between blood and feathers of the ferruginous pygmy owl (*Glaucidium brasilianum*) in Mexico

OCs	*r*	*p*
ΣHCH	0.26	0.67
ΣHeptachlor	−0.16	0.90
ΣDrines	0.38	0.41
ΣDDT	0.87	0.02*
ΣEndosulfan	−0.23	0.65

* Significance level, $p < 0.05$

In this study, the ΣDrines had the highest mean concentration in blood samples from the ferruginous pygmy owl (0.31 ppm; Table 1). This value is comparable to the dieldrin concentrations in great-horned owls in the United States (Frank and Lutz 1999); the deaths of 2 adults (0.34 ppm) and one juvenile (at 0.22 ppm) were also reported in this study. Because raptors are exposed to pesticides all year around, the major effects of dieldrin is thought to be through direct mortality of resident species. Certainly, it is always difficult to specify the cause of mortality or to identify causal relationships in a very small sample size. Other factors, such as organismal age, sex, and body condition also need to be considered and it is important to monitor OC concentrations in the organisms over a long period of time.

Despite the dieldrin concentrations observed in this study, the owls did not show any apparent ill effects. However, we would expect ferruginous pygmy owls to present alterations in their hematology values, because blood concentrations detected were higher than those reported for osprey chicks (Rivera-Rodríguez and Rodríguez-Estrella 2011). The mean triglyceride levels and corpuscular hemoglobin concentrations in osprey decreased with increasing concentrations of pp-DDE, β-HCH, dieldrin, and heptachlor epoxide (Table 2). Other studies have shown that OC concentrations negatively correlated with breeding success, infertility, impaired reproductive behavior, and parental care in birds of prey and aquatic birds (Fry 1995, Wilson et al. 1999). In addition to these effects, chronic neurotoxicity may occur, which implies more collisions, greater electrocution risk, a negative impact on flight cost, and more time spent on feeding trips (Bustnes et al. 2002, 2008).

This is the first empirical report of OCs in an owl species in Mexico. Our results showed variations in OC concentrations between the feather and blood tissues for seven chemical families. Thus, ferruginous pygmy owls are clearly exposed to these pesticides, and although some of these pesticides have been banned and others are now restricted, some continue to be sold in local stores (e.g., endosulfan). Organophosphate pesticides (e.g., Furadan and Semevin) are increasingly used because they degrade rapidly via hydrolysis upon exposure to soil, sunlight, and air; but when used in large amounts they cause severe toxicity and pose exposure risks of large duration and intensity. Organophosphate pesticide poisonings cause substantial morbidity and mortality. Studies have showed decrease in neuropsychological performance among individuals with previous intoxication, and also can affect the endocrine system (Rosenstock et al. 1991; Kaiser 2000). Further quantitative, long-term studies are thus needed to evaluate the cumulative and interactive effects of organochlorine concentrations in several species of Neotropical owls.

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Compliance with Ethical Standards

Ethical Approval All applicable international, national, and institutional guidelines for the care and use of animals were strictly followed. All procedures performed in the studies involving animals were in accordance with the ethical standards of the institution or practice where the studies were conducted. For instance, during handling, the owls were monitored to ensure that no signs of stress occurred (salivation and/or defecation). This work was carried out with permission from the Ethics Committee for Research at the Colegio de la Frontera Sur, Chiapas, Mexico, official number registration CEI-O-025/14.

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