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Long-term effects of lead poisoning on bone mineralization in vultures exposed to ammunition sources

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Bone lead accumulation decreases the degree of bone mineralization in vultures exposed to ammunition sources.

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Long-lived species are particularly susceptible to bioaccumulation of lead in bone tissues. In this paper we gain insights into the sublethal effects of lead contamination on Egyptian vultures (*Neophron percnopterus*). Our approach was done on the comparison of two populations (Canary Islands and Iberian Peninsula) differing in exposures to the ingestion of lead ammunition. Blood lead levels were higher in the island population (Canary Islands range: 5.10–1780 $\mu g\ L^{-1}$ $n = 137$; Iberian Peninsula range: 5.60–217.30 $\mu g\ L^{-1}$ $n = 32$) showing clear seasonal trends, peaking during the hunting season. Moreover, males were more susceptible to lead accumulation than females. Bone lead concentration increased with age, reflecting a bioaccumulation effect. The bone composition was significatively altered by this contaminant: the mineralization degree decreased as lead concentration levels increased. These results demonstrate the existence of long-term effects of lead poisoning, which may be of importance in the declines of threatened populations of long-lived species exposed to this contaminant.

1. Introduction

Lead is a highly toxic heavy metal, which can be released into the environment via numerous routes, but principally by industrial, mining and hunting activities (Fisher et al., 2006). The diffusion into the environment of lead produced by human activities has historically been shown to affect wildlife (Depledge et al., 1993; Eens et al., 1999). Nevertheless, the restrictions implemented over the last few decades have resulted in the current situation where contaminating sources, particularly those from industrial activities in developed countries are very limited. Consequently, the contamination deriving from hunting activities has increased in relative importance in recent decades (Fisher et al., 2006). Ingestion of lead shot and bullet fragments is currently the principal source of lead poisoning for numerous vertebrate species, primarily birds (Bellrose, 1959; Clark and Scheuhammer, 2003; Mateo, 1998; Pain et al., 2005; Pattee and Hennes, 1983; Redig et al., 1980).

Individual effects of lead poisoning in wild vertebrates have been repeatedly highlighted. Acute poisoning (exposure to a high concentration during a short time period) can lead to death (Mateo, 1998; Ramo et al., 1992), while chronic exposure (prolonged exposure at lower concentrations) can have sublethal effects which affect reproductive success (Buerger et al., 1986), behavior (Scheuhammer, 1987), immune response (Redig et al., 1991; Snoeijs et al., 2004) and physiology (Burger, 1995; Fair and Ricklefs, 2002). However, the majority of the studies have limited their scope to descriptive aspects or are based on experimental treatments under controlled laboratory conditions; in consequence, few studies have treated this problem in depth in populations of wild vertebrates (Mateo et al., 1999; Pain et al., 2005).

Bones are a long-term repository for lead, containing approximately 90% of the total body burden in mammals and birds (Ethier et al., 2007). The Pb^{2+} ion readily replaces the Ca^{2+} ion (Ehle, 1993; Scheuhammer, 1987) additionally altering the hormonal regulation of the calcium and the osteoblast function (Pounds et al., 1991; Ronis et al., 2001). This leads to long-term detrimental effects, which have regularly been demonstrated in clinical studies on mammals, and

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fundamentally in humans (Cheng et al., 2001; Latorre et al., 2003; Pounds et al., 1991). Nevertheless, very little information exists on the effects that the substitution of calcium by lead in bone tissue and its consequences in processes such as the mineralization and the alteration of compositional properties and bone mechanics may have (Bjorå et al., 2001; Gruber et al., 1997; Hamilton and O'Flaherty, 1995; Pain et al., 2007). It is foreseeable that these effects could be particularly severe for long-lived vertebrates, whose life-history traits make them prone to lead bioaccumulation over time. Thus, long-term effects of lead poisoning could play an until now unknown role threatening some populations and species of large birds of prey such as the Bald Eagle (*Haliaeetus leucocephalus*), Steller's Sea Eagle (*H. pelagicus*), White-tailed Sea Eagle (*H. albicilla*) and the critically endangered Californian Condor (*Gymnogyps californianus*) which are all known to be affected by lead ammunition sources (Behrens and Brooks, 2000; Church et al., 2006; Franson, 1996; Kenntner et al., 2001; Meretsky et al., 2000).

In this paper we gain insights into the sublethal effects of lead contamination on long-lived vertebrates. We studied the Egyptian Vulture (*Neophron percnopterus*), a small vulture considered as globally endangered (IUCN, 2007) whose most important populations in the Western Palearctic remain in Spain (Donázar, 1993, 2004; Perea et al., 1990). Some Spanish populations of this species have been monitored from the eighties until present, providing an adequate data source for the objectives proposed (Donázar, 1993; Donázar et al., 1996, 2002). Our approach is based on the comparison of two populations, one resident on islands (Canary Islands) and the other a migratory continental one (Iberian Peninsula) which are potentially differentially affected by this contaminant (see Donázar et al., 2002). Our first hypothesis is that the island population, being resident, suffers higher exposure than the migratory continental one to the ingestion of lead shot during the winter hunting season. Consequently we can predict that (a) we should find a higher frequency of lead shot in regurgitated pellets and of acute-type poisoning shown by blood analysis. As a corollary of the former prediction, (b) in the island population we should find a seasonal pattern with higher lead blood levels during the hunting season. Our next hypothesis is that the contamination has long-term effects on individuals. Given that the bone lead level reflects life time exposure, we predict that (c) the Canary Islands Egyptian Vultures will show higher contaminant levels in this tissue, and (d) there will be an effect of bioaccumulation with age. Finally, we expect to find negative effects on the affected individuals, such that (e) a negative relationship will exist between lead concentration and compositional properties of the bone tissue.

2. Materials and methods

2.1. Area and study populations

We analyzed samples of Egyptian Vultures from Fuerteventura, Canary Islands (30 breeding pairs; 1662 Km^2) and from the Iberian Peninsula. Within the latter area, individuals from Cádiz (30 b.p.; 9500 Km^2), the Ebro valley (100 b.p.; 19,000 Km^2) and Segovia (25 b.p.; 5000 km^2) were included. Throughout its distribution, Egyptian Vultures usually consume livestock carcasses in "vulture restaurants", but also scavenge heavily on wild prey (mainly rabbit *Oryctolagus cuniculus* and pigeon *Columba* spp. carcasses). In Iberia, garbage dumps also provide an important fraction of their trophic needs (see Donázar, 1993; Donázar et al., 2002). The Iberian populations remain in their distinct breeding areas between March and September and so consequently their presence does not overlap with the hunting season in Spain, which used to begin at the end of the summer, finishing in January–February before the arrival of the transaharan migrants (Cramp and Simmons, 1980; Del Hoyo et al., 1994). In Fuerteventura, the Egyptian Vultures are sedentary, and so are also present on the breeding grounds during the hunting season (August–November).

2.2. Field procedures

To determine the incidence of lead shot ingestion in the mainland population and compare it with that previously observed in the island one (see Donázar et al., 2002), we collected 327 regurgitated pellets at the communal roosts used by the species in the Ebro valley from 1999 to 2003. We determined the presence of ingested shot by X-rays. Pellets apparently containing shot were subsequently dissected to confirm the presence of lead.

From 1999 to 2005, we obtained 137 blood samples of Egyptian Vultures on the Canary Islands, both from fledglings ($n = 61$) and individuals of other ages trapped during cannon-netting ($n = 76$). In addition, we obtained 32 samples of immature and adult mainland birds (10 individuals in the Ebro valley in 2002, 19 in Segovia in 2003–2004 and three in Cádiz in 2003). The birds' ages were determined using plumage characteristics (Forsman, 1998) as 1 = fledglings, 2 = individuals of 1–2 years old, 3 = 2–3 years, 4 = 3–4 years, 5 = 4–5 years (adults) and 6 = individuals $\geq$5 years old). We extracted a small quantity of blood (1 ml) from each individual from the brachial vein. It was placed in a tube containing lithium-heparin and immediately frozen at −20 °C.

We extracted bone tissue samples from 39 Egyptian Vulture carcasses (28 from the Canary Islands and 11 from the Iberian Peninsula), all found dead in the field between 1999 and 2004. The age of 16 of the island birds and all of the continental birds was known. Age and sex was known for 14 island birds and five continental birds. We extracted the humerus of each individual, which was measured and weighed after meticulous clearing of all muscle material with a scalpel (right humerus 66.7% of cases, left humerus 33.3%). The humerus has frequently been used in studies monitoring the exposure of birds to lead (Ethier et al., 2007). As a pneumatic bone with little hemopoietic activity (Schepelmann, 1990), it may be less likely to be affected by acute exposures due to the absence of the bone marrow and as a result more reliably reflect chronic exposure to lead. Moreover, lead concentrations in the humerus are similar to and highly correlated with those of other long bones such as the femur (Mateo et al., 2003). We obtained from each humerus a fragment approximately 2 cm long from the diaphysis. We washed the sections obtained repeatedly in distilled water to remove any possible traces resulting from the dissection and cutting processes. Finally, we grounded the samples with a cryogenic mill (Freezer/Mill, SPEX) for later analysis.

2.3. Laboratory analyses

2.3.1. Recent exposure

We determined the blood lead concentration using atomic absorbtion spectrophotometry in graphite furnace (GF-AAS) (AAnalyst 800, Perkin Elmer) following the procedure described in Mateo et al. (1999). Lead concentrations in $\mu g L^{-1}$ are the result of the average of three replicates. Additionally, we triple processed a bovine blood sample CRM 196 with an average (SD) of 772 (11) $\mu g\ L^{-1}$, giving a result of 767 (8) $\mu g\ L^{-1}$, with an RSD of 1.04%. In compliance with the rules of use, the result is acceptable as it falls within the value of the average ± 2SD (of 95% of the laboratories which have participated in the analysis of the certified sample). The detection limit for lead was 0.5 $\mu g\ L^{-1}$. We interpreted blood lead levels as background (<200 $\mu g\ L^{-1}$), subclinical (200–1000 $\mu g\ L^{-1}$) and toxic levels ($\geq$1000 $\mu g\ L^{-1}$) as reviewed by Franson (1996) for Falconiformes.

2.3.2. Chronic exposure

We analyzed the lead content in the bones using inductively coupled plasma with optical emission spectrophotometry (ICP-OES, Perkin-Elmer Optima 5100 DV) and GF-AAS. The sample, 50 mg of ground bone, was digested with 1 ml of 70% nitric acid (HNO_3) and 1 ml of 30% nitrogen peroxide (H_2O_2) in a microwave oven and finally diluted with ultrapure water to give a 10 ml sample. Bone lead levels were interpreted as background (<10 $\mu g\ g^{-1}$) or abnormal exposure ($\geq$10 $\mu g\ g^{-1}$) according to the data reviewed by Mateo et al. (2003).

We investigated the effect of lead contamination on bone mineralization by means of Fourier transformed infrared spectrometry (FTIR), normally used to analyse bone chemical composition (Miller et al., 2001; Ou-Yang et al., 2001; Rey et al., 1991). We undertook it by mixing and homogenizing 5 mg of powdered bone sample with 90 mg of KBr. The tablets were prepared under a pressure of 10 metric Tons for 10 min. We registered the infrared spectra on a Magna IR200 Nicolet FTIR spectrometer. The spectra were acquired in the absorption mode between 400 and 4000 cm^{-1}, with a resolution of 2 cm^{-1} and a total of 128 scans per sample. A reference tablet (95 mg of BrK) was used for background correction.

We determined the organic and mineral material content of the bone samples measuring the area of the absorption bands associated with molecular groups of carbonate, phosphate, amide and C–H aliphatic in the infrared spectrum. We undertook the band analyses and deconvolution of peaks using the computer programs EZ-OMNIC® and PeakFIT®. The deconvolution of overlapping peaks was resolved using a second derivative method. This method allows a more accurate and more detailed quantitative analysis of the spectrum. A detailed description of the methodology employed is described elsewhere (Rodriguez-Navarro et al., 2006). We calculated the degree of mineralization of the bone as:

$$\text{Mineralization degree} = \text{phosphate mineral}\left(v_3PO_4 - \text{band area } 900-1200\ cm^{-1}\right) / \text{collagen}\left(\text{amide I} - \text{ band area A1660}cm^{-1}\right)$$

2.4. Statistical analyses

Lead concentrations, both in blood and bone samples were not normally distributed (Shapiro–Wilk, $p < 0.001$), so were normalized through logarithmic transformation in both cases.

We compared the percentage of pellets containing lead shot between the island and continental populations by a Chi-test.

We tested differences in blood lead concentrations between the populations through a *T* test for independent samples. We determined the relation between blood lead levels and hunting activity in the island population using a univariate analysis of variance with the lead concentration as the response variable. We included the capture date: during the hunting season (1) or outside it (0); the sex: female (1) or male (2); and the age of the individuals (1–6, see above for definition of age categories) as model factors. The possible interactions were considered in the model.

We analyzed the possible differences in bone lead concentrations between the populations by means of an analysis of the variance (ANOVA) considering all individuals combined. We included population: Canary Islands (1) or Iberian Peninsula (0); the sex: female (1), male (2) and the age (1–6) as factors. Secondly, we undertook a similar ANOVA analysis but only using adult individuals (≥5 years). We investigated the existence of a bioaccumulation effect through a univariate analysis of variance, considering the bone lead concentration as the response variable. The factors corresponding to age (1–6) and sex, as well as their possible interactions were considered in the model. We analysed the effect of age on the bone lead concentration only for the island population, given that all the continental individuals of known age, except for one, were adults ($n = 10$). Subsequently, we examined the relationship between bone lead concentrations and age through a Pearson correlation (one-tailed test).

We analysed the relationships between the degree of bone mineralization with the individual and population characteristics through a univariate analysis of variance, including population, sex and age as factors. The lead concentration was included as a covariate. Finally, we analysed the effect of lead on the compositional properties of the bone (degree of mineralization) through a Pearson correlation (one-tailed test).

3. Results

3.1. Lead intoxication in relation to geographical and seasonal patterns of hunting activities

We found no lead shot in the continental pellets examined ($n = 327$), which was significantly different ($\chi^2 = 14.9$, DF = 1, $p = 0.01$) from that found in a previous study carried out in the Canary Islands, where 5% of the pellets examined contained lead shot ($n = 424$; Donázar et al., 2002).

Although the mean blood lead concentration was significantly higher in the continental population: *T* test for unequal variances $t = 2.50$, DF = 66.56, $p = 0.01$, 95% CI = 0.03–0.30 (Lavene test of variance equality $F = 5.43$, $p = 0.021$), both populations presented different distributions of blood lead concentrations (Fig. 1). This different distribution stems from the fact that only one individual from the mainland population presented a concentration indicative of abnormal exposure (217.30 $\mu g\ L^{-1}$), whereas in the island population 10 individuals (7.30%) showed subclinical levels above 200 $\mu g\ L^{-1}$, and three of these (2.19%) showed blood lead levels of over 500 $\mu g\ L^{-1}$. One of these birds, captured in 2000 and which subsequently disappeared, showed a concentration of 1780 $\mu g\ L^{-1}$, considered to be a toxic level birds of prey (Franson, 1996).

Blood lead levels were significantly higher in the island population during the hunting season (geometric mean = 93.33, 95% CI = 70.79–123.03, $n = 47$) than outside it (geometric mean = 28.84, 95% CI = 24.55–34.67, $n = 90$) ($F_{1,\ 132} = 62.66$, $p < 0.001$), and differed marginally between the sexes ($F_{1,132} = 3.08$, $p = 0.08$) being higher in males (geometric mean = 44.67, 95% CI = 33.11, $n = 57$) than for females (geometric mean = 41.69, 95% CI = 33.11–51.29, $n = 79$). In addition, an interaction was apparent: males during the hunting season showed the highest levels of lead ($F_{1,\ 132} = 8.32$, $p = 0.005$). Age and its possible interactions showed no significant effect ($p > 0.05$ in all cases).

3.2. Lead accumulation in bone and its consequences on mineralization

Only one individual 3 years old from the island population showed bone lead levels >20 $\mu g\ g^{-1}$, considered to indicate excessive exposure and absorption of Pb at some stage. Moreover, Pb concentrations >20–30 $\mu g\ g^{-1}$ are often found in birds that have died of Pb poisoning (Pain et al., 2007).

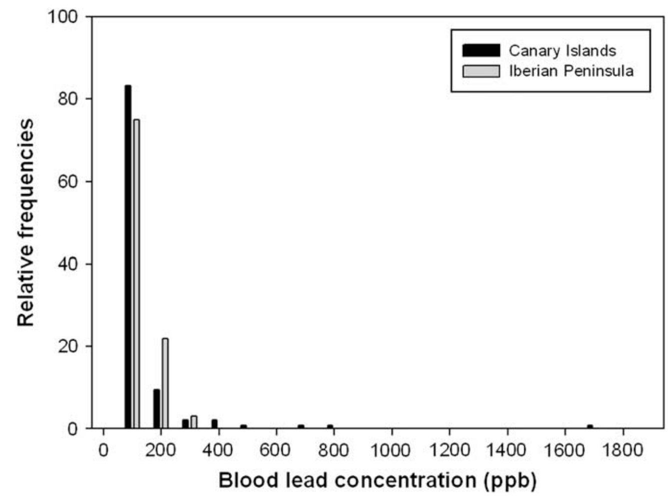

Fig. 1. Distribution of blood lead concentrations (ppb = $\mu g\ L^{-1}$) in the mainland ($n = 32$) and the island ($n = 137$) populations of Egyptian Vulture.

When all age classes were considered, bone lead levels did not differ significantly between the continent (geometric mean = 6.17, 95% CI = 4.27–8.91, $n = 11$) and the island (geometric mean = 7.42, 95% CI = 5.25–8.91, $n = 28$) (ANOVA, $F_{1,\ 37} = 0.21$, $p = 0.65$). However, bone lead levels increased with age (ANOVA, $F_{5,\ 22} = 4.85$, $p = 0.004$). Considering only adult birds (≥5 years), the lead concentration was significantly higher for the island population (geometric mean = 8.13, 95% CI = 6.03–10.72, $n = 11$) than for the mainland one (geometric mean = 5.49, 95% CI = 4.07–7.41, $n = 10$) (ANOVA, $F_{1,\ 19} = 4.5$, $p = 0.04$). Sex showed no significant effect in any of the analyses.

We found a bioaccumulation effect of bone lead with age in the island population (ANOVA $F_{5,\ 11} = 4.418$, $p = 0.019$, $n = 17$) (Fig. 2). The correlation between the variables was positive (Pearson $r = 0.54$, $p = 0.013$, $n = 17$).

Age or sex had no significant effects on the degree of bone mineralization. However, the degree of mineralization was negatively correlated with the bone lead concentration when we combined all data from both populations (Pearson $r = -0.30$,

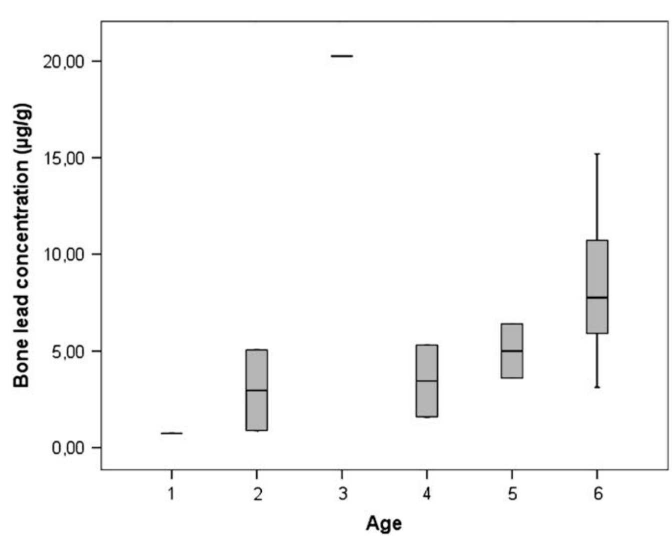

Fig. 2. Bioaccumulation effect of bone lead with age in the island population of Egyptian Vultures.

$p = 0.032$, $n = 39$). This correlation was even stronger when considering only adult birds (Pearson $r = -0.63$, $p = 0.001$, $n = 20$) (Fig. 3).

4. Discussion

Our study demonstrates that ingestion of lead ammunition is related to the accumulation of lead in bones of Egyptian Vultures. It ultimately affected the degree of bone mineralization, this being lowest in those birds with highest bone lead concentrations. Numerous studies indicate that heavy metals, and in particular lead, can affect the correct mineralization of the bone (Gruber et al., 1997; Hamilton and O'Flaherty, 1995), directly or indirectly altering the renewal rate by affecting osteoblasts and osteoclasts activity (Berglund et al., 2000; Carmouche et al., 2005; Kanti and Smith, 1997; Puzas et al., 1992) and playing an important role in the development of bone pathologies (Bjorå et al., 2001; Smits et al., 2005; Puzas, 2000). However, the relationship between bone lead and mineralization has never before been described in wild populations (but see Bjorå et al., 2001), even though it is reasonable to consider it is a logical consequence of repeated lead ingestion and accumulation in long-lived species. The reduction in bone mineralization could mean an increase in bone fragility (Fleming et al., 2000; Whitehead and Fleming, 2000). Indeed, in the island population of Egyptian Vulture an unusually high level of frequency of fractures and even leg amputations has been recorded (8% until 2007, $n = 150$) while this situation has not been detected in the mainland population ($n > 2000$, authors, unpublished data). Although on occasions these fractures are derived from entanglement on ground wire stabilizers (Gangoso and Palacios, 2002), very probably, these fractures may be facilitated by a greater bone fragility (Bjorå et al., 2001; Smits et al., 2005).

The ecology of the island population makes it particularly susceptible to poisoning from ammunition sources. The sedentary nature of the island birds leads to a higher probability of ingestion of lead shot as compared to the mainland population, since they coincide with the hunting season on the island. This appears to be supported given that the blood lead levels found in birds from the Canary Islands were much higher during the hunting season than outside it. The volcanic origin of the island makes the recovery of wounded game such as wild rabbits and Barbary Partridges (*Alectoris barbara*) extremely difficult. In addition, certain introduced species and of little hunting interest, such as feral pigeons (*Columba livia*) and barbary ground squirrels (*Atlantoxerus getulus*) are habitually shot but not retrieved, with the subsequent risk of them being ingested by scavengers (Knopper et al., 2006). In addition, the frequency of individuals in the island population with blood lead levels indicative of abnormal Pb exposure (>200 μg/L; Franson, 1996) was higher than that of mainland birds which are less exposed to the chance of consuming shot animals. However, the mainland populations were not free of the poisoning risk, given the frequency of individuals with blood lead levels of <200 μg L^{-1}. Although the Iberian Egyptian vultures are not present in Spain during the main hunting season, they are exposed to short hunting periods which takes place in August–September, as well as the ingestion of hunted game from shot at but not killed or crippled game from the previous hunting season. Besides, as found in other bird species, other localized contaminant sources could be operating, such as industrial activity or the ingestion of contaminated materials in rubbish tips where mainland Egyptian vultures feed regularly (Donázar et al., 1996; Blanco et al., 2004; Smits et al., 2005).

Male Egyptian Vultures were significantly more affected by lead contamination during the hunting season, which could be related to sexual differences in the food searching behavior, a phenomenon described in dimorphic raptors where spatial segregation or food niche partitioning is seen (Hedrick and Temeles, 1989; Lee and Severinghaus, 2004). Indeed, our data indicate that females concentrate on Fuerteventura at predictable food sources ("vulture restaurants") while males search for more prey but which are less predictable spatially (authors, unpublished data). Likewise, bioaccumulation of lead in animal tissues is a highly sex-linked process, given that the forms of ingesting and excreting the contaminant could differ notably between males and females (Tejedor and González, 1992).

The consequences at the population level from lead poisoning remain to be determined. As noted above, it is reasonable to assume that the probability of bone fracture is increased given alterations in the bone mineralization process, which undoubtedly lowers survival rates and/or at least the effective population size, given that no live individuals with apparent fractures has bred in the island population studied (authors, unpublished data). The island vultures also have a very low productivity rate (0.5 chicks/pair/year, Donázar et al., 2002) and it seems reasonable to consider that the lead could be playing an important role in this, especially given that the lead accumulated in the bones of the females is mobilized during eggshell formation (Taylor, 1970) and transmitted to both the eggshell and to the embryo (Edens and Garlich, 1983; Grandjean, 1976; Pattee, 1984). Moreover, lead may affect sperm quality and consequently the fertilization rate (Benoff et al., 2000; Castellanos et al., in press). Additionally, it has hypothesized that the accumulation of toxins at subclinical levels could be the cause of immunosuppression, which increases susceptibility to infectious diseases (Blanco et al., 2004; Daszak et al., 2001; Dobson and Foufopoulos, 2001; Snoeijs et al., 2004). In the island Egyptian Vulture population we find they show poor immunocompetence and a comparatively higher susceptibility to pathogen incidence (Gangoso, 2006), which, although it could have its origin in multiple causes, given the results of the contaminants analyses leads us to suspect that some form of interaction between these factors should exist.

Summarizing, our study demonstrates that a population of long-lived vertebrates, in this case an avian scavenger, exposed to the ingestion of lead shot can be affected over the long-term by the bioaccumulation of this heavy metal in the bone tissue through a loss of mineralization. The ultimate consequences of poisoning by lead can be readily underestimated given that the sublethal effects are difficult to detect and may only show over a very long time

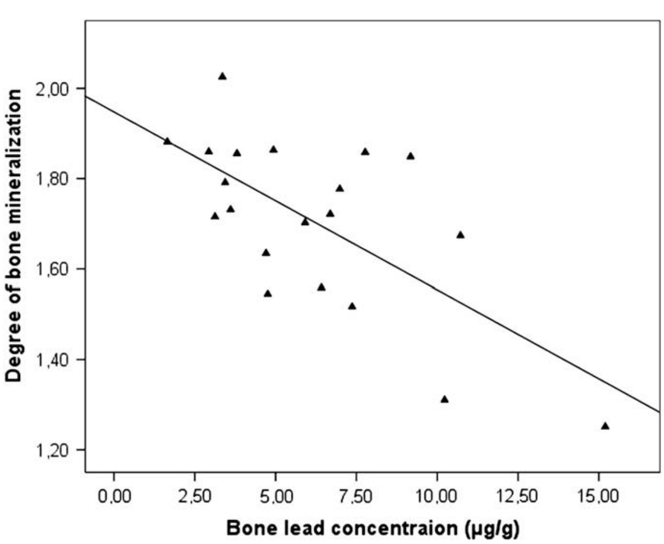

Fig. 3. Negative relation between bone lead (μg g^{-1}) and degree of bone mineralization in adult Egyptian Vultures (≥5 years old) from the island and mainland populations (fit line Sq r lineal = 0.45 is showed).

period and in combination with other factors (Franson et al., 1983; Mateo et al., 1997; Rocke and Samuel, 1991; Smits et al., 2007). Regular monitoring based on blood analyses from regular field sampling is consequently important to reveal recent exposure to lead, but provides no information regarding the duration of exposure, and, if it doesn't cause the death of the individual, even less information on the long-term effects on the organism (Fry, 2003). Without doubt, the hidden effects such as those revealed in this study, may be acting as the limiting factors in relation to the decline and recovery of populations of long-lived vertebrates exposed to lead poisoning (Fry, 2003; Pattee et al., 1990).

5. Conclusions

We analyzed the long-term effects of lead poisoning in two populations of Egyptian vultures (insular vs. mainland). The ecological context of the insular population determined an elevated exposition to lead shot, and consequently, higher levels of blood and bone lead than the mainland one. Moreover, blood lead levels were higher during the hunting season in the island population, especially for males. In addition, we found a clear effect of bioaccumulation of bone lead with age. The main conclusion drawn is that lead causes a reduction in bone mineralization. These hidden effects of lead poisoning may be of importance in the declines of threatened populations of long-lived species exposed to this contaminant.

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References

Behrens, J., Brooks, J., 2000. Wind in their wings: the condor recovery program. Endangered Species Bulletin 25, 8–9.

Bellrose, F., 1959. Lead poisoning as a mortality factor in waterfowl populations. Illinois Natural History Survey Bulletin 27, 235–288.

Benoff, S., Jacob, A., Hurley, I.R., 2000. Male infertility and environmental exposure to lead and cadmium. Human Reproduction Update 6, 107–121.

Berglund, M., Akesson, A., Bjellerup, P., Vahter, M., 2000. Metal-bone interactions. Toxicology Letters 112-113, 219–225, doi:10.1016/S0378-4274(99)00272-6.

Bjorå, R., Falch, J.A., Staaland, H., Nordsletten, L., Gjengedal, E., 2001. Osteoporosis in the Norwegian moose. Bone 29, 70–73, doi:10.1016/S8756-3282(01)00469-0.

Blanco, G., Jimenez, B., Frias, O., Millan, J., Davila, J.A., 2004. Contamination with nonessential metals from a solid-waste incinerator correlates with nutritional and immunological stress in prefledgling black kites (*Milvus migrans*). Environmental Research 94, 94–101.

Buerger, T.T., Mirarchi, R.E., Lisano, M.E., 1986. Effects of lead shot ingestion on captive mourning dove survivability and reproduction. Journal of Wildlife Management 50, 1–8.

Burger, J., 1995. A risk assessment for lead in birds. Journal of Toxicology and Environmental Health 45, 369–396.

Carmouche, J.J., Puzas, J.E., Zhang, X., Tiyapatanaputi, P., Cory-Slechta, D.A., Gelein, R., Zuscik, M., Rosier, R.N., Boyce, B.F., Keefe, R.J.O., Schwarz, E.M., 2005. Lead exposure inhibits fracture healing and is associated with increased chondrogenesis, delay in cartilage mineralization, and a decrease in osteoprogenitor frequency. Environmental Health Perspectives 113, 749–755, doi:10.1289/ehp.7596.

Castellanos, P., Mateo, R., Reglero, M.M., Esteso, M.C., Fernández-Santos, M.R., Garde, J.J., In press. In vitro effects of lead on fatty acid composition, oxidative stress biomarkers and quality of ram spermatozoa. Toxicological and Environmental Chemistry. doi:10.1080/02772240801936810.

Cheng, Y., Schwartz, J., Sparrow, D., Aro, A., Weiss, S.T., Hu, H., 2001. Bone lead and blood lead levels in relation to baseline blood pressure and the prospective development of hypertension the normative aging study. American Journal of Epidemiology 153, 164–171.

Church, M.E., Gwiazda, R., Risebrough, R.W., Sorenson, K., Chamberlain, C.P., Farry, S., Heinrich, W., Rideout, B.A., Smith, D.R., 2006. Ammunition is the principal source of lead accumulated by California condors re-introduced to the wild. Environmental Science and Technology 40, 6143–6150.

Clark, A.J., Scheuhammer, A.M., 2003. Lead poisoning in upland foraging birds of prey in Canada. Ecotoxicology 12, 23–30.

Cramp, S., Simmons, K.E.L., 1980. The birds of the Western Palearctic, Vol. II. Oxford University Press, Oxford.

Daszak, P., Cunningham, A.A., Hyatt, A.D., 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropica 78, 103–116.

Del Hoyo, J., Elliot, A., Sargatal, J., 1994. Handbook of the Birds of the World, Vol. II. España, Lynx Editions, Barcelona.

Depledge, M., Weeks, J., Bjerregaard, P., 1993. Heavy metals. In: Calow, P. (Ed.), Handbook of Ecotoxicology, Vol. 1. Blackwell Scientific, Oxford, pp. 79–105.

Dobson, A., Foufopoulos, J., 2001. Emerging infectious pathogens of wildlife. Philosophical Transactions of the Royal Society of London B 356, 1001–1002.

Donázar, J.A., 1993. Los buitres ibéricos: biología y conservación. In: Reyero, J.M. (Ed.), Madrid, España.

Donázar, J.A., 2004. Alimoche común *Neophron percnopterus*. In: Madroño, A., González, C., Atienza, J.C. (Eds.), Libro Rojo de las Aves de España. Dirección General para la Biodiversidad-SEO/BirdLife, Madrid, pp. 129–131.

Donázar, J.A., Ceballos, O., Tella, J.L., 1996. Communal roosts of egyptian vultures (*Neophron percnopterus*): dynamics and implications for the species conservation. In: Muntaner, J., Mayol, J. (Eds.), Biología y conservación de las rapaces mediterráneas, 1994. Monografías, n° 4. SEO, Madrid, pp. 189–201.

Donázar, J.A., Palacios, C.J., Gangoso, L., Ceballos, O., González, M.J., Hiraldo, F., 2002. Conservation status and limiting factors in the endangered population of egyptian vulture (*Neophron percnopterus*) in the Canary Islands. Biological Conservation 107, 89–97.

Edens, F.W., Garlich, J.D., 1983. Lead-induced egg production decrease in leghorn and japanese quail hens. Poultry Science 62, 1757–1763.

Eens, M., Pinxten, R., Verheyen, R.F., Blust, R., Bervoets, L., 1999. Great and blue tits as indicators of heavy metal contamination in terrestrial ecosystems. Ecotoxicology and Environmental Safety 44, 81–85.

Ehle, A.L., 1993. Lead. In: Macrae, R., Robinson, R.K., Sadler, M.J. (Eds.), Encyclopaedia of Food Science, Food Technology and Nutrition. Academic Press, London, pp. 2665–2673.

Ethier, A.L.M., Braune, B.M., Scheuhammer, A.M., Bond, D.E., 2007. Comparison of lead residues among avian bones. Environmental Pollution 145, 915–919, doi:10.1016/j.envpol.2006.05.001.

Fair, J.M., Ricklefs, R.E., 2002. Physiological, growth, and immune responses of japanese quail chicks to the multiple stressors of immunological challenge and lead shot. Archives of Environmental Contamination and Toxicology 42, 77–87.

Fisher, I., Pain, D.J., Thomas, V.A., 2006. Review of lead poisoning from ammunition sources in terrestrial birds. Biological Conservation 131, 421–432.

Fleming, R.H., McCormack, H.A., Whitehead, C.C., 2000. Prediction of breaking strength in osteoporotic avian bone using digitized fluoroscopy, a low cost radiographic technique. Calcified Tissue International 67, 309–313.

Forsman, D., 1998. The Raptors of Europe and the Middle East: A Handbook of Field Identification. T. and A.D. Poyser, Carlon.

Franson, J.C., 1996. Interpretation of tissue lead residues in birds other than waterfowl. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. SETAC CRC Lewis Publishers, Boca Raton, CA, pp. 265–279.

Franson, J.C., Sileo, L., Pattee, O.H., Moore, J.F., 1983. Effects of chronic dietary lead in american kestrels (*Falco sparverius*). Journal of Wildlife Diseases 19, 110–113.

Fry, D.M., 2003. Assessment of Lead Contamination Sources Exposing California Condors. Final Report. California Department of Fish and Game. Habitat Conservation Planning Branch, Sacramento, CA.

Gangoso, L., 2006. Insularidad y Conservación: el caso del alimoche (*Neophron percnopterus*) en Canarias. Doctoral Thesis. University of Seville, Spain.

Gangoso, L., Palacios, C.J., 2002. Endangered Egyptian vulture (*Neophron percnopterus*) entangled in a power line ground-wire stabilizer. Journal of Raptor Research 36, 328–329.

Grandjean, P., 1976. Possible effect of lead on egg-shell thickness in kestrels 1874–1974. Bulletin of Environmental Contamination and Toxicology 16, 101.

Gruber, H.E., Gonick, H.C., Khalil-Manesh, F., Sanchez, T.V., Motsinger, S., Meyer, M., 1997. Osteopenia induced by long-term, low- and high-level exposure of the adult rat to lead. Mineral and Electrolyte Metabolism 23, 65–73.

Hamilton, J.D., O'Flaherty, E.J., 1995. Influence of lead in mineralization during bone growth. Fundamental and Applied Toxicology 26, 265–271.

Hedrick, A.V., Temeles, E.T., 1989. The evolution of sexual dimorphism in animals: hypotheses and tests. Trends in Ecology and Evolution 4, 136–138.

IUCN, 2007. Red List of Threatened Species. http://www.iucnredlist.org.

Kanti, A., Smith, M.A., 1997. Effects of heavy metals on chondrogenic differentiation of embryonic chick limb cells. In Vitro Toxicology 10, 329–338.

Kenntner, N., Tataruch, F., Krone, O., 2001. Heavy metals in soft tissue of white-tailed eagles found dead or moribund in Germany and Austria from 1993 to 2000. Environmental Toxicology and Chemistry 20, 1831–1837.

Knopper, L., Mineau, P., Scheuhammer, A., Bond, D., McKinnon, D., 2006. Carcasses of shot Richardson's ground squirrels may pose lead hazards to scavenging hawks. Journal of Wildlife Management 70, 295–299, doi:10.2193/0022-541X200670 [295:COSRGS]2.0.CO,2.

Latorre, F.G., Hernández-Ávila, M., Tamayo Orozco, J., Albores Medina, C.A., Aro, A., Palazuelos, E., Hu, H., 2003. Relationship of blood and bone lead to menopause and bone mineral density among middle-age women in Mexico City. Environmental Health Perspectives 111, 631–636.

Lee, Y.F., Severinghaus, L.L., 2004. Sexual and seasonal differences in the diet of lanyu scops owls based on fecal analysis. Journal of Wildlife Management 68, 299–306, doi:10.2193/0022-541X2004068 [0299:SASDIT]2.0.CO,2.

Mateo, R., 1998. La Intoxicación por ingestión de perdigones de plomo en aves silvestres: aspectos epidemiológicos y propuestas para su prevención en España, Doctoral Thesis. Universitat Autònoma de Barcelona.

Mateo, R., Martínez-Vilalta, A., Guitart, R., 1997. Lead shot pellets in the Ebro delta, Spain: densities in sediments and prevalence of exposure in waterfowl. Environmental Pollution 96, 335–341.

Mateo, R., Estrada, J., Paquet, J.Y., Riera, X., Dominguez, L., Guitart, R., Martinez-Vilalta, A., 1999. Lead shot ingestion by marsh harriers *Circus aeruginosus* from the Ebro delta. Spain. Environmental Pollution 104, 435–440.

Mateo, R., Taggart, M., Meharg, A.A., 2003. Lead and arsenic in bones of birds of prey from Spain. Environmental Pollution 126, 107–114, doi:10.1016/S0269-7491(03)00055-1.

Meretsky, V.J., Snyder, N.F.R., Beissinger, S.R., Clendenen, D.A., Wiley, J.W., 2000. Demography of the California condor: implications for reestablishment. Conservation Biology 14, 957–967, doi:10.1046/j.1523-1739.2000.99113.x.

Miller, L.M., Vairavamurthy, V., Chance, M.R., Mendelsohn, R., Paschalis, E.P., Betts, F., Boskey, A.L., 2001. In situ analysis of mineral content and crystallinity in bone using infrared micro-spectroscopy of the ν_4 PO_4^{3-} vibration. Biochimica et Biophysica Acta 1527, 11–19, doi:10.1016/S0304-4165(01)00093-9.

Ou-Yang, H., Paschalis, E.P., Mayo, W.E., Boskey, A.L., Mendelsohn, R., 2001. Infrared microscopic imaging of bone: spatial distribution of CO32. Journal of Bone Mineral Research 16, 893–900, doi:10.1359/jbmr.2001.16.5.893.

Pain, D.J., Meharg, A.A., Ferrer, M., Taggart, M., Penteriani, V., 2005. Lead concentrations in bones and feathers of the globally threatened Spanish imperial eagle. Biological Conservation 121, 603–619.

Pain, D.J., Carter, I., Sainsbury, A.W., Shore, R.F., Eden, P., Taggart, M.A., Konstantinos, S., Walker, L.A., Meharg, A.A., Raab, A., 2007. Lead contamination and associated disease in captive and reintroduced red kites *Milvus milvus* in England. Science of the Total Environment 376, 116–127, doi:10.1016/j.scitotenv.2007.01.062.

Pattee, O.H., 1984. Eggshell thickness and reproduction in American kestrels exposed to chronic dietary lead. Archives of Environmental Contamination and Toxicology 13, 29–34.

Pattee, O.H., Hennes, S.K., 1983. Bald Eagles and Waterfowl: the Lead Shot Connection. 48th North American Wildlife Conference 1983. Wildlife Management Institute, Washington, pp. 230-237.

Pattee, O.H., Bloom, P.H., Scott, J.M., Smith, M.R., 1990. Lead hazards within the range of the California condor. The Condor 92, 931–937.

Perea, J.L., Morales, M., Velasco, J., 1990. El alimoche (*Neophron percnopterus*) en España. Población, distribución, problemática y conservación. Colección Temática, ICONA, Madrid, Spain.

Pounds, J.G., Long, G.J., Rosen, J.F., 1991. Cellular and molecular toxicity of lead in bone. Environmental Health Perspectives 91, 17–32.

Puzas, J.E., 2000. Osteotoxicology: the role of lead in bone diseases. Current Opinion in Orthopedics 11, 360–365.

Puzas, J.E., Sickel, M.J., Felter, M.E., 1992. Osteoblasts and chondrocytes are important target cells for the toxic effects of lead. Neurotoxicology 13, 783–788.

Ramo, C., Sánchez, C., Hernández, S.A., 1992. Lead poisoning of greater flamingos *Phoenicopterus ruber*. Wildfowl 43, 220–222.

Redig, P.T., Stowe, C.M., Barnes, D.M., Arent, T.D., 1980. Lead toxicosis in raptors. Journal of American Veterinary Medical Association 177, 941–943.

Redig, P.T., Lawler, E.M., Schwartz, S., Dunnette, J.L., Stephenson, B., Duke, G.E., 1991. Effects of chronic exposure to sublethal concentrations of lead acetate on heme synthesis and immune function in red tailed hawks. Archives of Environmental Contamination and Toxicology 21, 72–77.

Rey, C., Renugopalakrishman, V., Collins, B., Glimcher, M.J., 1991. Fourier transform infrared spectroscopic study of the carbonate ions in bone mineral during aging. Calcified Tissue International 49, 251–258, doi:10.1007/BF02556214.

Rocke, T.E., Samuel, M.D., 1991. Effects of lead shot ingestion on selected cells of the mallard immune system. Journal of Wildlife Diseases 27, 1–9.

Rodriguez-Navarro, A.B., Romanek, C.S., Alvarez-Lloret, P., Gaines, K.F., 2006. Effect of in ovo exposure to PCBs and Hg on clapper rail bone mineral chemistry from a contaminated salt marsh in coastal Georgia. Environmental Science and Technology 40, 4936–4942.

Ronis, M.J.J., Aronson, J., Gao, G.G., Hogue, W., Skinner, R.A., Badger, T.M., Lumpkin, C.K., 2001. Skeletal effects of developmental lead exposure in rats. Toxicological Sciences 62, 321–329.

Schepelmann, K., 1990. Erythropoietic bone marrow in the pigeon: development of its distribution and volume during growth and pneumatization of bones. Journal of Morphology 203, 21–34, doi:10.1002/jmor.1052030104.

Scheuhammer, A.M., 1987. The chronic toxicity of aluminium, cadmium, mercury and lead in birds: a review. Environmental Pollution 46, 263–295.

Smits, J., Bortolotti, G., Baos, R., Blas, J., Hiraldo, F., Xie, Q., 2005. Skeletal pathology in white storks (*Ciconia ciconia*) associated with heavy metal contamination in southwestern Spain. Toxicologic Pathology 33, 441–448, doi:10.1080/01926230590953097.

Smits, J., Bortolotti, G., Baos, R., Jovani, R., Tella, J.L., Hoffmann, W., 2007. Disrupted bone metabolism in contaminant-exposed white storks (*Ciconia ciconia*) in southwestern Spain. Environmental Pollution 145, 538–544, doi:10.1016/j.envpol.2006.04.032.

Snoeijs, T., Dauwe, T., Pinxten, R., Vandesande, F., Eens, M., 2004. Heavy metal exposure affects the humoral immune response in a free-living small songbird, the great tit (*Parus major*). Archives of Environmental Contamination and Toxicology 46, 399–404, doi:10.1007/s00244-003-2195-6.

Taylor, T.G., 1970. The role of the skeleton in eggshell formation. Annales de Biologie Animale, Biochimie. Biophysique 10, 83–91.

Tejedor, M.C., González, M., 1992. Comparison between lead levels in blood and bone tissue of rock doves (*Columba livia*) treated with lead acetate or exposed to the environment of Alcala de Henares. Bulletin of Environmental Contamination and Toxicology 48, 835–842.

Whitehead, C.C., Fleming, R.H., 2000. Osteoporosis in cage layers. Poultry Sciences 79, 1033–1041.