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Major lead exposure from hunting ammunition in eagles from Sweden

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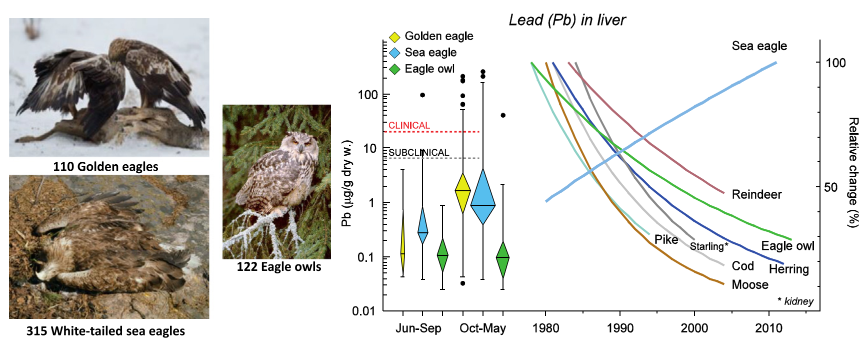
HIGHLIGHTS

- We studied distributions of liver Pb levels in sea eagle, golden eagle & eagle owl.
- Pb in scavenging eagles but not eagle owl correlate with exposure to spent ammunition.
- Lethal poisoning occurred in 15% of sea eagles, 7% golden eagles and 0.8% eagle owls.
- High-exposure areas had 24% lethality and Pb increased over 30 yrs. in sea eagle liver.
- An estimated new threshold for background Pb levels was exceeded by 81% of sea eagles.

GRAPHICAL ABSTRACT



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ABSTRACT

Exposure to lead (Pb) from ammunition in scavenging and raptorial birds has achieved worldwide recognition based on incidences of lethal poisoning, but exposure implies also sublethal levels with potential harmful effects. Background and elevated Pb levels in liver from 116 golden eagles (GE, *Aquila chrysaetos*) and 200 white-tailed sea eagles (WTSE, *Haliaeetus albicilla*) from Sweden 2003–2011 are here examined, with supporting data from a previous WTSE report and eagle owl (EO, *Bubu bubo*) report. GE and WTSE display seasonal patterns, with no Pb level exceeding a generally accepted threshold for subclinical effects during summer but strongly elevated levels from October. Fledged juveniles show significantly lower levels than all other age classes, but reach levels found in older birds in autumn after the start of hunting seasons. Pb levels in EO (non-scavenger) show no seasonal changes and indicate no influence from ammunition, and are close to levels observed in juvenile eagles before October. In all, 15% WTSE and 7% GE were lethally poisoned. In areas with high-exposure to hunting ammunition, 24% of WTSE showed lethal Pb levels, compared to 7% in both eagle species from low-exposure areas. Lethal poisoning of WTSE remained as frequent after (15%) as before (13%) a partial ban on use of Pb-based shotgun ammunition over shallow waters (2002). Pb levels increased significantly in WTSE 1981–2011, in contrast to other biota from the same period. A significant decrease of Pb in WTSE liver occurred below a threshold at 0.25 µg/g (dry weight), exceeded by 81% of the birds. Trend patterns in Pb isotope ratios lend further support to this estimated cut-off level for environmental background concentrations. Pb from spent ammunition affects a range of scavenging and predatory species. A shift to Pb-free ammunition to save wildlife from unnecessary harm is an important environmental and ethical issue.

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1. Introduction

Lead (Pb) is a naturally occurring, but relatively rare, trace element, occurring in varying concentrations in the Earth's crust and extracted for many uses since ancient time. Background Pb levels in biota are mainly a result of various emissions from human mining and smelting activities and use of Pb in products including gasoline with added Pb over many decades (Krone, 2018). Pb exposure in predatory and scavenging birds comes from a range of anthropogenic sources, including ammunition (Fisher et al., 2006; Behmke et al., 2015; Katzner et al., 2018). Pb is highly toxic and effects from ingestion of Pb by humans and wildlife draws much attention to this day. Observations recorded in humans, even at low levels of Pb exposure, span from effects on the brain with detrimental behavioral changes and lowered IQ in children to anemia, hypertension, immuno-suppression, renal and reproductive impairment. High exposure levels cause severe effects on the central nervous system leading to coma, convulsions and death (WHO, 2016). Moderate exposure of Pb in birds results in signs of sublethal effects over a wide range (summarized by Krone, 2018; Pain et al., 2019). Bald eagles (*Haliaeetus leucocephalus*) experimentally dosed with ten Pb shotgun pellets showed a range of escalating changes in blood chemistry parameters, with fatal results within 12–20 days (Hoffman et al., 1981; Pattee et al., 1981). This frequently occurs through direct ingestion of Pb-based shotgun pellets in birds that use grit in their digestive system, as waterfowl and granivorous species (Pain et al., 2019), but also from indirect ingestion of Pb-based shotgun pellets and bullet fragments in conjunction with food intake in a range of predatory and scavenging animals (e.g. Monclús et al., 2020, Mctee et al., 2019). Scavengers readily utilize discarded parts from hunted game with fragments of Pb-based ammunition, as well as carcasses of unretrieved shot animals. Game species hunted with Pb-based shotgun ammunition often contain pellets in their flesh when non-lethally hit (Kendall et al., 1996; Mateo et al., 2014; Pain et al., 2015). Pb-based shotgun pellets and bullet fragments ingested with meat from carcasses, offal and wounded prey end up in the bird's gizzard where it gradually dissolves and is distributed via the intestines to the blood and further to soft tissues (kidney, liver) and bone tissue where Pb is stored (summarized in Krone, 2018). Evidence of exposure and poisoning from ingestion of spent ammunition in free-ranging predatory and scavenging species exists from North America (e.g. Elliott et al., 1992; Kramer and Redig, 1997; Clark and Scheuhammer, 2003; Church et al., 2006; Craighead and Bedrosian, 2009; Johnson et al., 2013; Franson and Russel, 2014; Katzner et al., 2018; Behmke et al., 2015; Slabe, 2019; Simon et al., 2020), and from Europe (e.g. Krone et al., 2002, 2006, 2009a, 2009b; Kenntner et al., 2004, 2007; Helander et al., 2009; Berny et al., 2015; Jenni et al., 2015; Madry et al., 2015; Ecke et al., 2017; Kitowski et al., 2017; Gil-Sánchez et al., 2018; Isomursu et al., 2018; Bassi et al., 2021; Fuchs et al., 2021) as well as from other continents (e.g. Ueta and McGrady, 2000; Krone et al., 2004; Saito, 2009; Garbett et al., 2017; Ishii et al., 2017; Lohr et al., 2020; Pay, 2020).

In Sweden, white-tailed sea eagle (WTSE) *Haliaeetus albicilla* and golden eagle (GE) *Aquila chrysaetos* are facultative scavengers known to be readily attracted to carrion - a strong feature that was extensively explored for culling in old times (e.g. von Greiff, 1828; von Wright, 1833; Hahr, 1868). It was even suggested that eagles use the sense of smell to find carcasses that they could not possibly see (Wheelwright, 1860), but this has been proven only for the turkey vulture *Cathartes aura* (Grigg et al., 2017). A recent study demonstrated the effectiveness of scavenging raptors (Mctee et al., 2019). The scavenging habit of eagles has also been explored for management purposes with supplemental feeding (Helander, 1978, 1985; Hario, 1981). Surveillance at such feeding stations in the USA (Watson et al., 2019) and in Sweden (unpubl.) shows that eagles return to feed as long as food is available and thereby reduce energy expenditure for active hunting. Eagles are also selective hunters with a keen eye for aberrant behavior and are thus prone to go after crippled prey, including specimens wounded by ammunition. These features make WTSE and GE vulnerable to poisoning from ingestion of Pb-based ammunition fragments in discarded meat from hunted game, crippled prey and specimens shot dead but not retrieved. A comprehensive review of papers on Pb in 39 raptor species in Europe reported clearly higher concentrations in scavenging species (Monclús et al., 2020).

Total Pb has declined in a variety of biological and environmental matrices, primarily as result of reductions of Pb as additive to gasoline. In Sweden, voluntary initiatives to reduce Pb in gasoline commenced during the late 1970s. A full ban on Pb in gasoline was enforced from 1994. Pb levels in mosses have shown a marked reduction of 96% in Sweden between 1975 and 2015 (Danielsson and Karlsson, 2016) and 82% in Europe at large since 1990 (Frontasyeva et al., 2020). Mean annual decrease rates of Pb in the livers of Baltic Sea herring (*Clupea harengus*) and cod (*Gadus morrhua*) 1980–2013 were around 5% (Bignert et al., 2014) and 4.3% in freshwater pike (*Esox lucius*) 1968–2013 (Lind et al., 2006). In mammal and bird species, mean annual decrease in liver Pb rates reported by Lind et al. (2006) were 3.5% for reindeer (*Rangifer tarandus* 1983–2003), 8.8% for moose (*Alces alces* 1980–2003), and 6.2–12% in kidney of starlings (*Sturnus vulgaris* 1982–1999). The mean annual decrease rate in eagle owl (*Bubo bubo*) 1978–2013 was 5.3% (Helander et al., 2019).

An earlier study based on 118 WTSE in Sweden 1981–2004 showed clear evidence of poisoning by Pb from spent ammunition and Pb concentrations in eagle liver did not decrease over time in response to the phase-out of leaded gasoline (Helander et al., 2009). This stands in sharp contrast to the declines of Pb in a multitude of other free-ranging animals in Sweden. Tissue levels of Pb that exceed accepted thresholds associated with spent hunting ammunition are easily distinguished (Franson and Pain, 2011), but lower levels can also involve ingested ammunition remains. Although radiogenic Pb isotope ratios can be indicative of ammunition sources (e.g. Church et al., 2006), poorly constrained isotopic end members makes it nearly impossible to directly estimate the total contribution of Pb from ammunition in wildlife tissues. Here we propose a method to estimate the total contribution of ammunition Pb indirectly using patterns of temporal trends. We hypothesize that eagles that were not (or not recently) subject to ingestion of Pb from ammunition would show a decline of Pb levels at rates comparable with observations in other Swedish biota. Specifically, in such a low-contaminated subsample of the population, under no influence from ammunition, temporal trends in Pb concentrations and Pb isotope ratios should reflect trends observed in a sympatric population of eagle owl (EO), a non-scavenging raptor.

In this study we apply a multiple-indicator approach and explore (I) the incidence of lethal Pb poisoning of GE in Sweden, and in WTSE over nine years after a partial ban on use of Pb-based shotgun ammunition over shallow waters, enforced in 2002; (II) the distributions of liver Pb concentrations and Pb poisoning in WTSE and GE in relation to hunting season and in relation to spatial exposure based on hunting statistics of big game; (III) the overall extent of population exposure to Pb from ammunition, in addition to Pb from "background" sources including emissions of Pb as additive to gasoline. Based on temporal trends of total Pb and Pb isotope ratios, and on seasonal and age-related patterns of Pb in eagles, we aim to estimate an environmental background Pb level for WTSE in Sweden under which there is no influence of ammunition Pb.

2. Material & methods

2.1. Sample composition

Eagles found dead or moribund in Sweden belong to the State and are mandatory to report to the Police for transport to the National Veterinary Institute (SVA) and the Swedish Museum of Natural History (NRM) for postmortem investigation and archiving of samples. The time of death is often not attainable for this kind of material. Speculative estimations of dates for death were avoided and seasonal distributions were based strictly on the finding dates of individual specimens. Organ samples taken from all fresh carcasses are stored at −25 °C in National Specimen Banks at NRM and SVA. Livers of 200 full-grown WTSE and 110 GE collected in Sweden 2003–2011 (Table A1) were available for Pb analysis. To study long-term trends, 115 WTSE from 1981 to 2004 (previously analyzed and reported in Helander et al., 2009) and 122 EO from 1978 to 2013 (previously analyzed and reported in Helander et al., 2019) were included. Liver samples from three WTSE and six GE nestlings were added for comparisons of Pb levels. Supporting information on Pb concentration in kidney was available in some cases (data from Helander et al., 2009, and from some specimens analyzed at the SVA). The distribution of WTSE samples in Table A1 reflects a population increase over the longer sampling period for WTSE (not applicable for the GE sample). Sex was determined from measurements before dissection (total length, wing cord, tarsus width) and during necropsy from inspection of the reproductive organs. Ages were clear for 184 WTSE and 36 GE banded as nestlings. Ages of non-ringed WTSE specimens were determined from plumage characteristics and molt patterns from 1st through 5th calendar-year-of-life (Cyl) (Helander et al., 1989) and were classified as 6+ thereafter. Non-ringed GE of ages after the 1st and 2nd Cyl were classified as either subadult (when in transitional plumages) or adult (6+) (Tjernberg, 1988; Forsman, 2016).

To compare for a temporal change in Pb isotope ratios, archived blood samples of WTSE nestlings from 15 nest sites 1995–1997 and from the same sites 2012–2014 were analyzed. Nestlings were 4–8 weeks old at sampling that took place in connection with annual surveys of the breeding population within a national monitoring program (Helander et al., 2008). The skin over the brachial vein was wetted and cleaned with sterile alcohol swabs and blood samples drawn from the vein using a 10 ml plastic syringe and 25 × 0.6 mm needle. The blood samples were transferred to sterile 5 ml plastic test tubes, kept on ice in a cooler in the field and transported in a liquid nitrogen vessel to the Swedish Environmental Specimen Bank, and kept at −80 °C thereafter. We used whole blood for Pb analysis. The blood samples were collected with appropriate permits from the Swedish Board of Agriculture (licenses no. 35-4927/02, 31-302-08) and from the Ethical Review Committee under the Swedish Animal Welfare Agency (licenses no. N262/02, N104/06, N157/08).

2.2. Necropsy

Eagle carcasses were inspected visually for assessment of the cause of death, and subsequently weighed, measured, radiographed and finally opened. Traumatic causes of death are often indicated in notes on the site and circumstances at the site of discovery (collision with vehicles and wires, burn-marks from electrocution, entanglements, drowning, wounds from intraspecific fights etc.) and from radiographs. When identified in radiographs, visual inspection during dissections confirmed the presence of pellets from a shotgun shell and fragments of bullets from rifle ammunition. All metallic particles collected from the gastro-intestinal tract of the eagles during necropsy were identified visually as shotgun pellets or bullet fragments. Ammunition remains retrieved from individual eagles (20 samples of shotgun pellets and one bullet fragment) were thoroughly cleaned and saved for subsequent chemical analyses to verify if made of Pb and for Pb isotope analysis. Necropsy was performed at the SVA following the standard operating procedures of the institute. A subsample of 39 WTSE necropsies were performed at the Leibniz Institute for Zoo and Wildlife Research (IZW) in Berlin, Germany.

2.3. Preparation & chemical analyses

Sample preparation and analysis was performed in LAF benches in a room with HEPA-filtered incoming air by an accredited laboratory (ITM/ACES, Stockholm University). Subsamples of 0.5–1 g liver or 1–5 ml of blood were freeze-dried and prepared for analysis by microwave-assisted digestion in closed vessels with freshly distilled nitric acid and hydrogen peroxide (10:1). Blanks and internal control samples, including a certified reference material (DOLT-3, Dogfish liver, National Research Council, Canada), were appended to every digestion batch. The 21 samples of ammunition retrieved from individual eagles were dissolved in distilled nitric acid. After dilution with Milli-Q water, Pb concentrations and radiogenic Pb isotope ratios were determined by ICP-MS (Inductively Coupled Plasma Mass Spectrometry, Thermo X Series II).

Total Pb concentrations were measured as the sum of ^{206}Pb, ^{207}Pb and ^{208}Pb (50 sweeps of 20 ms dwell time per mass, three replicates) with rhenium as internal standard. Our mean value for DOLT-3 (0.30 ± 0.047 μg/g) compared well with the certified value (0.32 ± 0.050 μg Pb g^{-1}). Duplicate samples showed a mean percentage deviation of 0.99 ± 1.1 ($n = 13$). Pb concentrations are presented as μg/g on a dry weight (dw) basis. All samples had Pb concentrations above LOD = 0.004 μg/g.

The radiogenic Pb isotope ratios ^{206}Pb/^{207}Pb and ^{208}Pb/^{207}Pb were determined by ICP-MS in the same digested samples as for total lead, but in separate runs with optimized settings (100 ms of dwell time at 50 sweeps for ^{206,207}Pb and at 25 sweeps for ^{208}Pb). Based on measured total Pb concentrations, the digested samples were divided into 14 groups that were run in sequence from low to high Pb concentrations. Sample runs were interspersed with blanks and DOLT-3 (control for total lead). Samples were also bracketed with different dilutions of a common lead isotopic standard (NIST 981, National Institute of Standards and Technology, USA) matching the Pb concentration range of the sample groups. The NIST 981 solutions ranged from 0.12 to 30 ppb and samples with higher concentrations were diluted to about 30 ppb to avoid significant dead-time effects by allowing the detector to work below the point of switching to analog mode for ^{208}Pb. The acid-dissolved ammunition samples were also diluted to 30 ppb and bracketed with NIST 981 of the same concentration. Measured and certified Pb isotope ratios of NIST 981 were used for mass bias correction of the isotope ratios of the samples. A constant correction factor of 1.0048 was applied to ^{206}Pb/^{207}Pb that did not change significantly with Pb concentration, whereas a correction factor based on linear regression ($0.9954–1.045 \times 10^{-12} \times$ ^{208}Pb counts per second) was used for ^{208}Pb/^{207}Pb because of a significant increase with Pb concentration. The precision (RSD %) of NIST 981 isotopic ratios was 0.24% for ^{206}Pb/^{207}Pb and 0.25% for ^{208}Pb/^{207}Pb ($n = 41$). The precision was somewhat lower for digested liver samples than for the pure lead reference NIST 981. Estimated isotope ratios for DOLT-3 were 1.1394 ± 0.76% and 2.4075 ± 0.61% for ^{206}Pb/^{207}Pb and ^{208}Pb/^{207}Pb, respectively (mean ± RSD; $n = 13$), although there are no certified Pb isotope ratios for DOLT-3 for comparison. The precision for duplicate eagle liver samples was 0.72% for ^{206}Pb/^{207}Pb and 0.85% for ^{208}Pb/^{207}Pb (n = 13).

2.4. Handling of data

2.4.1. Statistical analysis

Temporal trends in Pb concentrations were checked for significance using OLS log-linear regression analysis. Before the regression analysis, the data was checked for potential influence from confounding factors including year, month of death, latitude, sex, age, total weight and percentage dry matter of the liver samples, by multiple regression analyses (Kleinbaum et al., 2008). The seasonal effect on Pb-concentrations is best described by a second-degree polynomial function, and the squared month was therefore included in the multiple regression model (Fig. A1). In a few cases, indicated in the text, the non-parametric Mann-Kendal trend test was used to avoid unbalanced influence from extreme values with high leverage effect. Differences between groups were tested with Mann-Whitney *U* test (MWU). For frequency distributions and contingency table analysis, the G-test with William's correction was applied (Sokal and Rohlf, 1995). The statistical tests were carried out using the software package PIA (Bignert, 2013). All tests were two-tailed and a significance level of 5% was applied; NS denotes non-significant.

2.4.2. Thresholds

Threshold levels in liver for classified Pb-effects as recommended by Franson and Pain (2011) for *Falconiformes* were applied, converted from a wet weight (ww) basis to a dry weight (dw) basis to control for variation in moisture content in dead specimens retrieved from the field (Adrian and Stevens, 1979). A conversion factor of 3.3 was used based on the mean value of dry matter content measured in our eagle liver samples (see Results below), yielding an upper threshold of 6.6 μg/g dw for suggested no-clinical-effect concentrations, 6.6–20 to indicate for subclinical effects, 20–33 for clinical poisoning and >33 μg/g for severe clinical poisoning.

3. Results

3.1. Spatial, sex and age relationships with Pb in eagles

Territorial (adult) WTSE and GE are mainly sedentary year-round, except for WTSE from northernmost Sweden that may stray as waters freeze in winter. Thousands of re-sightings of ringed birds show that immature as well as adult birds of both species reside mainly within Sweden year-round (unpublished material). The relative spatial density of collected samples reflect the core breeding areas for each species during the period of study (Fig. 1). The abundance of WTSE is higher along the eastern coast (counties B, C, D, E, H, X) and in the inland of Lapland (BD), and breeding GE are mainly spread inland from north of counties S, T, U, C, and on the island of Gotland (county I) and the county of Skåne (M) in the south. Aggregations of finds along lines in BD, X and C in Fig. 1 mainly illustrates railway kills.

There was no significant difference in Pb levels between the species or between sexes (Fig. A2). However, a bimodal distribution of concentrations appears in male WTSE. Pb concentrations >20 μg/g in males and females, respectively, were 4.9% vs 7.8% in GE and 10.9% vs 15.5% in WTSE (NS). Juvenile WTSE in their 1st calendar year of life (Cyl) and retrieved during July–September show a mean concentration of 0.224 μg/g ($n = 11$, Table 1), compared to 0.817 μg/g for older WTSE during this season ($n = 33$, excluding one bird with Pb > 6.6 μg/g; $p < 0.004$, MWU). In October through December, juveniles (1st Cyl) have attained about the same mean, median and range in Pb concentrations as observed in all older age classes. Thus, no apparent change in Pb concentrations (at below <6.6 μg/g) with increasing age is observed in eagles, including juveniles retrieved after September and adults of known ages of up to 30 years. Excluding juveniles retrieved during July–September, the range of median liver Pb per age class in the population segments with Pb < 6.6 μg/g was 0.47–1.3 μg/g in WTSE and 0.47–1.8 μg/g in GE (Table 1; Fig. A3). Variations in mean Pb levels between year classes are due mainly to random occurrence of Pb-poisoned specimens, including some extremes, with no apparent change with increasing age (Fig. A3). A very similar age-related seasonal pattern of Pb-levels occurs in GE (Table 1). In total, 20% of the 150 adult WTSE were Pb-poisoned, versus 10% among 94 juveniles and 68 immatures ($p < 0.012$). The age distribution among Pb poisoned GE ($n = 8$) was 3 juvenile, 2 subadult and 3 adult birds.

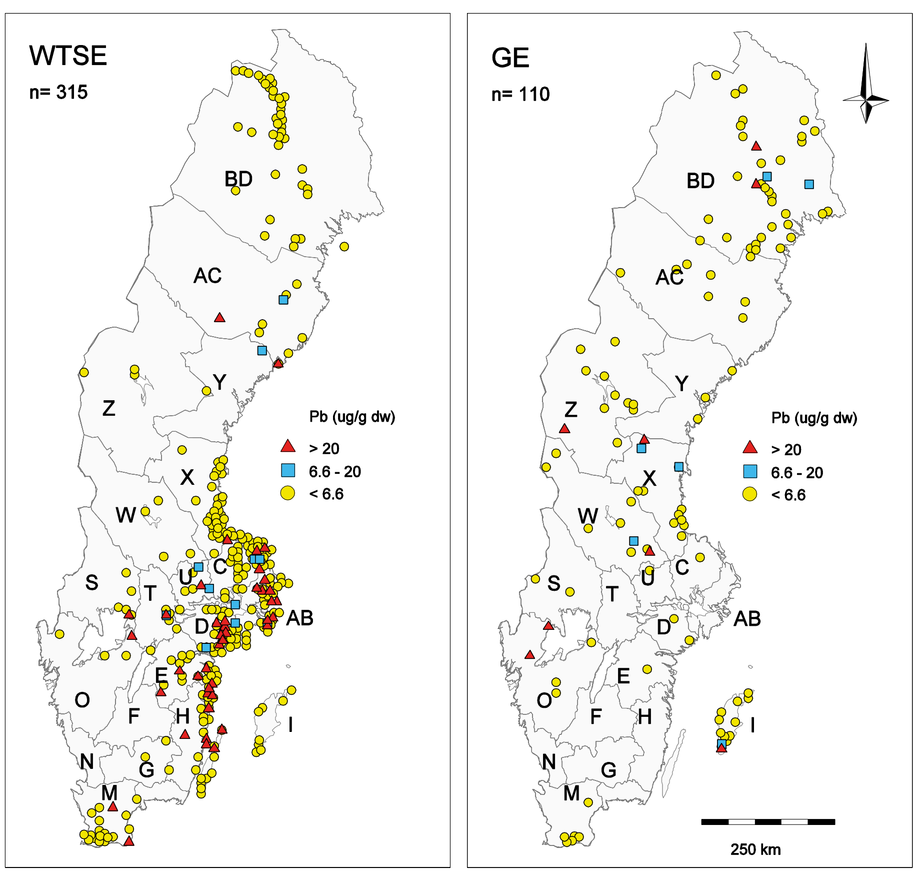

Fig. 1. Spatial distribution of WTSE (1981–2011) and GE (2003−2011) liver samples from specimens found dead and collected for necropsy and analyses. Symbols indicate individual liver Pb within three concentration intervals suggested to correspond to the degree of Pb poisoning (see text). Letters indicate county codes: AB = Stockholm; C = Uppsala; D = Södermanland; E = Östergötland; F = Jönköping; G = Kronoberg; H = Kalmar; I = Gotland; K = Blekinge; M = Skåne; N = Halland; O = Västra Götaland; S = Värmland; T = Örebro; U = Västmanland; W = Dalarna; X = Gävleborg; Y = Västernorrland; Z = Jämtland; AC = Västerbotten; BD = Norrbotten.

Table 1
Age and liver Pb distributions of eagles, including 244 white-tailed sea eagles (WTSE) and 66 golden eagles (GE) with known calendar year-of-life (Cyl) and subadults and adults with unknown Cyl. First-year birds subdivided into nestlings and fledged juveniles, retrieved before and after October, respectively. Pb concentrations (μg/g dw) are divided into three intervals based on published threshold values adapted after Franson and Pain (2011): <6.6 (no clinical effects), 6.6–20 (subclinical effects) and > 20 μg/g (clinical poisoning).

Eagle age		No clinical effects <6.6 μg Pb/g				Subclinical effects 6.6–20 μg Pb/g		Clinical poisoning >20 μg Pb/g		All Pb levels		
Age class	Cyl	n	Mean	Median	Range	n	Range	n	Range	n	Mean	Median
WTSE												
juv Jun (nestlings)	1	3	0.133	0.168	0.06–0.17					3	0.133	0.168
juv Jul-Sep	1	11	0.224	0.164	0.03–0.72					11	0.224	0.163
juv Oct-Dec	1	16	1.490	0.819	0.09–5.70	1	17.2	1	93.8	18	7.492	1.362
immature	2	57	1.137	0.563	0.03–6.54	2	12.7–18.9	6	21.3–74.6	65	5.603	0.770
immature	3	19	1.237	0.540	0.11–6.13	1	9.31	4	60.1–109	24	15.62	0.607
subadult	4	24	1.022	0.615	0.07–5.75			1	23.7	25	2.276	0.663
subad	5	8	0.822	0.601	0.23–2.24	2	8.26–11.0	2	72.8–95.7	12	16.92	1.111
subad/ad	6	9	1.540	1.305	0.47–4.33			3	20.5–72.4	12	11.90	1.688
ad	7	13	1.058	0.689	0.07–4.21	2	8.22–8.66			15	2.043	1.006
ad	8–9	16	1.047	0.843	0.10–2.93	1	12.8	3	35.9–77.8	20	10.24	0.877
ad	10–13	13	0.863	0.465	0.11–4.26			6	44.1–135	19	30.56	0.757
ad	14–19	9	0.908	0.534	0.09–3.19	1	16.9	1	94.2	11	10.84	0.588
ad	20–30	7	1.203	0.473	0.20–3.65			2	23.4–135	9	18.52	0.707
Subtotal		*205*				*10*		*29*		*244*		
imm/subad	2–5	7	0.841	0.214	0.06–4.59					7	0.841	0.214
ad (unspec.)	6+	48	1.216	0.570	0.04–5.35	2	9.62–19.4	14	29.8–256	64	23.04	1.074
Total		*260*				*12*		*43*		*315*		
GE												
juv Jun (nestlings)	1	6	0.067	0.056	0.01–0.14					6	0.067	0.056
juv Jul-Sep	1	3	0.065	0.047	0.03–0.12					3	0.065	0.047
juv Oct-Dec	1	9	1.423	0.567	0.10–6.27					9	1.423	0.567
subad	2	15	1.121	0.622	0.13–6.06			3	64.8–172	18	19.29	0.674
subad	3	5	2.253	1.553	0.35–5.71					5	2.253	1.553
subad	4	4	1.821	1.821	0.84–2.80					4	1.821	1.821
subad	5	6	0.980	0.716	0.24–2.17					6	0.980	0.716
subad/ad	6	4	0.382	0.472	0.03–0.55					4	0.382	0.472
ad	7–12	4	1.472	1.132	0.72–2.90	1	9.28			5	3.033	1.344
ad	13–29	5	2.607	2.607	1.75–3.47	1	7.00			6	1.978	3.468
Sub-total		*61*				*2*		*3*		*66*		
imm/subad	2–5	7	2.005	1.387	0.53–6.36	1	7.45	2	33.3–178	10	23.29	1.735
ad (unspec)	6+	27	2.256	1.981	0.13–5.97	2	9.43–11.0	3	33.7–211	32	12.20	2.372
Total		*95*				*5*		*8*		*108*		

3.2. Remains from ammunition in eagles

All metallic particles collected from the gastro-intestinal tract of the eagles during necropsy were verified as Pb either by chemical analysis or by morphological inspection (considering softness and colour of the material). Among the 300 radiographed WTSE specimens, 44 (15%) contained visible remains of Pb-based ammunition: 15 with Pb present in the gastrointestinal tract (GIT) (10 shotgun pellets, 5 bullet fragments) and 28 with visible embedded pellets (27) or bullet fragments (1) from poaching. One specimen was killed by a bullet through the abdomen without visible fragments. Nine out of 97 radiographed GE (9%) contained visible remains of Pb-based ammunition in their bodies (pellets = 4, bullet fragments = 5), but none in GIT. At least four GE with bullet fragments had died from being shot at. Specimens with pellets or bullet fragments in GIT (only for WTSE) had significantly higher liver Pb concentrations ($p < 0.001$). There was no statistically significant difference in liver Pb levels in eagles with or without visible remains from ammunitions in body outside GIT (Fig. 2).

3.3. Cause of death and Pb distributions

The most frequent cause of death in this sample of found and retrieved carcasses of WTSE and GE is collisions, constituting nearly half of all cases in both species (Table A2; Fig. 3). One third of all cases were railway kills, attracted to the location by carcasses of other collision casualties (moose, reindeer etc). The high occurrence of railway kills is due to (1) an obligation placed on train drivers to report all collisions for removal from the railway, and (2) an obligation to bring in specimens of all species that belong to the State (which includes eagles). Pb poisoning is the second most commonly recorded death cause in WTSE (15%), followed by other trauma (12%), collision with power lines (9%), electrocution (5%) and starvation (5%). Three WTSE diagnosed as Pb poisoned based on necropsy had liver Pb concentrations slightly below the suggested threshold of 20 μg/g for poisoning. Among GE, 7% had lethal levels of Pb. Other more common causes of death for GE were starvation (12%), electrocutions (11%) and other trauma (12%). The majority of Pb poisoned eagles had liver Pb above the threshold of 33 μg/g for severe clinical poisoning (Fig. 3). There is no apparent correlation between Pb level and the cause of death besides lethal poisoning, but 12 out of 15 specimens with concentrations in the subclinical range (6.6–20 μg/g)) died in collisions and other trauma-related incidents. Range, median and mean Pb concentrations per cause of death summarized in Table A2.

Sex and age distribution of liver Pb concentrations are summarized in Table A3. Nine WTSE (3%) and six GE (5%) had concentrations of 6.6–17 μg/g indicative of possible subclinical effects (Fig. 3). WTSE that died from starvation, infection and Pb poisoning had weight ranges of 2.17–4.23 kg (male) and 2.80–5.09 kg (female), whereas birds that died from other causes (mainly collisions and other trauma) weighed 2.89–6.15 kg (male) and 3.22–7.03 kg (female). GE showed similar distributions: males and females dying from starvation, infection and Pb poisoning weighed 1.80–2.50 kg and 2.37–4.80 kg, respectively, and from other causes 2.38–5.30 and 3.24–7.05 kg, respectively (Table A4). WTSE body weight (adjusted for sex) decreases significantly ($p < 0.001$) with liver Pb concentrations (Fig. A4).

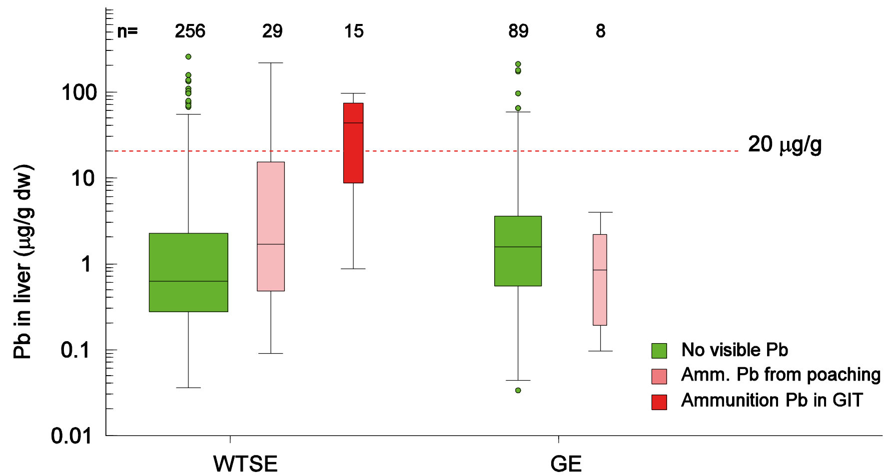

Fig. 2. Box and whisker plots of liver Pb concentrations of radiographed WTSE and GE, with no visible Pb fragments in body (left, green), ammunition Pb in body from poaching (middle, pink) and ingested shotgun pellets or bullet fragments in gastrointestinal tract (GIT, red). Sample sizes in upper row. The dashed red line indicates a threshold for clinical effects.

3.4. Lead in eagles after partial ban of Pb in shotgun ammunition

In order to save waterfowl from ingestion of Pb-based shotgun pellets (as grit), a ban of Pb-based shotgun ammunition over shallow wetlands was enforced in Sweden from 1 July 2002. Pellets embedded in tissues of prey as well as shotgun pellets ingested as grit by prey, such as ducks, are sources of Pb in eagles (Tavecchia et al., 2001; Guillemain et al., 2007; Pain et al., 2015). The date of legislation enforcement was close to the start of the hunting season for ducks and it appears reasonable to allow for some lag in adaptation to the new rules, so eagles from 2002 is here included in the "pre-ban" sample (Fig. A5A). Thirteen percent of WTSE had liver Pb levels exceeding ca 20 μg/g in 1981–2002 ($n = 92$) compared to 15.2% in 2003–2011 ($n = 223$, NS). The distribution among concentration intervals differs between WTSE and GE in the post-ban period: lethal poisoning was significantly more common in WTSE than in GE (15.2% vs 7.3%, $p < 0.033$; Fig. A5A), whereas concentrations within the no-clinical-effects range (<6.6 μg/g) averaged significantly higher in GE ($p < 0.002$; Fig. A5B).

3.5. Seasonal variation

There is a clear seasonal pattern of liver Pb levels in both eagle species (Fig. 4). All Pb concentrations exceeding a threshold at 6.6 μg/g for subclinical effects occur from Oct–May in WTSE, with a single acute poisoning found also in early June, and in GE from Nov–May (Fig. 4). There is a time lag with respect to the onset of hunting seasons: Pb concentrations in eagle livers rise sharply in October, with monthly geometric means of 1–4 μg/g during Oct–May. There is also an apparent, prolonged lag in response to the ending of most hunting seasons; geometric mean concentrations decline month by month in WTSE from May to August–September. It is noteworthy that the highest Pb concentrations in GE occur in May, with two out of six being Pb-poisoned (>20 μg/g) and no bird with less than 2.45 μg/g Pb in liver (Fig. 4). Pb concentrations

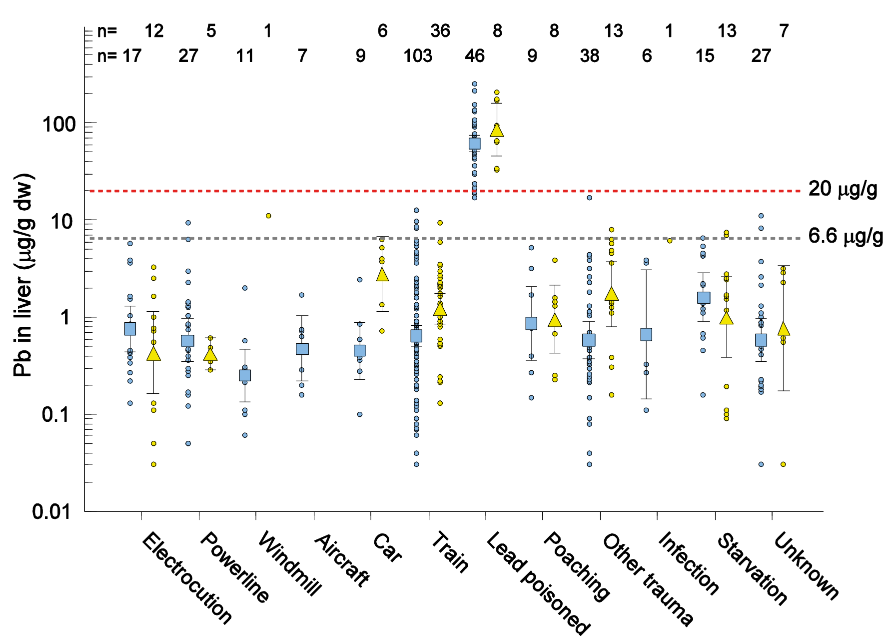

Fig. 3. Geometric mean values with 95% confidence interval for Pb concentrations in liver, distributed by assigned causes of death among WTSE (left, blue; $n = 315$) and GE (right, yellow; $n = 110$). Sample sizes for each cause-of-death category in upper rows. Thresholds for subclinical (6.6 μg/g) and clinical (20 μg/g) effects indicated with dashed lines.

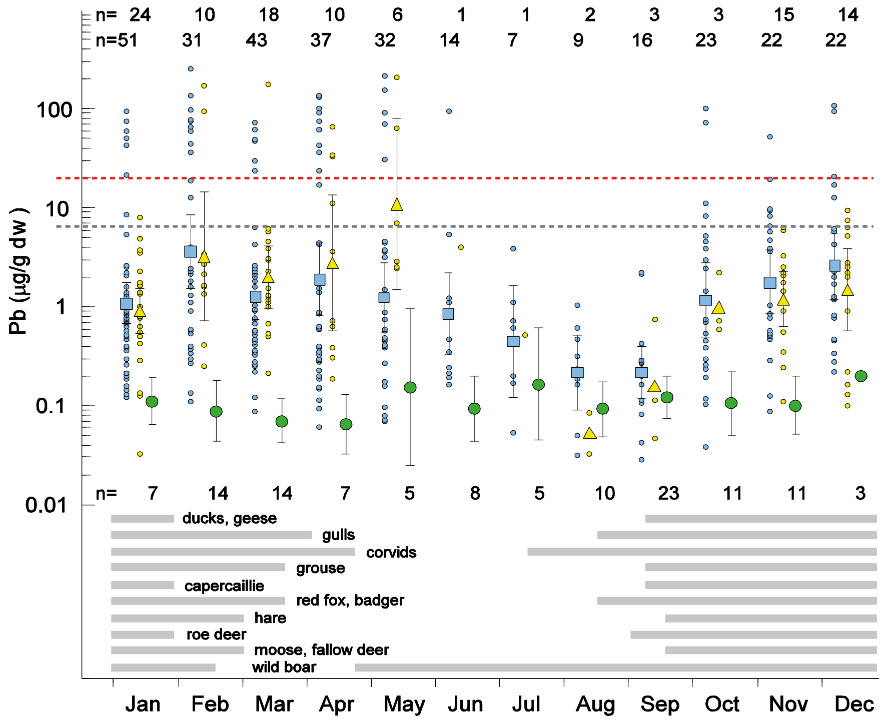

Fig. 4. Seasonal distributions of liver Pb levels in WTSE, GE and EO in relation to potential exposure to hunting ammunition. Concentrations per specimen (small circles) and geometric mean values (±95% CI, large symbols) of Pb concentrations in liver for each month in WTSE (left, blue squares; $n = 315$), GE (middle, yellow triangles; $n = 110$) and EO (right, green circles, $n = 121$ excluding one extreme value of 40.7 μg/g; individual points not shown; EO data from Helander et al., 2019). Monthly sample size for eagles in upper rows and for EO at the bottom. The dashed black and red lines, respectively, indicate Pb threshold levels at 6.6 μg/g for subclinical effects and 20 μg/g for clinical effects. Horizontal bars illustrate hunting seasons for wildfowl and game species preyed upon or scavenged by WTSE and GE.

in EO liver are included for comparison, with monthly geometric means of ca. 0.07–0.20 μg/g and no apparent seasonal variation throughout the year (Fig. 4).

3.6. Exposure to spent ammunition in big game

If exposure to Pb from spent hunting ammunition is an important cause of the elevated concentrations found in WTSE and GE, a spatial co-variation with hunting intensity would be expected. As an estimate of exposure to Pb from ammunition used for big game (moose, deer and wild boar), we defined an exposure index as the mean number of reported big game shot per year and km^2 unit area during 2004–2011 in Swedish counties (Table A5). This index was compared with the mean liver Pb concentrations in eagles in the same unit area and time period. There is a significant relationship between the exposure index and liver Pb concentrations in WTSE ($p = 0.002$) but not in GE (Fig. 5).

3.7. Temporal trends of Pb in WTSE

3.7.1. Total Pb

There is a statistically significant increase in median WTSE liver Pb levels between 1981 and 2011 ($p < 0.017$, Fig. 6). However, arithmetic as well as geometric mean values show considerable annual variation due to random occurrence of severely Pb poisoned specimens and show no significant trends. Nevertheless, overall Pb in WTSE has clearly not declined in response to the phase-out of Pb in gasoline, in contrast to the declines reported for EO (Helander et al., 2019) and other species in Sweden (Lind et al., 2006; Bignert et al., 2014). The green field in Fig. 6 illustrates an expected scenario in WTSE without an influence from Pb-based ammunition, based on the observed trend in EO with an addition of 0.07 μg/g for WTSE as indicated in Fig. A7B. The overall median (0.739 μg/g) and mean (12.2 μg/g) of total Pb for the study period are seven and 68 times higher, respectively, than the concurrent median (0.103 μg/g) and mean (0.179 μg/g) reported for EO 1978–2013 (Helander et al., 2019).

A significant annual decrease rate of 5.2% occurred in EO 1978–2013 (Helander et al., 2019). It is reasonable to assume that Pb levels in EO reflect an intake of background Pb, including from industrial and fuel emissions. We examined if a declining temporal trend could be observed in WTSE as well, in a population segment with concentrations that could represent eagles that had not, or not recently, been subject to ingestion of Pb from ammunition. WTSE with Pb below 6.6 μg/g (threshold for subclinical effects) rather show an annual increase of 4.6% over the study period, however not significant ($p < 0.074$; Fig. A6). If gradually restricting the total sample (excluding nestlings) by using lower cut-off thresholds (4 μg/g, 2 μg/g, 1.5 μg/g, etc), a statistically significant decrease is first attained at concentrations below 0.25 μg/g ($p < 0.013$, $n = 60$). This sub-sample include birds of all age classes (Table 1), from juveniles up to a known age of 20 years. However, a fixed threshold at 0.25 μg/g is probably not applicable, since lead has decreased in the environment over our 30-year study period. Comparing WTSE with Pb <0.25 μg/g from 1981 to 2011 (n = 60) with EO 1978–2013 ($n = 121$) a faster decline shows in EO, due to a few EO specimens from before 1995 with concentrations up to 0.5 μg/g (Fig. A7A). Applying a Pb threshold of 0.25 μg/g for both species gives nearly parallel trends, with WTSE consistently about 0.07 μg/g higher than in EO over the period of study (Fig. A7B). Differences in prey species and possibly a higher trophic level in WTSE may explain this systematic discrepancy in background concentrations between WTSE and EO. An alternate or additional possibility could be an influence of ingestion of Pb from ammunition even below the threshold found here of 0.25 μg/g for a statistically significant decline to appear. Notably, the observed annual decrease rate of liver Pb concentrations <0.25 μg/g appears slower in WTSE (ca 2%, Fig. A7) compared to the 3.5% to 8.8% as observed in a number of other vertebrates in Sweden within the period of study (Lind et al., 2006; Bignert et al., 2014; Helander et al., 2019).

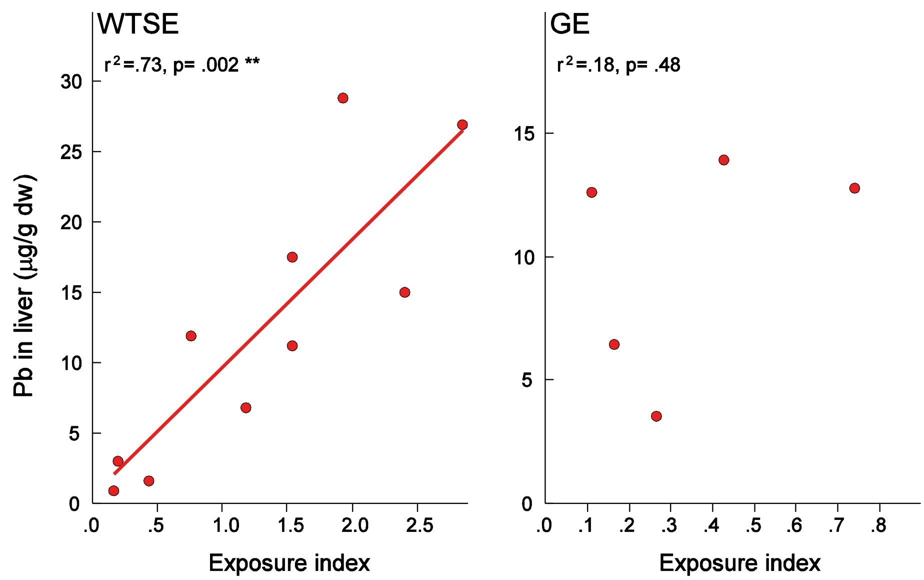

Fig. 5. Pb mean levels in WTSE and GE liver vs. exposure index based on number of shot big game/year/km^2 in Swedish counties during 2004–2011. Note difference in scales. Ordinary least squares regression. For reference, see Table A5. Regional hunting bag statistics from the Wildlife Monitoring Scheme of the Swedish Association for Hunting and Wildlife Management, Stockholm.

We postulate that the yearly mean Pb concentrations measured in EO liver represent an average annual decrease in a predatory bird exposed to Pb from anthropogenic sources including gasoline in addition to natural background, but not to Pb from ammunition. WTSE with Pb concentrations similar to the range in EO should have obtained Pb from the same background sources. Fig. A7C shows WTSE specimens within the same range of Pb concentrations as observed in EO, indicated by the dashed purple line. The dashed line also includes an addition of 0.07 μg/g between EO and WTSE as indicated in Fig. A7B. We can thus estimate an upper range for environmental background Pb concentrations for WTSE to about 0.6 μg/g (dw) at the beginning of the study period and decreasing to about 0.4 μg/g at the end (Fig. A7C).

3.7.2. Pb isotope ratios

The isotope ratios $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ have increased significantly in Sweden during the study period as observed in EO, apparently as a response to the phase-out of Pb in gasoline over the course of our study (Helander et al., 2019). We expect a similar response in other biota, including WTSE, but no significant time trend in Pb isotope ratios could be detected for the full sample from 1981 to 2011. As for total Pb, where the expected temporal change occurred only at low concentrations, the WTSE liver samples were split accordingly to study the temporal trends of Pb isotope ratios: above (I) and below (II) the threshold of 0.25 μg/g. For comparison, also included were samples of WTSE nestling blood (III), and EO liver (IV). The median Pb concentration in the nestling

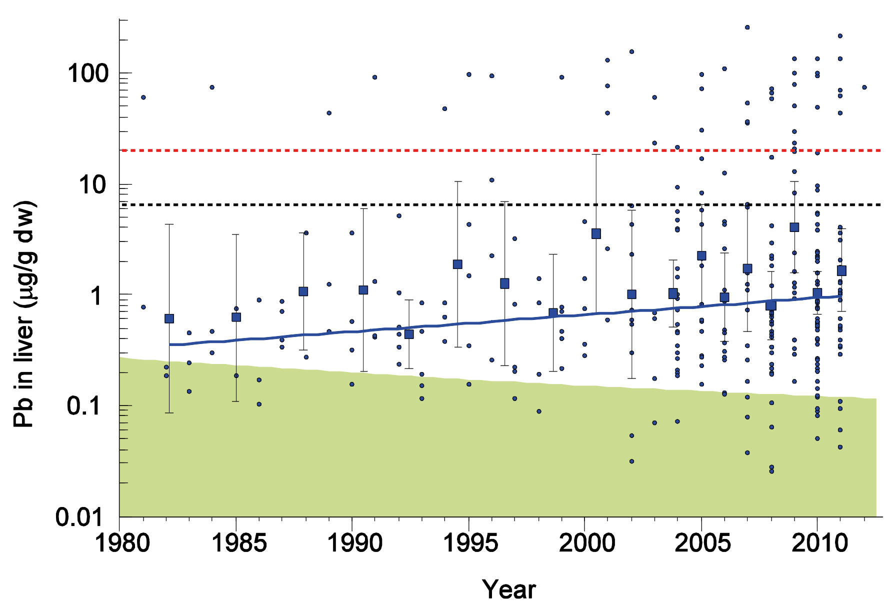

Fig. 6. Total Pb in WTSE 1981-2011 ($n = 315$). Green area at the bottom illustrates the estimated average annual change of contribution from leaded gasoline starting at 0.27 μg/g and decreasing to 0.12 μg/g over the course of 31 years, based on the slope of total Pb in EO (Fig. A7C) but with a constant addition of 0.07 μg/g for WTSE (see Fig. A7B). A minimum of seven observations are required to form a mean (±95% CI, blue squares) and to form medians. The blue line is the Theil-Sen slope for the medians with a significant increase over time ($p = 0.019$, Mann-Kendall trend test). The dashed lines indicate concentration thresholds for subclinical and clinical effects.

blood sample was 0.092 μg/g (corresponding to about 0.020 μg/g on a wet weight basis by a conversion factor of 4.6 according to Scanlon, 1982) and the median Pb level in EO liver was 0.103 μg/g. The studied Pb isotope ratios ^{206}Pb/^{207}Pb and ^{208}Pb/^{207}Pb increased significantly over time and at similar rates in all groups with low Pb levels (Fig. 7): WTSE with Pb < 0.25 μg/g (II; $n = 59$; $^{206/207}$Pb: slope = 0.21% per year, $p < 0.001$; $^{208/207}$Pb: 0.069%, <0.001), WTSE nestling blood (III: $n = 34$; ^{206}Pb/^{207}Pb: 0.092%, 0.047; $^{208/207}$Pb: 0.063%, 0.003), and EO (IV; $n = 122$; $^{206/207}$Pb: 0.13%, <0.001; $^{208/207}$Pb: 0.047%, <0.001). In contrast, there is no significant temporal change for the more contaminated sub-sample of WTSE (I; $n = 253$; $^{206/207}$Pb: 0.039%, 0.103; $^{208/207}$Pb: −0.01%, 0.274).

Pb isotope ratios of ammunition fragments retrieved from 21 individual eagles were very scattered, with median 1.162 and 2.444 for ^{206}Pb/^{207}Pb and ^{208}Pb/^{207}Pb, respectively (Fig. 7). These values agree well with ammunition Pb isotope ratios published by Sjåstad et al. (2014) with medians of 1.152 and 2438 ($n = 338$). Pb isotope ratios of ammunition largely overlap with the range of Pb isotope ratios in liver, especially for the eagles with liver Pb > 0.25 μg/g (Fig. 7).

4. Discussion

4.1. Seasonal and spatial exposure

A plausible explanation for the significantly elevated Pb levels observed in WTSE and GE liver during autumn, winter and spring (Fig. 4) is a response to a seasonal change in exposure to ammunition remains; i.e., shotgun pellets and bullet fragments. Changes in Pb levels in eagles and other raptors that correlate with hunting seasons have also been demonstrated elsewhere (e.g., Elliott et al., 1992; Pain et al., 1997; Bedrosian et al., 2012; Franson and Russel, 2014; Ecke et al., 2017; Garbett et al., 2017; Isomursu et al., 2018; Descalzo et al., 2021). In Tasmania with no seasonal restrictions in hunting, scavenging wedge-tailed eagles (*Aquila audax*) showed elevated Pb levels year-round (Pay, 2020). The significantly lower Pb levels in 1st Cyl juvenile eagles during June–September and their strongly elevated levels from October (Table 1, Fig. 4) indicates a rapid response to exposure of Pb from ammunition after the start of hunting season in both GE and WTSE. Pb levels in EO (Fig. 4) remain in the same range as in 1st Cyl

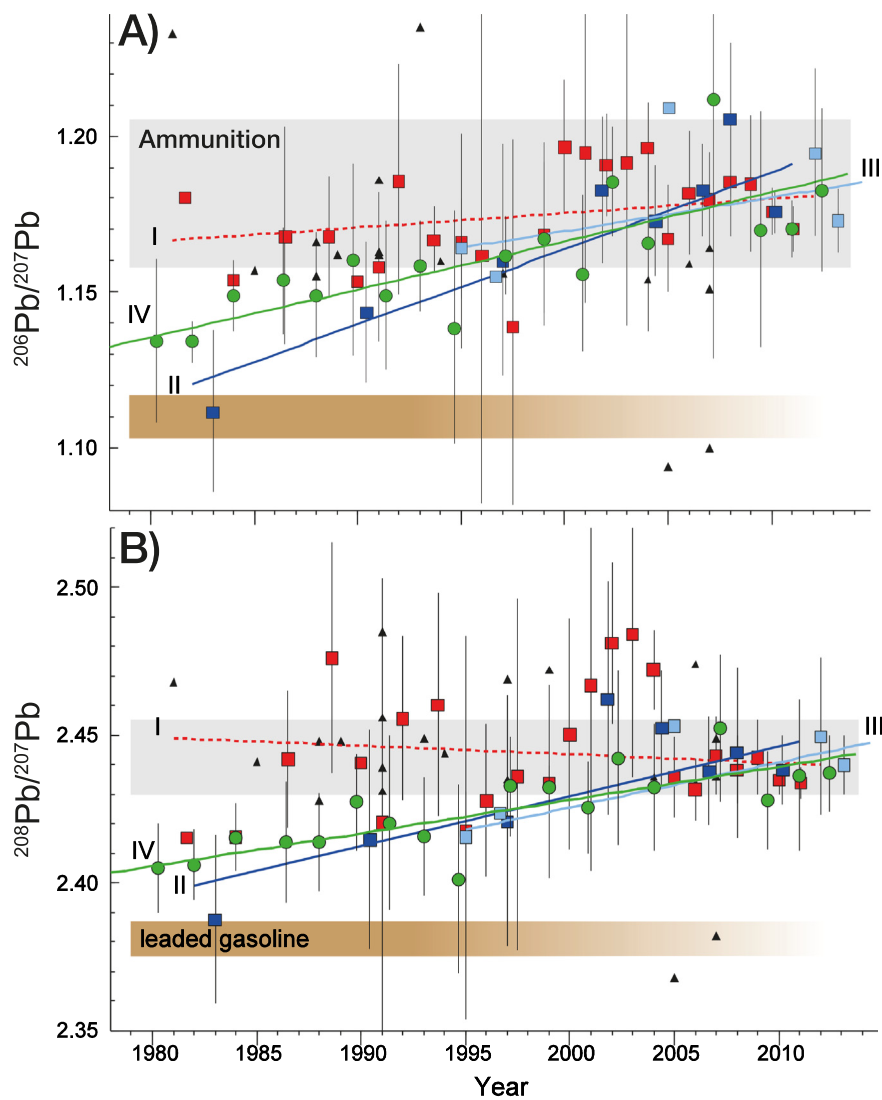

Fig. 7. Temporal trends of radiogenic Pb isotope ratios, ^{206}Pb/^{207}Pb (A) and ^{208}Pb/^{207}Pb (B) in (I) WTSE liver with Pb >0.25 μg/g (red squares); (II) WTSE liver with Pb <0.25 μg/g (dark blue squares); (III) nestling WTSE blood (light blue squares); (IV) EO liver (green circles). Large symbols represent annual mean (±95% CI). Small black triangles represent ammunition fragments found in individual eagles at necropsy and the gray range is the 95% CI of the mean. The brown range indicate isotope ratios (95% CI of the mean) for leaded gasoline in Europe (adapted from Hansmann and Köppel, 2000). See text for trend statistics.

WTSE and GE before October, i.e., before eagles being signifiantly exposed to Pb from spent ammunition for the first time in life. These concentrations should represent a range without an influence from ammunition Pb in Sweden. Already during October–December, juveniles attained about the same mean, median and range of Pb concentrations as observed in all older age classes (Table1, Fig. A3). Hunting seasons for common bird game species (waterfowl, grouse) and roe deer (*Capreolus capreolus*) commence in late August, and in early September for moose in the northern half of Sweden; in the southern half of the country moose hunting starts in mid-October. The lack of a seasonal pattern in liver Pb concentrations in EO (a non-scavenger) stands in sharp contrast to the marked seasonal patterns for eagles (Fig. 4, Table 1). Ecke et al. (2017) showed an almost immediate response of elevated Pb levels in blood from captured, live GE, after the start of small game and moose hunting season in Sweden. Our material shows a time lag of about one month in response to the start of hunting (Fig. 4). The slower response here probably reflects the use of different sample matrices. Blood drawn from live GE during the hunting season represents the day of sampling (Ecke et al., 2017) whereas our material was collected ad hoc upon occurrence of dead eagles in the field. These birds had often been dead for days and sometimes more when found which causes some bias. There is also a natural time lag in the elevation of concentrations after exposure between blood (rapid) and liver. The half-life of Pb in soft tissues such as liver is about 1–3 months (Pain, 1996). This also leads to an apparent lag in the response to decreased exposure in spring in our material (Fig. 4). Vultures and eagles in Spain showed a delayed effect of Pb exposure during the hunting season in liver of dead specimens as well as in blood from captured live birds (Descalzo et al., 2021). There are clear seasonal patterns relating to hunting seasons in our material of eagles, although some exposure to Pb from ammunition will prevail year-round in the presence of wounded survivors from shooting (e.g. Guillemain et al., 2007; Larsen and Nybakk, 2011). WTSE showed a significant positive correlation between liver Pb concentration and regional exposure to harvested big game, while GE did not (Fig. 5). The GE sample represents mainly inland areas with exposure indices within a narrower range (Fig. 5, Table A5). WTSE from inland locations (Fig. 1) show a similar rate of Pb-poisoning as for GE (ca 7%) whereas the rate of Pb-poisoned WTSE from the four coastal counties with the highest exposure indices (Table A5) is 24%. The observed discrepancies in rates of poisoning in our material thus relates largely to different regional exposure. The regional pattern does not coincide with mining activity as most mining in Sweden is located inland.

4.2. Long-term temporal changes

Contrary to a decrease in total Pb as reported for other biota in Sweden over recent decades, we observe a significant increase of median Pb levels in WTSE (Fig. 6). A decrease in Pb occurs for a subsample of 60 specimens with concentrations < 0.25 μg/g representing a population only exposed to background and anthropogenic sources other than ammunition (Fig. A7A). Along with an overall decrease in Pb in other biota following the phase-out of Pb in gasoline, the Pb isotopes have changed accordingly. As for total Pb, expected trends in Pb isotope ratios in WTSE liver can be demonstrated for the low-contaminated liver subsample (<0.25 μg/g) and the likewise low-contaminated blood samples from WTSE nestlings, but not for the larger subsample of more highly contaminated WTSE livers (Fig. 7). Manufacture of Pb-based ammunition includes a range of Pb sources on an international scale (Sjåstad et al., 2014), as well as recycled material. We observe also here a very scattered isotopic composition of the ammunition remains, mainly shotgun pellets, retrieved from found-dead eagles 1981–2007 (Fig. 7). Over time the Pb isotope ratios of WTSE with Pb > 0.25 μg/g do not change, while those of low-contaminated WTSE specimens and EO slowly appear to move away from a clear influence of leaded gasoline in the early years, toward an isotopic composition similar to the majority of the ammunition samples in later years (Fig. 7). However, this does not mean that ammunition has become the main source of Pb for these populations, but rather illustrates that the isotopic composition of ammunition and other sources, including natural, overlap, making it very difficult to define end-members for source apportionment using Pb isotopes. In addition, the analytical precision for isotope ratios obtained with single-quadrupole ICP-MS may be inadequate for source apportionment, as pointed out by Gulson et al. (2018). Nevertheless, here the time series of Pb isotope ratios provide independent and complementary evidence of the influence of ammunition on Pb concentrations in eagles. The consistent temporal patterns among the studied sub-groups, shown for both total Pb and Pb isotope ratios, indicate a common cause. A temporal decrease of Pb in WTSE, as observed for non-scavenging EO, would illustrate an expected development under no influence from ingestion of ammunition Pb during the study period (Fig. A7C). Of the total sample of WTSE livers in this study, 81% exceeded the fixed cut-off level at 0.25 μg/g needed to show an expected temporal decrease of Pb in the material. A majority of 90% of WTSE exceeds the estimated mean level trend curve for environmental background concentrations including Pb from gasoline but not from ammunition (Fig. 6). Instead, applying the purple line in Fig. A7C, for an estimated upper range of concentrations starting at 0.6 μg/g, the proportion exceeding the line is 64%. Whatever approach is applied, these results strongly suggest a major influence from exposure to Pb from ammunition for a majority of the WTSE population. The similar seasonal patterns of exposure for the two eagle species and the higher mean level shown for GE (Fig. A5) indicate a major exposure in GE in Sweden also, as previously shown for GE in the Swiss Alps by Madry et al. (2015).

Our results indicate no effect on the prevalence of lethal poisoning in WTSE over a nine-year period after the enforcement of a partial ban of Pb-based shotgun ammunition. This is surprising, since this was a period when sales of Pb-based shotgun ammunition decreased in Sweden, and when the hunting of most ducks decreased (Bergqvist et al., 2015). Sales of Pb-based shotgun ammunition decreased from 880 metric tons 1995 to 270 metric tons 2005 (KEMI, 2007). These amounts apparently also include Pb-based shotgun ammunition for sport shooting: an estimated amount of Pb entering the environment from hunting in 2005 was 100–160 metric tons (Naturvårdsverket, 2006). Winter-feeding of eagles with flesh from domestic animals, for management purposes, decreased after 2007 and should have contributed to an increased exposure of eagles to Pb from hunting ammunition. The winter-feeding season largely coincides with the hunting season. The number of moose shot in Sweden 1990–2010 averaged around 98,000 per year but tended to decline from 130,000–99,000 over the period (Swedish Association for Hunting and Wildlife Management, 2021). However, the increase in WTSE liver Pb levels coincides in time with a strong increase in the population of wild boar (*Sus scrofa*) in southern and central Sweden. The estimated number of shot wild boar in Sweden increased from ca. 300 in 1990 to ca. 5000 in 2000, 24,000 in 2005, 49,000 in 2008 and 98,000 in 2012 (Bergqvist and Elmhagen, 2016). A strong increase has also occurred in the national number of shot fallow deer (*Dama dama*), from ca 3000 to 24,000 between 1989 and 2011 (Swedish Association for Hunting and Wildlife Management, 2021). According to Stokke et al. (2017), 90% of hunters in Fennoscandia used Pb-based rifle ammunition for moose, and it is reasonable to assume a similar dominance of Pb-based rifle ammunition for wild boar and fallow deer also. This suggests a considerable increase in total exposure of scavengers to Pb-based ammunition in gut-piles from harvested big game shot in southern and central Sweden during the period of study. Our material shows a strong increase of liver Pb levels in WTSE after the 1990s from within the distribution range of wild boar (counties AB, C, D, E, F, G, H, K, L, M, O, T, U, W; Fig. 1) but no increase in counties without wild boar during the study period (Fig. 8). An increased exposure of Pb from hunting of wild boar may have overshadowed an anticipated decrease in Pb exposure following the partial ban of Pb-based shotgun ammunition enforced in 2002, although an extensive survey in Sweden also showed low compliance with this legislation and low consent to voluntary use of Pb-free ammunition (Widemo, 2021).

4.3. Frequency distibutions

4.3.1. Occurrence of spent ammunition in eagles

Shotgun pellets were twice as frequent in GIT compared to bullet fragments. This shows that ingestion of shot from crippled or dead prey specimens is frequent in WTSE, but is not evidence that poisoning from shotgun pellets was more common than from bullet fragments. Numerous small Pb fragments from a bullet will dissolve faster in GIT than a corresponding amount of Pb contained in a shotgun pellet. Ingested pellets and bullet fragments erode and dissolve under the influence of active digestion of food in the gizzard and its very acidic environment (pH 1.2–1.4; summarized in Krone, 2018). Scavengers have evolved extremely high gastric acidity to protect from pathogens in carcasses (Beasley et al., 2015). Thus, the rate of absorption must vary considerably depending on the amount, size and structure of ingested Pb particles. The majority (78%) of lethally Pb-poisoned specimens (>20 μg/g) showed no visible remains of solid Pb in GIT (Fig. 2). Similarly, 85% of Pb poisoned, dead bald eagles and golden eagles lacked visible remains of Pb ammunition (Franson and Russel, 2014) and only one bird among 138 Pb poisoned live eagles in the USA had radiographic evidence of Pb pellets in the ventriculus (Kramer and Redig, 1997). Here, ten out of 15 WTSE with pellets or bullet fragments in the GIT were lethally poisoned. Among the remaining five birds with ammunition Pb in GIT, two had elevated levels (8–10 μg/g) and three had 0.86, 1.3 and 1.3 μg Pb/g in liver, respectively. For one of those with 1.3 μg/g, there is also a recorded kidney concentration of 12 μg/g. Likely the Pb absorption had just started in those birds with low liver values.

4.3.2. Pb levels, poisoning and age

Repeated small intakes of Pb during the lifespan of an individual will typically result in elevated levels in the skeleton over time, whereas repeated ingestion over a short time will lead to elevated levels in soft organs as well (summarized in Krone, 2018). The data presented in Table 1 and Fig. A3 indicate no increase of liver Pb levels with age after the 1st year of life. Mean concentrations below a subclinical threshold (<6.6 μg/g) were equal in adult and subadult WTSE (1.09 vs 1.10 μg/g). Adult WTSE ($n = 150$) showed a significantly higher mean Pb level (18.1 μg/g) than subadults (6.97 μg/g; $n = 162$, $p = 0.008$ MWU), due to a significantly higher frequency of lethal poisoning (20% vs 10%; $p < 0.004$). Franson and Russel (2014) reported a similar result for bald eagles among age groups. Isomursu et al. (2014) observed higher frequency of Pb poisoning in 15 young WTSE (1–2 Cyl, 67%) than in 70 older birds (21%). Total Pb levels in our GE samples averaged significantly higher in 55 subadults (11.3 μg/g) than in 47 adults (9.01 μg/g; $p = 0.023$, MWU), whereas Pb < 6.6 μg/g averaged higher in 40 adults (1.94 μg/g) than in 49 younger birds (1.39 μg/g; $p = 0.015$, MWU). Among eight cases of acute Pb poisoning of GE in our study, only three were adult birds. Slabe (2019) reported similar contradictory differences from North America in liver Pb level patterns among age groups of GE and bald eagle. In all, these distributions of Pb concentrations and lethal poisoning among age classes of eagles appear inconclusive.

4.3.3. Environmental background concentrations

Pb concentrations in wildlife will vary depending on natural presence of Pb in the Earth's crust, the ubiquitous but varying presence of Pb from industrial sources and leaded gasoline, and from differences in food types. Excluding an influence from ingestion of Pb-based shotgun pellets and bullet fragments, we estimate an environmental background liver Pb concentration in WTSE in Sweden during the study period at a range up to about 0.6 μg/g (Fig. A7C). This is one order of magnitude below a previously suggested threshold at 6.6 μg/g for subclinical effects (Franson and Pain, 2011). Excluding a solitary Pb poisoned specimen (0.8% of the sample), EO samples show a log-normal distribution of Pb concentrations with a median of 0.103 μg/g (Fig. 9). GE and WTSE show bimodal distributions, indicating recent exposure to Pb from spent ammunition in the segment to the right, but with slightly overlapping segments in WTSE. In samples <6.6 μg/g (Fig. 9, WTSE 1981–2011, left-hand segment) the median concentration of 0.554 μg/g is twice as high as the level under which we could statistically detect the expected temporal changes in total Pb and in Pb isotope ratios. WTSE retrieved during June–September (i.e., outside the main hunting season, allowing for some lag in response to exposure) had significantly lower Pb levels (median 0.348 μg/g, $n = 33$ excluding 11 juveniles) than samples from the rest of the year (median 0.574 μg/g; $n = 208$, $p < 0.05$, MWU). The lack of expected temporal changes in Pb levels and isotope ratios over a wide range of low-contaminated samples suggest that these elevated concentrations <6.6 μg/g also stem from exposure to spent ammunition. Ingestion of gut piles and shot game can include various amounts of a very wide range of Pb particle sizes, from

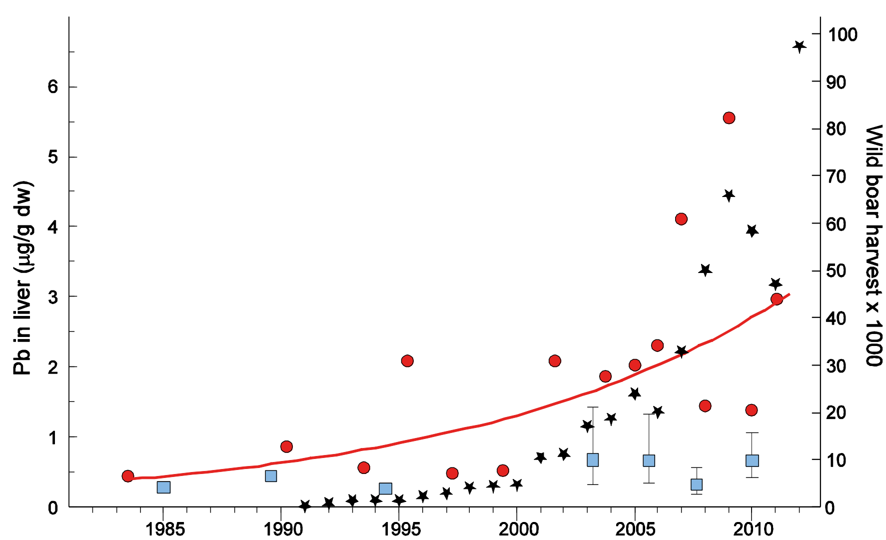

Fig. 8. Trends of mean liver Pb-levels in WTSE in Sweden in relation to reported total numbers of harvested wild boar from hunting. A minimum of 7 WTSE specimens was used to form mean values. Red circles represent WTSE from counties within the distribution range of wild boar ($p = 0.002$; $n = 211$ specimens) and blue squares from counties without wild boar ($p = 0.078$; $n = 93$). Specification of counties is given in the text. Black stars indicate approximate annual harvest of wild boar (from Bergqvist and Elmhagen, 2016).

chunks of bullets and shotgun pellets down to nanoparticles (Herring et al., 2020; Kollander et al., 2016). Repeated ingestion of small amounts will occur year by year by many survivors, in addition to birds that died from lethal poisoning. Such repeated sub-lethal intake of Pb from ammunition was indicated for individual GE based on distributions of Pb in feathers and bone (Jenni et al., 2015). Recurrent sub-lethal exposure to Pb-based ammunition in these scavenging and opportunistic raptors should show as elevated (above background) levels over a wide range below subclinical concentrations, and could then explain the absence of expected temporal changes in both total Pb and Pb isotope ratios for the majority of WTSE in this study. As noted in paragraph 3.7, this may also explain the higher subclinical liver Pb levels in WTSE compared with EO.

Pb levels <6.6 μg/g were significantly higher in GE than in WTSE during October-May (Fig. A5B). Liver Pb concentrations in three GE from June–September are low as in WTSE (Table 1) but the sample is too small to infer a "background" concentration range in GE in Sweden. The frequency distributions in Fig. 9 and in Fig. A5 indicate a higher background Pb level in GE than in WTSE. This may be natural, if Pb levels average higher in the predominantly terrestrial food web of GE than in the predominantly aquatic food chain of WTSE. However, published evidence for this is largely lacking. Alternatively, more elevated subclinical Pb levels in GE than in WTSE could result from a more frequent ingestion in GE of Pb from spent ammunition. In Sweden, a substantial part of WTSE food during the ice-free period of the year is fish (Helander, 1983) that does not contain any ammunition remains. GE in Fennoscandia feed mainly on terrestrial mammal and bird prey (Tjernberg, 1981; Sulkava et al., 1984). Small game is hunted extensively and wounded specimens that escape are a potential source of Pb. In Norway, shotgun pellets from previous shootings were found in 16–25% of rifle-killed mountain hare (*Lepus timidus*) (Larsen and Nybakk, 2011) and in 14% of snared willow ptarmigan (*Lagopus lagopus*) (Holmstad, 1998). In a conservative estimate of 795,400 game killed with Pb-based shotgun pellets in Sweden 2002/2003, 85% were from terrestrial habitats and predominantly of typical GE prey species (Naturvårdsverket, 2006). This suggests a more frequent exposure in GE to Pb from spent pellets embedded in crippled prey and carcasses. Higher subclinical Pb concentrations in GE than in WTSE is not contradictory to the fact of acute Pb poisoning being significantly higher in WTSE in our material (Fig. A5). The strong increase in Pb exposure from harvested wild boar (Fig. 8) limits itself to the southern and central part of Sweden, holding the majority of WTSE but only a small part of GE in our material (Fig. 1). The GE is also a more swift and agile flyer and possibly more successful in hunting live prey than WTSE and may be less prone to scavenge on leftovers from hunting of big game. Published evidence from North America have also indicated a higher rate of lethal Pb poisoning in bald eagle than in GE (Russel and Franson, 2014; Slabe, 2019).

It has been suggested that all Pb residue levels below an estimated threshold for subclinical poisoning can be considered as "background" concentrations, i.e. as "evidence of environmental exposure distant from any specific source of lead contamination" (Franson and Pain, 2011). Our results indicate that this is highly disputable as a general assumption. The environmental background liver Pb level estimated here is far below an applied threshold of 6.6 μg/g for subclinical poisoning. The temporal increase of Pb in all WTSE ($n = 315$, Fig. 5) is also mirrored by birds with Pb < 6.6 μg/g ($n = 255$, Fig. A6), indicating significant exposure to Pb from spent ammunition also at lower Pb levels.

4.3.4. Effect thresholds

Among eagles judged to have died from Pb poisoning, all GE and 80% of WTSE had levels exceeding 33 μg/g in their livers. Three WTSE diagnosed as lead poisoned, based on necropsy findings had liver Pb concentrations slightly below the suggested threshold of 20 μg/g for poisoning. Liver Pb levels (μg/g) in these birds were 17.2 (26.0 in kidney), 18.9 (19.3 in kidney) and 19.4 (22.8 in kidney). Another WTSE found dead from collision with a power-line had 9.3 μg/g Pb in the liver and 22.7 μg/g in kidney. Isomursu et al. (2018) reported similar cases of lethal poisoning from elevated Pb levels in kidney with corresponding sublethal levels in liver. The advantage of access to both liver and kidney data when studying the occurrence of Pb poisoning is obvious. Manning et al. (2019) also reported fatal Pb toxicity in some bald eagles with liver and kidney levels below 20 μg/g.

Evidence of how sublethal Pb concentrations might affect fitness and survival in free-ranging eagles is scarce. Studies on other birds showed signs of effects from moderate Pb exposure on delta-aminolevulinic acid-dehydratase (ALAD) activity and other blood parameters as well as on breeding success (Franson et al., 1983, 2000; Henny et al., 1991; Berglund et al., 2007, 2010; Gómez-Ramírez et al., 2011; Herring et al., 2020; Descalzo et al., 2021). Birds dosed with Pb-based shotgun pellets showed reduced hatching success (Buerger et al., 1986; Vallverdú-Coll et al., 2016), reduced egg and hatchling weight, reduced weanling survival and increased lesions in the weanling's liver and other organs (Williams et al., 2017). Experimental studies with gulls showed that

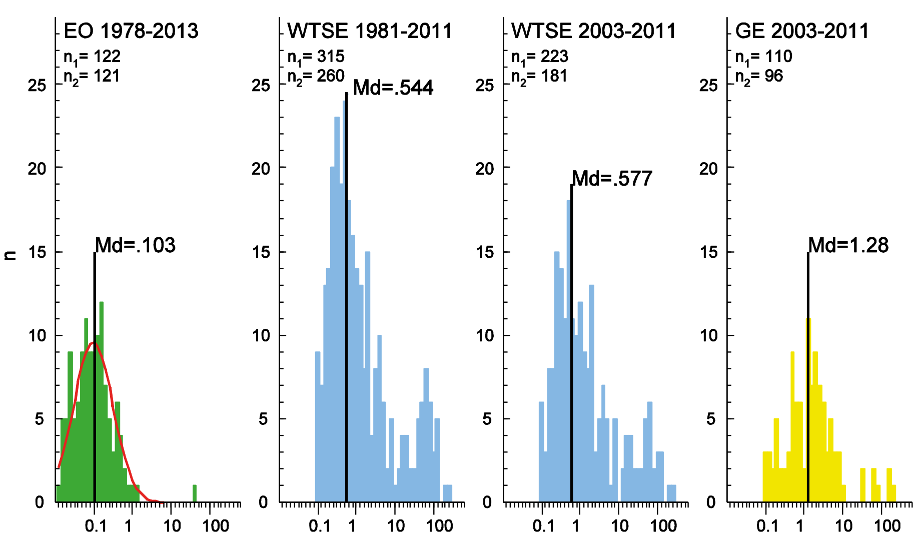

Fig. 9. Frequency distributions of liver Pb concentrations (μg/g dw, $\log_{10}$ scale) in EO (green), WTSE (blue), and GE (yellow). n_1 denotes total sample size; Md and n_2 denotes median and sample size for samples with Pb < 6.6 μg/g.

low levels of Pb affected locomotion, balance and depth perception (Burger and Gochfield, 2000), dysfunctions that could predispose birds to e.g. collisions. Among eagles with elevated but sublethal liver Pb levels in this study (9 WTSE with 8–17 μg/g and 6 GE with 7–11 μg/g, Fig. 3), ten died in collisions and two by other trauma, two were emaciated and one died by unknown cause. Excluding the single unknown cause of death, 50% of these eagles died in collision with train, compared to 40% among birds with Pb < 6.6 μg/g and known death cause ($n = 324$). A single GE died in a wind rotor collision with a liver Pb level of 10 μg/g; but 11 WTSE that died in rotor collisions had much lower levels (0.06–2.0 μg/g). Four out of 6 GE that died in collision with car had Pb levels of 3.7–6.4 μg/g – below a suggested threshold of 6.6 μg/g for subclinical effects, but still in the higher range there (Table A2, Fig. A5). Body mass is an important indicator for condition and decreases with liver Pb levels in WTSE but a statistically significant decrease can only be confirmed here at Pb concentrations >20 μg/g ($p < 0.001$) (Fig. A4). Our material of liver Pb levels in an intermediate range is too small for further inference of effects from sublethal Pb concentrations. Berny et al. (2015) demonstrated significantly higher median liver Pb concentrations even below a threshold of 6 μg/g in vultures and kites found dead from trauma and/or electrocution vs other death causes. Ecke et al. (2017) observed altered flight performance in GE at considerably lower Pb concentrations in blood than a previously suggested threshold for effects. The relative importance of sublethal Pb concentrations for animal health merits further studies (Hunt, 2012).

4.3.5. Bias in causes of death

Dead animals retrived from the field will naturally be biased toward those found more easily, notably victims from collisions, compared to most other causes of death. Eagles scavenge carcasses on the railway in the same way as they do carcasses and gut piles from hunting activities. As noted above, one third of both WTSE and GE samples in our material represent birds that were hit by trains (Table A2). Mandatory obligations to report these casualities and also to submit these "state game" specimens for investigation clearly leads to overestimating this cause of mortality in our material compared to other death causes. Birds killed on the railway (and road) are removed quickly and thus fresh and suitable for Pb analysis. Birds that die of other causes are often in various states of decay when found, and such specimens cannot be used for Pb analyses in soft tissues like the liver. Furthermore, birds that suffer from illness, such as from Pb poisoning, tend to hide away if they can and are consequently less likely to be found fresh for that reason alone (Krone et al., 2009b). Altogether, these sources of sampling bias could imply a considerable underestimate of Pb poisoned birds in the present material, obtained through passive collection of finds in the field.

5. Conclusions

By making a reasonable assumption that Pb concentrations and Pb isotope ratios in eagles would follow the temporal trajectories of other biota (not exposed to Pb from ammunition), we inferred environmental background Pb levels in Sweden at about an order of magnitude lower than a suggested threshold for subclinical effects. A majority of WTSE did not follow the expected trends and had liver Pb concentrations above our estimated background, indicating a strong influence from a Pb source other than natural background and large-scale historic Pb pollution such as leaded gasoline. The agreement between Pb concentrations and hunting seasons, the significant correlation with spatial exposure indices, and the significant increase of Pb in eagles along with the increase in wild boar harvesting provide additional evidence for this source being spent ammunition.

Eagles are monitored sentinel species, but exposure to Pb from spent ammunition will also affect a range of other scavenging and predatory bird species under no such surveillance, including corvids, buzzards, kites, goshawk (*Accipiter gentilis*) and also mammalian scavengers (e.g. Burco et al., 2012; Legagneux et al., 2014; Slabe, 2019; Monclús et al., 2020; Taggart et al., 2021; Fuchs et al., 2021). A decrease of nesting gyrfalcons (*Falco rusticolus*) has occurred in Sweden following an increase in grouse hunting after 1993. Gyrfalcons are selective hunters, and known also to feed readily on carrion (Tømmeraas, 1989; Nielsen, 2002), so poisoning from Pb-based shotgun pellets and bullet fragments could be a contributing reason, but will be hard to evaluate in this endangered species in Sweden due to its rare and remote occurrence. Some regulations and voluntary agreements to shift away from Pb-based ammunition have been at least partly successful in reducing Pb poisoning in birds (Kelly et al., 2011; Bedrosian et al., 2012: Mateo et al., 2014). However, most partial bans have had little effect in reducing exposure and poisoning of scavenging raptors (Scheuhammer and Thomas, 2011; Pain et al., 2019; Green et al., 2021; Widemo, 2021). A shift away from Pb-based ammunition is warranted in order to mitigate avoidable and unnecessary detrimental effects on wildlife from hunting (Arnemo et al., 2019). We recommend and encourage the use of Pb-free ammunition in all hunting.

CRediT authorship contribution statement

Björn Helander: Conceptualization, Methodology, Resources, Formal analysis, Validation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition. **Oliver Krone:** Conceptualization, Methodology, Validation, Writing – review & editing. **Jannikke Räikkönen:** Resources, Validation, Writing – review & editing. **Marcus Sundbom:** Conceptualization, Methodology, Validation, Writing – review & editing. **Erik Ågren:** Resources, Validation, Writing – review & editing. **Anders Bignert:** Conceptualization, Methodology, Formal analysis, Validation, Visualization, Software, Writing – review & editing.

Declaration of competing interest

The authors declare no known competing personal or other interests that could have had an influence on the work and conclusions in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.148799.

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