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ORIGINAL PAPER

Poisoning from lead gunshot: still a threat to wild waterbirds in Britain

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Abstract Lead is a highly toxic metal known to be an important cause of morbidity and mortality in waterbirds and terrestrial birds worldwide. The risk to birds of poisoning from lead has resulted in the introduction of legislation in many countries, such as UK restrictions on the use of lead in angling weights and lead gunshot. In this study, we examined data on current and historical trends in lead poisoning in British waterbirds and related these to the introduction of legislation restricting the use of lead. Our results indicate that lead poisoning has continued to affect a wide range of British waterbirds long after legal restrictions were introduced. Elevated levels of lead (i.e. >20.0 μg/dL) were found in the blood of 34 % (n=285) of waterbirds tested at four sites in Britain during the 2010/2011 winter and accounted for the deaths of at least 10.6 % (n=2,365) of waterbirds recovered across Britain between 1971 and 2010 and 8.1 % (n=1,051) between 2000 and 2010, with lead gunshot being the most likely source of poisoning. The proportion of birds dying from lead poisoning in England did not vary significantly after the introduction of legislation, accounting for 13.7 % of non-infectious causes of death between 1971 and 1987 (n=204), 20.8 % (n=360) between 1988 and 1999 and 11.8 % (n=423) between 2000 and 2010, despite a significant change in lead-related mortality in mute swans found during the same time period, 25 % (n=12) between 1971 and 1987, 4.6 % (n=65) between 1988 and 1999 and 2 % (n=100) between 2000 and 2010. Existing legislation needs review and extension to ensure the delivery of international commitments and a broad-scale transition to the use of non-toxic shot and angling materials in all environments.

Keywords Lead poisoning · Lead gunshot · Legislation · Waterbirds · Britain

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Introduction

Lead is a highly toxic metal that can affect virtually every physiological system in animals (Pokras and Kneeland 2009; EFSA 2010; Franson and Pain 2011). Poisoning caused by lead is a widely accepted and well-documented global problem for humans, domestic animals and wildlife and remains an important cause of morbidity, mortality and suffering in waterbirds and terrestrial birds (Pain 1996; Scheuhammer and Norris 1996; Beintema 2001; Mateo 2009; Pain et al. 2009). In Britain, lead poisoning has been studied since the 1960s and reported in many species of waterbird (Olney 1960; Thomas 1980; Birkhead 1982; Mudge 1983; Perrins et al. 2003; O'Connell et al. 2008).

Waterbirds are poisoned following ingestion of spent lead gunshot, either inadvertently or when mistaken for food particles or grit (Hall and Fisher 1985; Pain 1990b; Moore et al. 1998; Gionfriddo and Best 1999; Mateo and Guitart 2000). Predatory and scavenging birds, primarily raptors, are also exposed to embedded lead ammunition (gunshot, bullets or fragments thereof) in their prey or carrion (Pain et al. 2009). A high proportion of both legally and illegally shot birds can carry embedded shot. For example, studies have revealed shot in 25 and 36 %, respectively, of first year and adult live-trapped pink-footed geese (*Anser brachyrhynchus*) (Noer and Madsen 1996) and 31 % of protected Bewick's swans (*Cygnus columbianus bewickii*) (Newth et al. 2011). Some raptors may therefore be exposed to lead across the migratory routes of their prey.

Although more recent examples are not available, in the early 1990s in the UK, an estimated 160 tonnes of lead shot equating to about 1.6 billion individual shot was deposited in wetlands alone (Pain 1992). As lead gunshot generally degrades only slowly (except under acidic conditions; Rooney et al. 2007) and may persist for tens or hundreds of years, this represents a substantial accumulation of this heavy metal in the sediments and soils of hunted habitats around the world.

The risk to birds from poisoning from lead gunshot has resulted in many countries imposing legislative restrictions on its use (Avery and Watson 2009). In Europe, much legislation has resulted from a request for the phasing out of lead shot over wetlands within the action plan and subsequent resolutions of the Agreement on the Conservation of African-Eurasian Migratory Waterbirds (AEWA 1999, 2002, 2008). As a signatory to AEWA, the UK responded by banning the use of lead shot over the foreshore and specified (wetland) Sites of Special Scientific Interest (SSSIs); for hunting wildfowl, coot (*Fulica atra)* and moorhen (*Gallinula chloropus*) in all areas in England in 1999 and Wales in 2002; and for hunting over wetlands (for any type of shooting activity) in Scotland in 2004 and Northern Ireland in 2009 (HMSO 1999, 2002a, b, 2003, 2004, 2009). However, in England, there is evidence of non-compliance with the restrictions with 68 % (n=40) of ducks purchased from game outlets in 2001/2002 and 70 % (n=492) purchased between 2008 and 2010 having been shot illegally with lead (Cromie et al. 2002; Cromie et al. 2010). In addition to compliance issues, restrictions do not extend to all habitats used by waterbirds, and hence many species remain at risk of ingesting lead.

Despite restrictions on its use, waterbirds in the UK have continued to be poisoned by lead. Elevated blood lead levels (i.e. >20.0 μg/dL) were recorded in 79.7 % of whooper swans (*Cygnus cygnus*) caught at wintering sites in England 5 to 6 years after the introduction of legislation, and in 61.4 % of this species in Scotland, 1 year following legislation, with ingestion of spent lead shot attributed as the most likely cause (O'Connell et al. 2008).

The purpose of the present study was to provide further information on current and historical trends in lead poisoning in British waterbirds. Firstly, we investigated blood lead levels in live birds caught during the 2010/2011 winter, and the incidence of lead-poisoning-induced mortality of birds found dead between 1971 and 2010. Secondly, we examined variation in the incidence of lead-poisoning-induced mortality in waterbirds in England between three-time phases (1971–1987, 1988–1999 and 2000–2010) to investigate whether there was a detectable change in the temporal trend in lead-related mortality in relation to the introduction of legislation restricting the uses of lead in angling weights in 1986 (HMSO 1986, as amended 1993; EA 2012) and in ammunition in 1999 (HMSO 1999, as amended 2002 and 2003). The findings of this study provide evidence to help inform the development of UK Government policy on the risks to wildlife health from exposure to lead ammunition, and possible management measures.

Methods

Study sites and sample collection

Blood lead levels in waterbirds wintering in Britain during winter 2010/2011

All live birds tested for blood lead levels were caught at or near to four Wildfowl & Wetlands Trust (WWT) centres in Britain: Slimbridge, Gloucestershire (51°58′98″ N, 2°25′02″ W), Welney, Norfolk (52°31′50″ N, 0°16′98″ E) and Martin Mere, Lancashire (51°58′98″ N, 2°25′02″ W) in England and Caerlaverock, Dumfriesshire (54°58′02″ N, 3°25′02″ W) in Scotland. A total of 285 birds were caught between 16th December 2010 and 24th February 2011 (Table 1) in decoy-type 'swan-pipes' (275 birds) or by cannon-netting (10 birds). These included a dabbling species (pintail, *Anas acuta*), diving species (pochard*, Aythya ferina*) and grazing species (whooper and Bewick's swans), all of which are known to have been previously affected by lead poisoning (Mudge 1983; Pain 1990a, b, 1992).

Sightings of Bewick's swans identified by bill pattern recognition techniques (at Slimbridge) and whooper swans identified by coded leg-rings were analysed to assess the length of time spent at WWT sites prior to testing. Given that blood lead concentrations usually reflect recent exposure to lead, i.e. within 35–40 days of testing (O'Halloran et al. 1988a), swans seen at WWT reserves more than 40 days prior to testing were deemed likely to have been exposed to lead in the vicinity of the reserves.

Birds were sexed (by plumage characteristics or cloacal examination), ringed, aged (as either adults or juveniles by plumage characteristics) and weighed, and their skull, tarsus and wing lengths were measured.

A blood sample was taken from the medial metatarsal vein of each bird with a 2-mL syringe using a 23-gauge needle as part of on-going broader health studies. Samples of 500 μl of blood were then transferred into a 1.5-mL tube containing lithium heparin and chilled (at 4 °C) until analysis.

Table 1 Summary of blood lead concentrations (in micrograms per decilitre) for target species of waterbirds sampled at WWT reserves in Britain during winter 2010/2011 and outputs of a general linear regression showing interspecies differences (with Bewick's swan as the reference level)

Species	Blood lead concentration (μg/dL) summary statistics			Blood lead concentration model outputs			Elevated blood lead levels (% >20 μg/dL)
	N	Median	Range	Model coefficient	*t* statistic	*P* value	
Bewick's swan	39	5.9	1.6–62.6	0			12.8
Whooper swan	177	18.2	5.6–132.9	−0.007	−0.03	0.98	42.9
Pochard	29	15.0	4.3–38.4	−0.022	−0.09	0.93	20.7
Pintail	40	10.3	3.5–196.0	0.439	2.44	0.02	25.0
Overall	285	15.4	1.6–196.0				34.0

The proportion of each species with elevated lead levels (i.e. values >20.0 μg/dL) is also shown

Mortality from lead poisoning of waterbirds recovered at sites in Britain between 1971 and 2010

Between 1971 and 2010, *postmortem* examinations were routinely carried out on waterbirds found dead during regular (near daily) patrols by reserve wardens and other staff in the vicinity of WWT centres: at Arundel, West Sussex (50° 51′41.35″ N, 0°32′59.31″ W), Llanelli, Carmarthenshire (51°40′01.10″ N, 4°07′20.08″ W), London Wetland Centre (51°28′37.94″ N, 0°14′9.343″ W), Washington, Tyne and Wear (54°53′52.73″ N, 1°28′47.70″ W), Slimbridge, Welney, Caerlaverock and Martin Mere. Dead wild birds were recovered on a less frequent ad hoc basis at other sites. Post mortem examinations were not always possible for carcasses that had been scavenged or predated or for those that had decomposed to such an extent that tissue analysis was not possible. Only birds whose cause of death was determined were included in this analysis.

The primary cause of death was determined for 2,365 wild waterbirds recovered at sites across England, Scotland and Wales during this time period. A total of 28 different species of waterbird were represented from the six subfamilies Anatinae (724 birds), Anserinae (1,358 birds), Aythyinae (151 birds), Merginae (17 birds), Oxyurinae (2 birds) and Tadorninae (113 birds).

Laboratory analyses

Blood analysis

A subsample of 0.1 g was taken from each 0.5 mL blood sample, and 0.5 mL of concentrated nitric acid added. Samples were left overnight, after which 1.0 mL of hydrogen peroxide was added to each. The samples were then digested using a microwave digester (MARS, CEM) initially at 55 °C for 10 min, then at 75 °C for 10 min, and finally at 95 °C for 30 min. The digests were made up to 5 mL by mass with Milli-Q water. Samples were then analysed using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500 series), and as an internal standard, a continuous concentration of 10 μg/l Rh, prepared in 1 % nitric acid, was introduced into the sample stream via a T-piece.

Several methods were used to ensure quality control. Blanks (digests without blood, processed using the methods outlined above, in 'Blood analysis') were used to assess background levels of lead and its limit of detection (LOD= 0.275 μg/L). Spikes (digests without blood, processed using the methods outlined above, in 'Blood analysis', with a known concentration of calibration standard added to them) were used to assess any fluctuation of lead during the preparation process. A certified reference material (in this case, bovine liver) (National Institute of Standards and Technology SRM 1577b) was also used to validate results. The recovery for the certified reference material was 0.117±0.007 (mean±s.e.m) with an average percentage recovery of 90.8 %.

Standard additions of samples were conducted prior to analysis to check that there were no interferences. All samples were randomised and analysed in one batch. The sample data were divided by the internal standard to account for any drifting of the instrument and the standards were re-analysed every 30 samples and used to construct an external calibration curve.

Birds with blood lead exceeding 20.0 μg/dL were considered to have an elevated concentration above background levels, indicative of lead ingestion (O'Halloran et al. 1988a) and consistent with adverse physiological effects (Franson and Pain 2011).

Post mortem analysis

Pathological findings that are characteristic of lead poisoning include impaction of the gizzard and oesophagus with food, very little body fat, low body weight, atrophy of gizzard and liver, enlarged gall bladder and green staining of the vent. Birds which exhibited these signs and the presence of lead (shot, angling weights or fragments thereof) in the gizzard and/or intestines and/or had elevated lead levels in kidney tissues (i.e. >25 μmol/kg in dry matter) were determined to have died from lead poisoning.

For each bird tested, 2 g (wet weight) of kidney tissue was digested in a solution of perchloric, nitric and sulphuric acid. The samples were then made up to 12 mL with de-ionised water. A dry matter analysis was performed on each sample. An aliquot of the received samples was dried for 4 h at 100 °C and then reweighed. Lead concentrations were determined by atomic absorption spectrophotometry, using a GBC908 Atomic Absorption Spectrophotometer in flame mode (F-AAS). As tissue analysis was not conducted routinely on all birds at post mortem examination, some cases of lead poisoning without characteristic pathology are likely to have been missed.

Treatment of the data and statistical analyses

Blood lead data

A general linear regression model was used to partition the variation in log-transformed blood lead levels (the response variable), relative to species, site, sex and age (the factors). A general linear model (GLM) with binomial error distributions and logit link functions was used to identify any significant variation in the probability of elevated (i.e. values >20.0 μg/dL) blood lead levels (the response variable) relative to species, site, sex and age (the factors).

Mortality data

A GLM with binomial error distributions and logit link functions was used to investigate interspecific differences in lead poisoning mortality of all birds (whose cause of death was known) recovered at sites across Britain (including WWT centres), between 1971 and 2010.

There was variability in data collection effort over time at sites away from WWT centres (see 'Mortality from lead poisoning of waterbirds recovered at sites in Britain between 1971 and 2010'). Therefore, to investigate any association between the introduction of legislation in England, specifically restrictions in use of lead in angling weights in 1986 (HMSO 1986, as amended 1993; EA 2012) and in ammunition in 1999 (HMSO 1999, as amended 2002 and 2003), and the temporal trend in lead-related mortality rates amongst waterbirds, an additional GLM with binomial error distributions and logit link functions was used to examine only data derived from birds recovered at WWT centres in England. Dead bird surveillance efforts at WWT centres remained relatively consistent over time (with near daily patrols) and hence data collected during each time period were considered comparable. This model investigated variation in the frequency of mortality from lead poisoning (the response variable), relative to species, time period (1971–1987 vs 1988–1999 vs 2000–2010), site, sex and age (the factors). WWT centres were established in different years (at Slimbridge in 1946, Welney and Caerlaverock in 1970, Martin Mere and Washington in 1975, Arundel in 1976, Llanelli in 1991 and London Wetland Centre in 2000), and so site was included in the model to account for this.

For this additional analysis, we calculated mortality rates attributed to lead poisoning as proportions of all mortality events associated with non-infectious causes. We omitted all cases of mortality relating to infectious disease (plus avian botulism) from these analyses as outbreaks of such conditions varied substantially and would confound a consistent assessment of the relative importance of lead poisoning in space and time. This subset of data is hereafter referred to as the 'non-infectious data subset'. The model was run once for all species and separately for three species (mute swan, whooper swan and mallard) that had sufficiently large sample sizes (i.e. >100 birds) to permit species-specific analyses. All statistical analyses were carried out using MINITAB (version 14) and GenStat (version 14.1) software packages.

Results

Blood lead levels in waterbirds wintering in Britain during winter 2010/2011

Interspecific comparison

Single blood samples were collected from 285 live birds during the 2010/2011 winter. Lead was detected in all blood samples, with levels ranging from 1.6 to 196.0 μg/dL (Table 1). There was a significant interspecific variation in blood lead levels ($F_{3,\ 274}$=3.34, P<0.05), with the highest median blood lead levels found in whooper swans and the lowest in Bewick's swans (Table 1). The widest variation in blood lead levels was recorded in pintails and whooper swans (Table 1). Elevated blood lead levels (i.e. >20.0 μg/dL) were found in 34 % of all birds tested and ranged from 12.8 % in Bewick's swans to 42.9 % in whooper swans but did not vary significantly between species (logistic regression, P>0.1) (Table 1).

Twenty whooper swans found to have elevated blood lead levels had been previously leg-ringed. Of these, 17 (85 %) had been sighted at Martin Mere (five birds) or Caerlaverock (12 birds), more than 40 days prior to testing there. Seven of the birds tested at Caerlaverock had been sighted in fields near to the reserve during the same winter and five of those had used these sites within 40 days of testing. Of five Bewick's swans caught at Slimbridge with elevated blood lead levels, three had been recorded at the reserve (using bill pattern recognition techniques), more than 40 days prior to testing.

Site comparison

Blood lead levels varied significantly between sites ($F_{3,\ 274}$= 9.76, P<0.001) as did the proportion of birds with elevated blood lead levels: 45.2 % of birds caught at Martin Mere (n= 62), 41.4 % of birds at Caerlaverock (n=145), 13.2 % of birds at Slimbridge (n=68) and 0 % of birds at Welney (n=10) (χ_3^2=11.72, P=0.008).

Age and sex comparisons

Neither blood lead levels nor the proportion of birds with elevated blood lead levels varied significantly with age and sex (GLMs, P always >0.05).

Mortality from lead poisoning

Waterbirds recovered at sites across Britain between 1971 and 2010

Lead poisoning accounted for mortality in 251 (10.6 %) of 2,365 individual waterbirds (whose cause of death was determined), recovered at sites across Britain (including WWT Centres) from 1971 and 2010 inclusive. Of these, 74.9 % (n=188) had lead shot in varying states of dissolution in their gizzards. In addition, fragments of lead angling weights were present in the gizzards of three mute swans (*Cygnus olor*) that were also entangled in fishing tackle. A total of 14 species of waterbird were affected: mute swan, whooper swan, Bewick's swan, Canada goose (*Branta canadensis*), Western greylag goose (*Anser anser*), pink-footed goose, mallard (*Anas platyrhynchos*), northern pintail, gadwall (*Anas strepera*), common teal (*Anas crecca*), European pochard, tufted duck (*Aythya fuligula*), common shelduck (*Tadorna tadorna*) and shoveler (*Anas clypeata*). There was a significant interspecific variation in the proportion of birds that had died from lead poisoning (GLM: χ_{27}^2=147.60, P< 0.001). Those species with the highest proportion of deaths recorded from lead poisoning included whooper swans (27.3 %), Bewick's swans (23 %), Canada geese (16.7 %) and pochard (16.7 %) (Fig. 1). Amongst the 1,051 individual waterbirds recovered at sites around Britain between 2000 and 2010 and whose cause of death was known, lead poisoning accounted for mortality in 85 (8.1 %).

Other variables associated with lead poisoning mortality

When sources of variation in the frequency of mortality from lead poisoning were analysed using the non-infectious data subset (i.e. those recovered at WWT centres in England between 1971 and 2010 inclusive, see 'Mortality data'), adult birds (of over 2 years) were found to be significantly more likely to be recorded as dying from lead poisoning than juvenile birds (<1 year) (17 %, n=780, and 7.2 %, n= 153; χ_1^2=9.77, P=0.002). However, the probability of birds dying from lead poisoning did not vary significantly relative to site and sex (GLM: P always >0.05).

Three species (mute swan, whooper swan and mallard) had sufficiently large sample sizes (i.e. >100 birds) to permit species-specific analyses. When investigated separately, adult whooper swans found at WWT centres in England were significantly more likely to be recorded as dying from lead poisoning than juvenile birds (51.5 %, n=101, and 26.1 %, n= 23; GLM: χ_1^2=5.62, P=0.01). Also, female whooper swans were more likely to be recorded as dying from lead poisoning than males (56.6 %, n=76, and 33.9 %, n=56; GLM: χ_1^2= 5.54, P=0.01). No such age and sex effects were detected in mallards and mute swans (GLM: P always >0.05).

Lead-related mortality accounted for 13.7 % of deaths, unrelated to infectious disease, between 1971 and 1987 (n= 204), 20.8 % between 1988 and 1999 (n=360) and 11.8 % between 2000 and 2010 (n=423). However, once variation associated with other factors (species, site, sex and age) was taken into account, the probability of birds dying from lead poisoning did not vary significantly between the three time phases (GLM: P>0.1). However, a significant time effect was observed for the proportion of mute swans dying of lead poisoning (GLM: χ_2^2=8.36, P=0.01), which varied from 25 % (n=12) between 1971 and 1987 to 4.6 % (n=65) between 1988 and 1999 and 2 % (n=100) between 2000 and 2010. Significant temporal variations in lead-related mortality rates were not detected for whooper swans or mallards (GLM: P always >0.05).

Discussion

Despite legislation aimed at reducing the impact of lead on wildfowl in the UK, it continues to poison and kill waterbirds. Elevated levels of lead (i.e. >20.0 μg/dL) were found in 34 % of waterbirds tested at four sites in Britain during the 2010/2011 winter ranging from 12.8 % of Bewick's swans to 42.9 % of whooper swans. In addition, lead poisoning accounted for the deaths of at least 8.1 % of waterbirds, recovered across Britain between 2000 and 2010. Lead shot was found in varying states of mechanical grinding and dissolution in the gizzards of 74.9 % of waterbirds that were diagnosed as having died of lead poisoning between 1971 and 2010, the disease accounting for the death of 10.6 % of waterbirds during this period.

Post mortem data presented in this paper are likely to underestimate the true impact of lead poisoning on waterbirds for a number of reasons. For example, some birds may have died from lead poisoning without exhibiting typical pathology in which case the cause of death may have been attributed to something else (Beyer et al. 1998). Also, lead

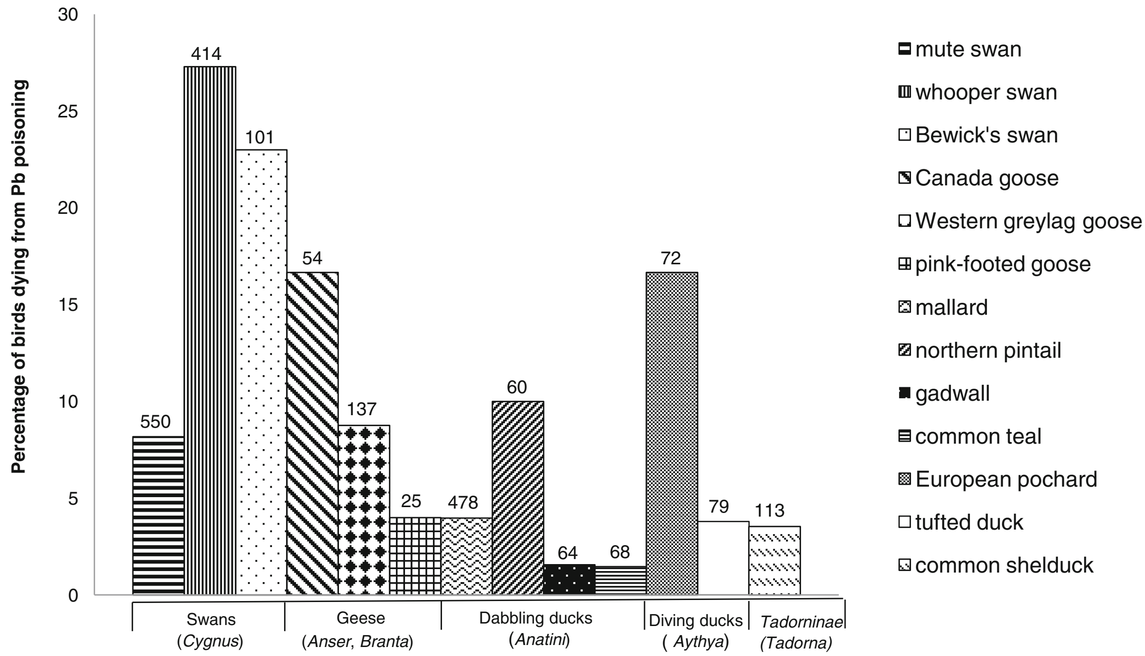

Fig. 1 Mortality from lead poisoning amongst waterbirds recovered at sites in Britain between 1971 and 2010. Sample sizes are indicated, and species for which less than 20 birds were available were omitted

poisoning may cause sublethal impacts on physiology and behaviour (Scheuhammer 1987; Pain et al. 2009) and so may contribute to premature death from other causes such as concurrent disease, starvation, predation and flying accidents (Scheuhammer and Norris 1996; Kelly and Kelly 2004). Furthermore, while large-scale die-offs occasionally occur, mortality from lead poisoning is generally less conspicuous and exhibited as day to day mortality which may result in the frequent and largely invisible losses of small numbers of birds that subsequently remain undetected (Stutzenbaker et al. 1986).

Moribund birds generally become increasingly reclusive, and after death, carcasses are not likely to be seen before being scavenged (Stutzenbaker et al. 1986; Pain 1991) and are thus likely to be under-represented in the post mortem dataset. Lead poisoning is understandably known as 'the invisible disease' (Pain 1991).

Interspecific variation in lead poisoning

In Britain, lead poisoning has been reported in a wide variety of waterbirds, including the 14 species recorded herein and others including goldeneye (*Bucephala clangula*), moorhen and snipe (*Gallinago gallinago*) (Thomas 1975; Mudge 1983). Many bird species feeding in an area where shooting with lead ammunition occurs illegally or legally are at some risk of exposure and potentially poisoning, but this is likely to be influenced by interspecific differences in behaviour.

In the present study, rates of mortality attributable to lead poisoning varied significantly between species, with whooper swans being more likely to be recorded as dying from lead poisoning (27.3 % of birds recovered in Britain between 1971 and 2010) and exhibiting the highest median blood lead levels (18.2 μg/dL). Lead poisoning was also diagnosed as having caused the deaths of 23 % of Bewick's swans and 16.7 % of Canada geese and pochard recovered in Britain between 1971 and 2010.

Swans and geese commonly forage on agricultural land, over which it is legal to shoot with lead gunshot (with the exception of wetlands and specified SSSIs, depending on UK country-specific legislative details). High mortality rates have been recorded in Canada geese in the USA, poisoned by lead gunshot ingested during foraging in crop fields (Szymczak and Adrian 1978). Whooper and Bewick's swans may require particularly large quantities of grit when feeding on more indigestible foods such as potatoes, corn and barley and are thus perhaps more likely to ingest spent gunshot (O'Connell et al. 2008). The poisoning and death of Bewick's, mute and whooper swans from lead in Britain and Ireland is well documented (O'Halloran et al. 1988b; O'Halloran, unpublished results; Brown et al. 1992; O'Connell at al. 2008), and lead gunshot have been frequently found in lead-poisoned swans (Owen and Cadbury 1975; Mudge 1983; Spray and Milne 1988; O'Halloran et al. 1988a, b; O'Halloran unpublished results; O'Connell et al. 2008).

Spent lead gunshot are also accessible to diving ducks such as pochard, when they gather food items from lake bottoms and sediments which are too compact for lead items to sink out of reach (Olney 1968; CWS 2001). During the winter, pintail may exploit fields that are temporarily available through flooding (JNCC 2012) which lie outside of permanent wetlands and that are hunted over with lead shot. Other studies elsewhere have found a particularly high prevalence of lead poisoning in pochard and pintail (e.g. values nearing or above 70 %; Mateo et al. 1997, 1998).

Spatial variation in lead poisoning

Blood lead concentrations and the proportion of birds with elevated levels (i.e. >20.0 μg/dL) varied significantly amongst sites in the present study. The highest proportions of birds with elevated levels of lead in the blood were from Martin Mere (45.2 %) and Caerlaverock (41.4 %), most likely to have resulted from current shooting with lead in areas surrounding these wetland sites (see 'Sources of lead' below), along with some residual lead from historical shooting (prior to the sites coming into WWT management in 1975 and 1970, respectively). This is consistent with previous studies which also recorded elevated lead levels (i.e. >1.21 μmol/L) in adult whooper swans caught at both sites (Spray and Milne 1988; O'Connell et al. 2008). In areas of high exposure (e.g. heavily hunted areas), the likelihood of picking up spent gunshot and repeated exposure increases (Pattee and Hennes 1983).

Location and timing of lead ingestion

Blood lead concentrations tend to reflect recent exposure of within 35–40 days of testing (O'Halloran et al. 1988a). The presence of lead in the blood of birds caught in the present study between December and February, having mostly arrived in Britain during the autumn (Rees et al. 1997; Kershaw et al. 1998; Kershaw 2002; Olgilvie 2002; Rees and Bowler 2002; Rees et al. 2002), is therefore consistent with exposure in Britain. Following arrival in Britain, long-distance winter movements are generally limited amongst Bewick's swans (Rees and Bowler 2002) although whooper swans may move between Britain and Ireland within a winter season (McElwaine et al. 1995). Sightings of swans (identified by bill pattern recognition techniques or leg rings) indicated that three of five Bewick's and 85 % (n=20) of whoopers found with elevated lead levels in the present study had been seen at WWT reserves more than 40 days prior to testing, suggesting that at least some lead is likely to have been ingested in the vicinity of reserves. Pochard are known to move from Northern Europe to Britain within the same winter although in small numbers (Keller et al. 2009). Within-winter movements for other waterbird species are less well understood (Owen and Black 1990) and hence the provenance of elevated lead levels in these species is less easily attributed.

Twelve of the 14 species of waterbird found dead from lead poisoning throughout Britain in the present study may have visited other countries during the preceding year (but is unlikely to be the case for Canada goose and mute swan). However, poisoned birds often die within 3 weeks (De Francisco et al. 2003) and sometimes within a few days of shot ingestion (Beintema 2001). Also, given the potentially debilitating nature of lead poisoning, affected birds may be less able to successfully migrate long distances. On balance, the evidence suggests that many birds dying of lead poisoning in Britain are likely to have ingested lead in Britain.

Sources of lead

In Britain, lead is still legally deposited in areas accessible to waterbirds, and thus many species remain at risk of exposure and ingestion, particularly those that graze on shot-over agricultural land. Although lead shot is still widely available and used extensively and legally for many shooting activities, birds are also likely to have ingested lead gunshot deposited following their illegal use. A recent study found that in the 10th and 11th seasons since the introduction of restrictive legislation in England (HMSO 1999, as amended in 2002 and 2003), 70 % of ducks sold by game suppliers throughout the country had been illegally shot using lead, with no apparent improvement since similar research was conducted in 2002 (Cromie et al. 2002; Cromie et al. 2010). Although questionnaire surveys of shooting participants found a good understanding of the spirit of the law, 45 % of the respondents indicated that they sometimes or never complied with the legislation (Cromie et al. 2010). To date, there has only been one prosecution relating to the legislation in England (R -v- Quince, Harrogate Magistrates Court, 16th May 2011), and this was a secondary offence, the primary offence being illegal shooting of a swan. The enforcement of local restrictions is difficult; lead shot is still legally available for use in other types of shooting, most of which occurs on privately owned land in England (Quy 2010). In the USA and Canada, compliance with legislation appears to be high which has been attributed to the support of waterfowl hunters and conservation police officers for the non-toxic shot programme (Anderson et al. 2000; Stevenson et al. 2005).

Lead gunshot may take tens or hundreds of years to break down and so can remain accessible to feeding waterbirds long after deposition (Mudge 1984; Rooney et al. 2007). While some of the lead recently ingested by waterbirds in Britain may have been deposited historically (Rocke et al. 1997; Quy 2010), it is perhaps more likely that the majority was deposited recently. Recently deposited lead gunshot is likely to be more readily available to waterbirds than historically deposited shot which may become increasingly inaccessible over time as it becomes incorporated into the substrate. However, rates of incorporation are likely to vary widely in space and time, dependent on prevailing local conditions. Research from the USA showed that of 1,345 mallard containing ingested shotgun pellets in the fifth and sixth year after a ban on the use of lead gunshot for waterfowl hunting, 68 % of birds contained only non-toxic shot

(Anderson et al. 2000). Given that the level of compliance was unknown (although it was assumed to be high; Anderson et al. 2000), this suggests that most shot ingested were those most recently deposited.

Efficacy of legislation in reducing lead-related mortality of waterbirds in England (HMSO 1986, as amended 1993; HMSO 1999, as amended 2002 and 2003; EA 2012)

The results of the present study indicate that lead poisoning continues to affect a wide range of waterbirds, long after restrictions on the use of lead shot came into force in Britain. Restrictions have worked in some countries. After a national ban on lead shot for waterfowl hunting in the USA in 1991, there was a relatively rapid decline in the rate of lead shot ingestion (Anderson et al. 2000). Consequently, 1.4 million of 90 million ducks in one year (1997) were estimated to have been spared from fatal lead poisoning (Anderson et al. 2000). The prevalence of elevated blood lead in American black ducks (*Anas rubripes*) wintering in Tennessee declined by 44 %, 6 to 8 years after the ban on lead shot (Samuel and Bowers 2000), and average bone lead concentrations in dabbling ducks and American black ducks in Canada decreased significantly following the establishment of a national regulation in 1997 prohibiting the use of lead shot for waterfowl hunting (Stevenson et al. 2005).

Results from the present study showed that the proportion of birds dying from lead poisoning in England (calculated from the noninfectious data subset) did not vary significantly after the introduction of legislation restricting the use of lead in ammunition, despite an anticipated reduction in lead deposition and subsequent exposure. Perhaps, this is not surprising given the observed lack of compliance with legislation reported in previous studies (Cromie et al. 2002 and Cromie et al. 2010) and the continued legal use of lead in areas used by waterbirds, where restrictions do not currently apply.

The proportion of mute swans dying of lead poisoning in England (calculated from the noninfectious data subset) was found to significantly change over time: from 25 % (1971–1987) to 4.6 % (1988–1999) and 2 % (2000–2010). In the 1970s and 1980s, lead angling weights were a major cause of mortality for mute swans in the UK (Birkhead 1982; Birkhead and Perrins 1986) probably because of their habit of frequenting urban rivers and lakes where fishing activity is high. These results support other evidence that legislation restricting the sale and use of lead fishing weights has had an effect in reducing lead poisoning in this species (Sears and Hunt 1991; Perrins et al. 2003).

Removing the threat of lead poisoning in waterbirds

Phasing out the use of lead shot is now widely recognised as a long-term solution for protecting waterbirds from lead poisoning, and across the world, a variety of regulations have been introduced to assist with this goal (Beintema 2001; Mateo 2009). The use of lead gunshot is banned for waterfowl hunting in the USA and Canada and banned completely in the Netherlands, Denmark and Norway (Avery and Watson 2009).

Considering the failure of current legislation in protecting waterbirds from lead poisoning in Britain, we suggest that the most practical and effective solution is likely to be an extension of current restrictions on the use of lead shot to cover all shooting. Non-toxic alternatives are widely available and their use would be consistent with the habitat goals and the 'wise use' and 'sustainable use' of wetlands principles of the Ramsar Convention (Ramsar 1971) and of the Convention on Biological Biodiversity (CBD 1992). Certain management practices (e.g. the provision of grit to reduce shot ingestion, cultivation of the soil to redistribute surface shot to deeper layers or the regulation of water levels to dissuade birds from feeding in particularly contaminated areas) should be considered in areas where lead gunshot densities are high and which are used by high concentrations of waterbirds. Such management techniques are, however, costly and time-consuming and can only be implemented on a local scale.

Given the long migrations that a number of these waterbird species undertake, during which political boundaries are repeatedly crossed, an international approach with measures taken across the distributional ranges is important if tackling the threat of lead poisoning is to be effective. A renewed commitment from international conventions, national legislation and the hunting community is needed to achieve a broad-scale transition to the use of non-toxic gunshot and angling materials in all environments and thus reduce unnecessary bird mortality and morbidity and environmental pollution.

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