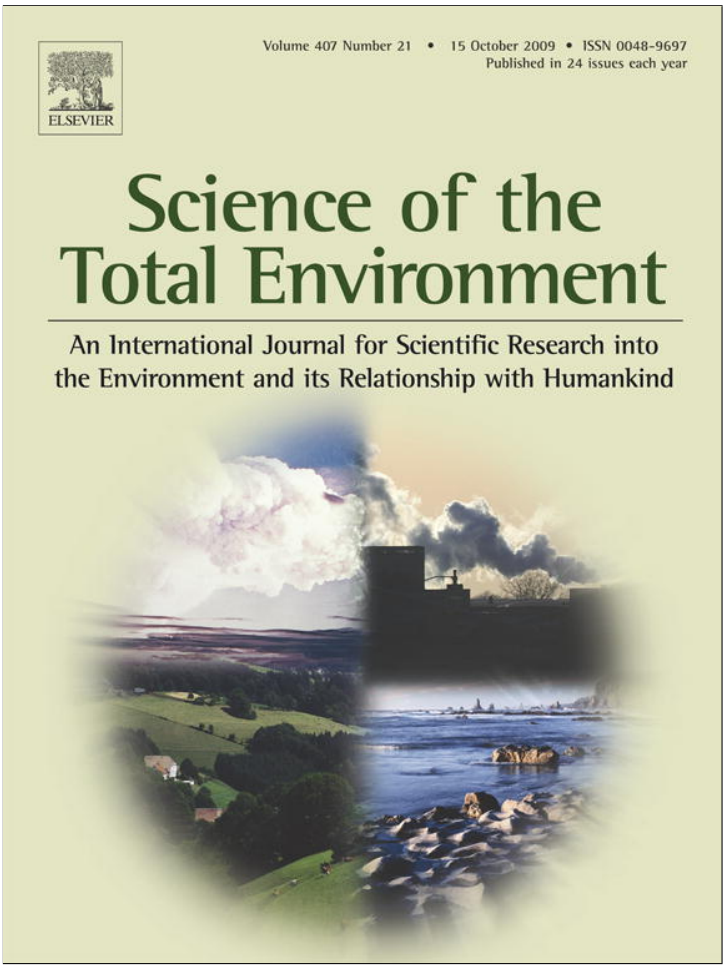

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Ingestion of lead from ammunition and lead concentrations in white-tailed sea eagles (*Haliaeetus albicilla*) in Sweden

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ABSTRACT

In this study we show for the first time that lead poisoning from ammunition is a significant mortality factor for white-tailed sea eagle (WSE) (*Haliaeetus albicilla*) in Sweden. We analyzed 118 WSEs collected between 1981 and 2004 from which both liver and kidney samples could be taken. A total of 22% of all eagles examined had elevated (>6 μg/g d.w.) lead concentrations, indicating exposure to leaded ammunition, and 14% of the individuals had either liver or kidney lead concentrations diagnostic of lethal lead poisoning (>20 μg/g d.w.). Lead concentrations in liver and kidney were significantly correlated. In individuals with lead levels <6 μg/g, concentrations were significantly higher in kidney than in liver; in individuals with lead levels >20 μg/g, concentrations were significantly higher in liver. The lead isotope ratios indicate that the source of lead in individuals with lethal concentrations is different from that of individuals exhibiting background concentrations of lead (<6 μg/g d.w.) There were no significant sex or age differences in lead concentrations. A study from the Baltic reported in principle no biomagnification of lead, but background lead concentrations in WSE liver in this study were still four to >10 times higher than concentrations reported for Baltic fish from the same time period. In contrast to other biota there was no decrease in lead concentrations in WSE over the study period. The proportion of lead poisoned WSE remained unchanged over the study period, including two years after a partial ban of lead shot was enforced in 2002 for shallow wetlands. The use of lead in ammunition poses a threat to all raptors potentially feeding on shot game or offal. The removal of offal from shot game and alternatives to leaded ammunition needs to be implemented in order to prevent mortality from lead in raptors and scavengers.

1. Introduction

Lead is a hazard to free ranging raptors feeding on game and offal containing lead shot or fragments of lead bullets (Pain and Amiard-Triquet, 1993; Pain et al., 1993; Pain et al., 1995; Kramer and Redig, 1997; Kim et al., 1999; Krone et al., 2004; Krone et al., 2006; Kenntner et al., 2007; Pain et al., 2007). Raptors are selective hunters and will catch crippled prey first-hand, and species with scavenging habits will be particularly at risk for exposure to lead ammunition. There are many reports of lead poisoning of raptorial birds around the world, and in some countries this has led to bans of lead shot ammunition in raptor habitats (Pain and Amiard-Triquet, 1993; Church et al., 2006; Kenntner et al., 2007).

Reports of lead poisoning of sea eagles have been published from several countries in Europe (Falandysz et al., 2001; Kenntner et al., 2004; Krone et al., 2004; Krone et al., 2006; Muller et al., 2007) as well as from Russia, Japan (Kim et al., 1999; Iwata et al., 2000; Kurosawa, 2000) and North America (USFWS, 1986; Wiemeyer et al., 1989; Elliott et al., 1992; Wayland and Bollinger, 1999; Miller et al., 2001; Wayland et al., 2003). The cause of the lead poisoning in sea eagles is usually unintentional consumption of lead-containing ammunition. Reported frequencies of white-tailed sea eagles (WSE) (*Haliaeetus albicilla*) found dead or moribund with lead concentrations diagnostic of lead poisoning are 17% in Greenland (Krone et al., 2004), 22 % in Finland (Krone et al., 2006) and 17 and 28% in Germany (Kenntner et al., 2001, 2004). Spent ammunition in the form of lead shot has been found in lead poisoned WSEs from Germany (Muller et al., 2007) and Greenland (Krone et al., 2004). Fragments of lead bullets have been found in lead poisoned individuals from Finland (Krone et al., 2006), Germany (Kenntner et al., 2004) and Japan (Iwata et al., 2000).

Lead poisoning has also been shown to be an important mortality factor for bald eagles (*Haliaeetus leucocephalus*) in North America. In a survey by the US Fish and Wildlife Service between 1963 and 1984, 6%

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of the mortality of investigated bald eagles was due to lead poisoning (USFWS, 1986). Wayland and Bollinger (1999) analyzed 127 dead or moribund bald and golden eagles (*Aquila chrysateos*) in the Canadian prairie provinces and in total, 16% of the eagles had elevated lead concentrations indicative of exposure to ammunition, and 12% had concentrations above 20 µg/g (d.w.) in kidney or 30 µg/g (d.w.) in liver, diagnostic of lethal lead poisoning. In a more recent study by Wayland et al. (2003) including 372 bald and golden eagles from western Canada, 10% were diagnosed as lead poisoned. In a study by Elliott et al. (1992), 37% of 65 analyzed bald eagles from British Columbia exhibited significant lead exposure and 14% were classified as lead poisoned. Lead levels in blood has also been shown to be higher in Canadian bald eagles during hunting season compared to other time points examined (Miller et al., 2001). As many as 71% of the regurgitated pellets from about 100 bald eagles wintering in Utah, USA, contained lead shot (Platt, 1976). These eagles were probably exposed to lead shot through the extensive hunt of rabbits in the Utah desert (Platt, 1976). The presence of lead shot in WSE pellets from Sweden 1964–1980 correlated with the hunting season, with a frequency of 9% shot-containing pellets during the winter hunting season, compared to 0.7% lead shot in pellets collected during the summer (Helander, 1983).

Lead in the form of ammunition becomes bioavailable in contact with the acidic gastric fluids of the stomach. Lead will be dissolved in the form of lead salts and can be absorbed systemically (Hoffman et al., 1981). Lead ammunition embedded in tissue that is not exposed to gastric acids will have much lower bioavailability and will not cause acutely toxic levels of lead (Guillemain et al., 2007; Martin et al., 2008). The exposure route causing lead poisoning from ammunition in birds is therefore either direct ingestion of leaded ammunition, which often is the case for waterfowl, (Pain, 1990; Mateo et al., 1998) or secondary ingestion of lead by raptors from eating shot prey, offal, or waterfowl with lead shot pellets in their gastrointestinal tract. Large raptors such as golden, bald and white-tailed sea eagles often scavenge on carcasses or offal when given the opportunity, which puts them in danger of lead poisoning from ammunition (Pain and Amiard-Triquet, 1993). A high incidence of metal retention was found in North-American deer (*Odocoileus* spp.) carcasses as a result of bullet fragmentation; all investigated whole or eviscerated deer killed with lead-based bullets contained fragments, suggesting a high potential exposure of scavengers to lead from this food source (Hunt et al., 2006).

Lead isotope ratios have often been used to identify the source of lead in lead exposed birds (Scheuhammer and Templeton, 1998; Meharg et al., 2002; Church et al., 2006; Svanberg et al., 2006) and humans (Levesque et al., 2003; Tsuji et al., 2008). This is possible since the ratio of the four stable lead isotopes ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb vary between different ores. Three of these isotopes are products of radioactive decay of other elements (^{235}U to ^{207}Pb, ^{238}U to ^{206}Pb and ^{232}Th to ^{208}Pb). Thus, the rate of radioactive decay, which depends on the time the radioactive elements were mixed into the ore, results in differences in Pb isotope ratios from different ores (reviewed in Scheuhammer and Templeton, 1998). Studies have shown that the lead isotope ratio of lead poisoned birds have closely matched that of ammunition (Meharg et al., 2002; Scheuhammer et al., 2003; Church et al., 2006; Svanberg et al., 2006).

Experimental studies where raptors were fed lead shot have demonstrated large individual variations in the susceptibility to lead poisoning, with liver lead concentrations at death ranging from 24 to 384 µg/g d.w. (Pattee et al., 1981; Carpenter et al., 2003; Pattee et al., 2006). The symptoms in the experimental studies closely matched those seen in lead poisoned raptors in the wild; anorexia, lethargy, muscle weakness, green-stained feces, emaciation and death. Lead concentrations above 5 µg/g on a wet-weight basis, approximately equal to 15 µg/g on a dry weight (d.w.) basis, in liver has been established as lethal for most raptorial species (Franson, 1996). In bald and golden eagles, the concentrations diagnostic of lethal poisoning have been proposed to be 30 µg/g d.w in liver and 20 µg/g d.w. in kidney (Wayland and Bollinger, 1999). Pain et al. (1995) suggested that concentrations above 6 µg/g (d.w.) are indicative of elevated exposure to lead in raptors; however, the concentration diagnostic of lead poisoning was suggested to be over 20 µg/g (d.w.).

In this study the lead concentrations in liver and kidney was examined in 118 dead or moribund WSEs turned in to the Swedish Museum of Natural History (SMNH) between 1981 and 2004. This is the first published survey of lead poisoning of WSE in Sweden. This material will also provide an important reference for future evaluations of the effects of the ban of lead shot in Swedish wetlands.

2. Materials and methods

2.1. Sample collection

In Sweden, the WSE is among game that belongs to the State, and finds of dead or moribund specimens must be reported to the police. Between 1981 and 2004, 374 specimens (or part of) were collected and sent to the SMNH in Stockholm. Organ samples were saved from all fresh specimens and stored at −80°°C in the museum's Environmental Specimen Bank. All individuals (118) from which both liver and kidney samples could be obtained were selected for this study. Lead concentrations in livers and kidneys of raptors are commonly used to assess diagnostic lead exposure. Ages were retrieved from bands identifying individual birds ($n=66$) or were determined from plumage characteristics (Helander et al., 1989). The distribution of ages (year of life) was: 1–2 = 54, 3–4 = 16, 5–25+ = 47. Sex was determined by ocular inspection of reproductive organs and from measurements (tarsus width, wing cord, total length). Two individuals were nestlings and are excluded when specified. X-rays were performed on 106 birds from this sample in order to determine if they were shot or had ingested shot or bullet fragments (Fig. 1).

2.2. Necropsy

All specimens received at the museum were visually inspected for determination of cause of death before being weighed, measured, X-rayed and finally opened. Traumatic causes of death were often indicated by finding circumstances (collision with vehicles and wires, burn-marks from electrocution, entanglements, drowning, intraspecific fights etc.) and from radiographs. When identified in radiographs, the presence of shot and fragments was confirmed visually in dissections. All shots found were lead shots. Veterinary inspection was not performed at the museum. The causes of death assigned to the specimens included in this study are summarized in Table 1.

2.3. Chemical analysis

The liver and kidney samples were freeze dried and pressure digested in concentrated ultra pure (quartz distilled) nitric acid and hydrogen peroxide in closed vessels in a microwave oven. Lead concentration and stable Pb isotopes in the digests were determined with ICP-MS (Thermo-Fisher X-series II). The Pb concentration was determined using the sum of ^{206}Pb, ^{207}Pb and ^{208}Pb, the quantification was made with external calibration, and Rh was used as internal standard. The analytical performance was checked by using three certified reference materials; DOLT-3 (dogfish liver), certified Pb-value 0.32 ± 0.6, found value 0.34 ± 0.053 µg/g (d.w.); oyster tissue (IK1566), certified 0.48 ± 0.04, found 0.45 ± 0.018 µg/g (d.w.); and bovine liver (IK1577a), certified 0.135 ± 0.015, found 0.124 µg/g (d.w.). The precision of the isotope ratios, as calculated from determinations of the calibration solutions, was found to be 0.4% for ^{206}Pb/^{207}Pb and 1.4% for ^{208}Pb/^{207}Pb, respectively. All analyses followed the quality assurance routines as specified in the accreditation of the laboratory at ITM.

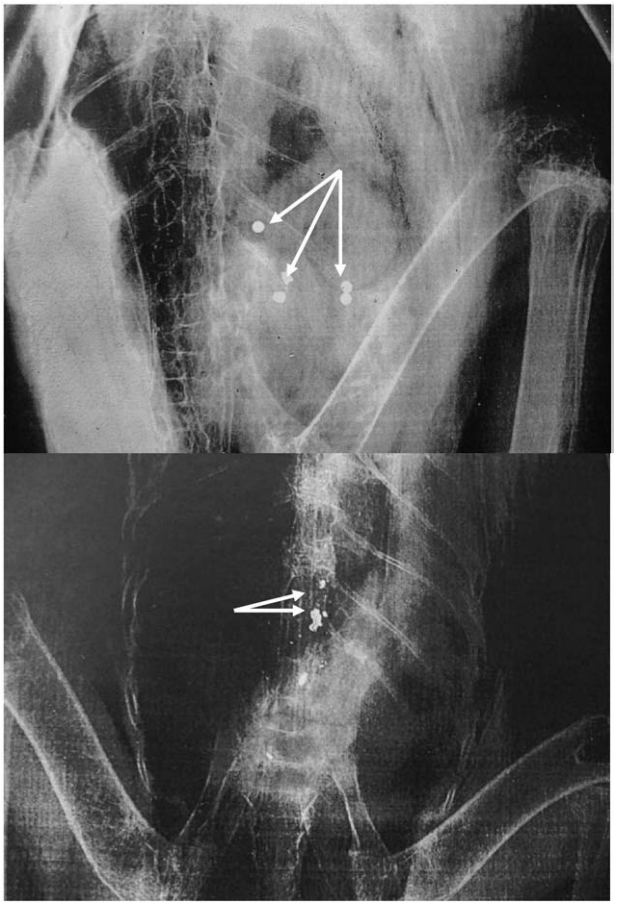

Fig. 1. Radiographs of white-tailed sea eagles with five lead shot (upper) and bullet fragments indicated by arrows in the gastrointestinal tract.

Concentrations are subsequently given as µg/g on a dry weight basis (d.w.). The mean and median percentages of dry matter in the samples were: *liver* 29.3 and 29.2, respectively (s.d. = 2.9, range 22.0–39.3, C.V. = 9.8); *kidney* 25.6 and 25.7, respectively (s.d. = 2.7, range 19.1–32.6, C.V. = 10.5). This corresponds very close to moisture values reported by Wayland et al. (2003) for bald and golden eagles from Canada.

We chose a concentration of 20 µg lead/g in liver or kidney as diagnostic of lead poisoning, according to Pain et al. (1995). Based on suggested thresholds given by Franson (1996) and Wayland and Bollinger (1999) concentrations exceeding 15 and 30 µg/g (d.w.) as thresholds, respectively, are also discussed.

2.4. Statistical analysis

Simple log-linear regression analyses were carried out to check for possible significant temporal trends. Log-linear rather than linear regression was used since we expect a percentual decrease after the ban of leaded petrol in accordance with other reported temporal trends in biota. For comparison of temporal trends of lead in eagles and prey species (reported elsewhere) only eagle specimens with background (subclinical) concentrations (<6 µg/g) were used. Inclusion of all specimens resulted in substantially increased random between-year variation and a corresponding loss in statistical power to detect trends. To check for significant differences between lead concentrations in liver and kidney in WSE with low and high concentrations, respectively, the non-parametric Wilcoxon's Signed-Ranks Test for paired observations was applied since the differences was deviating significantly from the normal distribution according to the Kolmogorov–Smirnov Test for goodness of fit. One-way Analysis of Variance (ANOVA) was used to check for significant differences between geographical regions (Table 2), for differences in the proportion of stable isotopes between groups, and also to test for differences in lead concentrations between three groups of specimens: two groups that contained lead from ammunition a) within their gastrointestinal tract b) in their flesh from being shot and c) the group where traces of lead was not found. Before the test was carried out, Bartlett's test was applied to check for possible heterogeneity between variances among the groups. No significant differences were found and homoscedasticity was therefore assumed. As a post-hoc test for pair-wise comparisons between individual groups, the GT2-test suggested by Hochberg (1974) for unequal sample sizes, was carried out. To check whether there was a significant difference in the proportion of lead poisoned specimen between two time periods, 1981–2002 and 2003–2004, respectively, a simple chi-square test was applied.

A significance level of 5% was generally applied for all tests.

3. Results

3.1. Concentrations

No significant differences in lead concentrations were detected between the sexes, neither with all individuals of reproductive age (lead poisoned individuals included) nor with individuals of reproductive age with background levels of lead only. No statistically significant correlation was found between age and lead concentration in liver or kidney. Thus, no further subdivision of the sample was made in relation to age or sex.

The geographic distribution of lead concentrations in liver tissue from sampled Swedish WSEs is presented in Fig. 2. The distribution of collected eagles reflects the distribution of WSE populations in Sweden during the study period (Helander, 2003). A regional distribution of background lead concentrations (<6 µg/g) in liver and kidney is given in Table 2. No significant differences between the regions could be detected (ANOVA, logged values). Considering the within region variances and the number of available specimens from each region, the statistical power is relatively low and the lowest

Table 1
Causes of death among 116 white-tailed sea eagles in this study (two nestlings excluded: one fell from a nest and one starved to death).

Lead poisoned	16
Collision with vehicle	25
Collision with wire, building	22
Shot, killed by conspecific	13
0ther trauma	9
Emaciated, illness	5
Unknown	26
Total	116

Table 2
Regional distribution of background lead concentrations in white-tailed sea eagle specimens retrieved in Sweden with <6 µg/g (d.w) in both liver and kidney ($n = 91$, two nestlings excluded).

Areas (counties)	*n*	Liver mean (s.d.)	Kidney mean (s.d.)	Liver median (range)	Kidney median (range)
BD + AC + Z	20	0.72 (1.18)	1.16 (1.50)	0.33 (0.07–4.6)	0.42 (0.20–5.9)
X	9	0.89 (1.16)	1.49 (1.72)	0.47 (0.19–3.8)	1.0 (0.33–5.9)
C	20	0.73 (0.91)	1.31 (1.20)	0.46 (0.18–4.4)	0.76 (0.20–3.8)
B	18	0.84 (1.14)	0.96 (0.86)	0.40 (0.03–4.3)	0.67 (0.05–3.5)
D + E + H + I	16	0.91 (1.70)	1.41 (1.35)	0.65 (0.05–3.9)	1.20 (0.09–4.8)
R + S + T + U + W	5	1.00 (0.93)	1.93 (1.51)	0.77 (0.14–2.6)	1.81 (0.21–4.4)
M	3	0.80 (0.55)	1.27 (0.52)	0.74 (0.30–1.4)	0.97 (0.97–1.9)

Area county-codes refer to Fig.2. Areas arranged from north to south.

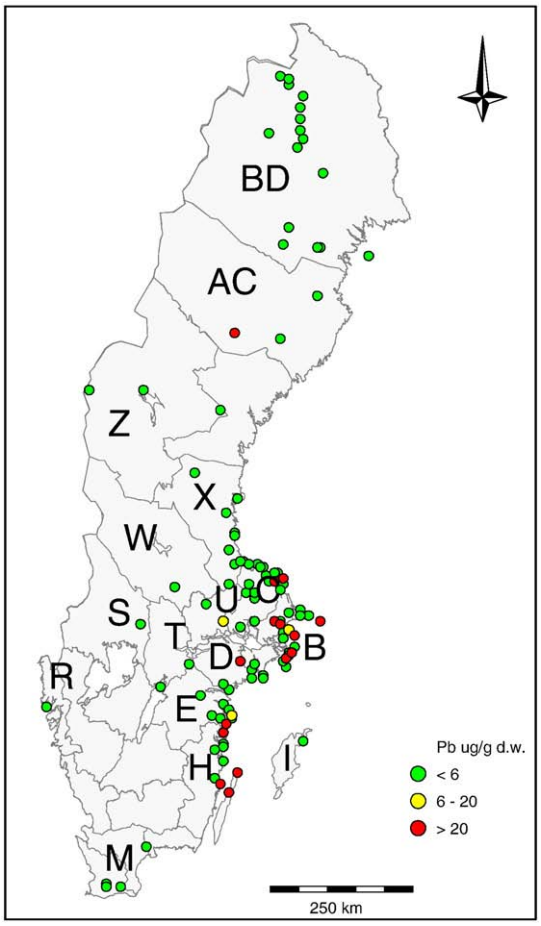

Fig. 2. Distribution of sampled white-tailed sea eagles grouped according to concentrations of lead in liver. Letters denote county-codes: AC = Västerbottens län, B = Stockholms län, BD = Norrbottens län, C = Uppsala län, D = Södermanlands län, E = Östergötlands län, H = Kalmar län, I = Gotlands län, M = Skåne län, R = Västra Götalands län, S = Värmlands län, T = Örebro län, U = Västmanlands län, W = Dalarnas län, X = Gävleborgs län, Z = Jämtlands län.

significant difference that would have been possible to detect would have been about four times the largest observed mean difference between regions.

We found no significant temporal trends during the investigated period in concentrations in liver, or in kidney (log-linear regressions, r^2-values in the range 0.01–0.2) based on specimens with background (subclinical) concentrations (<6 µg/g), neither in the material from all counties ($n^{liver} = 100$, $n^{kidney} = 93$) nor in the material excluding inland northern Sweden (counties BD, AC and Z, see Fig. 2 and Table 2) ($n^{liver} = 70$, $n^{kidney} = 86$) as exemplified in Fig. 3. No further subdivision of the sample was made in relation to time points.

The median concentration of lead in liver from all specimens was 0.601 µg/g with a range of 0.031–154. The distribution of lead in eagle liver and kidney grouped into concentration intervals is given in Table 3. Concentrations >6 µg/g, indicative of elevated lead exposure, was found in 25 kidney and 18 liver samples representing a total of 25 out of 116 birds (22%). Of these, concentrations diagnostic of lead poisoning (>20 µg/g) was found in 15 liver samples and 13 kidney samples from a total of 16 birds. Thus, concentrations of lead diagnostic of lead poisoning were found in either liver or kidney in 14% of the specimens in our sample. One specimen had a lead concentration of 22.7 µg/g in kidney and 9.3 µg/g in liver tissue. The three kidney samples that did not exceed 20 µg/g still had elevated concentrations (16.0–19.9 µg/g). The correlation between liver and kidney concentrations was significant ($r^2 = 0.72$ ***$P<0.001$). In individuals

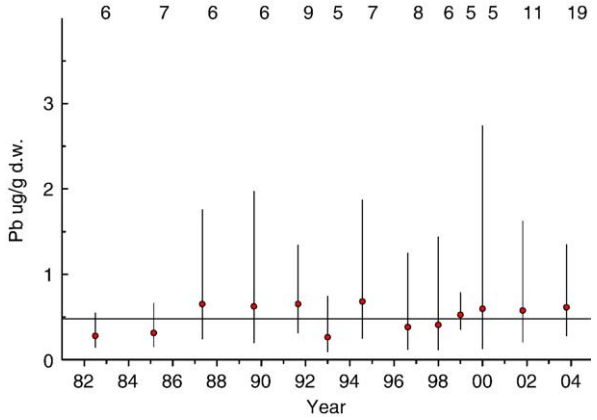

Fig. 3. Geometric mean concentrations of lead in WSE liver samples of background levels (<6 ug/g d.w.), 1982–2004. Vertical bars indicate 95% confidence intervals. Years with less than 5 specimens were fused with neighboring years and a corresponding mean year was calculated. The row of numbers at the top indicates the number of specimens for each year or year interval. No significant trend was detected.

with background levels of lead (<6 µg/g, see Table 2), concentrations were significantly higher in kidney tissue than in liver tissue (median kidney = 0.94 µg/g, median liver 0.46 µg/g, ***$P<0.001$, $n = 98$, Wilcoxon Signed-Rank test). For individuals with concentrations diagnostic of lead poisoning (>20 µg/g), however, the concentrations in liver tissue often greatly exceeded those found in kidney tissue (median liver = 74.6 µg/g, median kidney = 30.2 µg/g, ***$P<0.001$, $n = 15$, Wilcoxon Signed-Rank test). See Fig. 4.

Two nestlings are included in this study. One that died of starvation had lead concentrations 0.168 in liver and 0.124 in kidney and the other, found under a fallen nest, had concentrations of 0.061 in liver and 0.175 in kidney.

3.2. Lead shot and fragments

Six individuals had lead ammunition in their gastrointestinal tract; four with lead shots and two with bullet fragments. Four of them also had lead liver; kidney levels indicative of lead poisoning (9.31;22.7, 43.1;33.5, 60.1;28.4 and 74.6;56.1 µg/g) while two had elevated levels in kidney but not in liver tissue (0.851;6.4 and 1.30;11.6 µg/g). The lead concentrations in kidney tissue was significantly higher in individuals with lead shot or bullet fragments within their gastrointestinal tract, ($n = 6$) compared to individuals with lead shot in their flesh from being shot ($n = 10$; *$P<0.042$), and compared to individuals without lead shot or fragments ($n = 89$ [excluding specimens that were not X-rayed]; **$P<0.005$, ANOVA).

3.3. Pb-isotope ratio

The composition of stable Pb-isotopes was different in the group with background lead concentrations below 6 µg/g compared to the

Table 3
Distribution of lead concentrations (µg/g, d.w.) in liver and kidney from 118 white-tailed sea eagles in Sweden, 1981–2004, grouped into concentration intervals.

Concentrations	Liver	Kidney
<0.5	53	33
0.5<1.0	24	22
1.0<2.0	10	22
2.0<6.0	13	16
6.0<20	3	12
>20	15	13

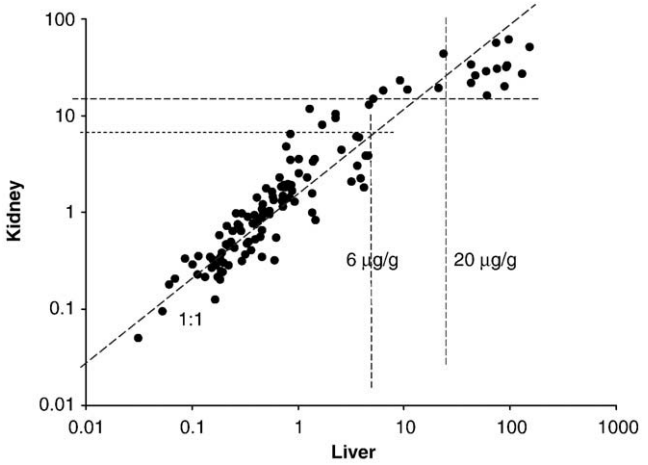

Fig. 4. Correlation between Pb-concentrations in liver and kidney from 118 white-tailed sea eagles (µg/g d.w.).

group with high concentrations above 10 µg/g. In the kidney samples, the $^{206}Pb/^{207}Pb$ ratio was 1.17 ± 0.033 in the background group ($n = 93$) and 1.20 ± 0.041 in the contaminated group ($n = 25$, $P < 0.01$) and the $^{208}Pb/^{207}Pb$ ratios were 2.44 ± 0.031 and 2.49 ± 0.032, respectively ($P < 0.001$). The differences of isotope ratios in the liver samples from the two groups were smaller and not statistically significant. The ratio of $^{206}Pb/^{207}Pb$ in liver was 1.17 ± 0.033 in the group with low Pb-levels and 1.18 ± 0.045 in the highly contaminated group. The $^{208}Pb/^{207}Pb$ ratios were 2.45 ± 0.034 and 2.45 ± 0.064, respectively.

4. Discussion

4.1. Threshold levels

The concentrations of lead and the finding circumstances for 18 eagles with elevated levels (>6 µg/g) in liver or kidney are given in Table 4. We chose the concentration of 20 µg lead/g in liver or kidney as diagnostic of lead poisoning, according to Pain et al. (1995). The concentrations diagnostic of lead poisoning in this study ranged between 21.2–154 µg/g in liver and 21.6–60.3 in kidney. Had we chosen 30 µg/g in liver or 20 µg/g in kidney as diagnostic of lead poisoning, according to Wayland and Bollinger (1999), the number of eagles deemed as lethally poisoned would have been reduced by one specimen (liver 21.2, kidney 19.0). This specimen, however, showed typical clinical symptoms of acute lead poisoning (see bird #11 in Table 4), and thus seems to support a threshold level 20 µg/g as diagnostic of lead poisoning in this species. If 15 µg/g in liver or kidney had been chosen as diagnostic of lead poisoning (according to Franson, 1996) another two (kidney) individuals would have been deemed as lethally poisoned (giving a total of 15% with concentrations diagnostic of lead poisoning). The background levels of lead are usually far below 6 µg/g. Among liver and kidney samples containing <6 µg/g lead, 87% and 83%, respectively, contained <2 µg/g (Table 3). The mean background levels, calculated from all birds with concentrations <6 µg/g in both liver and kidney ($n = 93$), was 0.80 ± 1.05 (liver) and 1.25 ± 1.26 (kidney).

4.2. Sex differences

There were no significant sex differences in lead concentrations in our study. Sex differences have been observed in ringed turtle doves (*Streptopelia risoria*), and this was proposed by the authors to be caused by the increased turnover of calcium during ovulation in females (Kendall and Scanlon, 1981). Female mallards have also been shown to have a 4–5 fold higher accumulation of lead during egg-laying compared to males (reviewed in Scheuhammer, 1987). For raptors, it has been suggested that a sex difference in size could influence the choice of prey, leading to increased exposure in females (Pain and Amiard-Triquet, 1993). WSE females are usually bigger and heavier than males (Cramp et al., 1980; Helander, 1988, Helander et al., 2007) but it has not been studied to what extent this has an influence on the choice of prey. In Egyptian vultures (*Neophron percnopterus*), a species with no sexual dimorphism in size (Cramp et al., 1980), the lead levels in blood were marginally ($P = 0.08$) higher in males than in females (Gangoso et al., 2009). Female American kestrels (*Falco sparverius*) fed dietary lead accumulated significantly higher concentrations in the liver than males on the same diet (Franson et al., 1983). However, sex differences in lead concentrations have not been noted in a majority of studies (Pain and Amiard-Triquet, 1993; Garcia-Fernandez et al., 1997; Wayland et al., 1999; Pain et al., 2007). If a sex difference in lead accumulation is largely dependent on increased turnover of calcium related to egg production the effect in WSE can be expected to be small, as a consequence of the small annual investment in egg production in this species—two eggs make up just about 4% of WSE female body weight (Isaksson and Helander, 2003).

Table 4
Finding circumstances and concentrations of lead and in 25 white-tailed sea eagles found in Sweden with levels exceeding 6 µg/g (d.w.) in liver or kidney.

Bird #	Finding circumstances and notes	Pb concentration	
		Liver	Kidney
1	F. d. with one lead shot in GIT	0.85	6.38
2	Killed by collision with aircraft	1.70	7.89
3	F. d. under powerline	2.26	9.38
4	Killed by collision with train	2.28	10.3
5	F. d. with two lead shot in GIT	1.30	11.6
6	Killed by collision with train	4.68	12.8
7	F. d., shot with rifle	5.17	14.6
8	F. d. under powerline	6.40	17.9
9	F. d. under powerline with bullet fragments in GIT	9.31	22.7
10	F. d.	11.0	18.4
11	F. d., good nutritional status, swollen liver, greatly enlarged gall bladder with greenish-black content	21.2	19.0
12	F. d.	23.7	43.3
13	F. d. with 6 lead shots in GIT	43.1	33.5
14	F. d. on eggs in nest	43.4	21.6
15	F. d. with wing injury	47.1	25.7
16	F. d. with bullet fragments in GIT	60.1	28.4
17	F. d. with healed gunshot in right wing	60.6	16.0
18	F. d. with lead shot in GIT	74.6	56.1
19	F. d.	76.2	30.2
20	F. d. under powerline	90.1	19.9
21	F. d., shot	92.7	31.6
22	Found alive, died one day after	93.8	32.9
23	F. d.	97.0	60.3
24	F. d.	132	26.7
25	F. d. beside reindeer carcass	154	50.9

F. d. = found dead; GIT = gastrointestinal tract.

4.3. Concentration relationships

The liver and kidney levels of lead have been shown to be correlated in other studies and this was the case also in this study. At background levels (<6 µg/g) the renal concentrations exceeded the hepatic concentrations, while in individuals with lethal lead concentrations the levels in liver were in most cases substantially higher than in kidney (Fig. 4). This was found also by Wayland et al. (1999), in wild golden and bald eagles, and by Pattee et al. (1981) in experimentally dosed bald eagles. Contrary to these results, the American kestrel, a species that has been shown to be very resistant to lead poisoning in experimental studies (Franson et al., 1983; Custer et al., 1984;

Stendell et al., 1989), was shown to accumulate more lead in their kidneys than in liver. American kestrels have been found to accumulate high concentrations of lead in bone tissue (Custer et al., 1984). The storage of lead in bone tissue seems to occur to a smaller extent in eagles, which may increase their susceptibility to the toxic effects of lead (Wayland et al., 1999).

If the absorbed dose of lead is high under a short period of time, acute lead toxicosis can sometimes occur without classical symptoms of lead poisoning. The affected birds die with a healthy appearance and in good body condition, as exemplified by bird #11 in Table 4. However, in most cases, lead poisoned birds become lethargic and anorexic. When food intake is decreased more lead can be absorbed, since intake of protein and/or calcium-rich foods decreases the absorption of lead (reviewed in Kendall et al., 1996; Pain, 1996). Intake of food is also necessary for the formation of pellets from hair and feathers (Tabaka et al., 1996) and in anorexic birds less regurgitation of shots occurs. This all leads to increased erosion of lead shots and systemic absorption of lead in poisoned individuals. Thus, at the time of death most individuals do no longer contain gun shot or fragments. Four specimens in our study contained lead shot in their gastrointestinal tract and two others contained bullet fragments. All six had lead levels in liver or kidney indicative of elevated exposure, and among them, four had levels diagnostic of lead poisoning. In the two individuals with elevated, but not diagnostically lethal concentrations of lead, the concentration was higher in kidney than in liver. This indicates that for individuals that have recently consumed metallic lead, the levels in kidney are more indicative of exposure than those in the liver, due to a delayed increase in lead concentration in this organ. Miller et al. (2001) also discussed this as a possible explanation for low lead concentrations in some individuals containing lead shot.

4.4. Lead in trauma-killed eagles

Birds under influence of high concentrations of lead become weakened and thus can be expected not to perform well. Two of the 16 eagles with concentrations of >20 µg/g in liver or kidney, diagnosed as lethal, were found under power-lines implying collision as possible ultimate death cause (see Table 4). One of these birds contained 9.31 and 22.7 µg/g and the other 90.1 and 19.9 µg/g in liver and kidney, respectively. Another bird found dead with lethal concentrations (47.1 and 25.7 µg/g in liver and kidney, respectively) had a broken wing, indicating trauma before death. Lower but elevated concentrations of lead were present in two eagles that had collided with wires (liver; kidney concentrations = 2.3; 9.4 and 6.4; 17.9 µg/g), in two that were killed by train (2.3; 10.3 and 4.7; 12.8 µg/g), in one bird that was found shot (5.2; 14.6 µg/g), one that collided with an aeroplane (1.7; 7.8 µg/g), and in two with unknown causes of death (1.3; 11.6 and 11.0; 18.4 µg/g). Whether the elevated concentrations of lead in those eagles contributed to their demise cannot be ascertained.

4.5. Exposure from lead ammunition

The significant differences in Pb-isotope ratios between individuals with background concentrations and with high concentrations of lead, indicates exposure to different sources of lead. The general $^{206}Pb/^{207}Pb$ ratio in Scandinavian aerosol particles, mainly derived from combustion of leaded petrol, has been around 1.14 during the last decades (Monna et al., 1997; Chiaradia and Cupelin, 2000), while natural lead in Swedish mineral soils generally show ratios above 1.4 (Bindler et al., 1999). The values in the eagle samples range from 1.1 to 1.25 which also include isotope ratios found in Pb shot pellets from N. American manufacturers (1.18 ± 0.05, Scheuhammer and Templeton, 1998). There is a deviating distribution in the highly contaminated individuals when $^{208}Pb/^{207}Pb$ is plotted vs $^{206}Pb/^{207}Pb$ (Fig. 5). This indicates that the lead in individuals with concentrations diagnostic of lead poisoning originates from other sources than long-range airborne pollution, probably from lead ammunition in their gastrointestinal tracts.

Studies within the National Environment Monitoring Program of metals in Swedish biota allow for comparison of concentrations in our WSE sample and in prey species. The mean background concentrations (<6 µg/g) of lead in liver of WSE in Table 2 are four to >10 times higher than concentrations reported by Lind et al. (2006) for herring (*Clupea harengus*) liver (0.064–0.17 µg/g d.w.) and perch (*Perca fluviatilis*) liver (0.029–0.048 µg/g d.w.) from the Baltic coast. A study from the southern Baltic indicates that there is in principle no biomagnification of trace metals, including lead, analyzed along successive trophic levels from potential prey to predators (Szefer, 1991). This implies that the substantially higher concentrations found in WSEs may primarily be a result of incidental ingestion of leaded ammunition. The metal deposition originating from long-range airborne pollution has decreased by 60% on average for southern Sweden during 1975–2000, and as much as 80–90% for lead, according to results from the repeated land moss surveys (Rühling and Tyler, 2001; Rühling and Tyler, 2004). This decline in lead exposure is also reflected in the concentrations in Swedish biota. Lind et al. (2006) reported significant reductions of lead concentrations in several species; the annual decrease in liver from herring and cod (*Gadus morrhua*) was 4.2–7.1% in 1981–2003, and 10–13% annually in perch liver and guillemot (*Uria aalge*) eggs 1995–2004. This is contrasted by the absence of a decrease in the WSE samples from the same time period. In Fig. 3 all samples with concentrations <6 ug/g are included. To investigate whether geographical differences could affect the result, a regression analysis was also carried out with only samples from the southern part of Sweden, however no trend was detected in either case. An explanation for this strikingly different picture in the eagles is probably the continuous exposure in this species to lead from ammunition in shot game and offal. It would be worthwhile to further investigate this hypothesis by studying other scavenging birds and mammals in Sweden.

A partial ban on the use of lead shot was introduced in Sweden on 1 July, 2002, for shallow wetlands. We found no significant difference between the distribution of lead poisoned WSE before and after this partial ban ($P>0.5$, $n_1 = 93$, $n_2 = 23$, chi-square-test). The temporal distribution of examined specimens is illustrated in Table 5. The partial ban on use of lead shot does not include hunting over deeper waters, including vast coastal areas, and it is notable that a majority of WSE with elevated lead concentrations were found on the coast

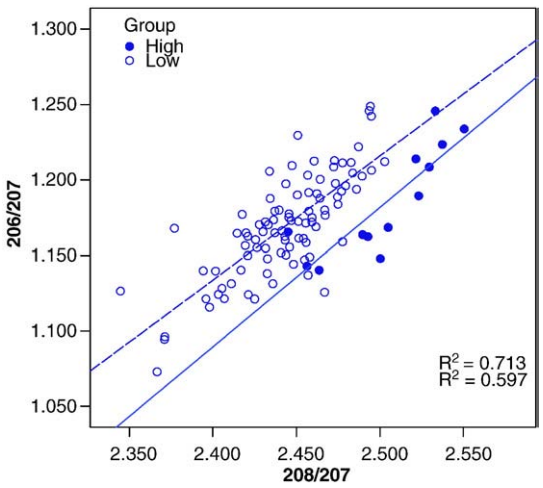

Fig. 5. Ratios of lead isotopes $^{206}Pb/^{207}Pb$ vs $^{208}Pb/^{207}Pb$ in kidney samples from specimens with high Pb-concentrations (>20 µg/g) and specimens with low (background) concentrations (<6 µg/g).

(Fig. 2). A study performed in the United States showed that 19% of all shot ducks and 15% of shot geese are never recovered (USFWS, 1986). National regulations prohibiting the use of lead ammunition for waterfowl hunting have been in effect since 1991 in the US and since 1996 in Canada and have led to dramatic declines in the average concentrations of lead in waterfowl tissues (Scheuhammer et al., 2008). These regulations for waterfowl hunting were in large part due to the hazard to another *Haliaeetus* species, the bald eagle, from secondary lead poisoning. Our study period includes only two years after the ban for shallow wetlands in Sweden was enforced and further investigations are necessary to evaluate its effects. The problem of lead poisoning of raptors is currently addressed under a new project in Germany, with the WSE as an umbrella species for other scavenging birds (Krone et al., 2009).

Clinical to lethal concentrations of lead was found in either liver or kidney from 22% of the WSEs examined, indicating that a large number of individuals are exposed to elevated concentrations of lead during their lifespan. It has recently been shown that incorporation of lead affects the mineralization of bone in Egyptian vultures (Gangoso et al., 2009). Other sub-lethal effects of lead shot recorded in birds include increased mortality in early embryos and hence decreased hatchability of eggs (Buerger et al., 1986), altered behavioral patterns, suppression of the immune response (Snoeijs et al., 2005) and altered blood serum chemistry (Hoffman et al., 1981). Exposure to lead shot poses an unnecessary stress on white-tailed sea eagle populations, given the availability of alternate non-toxic projectiles (Fig. 6).

4.6. Representativeness of samples

Lead poisoning caused by shots or bullet fragments has proved to be an important mortality factor for WSE in several other European countries. In this study about 14% of the examined dead eagles turned in to the SMNH had lead levels >20 μg/g, indicative of lead poisoning. It is of interest how representative this figure is for the mortality from lead intoxication in the population. Our material was chosen from the Environmental Specimen Bank at the SMNH by simply including all individuals from which both liver and kidney samples were available. This could lead to a certain bias in the material since the way the eagle died in some cases can have an impact on the status of the carcass upon arrival at the museum. It is probable that finds of dead eagles are biased in favor of victims from man-related traumas such as train- and road-kills, collisions with wires, electrocutions etc. Birds that are lead poisoned become anorexic (Pattee et al., 1981, 2006), and food exploratory behavior is decreased (Krone et al., 2009). Individuals that eventually die from lead poisoning may be under-represented in this material, since animals that sicken will often try to hide away (Krone et al., 2009). This might lead to less frequent recovery of lead poisoned carcasses, and also to increased time until discovery which causes decomposition of the body. This in turn will make the sampling of liver and kidney tissue impossible, which would also lead to an under-representation of lead poisoned individuals in this sample. Furthermore, a number of suspected WSE victims of lead intoxication were transferred from the museum to the National Veterinary Institute for investigation during the present study period, and are not included in this study. Out of 60 WSE that were submitted to the National Veterinary Institute 1986–2007 at least 23% had died from acute lead poisoning (Axelsson, 2009). Taken together, these circumstances imply that the magnitude presented in this study of lead as a mortality factor in Swedish WSE is an under-estimate.

Table 5
Temporal distribution of lead poisoned white-tailed sea eagles 1981–2004.

Time period	Sample size	Lead poisoned (numbers)	Lead poisoned (percentage)
1981–1990	28	3	10.7
1991–1995	24	3	12.5
1996–1999	20	2	10.0
2000–2002	21	4	19.0
1981–2002	*73*	*12*	*16.4*
2003–2004	23	4	17.4

A ban of lead shot ammunition was enforced for shallow wetlands in July 2002. The data from four time periods 1981–2002 is summarized in italics.

Fig. 6. Swedish adult female white-tailed sea eagle with lead poisoning, 48 h before death. Photo B.Helander.

5. Conclusions

This study shows that the use of lead in ammunition poses a threat to white-tailed sea eagles feeding on shot game or offal. Given the general behavior of selective hunting in raptors, this problem may include other species so far not investigated. The removal of offal from all shot game and a changeover from lead ammunition to non-poisonous alternatives need to be implemented in order to prevent mortality from lead in raptorial and scavenging birds and mammals.

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