Science of the Total Environment 748 (2020) 141437

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

Lead contamination in raptors in Europe: A systematic review and meta-analysis☆

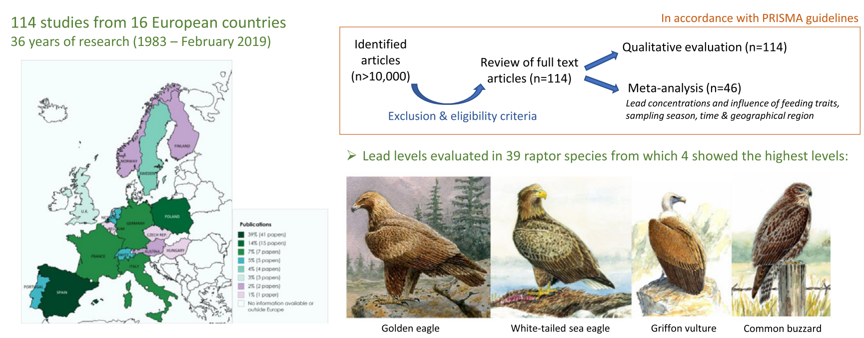
Laura Monclús [a,b,]*, Richard F. Shore [c,1], Oliver Krone [d]

[a] Department of Biology, Norwegian University of Science and Technology, Trondheim 7491, Norway
[b] Department of Animal Health and Anatomy, Autonomous University of Barcelona, Bellaterra 08193, Spain
[c] U.K. Centre of Ecology and Hydrology, Lancaster Environmental Centre, Lancaster LA1 4AP, UK
[d] Department of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research, Berlin 10315, Germany

HIGHLIGHTS

- We reviewed 114 studies on lead contamination in European raptors.
- We found a lack of homogenization in the monitoring schemes around Europe.
- Lead concentrations varied across feeding traits and between sampling seasons.
- Evidence for high occurrence of lead contamination was found, especially in scavengers.
- We urge studies relating lead exposure to quantitative impacts on European raptors.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 May 2020
Received in revised form 30 July 2020
Accepted 31 July 2020
Available online 8 August 2020

Editor: Filip M.G. Tack

Keywords:
Pb
Birds of prey
Poisoning
Ammunition
Heavy metal

ABSTRACT

Lead contamination is a widely recognised conservation problem for raptors worldwide. There are a number of studies in individual raptor species but those data have not been systematically evaluated to understand raptor-wide lead exposure and effects at a pan-European scale. To critically assess the extent of this problem, we performed a systematic review compiling all published data on lead in raptors (1983–2019) and, through a meta-analysis, determined if there was evidence for differences in exposure across feeding traits, geographical regions, between hunting and non-hunting periods, and changes over time. We also reviewed the impact of lead on raptors and the likely main source of exposure. We examined 114 studies that were unevenly distributed in terms of time of publication and the countries in which studies were performed. Peer-reviewed articles reported data for 39 raptor species but very few species were widely monitored across Europe. Obligate (vultures) and facultative scavengers (golden eagle, common buzzard and white-tailed sea eagle) accumulated the highest lead concentrations in tissues and generally were the species most at risk of lead poisoning. We found no evidence of a spatial or decadal trend in lead residues, but we demonstrated that high lead blood levels relate to hunting season. Exposure at levels associated with both subclinical and lethal effects is common and lead from rifle bullets and shot is often the likely source of exposure. Overall, our review illustrates the high incidence and ubiquity of lead contamination in raptors in Europe. However, we did not find studies that related exposure to quantitative impacts on European raptor populations nor detailed studies on the impact of mitigation measures. Such information is urgently needed and requires a more harmonised approach to quantifying lead contamination and effects in raptors across Europe.

☆ This paper is dedicated to the memory of Richard F. Shore

* Corresponding author at: Department of Biology, Norwegian University of Science and Technology, 7491 Trondheim, Norway.
E-mail address: laura.monclus@ntnu.no (L. Monclús).

[1] Deceased.

https://doi.org/10.1016/j.scitotenv.2020.141437

Contents

1. Introduction

Lead is one of the most toxic heavy metals affecting all living organisms, including humans and wildlife. It is widely distributed and persistent in the environment causing problems worldwide (Burger, 1995). Birds are one of the taxa most affected by lead and exposure and effects have been extensively studied and documented over the last half century (Benson et al., 1974; Hernández et al., 1988; Pain et al., 1993a, 2009; Pain et al., 1995; Fisher et al., 2006). Trends in the exposure to and effects of lead contamination in birds have been seen to reflect those in humans (Pain et al., 2019) and so raptors can be valuable indicators of environmental pollution as well as warning system of potential hazards to human health. Raptors are long-lived apex predators which accumulate contaminants, are distributed across large geographical areas and are relatively easy to sample in order to obtain non-destructive materials. They can, therefore, be used to track spatio-temporal trends of pollutants as well as to identify adverse effects (Furness, 1993; Gómez-Ramírez et al., 2014).

In general, exposure to lead can result from numerous sources such as lead-based gasoline, fishing sinkers, mining activities and industry. However, lead from ammunition used by hunters has been described as the most important source affecting birds and raptors in particular (Krone, 2018). Raptors ingest lead in their food in the form of gunshot and bullet fragments that are present in the viscera of prey and scavenge (including ingested shot) or embedded in tissues. Although raptors can eliminate lead via regurgitation of pellets, their digestive process facilitates rapid dissolution and absorption of lead into the bloodstream. It is transported around the body, reaching all organs and tissues including the liver and kidneys, bones and growing feathers (Pain et al., 2019). While bones retain elevated lead levels for long periods (years), thereby providing a measure of lifetime exposure, the half-life of lead is shorter in soft tissues (weeks to months) and blood (around two weeks) (Pain, 1996). Depending on the levels reached in the different organs, lead can cause effects that range from subclinical to lethality. Lead affects the vascular, nervous, renal, immune and reproductive systems, haematological parameters, and also impacts behaviour and survival (Eisler, 1988; De Francisco et al., 2003; Franson and Pain, 2011). When exposure results in acute toxicity, birds may die suddenly yet appear to be in good physical conditions (Krone, 2018).

Given the available evidence of the environmental impacts of lead, several countries have implemented national regulations to ban lead in ammunition. In Europe, the Convention on the Conservation of European Wildlife and Natural Habitats was the first to phase out lead shot ammunition in 1991 (Bern Convention). Subsequently, various International Agreements, Resolutions and Guidelines were adopted by the European Union (EU) and associated countries such as Norway and Switzerland. These led Member States to develop and implement their own regulations on the use of lead in rifle and shotgun ammunition (reviewed in Mateo and Kanstrup, 2019) and there is no harmonised legislation across the EU (ECHA, 2018; Mateo and Kanstrup, 2019). As a result, the European Chemicals Agency called for the need for EU-wide action to address the environmental risk of lead across all Member States, the aim of which would be to protect not only wildlife but also human health (ECHA, 2017).

Recent publications have suggested that lead is an important conservation problem for raptors (Pain et al., 2019; Plaza and Lambertucci, 2019). However, while there are a large number of studies on lead contamination in individual raptor species, typically in single countries, there has been no systematic evaluation of the available data on exposure and effects in raptors of lead at a pan-European scale. The aim of this systematic review was to compile all published data reporting lead contamination in European raptors and, through a meta-analysis, analyse spatio-temporal trends in contamination and explore how lead concentrations differ between species with different feeding traits and between hunting and non-hunting seasons. We also aimed to examine the scale of subclinical and lethal effects and evaluate the contribution of rifle and shotgun ammunition as a source of lead exposure.

2. Material and methods

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009).

2.1. Data sources and search strategy

We examined papers from the first published on the subject (1983) up to February 2019. Four different search engines were used: (1) Web of science (www.webofscience.com); (2) PubMed (www.ncbi.nlm.nih.gov/pubmed); (3) ScienceDirect (www.sciencedirect.com); and (4) Scopus (www.scopus.com). First, we performed two general searches without restriction on the year of publication and including all titles, abstracts and keywords. We used the following terms: (1) Lead OR plumbism OR Pb AND raptor* OR "bird* of prey" OR falcon* OR accipitr* OR strigiformes; (2) Lead OR plumbism OR Pb AND raptor* OR "bird* of prey" OR vulture* OR hawk* OR owl* OR eagle*. We then made additional searches using the words ammunition, heavy metals and raptors. We also examined the reference list of the main reviews on heavy metals in raptors to identify additional papers that had been missed.

2.2. Criteria for selection and eligibility of data

The study design and inclusion/exclusion criteria are defined in SI Appendix A1. The eligibility criteria are illustrated in the PRISMA flow diagram (SI Fig. A1).

The search returned more than 10,000 publications, from which we removed irrelevant (for example where "lead" was used as a verb) and non-English papers, duplicates and grey literature. Book chapters, technical reports and conference proceedings were also excluded (SI Fig. A1). This left 293 publications and we screened their title and abstracts, removing 179 because they covered non-European or non-raptor species (SI Fig. A1). We fully reviewed the remaining 114 papers, including 10 reviews and 1 modelling study. For our qualitative analysis, we included all 114 papers as our objective was to evaluate trends in the publications; the reviews and modelling studies were only included in this first part of the analysis (Section 2.3). We then conducted a quantitative meta-analysis and only included publications containing data that reported raw or statistical summary data on lead concentrations in raptors ($n = 46$ publications; see SI Excel file). We excluded publications in which: lead concentrations were only recorded per pool of species; lead contamination was reported only as pellets or bullets detected by X-ray; results were only expressed in graphical form; data only consisted of non-detected values; mean and standard deviation was not clearly shown or could not be estimated (see Section 2.4.2). We determined whether any papers overlapped in the data presented and found two such publications. We excluded one of these from the meta-analysis (SI Excel file) but included it in the qualitative analysis as it provided relevant new information. Finally, we also checked for papers that reported potential data outliers that would excessively influence our meta-analysis and excluded them from the analyses (see Section 2.5).

2.3. Data extraction

Extraction of data from the final set of studies ($n = 114$) was conducted by one reviewer (LM) using standardised forms (see SI Excel file). Reviews and modelling papers were only considered to gather general information and were excluded from the rest of the analyses. Key information was categorized as: (1) general information: year of publication and country (including 114 publications in the analysis); (2) individual information: species, source of animals (i.e., free-ranging individuals, individuals admitted to wildlife rehabilitation centres, museum specimens, captive birds) (103 publications); (3) sample information: type of sample analysed for lead (103 publications); (4) lead information: concentrations reported (46 publications); (5) source of contamination: presumed or confirmed lead source and use of isotopes (103 publications); (6) health effects of lead (103 publications).

2.4. Data synthesis

2.4.1. Description of variables

For the first part of the study (qualitative analysis), we analysed trends in publication, and when examining temporal trends, we used the year of publication of the paper. Other factors that we considered were country, species, types of matrix, lead levels above threshold concentrations, occurrence of subclinical health effects and mortality, and source of lead.

For the meta-analysis, we defined year as the year the study was undertaken (not published) and we selected the mid-year of the timespan in long-term studies. We then pooled studies into four decades: 1983–1989; 1990–1999; 2000–2009 and 2010–2019. With regards geographical region, we pooled countries into four European regions: West (The Netherlands, Switzerland, Germany, Austria, Belgium, England and France), North (Norway, Finland and Sweden), South (Spain, Portugal and Italy) and East (Hungary, Czech Republic and Poland). For the meta-analysis, we pooled species into two feeding trait groups: scavengers (including obligate and facultative) and non-scavengers (including the rest of birds of prey and owls) (see species classification in SI Table A1).

When considering whether concentrations exceeded subclinical threshold levels (e.g., when deleterious effects begin), we used the minimum lead concentrations that can cause subclinical symptoms as proposed by Franson and Pain (2011): liver > 6 mg/kg dw (2 mg/kg ww); kidney > 8 mg/kg dw (2 mg/kg ww); blood > 20 μg/dl; bone > 10 mg/kg dw. When considering if accumulated residues were likely to cause mortality, we used the lethal thresholds also proposed by Franson and Pain (2011): liver > 18 mg/kg dw (6 mg/kg ww); kidney > 25 mg/kg dw (mg/kg ww); blood > 50 μg/dl; bone > 20 mg/kg dw.

Nearly 60% of the studies we reviewed involved the analysis of lead in free-ranging birds trapped in the wild, while the rest were from animals sampled in captivity (30% wildlife rehabilitation centres, <7% zoos, <7% veterinary clinics and <5% museums). Half of the individuals analysed were found dead in the field or were euthanized for medical reasons. The other half was sampled alive. To avoid possible bias associated with individual origin, we checked each study for where and when individuals were sampled. In captivity (including wildlife rehabilitation centres, zoos and veterinary clinics), almost all individuals were sampled either on arrival or within the first weeks of admittance to the centre. Individuals maintained in captivity for long periods before being sampled were removed from the analysis to avoid bias. We also screened statistically to determine whether provenance and the time of sampling of the bird (i.e., on arrival, within the first weeks, time of screening unknown) affected lead levels. As we did not find any significant effect (all cases $P > 0.05$) we therefore removed this parameter from further analysis.

2.4.2. Lead calculations

We extracted the published data on lead concentrations by species and by study (SI Table B1). To compare lead concentrations between studies, we converted all tissue concentrations so that they were expressed as mg/kg dry weight (dw). Following Krone (2018), we used the following correction factors: 1 $\mu g\ g^{-1}$ ww = 4.6 $\mu g\ g^{-1}$ dw for blood, 3.1 $\mu g\ g^{-1}$ dw for liver, 4.3 $\mu g\ g^{-1}$ dw for kidney and 1.2 $\mu g\ g^{-1}$ dw for bone.

Lead concentrations were included in statistical models when the mean and variance (standard deviation; sd) from each study were given or could be derived. For studies that did not report the mean and sd but instead provided the median, range (minimum-maximum) and sample size, we estimated the mean and sd using the formulas described by Hozo et al. (2005). Studies not reporting the mean and sd, nor the median, range and sample size, were excluded from the meta-analysis (see SI Excel file).

2.5. Meta-analysis and statistics

We first ran forest plots to visualise how mean lead concentrations and confidence intervals (CIs) varied between the species covered in each study. The lead concentration data for individual species were not homogeneously distributed across decades and/or geographic region. Therefore, for the purposes of statistical evaluation of the data in the meta-analysis, we pooled species by feeding trait (see Section 2.4.1).

We used Metafor (Viechtbauer, 2019), a R package (R version 3.5.3, R Core Team, 2015), to estimate the differences in mean lead concentrations across feeding traits, decades and geographical regions. When examining lead concentrations in blood, we also explored differences between sampling seasons (4 levels: hunting, non-hunting, unknown and year-round). Lead concentrations were included as the response variable and feeding trait, decade, geographical region and sampling season (model for blood only) as explanatory variables; geographic region was not included in the model for bone lead because almost all the studies used were on raptors from southern Europe. We used multilevel random-effects models with REML (Restricted Maximum Likelihood) to account for within study variance caused by change and sampling error, and for between study variance caused by heterogeneity (Viechtbauer, 2010). We conducted four separate meta-analyses with the exposure measures being lead concentrations in: (i) liver, (ii) kidney, (iii) blood and (iv) bone.

The significance of the explanatory variables included in the models was assessed using the Akaike's Information Criterion (AIC) (Burnham and Anderson, 2002). We first included all variables in the models but later removed non-significant variables in a backward stepwise procedure. We ranked models using AIC and Akaike weight (w) (likelihood that a given model is the best among all candidate models). We selected the model with the greatest w and lowest AIC value as the model that best fitted the data without overparameterization (SI Table A2).

We computed the heterogeneity statistic I^2 which estimates (in percent) how much of the observed variation can be attributed to the actual difference between studies, rather than within-study variance (Higgins et al., 2003). We also computed the R^2 statistic, which is the amount (in percent) of the variation that is accounted for by the variables included in the model (Viechtbauer, 2010). The models for all four models (liver, kidney, blood and bone) had high heterogeneity (I^2 ~ 99%) indicating that most of the variation in lead levels was due to differences between rather than within individual studies (Higgins et al., 2003). The models explained circa 30% (R^2) of the variation in lead concentrations in the case of in liver, kidney and blood, and 15% in the case of bone.

To identify studies that were potential outliers and contributed excessively to model heterogeneity, we applied the Baujat plot and a set of diagnostic tools (Quintana, 2015; Viechtbauer and Cheung, 2010). The Baujat plot horizontal axis illustrates study heterogeneity and the vertical axis the influence of the study. Studies falling on the top right quadrant of the plot were rejected (see SI Excel file), as these exerted an excessive contribution to both factors. To investigate whether the studies included in our meta-analysis were a representative sample of all studies conducted on lead contamination in raptors, we checked for publication bias using the funnel and Egger's test (Egger et al., 1997). We found evidence of publication bias (Egger's test $P < 0.001$) for liver, kidney and blood (SI Figs. A2–A4). Publication bias dictates that studies with non-significant results are less likely to be published (Quintana, 2015). However, as our measure of interest was the concentrations of lead accumulated by raptors and our main aim was to study the variables that explained variation in residue magnitude, we did not expect publication bias to be a significant matter of concern.

Finally, Spearman's rank correlation tests were used to calculate correlations between lead concentrations in liver, kidney, blood and bone.

3. Results

3.1. Overview of lead publications in Europe

Of the 114 papers describing lead contamination in raptors in Europe, almost half (46%) were published between 2010 and 2019, while ~34% were published between 2000 and 2009, ~12% from 1990 to 1999 and < 8% from 1983 to 1989 (SI Fig. A5).

The number of studies and species monitored per country are shown in Table 1. The studies included information of 39 species consisting on 29 birds of prey (4 obligate scavengers, 5 facultative scavengers and 20 non-scavengers) and 10 owls. Among the birds of prey, the common buzzard (*Buteo buteo*) (23 papers), the white-tailed sea eagle (*Haliaeetus albicilla*) (20 papers) and the griffon vulture (*Gyps fulvus*) (15 papers) were the most studied species. The most studied owls were the barn owl (*Tyto alba*) and tawny owl (*Strix aluco*) (12 papers each), then the Eurasian eagle owl (*Bubo bubo*) and the little owl (*Athene noctua*) (11 papers each) (SI Table A1). The predominant species as well as the number of species studied often varied between countries (Table 1; SI Table A1). The species in which lead has been monitored most widely across Europe are the common buzzard and the Eurasian sparrowhawk (*Accipiter nisus*) (8 countries each), then the barn owl (7 countries) (SI Fig. A6).

3.2. Methods of lead analyses

Studies analysing lead concentrations in birds used a variety of different matrices and some studies analysed multiple matrices. The most commonly analysed matrix was the liver ($n = 53$ studies), followed by blood ($n = 36$), kidney ($n = 34$), bone ($n = 26$); other organs and sample types included lung, stomach, brain, intestine and heart ($n = 34$), feathers ($n = 24$), pellets ($n = 9$) and eggs ($n = 7$). The extent to which each matrix has been used has varied widely over time. The most marked change in the last decade has been the increase (~55%) in analysis of non-destructive samples, in particular blood and feathers, although liver has been the most extensively analysed matrix overall (SI Fig. A7).

There was a significant positive correlation between lead concentrations in the liver and kidney across all species combined (Spearman's correlation coefficient: $r = 0.66$, $P < 0.001$, $n = 25$; SI Fig. A8). There were no other significant correlations in lead concentrations between matrices (SI Table A3).

3.3. Lead concentrations in samples

All data on lead concentrations in different matrices and species are given in SI Table B1.

The griffon vulture, an obligate scavenger, had the highest mean lead concentrations in liver and kidney (Figs. 1–2).

Table 1
Overview of papers published per country, raptor groups monitored and most studied raptor species. Asterisk shows countries that have equal number of studies for more than two species. Species classification can be found in SI Table A1.

Country	Number of papers published	No. species studied					Most studied raptor species
		Birds of prey			Owls	Total species	
		Non-scavengers	Facultative scavengers	Obligate scavengers			
Spain	41	8	6	4	5	23	Griffon vulture
Poland	15	8	4	0	5	17	White-tailed sea eagle
Italy	7	5	2	1	4	12	Common buzzard/little owl
France	7	11	4	3	6	24	Western marsh harrier
Germany	7	1	2	0	0	3	White-tailed sea eagle
Switzerland	5	0	2	1	1	4	Golden eagle
Netherlands	5	1	1	0	2	4	Barn owl
Portugal	5	1	2	1	0	4	Griffon vulture
Sweden	4	1	2	0	1	4	*
U.K.	3	10	5	0	4	19	Red kite
Norway	2	1	0	0	1	2	Tawny owl/northern goshawk
Austria	2	0	2	0	0	2	White-tailed sea eagle/golden eagle
Finland	2	0	1	0	0	1	White-tailed sea eagle
Hungary	1	1	1	0	4	6	*
Belgium	1	1	0	0	2	3	*
Czech Rep.	1	0	0	2	0	2	Egyptian vulture/cinereous vulture

Facultative scavengers (common buzzard, golden and white-tailed sea eagle) had the next highest concentrations. Interestingly a single study showed that the marsh harrier (*Circus aeruginosus*) had the highest lead blood levels (Fig. 3) but the next recorded highest concentrations were in the griffon vulture.

The golden eagle (*Aquila chrysaetos*), the Eurasian eagle owl and the common buzzard were the species with the highest levels of bone lead (Fig. 4).

The most parsimonious model for liver lead included feeding trait, geographic region and decade ($QM_7 = 33.07$, $P < 0.0001$). The model for kidney lead also included feeding trait and decade but not geographic region ($QM_3 = 12.83$, $P < 0.01$), while the best model for blood lead included feeding trait and sampling season ($QM_4 = 18.55$, $P < 0.001$) (SI Table A2). Lead concentrations in all three matrices were higher in scavengers than non-scavengers ($P < 0.01$; Fig. 5; SI Tables A4–A6). Blood lead concentrations were higher in birds sampled during the hunting season than in those sampled in the non-hunting season, year-round or at an unknown time ($P < 0.01$ all, SI Table A7). Although geographic region was retained as a variable in best model for liver lead, *post-hoc* comparisons did not indicate any significant difference between individual regions (SI Fig. A9, Table A8). There was no overall consistent temporal trend across the decades in mean lead

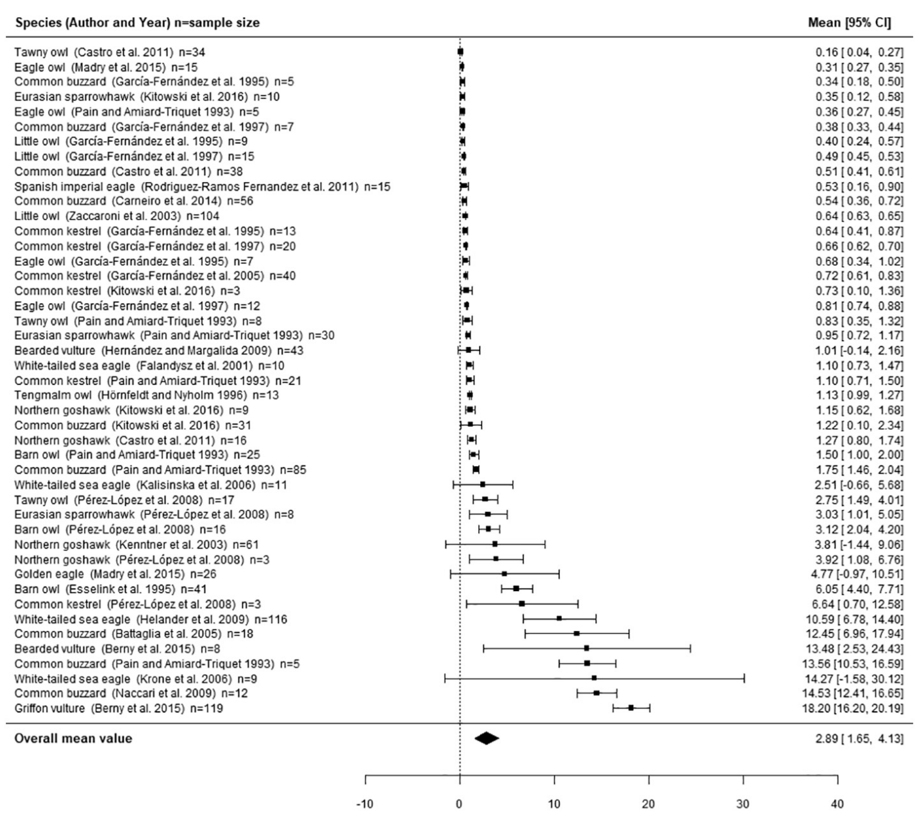


Fig. 1. Forest plot showing liver lead mean concentrations (mg/kg dw) in different raptor species. Error bars indicate 95% confidence intervals (CI). The diamond indicates the overall mean value across all the studies.

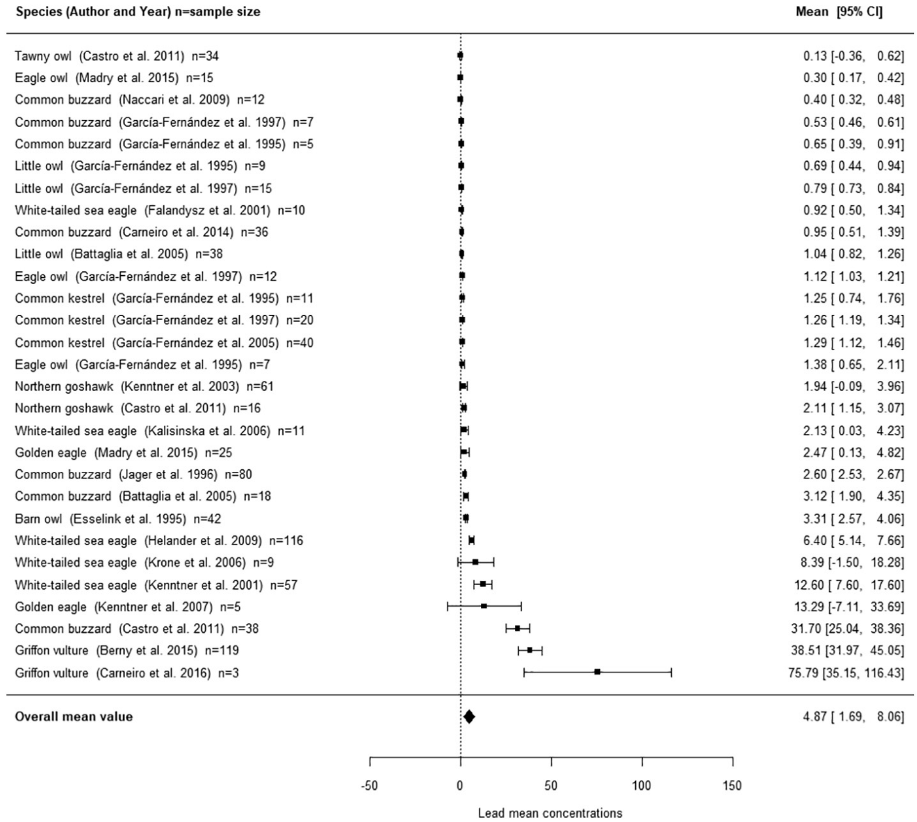


Fig. 2. Forest plot showing kidney lead mean concentrations (mg/kg dw) in different raptor species. Error bars indicate 95% confidence intervals (CI). The diamond indicates the overall mean value across all the studies.

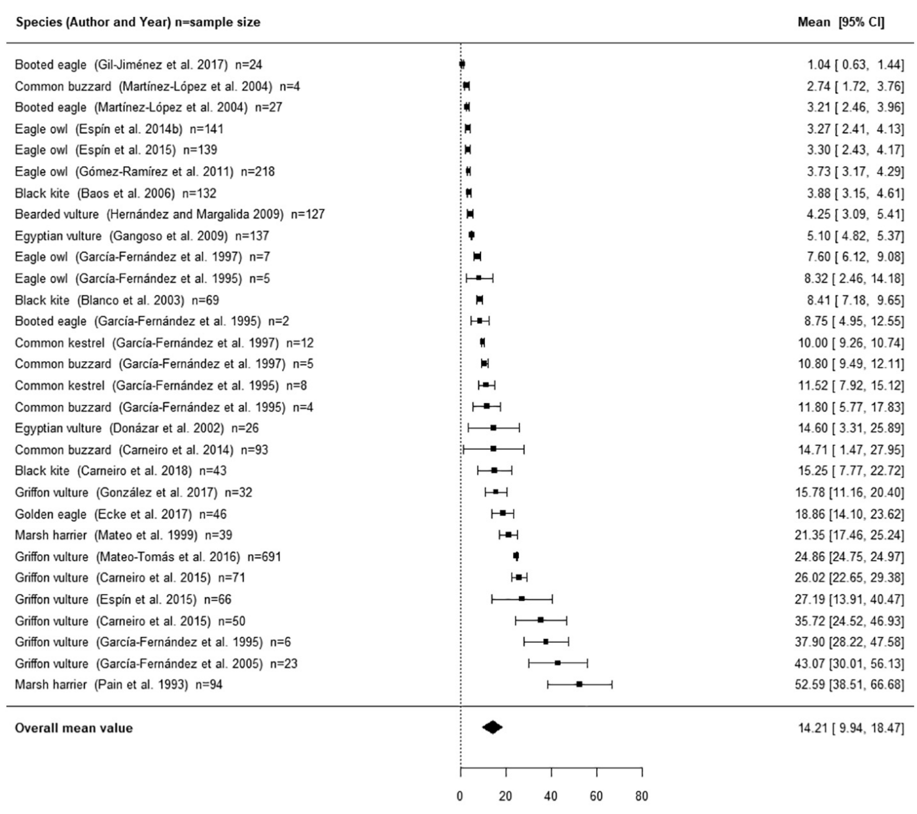


Fig. 3. Forest plot showing blood lead mean concentrations (μg/dl) in different raptor species. Error bars indicate 95% confidence intervals (CI). The diamond indicates the overall mean value across all the studies.

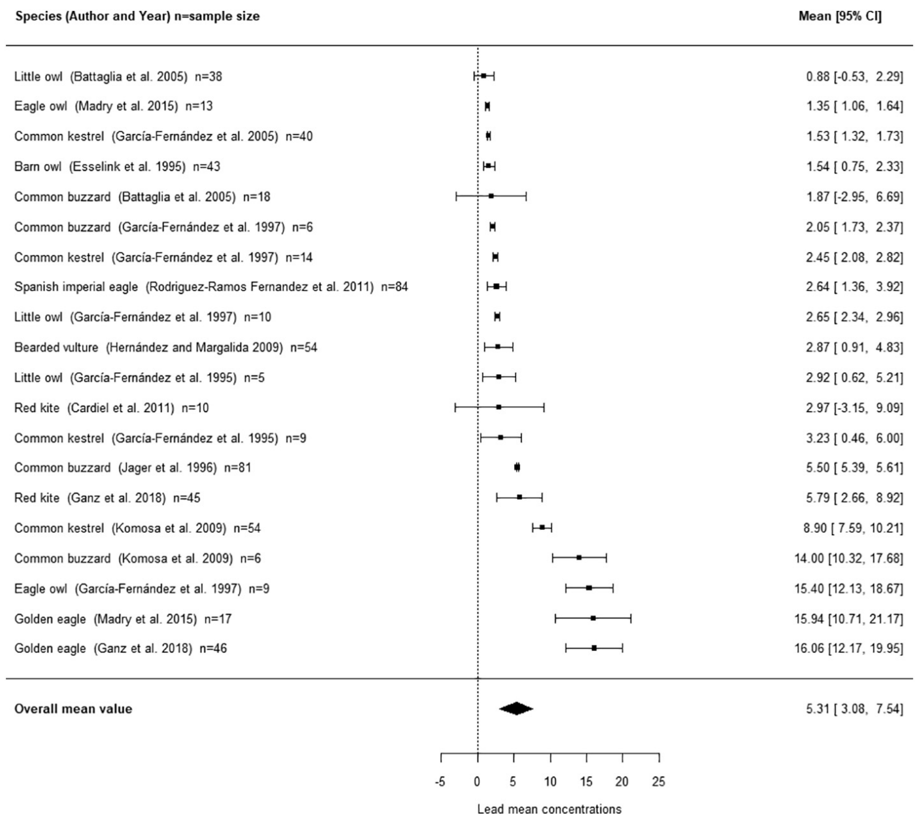


Fig. 4. Forest plot showing bone lead mean concentrations (mg/kg dw) in different raptor species. Error bars indicate 95% confidence intervals (CI). The diamond indicates the overall mean value across all the studies.

concentrations when assessed in kidney and blood (SI Fig. A10, Tables A9–A10) but there was a marginally significant decrease between 2000 and 2009 and 2010–2019 in liver lead ($P = 0.06$) (SI Fig. A10, Table A9).

The best model explaining lead variation in bone differed from those for the other matrices in that it was the null model closely followed by the one including feeding trait, geographic region and decade ($\Delta AIC < 2$) (SI Table A2). We did not continue further with any statistical analysis because the model was non-significant and explained little of the variation in bone lead ($QM_5 = 8.2$, $P > 0.05$). However, we included bone concentrations in Figs. 4 and 5 and SI Figs. A5 and A6 to enable visualisation of the distribution of data.

3.4. Effects of elevated lead and associated sources

3.4.1. Subclinical effects

In total, there were 226 species-specific datasets in the 114 papers reviewed. Just over half (51.8%) reported lead concentrations that exceeded subclinical threshold values (defined in Section 2.4.1) in at least some individual birds (SI Table B1), although in some cases, these included reports for multiple sample matrices (i.e., blood, liver, kidney and bone) from the same individuals. The species with the highest prevalence of exceedances (but excluding single case study reports) were the bearded vulture (*Gypaetus barbatus*) (100% of $n = 3$ datasets), the griffon vulture (92% of $n = 13$), the red kite (*Milvus milvus*) (75% of $n = 8$), the common buzzard (75% of $n = 20$) and the white-tailed sea eagle (67% of $n = 18$) (SI Table B1). The main source and route of exposure was ingestion of lead shot or ammunition (31 studies) although this was only presumed (with no confirmation) in most cases. Another fourteen studies found lead shot (9) or lead fragments (5) in the gastrointestinal tract of birds while four reported lead in regurgitated pellets. Nine studies undertook isotopic analyses and ten other studies found embedded shot in muscles of birds, suggesting a non-ingestion source of contamination but one that was still associated with hunting (SI Table B1). Mining and urban/industrial pollution were the other most frequently cited (but typically presumed) sources of lead contamination (12 studies).

A number of studies have examined associations between lead concentrations and subclinical effects. We found seven studies that determined if there was evidence that lead contamination could affect a range of effects on biomarkers in raptors. These included reports of adverse effects on oxidative stress, enzymatic activity, DNA damage and blood biochemistry (Table 2). The potential effect of lead on reproductive success in raptors has also been examined and the occurrence and nature of any effects has varied between studies (Table 2).

3.4.2. Lethality

Thirty-six (36) studies, encompassing 14 species, reported lead residues that exceeded the concentration thresholds for lethal lead contamination and lead-induced mortalities (Table 3). Most were from the last two decades (2000–09: 47.2% of studies; 2010–19: 33.3%) with only five (11%) conducted between 1990 and 1999 and three (8%) between 1983 and 1989 (Table 4). The proportion of studies on each species that reported exceedance of lethal concentration thresholds or mortality was calculated. The highest proportions were for bearded vulture, red kite, white-tailed sea eagle, golden eagle and common buzzard (Table 3). We also calculated the percentage of individuals in each of those studies that had lead concentrations above the lethal threshold level (Table 4). This was 2% and 40% for the bearded vulture (two studies only) while the median (range) was 12% (2%–24%) for the red kites (five studies), 26% (9%–100%) for the white-tailed sea eagle (nine studies), 24% (7%–43%) for the golden eagle (five studies) and 4.5% (1%–100%) for the common buzzard (eight studies). Those studies that reported the presence of lead in the gastrointestinal tract are also detailed in Table 4.

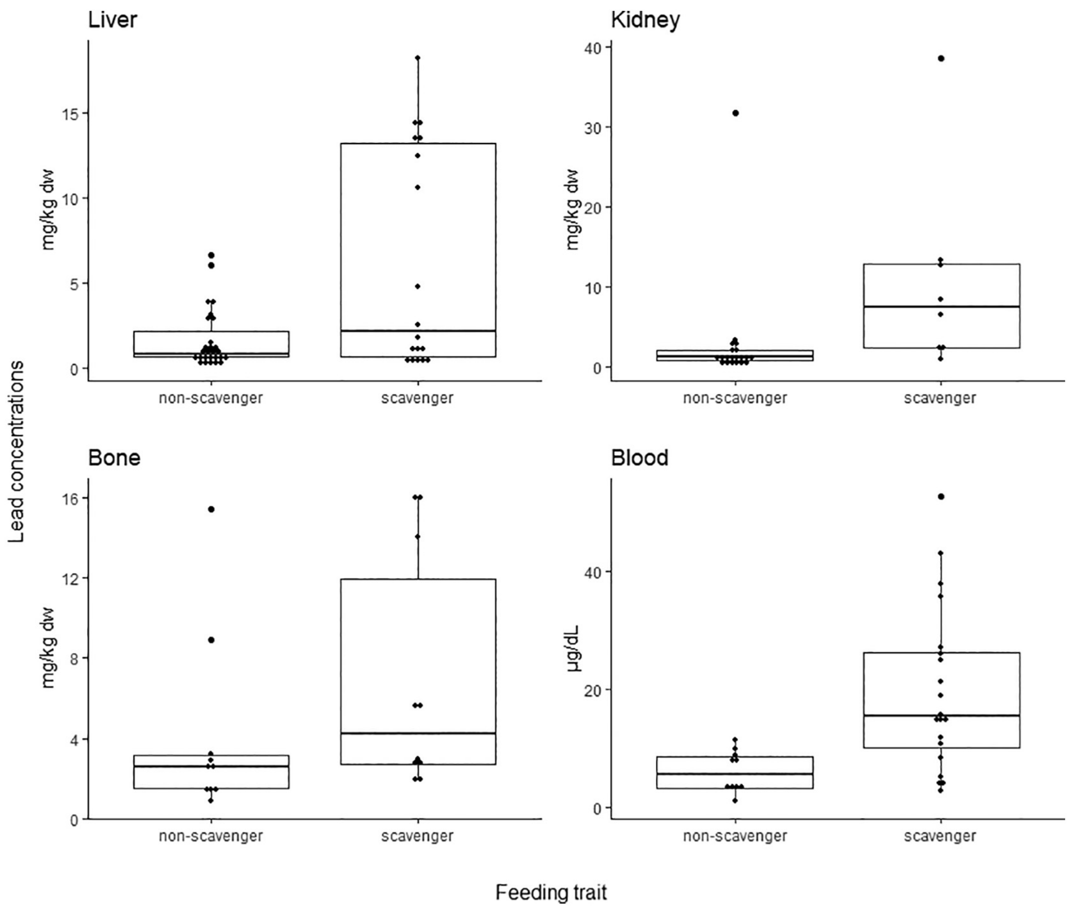


Fig. 5. Lead concentrations in liver, kidney, bone and blood of scavengers and non-scavengers. One high value for kidney lead in scavenger was eliminated for better visualisation of the figure but was included in the statistical analysis. Boxplots include the median value (thick line in the middle of the box), the 25th–75th interquartile range (top and bottom of the box) and the maximum and minimum values within 1.5 interquartile range (whiskers).

Table 2
Studies investigating subclinical lead effects. Matrix used (bl = blood; F = feathers; L = liver; E = eggs) and lead concentrations found associated with effects are shown.

Effects	Association with lead levels	Species	Year	n	Location	Ref.
Biomarkers						
Oxidative stress (GPx, CAT, TBARS)	bl: ≥15 µg/dl	Griffon vulture	2014	66	Spain	[1]
	bl: ≥2 µg/dl	Eurasian eagle owl	2014	141	Spain	[2]
δ-ALAD inhibition	bl: ≥10 µg/dl	Eurasian eagle owl	2011	218	Spain	[3]
	bl: ≥5 µg/dl	Booted eagle; common buzzard; northern goshawk	2004	27; 4; 3	Spain	[4]
	bl: ≥5 µg/dl	Eurasian eagle owl	2014	139	Spain	[5]
	≥8 µg/dl	Griffon vulture	2014	66	Spain	[5]
	bl: ≥30 µg/dl	Griffon vulture; Eurasian eagle owl	2014		Spain	[5]
DNA damage	No association bl: 3.88 (±4.3) µg/dl	Black kites	2006	132	Spain	[6]
Chronic stress (corticosterone)	No association F: <0.5 µg g^{-1}	Golden eagles	2018	24	Switzerland	[7]
Breeding parameters						
No. fledglings/breeding attempt	Decrease with ↑Pb F: 0.82 (±0.4) µg g^{-1}	Bonelli's eagle	2018	57	Spain	[8]
Nestling mortality	No association L: 1.13 (±0.25)	Tengmalm's owl	1996	13	Sweden	[9]
Fecundity	No association bl: 1.83 (±1.3) µg/dl	Booted eagle	2017	8	Spain	[10]
Viability eggs	No association E: 0.82 (±0.4) µg g^{-1} ww	Spanish imperial eagle	1988	10	Spain	[11]
Shell thickness	No association E: 0.037 µg g^{-1} ww	Marsh harrier	1999	13	France	[12]

References: [1] Espín et al. (2014a); [2] Espín et al. (2014b); [3] Gómez-Ramírez et al. (2011); [4] Martínez-López et al. (2004); [5] Espín et al. (2015); [6] Baos et al. (2006); [7] Ganz et al. (2018a); [8] Gil-Sánchez et al. (2018); [9] Hornfeldt and Nyholm (1996); [10] Gil-Jiménez et al. (2017); [11] Gonzalez and Hiraldo (1988); [12] Pain et al. (1999).

Table 3
Number and proportion (%) of studies reporting lead residues that exceeded the concentration thresholds for lethal lead contamination and lead-induced mortalities.

Species	Total studies	Studies relating mortality with lead poisoning (%)
Bearded vulture	3	2 (67)
Red kite	10	5 (50)
White-tailed sea eagle	21	10 (48)
Golden eagle	11	5 (45)
Common buzzard	23	8 (35)
Spanish Imperial eagle	7	2 (29)
Honey buzzard	4	1 (25)
Egyptian vulture	5	1 (20)
Peregrine falcon	10	2 (20)
Griffon vulture	15	3 (20)
Eurasian sparrowhawk	12	2 (17)
Northern goshawk	14	2 (14)
Marsh harrier	11	1 (9)
Eurasian eagle-owl	11	1 (9)

4. Discussion

4.1. Overview of lead publications in Europe

Our review of 36 years of research shows that 16 of 44 European countries have reported data on lead concentrations in raptors. The highest number of publications is from Spain. This perhaps reflects the fact that Spain's geographical position and geophysical diversity provides a matrix of suitable resources and habitats for raptors, of which there are some 26 bird of prey and 6 owl species; Spain supports a good proportion of the wintering populations of many species (García-Fernández et al., 2008). In addition, hunting is a widespread activity in Spain and constitutes an important conservation problem (Moleón et al., 2011; Mateo et al., 2013). Although there have been fewer (7) published studies reporting data from France, they contain data on lead concentrations in more raptor species (24) than reported by any other European country (Table 1). Monitoring of lead in raptors is relatively sparse in northern Europe (data for 6 bird of prey and 2 owl species) while only Poland in eastern Europe has monitored lead in a large number of species (12 birds of prey and 5 owl species). These results indicate that there is a major geographical bias in the reporting of lead contamination in raptors and this effectively hampers assessment of temporal and spatial trends in lead contamination at a pan-European scale. We also note that almost half of the information available about lead contamination in European raptors has been published in the last two decades. This publications trend has also been reported in other avian studies (e.g., Plaza and Lambertucci, 2019).

4.2. Raptor species used to monitor lead concentrations

The common buzzard, the griffon vulture and the white-tailed sea eagle are the species that have been most frequently monitored for lead contamination (SI Table A1) but such monitoring has not been geographically widespread across Europe, except perhaps for the common buzzard (data from 8 countries; SI Fig. A6). A previous assessment of the state of contaminant monitoring using raptors noted the need for a harmonised (species and matrices analysed) sampling strategy (Gómez-Ramírez et al., 2014; Espín et al., 2016) if spatial and temporal trends were to be detected at a pan-European scale. It has recently been argued that this could be achieved for lead through monitoring of the common buzzard or by monitoring the golden and white-tailed sea eagle in combination (Badry et al., 2020). The current geographical spread of available data on lead contamination is similar for common buzzard and for the golden/white-tailed sea eagle combination but both still only encompass some 20% of European countries and less than 30% of EU Member States. Given regulation of lead and other contaminants is centralised across the EU through such bodies as ECHA, there is clear need for wider-scale harmonised monitoring of lead to understand how environmental concentrations are changing and the associated impacts on raptors across Europe.

4.3. Value of matrices chosen to monitor lead concentrations

4.3.1. Use of matrices

Liver has been most extensively used for monitoring exposure to lead in recent decades, followed by blood, kidney and bone. Liver and kidney are useful proxies for medium-term exposure and bone constitutes a long-term depot for lead and therefore is used as a proxy for lifetime exposure (Pain et al., 2005; Rodriguez-Ramos Fernandez et al., 2011). Such analyses provide post-mortem confirmation of exposure and exceedance of threshold concentrations, but they can only be used in passive monitoring. Such monitoring comprised 50% of the studies we reviewed.

Feathers and blood can be sampled from live birds as part of active monitoring programmes. In the last 10 years, feathers have been increasingly analysed as a non- (or minimally-) invasive matrix and the number of such studies is likely to grow further. This is perhaps because feathers can be sampled opportunistically (during other sampling activities or ringing) and without disturbing the bird as shed feathers can be collected from the vicinity of the nests. Feathers can also be taken from dead birds and so the same sample type can be collected across both active and passive monitoring activities. Feathers act as an archive of exposure during the period of feather growth and thus reflect chronic levels of contamination (Burger, 1993). In the case of lead, exposure may be intermittent (e.g., when lead shot is ingested) and feather segments may be used to quantify variability in exposure (Rodriguez-Ramos Fernandez et al., 2011; Ganz et al., 2018b). However, feathers are moulted and re-grown during the summer months when, compared to winter, fewer game animals are shot. Thus, seasonal exposure peaks may be missed by feather analyses. Furthermore, there remains some uncertainty as to how lead is distributed between different feather sections and the extent to which external contamination may interfere with the analytical determination of internal lead concentrations (Cardiel et al., 2011; Ganz et al., 2018b). This may explain why blood remains the currently most widely used sample taken during active sampling and is frequently used for diagnosing poisoning in living birds.

Although eggs have been long used for monitoring several other types of contaminants such as persistent organic pollutants, perfluorinated compounds and mercury (Espín et al., 2016), very few studies have used eggs to monitor lead concentrations. This is because maternal transfer of lead to eggs is low (Walsh, 1990; Furness, 1993). Similarly, regurgitated pellets have been rarely used in monitoring studies even though they provide spatio-temporal information about both lead ingestion and overall diet (Mateo et al., 1999). Bullet fragments and shotgun pellets in regurgitated castings can be used as indicators of the ingestion of ammunition fragments by birds and can be used to non-invasively monitor the compliance of regulations with regards the use of lead-free ammunition.

4.3.2. Correlation in lead concentrations between matrices

We examined the available data from the publications we reviewed to determine if lead concentrations in different matrices were correlated with each other across multiple species and so whether concentrations in one sample type could be used to predict concentrations in another. Quantification of such relationships may facilitate future capability to compare data between studies that have measured residues in different sample types. We found there was a significant correlation between liver and kidney lead concentrations across species and that concentrations tended to be higher in the kidney. Our findings are consistent with those of other studies on individual species that have reported a similar association and higher levels in kidney than in liver (Esselink et al., 1995; Ek et al., 2004; Helander et al., 2009). The transfer

Table 4
Studies reporting lethal lead levels and mortality. Evidence of lead, matrix used (bl = blood; B = bone; L = liver; K = kidney) and percentage (%) of individuals showing lethal levels are shown. The lethal thresholds used are the following: Blood > 50 μg/dl; liver > 20 mg/kg dw; kidney > 25 mg/kg dw; bone > 20 mg/kg dw. Specific thresholds for some studies are detailed.

Species	Location	Year	Total animals[1]	N° animals Pb > lethal threshold	Death rate (%)[2]	Evidence of lead	Ref.
Bearded vulture	Spain	2009	87 (bl) 43 (B, L)	2 bl: Pb > 40 μg/dl[3] 1 L: Pb = 22 mg/kg	2		[1]
	Switzerland	2018	5 (B)	2 B: Pb = 59; 100 mg/kg	40		[2]
Common buzzard	Italy	2005	18 (L, K, B)	2 L: Pb > 20 mg/kg 1 B: Pb = 42 mg/kg	17		[3]
	Spain	2008	3 (L)	1 L: Pb = 18 mg/kg	34		[4]
	Portugal	2014	125 (93 bl; 56 L; 36 K)	2 bl (max. 631 μg/dl)	1		[5]
	Poland	2016	31 (L)	1 L: Pb = 15 mg/kg[3]	3		[6]
	U.K.	1995	56 (L)	1 L: Pb > 20 mg/kg	2		[7]
	France	1993	90 (L)	1 L: Pb > 20 mg/kg 3 L: Pb > 15 mg/kg[3]	4		[8]
	Italy	2008	19 (L)	1 L: Pb = 21 mg/kg	5		[9]
	U.K.	1983	1 (L, K)	1 L: Pb = 175 mg/kg 1 K: Pb = 66 mg/kg	100		[10]
Egyptian vulture	Spain	2009	39 (B)	1 B: Pb > 20 mg/kg	3		[11]
Eurasian eagle-owl	Spain	2003	42 (B)	1 B: Pb > 20 mg/kg	2		[12]
Eurasian	France	1993	32 (L)	1 L: Pb = 52 mg/kg	3		[8]
Sparrowhawk	U.K.	1983	1 (L, K)	1 L: Pb = 35.7 mg/kg 1 K: Pb = 31.2 mg/kg	100		[10]
Golden eagle	Alps Switzerland	2015	41 (26 L; 25 K; 17 B; 7 bl)	1 L: Pb = 77.4 mg/kg 1 K: Pb = 30.9 mg/kg 5 B: Pb > 20 mg/kg 3 bl: Pb = 32[4]; 56.3; 108 μg/dl	24		[13]
	Alps Switzerland, Germany, Austria	2007	7 (L, K)	2 L: Pb = 184; 21 mg/kg 1 K: Pb = 55 mg/kg	43		[14]
	Switzerland	2018	46 B 55 L	14 B: Pb > 20 mg/kg 2 L: Pb = 77.4; 80.4 mg/kg	30 (B)[a]		[2]
	Switzerland	2015	36 (26 L; 25 K; 17 B; 6 bl)	1 L: Pb = 77 mg/kg 1 K: Pb = 31 mg/kg 2 bl: Pb = 56; 108 μg/dl	8 3/36[b]		[15]
	Sweden	2017	111 (L) 46 (bl)	8 L: Pb = 27–177 mg/kg	7		[16]
Griffon vulture	Iberian Peninsula (Spain, Portugal)	2016	3 (bl, L, K)	3 bl: Pb = 969–1384 μg/dl 3 L: Pb = 309–1077 mg/kg 3 K: Pb = 36–100 mg/kg	100	Lead pellets in the stomach of 1 bird	[17]
	Spain	1997	1 (L)	1 L: Pb = 52 mg/kg	100	Lead shot/bullet in the gizzard	[18]
	Pyrenees (France)	2015	119 (L, K)	3 L: Pb max. 66 mg/kg 3 K: Pb max. 146 mg/kg	3		[19]
Honey buzzard	The Netherlands	1985	1 (bl)	1 bl: Pb = 80 μg/dl	100	Lead pellet in the gizzard	[20]
Northern	France	1993	1 (L)	1 L: Pb = 771 mg/kg	100		[8]
Goshawk	Germany	2003	62 (L, K)	1 L: Pb = 51 mg/kg	2		[21]
Peregrine	U.K.	1995	26 (L)	1 L: Pb > 20 mg/kg	4		[7]
Falcon	U.K.	1983	1 (L, K)	1 L: Pb = 64.3 mg/kg 1 K: Pb = 34 mg/kg	100		[10]
Red kites	U.K.	2007	86 (86 B, 44 L)	6 L: Pb > 15 mg/kg 18 B: Pb > 20 mg/kg	24 21/86[b]		[22]
	Spain	2003	12 (B)	1 B: Pb > 20 mg/kg	8		[12]
	Switzerland	2018	45 (45 B; 34 L)	1 B: Pb = 43 mg/kg	2		[2]
	Pyrenees (France)	2015	34 (L, K)	4 L: Pb max. 159 mg/kg 4 K: Pb max. 189 mg/kg	12		[19]
	U.K.	2017	87 (86 B, 44 L)	6 L: Pb > 15 mg/kg[3] 11 B: Pb = 30–188 mg/kg	20	1 bird with lead shot in the oral cavity	[23]
Spanish imperial eagle	Spain	2011	85 (84 B, 15 L)	3 B: Pb > 20 mg/kg	4		[24]
	Spain	2005	34 (B)	4 B: Pb > 50 mg/kg	12		[25]
Western marsh harrier	France	1993	94 (bl) 11 (7 L, 10 B)	1 L, B (with shot): Pb = 55 mg/kg L; 16 mg/kg B 13 bl: Pb > 60 μg/dl[5]	13 14/105[b]	1 bird with lead shot in the crop.	[26]
White-tailed sea eagle	Poland	2017	22 (L)	7 L: Pb > 30 mg/kg	32		[27]
	Germany	2001	61 (L, K)	16 L: Pb = 15[3]–192 mg/kg 13 K: Pb = 22[3]–73 mg/kg	26 16/61[b]		[28]
	Sweden	2009	118 (L, K)	15 L: Pb > 20 mg/kg 13 K: Pb > 20 mg/kg	14 16/118[b]	4 birds with lead shots and 2 with bullet fragments	[29]
	Finland	2018	123 (L, K)	38 L: Pb = 3.5–35 mg/kg	n.a.[c]		[30]
	Poland	2001	10 (L)	1 L: Pb = 40 mg/kg 1 K: Pb = 48 mg/kg	10		[31]
	Poland	1988	4 (B, L, K)	2 L: Pb > 50 mg/kg 2 K: Pb > 40 mg/kg 2 B: Pb > 12 mg/kg[3]	50 2/4[b]		[32]
	Germany	2007	87 (bl)	29 bl: Pb 39–572 μg/dl	34	11 birds with lead fragments in the gastrointestinal tract	[33]

Table 4 (*continued*)

Species	Location	Year	Total animals[1]	N° animals Pb > lethal threshold	Death rate (%)[2]	Evidence of lead	Ref.
	Finland	2006	11 (L, K)	2 L: Pb > 34 mg/kg 2 K: Pb > 27 mg/kg	18 2/11[b]	1 bird with bullet fragments in the gizzard	[34]
	Poland	2006	11 (L, K)	1 L: Pb = 48 mg/kg 1 K: Pb = 43 mg/kg	9		[35]
	Germany	2009	1 (L, K)	1 L: Pb = 48 mg/kg 1 K: Pb = 32 mg/kg	100	Metallic fragments in the oesophagus (RX)	[36]

References: [1] Hernández and Margalida (2009); [2] Ganz et al. (2018a); [3] Battaglia et al. (2005); [4] Pérez-López et al. (2008); [5] Carneiro et al. (2014); [6] Kitowski et al. (2016); [7] Pain et al. (1995); [8] Pain and Amiard-Triquet (1993); [9] Zaccaroni et al. (2008); [10] MacDonald et al. (1983); [11] Gangoso et al. (2009); [12] Mateo et al. (2003); [13] Jenni et al. (2015); [14] Kenntner et al. (2007); [15] Madry et al. (2015); [16] Ecke et al. (2017); [17] Carneiro et al. (2016); [18] Mateo et al. (1997); [19] Berny et al. (2015); [20] Lumeij et al. (1985); [21] Kenntner et al. (2003); [22] Pain et al. (2007); [23] Molenaar et al. (2017); [24] Rodriguez-Ramos Fernandez et al. (2011); [25] Pain et al. (2005); [26] Pain et al. (1993b); [27] Kitowski et al. (2017); [28] Kenntner et al. (2001); [29] Helander et al. (2009); [30] Isomursu et al. (2018); [31] Falandysz et al. (2001); [32] Falandysz et al. (1988); [33] Müller et al. (2007); [34] Krone et al. (2006); [35] Kalisińska et al. (2006); [36] Krone et al. (2009).

[1] Total number of birds is given as a single number if different matrices were sampled from the same individuals [matrices used are indicated using abbreviations within ()] or in different numbers if different birds were used for the different matrices sampled.

[2] Percentage (%) of birds with higher lead levels related to mortality. This percentage was calculated out of total number of birds studied except when indicated: [a]only bone residues were considered when calculating the %; [b]when some of the same individuals had lethal lead levels in multiple matrices, this was taken into account indicated underneath the % value; [c]% of individuals with Pb levels > 20 mg/kg was not reported.

[3] Considered lethal threshold.

[4] Showing acute poisoning symptoms.

[5] Lethal threshold concentration used in the study was 60 (not 50) mg/kg.

of lead into bone is slower than that to the soft tissues and bone lead is thought to reflect long-term chronic accumulation (Fisher et al., 2006). While some studies have reported positive correlations between bone and liver or bone and kidney lead concentrations (Esselink et al., 1995; Ganz et al., 2018a; Ishii et al., 2018), we found no such relationships nor any statistically significant association between levels in blood and in soft tissues or bone. This likely reflects the differences in the pharmacokinetics of lead between different body compartments. However, our results suggest that either the liver or kidney may be a suitable sample type for monitoring medium term exposure to lead across different raptor species. With additional data collection, it would be possible to generate read-across values between the two sample types, enabling comparison between studies reporting only liver or kidney lead concentrations.

4.4. Lead concentrations in raptors

4.4.1. Feeding traits associated with high exposure to lead

It has been argued that scavengers are at greater risk than active hunters of ingesting lead particles from spent ammunition as they feed on the unretrieved carcasses of hunted animals (Krone, 2018). Our findings are consistent with this concept insomuch that, within the studies we reviewed, obligate and facultative scavenger species generally had the highest lead concentrations in liver, kidney, blood and bone.

Of the obligate scavengers, the griffon vulture, which is the most abundant vulture in south Europe (BirdLife International, 2017), has been the only species in which lead contamination has been studied extensively (Table 3). The highest reported average liver, kidney and blood lead concentrations in the studies we reviewed were reported in this species, although we did not find extensive reporting of individuals exceeding lethal threshold concentrations. Other obligate scavenging birds of prey in Europe include the bearded vulture, Egyptian vulture (*Neophron percnopterus*) and cinereous vulture (*Aegypius monachus*), but they have been less well studied (Donázar et al., 2002; Hernández and Margalida, 2008; Gangoso et al., 2009; Hernández and Margalida, 2009; Berny et al., 2015; Ganz et al., 2018a; Pikula et al., 2013). However, Berny et al. (2015) found high lead liver concentrations in 8 bearded vultures and suggested lead may be a conservation problem for this scavenger because its highly acidic gastric juices, designed to facilitate digestion of bone, also enhances absorption of lead. Hernández and Margalida (2009) reported lead concentrations above lethal threshold levels in individual bearded vultures, albeit a small number (Table 4), and showed that lead concentrations were higher in individuals during the hunting than the non-hunting season. Lead concentrations in Egyptian vultures were also found to peak during the hunting season (Gangoso et al., 2009), suggesting that lead from hunting ammunition is a likely important contaminant source in both species.

Facultative scavenging species are also exposed to and accumulate relatively high lead concentrations. This is particularly true for the golden eagle, common buzzard and white-tailed sea eagle. All three are known to scavenge carcasses, including those of unretrieved shot animals, but the extent to which they do this may have been underestimated (Selva et al., 2005; Blázquez et al., 2009; Sánchez-Zapata et al., 2010). Five of the 11 studies on golden eagles that we reviewed were from Switzerland and bone lead concentrations in Swiss eagles were the highest of those reported in any species. The lead isotope signatures in those birds were similar to that of hunting ammunition (Madry et al., 2015; Ganz et al., 2018a) and the authors concluded there was a high risk of lead exposure in golden eagles in the Swiss Alps. There is less information for lead contamination in golden eagles elsewhere in Europe.

Compared with the golden eagle, less is known about lead exposure in the common buzzard and we did not find any published studies that reported lead isotope signatures in this species. However, opportunistic scavenging has been presumed to be the cause of lead contamination in buzzards (Battaglia et al., 2005; Carneiro et al., 2014). Furthermore, three studies on common buzzards have reported elevated liver and kidney lead levels that were of similar magnitude to those found in (obligate scavenging) vultures (Pain and Amiard-Triquet, 1993; Naccari et al., 2009; Castro et al., 2011). Scavenging of shot animals is likewise thought to account for high levels of lead accumulation in white-tailed sea eagles and marsh harriers. In a study on white-tailed sea eagles, the lead isotope ratios differed between individuals with lethal lead concentrations and those with only background lead concentrations (Helander et al., 2009). The authors concluded that lead in individuals with lethal concentrations likely originated from lead ammunition sources, thus suggesting that scavenging was a likely route of exposure. In marsh harriers from wetlands in France (Pain et al., 1993a) and Spain (Mateo et al., 1999), blood lead concentrations were among the highest reported for any raptor. This high level of exposure was also attributed to their scavenging of carcasses or capturing injured (shot) prey as shot was found in the pellets regurgitated by the marsh harriers.

Overall, these studies demonstrate that not only obligate but also facultative scavengers are vulnerable to lead exposure and poisoning.

However, it is notable that among the studies we reviewed, some reported only low lead concentrations in facultative scavenging species such as common buzzard, white-tailed sea eagle and marsh harriers (Figs. 1–4). This may reflect differences between studies in the extent of opportunistic scavenging exhibited by individuals and is likely a function of the intensity of hunting activity and subsequent availability of scavenge.

4.4.2. Spatio-temporal trends in lead residues

The use of lead-based ammunition for hunting has been regulated in 23 European countries, with some countries adopting total (e.g., The Netherlands and Denmark) and others partial (see Mateo and Kanstrup, 2019) bans. However, the effectiveness of such mitigations on reducing lead contamination in raptors has not been widely examined. Our review indicated that lead can still be found in high concentrations in European raptors, but concentrations vary markedly between species in different regions. This variation may reflect differences between countries in the scale and effectiveness of mitigation measures. Mateo et al. (2007) reported a decrease in the ingestion of lead shot by the Spanish imperial eagles in Doñana, an important Spanish wetland where legislation against the use of lead shot was adopted in 2001. In contrast, Helander et al. (2009) found that the proportion of white-tailed sea eagles poisoned by lead did not differ before and after a partial ban of lead shot in Sweden and suggested that this was because the ban did not cover coastal areas utilized by this species. Overall, our review found no clear evidence that lead concentrations in raptors have decreased over time across Europe. Although visually, there appeared to be some difference in lead concentrations between regions (SI Fig. A9), these differences were not statistically significant. However, data were scant for the northern and eastern regions and further studies are needed to investigate if there are, in fact, regional differences and, if so, what are the causes.

4.4.3. Influence of hunting season on lead concentrations

When evaluating blood lead concentrations, we found seasonal trends in risk of lead poisoning with blood lead levels higher during the hunting than the non-hunting season. Previous studies have already reported such an association (e.g., Berny et al., 2015; Carneiro et al., 2014; Gangoso et al., 2009; Mateo et al., 1999). However, our review provides the first systematic evaluation and evidence of this trend using data from 10 species. The generally high blood lead concentrations during the hunting season are probably associated with the consumption of lead-shot individuals of small or large game species and implicate spent lead ammunition as an important factor for the uptake of lead in raptors. Among the studies reviewed, almost all were from Spain and other southern countries. Thus, we were not able to evaluate how patterns for blood lead concentrations were related to hunting activities across different regions of Europe. The studies incorporated in the analysis were published in a range of years from the 1990s to the 2010s indicating that hunting is still a current important conservation problem for raptors and that future mitigation measures are necessary to avoid high lead exposure or lead poisoning.

4.5. Effects of lead

4.5.1. Threshold for subclinical exposure

More than half of the studies reviewed reported lead concentrations in raptors that exceeded the subclinical threshold for harm defined by Franson and Pain (2011). This threshold has been widely used to interpret the toxicological significance of lead contamination in raptors. However, both higher [e.g., 0.3 mg/kg dw (Pain et al., 1993a, 1993b)] and lower [0.15 mg/kg dw (Espín et al., 2015; Gómez-Ramírez et al., 2011; Martínez-López et al., 2004); 0.10 mg/kg dw (Gómez-Ramírez et al., 2011; Espín et al., 2014a, 2014b)] threshold levels for blood have been considered as subclinical in some studies (SI Table B1). In fact, critical thresholds may be species-specific as it has been shown that some raptor species are more sensitive to lead than others (Ecke et al., 2017; Pain et al., 2019). For instance, the griffon vulture is widely recognised as relatively tolerant to lead (García-Fernández et al., 2008; Mateo-Tomas et al., 2016) and Espín et al. (2015) showed that Eurasian eagle owls were more affected than griffon vultures with similar blood lead residues. Although 12 out of 15 studies in griffon vultures we reviewed showed some individuals exceeding subclinical thresholds (SI Table B1), it is possible that this may overestimate the likelihood of effects in this species. Other species in which a high proportion (60%) of studies indicated individuals exceeding subclinical threshold levels included the common buzzard, the red kite and the white-tailed sea eagle (SI Table B1) and these may be species particularly at risk of subclinical effects although species-specific thresholds for these species are not defined.

4.5.2. Subclinical and clinical effects

Exposures to lead that result in subclinical effects are of particular concern as such effects are often hard to recognise in free-living birds and their effects on populations remain unknown. In our review, we have highlighted studies that reported a relationship between lead residues in birds and biomarker responses. These studies provide evidence that antioxidant enzyme activity can be used as a biomarker of heavy metal exposure and effects in raptors (Martínez-López et al., 2004; Gómez-Ramírez et al., 2011; Espín et al., 2014a, 2014b, 2015). They demonstrated for the first time that low lead levels in blood or tissues (between “background” and subclinical concentrations) are associated with effects on the antioxidant system and that such effects may occur below the subclinical threshold concentrations suggested by Franson and Pain (2011).

Exposure to lead has also been reported to affect reproduction (sperm motility, organ development, egg hatching rate) in non-raptor species such as red-legged partridges (*Alectoris rufa*) and domesticated pigeons (*Columba livia domestica*) (Pain et al., 2019). Less is known about the potential reprotoxic effects of lead in raptors (Table 2). Although Gil-Sánchez et al. (2018) did observe an apparent direct negative relationship between high lead concentrations and the number of fledglings per breeding attempt in Bonnelli's eagle (*Aquila fasciata*), they could not suggest a likely mechanism of action. Pain et al. (1999) showed that lead did not affect shell thicknesses and suggested that any pathway by which lead might affect reproductive success would be through direct effects on the parents that resulted in impaired incubation or nestling care. Further studies are needed to establish how lead affects reproductive success and whether this is largely or exclusively driven by impacts on parent behaviour and condition.

In this review, we found a number of studies that related behavioural changes in raptors to chronic exposure to lead. For instance, Krone et al. (2009) recorded the long-term activity of a white-tailed sea eagle and noted a change in daily movement and activity patterns that was associated with clinical lead intoxication caused by incidental ingestion of lead fragments from a rifle bullet. Berny et al. (2015) found an association between lead concentrations and the proportion of trauma/electrocution. They found that long-term low lead levels (even below subclinical levels) could impair flight capabilities and individuals were more likely to hit obstacles. Ecke et al. (2017) found that blood lead concentrations higher than 2.5 μg/dl in golden eagles impaired flight performance in terms of decreased height and movement rate. These studies suggest that lead may well have the capacity to affect individual fitness in raptors. Such effects are rarely detected by conventional monitoring programmes examining lead contamination in raptors and the impacts of such exposure on raptor populations is likely to be underestimated.

4.5.3. Mortality

Lead poisoning has been identified as an important cause of death for wildfowl (Pain et al., 2019). Lead poisoning in raptors is less well-studied but individuals of many species have been reported as dying of lead poisoning (Mateo, 2009; Pain et al., 2009, 2019). In our review, we included all studies that reported lead-related mortality in different raptor species. By specifying both the proportion of studies in which mortality was reported and the proportion of individuals per study that had lead residues above the lethal threshold level, we were better able to evaluate the scale of lead poisoning in European raptors (Tables 3 & 4). Mortality was reported in 14 of 39 raptor species covered in the studies that we reviewed. Our review of the mortality data (Tables 3 & 4) suggests that the bearded vulture, the red kite, the white-tailed sea eagle and the golden eagle may be the species most at risk of being poisoned. Interestingly, reports of mortalities in griffon vultures were less prevalent than for these other species (Table 3), even though griffon vultures often had the highest accumulated lead residues of all species. This is consistent with the concept that this species may be relatively tolerant of lead (Section 4.5.1), although those studies reporting the highest lead levels in griffon vultures also reported that there were mortalities.

Overall, lead continues to cause mortality in many raptor species, as determined by diagnosed clinical cases and from the exceedance of lethal threshold levels. Circa 75% of all studies reporting lead poisoning are from the last 20 years (Table 4). This increase most likely reflects a rise in awareness of lead poisoning in raptors and a resultant increase in the number of investigations performed. In addition, it is important to consider that reported mortality events may be a small proportion of the real lead-related mortality that happens in the wild.

4.6. Identifying the sources of lead contamination

Most of the articles reporting lead concentrations above the thresholds suggested that hunting ammunition was the source. In many studies, the source of lead was only presumed but such attribution is difficult to confirm. One method has been to search for ammunition fragments and shot in the gastrointestinal tract (MacDonald et al., 1983; Lumeij et al., 1985; Mateo et al., 1997; Andreotti et al., 2017). Other studies have also reported lead ammunition/shot embedded in tissues but this represents a different exposure pathway and is associated with accidental or deliberate shooting of individuals; tissue concentrations associated with embedded shot are mostly lower than those caused by ingested lead (SI Table B1; Ganz et al., 2018a; Plaza and Lambertucci, 2019; but also Berny et al., 2015). Another attribution method that is gaining popularity is the analysis of isotopic signatures. This involves comparing the lead isotope signatures of raptor tissues with those of lead ammunition. For example, in red kites, isotope signatures for body tissues were similar to those for lead shot retrieved from regurgitated pellets (Pain et al., 2007). Other studies have used isotopic signatures more indirectly. For instance, hunting ammunition was inferred as the likely cause of lead poisoning in four vulture species because the lead isotopes in the vultures were different to those in soil, including soils from mining areas (Berny et al., 2015; Madry et al., 2015). Other studies using isotopes on griffon vultures and white-tailed sea eagles distinguished between background levels of lead that were derived from natural sources and elevated levels that were assumed to have come from ammunition sources (Helander et al., 2009; Mateo-Tomas et al., 2016). The characterisation and use of isotope signals for environmental samples and hunting ammunition may be a key tool for confirming when lead ammunition/shot is the cause of lead poisoning in raptors. Reliable quantification of the number of cases of poisoning that are due to ammunition lead would enable assessment of the importance of this issue across Europe.

Besides ammunition, some studies have hypothesised that highly polluted environments may be the source of lead exposure in the raptors that they studied. However, the number of these studies is small. They have generally been focused on mining areas (Baos et al., 2006; Gómez-Ramírez et al., 2011; Espín et al., 2014b, 2015) or where raptors are in close proximity to industrialised (García-Fernández et al., 1997; Espín et al., 2014b) or highly-polluted (i.e., solid-waste incinerator; Blanco et al., 2003) areas.

5. Conclusions: key findings and recommendations

Despite an increasing amount of research on lead in raptors, there is a geographical distribution bias in publications originating from Europe. Most studies come from western and southern Europe and few are from eastern and northern countries. Furthermore, few raptor species are widely monitored for lead. As a result, we had to pool our analyses by feeding trait, geographical region and across decades. We also found that there are large-scale variation in lead concentrations within and between raptor species. However, through the meta-analysis we were able to conclude four main points: (1) scavengers, both obligate and facultative species, are more prone to lead contamination than non-scavengers including birds of prey and owls, (2) lead contamination in raptors is still widely detected across Europe despite partial bans on the use of lead in ammunition and shot, (3) there is a seasonal peak in blood lead concentrations related to hunting season in southern European countries, (4) the levels of exposure in several species are generally relatively high and exceedance of subclinical threshold levels is widespread.

By conducting this meta-analysis, we have identified a range of gaps in information provision and knowledge. We make a number of recommendations about future approaches that are needed if our understanding of the impacts of lead on raptors is to be improved. These are:

- Monitoring the same species across different monitoring schemes and countries. Suitable candidate species have been suggested elsewhere (Badry et al., 2020). In agreement, we suggest the common buzzard and the golden/white-tailed sea eagle combination as potential species to monitor lead trends in Europe. Our review indicates these species are more prone to lead poisoning and are also most widely monitored across Europe. Their distribution is also suitable covering most of Europe.
- Monitoring the same sample matrices across schemes. This review shows that lead levels in kidney and liver are highly correlated and these matrices could be used interchangeably to some extent, although this would require further studies to generate species-specific read-across values. Monitoring of lead using non-invasive samples, such as feathers, has the potential to increase our breadth of knowledge about exposure of raptors to lead but further studies are required to determine adverse-effects threshold values.
- This review shows that there is a scarcity, and in some cases complete absence, of data on lead exposure and monitoring in the northern and eastern Europe, and increased sampling and measurements are recommended for these regions.
- As hunting ammunition is among the most important causes of lead poisoning in raptors, publications should always specify when samples are collected (winter, breeding, hunting, non-hunting season).
- Lead-based ammunition is a well-recognised source of lead exposure and poisoning in raptors, yet it is difficult to confirm. More routine measurement of tissue isotopic signatures, searching for ammunition fragments in the gut, and non-invasive monitoring of regurgitated pellets would all enable quantification of the true scale of exposure. Where possible, we recommend analysis of lead isotopic ratios as well as determination of total lead concentrations.
- When reporting results, studies need to publish the data for individuals, either as part of the supporting information of the paper or preferentially as published datasets that can be downloaded from data archives. Metadata should include information of the quality assurance measurements associated with the lead concentration data. Provision of such information, and inclusion within publications of the full range of summary statistics (mean, median, variance and range of values) would enhance data comparability and the value of such studies for future systematic evaluations.

- When mortality or subclinical effects are reported, it is crucial to provide individual information on the tissue lead levels that are related to the effects.
- This review shows that there are few studies on the subclinical effects of lead in raptors and the consequences and significance of subclinical exposure remains largely unknown. We highlight the need for such studies which should focus on subclinical effects that may directly or indirectly affect survival and reproduction.

Finally, it is evident from this review that lead-induced mortalities and subclinical effects occur in European raptors. However, the impacts on population demography appear to have been little studied. This is in stark contrast to studies on species such as the Californian condor (*Gymnogyps californianus*) in North America which have resulted in real understanding of the population impacts of lead and the need for, and effectiveness of, mitigation options in this species (Finkelstein et al., 2012; Herring et al., 2018). In our review, we did not find any studies that related exposure to lead in raptors to predicted quantified impacts on European raptor populations. While the death of individual raptors from lead poisoning is clearly undesirable (and in some cases may be sufficient to elicit mitigation), an understanding of the risk at the population level is typically the evidence that guides the need for mitigation. Improved assessment of the extent and scale of lethal and subclinical effects, modelling of how such effects may alter population demography, and evidence of how bans (where these have been implemented) have reduced exposure, are all needed. Such evidence at a pan-European scale is likely to require increased harmonisation between national contaminant raptor monitoring schemes in Europe (Gómez-Ramírez et al., 2014).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.141437.

Funding sources

This paper arises from work carried out by the European Raptor Biomonitoring Facility (ERBF) COST Action (CA16224). A short-term scientific mission was granted to Laura Monclús to investigate published data on lead monitoring in European raptors in the Leibniz Institute for Zoo and Wildlife Research in Berlin, Germany. Richard Shore was supported by the Natural Environment Research Council National Capability UK-SCAPE programme [NERC grant reference number NEC06550].

CRediT authorship contribution statement

Laura Monclús: Conceptualization, Methodology, Formal analysis, Writing-original draft. **Richard F. Shore:** Conceptualization, Writing-review & editing. **Oliver Krone:** Conceptualization, Supervision, Writing-review & editing.

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.

Acknowledgments

This paper is dedicated to the memory of Richard Shore. Richard was an expert toxicologist and a great person, who contributed enormously to the realization of this study. We are honoured to have worked with him. The authors would like to thank Alexandre Courtiol for his help and useful input in statistics and the artist Nikolai Kraneis (www.nikolai-kraneis.com) for the drawings of the raptors of the graphical abstract.

References

Andreotti, A., Fabbri, I., Menotta, S., Borghesi, F., Menotta, S., 2017. Lead gunshot ingestion by a peregrine falcon. Ardeola 65, 53–58. https://doi.org/10.13157/arla.65.1.2018.sc1.

Badry, A., Krone, O., Jaspers, V.L.B., Mateo, R., García-Fernández, A., Leivits, M., Shore, R.F., 2020. Towards harmonisation of chemical monitoring using avian apex predators: identification of key species for pan-European biomonitoring. Sci. Total Environ. 731, 139–198. https://doi.org/10.1016/j.scitotenv.2020.139198.

Baos, R., Jovani, R., Pastor, N., Tella, J.L., Jiménez, B., Gómez, G., González, M.J., Hiraldo, F., 2006. Evaluation of genotoxic effects of heavy metals and arsenic in wild nestling white storks (*Ciconia ciconia*) and black kites (*Milvus migrans*) from Southwestern Spain after a mining accident. Environ. Toxicol. Chem. 25, 2794–2803. https://doi.org/10.1897/05-570R.1.

Battaglia, A., Ghidini, S., Campanini, G., Spaggiari, R., 2005. Heavy metal contamination in little owl (*Athene noctua*) and common buzzard (*Buteo buteo*) from northern Italy. Ecotoxicol. Environ. Saf. 60, 61–66. https://doi.org/10.1016/j.ecoenv.2003.12.019.

Benson, W.W., Pharaoh, B., Miller, P., 1974. Lead poisoning in a bird of prey. Bull. Environ. Contam. Toxicol. 11, 105–108. https://doi.org/10.1007/BF01684587.

Berny, P., Vilagines, L., Cugnasse, J.M., Mastain, O., Chollet, J.Y., Joncour, G., Razin, M., 2015. VIGILANCE POISON: illegal poisoning and lead intoxication are the main factors affecting avian scavenger survival in the Pyrenees (France). Ecotoxicol. Environ. Saf. 118, 71–82. https://doi.org/10.1016/j.ecoenv.2015.04.003.

BirdLife International, 2017. Gyps fulvus (amended version of 2016 assessment). The IUCN Red List of Threatened Species 2017: eT22695219A118593677 https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T22695219A118593677.en.

Blanco, G., Frías, O., Jiménez, B., Gómez, G., 2003. Factors influencing variability and potential uptake routes of heavy metals in black kites exposed to emissions from a solid-waste incinerator. Environ. Toxicol. Chem. 22, 2711–2718. https://doi.org/10.1897/02-519.

Blázquez, M., Sánchez-Zapata, J.A., Botella, F., Carrete, M., Eguía, S., 2009. Spatio-temporal segregation of facultative avian scavengers at ungulate carcasses. Acta Oecol. 35, 645–650. https://doi.org/10.1016/j.actao.2009.06.002.

Burger, J., 1993. Metals in avian feathers: bioindicators of environmental pollution. Rev. Environ. Toxicol. 5, 203–311.

Burger, J., 1995. A risk assessment for lead in birds. J. Toxicol. Environ. Health 45, 369–396. https://doi.org/10.1080/15287399509532003.

Burnham, K., Anderson, D., 2002. MOdel Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Second ed. Springer-Verlag, New York.

Cardiel, I.E., Taggart, M.A., Mateo, R., 2011. Using Pb-Al ratios to discriminate between internal and external deposition of Pb in feathers. Ecotoxicol. Environ. Saf. 74, 911–917. https://doi.org/10.1016/j.ecoenv.2010.12.015.

Carneiro, M., Colaço, B., Brandão, R., Ferreira, C., Santos, N., Soeiro, V., Colaço, A., Pires, M.J., Oliveira, P.A., Lavín, S., 2014. Biomonitoring of heavy metals (Cd, Hg, and Pb) and metalloid (As) with the Portuguese common buzzard (*Buteo buteo*). Environ. Monit. Assess. 186, 7011–7021. https://doi.org/10.1007/s10661-014-3906-3.

Carneiro, M.A., Oliveira, P.A., Brandão, R., Francisco, O.N., Velarde, R., Lavín, S., Colaço, B., 2016. Lead poisoning due to lead-pellet ingestion in griffon vultures (*Gyps fulvus*) from the Iberian Peninsula. J. Avian Med. Surg. 30, 274–279. https://doi.org/10.1647/2014-051.

Castro, I., Aboal, J.R., Fernández, J.A., Carballeira, A., 2011. Use of raptors for biomonitoring of heavy metals: gender, age and tissue selection. Bull. Environ. Contam. Toxicol. 86, 347–351. https://doi.org/10.1007/s00128-011-0220-4.

De Francisco, N., Ruiz Troya, J.D., Agüera, E.I., 2003. Lead and lead toxicity in domestic and free living birds. Avian Pathol 32, 3–13. https://doi.org/10.1080/03079450301777.

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. Biol. Conserv. 107, 89–97. https://doi.org/10.1016/S0006-3207(02)00049-6.

ECHA, 2017. Annex XV Restriction Report Proposal for a Restriction Substance Name: Lead IUPAC NAME: Not Applicable CAS NUMBER: 7439-92-1.

ECHA, 2018. ECHA Annex XV Investigation Report (ECHA/PR/18/14). A Review of the Available Information on Lead in Shot Used in Terrestrial Environments, in Ammunition and in Fishing Tackle. Helsi.

Ecke, F., Singh, N.J., Arnemo, J.M., Bignert, A., Helander, B., Berglund, Å.M.M., Borg, H., Bröjer, C., Holm, K., Lanzone, M., Miller, T., Nordström, Å., Räikkönen, J., Rodushkin, I., Ågren, E., Hörnfeldt, B., 2017. Sublethal lead exposure alters movement behavior in free-ranging golden eagles. Environ. Sci. Technol. 51, 5729–5736. https://doi.org/10.1021/acs.est.6b06024.

Egger, M., Davey Smith, G., Schneider, M., Minder, C., 1997. Bias in meta-analysis detected by a simple, graphical test. BMJ 315, 629–634.

Eisler, R., 1988. Lead hazards to fish, wildlife, and invertebrates: a synoptic review. Contaminant Hazard Reviews. U.S. Fish and Wildlife Service Biological Report.

Ek, K.H., Morrison, G.M., Lindberg, P., Rauch, S., 2004. Comparative tissue distribution of metals in birds in Sweden using ICP-MS and laser ablation ICP-MS. Arch. Environ. Contam. Toxicol. 47, 259–269. https://doi.org/10.1007/s00244-004-3138-6.

Espín, S., Martínez-López, E., Jiménez, P., María-Mojica, P., García-Fernández, A.J., 2014a. Effects of heavy metals on biomarkers for oxidative stress in Griffon vulture (*Gyps fulvus*). Environ. Res. 129, 59–68. https://doi.org/10.1016/j.envres.2013.11.008.

Espín, S., Martínez-López, E., León-Ortega, M., Martínez, J.E., García-Fernández, A.J., 2014b. Oxidative stress biomarkers in Eurasian eagle owls (*Bubo bubo*) in three different scenarios of heavy metal exposure. Environ. Res. 131, 134–144. https://doi.org/10.1016/j.envres.2014.03.015.

Espín, S., Martínez-López, E., Jiménez, P., María-Mojica, P., García-Fernández, A.J., 2015. Delta-aminolevulinic acid dehydratase (δALAD) activity in four free-living bird species exposed to different levels of lead under natural conditions. Environ. Res. 137, 185–198. https://doi.org/10.1016/j.envres.2014.12.017.

Espín, S., García-Fernández, A.J., Herzke, D., Shore, R.F., van Hattum, B., Martínez-López, E., Coeurdassier, M., Eulaers, I., Fritsch, C., Gómez-Ramírez, P., Jaspers, V.L.B., Krone, O., Duke, G., Helander, B., Mateo, R., Movalli, P., Sonne, C., van den Brink, N.W., 2016. Tracking pan-continental trends in environmental contamination using sentinel raptors—what types of samples should we use? Ecotoxicology 25, 777–801. https://doi.org/10.1007/s10646-016-1636-8.

Esselink, H., van der Geld, F.M., Jager, L.P., Posthuma-Trumpie, G.A., Zoun, P.E.F., Baars, A.J., 1995. Biomonitoring heavy metals using the barn owl (*Tyto alba guttanata*): sources of variation especially to body condition. Arch. Environ. Contam. Toxicol. 28, 471–486.

Falandysz, J., Jakuczun, B., Mizera, T., 1988. Metals and organochlorines in four female white-tailed eagles. Mar. Pollut. Bull. 19, 521–526.

Falandysz, J., Ichihashi, H., Szymczyk, K., Yamasaki, S., Mizera, T., 2001. Metallic elements and metal poisoning among white-tailed sea eagles from the Baltic south coast. Mar. Pollut. Bull. 42, 1190–1193. https://doi.org/10.1016/S0025-326X(01)00217-X.

Finkelstein, M.E., Doak, D.F., George, D., Burnett, J., Brandt, J., Church, M., Grantham, J., Smith, D.R., 2012. Lead poisoning and the deceptive recovery of the critically endangered California condor. Proc. Natl. Acad. Sci. 109, 11449–11454. https://doi.org/10.1073/pnas.1203141109.

Fisher, I.J., Pain, D.J., Thomas, V.G., 2006. A review of lead poisoning from ammunition sources in terrestrial birds. Biol. Conserv. 131, 421–432. https://doi.org/10.1016/j.biocon.2006.02.018.

Franson, J.C., Pain, D.J., 2011. Lead in birds. In: Beyer, W.N., Meador, J.P. (Eds.), Environmental Contaminants in Birds: Interpreting Tissue Concentrations. Taylor and Francis Group, Boca Raton, pp. 563–593.

Furness, R., 1993. Birds as Monitors of Environmental Change (London, UK).

Gangoso, L., Álvarez-Lloret, P., Rodríguez-Navarro, A.A.B., Mateo, R., Hiraldo, F., Donázar, J.A., 2009. Long-term effects of lead poisoning on bone mineralization in vultures exposed to ammunition sources. Environ. Pollut. 157, 569–574. https://doi.org/10.1016/j.envpol.2008.09.015.

Ganz, K., Jenni, L., Madry, M.M., Kraemer, T., Jenny, H., Jenny, D., 2018a. Acute and chronic lead exposure in four avian scavenger species in Switzerland. Arch. Environ. Contam. Toxicol. 75, 566–575. https://doi.org/10.1007/s00244-018-0561-7.

Ganz, K., Jenny, D., Kraemer, T., Jenni, L., Jenni-Eiermann, S., 2018b. Prospects and pitfalls of using feathers as a temporal archive of stress events and environmental pollutants: a review and case study. J. Ornithol. 159, 771–783. https://doi.org/10.1007/s10336-018-1547-y.

García-Fernández, A.J., Motas-Guzmán, M., Navas, I., María-Mojica, P., Luna, A., Sánchez-García, J.A., 1997. Environmental exposure and distribution of lead in four species of raptors in Southeastern Spain. Arch. Environ. Contam. Toxicol. 33, 76–82.

García-Fernández, A.J., Calvo, J.F., Martínez-López, E., María-Mojica, P., Martínez, J.E., 2008. Raptor ecotoxicology in Spain: a review on persistent environmental contaminants. AMBIO A J. Hum. Environ. 37, 432–439. https://doi.org/10.1579/0044-7447(2008)37[432:reisar]2.0.co;2.

Gil-Jiménez, E., Manzano, J., Casado, E., Ferrer, M., 2017. The role of density-dependence regulation in the misleading effect of the Aznalcollar mining spill on the booted eagle fecundity. Sci. Total Environ. 583, 440–446. https://doi.org/10.1016/j.scitotenv.2017.01.098.

Gil-Sánchez, J.M., Molleda, S., Sánchez-Zapata, J.A., Bautista, J., Navas, I., Godinho, R., García-Fernández, A.J., Moleón, M., 2018. From sport hunting to breeding success: patterns of lead ammunition ingestion and its effects on an endangered raptor. Sci. Total Environ. 613–614, 483–491. https://doi.org/10.1016/j.scitotenv.2017.09.069.

Gómez-Ramírez, P., Martínez-López, E., María-Mojica, P., León-Ortega, M., García-Fernández, A.J., 2011. Blood lead levels and δ-ALAD inhibition in nestlings of Eurasian Eagle Owl (*Bubo bubo*) to assess lead exposure associated to an abandoned mining area. Ecotoxicology 20, 131–138. https://doi.org/10.1007/s10646-010-0563-3.

Gómez-Ramírez, P., Shore, R.F., van den Brink, N.W., van Hattum, B., Bustnes, J.O., Duke, G., Fritsch, C., García-Fernández, A.J., Helander, B.O., Jaspers, V., Krone, O., Martínez-López, E., Mateo, R., Movalli, P., Sonne, C., 2014. An overview of existing raptor contaminant monitoring activities in Europe. Environ. Int. 67, 12–21. https://doi.org/10.1016/j.envint.2014.02.004.

Gonzalez, L.M., Hiraldo, F., 1988. Organochlorine and heavy metal contamination in the eggs of the Spanish Imperial Eagle (*Aquila (heliaca) adalberti*) and accompanying changes in eggshell morphology and chemistry. Environ. Pollut. 51, 241–258. https://doi.org/10.1016/0269-7491(88)90165-0.

Helander, B., Axelsson, J., Borg, H., Holm, K., Bignert, A., 2009. Ingestion of lead from ammunition and lead concentrations in white-tailed sea eagles (*Haliaeetus albicilla*) in Sweden. Sci. Total Environ. 407, 5555–5563. https://doi.org/10.1016/j.scitotenv.2009.07.027.

Hernández, M., Margalida, A., 2008. Pesticide abuse in Europe: effects on the cinereous vulture (*Aegypius monachus*) population in Spain. Ecotoxicology 17, 264–272. https://doi.org/10.1007/s10646-008-0193-1.

Hernández, M., Margalida, A., 2009. Assessing the risk of lead exposure for the conservation of the endangered Pyrenean bearded vulture (*Gypaetus barbatus*) population. Environ. Res. 109, 837–842. https://doi.org/10.1016/j.envres.2009.05.001.

Hernández, L.M., González, M.J., Rico, M.C., Fernández, M.A., Aranda, A., 1988. Organochlorine and heavy metal residues in Falconiforme and Ciconiforme eggs (Spain). Bull. Environ. Contam. Toxicol. 40, 86–93. https://doi.org/10.1007/BF01689392.

Herring, G., Eagles-Smith, C.A., Varland, D.E., 2018. Mercury and lead exposure in avian scavengers from the Pacific Northwest suggest risks to California condors: implications for reintroduction and recovery. Environ. Pollut. 243, 610–619. https://doi.org/10.1016/j.envpol.2018.09.005.

Higgins, J.P.T., Thompson, S.G., Deeks, J.J., Altman, D.G., 2003. Measuring inconsistency in meta-analyses. BMJ 327, 557–560. https://doi.org/10.1136/bmj.327.7414.557.

Hornfeldt, B., Nyholm, N.E.I., 1996. Breeding performance of Tengmalm's owl in a heavy metal pollution gradient. J. Appl. Ecol. 33, 377–386. https://doi.org/10.2307/2404759.

Hozo, S.P., Djulbegovic, B., Hozo, I., 2005. Estimating the mean and variance from the median, range, and the size of a sample. BMC Med. Res. Methodol. 5. https://doi.org/10.1186/1471-2288-5-13.

Ishii, C., Nakayama, S.M.M., Kataba, A., Ikenaka, Y., Saito, K., Watanabe, Y., Makino, Y., Matsukawa, T., Kubota, A., Yokoyama, K., Mizukawa, H., Hirata, T., Ishizuka, M., 2018. Characterization and imaging of lead distribution in bones of lead-exposed birds by ICP-MS and LA-ICP-MS. Chemosphere 212, 994–1001. https://doi.org/10.1016/j.chemosphere.2018.08.149.

Isomursu, M., Koivusaari, J., Stjernberg, T., Hirvelä-Koski, V., Venäläinen, E.R., 2018. Lead poisoning and other human-related factors cause significant mortality in white-tailed eagles. Ambio 47, 858–868. https://doi.org/10.1007/s13280-018-1052-9.

Jenni, L., Madry, M.M., Kraemer, T., Kupper, J., Naegeli, H., Jenny, H., Jenny, D., 2015. The frequency distribution of lead concentration in feathers, blood, bone, kidney and liver of golden eagles *Aquila chrysaetos*: insights into the modes of uptake. J. Ornithol. 156, 1095–1103. https://doi.org/10.1007/s10336-015-1220-7.

Kalisińska, E., Salicki, W., Jackowski, A., 2006. Six trace metals in white-tailed eagle from northwestern Poland. Polish J. Environ. Stud 15, 727–737.

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. Environ. Toxicol. Chem. 20, 1831–1837. https://doi.org/10.1002/etc.5620200829.

Kenntner, N., Krone, O., Altenkamp, R., Tataruch, F., 2003. Environmental contaminants in liver and kidney of free-ranging northern goshawks (*Accipiter gentilis*) from three regions of Germany. Arch. Environ. Contam. Toxicol. 45, 128–135. https://doi.org/10.1007/s00244-002-2100-8.

Kenntner, N., Crettenand, Y., Fünfstück, H.J., Janovsky, M., Tataruch, F., 2007. Lead poisoning and heavy metal exposure of golden eagles (*Aquila chrysaetos*) from the European Alps. J. Ornithol. 148, 173–177. https://doi.org/10.1007/s10336-006-0115-z.

Kitowski, I., Sujak, A., Wiącek, D., Strobel, W., Komosa, A., Stobiński, M., 2016. Heavy metals in livers of raptors from Eastern Poland –the importance of diet composition. Belgian J. Zool. 146, 3–13.

Kitowski, I., Jakubas, D., Wiącek, D., Sujak, A., 2017. Concentrations of lead and other elements in the liver of the white-tailed eagle (*Haliaeetus albicilla*), a European flagship species, wintering in Eastern Poland. Ambio 46, 825–841. https://doi.org/10.1007/s13280-017-0929-3.

Krone, O., 2018. Lead poisoning in birds if prey. Birds of Prey: Biology and Conservation in the XXI Century, pp. 251–271 https://doi.org/10.2307/j.ctt5hjq5s.44.

Krone, O., Wille, F., Kenntner, N., Boertmann, D., Tataruch, F., 2006. Mortality factors, environmental contaminants, and parasites of white-tailed sea eagles from Greenland. Avian Dis. 48, 417–424. https://doi.org/10.1637/7095.

Krone, O., Berger, A., Schulte, R., 2009. Recording movement and activity pattern of a white-tailed sea eagle (*Haliaeetus albicilla*) by a GPS datalogger. J. Ornithol. 150, 273–280. https://doi.org/10.1007/s10336-008-0347-1.

Lumeij, J.T., Wolvekamp, W.T., Bron-Dietz, G.M., Schotman, A.J., 1985. An unusual case of lead poisoning in a honey buzzard (*Pernis apivorus*). Vet. Q. 7, 165–168. https://doi.org/10.1080/01652176.1985.9693976.

MacDonald, J.W., Randall, C.J., Ross, H.M., Moon, G.M., Ruthven, A.D., 1983. Lead poisoning in captive birds of prey. Vet. Rec. 113, 65–66.

Madry, M.M., Kraemer, T., Kupper, J., Naegeli, H., Jenny, H., Jenni, L., Jenny, D., 2015. Excessive lead burden among golden eagles in the Swiss Alps. Environ. Res. Lett. 10, 34003. https://doi.org/10.1088/1748-9326/10/3/034003.

Martínez-López, E., Martínez, J.E., María-Mojica, P., Peñalver, J., Pulido, M., Calvo, J.F., García-Fernández, A.J., 2004. Lead in feathers and δ-aminolevulinic acid dehydratase activity in three raptor species from an unpolluted Mediterranean forest (Southeastern Spain). Arch. Environ. Contam. Toxicol. 47, 270–275. https://doi.org/10.1007/s00244-004-3027-z.

Mateo, R., 2009. Lead Poisoning in Wild Birds in Europe and the Regulations Adopted by Different Countries. , pp. 1–28 https://doi.org/10.4080/ilsa.2009.0107.

Mateo, R., Kanstrup, N., 2019. Regulations on lead ammunition adopted in Europe and evidence of compliance. Ambio 48, 989–998. https://doi.org/10.1007/s13280-019-01170-5.

Mateo, R., Molina, R., Grífols, J., Guitart, R., 1997. Lead poisoning in a free ranging griffon vulture (Gyps fulvus). Vet. Rec. 140, 47–48. https://doi.org/10.1136/vr.140.2.47.

Mateo, R., Estrada, J., Paquet, J.-Y., Riera, X., Domínguez, L., Guitart, R., Martínez-Vilalta, A., 1999. Lead shot ingestion by marsh harriers *Circus aeruginosus* from the Ebro delta, Spain. Environ. Pollut. 104, 435–440. https://doi.org/10.1016/S0269-7491(98)00169-9.

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

Mateo, R., Green, A.J., Lefranc, H., Baos, R., Figuerola, J., 2007. Lead poisoning in wild birds from southern Spain: a comparative study of wetland areas and species affected, and trends over time. Ecotoxicol. Environ. Saf. 66, 119–126. https://doi.org/10.1016/j.ecoenv.2005.12.010.

Mateo, R., Vallverdú-Coll, N., Ortiz-Santaliestra, M.E., 2013. Intoxicación por munición de plomo en aves silvestres en España y medidas para reducir el riesgo. Ecosistemas 22, 61–67. https://doi.org/10.7818/ecos.2013.22-2.10.

Mateo-Tomas, P., Olea, P.P., Jiménez-Moreno, M., Camarero, P.R., Sánchez-Barbudo, I.S., Rodríguez Martín-Doimeadios, R.C., Mateo, R., 2016. Mapping the spatio-temporal risk of lead exposure in apex species for more effective mitigation. Proc. R. Soc. B Biol. Sci. 283. https://doi.org/10.1098/rspb.2016.0662.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 21 (6), e1000097. https://doi.org/10.1374/hournal.pmed.1000097.

Molenaar, F.M., Jaffe, J.E., Carter, I., Barnett, E.A., Shore, R.F., Rowcliffe, J.M., Sainsbury, A.W., 2017. Poisoning of reintroduced red kites (*Milvus milvus*) in England. Eur. J. Wildl. Res. 63, 94. https://doi.org/10.1007/s10344-017-1152-z.

Moleón, M., Sánchez-Zapata, J.A., Gil-Sánchez, J.M., Barea-Azcón, J.M., Ballesteros-Duperón, E., Virgós, E., 2011. Laying the foundations for a human-predator conflict solution: assessing the impact of Bonelli's eagle on rabbits and partridges. PLoS One 6, e22851. https://doi.org/10.1371/journal.pone.0022851.

Müller, K., Altenkamp, R., Brunnberg, L., 2007. Morbidity of free-ranging white-tailed sea eagles (*Haliaeetus albicilla*) in Germany. J. Avian Med. Surg. 21, 265–274. https://doi.org/10.1647/2007-001r.1.

Naccari, C., Cristani, M., Cimino, F., Arcoraci, T., Trombetta, D., 2009. Common buzzards (*Buteo buteo*) bio-indicators of heavy metals pollution in Sicily (Italy). Environ. Int. 35, 594–598. https://doi.org/10.1016/j.envint.2008.11.002.

Pain, D.J., 1996. Lead in waterfowl. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife - Interpreting Tissue Concentrations. South Carolina, pp. 251–264.

Pain, D.J., Amiard-Triquet, C., 1993. Lead poisoning of raptors in France and elsewhere. Ecotoxicol. Environ. Saf. 25, 183–192.

Pain, D.J., Amiard-Triquet, C., Bavoux, C., Burneleau, G., Eon, L., Nicolau-Guillaumet, P., 1993a. Lead poisoning in wild populations of marsh harriers *Circus aeruginosus* in the Camargue and Charente-Maritime, France. Ibis (Lond. 1859) 135, 379–386. https://doi.org/10.1111/j.1474-919X.1993.tb02109.x.

Pain, D.J., Metayer, C., Amiard, J.C., 1993b. Lead determination in avian blood: application to a study of lead contamination in raptors from France. Int. J. Environ. Anal. Chem. 53, 29–35. https://doi.org/10.1080/03067319308045980.

Pain, D.J., Sears, J., Newton, I., 1995. Lead concentrations in birds of prey in Britain. Environ. Pollut. 87, 173–180.

Pain, D.J., Burneleau, G., Bavoux, C., Wyatt, C., 1999. Levels of polychlorinated biphenlyls, organochlorine pesticides, mercury and lead in relation to shell thickness in marsh harrier (*Circus aeruginosus*) eggs from Charente-Maritime, France. Environ. Pollut. 104, 61–68. https://doi.org/10.1016/S0269-7491(98)00154-7.

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. Biol. Conserv. 121, 603–610. https://doi.org/10.1016/j.biocon.2004.06.012.

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. Sci. Total Environ. 376, 116–127. https://doi.org/10.1016/j.scitotenv.2007.01.062.

Pain, D., Fisher, I.J., Thomas, V.G., 2009. A global update of lead poisoning in terrestrial birds from ammunition sources. In: Watson, R.T., Fuller, M., Pokras, M., Hunt, W.G. (Eds.), Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA, pp. 99–118 https://doi.org/10.4080/ilsa.2009.0108.

Pain, D.J., Mateo, R., Green, R.E., 2019. Effects of lead from ammunition on birds and other wildlife: a review and update. Ambio 48, 935–953. https://doi.org/10.1007/s13280-019-01159-0.

Pérez-López, M., Hermoso de Mendoza, M., López Beceiro, A., Soler Rodríguez, F., 2008. Heavy metal (Cd, Pb, Zn) and metalloid (As) content in raptor species from Galicia (NW Spain). Ecotoxicol. Environ. Saf. 70, 154–162. https://doi.org/10.1016/j.ecoenv.2007.04.016.

Pikula, J., Hajkova, P., Bandouchova, H., Bednarova, I., Vojtech, A., Beklova, M., Kral, J., Ondracek, K., Osickova, J., Pohanka, M., Sedlackova, J., Skochova, H., Sobotka, J., Treml, F., Kizek, R., 2013. Lead toxicosis of captive vultures: case description and responses to chelation therapy. BMC Vet. Res. 9, 11. https://doi.org/10.1186/1746-6148-9-11.

Plaza, P.I., Lambertucci, S.A., 2019. What do we know about lead contamination in wild vultures and condors? A review of decades of research. Sci. Total Environ. 654, 409–417. https://doi.org/10.1016/j.scitotenv.2018.11.099.

Quintana, D.S., 2015. From pre-registration to publication: a non-technical primer for conducting a meta-analysis to synthesize correlational data. Front. Psychol. 6, 1–9. https://doi.org/10.3389/fpsyg.2015.01549.

R Core Team, 2015. R: A Language and Environment for Statistical Computing. Version 3.2.2. R Foundatin for Statistical Computing, Vienna.

Rodriguez-Ramos Fernandez, J., Höfle, U., Mateo, R., Nicolas De Francisco, O., Abbott, R., Acevedo, P., Blanco, J.M., 2011. Assessment of lead exposure in Spanish imperial eagle (*Aquila adalberti*) from spent ammunition in central Spain. Ecotoxicology 20, 670–681. https://doi.org/10.1007/s10646-011-0607-3.

Sánchez-Zapata, J.A., Eguía, S., Blázquez, M., Moleón, M., Botella, F., 2010. Unexpected role of ungulate carcasses in the diet of golden eagles *Aquila chrysaetos* in Mediterranean mountains. Bird Study 57, 352–360. https://doi.org/10.1080/00063651003674946.

Selva, N., Jędrzejewska, B., Jędrzejewski, W., Wajrak, A., 2005. Factors affecting carcass use by a guild of scavengers in European temperate woodland. Can. J. Zool. 83, 1590–1601. https://doi.org/10.1139/z05-158.

Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. J. Stat. Softw. 36, 1–48. https://doi.org/10.18637/jss.v036.i03.

Viechtbauer, W., 2019. The metafor package: a meta-analysis package for R. http://www.metafor-project.org/.

Viechtbauer, W., Cheung, M.W.L., 2010. Outlier and influence diagnostics for meta-analysis. Res. Synth. Meth. 10, 112–125. https://doi.org/10.1002/jrsm.11.

Walsh, P.M., 1990. The use of seabirds as monitors of heavy metals in the marine environment. In: Furness, R.W., Rainbow, P.S. (Eds.), Heavy Metals in the Marine Environment. CRC Press, Boca Raton, FL, pp. 183–204.

Zaccaroni, A., Andreani, G., Ferrante, M.C., Carpenè, E., Isani, G., Lucisano, A., 2008. Metal concentrations in the liver and kidney of raptor species from the Calabria region, Italy. Acta Vet. Brno. 58, 315–324. https://doi.org/10.2298/avb0804315z.