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Blood lead levels in an endangered vulture decline following changes in hunting activity

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ABSTRACT

Lead ammunition stands out as one of the most pervasive pollutants affecting wildlife. Its impact on bird populations have spurred efforts for the phase-out of leaded gunshot in several countries, although with varying scopes and applications. Ongoing and future policy changes require data to assess the effectiveness of adopted measures, particularly in the current context of biodiversity loss. Here, we assessed the long-term changes in blood lead (Pb) levels of Egyptian vultures from the Canary Islands, Spain, which have been severely affected by Pb poisoning over the past two decades. During this period, the reduction in hunting pressure and changes in legislation regarding firearms usage for small game hunting likely contributed to a decrease in environmental Pb availability. As anticipated, our results show a reduction in Pb levels, especially after the ban on wild rabbit hunting with shotgun since 2010. This effect was stronger in the preadult fraction of the vulture population. However, we still observed elevated blood Pb levels above the background and clinical thresholds in 5.6% and 1.5% of individuals, respectively. Our results highlight the positive impact of reducing the availability of Pb from ammunition sources on individual health. Nonetheless, the continued use of Pb gunshot remains an important source of poisoning, even lethal, mainly affecting adult individuals. This poses a particular concern for long-lived birds, compounding by potential chronic effects associated with Pb bioaccumulation. Our findings align with recent studies indicating insufficient reductions in Pb levels among European birds of prey, attributed to limited policy changes and their uneven implementation. We anticipated further reductions in Pb levels among Egyptian vultures with expanded restrictions on hunting practices, including a blanket ban on Pb shot usage across all small game species.

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1. Introduction

Understanding the impacts of pollutants on the environment is crucial for driving policy changes aimed at mitigating or eliminating these effects. A clear example of policy changes driven by human health concerns is found in lead (Pb) contamination, a heavy metal released through various anthropogenic activities such as hunting, fishing (using Pb-based sinkers), mining, household practices (e.g., lead-based plumbing, kitchen utensils, and paint), industrial processes, and motor vehicle emissions (Pb-based gasoline) (Harrison, 2012; Kamenov and Gulson, 2014; Stroud, 2015). Lead intoxication in humans occurs through inhalation of contaminated dust particles and aerosols, or ingestion of contaminated food and water. Lead poisoning can adversely affect the kidneys, liver, heart, brain, skeleton, and nervous system (Flora et al., 2006). The adverse effects of Pb toxicity on human health led to the gradual adoption of measures to remove it from our immediate environment (Stroud, 2015; Pain et al., 2019), leading to a reduction in Pb levels within human populations in certain regions (e.g., some European countries, Lermen et al., 2021). However, the global challenge persists unabated (Njati and Maguta, 2019; McFarland et al., 2022; Mielke et al., 2022). World Health Organization (WHO) and several health agencies throughout the world have recognized that a 'no-effect' threshold in humans cannot be defined for Pb and, therefore the efforts to reduce the remaining sources of Pb pollution must be incremented (Green and Pain, 2019).

However, Pb remains a major problem in wildlife conservation (Pain et al., 2019). Wild birds are probably the vertebrates most severely impacted by Pb poisoning, with millions of deaths reported annually, including birds of prey from both American and European continents (see review in Pain et al., 2019; Plaza and Lambertucci, 2019). Lead poisoning in birds of prey primarily occurs through the ingestion of Pb from hunting ammunition, particularly affecting avian scavengers that feed on shot animals (Bassi et al., 2021; Pain et al., 2019). High levels of Pb in various tissues have been documented in vulture and condor populations worldwide (see Plaza and Lambertucci, 2019 and references therein). Lead concentrations are typically higher during the hunting season compared to other times of the year (Descalzo et al., 2021; Kelly and Johnson, 2011; Monclús et al., 2020). For example, in Egyptian vultures (*Neophron percnopterus*) blood Pb levels were significantly higher during the hunting season (geometric mean = 93.33, 95% CI = 70.79–123.03, $n = 47$) than in the rest of the year (geometric mean = 28.84, 95% CI = 24.55–34.67, $n = 90$) (Gangoso et al., 2009). Indeed, in the meta-analysis conducted by Monclús et al. (2020) using data from ten raptor species, it was found that blood Pb levels were significantly higher in birds sampled during the hunting season compared to those sampled during the non-hunting season, year-round or at unspecified times. In addition, the exposure of scavenger species depends on their foraging behaviour and the primary prey they consume (Green et al., 2022; Monclús et al., 2020; Nadjafzadeh et al., 2013). For example, larger scavengers often ingest Pb from bullet fragments present in large game carcasses (Kelly and Johnson, 2011; Lambertucci et al., 2011; Mateo-Tomás et al., 2016), while smaller vultures and facultative scavengers may ingest Pb shot pellets from unretrieved small game (Gangoso et al., 2009; Gil-Sánchez et al., 2018; Mateo et al., 1999; Slabe et al., 2020). Small game, including rabbits, squirrels, partridges, and quail, are crucial prey for many raptor species, including small vultures, heightening the risk of Pb poisoning (Golden et al., 2016). However, the role of small game as a source of Pb ammunition and the effects of ongoing legislative changes on scavenger exposure to this source remain uncertain.

The harmful impact of Pb on wild birds, particularly vultures, is firmly established (Haig et al., 2014; Pain et al., 2009; Plaza and Lambertucci, 2019). Vultures' digestive system rapidly dissolves and absorbs ingested Pb (Pain et al., 2019), meaning that even low Pb levels can disrupt nearly all organic functions (Burger, 1995; Descalzo et al., 2021; Espín et al., 2014, 2015; Haig et al., 2014; Kendall et al., 1996; Pain et al., 2009). Acute poisoning may cause multi-organ failure and death, as experimentally demonstrated in turkey vultures (*Cathartes aura*) (Carpenter et al., 2003), while chronic exposure and bioaccumulation can result in various adverse effects on physiology and behaviour, including anaemia, muscle wastage and weight loss, lethargy, coordination issues, limb paralysis, and convulsions (Burger, 1995; Ecke et al., 2017; Kendall et al., 1996; Krone, 2018; Pain et al., 2019). Chronic exposure may also affect breeding success and population dynamics of raptors (Slabe et al., 2022), with estimated reductions in population sizes of European raptors ranging from 0.2% to 14.4% (Green et al., 2022). In the case of vultures, Pb poisoning is now recognized as a significant global conservation threat (Lambertucci et al., 2011; Pain et al., 2019; Plaza and Lambertucci, 2019; Rajamani and Subramanian, 2015). This is illustrated by the critically endangered California condor (*Gymnogyps californianus*), whose population recovery depends on active management and the reduction of Pb-poisoning rates. Management interventions involve the regular provision of Pb-free food, removal of hazardous trash from and around nesting sites, and even the regular recapture of free-ranging individuals for physical control and treatment (Finkelstein et al., 2012).

Lead ammunition remains largely unregulated worldwide for hunting in terrestrial ecosystems (Avery and Watson, 2009; Mateo and Kanstrup, 2019; Plaza et al., 2018), although some countries have implemented partial or total bans. In Europe, 23 countries have enacted regulations governing its use, with Denmark having imposed a total ban on Pb shot, which will be extended to include Pb bullets in 2024 (reviewed in Mateo and Kanstrup, 2019, see also Sonne et al., 2022). However, the effectiveness of these measures remains uncertain, as contrasting outcomes have been observed in different countries (Helander et al., 2009; Kanstrup, 2019; Kanstrup and Balsby, 2019; Mateo et al., 2014). For instance, while the prevalence of clinical levels of Pb in the livers of raptors substantially decreased in Denmark following the ban on Pb ammunition (Kanstrup and Balsby, 2019), partial bans in Sweden have shown limited success in reducing Pb poisoning in white-tailed sea eagles (*Haliaeetus albicilla*) (Helander et al., 2009). In Southern European countries like Spain, Pb bullet regulations are largely absent, contributing to Pb remaining a serious threat to populations of birds of prey, especially vultures (Berny et al., 2015; Descalzo et al., 2021; Gangoso et al., 2009; Mateo-Tomás et al., 2016; Rodríguez-Ramos et al., 2009). Current regulations primarily target specific game species or habitats (e.g., waterbirds/wetlands, ECHA, 2018), resulting in divergent outcomes due to discrepancies in compliance among regions and the extensive mobility and dietary diversity of raptors and vultures. Despite global resolutions advocating for the phase-out of leaded gunshot across all habitats, as endorsed by the Convention on the Conservation of Migratory Species of Wild Animals (UNEP/CMS, 2014), evidence indicates no significant reduction in Pb levels among European raptors over the past three decades (Monclús et al., 2020). Consequently, there is an urgent need for assessment of recent hunting and conservation policy changes, with a particular focus on high-risk populations such as vultures, which serve as key indicators of the availability of Pb in the environment (Gómez-Ramírez et al., 2014; Kelly and Johnson, 2011; Monclús et al., 2020).

Here, we assessed long-term changes in blood Pb levels of Egyptian vultures (*N. p. majorensis*) from the Canary Islands, Spain, spanning the past two decades. This subspecies is endangered and endemic to the archipelago (Donázar et al., 2002a). Research conducted in the early 2000s revealed a high incidence of Pb poisoning: 13.5% and 2.7% of individuals showed sub-clinical and clinical intoxication levels, respectively, likely attributed to the ingestion of hunting ammunition (Donázar et al., 2002b). Moreover, bioaccumulation of Pb in bones and its detrimental effects on bone mineralization degree was also documented in this population (Gangoso et al., 2009). We hypothesized that variations in annual Pb levels in Canarian Egyptian vultures would correspond to long-term changes in hunting activity and policy occurred since 1998 to the present. Although Pb-based ammunition persists for upland game hunting on the islands, notable alterations in hunting activity and policy have occurred over time, including the prohibition of wild rabbit (*Oryctolagus cuniculus*) hunting with shotguns from 2010 onwards and a reduction in the number of hunting licenses issued annually (see methods). These changes are likely to have reduced exposure to Pb in the environment. Consequently, we predict: i) an overall decline in vultures' blood Pb levels over the years. Since blood Pb reflects recent exposure (Fry et al., 2009; Pain et al., 2019), it allows us to test for temporal associations between Pb levels and hunting pressure. Therefore, we tested whether individual blood Pb levels were related to: ii) the number of hunting licenses issued annually, iii) the change in wild rabbit hunting policy since 2010, and iv) the season, with higher exposure expected during the hunting season (Gangoso et al., 2009).

In this study, our focus was on an endangered vulture population severely impacted by lead poisoning. We aimed to explore the correlation between changes in hunting pressure and policy over the past two decades and the resulting shifts in the blood lead levels of these vultures, which serve as key indicators of lead exposure in the environment. The extensive monitoring of this population over time makes it an ideal study system for addressing these critical issues, which hold significant importance within the context of conserving this critically endangered island population. Additionally, we delved into the role of small game as a significant source of lead poisoning, an aspect that has been underexplored in previous studies examining its impact on wild populations.

2. Methods

2.1. Study population and sampling

The Egyptian vulture is a medium-sized avian scavenger inhabiting arid and mountainous regions across Africa, Asia, and Europe. Our study focuses on a relict population of an endemic subspecies (Donázar et al., 2002a) inhabiting the eastern Canary Islands, specifically Fuerteventura and Lanzarote. The local population of Egyptian vultures, numbering approximately 400 individuals in 2022, with the majority (around 90%) residing on Fuerteventura (own unpublished data), is classified as "Endangered", mainly due to non-natural mortality, including Pb poisoning (Badia-Boher et al., 2019). Currently, the population heavily relies on carcasses of domestic livestock, mainly goats and pigs, which are provided at supplementary feeding stations. In addition, Egyptian vultures frequently consume carcasses of small vertebrates, mainly wild rabbits and rock pigeons (*Columba livia*), which they locate in the field during their foraging expeditions across the island (Donázar et al., 2002b; Medina, 1999). A previous study using x-ray analyses of 424 regurgitated pellets collected at the roost-site revealed that ingested Pb shot is the primary source of Pb in Canarian Egyptian vultures (Donázar et al., 2002b). Pellets containing Pb shots (3.1%, including a single pellet containing 10 Pb shots) were dissected and examined with a magnifying lens, confirming the simultaneous presence of remains of small game. More than 99% of the mass of the lead shot pellets is Pb (Hall and Fisher, 1985; Potysz et al., 2023), while the remaining 1% consist of antimony and arsenic traces. Due to the absence of Pb sources from industries, major cities, or mining on Fuerteventura, it is unlikely that Pb exposure in Egyptian vultures is related to environmental pollution of Pb in the atmosphere or soil.

The primary game species on Fuerteventura include wild rabbits and Barbary partridges (*Alectoris barbara*), but invasive rock pigeons and Barbary ground squirrels (*Atlantoxelus getulus*) are also shot without restrictions. Goats, both domestic and feral, are not targeted for hunting on the island. Although the hunting season in the study area spans from August to December, the number of authorized hunting days varies depending on prey availability. In some cases, there may even be a total hunting ban when the populations of the main target species are extremely low, as observed in 2001, 2012, 2014, and 2019. The number of hunting licenses (i.e., specific permits to hunt animals included in the catalogue of game species with a shotgun, during the authorized periods) issued on the islands fluctuated annually, but show a negative trend of approximately 40% between 1998 and 2022 ($r_s = -0.44855$, p (two-tailed) $= 0.02452$, $n = 25$) (Fig. 1). In addition, hunting wild rabbits with shotguns was prohibited in 2010, permitting capture only through the use of dogs and ferrets (*Mustela putorius*).

From 1999 to 2022, we collected 344 blood samples from Canarian Egyptian vultures trapped using cannon-netting in baited locations. Additionally, two adults displaying clear symptoms of Pb poisoning (e.g., dropping wings, see Fig. 2) were found and captured by locals by hand, then sent to a raptor recovery centre. These individuals were sampled separately, and their results are presented separately as well. Birds were classified as preadult ($n = 226$) and adult ($n = 118$) on the basis of plumage characteristics and ring recovery data. We classified birds as preadults if they were below six years old and had not yet acquired full adult plumage (see Donázar et al., 2002a). We extracted 1 ml of blood from each individual from the brachial vein, which was then stored in a tube containing lithium-heparin and immediately frozen at −20 °C for blood Pb analyses (see details below). Additionally, a few drops were preserved in Eppendorf tubes filled with absolute ethanol for molecular sexing purposes. Sex was determined following Griffiths et al. (1998).

All procedures underwent ethical review and were carried out in accordance with approved guidelines set out by the Bioethics and Animal Welfare Committee (CEEA-EBD-CSIC). The vulture trapping and marking procedures were approved by the Canarian Government.

2.2. Pb analysis in blood

Samples were analysed by atomic absorption spectroscopy with graphite furnace (GF-AAS; AAnalyst 800, PerkinElmer) after diluting whole blood samples in Triton at 0.1% with standard addition calibration following Gangoso et al. (2009). The limits of quantification of Pb obtained in the different years of the analyses ranged between 0.1 and 0.6 μg/dL. Blanks, certified reference materials (ERM-CE195 and ERM-CE 196 Bovine Blood with 41.6 ± 0.9 and 77.2 ± 1.1 μg/dL, respectively; n in different years = 2–16) or avian blood spiked with 40 μg/dL ($n = 5$) were processed together with blood samples analysed in each batch during the different years of the study period. The obtained recoveries (mean ± RSD) for Pb analyses ranged between 86.8 ± 9.6%

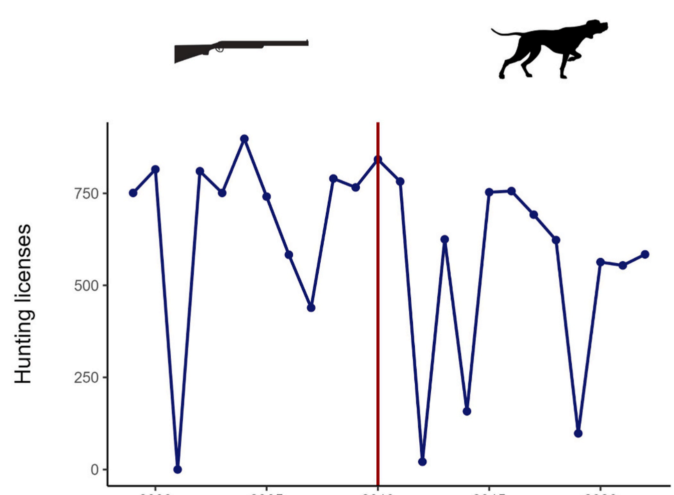

Fig. 1. Changes in hunting activity in the study area (Fuerteventura Island). Note the strong long-term decline in the number of hunting licenses. Years with zero or very low numbers of licenses correspond to periods of ban determined by low populations of main game species. From 2010 onwards, wild rabbit hunting with shotguns (left) was banned to be authorized only with dogs (right).

Fig. 2. Adult Canarian Egyptian vulture (ringed with plastic ring code [25M]) observed in the landfill of Fuerteventura in (A) October 2014 and (B) June 2015. Drooping wings is a clinical sign of chronic lead poisoning. This bird was captured alive by hand the 26^{th} January 2016 and showed a lead level in blood of 764.8 μg/dL. It died shortly after hospitalization. Photos: authors.

and 94.5 ± 3.3% for the certified blood and 101.8 ± 8.6% for the spiked blood. For blood samples collected in 2021 and 2022, analyses were performed by ICP-MS (7800 Quadrupole, Agilent) after the digestion in a microwave oven (Ethos E, Milestone) with nitric acid and hydrogen peroxide as described by Arrondo et al. (2020). The limit of quantification of Pb obtained with this technique was 0.1 μg/dL. Blanks and a certified reference material (ERM-CE195 Bovine Blood, n = 4), were processed with each batch of samples and the obtained recovery was 94.6 ± 8.8%.

2.3. Statistical analyses

As a first step, and taking into account the sex and age (immature/adult) of individuals, we calculated the proportion of Egyptian vultures with blood Pb levels above the background (>20 μg/dL) and clinical levels (>50 μg/dL) following Pain et al. (2019). We assessed overall changes in blood Pb levels along the study period and whether these changes were consistent across individuals by fitting a Linear Model (LM) with Pb concentration (ug/dl) as the response variable. Since this variable was not normally distributed, we used an Ordered Quantile (ORQ) normalization transformation (*orderNorm* function, package "bestNormalize"; Peterson and Cavanaugh, 2020; Peterson, 2021). Because the exposure of vultures to Pb may differ between sexes and age classes due to slight differences in foraging behaviour (van Overveld et al., 2018) we accounted for the potential effect of individual traits in the addressed relationships. We included *Year* of capture, *Age* (immature/adult) and *Sex* (male/female) and their two-way interactions as explanatory variables. Secondly, we tested whether changes in blood levels were influenced by long-term changes in hunting activity by fitting another LM with the same response variable. In this model, we included *Season*, indicating if samples were taken during the hunting (August–December) or non-hunting (January–July) season, *Licenses,* representing the number of hunting licenses issued in the season immediately prior to the bird's capture, and *Shotgun,* indicating whether rabbits were hunted with Pb shotgun pellets (yes - <2010/no ≥ 2010) as explanatory variables. We also included the individual variables *Age* and *Sex*, and their two-way interactions with the three hunting-related variables.

We used Akaike Information Criterion corrected for small sample sizes (AICc) for model selection. We considered as statistically equivalent those models with Delta AICc <2.0 and used model averaging to deal with model selection uncertainty (Burnham and Anderson, 2002). All analyses were carried out in R statistical software (R CoreTeam, 2019) using AICcmodavg (Mazerolle, 2019) for model ranking and MuMIn (Barton, 2017) for model averaging.

3. Results

Blood Pb levels above the background and clinical thresholds (>20 μg/dL and >50 μg/dL, respectively, Pain et al., 2019) were detected in 5.6% and 1.5% of individuals, respectively (Table 1, Fig. 3). Overall, 6.2% of preadults and 4.2% of adults showed Pb levels >20 μg/dL. Furthermore, potentially lethal levels (>100 μg/dL) were observed in one immature bird and two adult females (129.0, 178.0 and 260.4 μg/dL). None of the three individuals with potentially lethal Pb levels died within at least two years after capture. Although they were not included in the sample of vultures surveyed for this study, it is important to note that two adult males were discovered in a moribund state and captured manually by locals in the field (Fig. 2). They showed Pb levels of 764.8 μg/dL and 2000 μg/dL, respectively, and died shortly after hospitalization.

We found a general long-term reduction in blood Pb levels over time, with a more notable decline observed in preadult vultures, as indicated by the interaction between *Year* and *Age* (Table 2 and Table S-1). In addition, blood Pb levels were associated with both *Season* and the interaction between *Shotgun* and *Age.* Higher blood levels were observed during the hunting season, and levels decreased after 2010, following the prohibition of wild rabbit hunting with shotguns. This effect was stronger in the preadult fraction of the population (Fig. 4). Nonetheless, it is important to acknowledge that the R^2 values for both models were relatively low, especially for the model incorporating the year of capture (model a, see Table 2). This indicates that there may be other factors beyond those considered in our modelling approach that are important in explaining the variation in blood Pb levels among Canarian Egyptian vultures over time.

4. Discussion

Our findings reveal a significant decline in blood Pb levels within a vulnerable population of scavenger birds over the course of two decades. This decline corresponds with concurrent changes in hunting practices, particularly the prohibition of shotguns for wild rabbit hunting and, more broadly, the decrease in hunting pressure. However, this overall trend did not affect all individuals uniformly, as the reduction in blood Pb levels was less pronounced in adult birds compared to preadults. In addition, we found that some individuals still exhibit elevated blood Pb concentrations. These findings suggest that mitigating Pb exposure in avian scavengers necessitates more proactive measures to entirely eliminate Pb ammunition from the environment.

The Pb values observed in the Canarian Egyptian vulture have notably decreased over the span of 20 years. In 2009, Gangoso et al. reported mean values of 14.6 ± 29.4 μg/dL (range = 0–178.0 μg/dL), whereas after 2010, mean levels halved (mean = 5.9 ± 18.3 μg/dL, range = 0–260.4 μg/dL). However, despite this decline, some individuals still showed elevated Pb levels. Nevertheless, blood Pb levels were notably lower than those reported in other populations of obligate scavenger birds exposed to Pb ammunition ingestion. For example, 45–93% of griffon vultures (*Gyps fulvus*) in Spain showed values surpassing background levels (Arrondo et al., 2020; Descalzo et al., 2021; Mateo-Tomás et al., 2016). In particular, Arrondo et al. (2020) reported that 37.9% of individuals (N = 58, both sexes and study populations grouped) showed blood Pb values potentially indicating sublethal effects (20–50 μg/dL), while 39.7% showed values suggestive of clinical effects (50–100 μg/dL), and 8.6% showed potentially lethal values (>100 μg/dL). Similarly, Descalzo et al. (2021) reported that 73.7%, 17.8% and 4.2% (N = 118) of griffon vultures admitted to wildlife rehabilitation centres showed blood Pb levels within these reference thresholds,

Table 1
Lead concentrations (original raw values, μg/dL) in blood of Egyptian vultures of different age and sex. The right columns show the percentage of individuals in each of the categories of lead exposure defined by Pain et al. (2019).

	N	mean	s.d.	range	% with lead concentration (μg/dL)			
					<20	20–50	50–100	>100
					Background	Sublethal	Clinical	Lethal
Preadult	226	6.9	14.6	0–178	93.8	4.9	0.9	0.4
Male	117	6.9	19.9	0–75.8	93.2	5.1	1.7	0
Female	109	6.8	17.7	0–178	94.5	4.6	0	0.9
Adult	118	8.3	26.5	0–260.4	95.8	2.5	0	1.7
Male	38	4.7	4.4	0–23.8	97.3	2.6	0	0
Female	80	9.9	31.9	0–260.4	95	2.5	0	2.5
Total	344	7.4	19.5	0–260.4	94.5	4.1	0.6	0.9

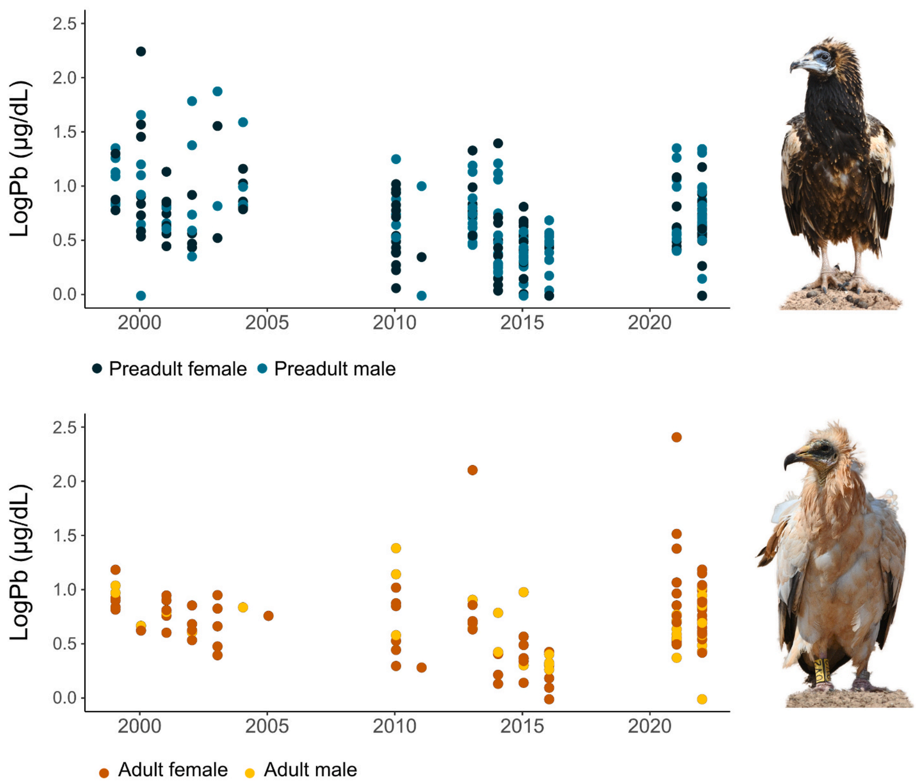

Fig. 3. Long-term changes in blood lead levels of Egyptian vultures in Fuerteventura expressed as log10 of raw concentrations. Above: preadults, below: adults. Sex identity is indicated by dots colours.

respectively. The values reported in the study by Mateo-Tomás et al. (2016) were 44.9%, 5% and 1.4%, respectively (N = 691). Elevated blood Pb values were also documented in other studies involving the same species in the Iberian Peninsula. Carneiro et al. (2015) reported that 65.3% of individuals had levels between 20 and 200 μg/dl, with one individual reaching 300.23 μg/dl (N = 121). García-Fernández et al. (2005) reported mean levels of 43.07 μg/dl (N = 23), with two individuals reaching values close to 150 μg/dl. Additionally, for other *Gyps* species in Africa, Garbett et al. (2018) found that 32% of individuals (N = 566) had Pb concentrations above background levels. Lead concentrations in New World vultures are notably high, as evidenced in studies involving Andean condors (*Vultur gryphus*) (Lambertucci et al., 2011) and, particularly, California condors. Finkelstein et al. (2012) reported that 20% of California condors each year had blood Pb levels ≥45 μg/dl, necessitating chelation treatment. In addition, Turkey vultures (*Cathartes aura*), also exhibited elevated Pb levels, with 48% of individuals (N = 172) showing levels >10 μg/dl, and some birds reaching ≥100 μg/dl (Kelly and Johnson, 2011).

Despite the comparatively lower values exhibited by Canarian Egyptian vultures in contrast to *Gyps* vultures, the data presented here underscore that Pb exposure remains high in certain instances on the island. Notably, three individuals -all females, two of whom were adults-showed lethal values (>100 μg/dL) after 2010, indicating that a fraction of the breeding population may still face a heightened risk of Pb intoxication. In fact, since 2010, two adult Canarian Egyptian vultures have perished with evident symptoms of Pb poisoning, such as dropping wings (Pain et al., 2019, see Fig. 2). The impact of lethal poisoning, however, could be underestimated, as other individuals may have perished without displaying pathological symptoms and remained undetected, as observed in other bird species (Newth et al., 2013). Moreover, poisoned or moribund birds tend to become increasingly reclusive, and their carcasses are unlikely to be discovered during routine field monitoring following their demise. Furthermore, the sublethal effects of Pb poisoning on physiology and behaviour may predispose affected birds to other causes of mortality, such as accidents with power lines (Kelly and Kelly, 2005), which currently represent the primary cause of mortality among Canarian Egyptian vultures (Donázar et al., 2002b; Badia-Boher et al., 2019). The prolonged decrease in Pb levels within the adult

Table 2
Parameter estimates of the averaged model assessing variation in blood lead values of Egyptian vultures with respect to (a) individual traits (*Age* and *Sex*) and *Year* of capture and (b) changes in hunting activity (*Season, Licenses* and *Shotgun*). Teste interactions are indicated with ":". 85% confidence intervals are shown.

(a) Model: Year	Estimate	Std. Error	7.5%	92.5%
(Intercept)	20.4998	22.0027	−11.1738	52.1733
Age (Immature)	65.6980	27.7263	25.7852	105.6109
Year	−0.0101	0.0109	−0.0259	0.0056
Age (Immature):Year	−0.0327	0.0138	−0.0525	−0.0129
R^2	0.0756			
AICc	957.93			
(b) Model: Hunting activity				
Intercept	0.2353	0.1772	−0.0197	0.4903
Season (No hunting)	−0.2591	0.1133	−0.4223	−0.0960
Age (Immature)	−0.2601	0.1246	−0.4394	−0.0807
Licenses	−0.0002	0.0002	−0.0005	0.0001
Sex (Male)	0.0378	0.1040	−0.1119	0.1875
Shotgun (Yes)	0.3215	0.2085	0.0213	0.6217
Age (Immature):Shotgun (Yes)	0.5274	0.2486	0.1695	0.8853
R^2	0.1338			
AICc	941.00			

fraction of the population aligns with trends observed in other studies involving long-lived birds of prey (Helander et al., 2021). Additionally, this trend may be attributed to the behaviour of preadult Egyptian vultures, which tend to concentrate their foraging activities at predictable locations, such as supplementary feeding sites (van Overveld et al., 2018). This behaviour could potentially render them less susceptible to Pb ammunition ingestion through the consumption of game species.

An important finding is the greater decrease in Pb concentrations observed in young birds compared to adults. While a portion of ingested Pb can be eliminated through growing feathers and excrement (Leonzio et al., 2009), Pb accumulates in bones throughout life (Gangoso et al., 2009) and can be further mobilized, particularly in females, during reproduction. In human studies, skeletal Pb contributing to elevated blood Pb concentration during pregnancy has been estimated at 33% (Gulson et al., 2003), but similar studies have not been conducted in birds during egg laying. Previous research has demonstrated high bone Pb concentrations in birds of prey in Spain, with geometric means ranging between 0.58 and 6.00 μg/g and maximum values of 185 μg/g (Mateo et al., 2003). Canarian Egyptian vultures also exhibited high bone Pb concentrations in adult birds (geometric mean = 8.13 μg/g, Gangoso et al., 2009). Since over 95% of Pb body burden is accumulated in bones, the resorption of bone tissue during periods of high calcium demand could potentially elevate blood Pb concentrations in adult vultures, especially in females.

Our findings suggest that the observed decrease in blood Pb levels is attributed to changes in hunting practices, notably the prohibition of rabbit hunting with Pb ammunition from 2010 onward. All studies on the diet of the Egyptian vulture in Fuerteventura indicate that, besides livestock, wild rabbits constitute the primary prey, followed by rock pigeons (Gangoso et al., 2006; Medina, 1999). This preference for rabbit and small vertebrate remains, even in the presence of ample livestock carcasses, is also observed in other European populations of the species, potentially driven by the necessity to obtain micronutrients essential for the development of nestlings. Concurrently, the count of small game hunters on the island has steadily dwindled, as evidenced by the issuance of annual licenses, although there exists considerable year-to-year variability due to changes in closure orders. Nonetheless, our findings suggest that this factor does not exert as significant an impact as changes in rabbit hunting, likely due to the wild rabbit being the primary hunting target on the island, boasting substantially higher densities than other game species (Cabildo Insular de Fuerteventura, unpublished). It is important to note the observed seasonal effect, with higher blood Pb levels during the hunting period. Lead is relatively rapidly cleared from the bloodstream (with an estimated elimination half-life of approximately 13 days in California condors, Finkelstein et al., 2012). Therefore, our analysis of blood Pb levels, reflecting recent exposure, along with previous evidence from analyses of regurgitated pellets (Donázar et al., 2002b), supports the notion that ammunition serves as the primary source of Pb, a conclusion consistent with numerous studies (Descalzo et al., 2021; Gangoso et al., 2009; Helander et al., 2021; Monclús et al., 2020).

It is worth considering that the relationships we found may be influenced by other factors not accounted for in this study. It could be argued that our findings may be influenced by the overall food availability on the island, whereby the presence of Pb-free food has increased over the past decade. Goat carcasses constitute the primary food source for Canarian Egyptian vultures, and although the livestock population peaked around 2010, it has since declined by nearly 50% (Donázar et al., 2020; Fernández-Gómez et al., 2024). Conversely, supplementary feeding, which relies on slaughterhouse remains of goats and pigs (van Overveld et al., 2018), has remained consistent throughout the study period (own unpublished data). A more plausible explanation for the decrease in blood Pb levels could be linked to the surge in Canarian

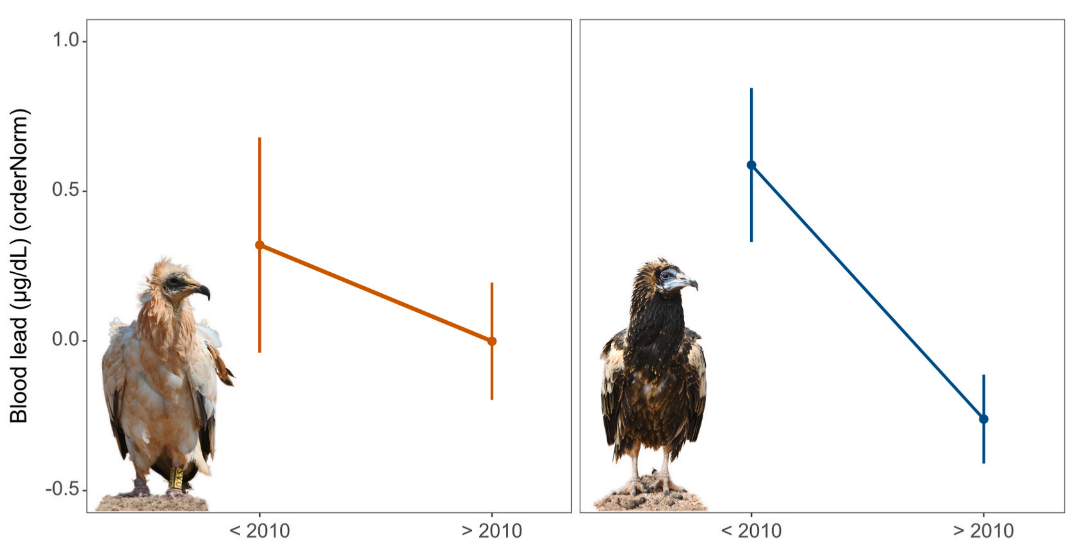

Fig. 4. Model projection (estimate ± standard deviation) of the changes in blood lead values of Egyptian vultures (μg/dL, normalized) with respect to the individual's age (adults: left panel, immatures: right panel) and hunting activity: before (<2010) and after (≥2010) the ban of wild rabbit hunting with shotgun.

Egyptian vulture numbers. The local breeding population has quadrupled over the study period, expanding from 21 territories in 1998 to 90 in 2022 (Badia-Boher et al., 2019, own unpublished data). Concurrently, with the observed decline in hunting pressure, the rising vulture population might lower the per capita likelihood of poisoning through the consumption of shot prey.

While changes in hunting practices may contribute to the observed variations in Pb levels, it is essential to consider other potential sources of Pb and their alterations over time. For example, Canarian Egyptian vultures frequently scavenge in the landfill situated at the island, visiting up to 40% of days per month (authors, unpublished data). This landfill receives a mixture of livestock remains, organic waste, and solid waste, including plastics, welding remnants, and e-waste, all potential sources of Pb (Korzun and Heck, 1990; Pascale et al., 2016). Previous studies have shown that scavengers foraging in landfills are more likely to show high Pb levels (Blanco et al., 2003; De la Casa-Resino et al., 2014; Plaza and Lambertucci, 2017). However, it is worth noting that high Pb levels have not been observed in nestling Egyptian vultures from more urbanized territories in Catalonia, northeastern Spain (Ortiz-Santaliestra et al., 2019). Finally, changes in the availability of Pb in the environment unrelated to hunting practices, such as those associated with policies limiting leaded petrol and paints (Stroud, 2015), could have contributed to the observed declining trend in blood Pb levels in Canarian Egyptian vultures. Nonetheless, the potential effect of these alternative Pb sources is probably smaller than the already reported effect of Pb from ammunition sources (Donázar et al., 2002b; Gangoso et al., 2009) because the island has a relatively low population density and lacks heavily polluting industries, mining, and waste incinerators (Donázar et al., 2002b; Schmitz et al., 2018). Furthermore, neither landfill feeding nor industrial pollution sources would explain the seasonal pattern we have found.

5. Conclusions

Despite widespread awareness of the harmful effects of Pb derived from hunting ammunition on both organisms and ecosystems, its use remains unregulated in many regions. Wild birds are particularly vulnerable to Pb poisoning, with millions of deaths occurring annually, including birds of prey and avian scavengers across the Americas and Europe (Pain et al., 2019; Plaza and Lambertucci, 2019). Current restrictions on Pb ammunition, particularly in areas like wetlands, have proven insufficient in preventing the poisoning of birds of prey and vultures (Descalzo et al., 2021; Helander et al., 2021; Monclús et al., 2020). Therefore, there is an urgent need to evaluate recent changes in hunting policies, with a specific focus on high-risk populations such as vultures, which play a crucial role as indicators of Pb contamination in the environment (Monclús et al., 2020).

Consistent with findings from previous studies (Mateo et al., 2007), our research underscores the significant impact of a simple hunting regulation measure on the concentration of blood Pb in Egyptian vultures. This regulation, while not directly aimed at conserving birdlife, has played a crucial role in mitigating one of the primary causes of non-natural mortality in this endangered population (Donázar et al., 2002b). Nevertheless, this single measure targeting one prey species proves inadequate. Despite the absence of Pb in wild rabbits consumed by vultures, these birds must still feed on other small game species, such as Barbary partridges and rock pigeons, which are still shot without restriction. This highlights the importance of addressing small game, including non-target prey species, as potential sources of Pb ammunition. Further reductions in Pb levels among vultures could be achieved by expanding hunting policy restrictions, such as implementing a ban on Pb hunting for other small game species.

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CRediT authorship contribution statement

L. Gangoso: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **R. Mateo:** Writing – review & editing, Formal analysis. **C. Santamaría-Cervantes:** Writing – review & editing, Formal analysis. **M. García-Alfonso:** Writing – review & editing, Methodology, Investigation. **C. Gimeno-Castellano:** Writing – review & editing, Methodology. **E. Arrondo:** Writing – review & editing, Methodology. **D. Serrano:** Writing – review & editing, Methodology. **T. van Overveld:** Writing – review & editing, Methodology. **M. de la Riva:** Writing – review & editing, Methodology, Data curation. **M.A. Cabrera:** Writing – review & editing, Resources, Funding acquisition. **J.A. Donázar:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Raw data used in this study is available in Gangoso et al., 2024.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.118712.

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