Science of the Total Environment 407 (2009) 3494–3502

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 4 March 2009

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).

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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.