Environment International 63 (2014) 163–168

Contents lists available at ScienceDirect

Environment International

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

Reducing Pb poisoning in birds and Pb exposure in game meat consumers: The dual benefit of effective Pb shot regulation

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ARTICLE INFO

Article history:
Received 26 July 2013
Accepted 6 November 2013
Available online xxxx

Keywords:
Dietary exposure
Environmental policy
Regulatory compliance
Pb poisoning
Non-toxic ammunition
Waterfowl

ABSTRACT

The use of lead (Pb) ammunition in the form of shot pellets has been identified as a Pb exposure risk in wildlife and their human consumers. We explore the hypothesis that Pb shot ban enforcement reduces the risk of avian Pb poisoning as well as Pb exposure in game meat consumers. We assessed compliance with a partial ban on Pb shot commencing in 2003 by examination of 937 waterbirds harvested by hunters between 2007 and 2012 in the Ebro delta (Spain). Prevalence of Pb shot ingestion was determined, as were Pb concentrations in liver and muscle tissue to evaluate the potential for Pb exposure in game meat consumers. Hunted birds with only embedded Pb shot (no steel) declined from 26.9% in 2007–08 to <2% over the following three hunting seasons after ban reinforcement. Pb shot ingestion in mallards decreased from a pre-ban value of 30.2% to 15.5% in the post-ban period. Liver Pb levels were predominantly defined by the presence of ingested shot, whereas muscle levels were defined by the presence of both ingested and embedded shot. Only 2.5% of mallard muscle tissue had Pb levels above European Union regulations for meat (0.1 μg/g wet weight) in the 2008–09 season, when Pb shot ingestion prevalence was also at a minimum (5.1%). Effective restrictions in Pb ammunition use have a dual benefit since this reduces Pb exposure for game meat consumers due to embedded ammunition as well as reducing Pb poisoning in waterbirds.

1. Introduction

The use of lead (Pb) ammunition has been clearly identified as a Pb exposure risk in wildlife and their human consumers (Green and Pain, 2012; Gustavsson and Gerhardsson, 2005; Lévesque et al., 2003). While the first articles regarding Pb poisoning in wild birds date back to the 19th century (Friend et al., 2009), it was not until the end of the 20th century that the use of Pb shot for waterfowl hunting was banned in several North American and European countries (Avery and Watson, 2009; Thomas and Guitart, 2010). In Spain, the use of Pb shot for hunting in wetlands was banned in protected areas in October 2001, but, in some wetlands (such as the Ebro delta) this ban was not fully implemented until the 2003–04 hunting season (Mateo et al., 2013).

Long use of Pb shot in wetlands has left a significant legacy because Pb shot can remain virtually unaltered in the environment for decades (Jorgensen and Willems, 1987; Takamatsu et al., 2010), as is the case in the Ebro delta. Here, waterfowl hunting has taken place for over a century, resulting in Pb shot densities (in the upper 20 cm of sediment) of 97–266 shot/m^2 in wetland lagoons and 6–83 shot/m^2 in surrounding rice fields (Guitart et al., 1994; Mateo et al., 1997, 2013). As a consequence, prevalence of Pb shot ingestion in this area has been very high. For example, between 1991 and 1996 it was 74.2% in Northern pintail (*Anas acuta*), 69.2% in common pochard (*Aythya ferina*) and 30.2% in mallard (*Anas platyrhynchos*) (Mateo et al., 2000). These rates of Pb shot ingestion in the Ebro delta and other Spanish wetlands meant that up to 40.4% of hunted waterbirds have been shown to hold liver Pb levels above the maximum residue level (MRL, 0.5 μg/g wet weight, w.w.) established for offal for human consumption in the European Union (EU) (Guitart et al., 2002).

It is now recognized that consumption of waterbirds with embedded Pb shot in their muscle tissue, or, that have ingested Pb shot, may pose a significant risk to human health (Green and Pain, 2012; Mateo et al., 2011). Indeed, regular consumption of meat from game animals hunted with Pb ammunition has been associated with increased human blood Pb levels (Bjerregaard et al., 2004; Iqbal et al., 2009); and recently, the Spanish Agency for Food Safety and Nutrition recommended that children under six years old and pregnant women do not eat meat from animals killed with Pb ammunition because of the potential for negative effects on the developing central nervous system (AESAN, 2012).

The most widely used alternative to Pb shot is steel, but, as noted by Jules Verne (1874) in his book "The Mysterious Island", its ballistic

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0160-4120/$ – see front matter
http://dx.doi.org/10.1016/j.envint.2013.11.006

properties do differ to that of Pb: "*As Smith had not discovered any lead in the island he substituted iron shot, which were easily made. As they were not so heavy as leaden ones they had to be made larger, and the charges contained a less number, but the skill of the hunters counterbalanced this defect*". Such ballistic differences between Pb and steel can also reduce the tendency for hunters to comply with regulations regarding Pb shot use (Friend et al., 2009). With this in mind, comparisons between existing data regarding Pb shot prevalence in waterfowl with new data obtained after a regulatory ban could be informative (as recently recommended by the African–Eurasian Waterbird Agreement; AEWA, 2012). Hence, between 2007 and 2012 we monitored the presence of embedded shot in hunted birds in the Ebro Delta. We recorded the prevalence of Pb shot and non-toxic shot ingestion, and Pb concentrations in liver and muscle tissue of waterfowl. In considering this information, the aim of this study was to (i) assess the degree of compliance with a ban on Pb ammunition, (ii) study the effect of the partial ban restricted to the protected areas on the prevalence of Pb shot ingestion in waterbirds, and (iii) evaluate the impact of this measure on Pb levels in game meat from a food safety perspective. The annual results of this study were also passed to the Regional Government (Department of Environment, Generalitat de Catalunya) which allowed them to assess on-going compliance with the Pb shot ban, and where needed, improve associated enforcement measures.

2. Methods

2.1. Study area

The Ebro delta is an alluvial plain situated in the NE of Spain with a surface area of 320 km^2 (see Supplementary data, Fig. S1). It is designated as an Important Bird Area (IBA) and included in the Ramsar Convention List of Wetlands of International Importance. The use of Pb ammunition is banned in the protected areas (lagoons and marshes) of the Ebro delta (24%), but it is still allowed in adjacent unprotected crop fields (76%, mostly rice fields).

2.2. Sampling and Pb analysis

A total of 523 waterfowl carcasses from 11 waterbird species (Table 1) were collected from hunting bags between 2007 and 2011. The hunting season in the Ebro delta begins in mid-October and ends on the first week of March. Carcasses were all X-rayed to detect shot pellets, which were then removed during necropsy (Fig. S2). Steel shot was easily distinguished from Pb shot because the former is usually larger, rounder and is attracted to a magnet. Steel shot is the only non-toxic alternative used in the study area at the moment. The embedded pellet data were used as an indicator of compliance with the ban on Pb use. Some animals had embedded steel and Pb shot, which is a likely consequence of repeated encounters with different hunters (Guillemain et al., 2007). In addition, 414 gizzards were collected by hunters during the 2011–12 season, which increased our sample size regarding shot pellet ingestion. Gizzard examinations (n = 885 (some gizzards were lost)) were performed as described by Mateo et al. (1997) in order to obtain comparable data with that reported for Pb shot ingestion between 1991 and 1996 (Fig. S3). Liver and pectoral muscle samples were also obtained and analyzed for Pb concentrations. Muscle samples were only collected in three of the hunting seasons (2007–08, 08–09, 10–11). Livers and muscles were stored at −20 °C until analysis. A total of 465 liver (58 were too damaged by shot to be analyzed) and 327 muscle samples were dried and analyzed for Pb following the methodology described by Mateo et al. (2007); using a graphite furnace-atomic absorption spectroscopy system (AAnalyst 800, Perkin Elmer). Blanks (n = 31) were analyzed in each batch of digestions. Limit of detection (LOD, back-calculated in tissue concentrations using 3 × SD of the blanks) was 0.030 μg/g dry weight (d.w.). Values <LOD were assigned as LOD/2 (0.015 μg/g d.w.) within statistical analysis. Bovine liver (BCR 185R, Community Bureau of Reference) with a certified Pb level (mean ± S.D.) of 0.172 ± 0.012 μg/g d.w. was analyzed with samples and the Pb recovery was 94.5% (n = 18, mean ± S.E. = 0.162 ± 0.007 μg/g d.w.). All Pb concentrations are presented as μg/g d.w.

2.3. Statistical analyses

All the X-rayed waterbirds were hunted in the protected lagoons. Hence, the presence of embedded Pb shot alone, without steel shot, was considered as a failure to comply with the ban. The presence of steel alone was considered compliance. We conservatively assumed that birds with steel and Pb were most probably killed with the latter, and were only carrying Pb shot as a result of previous hunter encounters in non-protected areas. Therefore, the data used to evaluate the trend in ban compliance was the percentage of birds with embedded Pb shot only. Percentages for non-compliance were compared among seasons (2007–08 to 2010–11) for the whole sample and for mallards only (as a bioindicator species) with chi-square (χ^2) tests. The number of embedded pellets was compared between mallards shot with steel only, or, with Pb only, with a Mann–Whitney test. This non-parametric test was used because the data did not fit a normal distribution (even after a logarithmic transformation). As the number of shot that impacts a bird may depend on the size of the animal, we also studied this relationship with a Spearman correlation coefficient (r_s). Differences in the prevalence of Pb shot ingestion and in liver Pb levels >5 μg/g d.w. were compared with χ^2 tests among species, between pre and post-ban periods for all species, and among the post-ban hunting seasons

Table 1
Prevalence of Pb and steel shot ingestion and liver Pb levels >5 μg/g d.w. in waterbirds before and after Pb shot ban.

Waterbird species		Pre-ban (1991–1996)[a]				Post-ban (2007–2012)[b]					
		Shot ingestion		Liver Pb		Shot ingestion				Liver Pb	
Common name	Scientific name	N	Pb (%)	N	>5 μg/g (%)	N	Pb (%)	Steel (%)	Either (%)	N	>5 μg/g (%)
Eurasian wigeon	*Anas penelope*	25	4.0	20	10.0	16	12.5	0.0	12.5	11	9.1
Gadwall	*Anas strepera*	25	8.0	24	8.3	40	0.0	2.5	2.5	9	0.0
Common teal	*Anas crecca*	35	22.9	31	19.3	170	10.6*	0.6	11.1	77	10.4
Northern pintail	*Anas acuta*	97	74.2	24	75.0	25	76.0	20.0	84.0	15	100
Mallard	*Anas platyrhynchos*	86	30.2	43	27.9	380	15.5*	10.0	21.0	216	20.4
Northern shoveler	*Anas clypeata*	36	27.8	36	22.2	102	7.8*	2.9	7.9	37	5.4*
Red-crested pochard	*Netta rufina*	21	19.0	21	9.5	16	12.5	25.0	31.3	2	0.0
Common pochard	*Aythya ferina*	26	69.2	26	53.9	20	35.0*	30.0	45.0	6	33.3
Tufted duck	*Aythya fuligula*	5	80.0	5	60.0	5	20.0	0.0	0.0	1	0.0
Eurasian coot	*Fulica atra*	28	3.6	28	3.6	93	2.2	23.7	25.8	91	0.0
Common snipe	*Gallinago gallinago*	–	–	2	0.0	18	5.6	0.0	5.6	–	–

[a] Guitart et al. (1994), Mateo et al. (1997, 2000).
[b] Present study.
* Significantly different between 1991–1996 and 2007–2012 periods (χ^2 tests, $p \leq 0.05$).

for mallards. The percentage of waterbirds with liver and muscle Pb concentrations above the MRL set for offal (0.5 μg/g w.w. ≈ 1.5 μg/g d.w.) and meat (0.1 μg/g w.w. ≈ 0.32 μg/g d.w.) for human consumption in the EU (European Commission, 2006) was calculated and compared among species and among seasons for mallards with χ^2 tests. One-way analysis of variance (ANOVA) was used to determine differences among species and hunting seasons in ln-transformed (natural logarithm) liver and muscle Pb concentrations. This transformation was used to adjust the data to a normal distribution to permit the use of parametric tests. Tukey tests were used to establish post-hoc differences among groups. The influence of the presence of ingested or embedded Pb pellets on Pb concentrations in liver and muscle was analyzed with general linear models (GLMs) using Pb concentration as the dependent variable, and the presence/absence of embedded Pb shot, the presence/absence of ingested Pb shot, and species as factors. The level of significance was set at $p \leq 0.05$. Statistical procedures were carried out with IBM SPSS Statistics 19.

3. Results

Compliance with the ban on Pb shot increased during our study period. In 2007–08, the percentage of waterbirds shot with Pb pellets only was 26.9%, and this declined significantly in the following three seasons to ≤2% ($\chi^2_3 = 86.8$, $p < 0.001$; Fig. 1). In mallards specifically, these values dropped from 35.7% in 2007–08 to ≤3.1% ($\chi^2_3 = 55.8$, $p < 0.001$; Fig. S4). The number of embedded shot also depended on species body mass ($n = 9, r_s = 0.683, p = 0.042$; Fig. S5). The number of embedded pellets was similar in mallards shot with Pb only ($n = 24$, median = 4, range = 1–24) and in those shot with steel only ($n = 164$, median = 5, range = 1–30) (Mann–Whitney test, $Z = 1.186, p = 0.236$).

The prevalence of Pb shot ingestion decreased significantly after the ban in Northern shoveler (*Anas clypeata*), common teal (*Anas crecca*), common pochard and mallard (χ^2 tests, all $p \leq 0.047$) and a similar trend was observed for the percentage of birds with liver Pb levels >5 μg/g d.w. (Table 1). Shot ingestion differed among species ($\chi^2_{13} = 116$, $p < 0.001$, Table 1). For mallard, during the 2007–08 hunting season shot ingestion was 28.6%, not significantly different to the pre-ban value of 30.2% (Table 1 and Fig. 2). However, a significant decrease was found in the 2008–09 season (5.1%) ($\chi^2_1 = 8.227$, $p = 0.004$) after an increase in ban compliance (Fig. 2). During the post-ban period, steel shot ingestion was found in all species except 3 (of 11; Table 1). The mean number of pellets (±SD, range) detected in the gizzards examined was 4.74 (±10.54, 1–86) for Pb, and 2.57 (±4.83, 1–33) for steel.

Muscle Pb concentrations decreased significantly between 2007–08 and the subsequent seasons in mallard ($F_{2,132} = 12.7$, $p < 0.001$) (Fig. 3) and in the entire pool of hunted birds ($F_{2,324} = 21.3$, $p < 0.001$). Liver Pb was significantly lower in 2008–09 compared to other seasons in mallards ($F_{3,212} = 3.12$, $p = 0.027$). In terms of EU MRL values, the mean dry mass of the muscle analyzed was (mean ± SD) 30.9 ± 2.6%. Pb concentrations differed among waterbird species in muscle ($F_{9,317} = 6.1$, $p < 0.001$) and in liver ($F_{9,455} = 18.6$, $p < 0.001$; Table 2). Muscle Pb was largely explained by the presence of ingested Pb shot ($F_{1,311} = 95.5$, $p < 0.001$; Fig. 4), but there was also a significant effect due to the presence of embedded Pb shot ($F_{1,311} = 15.7$, $p < 0.001$). Liver Pb was explained only by the presence of ingested Pb shot in the gizzard ($F_{1,449} = 175.6$, $p < 0.001$; Fig. S6).

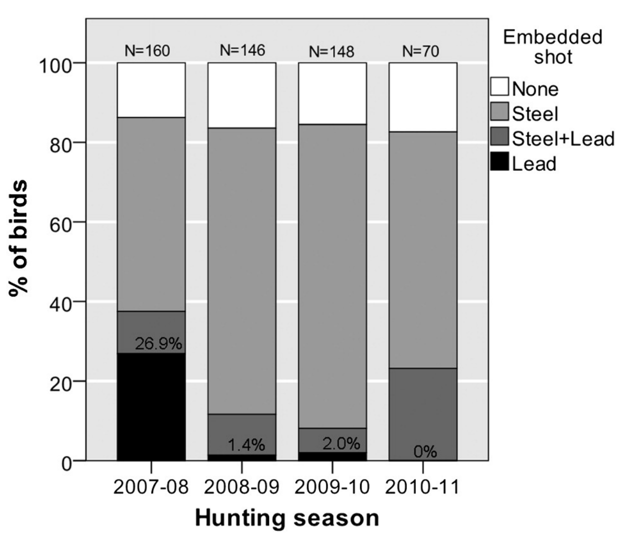

Fig. 1. Percentage of waterbirds with embedded steel and/or lead shot after the partial ban on Pb shot in the Ebro delta. Sample size (N) and % with only embedded Pb shot are shown for each season.

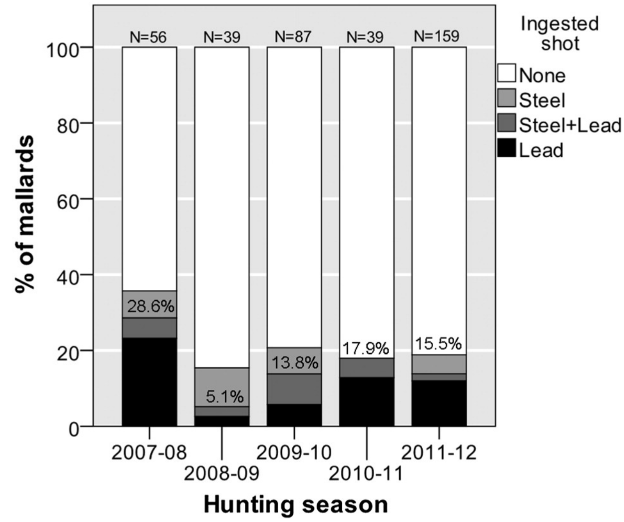

Fig. 2. Prevalence of steel and/or lead shot ingestion in mallards after the partial ban on Pb shot in the Ebro delta. Sample size (N) and % with ingested Pb shot (Pb and Pb + steel) are shown for each season.

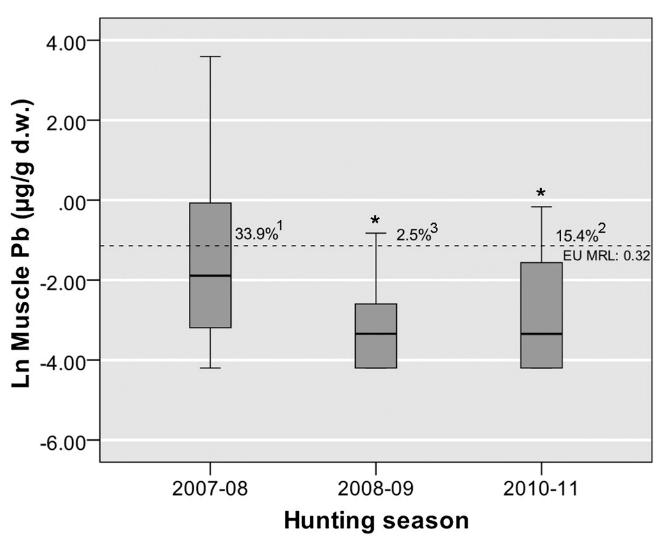

Fig. 3. Box-plots (median, 25–75%, range) of muscle Pb levels (ln-transformed) in mallards hunted during three different hunting seasons. The dashed line represents the maximum residue level set for meat for human consumption in the European Union (European Commission, 2006). The percentage of samples above this MRL is shown for each season. *Significant differences with 2007–08 season ($F_{2,132} = 12.7$, $p < 0.001$). [1,2,3]Differences in superscripts show differences among seasons in percentages above MRL ($\chi^2_2 = 15.35$, $p < 0.001$).

Table 2
Concentrations of Pb (μg/g d.w.) in liver and muscle of waterbirds after the Pb shot ban in the Ebro delta.

Species	Liver					Muscle				
	N	GM[a]	95% CI[b]	Min–max	>1.5 μg/g (%)[c]	N	GM	95% CI	Min–max	>0.3 μg/g (%)[c]
Eurasian wigeon	11	0.30[2]	0.09–0.88	<LOD[d]–12.0	9.1	12	0.07[2]	0.02–0.19	<LOD–1.14	16.7
Gadwall	9	0.37[2]	0.22–0.60	0.18–1.49	0.0	10	0.09[2]	0.04–0.20	<LOD–1.38	10.0
Common teal	77	0.19[2]	0.10–0.28	<LOD–52.9	16.9	43	0.09[2]	0.05–0.19	<LOD–20.8	30.2
Northern pintail	15	41.6[1]	25.6–68.8	6.95–166	100	15	1.43[1]	0.79–2.59	0.12–5.60	86.7
Mallard	216	1.10[2]	0.84–1.39	<LOD–180	42.1	135	0.08[2]	0.06–0.11	<LOD–36.3	19.3
Northern shoveler	37	0.30[2]	0.16–0.61	<LOD–175	16.2	25	0.05[2]	0.02–0.10	<LOD–2.89	12.0
Red-crested pochard	2	0.37[2]		0.26–0.52	0.0	2	0.10[2]		0.06–0.15	0.0
Common pochard	6	1.44[2]	0.17–12.1	0.08–23.7	50.0	6	0.37[1,2]	0.10–1.35	0.07–1.69	50.0
Tufted duck	1	0.63			0.0	1	0.10			
Eurasian coot	91	0.40[2]	0.31–0.46	<LOD–2.25	1.1	78	0.04[2]	0.03–0.06	<LOD–444	6.4

[1,2]Means sharing a number do not differ significantly.
[a] GM: geometric mean.
[b] CI: confidence interval.
[c] Percentage of samples above maximum residue levels set for offal (1.5 μg/g) and muscle (0.3 μg/g) for human consumption in the European Union (European Commission, 2006).
[d] LOD: limit of detection.

The number of ingested shot positively correlated with Pb levels in muscle ($r_s = 0.521$, $p < 0.001$) and in liver ($r_s = 0.505$, $p < 0.001$).

4. Discussion

4.1. Pb shot ban compliance

During the 2007 to 2011 period there was a significant increase in compliance with a ban on the use of Pb shot in the Ebro delta. Non-compliance values declined from 26.9% in 2007–08 to ≤2% in the three subsequent seasons (Fig. 1). The Pb shot ban has thus translated into a significant reduction in the prevalence of Pb shot ingestion in four waterfowl species, and a significant decrease in Pb levels in game meat. This latter decrease can be attributed to both (a) a reduction in the prevalence of Pb shot ingestion, and (b) the reduced risk of Pb ammunition contamination of meat around wounds because steel shot is being used instead.

The contribution of non-toxic shot regulations to waterfowl conservation has been evaluated in the USA, where the use of Pb shot for waterfowl hunting was completely banned in 1991. There, compliance values based on counts of Pb and steel shot shell wads found in the field ranged from 54.8 to 92.2% in different USA locations, and five years after the Pb ban in Illinois, hunter compliance based on embedded shot was 98.9% in mallard and 96.5% in Canada goose (*Branta canadensis*) (Havera et al., 1994). Minimum hunter non-compliance was just 1.1% (for mallard) and 1.8% (for goose), which is similar to the compliance values observed here for the Ebro delta. In Canada, where ban compliance based on anonymous hunter surveys was >80%, bone Pb levels in hunted waterfowl declined significantly from 1989–90 to 2000 (Stevenson et al., 2005). In the USA and Canada, legislative compliance appears to be high, which has been attributed to the general support of waterfowl hunters for the non-toxic shot program and to active enforcement led by conservation police officers (Anderson et al., 2000; Stevenson et al., 2005). Compliance values from North America contrast quite starkly against the low level of compliance recently documented in England, where 68% (in 2001–2002) and 70% (in 2008–2010) of mallards had been shot with Pb despite the fact that this ammunition was banned for hunting over wetlands in 1999 (Cromie et al., 2010). During our first season of monitoring, we also detected relatively high non-compliance values in the Ebro delta (26.9%, Fig. 1). However, this was corrected in subsequent seasons as enforcement and vigilance from park rangers increased, and, as local authorities threatened to ban hunting in the protected areas if non-compliance persisted. Improved enforcement was undertaken without any new laws or changes to regulations regarding Pb shot use. Instead, stricter controls on ammunition carried by hunters at entry points to hunting areas were put in place, random carcass sampling was undertaken at the end of shoots, and, national ID numbers were recorded for the hunters who harvested each bird. Such measures acted as simple but effective deterrents against non-compliance. Carcasses were also X-rayed for the present study, and results obtained were regularly passed to the regional government so that the effectiveness of enforcement actions could be assessed and, if needed, adjusted during the subsequent hunting season. As a consequence, a marked decrease in the percentage of embedded Pb shot (≤2%) was observed in 2008–2009, and in subsequent hunting seasons (Fig. 1).

One argument often used by hunters against replacing Pb ammunition with non-toxic alternatives is that Pb shot tends to remain embedded in the target, while steel may pass through and thus leave badly injured birds but not kill them. However, this was not supported by our data as we found that the number of embedded pellets in mallards was similar in individuals shot with Pb only and in those shot with steel only. Moreover, the performance of steel shot appears to be entirely acceptable for waterfowl hunting, in as far as the number of harvested birds was similar before and after the Pb shot ban in the Ebro delta (Mateo et al., 2013). Likewise, the crippling rate found in the USA was only found to increase temporarily, during ban implementation (Schulz et al., 2009); and presumably, after this point, hunters had

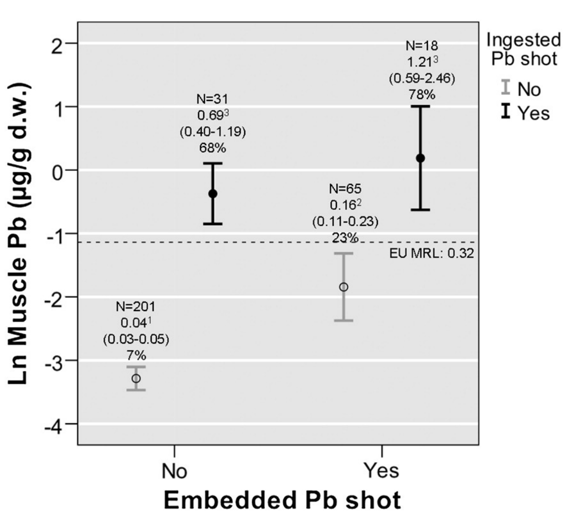

Fig. 4. Muscle Pb levels (ln-transformed, mean with 95% CI) in waterbirds with ingested and/or embedded Pb shot. The dashed line represents the maximum residue level (MRL) set for meat for human consumption in the European Union (European Commission, 2006). Values adjacent to bars are N, back-transformed geometric mean with 95% CI and % of samples above the EU MRL (0.32 μg/g d.w. ≈ 0.1 μg/g w.w.). Means sharing a superscript do not differ significantly.

made slight adaptations to their style and technique which facilitated the effective use of the steel shot alternative.

4.2. Risk for waterbirds

Inter-specific differences observed here in the prevalence of Pb shot ingestion are most likely a function of variability among species in terms of diet, and the type of grit ingested (Figuerola et al., 2005; Mateo et al., 2000; Pain, 1990). In this regard, mallard is a very useful biomonitoring species for Pb poisoning, since it holds a near worldwide and abundant distribution (Guitart et al., 1994), and the prevalence of Pb shot ingestion in this species is commonly moderate to high among waterfowl (Mateo, 2009). Here, the prevalence of Pb shot ingestion in the 2007–08 hunting season (28.6%, Fig. 2) did not differ when compared to the pre-ban value (30.2%; Mateo et al., 2000). However, a significant decrease in Pb shot ingestion was found in the following seasons (mean 2008–12: 15.5%, Fig. 2), after ban reinforcement. Similar reductions in Pb shot ingestion in waterbirds after a Pb shot ban have also been observed in the Mississippi flyway (USA), where rates of Pb shot ingestion declined from 7.8–8.4% for the pre-ban period (1938–79) to 2.8% (1996–97) after the 1991 nationwide ban (Anderson et al., 2000). Samuel and Bowers (2000) also found a reduction in the prevalence in waterfowl with elevated blood Pb levels (>20 μg/dL) from 11.7% before the ban to 6.5% six years after the ban in Tennessee (USA). In contrast, the proportion of waterbirds birds dying due to Pb poisoning has yet to decline in England despite a Pb shot ban (Newth et al., 2013).

An on-going risk for waterbirds exists due to the high density of Pb shot pellets already accumulated in sediments, even in the protected areas within the Ebro delta. In addition to this, Pb shot pellets can still be used legally in areas regularly used by waterfowl for feeding such as in adjacent rice fields, which are not considered wetlands by the Catalan authorities. This essentially means that a partial Pb shot ban is in force which is only commonly observed in protected wetlands. With this in mind, it is unsurprising that overall Pb shot ingestion rates in mallards only decreased by ~50% during the period from 1991–96 (30.2%) to 2011–12 (13.8%) (Fig. 2).

4.3. Risk for human consumers

Pb exposed animals represent a significant hazard for human consumers due to the elevated Pb levels held in their tissues (Guitart et al., 2002; Taggart et al., 2011). Here, 68–78% of birds (Fig. 4) with ingested Pb shot also had muscle Pb levels above EU MRLs established for livestock meat (0.1 μg/g w.w., European Commission, 2006). In addition, birds that had been killed with Pb shot tended to have higher muscle Pb than birds shot with steel, or, without pellets embedded in the flesh (Fig. 4). It is well recognized that Pb shot and bullets can suffer from fragmentation on impact with game animals. This can result in quite widespread contamination around wounds and increase the risk of exposure to Pb in human consumers of game meat (Hunt et al., 2009; Johansen et al., 2004; Pain et al., 2010; Scheuhammer et al., 1998). Moreover, common recipes that use acidic ingredients, like vinegar or wine, to cook game meat can further increase the transfer of Pb from ammunition to the meat and enhance the subsequent bioavailability of that Pb (Mateo et al., 2007, 2011). Logically, biologically incorporated Pb held within poisoned bird tissues might also be expected to have a higher bioavailability for consumers when compared to embedded Pb in a metallic form. In addition to dietary intake, regular physical contact with Pb ammunition during hunting is also an additive and important route of Pb exposure for hunters (Tsuji et al., 2008).

In broader terms, restrictions in Pb ammunition use can have an immediate effect on the reduction of Pb exposure in game consumers from embedded ammunition, and, a mid-term effect due to the decrease in the prevalence of Pb poisoning in waterbirds harvested by hunters. Here, we have found that the percentage of muscle samples of mallards above the EU MRL for Pb declined from 33.9% in 2007–08 to 2.5% in 2008–09 (Fig. 3); and this reduction directly coincided with increased Pb shot ban compliance and the lowest observed prevalence of Pb shot ingestion (5.1%, Fig. 2). However, there was an increase in the 2010–11 season in the prevalence of lead shot ingestion (17.9%, Fig. 2) and the muscle Pb levels recorded above EU MRL (15.4%, Fig. 3). This finding is difficult to explain, because the compliance with the Pb shot ban in the protected areas was high in all seasons after 2008–09. Several factors may have acted to increase the prevalence of Pb shot ingestion in 2010–11. One issue may relate to the increasing number of permits given to scare birds away from the rice fields during spring and summer. This may have had a negative effect on efforts to reduce the impact of Pb shot in surrounding protected areas, especially since farmers and hunters predominantly used Pb shot gun fire for this purpose.

Although consumption of game meat in the general population within Spain (and elsewhere in Europe) is generally low, it can be significant among hunters, their families and their social group (Sevillano Morales et al., 2011). Unlike in the USA, wild game meat in Spain (and other EU countries) can be/is widely sold and is available on the menus of restaurants. Likewise, even sporadic/occasionally elevated intake of Pb ammunition fragments in particularly susceptible groups within the general population (pregnant women, children) may well be toxicologically relevant. In cases where gamebird consumption is greater than once per week, an effective ban on Pb shot may reduce significantly the risk of dietary exposure to elevated Pb. For example, Green and Pain (2012) found that consumption of <1 meal per week may be associated with a reduction in intelligence quotient (IQ) in children, and that 1.2–6.5 meals per week may be associated with increased systolic blood pressure, occurrence of chronic kidney disease, and rates of spontaneous abortion.

Although the results of this study demonstrate the clear beneficial effects that can occur when a ban on Pb ammunition in a protected wetland is effectively enforced, the partial implementation of this ban (which excludes adjacent rice fields/feeding grounds) will not fully and effectively eliminate the risk of Pb exposure in humans due to consumption of contaminated meat from poisoned birds or those physically containing Pb ammunition residues. It is critical that hunters themselves are engaged in efforts to ameliorate the impacts of Pb on wildlife in general, and on game species in particular. Collaboration with, and educational programs for, the hunting community should therefore be promoted (Friend et al., 2009); and these programs must highlight the dual benefits of using non-toxic shot, as well as the potential risk that Pb contaminated food poses to human health, especially to fetuses and to young children (Carlisle et al., 2009; Lanphear et al., 2005). We suggest that in order to effectively reduce the risk of Pb exposure in humans and poisoning in waterbirds, "protected wetland only" bans on Pb shot should be extended to adjacent feeding grounds. The fact that reasonably good ban compliance was obtained in protected areas within a short period, and, that hunting bag counts were apparently unaffected by a change to steel ammunition (Mateo et al., 2013), also supports the goal to extend the Pb ban to other ecologically important habitats.

Acknowledgments

This study was supported by the Spanish Ministry of Science and Innovation (grants CGL2007-62797 and CGL2010-17030). NVC benefits from an FPI Fellowship (BES-2011-045670), MAT and MEOS were contracted under the Juan de la Cierva program and MMH benefits from a Marie Curie Fellowship (PIEF-GA-2011-299747). We thank the Ebro Delta Natural Park for their support, the Hunting Association "Terres de l'Ebre" for their help with sampling and Pablo R. Camarero for his analytical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2013.11.006.

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