Science of the Total Environment xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

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

Bone lead levels and lead isotope ratios in red grouse from Scottish and Yorkshire moors

Vernon G. Thomas [a,*], Anton M. Scheuhammer [b], Della E. Bond [b]

[a] Department of Integrative Biology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[b] Metals Toxicology Laboratory, National Wildlife Research Centre, Carleton University, Ottawa, Ontario, Canada K1A 0H3

ARTICLE INFO

Article history:
Received 14 November 2008
Received in revised form 1 February 2009
Accepted 2 February 2009
Available online xxxx

Keywords:
Lead
Bones
Isotope ratios
Red grouse
Shot
Toxicity

ABSTRACT

Leg and foot bones of adult and juvenile red grouse (*Lagopus lagopus scoticus*) were collected from hunter-shot birds on two Scottish estates (Glendye and Invermark) and one Yorkshire estate in September, 2003. The lead content of bones was measured by atomic absorption spectrophotometry, and corresponding stable lead isotopes ($Pb^{204,\ 206,\ 207,\ 208}$) by inductively coupled plasma mass spectrometry. At the Glendye (N=111) and Invermark (N=85) estates, relatively few birds (5.4% and 3.5%, respectively) had highly elevated bone lead concentrations (>20 µg/g dry weight). In bones of these highly exposed birds, a combination of Pb^{206}:Pb^{207} and Pb^{208}:Pb^{207}ratios was consistent with ingestion of lead gunshot available in Europe. By contrast, Yorkshire grouse experienced a high incidence (65.8%) of bone lead >20 µg/g. The Pb^{206}:Pb^{207} and Pb^{208}:Pb^{207}ratios in bones of these highly exposed birds were consistent with a combined exposure to ingested lead gunshot and lead from galena mining in the region. Lead isotope ratios also indicated that lead from UK gasoline combustion and fallout from atmospheric particles was not a likely source of elevated lead in bones of either Scottish or Yorkshire grouse. Suggested management options for the three moors include adopting nontoxic shot for all game shooting on the estates, allowing heather (*Calluna vulgaris*) vegetation to grow tall in lead shot fall-out zones to reduce physical access to high densities of lead shot already present, and provision of calcareous grit across moors to reduce lead assimilation from all ingested sources of lead.

1. Introduction

Lead toxicosis in birds due to the ingestion of discharged lead gunshot has been known for over a century (Calvert, 1876). While regulations to remediate this problem were initially based on waterfowl studies, more recent research has also revealed a substantial incidence of lead shot ingestion and toxicosis among upland game bird species (mainly Galliformes) and their predators, both in North America and Europe (Pain et al., 1993; Kendall et al., 1996; Clark and Scheuhammer, 2003; Scheuhammer et al., 2003; Butler et al., 2005; Fisher et al., 2006). Identification of shot as a primary source of lead exposure in hunted avian species has been based in part on the ratios of stable lead isotopes in tissues of lead-exposed birds, and their similarity with ratios characteristic of metallic shot, versus a dissimilarity with background lead in the birds' general environment (Scheuhammer and Templeton, 1998; Scheuhammer et al., 2003; Pain et al., 2007).

Red grouse (*Lagopus lagopus scoticus*) is a wild non-migratory species found on the heather-dominated (*Calluna spp*) moors of the UK. They are a highly-valued game bird and their habitat on moors has been managed for well over a century to promote high population numbers for shooting (Thirgood et al., 2000). The moors have thus received much lead shot deposition over time. Lead shot is still allowed for this type of bird shooting in the UK. The lead in the vicinity of the traditional grouse shooting sites has not been deliberately removed nor covered, so there is a considerable potential for grouse to ingest lead shot, especially since this species uses grit to assist physical digestion of its food. Given the concerns about other upland galliform species ingesting lead shot as though it were grit (Butler et al., 2005), and concerns about birds of prey feeding on lead poisoned birds (Pain et al., 1993, 2005; Mateo et al., 2001), this study was performed to determine the degree of lead exposure of wild red grouse. A bird's exposure to ingested lead can be determined by sampling various tissues. Levels of lead in the blood and liver provide an indication of recent exposure and potential lethality of the accumulated lead (Franson, 1996; Pain, 1996). Lead in the bones has a long biological half-life (see Harrison and Laxen (1981) for human bone) and provides an indication of a bird's chronic exposure to lead over its lifetime (Tejedor and Gonzalez, 1992). Red grouse taken by shooters are sold to the restaurant trade, thus limiting the soft tissue material that can be taken from a bird for metal analysis. However, the bone of the lower leg is often available for analysis. The studies of Gjerstad and Hanssen (1984) and Fimreite (1984) indicate that captive willow ptarmigan

* Corresponding author. Tel.: +1 519 824 4120x52738; fax: +1 519 767 1656.
E-mail address: vthomas@uoguelph.ca (V.G. Thomas).

0048-9697/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.scitotenv.2009.02.003

(*Lagopus lagopus lagopus*) dosed orally with different amounts of lead gunshot can die of induced lead toxicosis, and that the amount of lead deposited in the leg bones is dose-related. However, although the majority of the body burden of lead may reside in bone, the bone lead level, alone, does not accurately indicate the potential for lead toxicity (Pain, 1996).

The habitats of red grouse have received, in addition to gunshot, varying amounts of lead from aerial deposition related to lead mining and smelting, leaded gasoline combustion, and the burning of coal in power stations (Lee and Tallis, 1973; Sturges and Harrison, 1986; Farmer et al., 1999; Hudson-Edwards and Macklin, 1999; Bellis et al., 2004). These are superimposed upon a background of indigenous lead in the soil. Some of the grouse shooting moors in Yorkshire are located near sites of historic lead mining and smelting that flourished from post-mediaeval times to the beginning of the 20th century and which still contribute lead to the local environment (Macklin et al., 1997; Hudson-Edwards and Macklin, 1999; Hudson-Edwards et al., 1999; Gill, 2001; Dennis et al., 2003). By contrast, grouse moors from north-eastern Scotland have not been associated with this local form of lead smelting activity, but have been subject to a considerable atmospheric deposition of lead (including smelting and coal burning) from other more distant locations (Farmer et al., 2005). The task is then to apportion the lead in the avian body to these different anthropogenic and natural background sources. A number of researchers have used the relative abundance of stable lead isotopes (Pb^{204}, Pb^{206}, Pb^{207}, Pb^{208}) to help distinguish contributions of lead from different sources to the abiotic and biotic compartments of the environment (Sugden et al., 1993; Farmer et al., 2000, 2002; Bellis et al., 2004; Komárek et al., 2008; Gulson, 2008), including lead in avian bones or livers (Scheuhammer and Templeton, 1998; Scheuhammer et al., 2003; Svanberg et al., 2006; Pain et al., 2007). By determining both the total lead concentrations and the lead isotope ratios in red grouse bones, we proposed to identify the source(s) of lead present in a sample of hunter-killed grouse from 3 different locations in the UK.

2. Materials and methods

2.1. Sample collection

Legs and feet of hunter-killed red grouse were obtained from two locations in Scotland, and one location in Yorkshire, UK. The Scottish samples were obtained from the moors of the Dalhousie, Invermark Estate, Angus (56° 54′N; 2° 56′W) and the Gladstone, Glendye Estate, Aberdeenshire (57° 1′N; 2° 37′W). The Yorkshire samples were from an anonymous private estate in Swaledale. All three estates had experienced shotgun shooting with traditional lead shot for over a century. Shooting occurred from fixed-position shooting butts (driven shooting), and also where birds were found across the moors (walked-up shooting) (Thirgood et al., 2000; Ferrandis et al., 2008). All samples were obtained during the shooting season in September, 2003. The two Scottish moors were not associated with the mining of lead ores at any time, but the Yorkshire estate was near areas where lead ore mining and smelting had been practiced historically (Fig. 2 in Dennis et al., 2003).

After a day's shooting, the birds were collected and categorized as either hatch year (juvenile), or after hatch year (adult), based on the degree of ossification of the lower mandible and skull (Linduska, 1945). It is assumed that the collections of grouse represent a random, representative sampling of the local, free-flying, population. Ethier et al. (2007) showed that the tarso-metatarsus was a suitably representative bone to use for sampling the cumulative lead exposure of birds. The entire tarso-metatarsus and foot was removed unilaterally from each bird, using stainless steel scissors, and inspected to determine if it had been damaged by impact from shot, or if it contained obvious shot pellets. Only undamaged tarsi and feet were sampled. No other tissues were taken from the birds. The entire collection of lower legs was placed in plastic bags and held frozen at −30 °C until analyzed.

2.2. Preparation of samples for analysis

Bone lead levels and lead isotope ratios were measured at the National Wildlife Research Centre, (Environment Canada, Ottawa, Ontario, Canada). The grouse legs were thawed and the tarso-metatarsal bone removed from the foot and cleaned of all adhering tissue using a stainless steel scalpel. The collection of the lower leg from the Yorkshire grouse had resulted in the tarso-metatarsus being cut too far towards the foot to provide a large enough sample of bone for lead analysis. For these birds, the middle phalanx, the largest of the remaining bones, was removed for subsequent lead analyses. To test the comparability of lead in different foot bones, 11 samples had both the tarso-metatarsus and one phalanx removed and analyzed for comparison. Twenty eight further samples had two different phalanges removed from the same foot for comparison. If on closer inspection, it appeared that a bone had been injured by a fragment of shot, that bone was discarded. All isolated bones were placed in acid-washed, polypropylene vials and stored frozen until analyzed. A total of 234 separate samples were obtained, finally, from the three UK sites.

All bone samples were freeze-dried for 48 h and the moisture content was recorded. Standard Reference Materials (SRMs) were prepared in the same manner as the bones. The SRMs included with each digestion set were NIST[1] RM 1486 bonemeal and NRCC DOLT-3[2]. Because the certified levels of lead for both the bonemeal and the DOLT-3 were too low for flame Atomic Absorption Spectrophotometry (AAS), some bonemeal samples and all DOLT-3 were spiked with lead solutions. Either 5 µg or 10 µg of lead was added in 0.5 mL volume made by diluting a Fisher Scientific® certified 1000 µg/mL lead solution.

Prior to digestion, 0.5 mL reverse osmosis (RO) water was added to each sample and 0.5 mL of spike solution was added to the SRMs. Nitric acid (70%, Fisher Trace Metals Grade®) was added to all at a minimum of 0.5 mL/0.1 g dry weight (sample weights ranged from 0.1 to 0.4 g). The tubes were left loosely capped overnight, gradually heated to 100 °C, and held at that temperature for 3 h. After cooling overnight, digests were adjusted to a known volume (usually 5 mL) with RO water, transferred to capped, acid-washed glass tubes and held until analysis.

2.3. Chemical analyses of lead and lead isotopes

Total lead was determined in digests by standard flame AAS using a Perkin Elmer Analyst 800 (Woodbridge, ON, Canada), and digests having <0.3 µg/mL were reanalyzed by Graphite Furnace AAS (GFAAS). The average Theoretical Method Detection Limit (TMDL) by flame AAS was 0.06 µg/mL, corresponding to a detection limit in bone of ≈1.2 µg/g. The average GFAAS TMDL was 0.0005 µg/mL, corresponding to a detection limit in bone of ≈0.01 µg/g.

For flame AAS, overall recovery of lead from all SRMs combined was 89.3 ± 8.6% ($N=55$). For GFAAS, recovery of lead from NIST 1486 bonemeal was 89.2 ± 4.2% ($N=13$). Overall analytical variability of duplicate sample determinations (flame AAS and GFAAS combined) averaged 9.9% Relative Standard Deviation (RSD) ($N=24$).

All lead concentrations are expressed on a dry weight basis, unless otherwise specified.

Stable lead isotopes (^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb)[3] were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) using a Perkin Elmer Elan9000, equipped with an AS-93plus autosampler. Instrument parameters were as follows: timing—75 sweeps/reading, 1 reading/replicate, 3 replicates/sample, peak hopping scan mode, total sample measurement time = 8.4 min; signal processing—dual detector mode, autolens off, average spectral peak processing, and average

[1] National Institute of Standards and Technology.
[2] National Research Council of Canada dogfish liver.
[3] The 204, 206, 207, and 208 isotopes of lead comprise approximately 1.4%, 24.1%, 22.1% and 52.4%, respectively, of naturally-occurring lead.

Please cite this article as: Thomas VG, et al, Bone lead levels and lead isotope ratios in red grouse from Scottish and Yorkshire moors, Sci Total Environ (2009), doi:10.1016/j.scitotenv.2009.02.003

signal profile processing. The instrument was optimized before each set of analyses. Blanks and a standard solution were read every 8 samples for calibration correction. This calibration standard solution was made by digesting NIST SRM 981 lead metal (certified for isotopic composition) in nitric acid, following the same procedure as for the bones, and diluting the resulting digest to ≈10 μg/mL. Based on these periodic calibration readings, mass discrimination correction factors were automatically calculated for ^{204}Pb, ^{206}Pb, and ^{208}Pb by the Elan9000, and all isotopes were automatically corrected to ^{207}Pb to account for mass bias and instrument drift. Correction factors were 0.9814±0.0190, 1.0013±0.0160, and 0.9919±0.0099 for ^{204}Pb, ^{206}Pb and ^{208}Pb respectively (N=71). This same calibration standard solution was also read as a sample at a rate of 10% in order to verify the analytical accuracy of the isotope abundances. Data for ^{204}Pb were also corrected for possible interference by ^{204}Hg, by monitoring ^{202}Hg and correcting for ^{204}Hg using the natural ^{204}Hg/^{202}Hg abundance ratio. All samples were diluted at least 50-fold to fall within the recommended matrix concentration, and to ensure that counts would be within an acceptable range. Acceptable ranges of counts corresponded to 0.27–25 μg Pb/L, depending on the isotope. The accuracy of isotope ratios was determined to be greatest within this concentration range. When analyses failed to meet these criteria, new dilutions were prepared and analyses repeated. Only when the analytical variability was <1.0% RSD for all isotope ratios, and counts were within the indicated limits, were results accepted.

In addition to the NIST SRM 981, accuracy of our bone isotope ratio analyses were tested using NIST RM 1486 bonemeal digests. Although NIST RM 1486 is not certified for stable lead isotopes, values published by Aung et al. (2004) and Yoshinga (1996) were averaged and used for comparison with our results as a measure of accuracy. Isotope ratios determined for NIST 981 samples (N=60) varied by ≤1.4% of the expected values for all ratios, and on average were within 0.13% of expected values. For NIST 1486 bone meal, all ratios were within a maximum of 1.8% of the expected values, and on average were within 0.50% of expected values. Analytical precision was within 0.31% RSD for all ratios, for all samples and SRMs.

2.4. Statistical analyses of data

Statistical analyses were conducted using SAS (Version 9.1, SAS Institute, Cary, NC, USA). Data were first tested for normality using the method of D'Agostino et al. (1990). An ANOVA, using the GLM procedure, or a T-test, using the T-TEST procedure, was used to test normally distributed variables. Non-normal data were log or square root transformed to attempt normalization. If transformation failed to normalize data, variables were tested using the NPAR1WAY procedure with the Wilcoxon option. This test uses Wilcoxon scores, which are the actual ranks of the data, in the one-way ANOVA statistic to produce the Kruskal–Wallis test. Regressions were tested using the REG procedure. Statistical significance was chosen as P≤0.05.

2.5. Sources of information on the isotopic composition of environmental lead in Scotland and Yorkshire

Data on the ^{206}Pb:^{207}Pb ratio for leaded gasoline used in eastern Scotland (Edinburgh) were taken from Sugden et al. (1993) for 1989–1991, and from Farmer et al. (2000) for 1989–1998. These data sets were presumed to be representative of the ^{206}Pb:^{207}Pb ratios in gasoline used in the vicinity of Glendye and Invermark estates. The ^{206}Pb:^{207}Pb ratios for rainwater falling at Tarland and Glensaugh, both near the two Scottish grouse moors, were also taken from Farmer et al. (2000) for the period December 1997–January 1998, and were assumed to be representative of rain falling at the Glendye and Invermark estates. The same study included lead isotope ratios for atmospheric particulates collected at Banchory, Scotland, approximately 30–40 km from the grouse collection sites. Farmer et al. (2002) presented data on three lead isotope ratios in *Sphagnum* mosses from northern and eastern Scotland, and showed how the lead content of such vegetation has changed over time. The lead isotope ratios for other environmental sources of lead in Scotland, including soil and coal, were taken from Sugden et al. (1993) and Farmer et al. (1999, 2000, 2005). Data on the concentrations of lead and lead isotope ratios in soil and parts of trees from South Yorkshire and Derbyshire, UK were derived from Bellis et al. (2002, 2004), together with the isotope ratio for Derbyshire galena (PbS), the principal source of the regional lead mining industry. Lead isotope ratios for galena from two North Yorkshire and 23 Derbyshire mine sites were taken from Rohl (1996). The lead isotope ratio for gasoline from Edinburgh was considered to represent gasoline used in the Yorkshire region. The ratios of lead isotopes in lead gunshot in major brands of cartridges of known European manufacture were taken from Stevenson (2002), and were also measured in several pieces of shot found embedded in the feet of red grouse used in the present study.

3. Results

3.1. Deriving the relationship between levels of lead in phalanges and tarsi

For 12 adult birds collected from Glendye, lead levels were measured in both the tarso-metatarsus and the mid phalanx of the same foot. The mean lead levels were 11.8±5.0 (SD) μg/g, and 9.0±3.5 μg/g in the tarso-metatarsus and mid phalanx, respectively. A Pearson correlation between the two variables revealed the following significant relation:

Phalanx lead = 1.658 + 0.6206 (tarso-metatarsal lead); (r=0.884; P<0.01).

For the Yorkshire grouse, this relationship was used to convert phalange lead levels to tarso-metatarsal lead levels, to enable direct comparison of lead in bones of grouse from all 3 estates.

3.2. Lead levels in bones of grouse from different estates

Our interpretation of bone lead levels is based on Pain (1996), who suggested that, in general, avian bone lead levels <10 μg/g reflect background lead exposure; 10–20 μg/g reflect higher-than-background exposure; and >20 μg/g denote highly elevated exposure. While the majority of grouse bones sampled at the Invermark and Glendye estates demonstrated background (<10 μg/g) lead levels, there was a markedly greater average accumulation of lead in the bones of grouse (both adult and juvenile) from the Yorkshire estate (Tables 1, 2). Proportionately more adults from the Invermark and Glendye estate had elevated bone lead levels compared to juveniles, but this was not the case for the Yorkshire site where a high proportion of both adult and juvenile grouse had bone lead levels above background (Table 1). Using the exposure categories of Pain (1996), 84.2% of birds (adult and juvenile combined) from the Yorkshire estate were lead-exposed. Corresponding values for the Invermark and Glendye estates were 16.5% and 30.6%, respectively (Table 1). Fewer grouse from the two Scottish estates had

Table 1
Numbers of juvenile and adult red grouse from Glendye, Invermark, and Yorkshire estates having bone lead levels in 3 exposure categories: Low (background), Elevated, and Very elevated (based on Pain, 1996)

Population	Bone lead level		
	Low ≤10 μg/g	Elevated 10–20 μg/g	Very elevated >20 μg/g
Glendye adults (58)	31	24	3
Glendye juveniles (53)	46	4	3
Invermark adults (45)	33	10	2
Invermark juveniles (40)	38	1	1
Yorkshire adults (8)	2	0	6
Yorkshire juveniles (30)	4	7	19

Sample sizes are given in parentheses.

Please cite this article as: Thomas VG, et al, Bone lead levels and lead isotope ratios in red grouse from Scottish and Yorkshire moors, Sci Total Environ (2009), doi:10.1016/j.scitotenv.2009.02.003

Table 2
Mean and range of lead levels in the leg bone of red grouse from two Scottish and one Yorkshire moor

	[Pb] in bone (μg/g dry weight)			
	All birds		Birds with elevated Pb	
Moor	Adults	Juveniles	Adults	Juveniles
Invermark	8.52±4.73[a] Range 2.65–24.28 (N=45)	6.01±7.88[b] Range 2.07–53.06 (N=40)	15.04±4.39[f] Range 10.74–24.28 (N=12)	32.17±29.53[fg] Range 11.29–53.06 (N=2)
Glendye	10.65±5.66[c] Range 2.59–35.47 (N=58)	7.59±7.37[d] Range 1.81–47.85 (N=53)	14.48±6.06[f] Range 10.01–35.47 (N=27)	21.93±12.72[g] Range 12.68–47.85 (N=7)
Yorkshire	85.35±114.7[e] Range 8.58–339.2 (N=8)	75.28±107.9[e] Range 1.13–444.2 (N=30)	110.9±123.6[h] Range 20.79–339.2 (N=6)	86.03±112.2[gh] Range 10.40–444.2 (N=26)

Values are μg Pb/g bone, dry weight, ±1 SD. Results for the Yorkshire moor are transformed from lead levels in the phalanges. The threshold value of 10 μg/g bone was chosen as representative of elevated lead exposure. Sample sizes are given in parentheses. Data were analyzed separately for birds with elevated bone-lead levels and then for all birds combined. Different superscripts indicate significant differences (P<0.05) between adults and juveniles of a given region, and birds of given age among regions.

highly elevated (>20 μg/g) bone lead levels compared to birds from the Yorkshire estate (Table 1).

When grouse with elevated (>10 μg/g) bone lead levels were compared by location, adult grouse from the two Scottish estates had similar mean bone lead levels (15.0 and 14.5 μg/g, respectively), as did juvenile grouse from the same estates (32.2 and 21.9 μg/g, respectively) (Table 2). The mean bone lead levels of adult and juvenile grouse with elevated (>10 μg/g) lead accumulation from the Yorkshire site were much higher than from the two Scottish estates, but the difference was statistically significant only for adults (P<0.001) (Table 2). The highest bone lead levels measured in grouse from the Invermark and Glendye estates (53.1 μg/g and 47.9 μg/g, respectively) were far lower than the highest levels found in juvenile (444.2 μg/g) and adult (339.2 μg/g) grouse from the Yorkshire estate (Table 2).

3.3. Lead isotope ratios of common environmental components of Scotland and Yorkshire

Table 3 presents the ^{206}Pb:^{207}Pb ratios for different environmental components of the Scottish and Yorkshire regions. Bone ^{206}Pb:^{207}Pb ratios for grouse with low lead exposure from the Yorkshire moor coincided well with the ratio for regional soils (~1.15–1.16). The ^{206}Pb:^{207}Pb ratio for gasoline (a major source of environmental lead near urban industrialized areas for many years) fell far below the ratios encountered for grouse bones at any level of exposure from any estate, as did the ratio for atmospheric particles measured in eastern Scotland (Table 3 and Figs. 1 and 2). The ^{206}Pb:^{207}Pb ratios for Scottish rainwater, organic soil, and coal overlapped with the bone ^{206}Pb:^{207}Pb ratios for the two Scottish estates and also that of commonly used European lead gunshot. The ^{206}Pb:^{207}Pb ratios for Yorkshire regional galena (PbS) and coal fell above the ratios observed for almost all of the grouse bones (Table 3, Figs. 1 and 2). The range of ^{206}Pb:^{207}Pb found in gunshot of European manufacture, although varying widely (1.08–1.17), overlapped with the range of values found in bones of lead-exposed adult grouse from all three locations (Table 3, Fig. 2). A wide range of ^{206}Pb:^{207}Pb ratios can be found in the shot used on a given day at any moor. For example, two pieces of lead shot recovered from the legs of two grouse taken for the present study at the Invermark estate on the same day had ^{206}Pb:^{207}Pb ratios of 1.118 and 1.157.

Bone lead levels for adult and juvenile grouse were regressed against their corresponding ^{206}Pb:^{207}Pb ratios to determine whether, as the lead content of the bone increased beyond background levels, a different source of lead with a different isotope ratio could be distinguished (Fig. 1). Only adult grouse from the Yorkshire estate demonstrated a significant relationship (R^2=0.5; p<0.05). This indicates that higher concentrations of bone lead tend to come from sources of lead having a higher ^{206}Pb content relative to the normal background soil and plant material from this site. Bone lead concentrations were also regressed against ^{204}Pb:^{207}Pb and ^{208}Pb:^{207}Pb, but none of these relationships was significant, except the regression using ^{204}Pb:^{207}Pb for juvenile birds from the Glendye estate (R^2=0.100, P=0.021). Note that the regression that was significant for the variable ^{206}Pb:^{207}Pb (adult grouse from the Yorkshire estate) became non-significant when the ^{204}Pb:^{207}Pb variable was used.

When bone lead concentrations for adults and juveniles were combined and regressed against ^{206}Pb:^{207}Pb ratios, a significant negative relationship was observed for Glendye, and a significant positive relationship was observed for Yorkshire (Fig. 2). Extending this regression to a background bone lead level of 1 μg/g coincides with an isotope ratio of approximately 1.14–1.15. Fig. 1A for grouse of the Invermark estate reveals an intercept at a similar lead isotope ratio value. For the Glendye and Invermark estates, this ratio coincides with recent isotope signatures for rain, organic soil, and coal (Table 3), and for the Yorkshire estate, the

Table 3
Lead isotope ratios for different environmental components in the vicinity of the Scottish and Yorkshire moors

Location and component	Mean ratio (^{206}Pb:^{207}Pb)	Source reference
Scotland		
Gasoline:		
Edinburgh, 1989–1998	1.076±0.011	Farmer et al. (2000)
Edinburgh, 1989–1991	1.082±0.024	Sugden et al. (1993)
Atmospheric particles:		
Edinburgh, 1990–1991	1.092±0.011	Sugden et al. (1993)
Banchory, 1985	1.107±0.007–1.113±0.003	Farmer et al. (2000)
Rainwater:		
Tarland 1997–98	1.144±0.0079–1.160±0.0035	Farmer et al. (2000)
Glensaugh 1997–98	1.144±0.008	Farmer et al. (2000)
Organic soil:		
Glensaugh, 2002	1.144±0.001–1.169±0.003	Farmer et al. (2005)
Pine needles:		
Darnaway, NE Scotland, 1985	1.111±0.005–1.117±0.009	Farmer et al. (2000)
Coal:		
Edinburgh	1.185±0.002	Sugden et al. (1993)
Diverse Scottish mines	1.167±0.0008–1.213±0.0031	Farmer et al. (1999)
Yorkshire–Derbyshire region		
Coal:		
Diverse mines in Yorkshire and Nottinghamshire	1.182±0.0022–1.191±0.0023	Farmer et al. (1999)
Soil:		
Longshaw, Derbyshire, 2003	1.152±0.001	Bellis et al. (2004)
Swinton, Yorkshire, 2003	1.157±0.002	Bellis et al. (2004)
Galena (PbS):		
Derbyshire mines	1.180±0.002	Bellis et al. (2004)
Derbyshire mines: mean of 23 mines	^{206}Pb:^{207}Pb=1.1813 ^{208}Pb:^{207}Pb=2.4585	Rohl (1996)
North Yorkshire mines, mean of 2 mines	^{206}Pb:^{207}Pb=1.1882 ^{208}Pb:^{207}Pb=2.4630	Rohl (1996)
Tree-bark surface (indicative of aerial deposition):		
Sheffield, Notts., 1998	1.130±0.001	Bellis et al. (2002)
Longshore, Derbs., 1998	1.122±0.002	Bellis et al. (2004)
Longshore, 2003	1.121±0.002	Bellis et al. (2004)
Swinton, Yorks., 1998	1.113±0.001	Bellis et al. (2004)
Swinton, 2003	1.113±0.002	Bellis et al. (2004)
European gunshot:		
European manufacturers*	Mean 1.1468±0.0294, range 1.08–1.17	Stevenson (2002)

*Mean of 9 values from shot made in UK, Spain, Czech Republic, France, Poland, and Hungary, and available for use in UK.
Values are the mean±1 SD.

Please cite this article as: Thomas VG, et al, Bone lead levels and lead isotope ratios in red grouse from Scottish and Yorkshire moors, Sci Total Environ (2009), doi:10.1016/j.scitotenv.2009.02.003

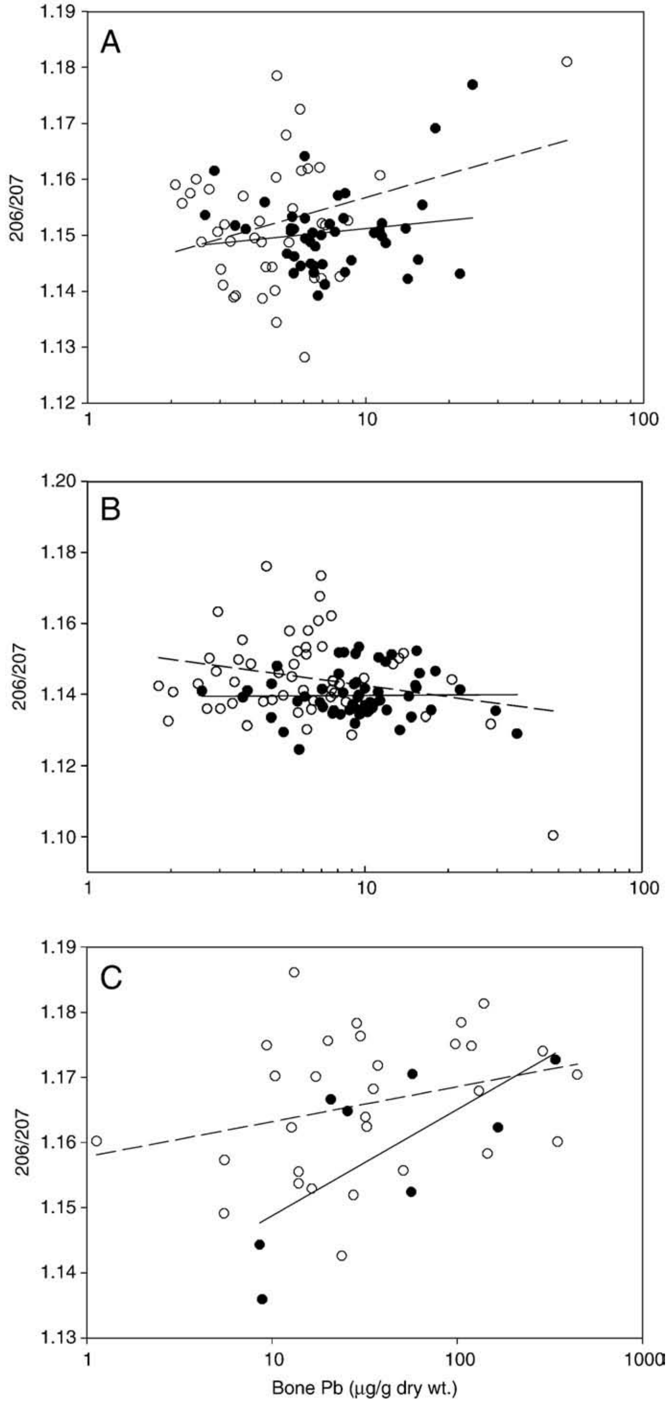

Fig. 1. Relationships between bone lead and bone ^{206}Pb:^{207}Pb ratio for adult (•) and juvenile (○) red grouse from three locations. A—Glendye estate; adults (R^2=0.000. P=0.916); juveniles (R^2=0.058, P=0.084). B—Invermark estate: adults (R^2=0.022, P=0.329); juveniles (R^2=0.092, P=0.057). C—Yorkshire estate: adults (R^2=0.500, P=0.050); juveniles (R^2=0.001, P=0.0869).

isotopic signature for soil (Table 3). These could be considered to be the natural environmental background isotope values on which other sources of lead are superimposed. However, the range of isotope ratios for commonly-used lead shot also encompasses the 1.14–1.15 ratio.

Each of the mean lead ratios for ^{204}Pb:^{207}Pb, ^{206}Pb:^{207}Pb, and ^{208}Pb:^{207}Pb was compared against the mean levels of lead in bones of adult and juvenile grouse from the three locations that demonstrated background, elevated (>10 μg/g bone) and very elevated (>20 μg/g bone) levels (Table 4). This was also performed to determine if the mean isotope ratio changed with increasing bone lead levels. For both the Glendye and Invermark estates, there was no significant difference among the means, whichever lead isotope ratio was selected. For grouse from the Yorkshire estate, the only significant difference was between the means of background and very elevated lead levels of adult grouse measured by the ^{206}Pb:^{207}Pb ratio (Table 4). This specific result confirms the significant regression observed in the adult grouse from the Yorkshire estate generated from the ^{206}Pb:^{207}Pb ratios (Fig. 1C). The three different lead isotope ratios in the European shot (taken from Stevenson, 2002), coincided with the ranges of all three bone lead category means (Table 4).

To further test for differences in sources of lead exposure for grouse with highly elevated lead concentrations,^{208}Pb:^{207}Pb ratios for bones with highly elevated lead (>20 μg/g) were plotted against their corresponding ^{206}Pb:^{207}Pb ratios. For grouse from the two Scottish estates, almost all highly exposed birds fell within the boundaries measured for European lead shot (Fig. 3). For the Yorkshire grouse, some bone values coincided with European lead shot, but there was additionally a pronounced grouping of individuals outside the lead shot boundaries, but closer to the value for galena (Fig. 3). From this analysis, it can be conservatively estimated that 3 of 85 (3.5%) grouse from the Invermark estate and 5 of 111 (4.5%) grouse from the Glendye estate probably accumulated their very elevated bone lead levels from ingestion of lead gunshot. Similarly, a minimum of 7 of 38 (18.4%) grouse from the Yorkshire probably ingested lead shot; however a higher proportion (81.6%) appear to have accumulated elevated bone lead from exposure to galena, probably in the soil.

4. Discussion

4.1. Interpreting sources of lead in red grouse bones

All the birds used in this analysis were part of local, free-flying, populations engaged in apparently normal behaviour at the time of collection. However, there was considerable exposure to lead, especially in some grouse from both Scottish estates, and both adult and juvenile grouse from the Yorkshire estate. Red grouse, like other birds, are exposed to environmental lead from a number of different sources, all of which contribute to current bone lead levels. The major source of lead for birds in uncontaminated environments is the diet. Additionally, ground-feeding birds frequently ingest a significant amount of soil while

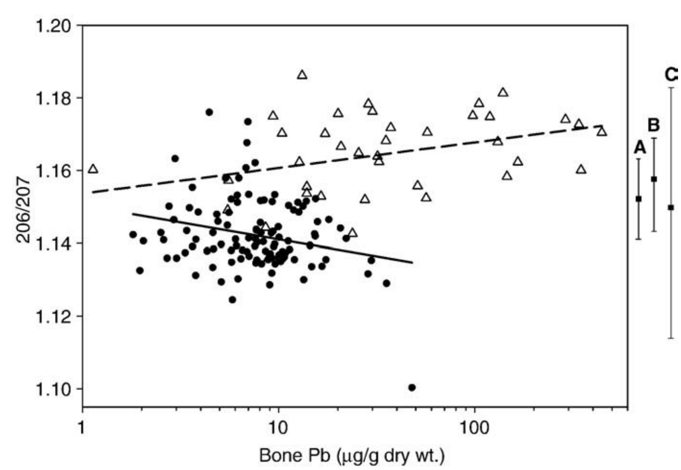

Fig. 2. Relationships between bone lead and ^{206}Pb:^{207}Pb ratios for adult and juvenile grouse combined from the Glendye (•, solid line, N=111) and Yorkshire (Δ, dashed, line, N=38) estates. Glendye: R^2=0.06, P<0.05; Yorkshire: R^2=0.13, P<0.05; Invermark (data not shown): R^2=0.035, P>0.05 (ns). Extension of the regression lines to a bone lead level of 1 μg/g gives a ^{206}Pb:^{207}Pb ratio ~1.15 for both estates, similar to the mean for rainwater and probably characteristic of background environmental lead in these areas. Also included for reference, from Table 3, are approximate ^{206}Pb:^{207}Pb means ± SD for: A—Scottish rainwater; B—Glensaugh soil; and C—lead shot available in Europe.

Please cite this article as: Thomas VG, et al, Bone lead levels and lead isotope ratios in red grouse from Scottish and Yorkshire moors, Sci Total Environ (2009), doi:10.1016/j.scitotenv.2009.02.003

Table 4
^{204}Pb: ^{207}Pb, ^{206}Pb: ^{207}Pb, and ^{208}Pb: ^{207}Pb in analyzed European gunshot and bones from adult (Ad) and juvenile (Juv) red grouse inhabiting the three estates in 3 lead exposure categories: 1 = Low Pb (<10 µg/g); 2 = Elevated Pb (10–20 µg/g): 3 = Very elevated Pb (>20 µg/g)

Isotope ratio	European shot	Age	Glendye bone			Invermark bone			Yorkshire bone		
			1	2	3	1	2	3	1	2	3
^{204}Pb: ^{207}Pb	0.0644 ± 0.0002	Ad	0.0643 ± 0.0002 (N = 35)	0.0643 ± 0.0002 (N = 29)	0.0642 ± 0.0003 (N = 8)	0.0645 ± 0.0004 (N = 33)	0.0646 ± 0.0005 (N = 10)	0.0647 ± 0.0002 (N = 2)	0.0644 ± 0.0001 (N = 2)	0.0642 (N = 1)	0.0642 ± 0.0003 (N = 9)
		Juv	0.0645 ± 0.0004 (N = 46)	0.0645 ± 0.0003 (N = 4)	0.0643 ± 0.0001 (N = 3)	0.0645 ± 0.0004 (N = 38)	0.0647 (N = 1)	0.0649 (N = 1)	0.0643 ± 0.0002 (N = 13)	0.0641 ± 0.0003 (N = 11)	0.0642 ± 0.0003 (N = 35)
^{206}Pb: ^{207}Pb	1.1468 ± 0.0294	Ad	1.1398 ± 0.0002 (N = 35)	1.1396 ± 0.0002 (N = 29)	1.1376 ± 0.0003 (N = 8)	1.1497 ± 0.0056 (N = 33)	1.1515 ± 0.0071 (N = 10)	1.1600 ± 0.0240 (N = 2)	1.1444 ± 0.0001* (N = 2)	1.1623 (N = 1)	1.1624 ± 0.0003* (N = 9)
		Juv	1.1460 ± 0.0004 (N = 46)	1.1459 ± 0.0003 (N = 4)	1.1253 ± 0.0001 (N = 3)	1.1512 ± 0.0105 (N = 38)	1.1607 (N = 1)	1.1810 (N = 1)	1.1644 ± 0.0002 (N = 13)	1.1649 ± 0.0003 (N = 11)	1.1665 ± 0.0003 (N = 35)
^{208}Pb: ^{207}Pb	2.4120 ± 0.0292	Ad	2.4146 ± 0.0059 (N = 35)	2.4162 ± 0.0059 (N = 29)	2.4174 ± 0.0061 (N = 8)	2.4206 ± 0.0072 (N = 33)	2.4165 ± 0.0065 (N = 10)	2.4221 ± 0.0242 (N = 2)	2.4276 ± 0.0020 (N = 2)	2.4436 (N = 1)	2.4419 ± 0.0158 (N = 9)
		Juv	2.4234 ± 0.0131 (N = 4)	2.4201 ± 0.0077 (N = 4)	2.4005 ± 0.0216 (N = 3)	2.4287 ± 0.0091 (N = 38)	2.4331 (N = 1)	2.4199 (N = 1)	2.4552 ± 0.0122 (N = 13)	2.4546 ± 0.0090 (N = 11)	2.4492 ± 0.0107 (N = 35)

Statistical comparisons among the three lead exposure categories performed separately for each age group and location were not significant, except for the ^{206}Pb:^{207}Pb values for adult grouse from the Yorkshire estate, shown by *(P < 0.05).

feeding (Beyer et al., 1994). Bendell-Young and Bendell (1999) showed that the grit selected by wild spruce grouse (*Dendragapus canadensis*) provided about 60% of the ingested lead for this species in central Ontario, Canada. Thus, in the absence of environmental lead pollution, lead concentrations in bones of hunted upland bird species should generally be low (<10 µg/g), and lead isotope ratios of bone tissue should be similar to the ratios of native soils and food items found in the local environment. Scheuhammer et al. (2003) observed that American woodcock bones with low lead concentrations had ^{206}Pb:^{207}Pb ratios similar to soil and earthworms collected from the same locations. For grouse with low (<10 µg/g) bone lead accumulation, lead isotope ratios in their bones should be similar to ratios in uncontaminated local soils. This was indeed the case for Yorkshire grouse, for which ^{206}Pb:^{207}Pb ratios of those few birds with low lead exposure were similar to ratios reported for uncontaminated Derbyshire and Yorkshire soils (~1.15) (Bellis et al., 2004; Table 3). The same correspondence existed for grouse bones and soil from the Scottish estates (Fig. 2).

Birds with more elevated bone lead levels (>10 µg/g) can confidently be considered to have experienced significant exposure to one or more sources of environmental lead contamination, which may include lead from many years of leaded gasoline combustion, lead from nearby base metal mining and smelting activity (Henny, 2003), and/or lead from spent lead gunshot (Kendall et al., 1996; Sanderson and Bellrose, 1986). To help distinguish among different potential sources of elevated environmental lead exposure in wildlife, some researchers have employed stable lead isotope signatures, which are often characteristic of different lead sources (Church et al., 2006; Pain et al., 2007; Scheuhammer et al., 2003; Scheuhammer and Templeton, 1998). In the present study, the ^{206}Pb:^{207}Pb ratio for UK gasoline (~1.08; Table 3) was much lower than ^{206}Pb:^{207}Pb ratios for all grouse bones, effectively ruling out a significant contribution from this source to elevated bone lead in grouse from any of the three estates. Similarly, Scheuhammer et al. (2003) concluded that the range of ^{206}Pb:^{207}Pb ratios in wing bones of woodcock with elevated lead concentrations in eastern Canada was not consistent with exposure to environmental lead from past Canadian gasoline combustion. Pain et al. (2007) were also able to dissociate the isotope signatures of gasoline and coal from that of lead in the bones of lead-poisoned red kites (*Milvus milvus*) in the UK. These findings are not surprising given that most wild bird habitats, including the moors in the present study, are typically remote from heavily trafficked roads and urban centers with high traffic densities where the deposition of lead from gasoline combustion was greatest. Additionally, in the UK, a transition to gasoline with a lower lead content began in 1986, with a complete ban on its use by 2000 (Farmer et al., 2000, 2005).

On the Yorkshire moor, a high percentage of grouse exhibited elevated (>10 µg/g) or highly elevated (>20 µg/g) bone lead concentrations (Table 1), as well as a significant positive relationship between bone lead levels and bone ^{206}Pb:^{207}Pb ratios (Fig. 2). Given the prevalence of past lead (galena) mining and smelting in the Yorkshire region, it may be expected that many of the Yorkshire grouse with the highest bone lead concentrations had isotope signatures (^{206}Pb:^{207}Pb and ^{208}Pb:^{207}Pb) close to that of lead from galena (Fig. 3). However, if lead from local lead mining and smelting were the sole source of elevated lead ingestion at the Yorkshire estate, the isotope ratios of all bones with high lead values from the Yorkshire moor would have overlapped almost exactly with the ratios for regional galena, yet this was not the case. In fact, lead isotope ratios for bones were always lower than ratios for galena lead, and approximately 30% of Yorkshire grouse with highly elevated bone lead had lead isotope ratios much lower than those of galena lead, suggestive of a second significant source of elevated lead exposure for these birds. These birds had lead isotope signatures falling within the range for lead shot available to UK hunters. Thus, a combination of exposure to ingested lead shot, along with lead in soil and diet items contaminated by lead from galena mining, together provide a plausible explanation for the pattern of lead isotope ratios observed in Yorkshire grouse with highly elevated bone lead levels (Fig. 3). Grouse and other upland birds with similar feeding habits are known from previous studies to ingest spent

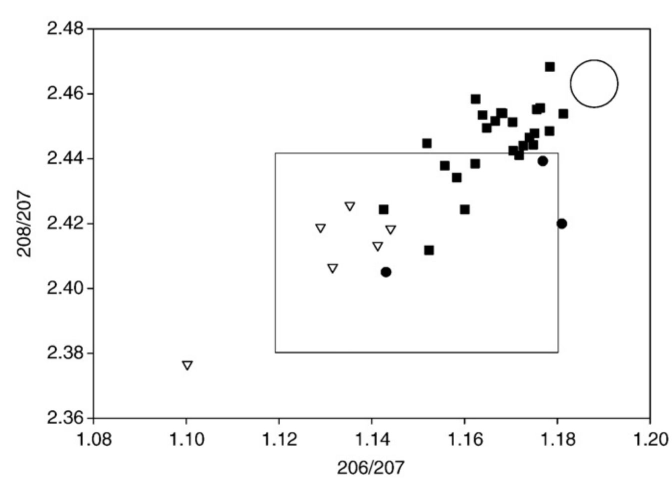

Fig. 3. Bone ^{208}Pb:^{207}Pb ratios plotted against bone ^{206}Pb:^{207}Pb ratios for grouse having very elevated bone lead levels (>20 µg/g) from Invermark (●), N = 3; Glendye (▽), N = 6; and Yorkshire (▪), N = 25. The rectangle indicates approximate ranges of ratios for Pb shot ammunition available in Europe (adapted from Stevenson, 2002, and including ratios for 3 embedded shot found and analyzed in the present study). The large open circle indicates the isotopic ratio signature for Pb from galena mined in Yorkshire (adapted from Rohl, 1996). The isotope signature for UK gasoline (see Table 3) is off the scale to the lower left. For grouse from the Yorkshire sample, a given bird's bone Pb isotope ratio will be determined by the relative amounts of lead from galena, lead gunshot and, possibly, lead from other sources that have been ingested over the bird's life.

Please cite this article as: Thomas VG, et al, Bone lead levels and lead isotope ratios in red grouse from Scottish and Yorkshire moors, Sci Total Environ (2009), doi:10.1016/j.scitotenv.2009.02.003

lead shot, probably mistaking small lead pellets for grit or seeds (Butler et al., 2005; Hunter and Rosen, 1965; Kreager et al., 2008; Rodrigue et al., 2005; Westermeier, 1966).

On the two Scottish moors, elevated lead exposure from mining and smelting activity can probably be ruled out because these moors are remote from such industrial activity. Indeed, unlike grouse bones from the Yorkshire estate, bones with highly elevated lead from the Scottish moors demonstrated no tendency towards isotope signatures characteristic of galena (Fig. 3). A lower percentage of grouse from the Scottish moors had highly elevated bone lead concentrations compared with the Yorkshire moor (Table 1), and almost all of the Scottish birds with highly elevated bone lead had lead isotope ratios falling within the boundaries for European lead shot (Fig. 3). A slight shift in the isotope signature of bone lead from the two Scottish estates is apparent (Fig. 3). Given the close proximity of the two estates and their similar rainfall and soil types, the most plausible explanation is that different brands of ammunition have been used on the two estates. The large variance in bone lead levels among grouse within each moor is consistent with grouse encountering and ingesting lead-dense particles distributed in a non-homogenous manner across their habitats. Butler et al. (2005) also reported a very large range of bone lead levels (7–445 µg/g, Mean 48.8 ± 8.8 µg/g) of ring-necked pheasants (*Phasianus colchicus*) sampled on British shooting estates, where, again, lead shot would not be distributed uniformly across habitats. We judge that, for grouse with highly elevated bone lead levels having lead isotope ratios inconsistent with environmental lead from mining and smelting, lead shot ingestion is the most likely source of elevated lead exposure.

Although the present analysis cannot distinguish, definitively, among different sources of lead accumulated in grouse bones, it is highly likely that, after many decades of concentrated shooting on hunted estates, discharged lead gunshot comprises the single most abundant source of lead in many red grouse habitats in the UK. The discharged lead has not been removed and has accumulated over time, remaining continually available to grouse. Given the acidic nature of many moorland soils, it is highly likely that some of the lead deposited decades ago has been mobilized and is now part of the general lead background of the moor. It is also possible that this form of molecular lead now comprises a substantial component of background lead in soils and plants, such that the environmental lead isotope signatures for shot-over moors may have moved away from historic geological background sources and toward signatures more characteristic of metallic lead gunshot. This may, in part, explain the absence of dramatic trends towards different lead isotope ratios in bones with increasing bone lead levels, especially for birds from the two Scottish moors.

Major declines in the aerial deposition of lead on the Scottish environment have occurred in recent decades (Farmer et al., 1999; Fig. 5 in Farmer et al., 2002), and a similar situation has been described for Yorkshire (Bellis et al., 2002, 2004). These declines represent diminished emissions from gasoline and coal combustion, and from lead smelting and mining. However, metallic lead from gunshot deposited on British grouse moors currently continues unabated, because lead shot is preferred by hunters, and its use is still allowed by law. Furthermore, the remnants of lead ore mining and processing from past centuries are still scattered over regions of the Yorkshire landscape and continue to be available as ingestible grit and as soil ingested incidentally while feeding. Future research on this issue should focus on better elucidating the relative contribution of lead gunshot versus other forms of environmental lead pollution to elevated lead exposure in red grouse. It should be noted that the isotopic ratios of European shot provided in the present study are only representative of gunshot available in the UK, that may have been used on the studied moors; mean isotope ratios for shot actually consumed by grouse on each moor may be somewhat different, especially if certain brands of ammunition are favored on some estates and not others. Future studies should include analyses of lead and stable lead isotopes in surface soils and local grits, in actual lead gunshot recovered from grouse habitats, and in crop and gizzard contents of grouse from each moor.

4.2. Potential health effects of elevated lead exposure in grouse

In most wild bird species, bone lead levels >20 µg/g indicate a highly elevated exposure to lead, and a potential for lead poisoning, especially in situations where acute exposure has occurred, such as ingestion of gunshot. Bone lead levels >20 µg/g are observed frequently in birds that have died from lead toxicosis (Franson, 1996; Pain, 1996). Although bone lead concentrations cannot, in the absence of other data, be used to diagnose lead poisoning in birds, nevertheless a high proportion of both adult and juvenile grouse from the Yorkshire moor, and a smaller proportion of grouse from the two Scottish estates, had bone lead levels sufficiently high to be of concern with respect to their long-term health. In addition to severe toxicosis and mortality in birds after lead shot ingestion (Fisher et al., 2006; Rattner et al., 2008), significant sublethal impacts on avian health and fitness can occur subsequent to elevated lead exposure. Franson (1986) suggested that increased susceptibility to infectious diseases could be a consequence of sublethal lead exposure in waterfowl. Deleterious impacts of lead on the immune system may occur at exposure levels lower than those associated with more overt toxic effects (Dietert and Piepenbrink, 2006; Grasman and Scanlon, 1995), and an interaction between ingested lead and impairment of the immune system has been established in a range of avian species (Redig et al., 1991; Rocke and Samuel, 1991; Grasman and Scanlon, 1995). Administering one lead shot of about 3.4 mm diameter (0.2 g mass) to mallard ducks (*Anas platyrhynchos*) significantly reduced antibody production within 7 days, as shown by reduced hemagglutination titers (Trust et al., 1990). In a similar study on mallard ducks, two such ingested lead shot reduced B-lymphocytes, a source of antibody production (Rocke and Samuel, 1991). Populations of red grouse are known to be afflicted severely by infections of louping-ill virus transmitted by ticks (*Ixodes ricinus*) (Duncan et al., 1978; Kirby et al., 2004), and infestation by the caecal threadworm parasite *Trichostrongylus tenuis* (Newborn and Foster, 2002). These infections affect both immature and adult birds and may contribute to substantial population declines. We hypothesize that elevated lead burdens in red grouse, regardless of source, could impair normal immune system functions and, quite apart from other signs of lead toxicosis, increase the susceptibility of individual birds to prevalent infections. Future research on the effects of elevated lead ingestion by red grouse should address the question of the possible immunotoxicity of such exposure, requiring blood sampling from live birds, measurement of discrete blood cellular components (as suggested in Dietert and Piepenbrink, 2006), measurements of blood lead levels, and correlations with chick survival/recruitment and productivity of breeding adults.

4.3. Implications for management of red grouse habitats

Given the probability that red grouse are ingesting discharged lead shot (at rates of 3.5%, 5.4%, and 18.4% of grouse from the Invermark, Glendye and Yorkshire estates, respectively), and in addition are sometimes ingesting significant levels of lead from other sources, such as lead from mining and smelting emissions in Yorkshire, a number of practices can be implemented on estates to reduce both the ingestion and assimilation of lead. The first would require that all grouse shooters use acceptable forms of nontoxic, lead-free shot, whether from fixed butts or walked-up situations. While this practice would not lower the prevalence of existing lead shot on the soil of the moors, it should gradually reduce the frequency of lead shot ingestion (see Anderson et al., 2000, for a discussion of what this practice has meant to US waterfowl populations that ingest nontoxic, rather than toxic lead shot). The ammunition industries already manufacture suitable cartridges containing an array of nontoxic shot that could be used for this sport. A

Please cite this article as: Thomas VG, et al, Bone lead levels and lead isotope ratios in red grouse from Scottish and Yorkshire moors, Sci Total Environ (2009), doi:10.1016/j.scitotenv.2009.02.003

decision by estate owners to require nontoxic shot for grouse shooting would reflect an appreciation of the importance of the consequences of lead ingestion relative to the reluctance of clients to use lead-free shot. This management option could be combined with heather management that would restrict access of grouse to areas with high lead shot densities. Where shooting occurs from fixed butts, the zone of shot fall-out having the highest spent shot densities probably extends to approximately 150 m from either side of the butts. See Ferrandis et al. (2008) for confirmation of this in driven partridge shooting in Spain. If the heather in this area were allowed to grow tall and dense, it would appear nutritionally unattractive to grouse, and the density of the vegetation might inhibit grouse from entering fall-out zones thus reducing their chances of encountering high densities of spent lead shot.

A further management option is to deploy piles of crushed grit high in calcium (such as crushed oystershell) throughout moors that lack calcareous soils, and especially in the vicinity of preferred feeding sites. The rationale for this practice is that the assimilation of dietary lead is reduced when dietary calcium intake is high (Scheuhammer, 1996; Snoeijs et al., 2005). The intake of more calcium would inhibit the uptake of lead from any source in the environment, not just that from lead shot. The benefits of calcareous grit would also extend to the breeding season, when exogenous calcium is required by breeding females. To be most effective, this practice would require that grit be readily accessible to grouse in many different locations on the moors. The three management options we have presented would have greatest effect when used in combination, curtailing the addition of new lead while simultaneously reducing the intake and dietary absorption of lead already present in the environment.

Acknowledgements

We are grateful to Lord Dalhousie and Mr. C. Gladstone for permission to use red grouse from their Invermark and Glendye estates for this study. Mr. Archie Dykes, Mr. Fred Taylor and other staff members of the Scottish estates provided tremendous assistance with logistic support and the collection of the material. Mr. James Ivory graciously provided accommodation for V.G. Thomas while in Scotland. Drs. Nicholas Aebischer and Dick Potts, and Mr. Ian McCall of The Game and Wildlife Conservation Trust at Fordingbridge, Hampshire, assisted with the collection of the grouse legs from the Yorkshire and Scottish estates. Mrs. Robyn Lima and Mr. Leigh Martin provided excellent technical assistance, dissecting and preparing the bones for Pb determination. We are grateful to the journal reviewers for their constructive comments. This research was supported in part by grants to V.G. Thomas and A.M. Scheuhammer from the Toxic Substances Research Initiative (TSRI) of the Government of Canada. The interpretations expressed in this paper are those of the authors, alone.

References

Anderson WL, Havera SP, Zercher BW. Ingestion of lead and nontoxic shotgun pellets by ducks in the Mississippi flyway. J Wildl Manage 2000;64:848–57.

Aung NN, Uryu T, Yoshinaga J. Lead isotopic compositions of environmental certified reference materials for an inter-laboratory comparison of lead isotope analysis. Anal Sci 2004;20:195–8.

Bendell-Young LI, Bendell JF. Grit ingestion as a source of metal exposure in the spruce grouse, *Dendragapus canadensis*. Environ Pollut 1999;106:405–12.

Bellis DJ, McLeod CW, Satake K. Pb and ^{206}Pb/^{207}Pb isotopic analysis of a tree bark pocket near Sheffield, UK recording historical change in airborne pollution during the 20th century. Sci Total Environ 2002;289:169–76.

Bellis DJ, Satake K, McLeod CW. A comparison of lead isotope ratios in the bark pockets and annual rings of two beech trees collected in Derbyshire and South Yorkshire, UK. Sci Total Environ 2004;321:105–13.

Beyer WN, Connor EE, Gerould S. Estimates of soil ingestion by wildlife. J Wildl Manage 1994;58:375–82.

Butler DA, Sage RB, Draycott RAH, Carroll JP, Potts D. Lead exposure in ring-necked pheasants on shooting estates in Great Britain. Wild Soc Bull 2005;33:583–9.

Calvert JH. Pheasants poisoned by swallowing shots. Field 1876;47:189.

Church ME, Gwiazda R, Risebrough RW, Sorenson K, Chamberlain CP, Farry S, et al. Ammunition is the principal source of lead accumulated by California condors re-introduced to the wild. Environ Sci Technol 2006;40:6143–50.

Clark AJ, Scheuhammer AM. Lead poisoning in upland-foraging birds of prey in Canada. Ecotoxicology 2003;12:23–30.

D'Agostino RB, Belanger A, D'Agostino Jr RB. A suggestion for using powerful and informative tests of normality. Am Stat 1990;44:316–21.

Dennis IA, Macklin MG, Coulthard TJ, Brewer PA. The impact of the October–November 2000 floods on contaminant metal dispersal in the River Swale catchment, North Yorkshire, UK. Hydrol Process 2003;17:1641–57.

Dietert RR, Piepenbrink MS. Lead and immune function. Crit Rev Toxicol 2006;36:359–85.

Duncan JS, Reid HW, Moss R, Phillips JDP, Watson A. Ticks, louping ill and red grouse on moors in Speyside, Scotland. J Wildl Manage 1978;42:500–5.

Ethier ALM, Braune BM, Scheuhammer AM, Bond DE. Comparison of lead residues among avian bones. Environ Pollut 2007;145:915–9.

Farmer JG, Eades LJ, Graham MC. The lead content and isotopic composition of British coals and their implications for past and present releases of lead to the UK environment. Environ Geochem Health 1999;21:257–72.

Farmer JG, Eades LJ, Graham MC, Bacon JR. The changing nature of the ^{206}Pb/^{207}Pb isotopic ratio of lead in rainwater, atmospheric particulates, pine needles and leaded petrol in Scotland, 1982–1998. J Environ Monit 2000;2:49–57.

Farmer JG, Eades LJ, Atkins H, Chamberlain DF. Historical trends in the lead isotopic composition of archival *Sphagnum* mosses from Scotland (1838–2000). Environ Sci Technol 2002;36:152–7.

Farmer JG, Graham MC, Bacon JR, Dunn SM, Vinogradoff SI, MacKenzie AB. Isotopic characterization of the historical lead deposition record at Glensaugh, an organic-rich, upland catchment in rural N.E. Scotland. Sci Total Environ 2005;346:121–37.

Ferrandis P, Mateo R, Lopez-Serrano FR, Martinez-Haro M, Martinez-Duro E. Lead shot exposure in red-legged partridge *(Alectoris rufa)* on a driven shooting estate. Environ Sci Technol 2008;42:6271–7.

Fimreite N. Effects of lead shot ingestion in willow grouse. Bull Environ Contam Toxicol 1984;33:121–6.

Fisher IJ, Pain DJ, Thomas VG. A review of lead poisoning from ammunition sources in terrestrial birds. Biol Conserv 2006;131:421–32.

Franson JC. Immunosuppressive effects of lead. In: Feierabend JS, Russel AB, editors. Lead poisoning in wild waterfowl—A workshop. Washington (DC): National Wildlife Federation; 1986.

Franson JC. Interpretation of tissue lead residues in birds other than waterfowl. In: Beyer WN, Heinz GH, Redmon-Norwood AW, editors. Environmental Contaminants in Wildlife: Interpreting tissue concentrations. Boca Raton (FL): SETAC CRC Lewis Publishers; 1996. p. 265–79.

Gjerstad KO, Hanssen I. Experimental lead poisoning in willow ptarmigan. J Wildl Manage 1984;48:1018–22.

Gill MC. Swaledale: its mines and smelt mills. Ashbourne, Derbyshire, UK: Landmark Publishing; 2001. 174 pp.

Grasman KA, Scanlon PF. Effects of acute lead ingestion and diet on antibody and T-cell-mediated immunity in Japanese quail. Arch Environ Contam Toxicol 1995;28:161–7.

Gulson B. Stable lead isotopes in environmental health with emphasis on human investigations. Sci Total Environ 2008;400:75–92.

Harrison RM, Laxen DPH. Lead Pollution: Causes and Control. London (UK): Chapman and Hall; 1981. 168 pp.

Henny CJ. Effects of mining lead on birds: a case history at Coeur d' Alene Basin, Idaho. In: Hoffman DJ, Rattner BA, Burton GA, Cairns J, editors. Handbook of Ecotoxicology. 2nd edition. Boca Raton, FL: Lewis Publishing; 2003. p. 755–66.

Hudson-Edwards KA, Macklin MG. Mediaeval lead pollution in the River Ouse at York, England. J Archaeol Sci 1999;26:809–19.

Hudson-Edwards KA, Macklin MG, Taylor MP. 2000 years of sediment-borne heavy metal storage in the Yorkshire Ouse basin, NE England, UK. Hydrol Process 1999;13:1087–102.

Hunter BF, Rosen MN. Occurrence of lead poisoning in a wild pheasant (*Phasianus colchicus*). Calif Fish Game 1965;51:207.

Kendall RJ, Lacher TE, Bunck C, Daniel B, Driver C, Grue CE, et al. An ecological risk assessment of lead shot exposure in non-waterfowl avian species: upland game birds and raptors. Environ Toxicol Chem 1996;15:4-20.

Kirby AD, Smith AA, Benton TG, Hudson PJ. Rising burden of immature sheep ticks (*Ixodes ricinus*) on red grouse (*Lagopus lagopus scoticus*) chicks in the Scottish uplands. Med Vet Entomol 2004;18:67–70.

Komárek M, Ettler V, Chrastný V, Mihaljevič M. Lead isotopes in environmental sciences: a review. Environ Int 2008;34:562–77.

Kreager N, Wainman BC, Jayasinghe RK, Tsuji LJS. Lead pellet ingestion and liver-lead concentrations in upland game birds from southern Ontario, Canada. Arch Environ Contam Toxicol 2008;54:331–6.

Lee JA, Tallis JH. Regional and historical aspects of lead pollution in Britain. Nature 1973;245:216–8.

Linduska JP. Age determination in the ring-necked pheasant. J Wildl Manage 1945;9:152–4.

Macklin, MG, Hudson-Edwards, KA, Dawson, EJ. The significance of pollution from historic metal mining in the Pennine orefields on river sediment contaminant fluxes to the North Sea. Sci Total Environ 1997;194/195:391–7.

Mateo R, Cadenas R, Manez M, Guitart R. Lead shot ingestion in two raptor species from Doñana, Spain. Ecotoxicol Environ Saf 2001;48:6-10.

Newborn D, Foster R. Control of parasite burdens in wild red grouse *Lagopus lagopus scoticus* through the indirect application of anthelmintics. J Appl Ecol 2002;39:909–14.

Pain DJ. Lead in waterfowl. In: Beyer WN, Heinz GH, Redmond-Norwood AW, editors. Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. Boca Raton (FL): SETAC CRC Lewis Publishers; 1996. p. 251–64.

Pain DJ, Amiard-Triquet C, Bavoux C, Burneleau G, Eon L, Nicolau-Guillaumet P. Lead poisoning in wild populations of marsh harriers *Circus aeruginosus* in the Camargue and Charente-Maritime, France. Ibis 1993;135:379–86.

Pain DJ, Meharg AA, Ferrer M, Taggart M, Penteriani V. Lead concentrations in bones and feathers of the globally threatened Spanish imperial eagle. Biol Conserv 2005;121: 603–10.

Pain DJ, Carter L, Sainsbury AW, Shore RF, Eden P, Taggart MA, et al. Lead contamination and associated disease in captive and reintroduced red kites (*Milvus milvus*) in England. Sci Total Environ 2007;376:116–27.

Rattner BA, Franson JC, Sheffield SR, Goddard CI, Leonaed NJ, Stang D, et al. Sources and implications of lead-based ammunition and fishing tackle to natural resources. Tech Rev, vol. 08-01. Bethesda (MD): The Wildlife Society; 2008. 62 pp.

Redig PT, Lawler EM, Schwartz S, Dunnette JL, Stephenson B, Duke GE. Effects of chronic exposure to sublethal concentrations of lead acetate on heme synthesis and immune function. Arch Environ Contam Toxicol 1991;21:72–7.

Rocke TE, Samuel MD. Effects of lead shot ingestion on selected cells of the mallard immune system. J Wildl Dis 1991;27:1–9.

Rodrigue J, McNicoll R, Leclair D, Duchesne JF. Lead concentrations in ruffed grouse, rock ptarmigan, and willow ptarmigan in Quebec. Arch Environ Contam Toxicol 2005;49: 97-104.

Rohl BM. Lead isotope data from the isotrace laboratory, Oxford: *ARCHAEOMETRY* data base 2, galena from Britain and Ireland. Archaeometry 1996;38:165–80.

Sanderson GC, Bellrose FC. A review of the problem of lead poisoning in waterfowl. Ill Nat Hist Surv Spec Publ 1986;4 34 pp.

Scheuhammer AM. Influence of reduced dietary calcium on the accumulation and effects of lead, cadmium, and aluminum in birds. Environ Pollut 1996;94:337–43.

Scheuhammer AM, Templeton DM. Use of stable isotope ratios to distinguish sources of lead exposure in wild birds. Ecotoxicol 1998;7:37–42.

Scheuhammer AM, Bond DE, Burgess NM, Rodrigues J. Lead and stable lead isotope ratios in soil, earthworms, and bones of American woodcock (*Scolopax minor*) from eastern Canada. Environ Toxicol Chem 2003;11:2585–91.

Snoeijs T, Dauwe T, Pinxten R, Darras VM, Arckens L, Eens M. The combined effect of lead exposure and high or low dietary calcium on health and immunocompetence in the zebra finch (*Taeniopygia guttata*). Environ Pollut 2005;134:123–32.

Stevenson, A.L. Lead levels and sources of exposure in migratory game birds after the implementation of lead-free shot in Canada. MSc Thesis, 2002, McGill University, Montreal, Quebec.

Sturges WT, Harrison RM. The use of Br/Pb ratios in atmospheric particles to discriminate between vehicular and industrial lead sources in the vicinity of a lead works—I. Thorpe, West Yorkshire. Atmos Environ 1986;20:833–43.

Sugden CL, Farmer JG, MacKenzie AB. Isotopic ratios of lead in contemporary environmental material from Scotland. Environ Geochem Health 1993;15:59–65.

Svanberg F, Mateo R, Hillstrom L, Green AJ, Taggart MA, Raab A, et al. Lead isotopes and lead shot ingestion in the globally threatened marble teal *(Marmaronetta angustirostris)* and white-headed duck *(Oxyura leucocephala)*. Sci Total Environ 2006;370:416–24.

Tejedor MC, Gonzalez M. Comparison between lead levels in blood and bone tissue of rock doves (*Columba livia*) treated with lead acetate or exposed to the environment of Alcala de Henares. Bull Environ Contam Toxicol 1992;48:835–42.

Thirgood S, Redpath S, Newton I, Hudson P. Raptors and red grouse: conservation conflicts and management solutions. Conserv Biol 2000;14:95-104.

Trust KA, Miller MW, Ringelman JK, Orme IM. Effects of ingested lead on antibody production in mallards (*Anas platyrhynchos*). J Wildl Dis 1990;26:316–22.

Westermeier RL. Apparent lead poisoning in a wild bobwhite. Wilson Bull 1966;78:471–2.

Yoshinga J. Isotope ratios analyses of lead in biological materials by inductively coupled plasma mass spectrometry. Tohoku J Exp Med 1996;178:37–47.

Please cite this article as: Thomas VG, et al, Bone lead levels and lead isotope ratios in red grouse from Scottish and Yorkshire moors, Sci Total Environ (2009), doi:10.1016/j.scitotenv.2009.02.003