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Factors that influence mercury concentrations in nestling Eagle Owls (*Bubo bubo*)

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HIGHLIGHTS

- Hg concentrations in blood and feathers of Eagle owl chicks can be considered low.
- We found a positive correlation between Hg levels in blood and feathers of chicks.
- Blood Hg concentrations in Eagle owls reflect Hg levels in muscle of rabbits.
- Spatial differences in Eagle owl Hg levels are related to local contamination.
- Temporal differences in Eagle owls Hg concentrations may be related to rainfalls.

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ABSTRACT

Mercury (Hg) is a global pollutant that bioaccumulates and biomagnifies in food chains, and is associated with adverse effects in both humans and wildlife. The Hg levels detected in blood obtained from Eagle Owl (*Bubo bubo*) chicks in Southeast Spain (Murcia) can be considered low (mean Hg concentration in blood from 2006 to 2012 was 36.83 ± 145.58 μg/l wet weigh, $n = 600$), and it is therefore unlikely that Hg pollution can negatively affect their breeding. Positive correlation ($r = 0.339$, $p < 0.001$, $n = 229$) was found to exist between the Hg concentrations in the blood and back feathers of the chicks. We provide a regression equation that could be helpful to estimate blood Hg levels when analyzing Hg concentrations in back feathers. Blood Hg concentrations in Eagle Owls have shown positive correlations with Hg levels in rabbit muscles, more evident in nests from the Northern area ($r = 0.600$, $p = 0.014$, $n = 16$), where rabbits are the main prey of Eagle Owls. The best Linear Mixed Model to explain variations in blood Hg concentrations in nestling Eagle Owls includes year and location within the mining area as variables. The variable year is assigned the largest value of relative importance, followed by the location in the ancient mining sites and then the zone. Rainfalls may have an effect on the temporal differences in the blood Hg concentrations of nestling Eagle Owls. Although the studied region is not considered Hg polluted, the Hg levels were higher in Eagle Owls and European Rabbits from areas within the ancient mining sites as compared to those in the entire region. This result shows that spatial differences in Hg concentrations in Eagle Owls may be affected by local contamination, and that the role of diet composition may be less significant.

1. Introduction

Mercury (Hg) is a global pollutant that bioaccumulates and biomagnifies in food chains, and is associated with adverse effects such as neurological, immunological and reproductive impacts in both humans and wildlife (Burger and Gochfeld, 1997; Evans et al., 1982). Primary sources of anthropogenic Hg emissions include the combustion of fossil fuels, mining and reprocessing of ores (gold, copper, lead and zinc), iron, steel and cement production, operation of Chlor-Alkali plants, and waste incineration and disposal (Driscoll et al., 2007; Pacyna et al., 2006). The persistence of this metal in the atmosphere and its ability to travel great distances have allowed it to become a global pollutant (Seewagen, 2010).

Inorganic Hg is converted into the organic form (methylmercury, MeHg), especially in aquatic ecosystems. This organic form of Hg is most harmful and able to bioaccumulate in food chains (Driscoll et al., 2007; Thompson and Furness, 1989b). Due to the methylation and bioaccumulation of MeHg in aquatic systems (Espín et al., 2012), much of the research effort involving Hg contamination in birds has

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focused mostly on waterbirds and piscivore species living in aquatic ecosystems (Seewagen, 2010). However, these are not necessarily the only groups of birds and ecosystems affected. Although it may appear to be smaller than in aquatic systems (Lindqvist, 1991), evidence suggests that methylation may also occur in terrestrial systems (Driscoll et al., 2007; Rimmer et al., 2005, 2010). Previous studies (quite recently, in the Iberian Peninsula (Ortego et al., 2006; Lourenço et al., 2011)) have analyzed Hg concentrations in Eagle Owl feathers. Some studies have found large accumulations of this contaminant in birds of prey that feed at the top of terrestrial trophic chains (Broo and Odsjö, 1981; Palma et al., 2005). Also, Zolfaghari et al. (2007) found significant differences in feather Hg concentrations in relation to trophic level in 18 bird species from southwest Iran, and raptors (including Eagle Owl) that feed on vertebrates (except fish) showed the highest levels of Hg.

The Eagle Owl is a large nocturnal raptor that is also a suitable biomonitor of Hg in terrestrial ecosystems because of its relatively long-life span as a top predator in food chains. Its resident and territorial habit makes it easy to localize environmental contamination. The Eagle Owl in our study area feeds primarily on European Rabbit (*Oryctolagus cuniculus*), showing geographical variation in diet composition depending on local habitat conditions (authors' unpublished data). Several studies have shown that diet composition bears significant influence on the concentration of contaminants in top predators, regardless of spatial or temporal variation in environmental contamination (Lindberg and Odsjö, 1983; Mañosa et al., 2003; Palma et al., 2005). Palma et al. (2005) found great spatial variation in Hg levels in Bonelli's Eagle (*Hieraaetus fasciatus*), in relation to diet composition and food chain biomagnification. It is therefore necessary to consider the effect of diet composition on the pollutant burdens of birds of prey that feed on terrestrial food chains, to correctly interpret spatial or temporal changes in environmental contamination (Lourenço et al., 2011; Mañosa et al., 2003; Palma et al., 2005).

In this study, we have evaluated Hg levels in blood and feathers of Eagle Owl chicks and in muscle samples from carcasses of its main prey, the European Rabbit, collected during seven breeding seasons (2006–2012) from Southeast Spain. The study area is located in the east of Murcia province (37°45′ N, 0°57′ W) (Fig. 1), and is divided into two subareas: some ancient mining sites (Sierra Minera Cartagena-La Unión) and an industrial area in Cartagena City are located in the Southern subarea (Fig. 1). Heavy industrial activity in Cartagena is represented by electric power plants, explosive factories, shipbuilding, and waste incineration. Moreover, the city is an active military and commercial port, with an accumulation of slag and sludge from mines, refineries, and other sources, on the outskirts (García-Fernández et al., 1995). For 2500 years, lead, zinc, copper, tin, iron, manganese and silver were extracted from the ancient mining sites until 1991 when the mines were closed (Pavetti et al., 2006). This mining area shows very high concentrations of heavy metals (Martínez-Sánchez and Pérez-Sirvent, 2009) and soil acidity at some points, which leads to a high bioavailability of metals, especially after rainfalls (Pavetti et al., 2006).

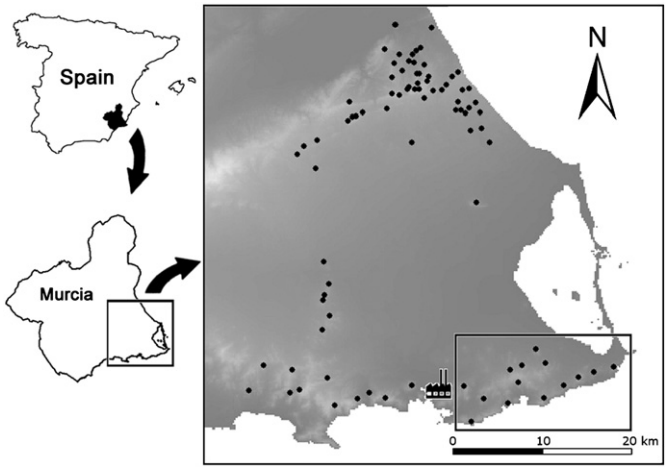

Fig. 1. Map showing the study area (Murcia, Spain). Circles represent nests in Northern and Southern areas. Industrial area is represented by a factory figure and area under mining influence is framed by a square.

The main aims of the present study are: (i) to provide data on Hg concentrations in blood and feathers of the terrestrial bird species, Eagle Owl, and in European Rabbit muscle, (ii) to study the relationship between Hg concentrations in blood and feathers of nestling Eagle Owl, (iii) to study the relationship between Hg concentrations in Eagle Owl blood and European Rabbit muscle, (iv) to evaluate spatial and temporal differences in Hg concentrations in Eagle Owl, and (v) to test local contamination, dietary composition and rainfalls as factors affecting Hg concentrations in Eagle Owl chicks.

2. Materials and methods

2.1. Study area and species

The study area is in the east of the province of Murcia (37°45′ N, 0°57′ W) (Fig. 1). The climate is meso-arid Mediterranean with 275–400 mm of annual rainfall and a high average annual temperature of 19 °C. Since the study area is relatively large and with known differences in land use, it was divided into two subareas. The northern subarea comprises the mountains Escalona, Altaona, Monte el Valle, and Columbares. In this subarea, land is mainly dedicated to citrus and dry farming, and is home to abundant European Rabbits, accounting for 71% of the prey consumed by Eagle Owls (authors' unpublished data). In the southern subarea, the European Rabbit is less abundant (35% of the Eagle Owl's diet), and the raptor consumes a similar proportion (23% of the diet) of rats (*Rattus rattus* and *Rattus norvegicus*), in addition to pigeons (*Columba* spp.), partridges (*Alectoris rufa*), hedgehogs (*Erinaceus europaeus* and *Atelerix algirus*) and yellow-legged gulls (*Larus michahellis*) (authors' unpublished data). This area is delimited by Sierra Minera Cartagena-La Unión, La Muela-Cabo Tiñoso and Almenara. Irrigation farming is predominant, but this subarea remarkably includes some ancient mining sites (Sierra Minera Cartagena-La Unión) and an industrial area, in Cartagena (Fig. 1). In our study area, the Eagle Owl prefers low to medium cliffs (Martínez and Calvo, 2000). This species is the largest nocturnal raptor in Spain; it is sedentary and highly territorial all year round, and occupies home ranges that vary in size depending on prey availability. Eagle Owl population in the province of Murcia is abundant and estimated to be approximately 240–270 pairs (Martínez and Calvo, 2006; Martínez and Zuberogoitia, 2003).

2.2. Sample method

A total of 600 blood samples from 2006 to 2012 (415 from Northern area and 185 from Southern area), and 229 back feathers from 2006 to 2008 (167 from Northern area and 62 from Southern area) were analyzed for Hg concentrations in Eagle Owl chicks of approximately 30-days old from Murcia (Fig. 1). In most cases, more than one chick was sampled per nest (from 1 to 5 chicks per nest), and some nests were sampled for more than one year. Eagle Owl chicks belonged to 231 broods from 89 different territories (58 from Northern area and 31 from Southern area) and sampling was done in February–March. In addition, we obtained 40 muscle samples from carcasses of European Rabbit from 2009, 2011 and 2012 collected at the sampling nests (25 from Northern area and 15 from Southern area). Preys were returned to the nest after the sampling.

Blood samples were obtained by puncturing the brachial vein with a 23G needle and syringe, and then conserved in Eppendorf tubes under refrigerated conditions during transport to the laboratory. Heparine was added to the tubes as anticoagulant. We collected 3–5 ml of

blood, depending on the stage of development of the nestling. Growing feathers were plucked from the back of chicks. We used body feathers because their sampling does not affect the chick's future flight performance. After sampling, the nestlings were returned to their nest. Feathers were stored in sterile plastic bags at room temperature, whole blood samples were stored in Eppendorf tubes and muscle samples were stored in plastic bags at −80 °C until analysis. The sampling in Murcia was authorized by the General Directorate of Natural Heritage and Biodiversity from the Autonomous Community of Murcia.

2.3. Mercury analysis

Total Hg was analyzed in Milestone DMA-80 direct Hg analyzer by atomic absorption spectrophotometry with a detection limit of 0.005 ng. Samples (0.1 g wet weight for blood, 0.01 g dry weight for feathers and 0.2–0.3 g wet weight for rabbit muscles, approximately) were loaded in a nickel boat and analyzed. Calibration curve was traced with ten points (in duplicate) from 0 to 1004 ng of Hg.

The method was tested for precision and accuracy using certified reference material (CRM) (Hg Standard for AAS, Fluka, 1000 mg/l Hg in 12% nitric acid, prepared with high purity Hg metal, HNO_3, TraceSELECT® and water TraceSELECT®Ultra). Recovery of total Hg from 5 replicates of CRM diluted to 1 ppm was 104.2 ± 11.8% (mean ± standard deviation). The coefficient of variation for the repeatability was 11.4%.

To remove external contamination from the feathers, which could otherwise alter the results of the Hg sequestrated in them, they were washed with tap water, distilled water, Milli-Q water and acetone, and then subsequently dried at room temperature overnight, before proceeding with the analytical determination.

2.4. Statistical analysis

We performed statistical analyses using the R v. 3.0.1 (R Core Team, 2013) software, which is distributed free under the GNU General Public License and available at the URL http://www.r-project.org/. Reported Hg values represent mean ± standard deviations. Since the Hg concentrations were not normally distributed, the data was log-transformed. Statistical tests were performed with Linear Mixed Models (LMM), using the "nlme" package (Pinheiro et al., 2013). Territory and brood (nested within territory) and/or chick were considered as random factors, while a number of biological and environmental covariates (see below) were taken as fixed factors.

First, we examined the differences between mean Hg concentrations in blood and feather samples using chick as random factor (an analysis equivalent to a classical paired t-test). We used a simple linear model and estimated Pearson correlation coefficient to analyze the relationship between Hg concentrations in blood and back feathers of individual chicks.

We applied the information-theoretic methodological approach outlined by Burnham and Anderson (2002) to analyze the variations in blood Hg concentrations in relation to environmental factors, and then performed the LMM analyses using territory and brood (nested within territory) as random factors, and the following covariates as fixed factors: (1) year, which represents the environmental stochasticity related to annual weather (rainfall) conditions; (2) zone (North/South), representing differences in dietary composition; (3) location of territory within the ancient mining area (1/0); and (4) linear distance (km) from territory to industrial area. Using these four variables, we developed a set of 21 ecologically plausible a priori candidate models (Table 1), including the 4 univariate models, 16 additive and interaction models, and the null (intercept only) model as a baseline for comparison. We avoided considering complex interaction models (>2 factors) due to the very large number of parameters to be estimated and because they have no clear ecological support.

Table 1
A priori hypothesized mixed models used to examine the spatio-temporal variations in blood Hg concentrations of nestling Eagle Owl. The asterisk (*) denotes interaction models (models including the interaction term between the two factors).

Model notation	Model description
g_{year}	Year (2006–2012)
g_{zone}	Zone (North vs. South)
g_{mining}	Location of the territory within the ancient mining area
$g_{distance}$	Distance (km) of the territory to the industrial area
$g_{year + zone}$	Year + zone
$g_{year + mining}$	Year + mining
$g_{year + distance}$	Year + distance
$g_{zone + mining}$	Zone + mining
$g_{zone + distance}$	Zone + distance
$g_{mining + distance}$	Mining + distance
$g_{year + zone + mining}$	Year + zone + mining
$g_{year + zone + distance}$	Year + zone + distance
$g_{year + zone + distance}$	Year + mining + distance
$g_{zone + mining + distance}$	Zone + mining + distance
$g_{year + zone + mining + distance}$	Year + zone + mining + distance
$g_{year * zone}$	Year * zone
$g_{year * mining}$	Year * mining
$g_{year * distance}$	Year * distance
$g_{zone * distance}$	Zone * distance
$g_{mining * distance}$	Mining * distance
g_{null}	Intercept only

Model comparisons were based on Akaike's information criterion (AIC; Burnham and Anderson, 2002). We ranked models based on AIC differences (Δ_i) and Akaike weights (w_i). Δ_i was calculated as the difference between the AIC of each model and the AIC of the best model. Models with $\Delta_i < 2$ can be considered as alternatives to the selected model, although models in which Δ_i is in the 2–7 range have some support (Burnham et al., 2011). Akaike weights may be interpreted as the probability that model i is the actual best model of the set. The evidence ratio (ER), a measure of the relative strength of each candidate model, was estimated as

$$ER = \frac{\exp\left(-\frac{1}{2}\Delta_{best}\right)}{\exp\left(-\frac{1}{2}\Delta_i\right)}$$

and provides a measure of how much more likely the best model is than model i (Symonds and Moussalli, 2011). The relative importance of each predictor, w + (*j*), was estimated by summing the Akaike weights across all the models in the set where variable *j* occurs (Burnham and Anderson, 2002).

We used the Pearson correlation test to examine the relationship between mean annual Hg concentrations in blood of nestlings from all territories in the study area and total rainfalls in the study area in the six months prior to sampling (September–February; weather station TP52 San Javier-El Mirador; SIAM, 2013). Finally, we also used simple linear models and estimated the Pearson correlation coefficient to analyze the relationship between Hg concentrations in blood samples of nestling Eagle Owls and muscle samples of the rabbit carcasses found in nests. In such cases, to avoid pseudo-replication, we calculated the mean Hg concentration of muscles from rabbits in the same nest ($n = 25$).

3. Results

3.1. Mercury levels in blood and feathers of nestling Eagle Owls

Table 2 shows Hg concentrations in blood and feathers of Eagle Owl, and Table 3 provides a review of Hg concentrations in feathers of Strigiformes. Hg concentration (mean ± SD) in blood from 2006 to 2012 was 36.83 ± 145.58 μg/l wet weigh ($n = 600$), and in feathers from 2006 to 2008 was 328.88 ± 447.15 μg/kg ($n = 229$). This shows

Table 2
Concentrations of mercury in the blood and feathers of Eagle Owl (*Bubo bubo*) and in rabbit muscles (*Oryctolagus cuniculus*).

Concentrations of Hg in Eagle Owl (*Bubo bubo*) and their main prey (*Oryctolagus cuniculus*)						
	Chick blood (μg/l, ww)		Chick feathers (μg/kg)		Rabbit muscle (μg/kg, ww)	
	Mean ± SD, median (range)	*n*	Mean ± SD, median (range)	*n*	Mean ± SD, median (range)	*n*
Total	36.83 ± 145.58, 14.69 (1.45–2886.20)	600	328.88 ± 447.15, 238.09 (66.50–4891.62)	229	13.71 ± 12.83, 9.26 (3.24–72.81)	40
Year						
2006	18.10 ± 22.52, 11.20 (3.82–140.09)	71	243.58 ± 144.24, 210.73 (85.89–970.31)	69	–	
2007	50.88 ± 75.98, 20.38 (3.31–466.71)	93	409.38 ± 670.11, 246.51 (66.50–4891.62)	93	–	
2008	41.41 ± 198.69, 10.16 (1.81–1635.98)	67	304.97 ± 161.28, 257.74 (84.77–887.342)	67	–	
2009	60.45 ± 270.69, 23.84 (3.08–2886.20)	116	–		15.04 ± 11.75, 8.57 (5.57–40.48)	12
2010	35.43 ± 98.85, 14.53 (2.33–840.48)	75	–		–	
2011	19.76 ± 40.35, 10.54 (1.45–384.36)	112	–		12.47 ± 7.23, 9.58 (6.34–28.18)	9
2012	21.53 ± 22.97, 14.57 (5.64–125.03)	66	–		13.46 ± 15.71, 7.85 (3.24–72.81)	19
Area						
North	34.08 ± 152.29, 12.27 (1.81–2886.20)	415	303.79 ± 375.22, 225.41 (66.50–3485.57)	167	11.32 ± 7.14, 9.21 (3.24–32.57)	25
South (mine area included)	42.99 ± 129.43, 18.56 (1.45–1635.98)	185	396.44 ± 598.20, 295.70 (85.90–4891.62)	62	17.70 ± 18.54, 10.02 (4.22–72.81)	15
Only ancient mine site	56.50 ± 175.60, 26.989 (4.85–1635.98)	87	373.09 ± 142.05, 334.68 (188.89–696.87)	37	24.98 ± 12.76, 24.70 (10.02–40.48)	4

significant higher Hg concentrations in feathers than in blood ($F = 1590.61$; *d.f.* = 1228; $p<0.001$) of nestling Eagle Owls (Table 2). Positive correlation exists between Hg concentrations in blood of chicks and in back feathers ($r = 0.339$, $p = 0.001$, $n = 229$) (Fig. 2). The equation estimated by simple linear regression is: log (Hg) in blood (μg/l, ww) = −0.588 + 0.617 ∗ log (Hg) in feathers (μg/kg). This equation was calculated considering Hg concentrations in blood and feathers of 229 nestling Eagle Owls from Southeast Spain.

3.2. Spatio-temporal models

Table 4 shows the ranking of the models used to explain variations in blood Hg concentrations in nestling Eagle Owls. The best models ($\Delta_i < 7$) include different combinations of three of the four predictors considered: year, mining and zone (see Table 1). The best approximating model is $g_{\text{year + mining}}$ ($w_i = 0.499$), but models $g_{\text{year + zone + mining}}$ ($w_i = 0.304$) and $g_{\text{year + zone}}$ ($w_i = 0.149$) also have important support. Therefore, the evidence ratio between the best model and the two alternative models is very low (1.6 and 3.3, respectively), which suggest that these three variables are determinant factors related to Hg concentrations.

Regarding the relative importance of each predictor, the variable year has the largest value of relative importance ($w_+ = 1.000$), followed by mining ($w_+ = 0.831$) and then zone (North vs. South) ($w_+ = 0.463$). The linear distance of the territory to the industrial area does not appear in any of the top ranked models and its relative importance ($w_+ = 0.029$) is very low. Table 2 shows that blood Hg concentrations in Eagle Owls change notably among years. However, Hg concentrations found in European Rabbit muscles were very similar among years ($F = 0.379$, $p = 0.687$, $n = 40$) (Table 2). In the study area, there are important fluctuations in rainfalls among years. Fig. 3 together represents the temporal trend in Hg concentration in blood of Eagle Owls and total precipitations in the province of Murcia. No significant correlation was found between mean Hg concentration in blood of nestlings and total precipitation ($r = 0.534$, $p = 0.216$, $n = 7$). With regard to the spatial variations, Hg levels were 34.08 ± 152.29 μg/l in the North ($n = 415$) and 42.99 ± 129.43 μg/l in the South ($n = 185$) (Table 2). When the Eagle Owls from the mining area ($n = 87$) were excluded, Hg levels in the South decreased to 31.00 ± 64.29 μg/l ($n = 98$). However, the mean Hg level in blood of chicks from the ancient mining site was notably higher (56.50 ± 175.60 μg/l, $n = 87$) (Table 2).

3.3. Prey study

Table 2 shows Hg concentrations in muscle of European Rabbit. Hg concentrations found in rabbit muscles showed no location-related differences ($F = 3.42$; *d.f.* = 1,23; $p = 0.078$) (Table 2). However, when we excluded the rabbits from the ancient mining site, the mean Hg levels in rabbit muscles were significantly lower (12.46 ± 12.38 μg/kg ww) than those in rabbits from the mining area (24.98 ± 12.76 μg/kg ww) ($F = 6.83$; *d.f.* = 1,23; $p = 0.019$) (Table 2).

Samples collected in 2009 ($r = 0.380$, $p = 0.029$), 2011 ($r = 0.614$, $p = 0.001$) and 2012 ($r = 0.556$, $p = 0.020$) respectively, showed positive correlations between Hg concentrations in blood of nestling Eagle Owls and muscles of their prey. Moreover, samples collected in 2011 ($F = 0.017$, $p = 0.897$) and 2012 ($F = 1.656$, $p = 0.202$) (Table 2) showed no significant differences between Hg levels in blood of Eagle Owls and rabbit muscles. However, samples collected in 2009 showed significant differences ($F = 8.194$, $p = 0.005$), and the highest concentrations were found in blood of chicks (Table 2).

In addition, it also showed significant positive correlation between Hg levels in the blood of Eagle Owl and rabbit muscles in the Northern area ($r = 0.600$, $p = 0.014$, $n = 16$) where the availability of rabbit is higher, but no significant correlation in the south ($r = 0.515$, $p = 0.156$, $n = 9$) (Fig. 4).

4. Discussion

4.1. Mercury levels in blood and feathers of nestling Eagle Owls

No previous data is available on Hg concentrations in blood of Eagle Owls. However, levels found in this study (Table 2) were much lower than reported for nestlings of fish-eating raptors such as Bald eagles (*Haliaeetus leucocephalus*) and Ospreys (*Pandion haliaetus*) (Jagoe

Table 3
Literature review on mercury concentrations in feathers of Strigiformes from different areas.

	Literature mercury levels (μg/g) in feathers of Strigiformes from different areas					
Species	n	Mean mercury levels (μg/g)	Sample type	Sampling area	Year	References
Eagle Owl (*Bubo bubo*)	229	0.32	Chicks	SE Spain	2006–2008	Present study
Eagle Owl (*Bubo bubo*)	32	0.12 (year 2002) and 0.09 (year 2003)	BF. Chicks 20–30 days old	Toledo, C Spain	2002–2003	Ortego et al. (2006)
Eagle Owl (*Bubo bubo*)	61	1.29	BF. Adult birds	SW Iberian Peninsula	2003–2007	Lourenço et al. (2011)
Barn owl (*Tyto alba*)	13	1.22	BF			
Tawny owl (*Strix aluco*)	3	0.48	BF			
Little owl (*Athene noctua*)	15	0.64	BF			
Little owl (*Athene noctua*)	7	0.12–0.36	P9-P3[a]	Belgium	2001	Dauwe et al. (2003)
Barn owl (*Tyto alba*)	5	0.77–0.90	P4-P2[b]			
Little owl (*Athene noctua*)	3	0.50 and 1.10	SF and TF	SW Iran	2005	Zolfaghari et al. (2007)
Tawny owl (*Strix aluco*)	2	0.56 and 0.85	SF and TF			
Eagle Owl (*Bubo bubo*)	3	0.30 and 0.71	SF and TF			
Eagle Owl (*Bubo bubo*)	39	3.20 (IP) and 6.51 (CP)	Adult birds	SW Sweden	1971–1976	Broo and Odsjö (1981)
Eagle Owl (*Bubo bubo*)	9	2.84 (IP) and 1.23 (CP)	Young birds, 1–3 months old		1975–1977	
Eagle Owl (*Bubo bubo*)	29	4.08 (IP) and 8.00 (CP)	Adult birds	SE Sweden	1970–1973	Odsjö and Olsson (1975)
Eagle Owl (*Bubo bubo*)	–	2.14 (IP) and 5.80 (CP)	Young birds, 1–3 months old		1970–1974	
Eagle Owl (*Bubo bubo*)	10	2.50 and 1.30	TF and InP	Sweden	1829–1933	Berg et al. (1966)
Eagle Owl (*Bubo bubo*)	4	0.70	SC. Chicks	Southern Finland	1984–1987	Solonen and Lodenius (1990)
Tawny owl (*Strix aluco*)	16	0.50	SC. Chicks			
Ural Owl (*Strix uralensis*)	4	0.60	SC. Chicks			
Great Gray Owl (*Strix nebulosa*)	1	0.60	SC. Chicks			
Long-eared Owl (*Asio otus*)	3	0.30	SC. Chicks			
Boreal Owl (*Aegolius funereus*)	3	1.50	SC. Chicks			
Little owl (*Athene noctua*)	7	17.00 (CF) and 10.00 (RS)[c]		The Netherlands	–	Van den Brink et al. (2003)

IP = inland population, CP = coastal population, TF = Tail feathers, SF = Secondary feathers, InP = Inner primaries, BF = Body feathers, SC = Secondary coverts, CF = Contaminated floodplains, RS = Reference site.
[a] P3 is the 3rd innermost primary feather and P9 is the 2nd outermost primary feather.
[b] P2 is the 2nd innermost primary feather and P4 is the 4th innermost primary feather.
[c] Geometric mean.

et al., 2002; Langner et al., 2012). Few studies have analyzed Hg concentration in feathers of owls, and more specifically Eagle Owl (Broo and Odsjö, 1981; Dauwe et al., 2003; Lourenço et al., 2011; Ortego et al., 2006; Zolfaghari et al., 2007) (Table 3). This study showed that the mean Hg concentrations in back feathers of Eagle Owl chicks were slightly higher than reported by Ortego et al. (2006) in Eagle Owl chicks from Central Spain, but similar to that reported in nestling Long-eared Owl (*Asio otus*) from Finland (Solonen and Lodenius, 1990) (Table 3). However, in this study Hg concentrations were lower than those reported by Lourenço et al. (2011) in adult Eagle Owls (Table 3). This may be due to the differences in the diets of young and adult individuals, or the differences in the local contamination sources, or because older individuals show higher Hg concentrations reflecting an accumulation of Hg in their tissues over a longer period of time, as observed in several bird species (Espín et al., 2012; Kojadinovic et al., 2007). Other authors have also found Hg levels in Eagle Owl to be higher than in this study, probably due to the use of alkyl Hg as a seed-dressing agent in terrestrial habitats in the areas studied between 1940 and 1966 (Broo and Odsjö, 1981; Odsjö and Olsson, 1975) (Table 3). Judging from such findings and in comparison with other studies that analyze Hg in chick feathers of other bird species (Barata et al., 2010; Goutner, 2011), Hg levels detected in Eagle Owl chicks from Southeast Spain can be considered low.

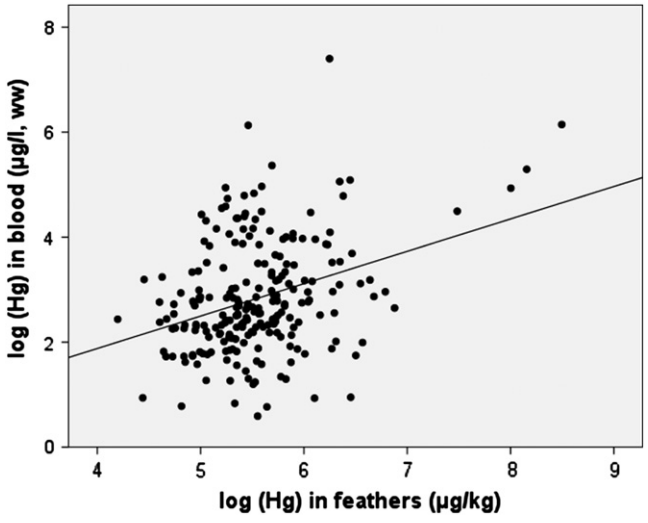

Fig. 2. Relation between mercury blood levels (μg/l, ww) and mercury feather levels (μg/kg) in Eagle Owls ($r = 0.339$, $p < 0.001$, $n = 229$). Log (Hg) in blood (μg/l, ww) = $-0.588 + 0.617 * \log$ (Hg) in feathers (μg/kg).

Besides, Hg levels found in Eagle Owls fell below toxic levels. The highest Hg concentrations in feathers were found in three chicks sampled in 2007 (1.3% of total studied population), with levels between 3 and 5 mg/kg, close to but below the critical level described by NAS (1978) (5 mg/kg in feathers) associated with reproductive impairment (lower clutch and egg size, reduced hatching rate and decreased chick survival). Hence, it is unlikely that Hg pollution can negatively affect the breeding performance of the overall population.

The reason why the Hg concentrations could be higher in feathers than in blood of nestling Eagle Owls (Table 2) may be due to the fact that feathers are important excretion routes for Hg in birds (Kenow et al., 2007). Thus, Burger (1993) considered that >90% of Hg total body burden may be sequestered into feathers during molt, and Hg binds with feather keratin as MeHg (Thompson and Furness, 1989a, 1989b).

In this study, feathers were still growing when they were sampled. Experimental studies have shown that Hg levels in feathers reflect the Hg concentrations in the blood during the feather growth (Lewis and Furness, 1991). Therefore, as expected, positive correlation exists between Hg concentrations in the blood of chicks and in back feathers (Fig. 2). Although the correlation may not be very strong, considering that there is no published data on Hg concentrations in the blood of Eagle Owls, we provide an equation that may be helpful to estimate Hg concentrations in the blood of Eagle Owl in studies that only analyze growing back feathers (Fig. 2).

Table 4
Ranking of the models used to explain variations in blood Hg concentrations in nestling Eagle Owls, based on Akaike's information criterion (AIC).

Tested model	*K*	AIC	Δ_i	w_i	ER
$g_{\text{year + mining}}$	10	1495.54	0.00	0.499	1.0
$g_{\text{year + zone + mining}}$	11	1496.53	0.99	0.304	1.6
$g_{\text{year + zone}}$	10	1497.95	2.41	0.149	3.3
$g_{\text{year * mining}}$	16	1502.61	7.07	0.015	34.2
$g_{\text{year + distance}}$	10	1502.95	7.41	0.012	40.7
$g_{\text{year + zone + distance}}$	11	1503.31	7.77	0.010	48.7
$g_{\text{year + zone + distance}}$	11	1505.51	9.97	0.003	146.3
$g_{\text{year * zone}}$	16	1505.60	10.06	0.003	153.2
$g_{\text{year + zone + mining + distance}}$	12	1505.71	10.17	0.003	161.5
g_{year}	9	1508.96	13.42	0.001	820.3
g_{mining}	4	1511.22	15.68	0.000	2539.3
$g_{\text{zone + mining}}$	5	1513.32	17.78	0.000	7258.2
g_{zone}	4	1514.79	19.25	0.000	1.51×10^4
$g_{\text{mining * distance}}$	6	1518.98	23.44	0.000	1.23×10^5
g_{distance}	4	1519.74	24.19	0.000	1.79×10^5
$g_{\text{mining + distance}}$	5	1519.83	24.29	0.000	1.88×10^5
$g_{\text{zone + mining + distance}}$	6	1522.37	26.83	0.000	6.70×10^5
$g_{\text{zone + distance}}$	5	1522.40	26.86	0.000	6.79×10^5
$g_{\text{zone * distance}}$	6	1529.86	34.32	0.000	2.84×10^7
$g_{\text{year * distance}}$	16	1548.85	53.31	0.000	3.76×10^{11}
g_{null}	2	2061.27	565.73	0.000	7.03×10^{122}

K = number of parameters estimated. Models were ranked using AIC differences (Δ_i) and Akaike weights (w_i). Models with $\Delta_i < 2$ can be considered to be alternative models to the selected model, although models where Δ_i is in the 2–7 range have some support (Burnham et al., 2011).

4.2. Temporal variations in mercury concentrations in Eagle Owl

Year was the most important evaluated factor influencing Hg levels in the blood of Eagle Owl. The study area showed variable rainfalls for the seven years of the study (Fig. 3), probably contributing to a higher removal of Hg from the atmosphere and local wet deposition. Moreover, the runoffs of storm water also wash off surfaces that may contain Hg and contribute to its transportation. The two years with the highest Hg levels in the blood of Eagle Owls were 2007 and 2009, precisely two years of high rainfalls (221 and 254 l/m^2, respectively) (Fig. 3). The year 2010 also had high rainfalls (260 l/m^2), but being that the previous year was rainy too, it is possible that less Hg was available for mobilization and deposition, and consequently lower concentrations were found in blood of Eagle Owls in 2010. The lack of differences in rabbit Hg levels among years seems to conflict with this explanation. However, the number of rabbit samples was smaller and only samples of 3 years were available for analysis, making it difficult to observe a trend. Fig. 3 shows a trend indicative of a possible effect of rainfall in Hg concentration in owls. The lack of significant correlation between mean Hg concentration in blood of nestlings and total precipitation could be explained by the low number of years ($n = 7$).

In addition, even though rabbits are the main prey of Eagle Owls in the studied area, when faced with prey scarcity, this generalist predator diversifies its diet to include other preys and predators (Lourenço et al., 2011). This fact could also affect the differences found among years in Hg blood concentrations, since it seems logical that birds did not feed on exactly the same proportion of rabbits during the period under study.

Positive correlations between Hg concentrations in blood of nestling Eagle Owls and in the muscles of their prey were found in samples collected in 2009, 2011 and 2012. Moreover, Hg levels in blood of Eagle Owls and in rabbit muscles were similar in samples collected in 2011 and 2012 (Table 2). However, significant higher concentrations were found in blood of chicks in comparison with rabbit muscles in 2009 (Table 2). This may be because almost all nests sampled in 2009 containing carcasses of rabbits were from the Northern area, and owls from this area have lower Hg concentrations than those in the Southern area, as discussed below (see Section 4.3). These results suggest that blood Hg levels are greatly influenced by Hg ingested through the consumption of rabbit.

4.3. Spatial variations in mercury concentrations. Are they affected by Eagle Owl feeding habits or by the contamination sources?

Some authors (Lourenço et al., 2011) have noted that spatial differences in Hg concentrations in Eagle Owls appear to be largely related to diet composition rather than to local contamination. In this study, lower Hg concentrations in Eagle Owl blood were found in the Northern area in comparison with the Southern area (Table 2). There is abundance of rabbit in the Northern area and it is therefore the main prey of Eagle Owls (71% of the diet), with partridges (6.74%) and pigeons (2.81%) as supplements. By contrast, in the Southern area rabbit accounts for only 35% of the diet, with rats (23%), pigeons (14%), partridges (5.26%), hedgehogs (5.26%) and yellow-legged gulls (3.16%) as supplements (authors' unpublished data). Even Stone Curlew (*Burhinus oedicnemus*) and Peregrine Falcon (*Falco peregrinus*) have been observed as preys in the Southern area (authors' unpublished data). These differences in diet composition are reinforced by the positive correlations found between Hg levels in the blood of Eagle Owl and rabbit muscles in the Northern area and the lack of correlation in the south (Fig. 4).

Species such as rabbits, partridges and pigeons feed on plants, and are classified as primary consumers (Lourenço et al., 2011). However, omnivorous species such as rats, hedgehogs and gulls could be classified as secondary consumers (Lourenço et al., 2011). Although less important in terms of ingested biomass, these prey are expected to have higher Hg levels than primary consumers. Studying Hg levels in the Eagle Owl and its prey, Lourenço et al. (2011) found that primary consumers had significantly lower Hg concentrations than secondary consumers, and that mesopredators had significantly higher Hg levels than secondary consumers. Palma et al. (2005) also found that the highest Hg concentrations were recorded in Bonelli's Eagles (*Hieraaetus fasciatus*) with a high dietary proportion of secondary consumers, while much lower Hg levels were recorded in eagles feeding almost exclusively on herbivores such as rabbits, pigeons and partridges. Following this reasoning, it seems logical that Eagle Owls in the Southern area, with a higher proportion of secondary consumers (31.58%) and even with mesopredators in their diet, are expected to have higher Hg concentrations in their tissues.

However, local contamination sources also contribute to the highest concentrations found in Eagle Owls from the south. The Southern area includes a subarea that is an ancient mining site (Fig. 1). The location within this mining area is the second variable with the largest value of relative importance, followed by the zone. When Eagle Owls from this mining area were excluded, Hg concentrations in blood of Eagle Owls were similar between Northern and Southern areas. However, mean Hg concentrations in the blood of Eagle Owl from the ancient mining site were higher than those found in the rest of the sampled population (Table 2). Hence, it seems that diet composition cannot explain the higher Hg concentrations in Eagle Owls from the Southern area in comparison with those from the Northern area. Similarly, no location-related differences were found for Hg concentrations in muscles of European Rabbit (Table 2), but when rabbits from the ancient mining site were excluded, the mean Hg levels in rabbit muscles were significantly higher in the mining area compared to Hg levels found in rabbits from the rest of the sampled population (Table 2). Given that the southeast of the Iberian Peninsula has a long history of the extraction of its mineral resources, there are still high amounts of waste in the mining areas. These materials are extremely rich in heavy metals, such as lead, copper and zinc, and also evidence of cadmium, arsenic and mercury (Faz-Cano et al., 2001). More than 50 years ago there were many chemical products manufacturing factories near the city of Cartagena (Fig. 1). As a consequence, soils in this area have also been affected by industrial wastes, and display a rather high metal

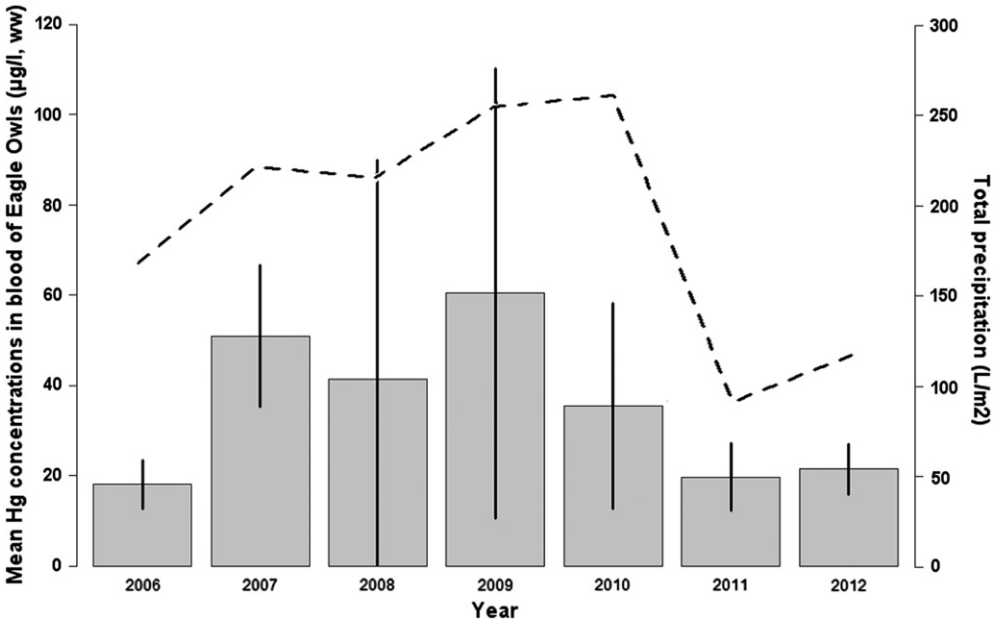

Fig. 3. Temporal trends in mercury levels (µg/l, ww) in blood of Eagle Owls and precipitations (l/m^2) in the province of Murcia. Bars represent Confidence Intervals (CI) stated at 95% confidence level. The dash represents the total annual rainfall.

pollution level, mainly of copper, zinc, cadmium, lead, and mercury (Faz-Cano et al., 2001). Besides the large amounts of metals and other contaminants present in this environment, the geographical and climatic factors may prevent the efficient dispersion of these contaminants (García-Fernández et al., 1995). A study conducted in the mid 90s showed that the highest concentrations of metals were found in the blood of wild birds (Eagle Owl included) from Cartagena in comparison to that of those from the rest of the province of Murcia (García-Fernández et al., 1995). Tests from 2003 to 2007 showed that Eagle Owl chicks from the "Sierra Minera Cartagena-La Unión" mining area or the surroundings had higher lead concentrations than the rest of the population (Gómez-Ramírez et al., 2011).

Therefore, although the studied area is not considered Hg polluted, these results show that spatial differences in Hg concentrations in Eagle Owls appear to be related to local contamination by the ancient mining sites, with diet composition probably playing a minor role.

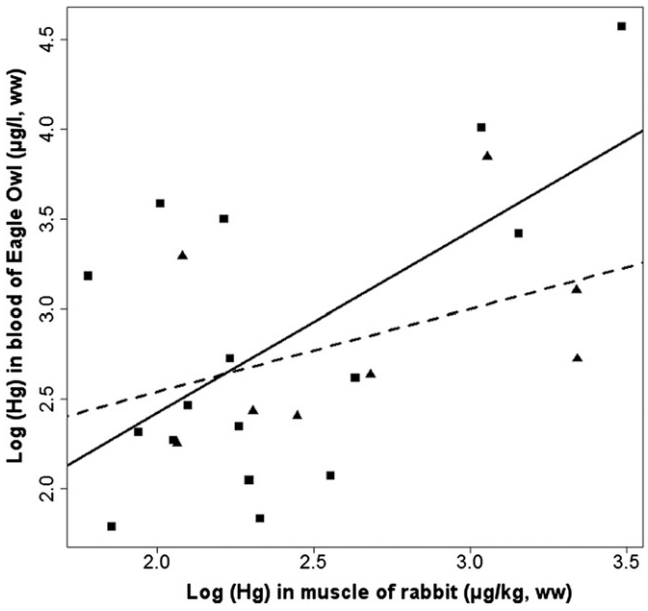

Fig. 4. Relation between mercury blood levels (µg/l, ww) in nestling Eagle Owl and mercury concentrations in rabbit muscles (µg/kg, ww) in Northern and Southern areas. Squares represent the correlation in the Northern area (solid trendline) and triangles represent the correlation in the Southern area (dashed trendline). European Rabbit samples were collected from the same nests as the nestling blood.

5. Conclusions

Hg levels in Eagle Owl chicks from Southeast Spain can be considered low, and it is unlikely that Hg pollution can negatively affect their breeding performance. A positive correlation was found between Hg concentrations in the blood of chicks and in back feathers. Thus, we provide a regression equation that may be helpful to estimate Hg concentrations in the blood of this species in studies that only analyze growing back feathers.

Blood Hg concentrations in Eagle Owls reflect Hg levels in rabbit muscles, more evidently in the Northern area, where rabbits are the main prey. In the Linear Mixed Models used to explain variations in blood Hg concentrations in nestling Eagle Owls, the best model includes year and location within the mining area as variables. The variable year is assigned the largest value of relative importance, followed by the location in the ancient mining sites and then the zone. Rainfalls may have an effect on the temporal differences in blood Hg concentrations of nestling Eagle Owls. Although the studied region is not considered Hg polluted, the mining influence seems to contribute to the higher Hg levels in Eagle Owls and European Rabbits from the areas near the ancient mining sites in comparison with the rest of the region under study. This result shows that spatial differences in Hg concentrations in Eagle Owls appear to be affected by local contamination, with diet composition probably playing a minor role.

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