Lead-Shot Exposure in Red-Legged Partridge (*Alectoris rufa*) on a Driven Shooting Estate

PABLO FERRANDIS,*^{,†} RAFAEL MATEO,[‡] FRANCISCO R. LÓPEZ-SERRANO,[†] MÓNICA MARTÍNEZ-HARO,[‡] AND ESMERALDA MARTÍNEZ-DURO[†]

ETS Ingenieros Agrónomos y Forestales, University of Castilla-La Mancha, Campus Universitario s/n, 02071 Albacete, Spain, and Instituto de Investigación en Recursos Cinegéticos IREC (CSIC-UCLM-JCCM), Ronda de Toledo s/n, 13071 Ciudad Real, Spain

Received January 29, 2008. Revised manuscript received June 3, 2008. Accepted June 4, 2008.

The goal of the study was to investigate the accumulation and spatial patterns of spent lead (Pb) shot pellets and the prevalence of shot ingestion in red-legged partridge in a driven shooting estate. Soil was collected using a regular sampling design perpendicular to three shooting lines. Factors involved in shot spatial distribution were investigated by a causal structural equation model (SEM). Shot ingestion prevalence and liver and bone Pb concentrations were studied in partridges hunted in 2004 and 2006. Shot soil-burden averaged 73 600 units/ ha (i.e., 8.1 kg/ha). Shot density was significantly higher in front of than behind shooting lines, with greatest accumulation occurring at between 40-110 m and in certain ecotones (i.e., shrubland-dry cropland). Analyses revealed 7.8% of partridges with evidence of Pb shot ingestion. Particle size in diet, gritsize composition, and shot ingestion prevalence were significantly higher in 2004 than in 2006, indicating that supplying partridges with large seeds (i.e., corn) may increase the risk of Pb shot ingestion. Moving shooting lines into croplands and controlling seed size used for diet supplementation may reduce shot ingestion.

Introduction

Lead (Pb) poisoning of waterfowl by ingestion of shot pellets spent in hunting activities has been observed for well over a century and is currently recognized as a major ecological problem in many European countries and in North America (1, 2). There can often be a high accumulation of pellets in flooded sediments where shooting has taken place in the same places year after year. In addition, if there is also a scarcity of adequate grit to serve as gastrolites, certain wetlands can pose a high-risk of Pb exposure as waterbirds are highly likely to ingest Pb pellets instead of grit (3, 4).

Unfortunately, shot ingestion is not restricted to wetland ecosystems. Locke and Friend (5) reviewed available information and reported that 31 free-ranging nonwaterfowl avian species had died due to Pb pellet ingestion. Reports on galliform species poisoned by Pb shot ingestion have proliferated in recent years (6–10). Kendall et al. (11) warned that there was increasing evidence of widespread deposition of Pb in terrestrial ecosystems, and recommended that further work be done to investigate the prevalence of Pb shot ingestion in birds and to assess the risk of toxic effects from hunting activities outside wetlands. Recent reports have noted the incidence of Pb poisoning in grey partridge (*Perdix perdix*) over the last four decades in the UK (9), and high bone Pb concentrations in American woodcock (*Scolopax minor*) in Canada (12). Despite this scenario, few detailed studies on spent-shot burden in upland habitats are available (13–17).

In Spain, the risk of Pb poisoning in upland gamebirds may increase because recreational upland hunting estates have proliferated rapidly in recent times. There has been a growing demand for red-legged partridge (*Alectoris rufa*; hereafter partridge), and this has led to an artificial increase in hunting bags (>4 million/year (7)) by the release of farm-reared partridges in many areas. Although hunting partridge is very important in southern European countries, there is currently little information on Pb shot prevalence in this species (7, 8).

The main goals of the present work were focused on the analysis of Pb-shot burden in a terrestrial system representative of small-game estates in Europe and the estimate of Pb prevalence in the most important upland gamebird species in Spain. Specifically, we aimed to define spatial patterns of spent-shot accumulation in the soil and the factors influencing this, as well as to determine the real risk that the degree of exposure observed may pose.

Material and Methods

Study Area. The study was carried out on a private upland small-game hunting estate located in a farm called Orán with a surface area of 3500 ha, in central-southeastern Spain. Two thirds of the land is occupied by irrigated and dry cereal crop fields (corn, Zea mays, and barley, Hordeum vulgare, respectively). Dry cropland is managed traditionally, by alternating yearly cultivation (November-June) with fallow. For the last two decades, cornfields have been irrigated using center-pivots, which has resulted in a highly productive agrosystem allowing one April-November cultivation cycle every year. The remaining surface area is covered by natural vegetation, consisting of sparse middle-height shrubland dominated by Stipa tenacissima, Rosmarinus officinalis, Thymus vulgaris, Quercus coccifera, and Juniperus oxycedrus. The soil is thin, stony, and formed of basic calcareous material. The climate is Mediterranean (annual mean temperature = 13.6 °C, annual rainfall = 367 mm), with a pronounced summer drought during July and August.

The partridge hunting season extends from October to February. On the estate studied, partridges are hunted by driven shooting ("ojeo" in Spanish), a common and widespread technique in Spain. In this hunting technique, gamebirds are flushed from plant cover over a line of hunters. In the study area used, there were 30 fixed shooting lines, with 16 stations at each one. Stations consisted of 2 m wide stony parapets, which were spaced around 40 m apart, usually hiding one hunter each. Ammunition employed were cartridges of caliber no. 7 (or no. 6 to a lesser extent). The partridge population (3000-4000; A. Sánchez, Pers. comm.) was wild (i.e., stocks for shooting were not supplemented by reared-gamebird releases), although birds were supplied with water by means of small artificial water-holes during summer, and with additional food by spreading corn and barley seeds on paths crossing the shrubland areas. Hunting on the estate has been practiced since the 1950's. The intensity of shooting,

^{*} Corresponding author phone: 34 967 599200; fax: 34 967 599238; e-mail: pablo.ferrandis@uclm.es.

[†] University of Castilla-La Mancha.

[‡] Instituto de Investigación en Recursos Cinegéticos IREC

however, is markedly variable. The frequency of partridge hunting events (i.e., several driven shootings in a day) per year ranges 0–2, depending on the reproductive success of partridge in the previous brooding season. The number of hunter ranges could vary from 6 to 16 per line, and the selection of lines for hunting was not systematic (although central zones were used more frequently). Hence, gathering precise data on hunting history was not possible. European rabbits (*Oryctolagus cuniculus*) are abundant in the area. Their population is controlled by hunting, which concentrates on the shrubland–cropland ecotones, the preferred habitat of this species. Here, stones removed from croplands are piled at the edges, forming brood refuges for rabbits.

Spent-Shot Estimate. Three shooting lines (zones) were selected, each having similar features, except for the distance to the croplands. The entire line and its influence area (i.e., 200 m around it, the maximum trajectory of no. 6 and no. 7 pellets (*18*)) were located within the shrubland zone and did not interfere with other lines or their influence areas. Orientation of lines was always SW–NE, and the territory was flat at all three locations.

In each shooting line, five perpendicular linear transects were established. Transects were 300 m long: 200 m ahead and 100 m behind the shooting line. The separation between two consecutive parallel transects in the line was three shooting stations (around 120 m).

Sampling was carried out during September 2004. At each transect, 30 plots were arranged at fixed intervals of 10 m, i.e., 20 plots ahead and 10 behind the shooting line. This design is justified since hunters shoot ahead much more frequently than they do behind. Plots were 1×2 m in area, positioning the longer plot-side parallel to the shooting line. In each plot, five soil quadrants of 15×15 cm were randomly placed and excavated to 1 cm depth (16), using a garden spade. Soil-depth sampling is justified since partridges often scratch the soil surface with the beak and feet when looking for food (19), and so, slightly buried pellets are potentially available. The five soil samples from each plot were gathered together in one labeled plastic bag forming a composite soil sample. Hence, we collected 150 soil samples at each shooting line. Distances between transects, sampling plots, and from plots to ecotones (if changes occurred), were confirmed by means of digital ortho-photographs using ArcMap 9.1 software (ESRI, 2001) and/or GPS geo-references.

In the laboratory, soil samples were poured through 1 cm and 1 mm mesh sieves, in order to eliminate big elements and to reduce soil bulk and aggregation, respectively. The remaining soil fraction was checked with a binocular microscope for the presence of Pb shot. Noneasily recognizable or surface-degraded Pb pellets in the soil were scratched with a scalpel to confirm their presence in the sample. A random sample of spent shot pellets recovered from the soil (n = 30) was weighed and sieved at different mesh sizes (those used in the granulometric analysis of grit; see below) to determine their dimensions.

Prevalence of Pb Shot Ingestion in Partridge. The owners of the estate donated 10 and 54 gamebirds that were shot and killed during two driven shootings at the beginning of the hunting season (at the end of October) in 2004 and 2006, respectively (in 2005, partridge hunting was not practiced on the estate). Twelve additional partridge gizzards taken from birds that were also driven-shooting hunted were received for analysis in 2006. Partridges were weighed and wing and tarsus were measured before necropsy. Sex was determined by gonad examination, and juveniles (<1 year old) were identified by the presence of the Fabricius bursae. Samples of liver and femur were collected for Pb analysis. Crop content was examined by counting and identifying elements (seeds for the most part). Samples of seeds (n = 10 for each species, if possible) recovered from the crops were

weighed. The gizzard examination followed the protocol established by Pain and Eon (20) and Butler (8). Gizzards (n = 76) were checked for shot entry holes and dissected. Content was washed and examined under a binocular microscope. All pellets found were checked for signs of being shot into the gizzard. Such shot tended to be deformed by impacts and was therefore not round in shape. Likewise it had fresh, exposed Pb surfaces which were shiny. Ingested shot tends to be very round and dull in appearance. Grit in gizzards (excluding pellets) was sieved through 5, 4, 3, 2, 1, and 0.5 mm mesh-sizes.

Lead Determination. Samples of liver and femur were freeze-dried and 0.3-0.5 g were digested with 3 mL of HNO₃ (69% Analytical grade, Panreac, Spain), 1 mL of H₂O₂ (30% v/v Suprapur, Merk, Germany) and 4 mL of H₂O (Milli-Q grade) with a microwave digestion system (Ethos E, Milestone, Italy). Samples were diluted to a final volume of 50 mL with Milli-Q H₂O. The determination of Pb was achieved using a graphite furnace-atomic absorption spectrometer (AAnalyst 800; Perkin-Elmer, U.S.) equipped with an autosampler AS 800 (Perkin-Elmer) and used 50 μ g NH₄H₂PO₄ and 3 μ g Mg(NO₃)₂ as a matrix modifier (Merck) in each atomization for Pb. Calibration standards were prepared from a commercial solution with 1 g/l of Pb (Panreac). The limit of detection was 0.073 μ g/g dry weight (d.w.). Blanks were processed in each batch of digestions. Reference samples of bovine liver (BCR 185R, Community Bureau of Reference) and bone ash (SRM 1400, National Institute of Standards and Technology, U.S.) were analyzed (n = 8 and 12, respectively) and the recovery (mean \pm SE) of Pb was 94.4 \pm 5.8% and 94.5 \pm 1.8%, respectively. Concentrations are given in dry weight (d.w.).

Data Analysis. Data on Pb-shot density in soil (log transformed) were subjected to a multifactor ANOVA, in order to test significant differences depending on zone and shooting direction. Cases responsible for differences were detected using the Fisher least significant difference test (LSD test) at p < 0.05.

To show a smoothed spatial distribution of the number of pellets within each zone, we carried out a local deterministic interpolation technique based on the extent of similarity (Inverse Distance Weighted, IDW (21)), using ArcMap 9.1 software (ESRI, 2001). IDW interpolation explicitly implements the assumption that things that are close to one another are more alike than those that are farther apart. Thus, IDW assumes that each measured point has a local influence that diminishes with distance. It works better if sample points are evenly distributed throughout the area and if they are not clustered (21), such as in our sampling design.

Factors determining the spatial distribution of spent shot pellets were investigated by structural equation modeling (SEM), also called "path analysis" (22). SEM is an advanced statistical method which operates on the basis of causal relationships, in contrast to classical statistical methods (e.g., ANOVA), where the data usually come from controlled experimental design with a predictive or explicative goal. Therefore, SEM can be used to confirm or discard a hypothesized causal model (i.e., causal relationships in observed data). For the estate studied, available information on variables related to hunting activity (e.g., abundance and distribution of hunting species, number of individuals killed, number of cartridges fired) was incomplete. We neither found data about flight height of partridges during driven shootings, average angle (azimuthal and zenithal) of shootings, wind effect on deviation of partridge flight, etc. Consequently, we were forced to define some variables subrogated to factors to be investigated. We hypothesized that (i) the spatial distribution of pellets depends directly on the driven shooting practice, exerting an uncontrolled (unknown) effect on partridge flight features, and on the physical characteristics

(angles) of shooting; (ii) the spatial distribution imposed by driven shooting practice is broken when, in addition, other hunting techniques are carried out on the same estate; and (iii) the density of pellets depends directly on shooting quantity, which in turn depends on the abundance of game animals.

As a consequence of Hypothesis 1, some places within a shooting-line area should have a higher probability of containing Pb shot pellets than others. To locate these places, we have defined two variables, i.e., the distance from any sampling point to the central shooting station (DC), and to the northern shooting station (DN). Regarding Hypothesis 2, it is well-known that rabbits are especially abundant near certain ecotones (see the study area description). Here, there is a higher probability that shots will be fired straight at the soil, when rabbits are hunted. The distance from sampling point to the nearest ecotone (D_{eco}) is a reasonable way of quantifying the unexpected high concentration of Pb shot due to rabbit hunting. Hypothesis 3 is based on the knowledge that partridges do not reproduce within irrigated croplands. Consequently, where there is a higher amount of irrigated surface, there will be a lower partridge density in a zone. Conversely, the higher the distance is to irrigated croplands, the higher the partridge population. We have quantified this hypothesis by the ratio "irrigated surface (ha)/square distance (km²) from gravity center of the irrigated surface to the shooting station". There were two important irrigated surfaces on the estate: one located to the north of our study zones (the ratio was denoted as R_1) and other one to the southeast (R_2) . Moreover, we have added an indicator variable to denote the direction of shooting (S, with value equal to 1 to refer to the area in front of the shooting line, and equal to 0 to refer to the area behind). All possible covariants between exogenous (independent) variables were also considered in the modeling. SEM was performed using AMOS 6.0 software.

The dependence of Pb concentration (log-transformed) in liver and femur on shot ingestion (i.e., shot pellets in gizzards), year of sampling, sex, and age was investigated with a general linear model (GLM). Pb-shot ingestion was compared between years by a contingency table with the Fisher's exact test. The size-class composition of grit in gizzards, obtained in the granulometric analysis, was compared between years by a MANOVA. Normality and homoscedasticity of samples were previously confirmed. The effect of Pb on body condition of birds was studied with a GLM using the ratio between body mass and wing length as a dependent variable, sex as a factor and lead concentration in liver or femur as covariants. The effect of Pb on the mass of the liver, spleen or abdominal fat was also studied including these dependent variables instead of body condition in the model.

Results

Spent-Shot Availability and Spatial Patterns. The average number of pellets in the soil was 73 600 units/ha, which is equivalent to 8.1 kg/ha (assuming a mean weight of 0.110 g per pellet; SD = 0.021; n = 30). Pellet density did not differ significantly among zones in spite of the interzone variability registered (range: 59 400–96 000 pellets/ha). Shooting direction affected shot pellet accumulation significantly, which was 3-fold higher in front of than behind shooting lines. The highest shot-pellet density was located between 40 and 110 m in front of lines (Figure 1). All spent shot pellets (n = 30) were sized within the 2–3 mm range.

Different patterns in the spatial Pb shot distribution were detected among zones (Figure 1). Shot distribution is well explained by the hypothesized path diagram (Figure 2). The reduced model explained adequately the variance-covariance matrix of the sample (p = 0.74) and did not differ from the original one ($\chi^2 = 3.65$, p = 0.30). All causal variables except

 R_2 had a relative high importance in explaining shot content in sampling points (Figure 2). Zones 2 and 3 showed similar general patterns in spatial shot distribution, but greatly differed from that in Zone 1 (Figure 1). This zone also differed in the magnitude of subrogated variables D_{eco} (lower) and R_1 (higher), which contrasts with the similarity between the other two zones.

Lead Shot Ingestion and Tissue Pb Concentrations in Partridges. Liver Pb concentration depended on shot ingestion (F = 10.77; df = 1; p < 0.05; Table 1), but not on year of sampling, sex, age, or any interaction of factors. In contrast, femur Pb concentration did not correspond with shot ingestion (Table 1), but increased with age (F = 6.11; df = 1; p < 0.017) and was higher in 2004 than in 2006 (F = 9.33; df = 1; p < 0.004; Juveniles 2004 (mean \pm SE): 4.64 \pm 3.16, Juveniles 2006: 0.74 \pm 0.32, Adults 2004: 18.68 \pm 17.31, Adults 2006: 1.74 \pm 0.37 μ g/g).

The prevalence of Pb pellet ingestion varied between years (Fisher's exact test; p < 0.05). In 2004, 20% of partridges sampled (n = 10) had ingested Pb shot-pellets in the gizzard in contrast with only 1.5% in 2006 (n = 66; 54 complete partridges analyzed plus 12 additional gizzards; Table 1). No effect of Pb prevalence on body condition, weight of liver and spleen, or abdominal fat was detected.

Partridges from 2004 had a higher weight of total grit in gizzards (mean = 2.98 g; SD = 0.60; n = 10) than those from 2006 (mean = 2.28 g; SD = 0.69; n = 66; $t_{74} = 3.02$; p < 0.01). In addition, the size-class composition of grit significantly differed between years (MANOVA: Wilks Lambda = 0.75; F = 3.68; p < 0.01), whereby the 2–3 mm fraction was higher in 2004 (20.4%) than in 2006 (11.9%; F = 11.85; df = 1, 73; p = 0.001).

The analysis of seed content in crops revealed interyear variations in the diet of partridges. In 2004, the diet was predominantly formed of corn seeds (90.7% of total biomass recovered from crops). In 2006, barley (72%) and smaller seeds (*Teucrium capitatum* and *Datura stramonium*) were the major component, whereas corn represented 21,5%.

Discussion

Spent-Shot Availability and Spatial Patterns. Although comparisons with other studies are difficult due to differences in methodological aspects, two general remarks can be made. Our results are in accordance to the general trend deduced from the literature: Pb shot densities are often lower in terrestrial than in wetland habitats (see for instance Mateo et al. (23, 24), who found 1.5-3.3 million pellets per ha in the upper 20 cm of sediment of Spanish wetlands). This would reflect the extremely high hunting pressure that many wetlands have suffered historically. Second, the estimates assessed in the present study were within the range of magnitudes usually recorded in upland ecosystems dedicated to intensive game hunting (13-17, 25). However, our records were higher than many prehunt (13, 14, 16) shot densities estimated in mourning dove (Zenaida macroura) fields, even though these systems accommodate more intense hunting regimes. Such differences may be explained because the soil in the study area is not tilled, as opposed to the cultivated mourning-dove fields. Tillage periodically redistributes particles into the soil, reducing the density of pellets accumulated near the surface throughout the hunting season (13, 14, 26). In addition, the compact nature of the soil analyzed (particularly dry and stony) may significantly attenuate the shot settlement rate (27, 28). Such upper soil-layer shot accumulation, in combination with the scratching feeding activity of partridges (19), may be critical as regards to Pb exposure risk for this species.

Most subrogated variables were included in the significant causal model for dispersion of shot pellets. In terms of the three hypotheses underlying the analysis, as regards Hy-

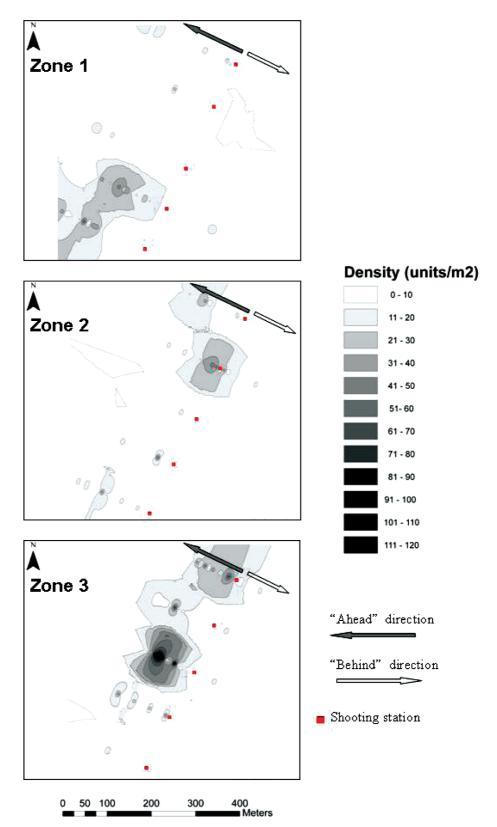


FIGURE 1. Spatial distribution mapping of shot pellets within each shooting line, resulting from the inverse distance weighted interpolation. Arrows show shooting direction from lines. Quadrants denote location of shooting stations included in sampling transects.

pothesis 1, the driven shooting technique induces shot pellets to accumulate in the central area in front of the shooting lines, reaching the highest density between 40 and 110 m from the stations. Bonet et al. (29) found a similar pattern in sediments of two lagoons in SE Spain, whereby the highest shot concentration was found between 70 and 120 m in front of shooting stations, and the lowest between 180 and 200 m. Our study also demonstrates that other techniques, apart from driven shooting, can affect spatial patterns (Hypothesis 2). Rabbit hunting is widespread in the Iberian Peninsula, and here, caused increased shot concentrations in drylandshrubland ecotones. In addition, unsuitable habitat for game

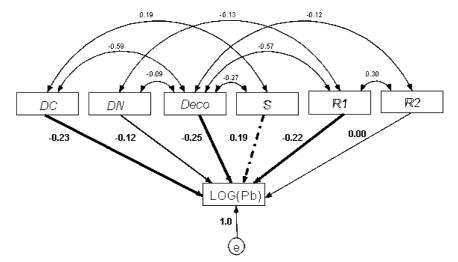


FIGURE 2. Path diagram for variables affecting dispersion of Pb spent shot-pellets in hunting zones studied. Shot number was log transformed (LOG9(Pb)). Two headed curved arrows depict standardized covariances (correlations). One headed arrows depict causal relationships. Positive effects are showed by dashed lines and negative ones by solid lines. Numbers near arrows indicate standardized path coefficients. Widths arrows are proportional to path coefficients. Likelihood Chi-square = 5.98, df = 9, p = 0.74. Subrogated variables: DC = distance from any sampling point to the central station of the shooting line, in meters; DN = distance from any sampling point to the northern station of the shooting line, in meters; Deco = distance from any sampling point to the nearest ecotone (dry cropland-shrubland), in meters, R1 = surface of irrigated croplands in Area 1 divided by the square distance from gravity center of the irrigated surface to the shooting station, in ha/km2; R2 = same for Area 2 (see the text for more details).

TABLE 1. Lead Concentration in Liver and Femur of Partridges

year	Pb shot ingestion ^a	п	liver Pb (µg/g)		femur Pb (µg/g)	
			mean \pm SE	range	mean \pm SE	range
2004	_	8	$\textbf{2.17} \pm \textbf{1.18}$	ND ^b -10.09	14.21 ± 10.71	0.87-87.90
	+	2	21.51	0.19-42.83	1.46	0.70-2.21
2006	_	53 ^c	0.58 ± 0.15	ND-5.73	0.84 ± 0.28	ND-14.62
	+	1	30.73		3.47	

^{*a*}+, Partridges with Pb shot ingested; –, partridges without Pb shot ingested. ^{*b*} ND = nondetectable. ^{*c*} Plus 12 additional gizzards examined.

animals may be used less, i.e., shot pellets in areas close to irrigated cornfields were less abundant (Hypothesis 3). The strong influence of these factors in Zone 1, rather than in Zones 2 and 3 (reflected by the contrasting values in subrogated variables D_{eco} and R_1) may explain the different spatial patterns detected. In Zone 1 shot was concentrated in the SW area, whereas Zones 2 and 3 showed higher relative densities in the central and NE areas. Dryland-shrubland ecotones were more abundant and close to Zone 1, in the S and W areas. In addition, partridges may fly in this direction rather than toward the irrigated cornfield area (to the north) when escaping during driven shooting, seeking the cover of natural shrubland. From these observations, it can be concluded that the spatial distribution of spent shot pellets in an upland area is quite variable, even among locations that are near each other. Likewise, it is strongly influenced by local traits such as the proximity of croplands and, of course, the hunting techniques practiced.

Lead Shot Ingestion and Tissue Pb Concentrations in Partridges. Our data show that 7.8% of partridges had either shot in the gizzard, and/or $> 5 \mu g/g$ d.w. of Pb in liver. There is little information on shot ingestion in *A. rufa*, in spite of the interest in this species as a gamebird. Soler et al. (7) found one partridge with ingested shot when analyzing a small sample (n = 7) from eastern Spain. The prevalence assessed in the present study can be considered high if compared with Butler's (β) results, who determined 0.16% and 1.4% prevalence when analyzing historical and current data on British partridge populations, respectively. Walter and Reese (β) detected 5.7% Pb shot prevalence in gizzards

of chukars (A. chukar) in a shrub-steppe community. Our study also demonstrates that ingestion is a major pathway for Pb exposure in partridge. Indeed, elevated Pb levels in partridge livers were positively related to shot ingestion. Neither age nor sex influenced Pb concentrations in liver, which is in contrast to several studies on waterfowl species (e.g., refs 30and 31). The good concordance between the presence of shot in gizzards and Pb concentrations in livers suggests recent exposure to Pb shot (23). In addition, the femur analysis would indicate long-term Pb exposure of partridges in the study area. Lead absorbed tends to accumulate in bones (30). Thus, significantly higher femur Pb concentration in adults than in juvenile partridges is consistent with the idea that the partridge population analyzed has been exposed to shot for long time. These results highlight the risk of A. rufa being exposed to Pb shot in upland game estates, and there is now a need for further research and monitoring in order to evaluate the risk posed and the scale of the issue for this important gamebird species.

We found no evidence of a link between elevated Pb levels in tissues and any health indicators (i.e., body condition, organ weight, abdominal fat). Partridge and other galliforms may be more resistant to Pb toxicosis than other species (7, 32) but Pb-pellet dosing studies on partridges are needed to confirm this. In addition, a certain percentage of upland birds may ingest large numbers of pellets (\geq 2) but may be unavailable to hunters because they exhibit sickly sheltering behavior, or die quickly as a consequence of the acute lead toxicosis (11, 16). It is possible that our study did not detect these partridges with pathological symptoms and that there has then been an underestimation in the level of pellet ingestion, and its impact at the population level.

The study revealed trends in shot ingestion prevalence, grit-size composition, and particle size in diet with time, in that all three traits were significantly higher in the sample from 2004, than in that from 2006. The consumption of harder and coarser foods is generally associated with the use of larger grit by birds (33), and the corn supplemented in 2004 may have resulted in a higher requirement for 2-3 mm sized grit. The size of the spent shot-pellets recovered from the soil in our study was 2-3 mm, and this change in grit selection may have increased the risk of Pb shot ingestion. Pain (3) and Mateo et al. (4) found an interspecific relationship between lead shot ingestion and the presence of grit >2 mm in Anatidae. In an experimental study, Trost (34) observed a similar selection by mallards of grit and shot mixed in a feeder, but they had the ability to discriminate between shot and food (corn or sorghum). Although these studies indicate the confusion of Pb shot with grit particles in waterbirds, another reason for shot-ingestion should be also considered in upland birds living in habitats where grit is not limiting. Schulz et al. (16) suggested that pellet ingestion by mourning doves may be due to confusion with seeds, since a high percentage of birds contained large numbers of pellets in the gizzard. Soler et al. (7) found a partridge with 14 shot pellets ingested. Indeed, Fumaria fruits resembling the shape (spherical) and size (2-2.25 mm) of shot pellets were recovered from crops of partridges analyzed in the present study. If partridges actively select spent pellets by misidentification with seeds, then the risk of Pb exposure would be higher than that deduced only from pellet density in the soil. Experimental research is urged in this direction.

Conservation and Management Implications. Physical characteristics of partridge shrubland-habitat severely restrict the practice and effectiveness of most common remediation measures suggested in the literature for alleviating shot ingestion. An eventual solution however should be considered: shooting lines and their influence area (i.e., 200 m around) could be moved into dry-crop fields, next to shrubland areas from where partridges would be driven. There, tillage (*26*) and other practices (e.g., removal of upper soil (*29*), gunshot removal by hand (*24*)) are possible. It should also be stressed that artificial supplementation of partridge diet by offering seed supply rich in corn should be avoided, since it is likely to increase shot ingestion.

However, the substitution of Pb by nontoxic ammunition is the only unique, effective, long-term solution, and is probably less expensive than the periodic operations cited above. Most national legislation has systematically ignored ecosystems other than wetlands thus far, in restricting the use of Pb ammunition. Recent studies, including this one, should encourage governments to consider terrestrial ecosystems that are vulnerable to this problem, and priority should be given to monitoring and evaluation of the potential risks on terrestrial estates, extending current restrictions on Pb shot use where necessary.

Acknowledgments

We sincerely thank Alejandro Sánchez and his family for permission to carry out the study in their farm, for providing all partridges analyzed in the study, and in general, for their interest in the research. We are also grateful to Cristina Garijo, Pilar Martínez, and Pablo Camarero for their assistance in soil sampling and laboratory work, to Francisco Cerdán and David Sanz for helping us in the performance of spatial interpolation maps of spent gunshot, and to Juan José Martínez-Sánchez for his suggestions on the early phase of the study. Three anonymous referees made valuable comments on an early version of the manuscript. Kathy Walsh

Supporting Information Available

Additional information on the study site, shot-density values in the soil, subrogated-variable averages, partridge diet, and size-class composition of grit in gizzards. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

- (1) Pain, D. J. *Lead poisoning of waterfowl: a review. In Lead Poisoning in Waterfowl*; Pain, D. J., Ed.; International Waterfowl and Wetlands Research Bureau: Slimbridge, 1992.
- (2) Scheuhammer, A. M.; Norris, S. L. a Review of the Environmental Impacts of Lead Shotshell Ammunition and Lead Fishing Weights in Canada. Occasional Paper 88; Canadian Wildlife Service: Ottawa, 1995.
- (3) Pain, D. J. Lead shot ingestion by waterbirds in the Camargue, France: an investigation of levels and interspecific differences. *Environ. Pollut.* **1990**, *66*, 273–285.
- (4) Mateo, R.; Green, A. J.; Guitart, R. Determinants of lead shot, rice, and grit ingestion in ducks and coots. *J. Wildl. Manage.* 2000, 64, 939–947.
- (5) Locke, L. N.; Friend, M. Lead Poisoning of Avian Species Other than Waterfowl. In Lead Poisoning in Waterfowl; Pain, D. J., Ed.; International Waterfowl and Wetlands Research Bureau: Slimbridge, 1992.
- (6) Walter, H.; Reese, K. P. Fall diet of chukars (*Alectoris chukar*) in eastern Oregon and discovery of ingested lead pellets. *West. North Am. Nat* 2003, 63, 402–405.
- (7) Soler, F.; Oropesa, A. L.; García, J. P.; Pérez, M. Lead exposition by gunshot ingestion in red-legged partridge (*Alectoris rufa*). *Vet. Hum. Toxicol.* **2004**, *46*, 133–134.
- (8) Butler, D. A. Incidence of lead shot ingestion in red-legged partridges (*Alectoris rufa*) in Great Britain. *Vet. Rec.* 2005, 157, 661–662.
- (9) Potts, G. R. Incidence of ingested lead gunshot in wild grey partridge (*Perdix perdix*) from the UK. *Eur. J. Wildl. Res.* **2005**, *51*, 31–34.
- (10) Rodrigue, J.; McNicoll, R.; Leclair, D.; Duchesne, J. F. Lead concentration in ruffed grouse, rock ptarmigan, and willow ptarmigan in Québec. *Arch. Environ. Con. Toxicol.* 2005, 49, 97–104.
- (11) Kendall, R. J.; Lacher, T. E.; Bunch, C.; Daniel, B.; Driver, C.; Grue, C. E.; Leighton, F.; Stansley, W.; Watanabe, P. G.; Withworth, M. 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.
- (12) Stevenson, A. L.; Scheuhammer, A. M.; Chan, H. M. Effects of non-toxic shot regulations on lead accumulation in ducks and American woodcock in Canada. *Arch. Environ. Contam. Toxicol.* 2005, 48, 405–413.
- (13) Lewis, J. C.; Legler, E. Lead shot ingestion by mourning doves and incidence in soil. *J. Wildl. Manage.* **1968**, *32*, 476–482.
- (14) Castrale, J. S. Availability of spent lead shot in fields managed for mourning dove hunting. *Wildl. Soc. Bull.* **1989**, *17*, 184–189.
- (15) Best, T. L.; Garrison, T. E.; Schmitt, C. G. Availability and ingestion of lead shot by mourning doves (*Zenaida macroura*) in southeastern New Mexico. *Southwest. Nat.* **1992**, *37*, 287–292.
- (16) Schulz, J. H.; Millspaugh, J. J.; Washburn, B. E.; Wester, C. R.; Lanigan, J. T.; Franson, J. C. Spent-shot availability and ingestion on areas managed for mourning doves. *Wildl. Soc. Bull.* 2002, 30, 112–120.
- (17) Imre, Á. Fácánok sörét eredetű ólommérgezése [Lead poisoning of pheasants caused by lead shots, in Hungarian]. *Magy. Állatorv. Lapja* **1997**, *119*, 328–330.
- (18) National Rifle Association of America. *Firearms Fact Book*, 3rd ed.; National Rifle Association: WA, 1991.
- (19) Pintos, R.; Braza, F.; Álvarez, F. Etograma de la perdiz roja (*Alectoris rufa*) en libertad. *Doñana, Acta Vert.* 1985, *12*, 231– 250.
- (20) Pain, D. J.; Eon, L. Methods of investigating the presence of ingested lead shot in waterfowl gizzards: an improved visual technique. *Wildfowl* **1993**, *44*, 184–187.
- (21) Johnston, K.; Ver Hoef, J. M.; Krivoruchko, K.; Lucas, N. Using ArcGIS Geostatistical Analyst; ESRI: New York, 2001.
- (22) Shipley, B. Cause and Correlation in Biology: A User's Guide to Path Analysis, Structural Equations and Causal Inference, Cambridge University Press: Cambridge, 2000.

- (23) Mateo, R.; Martínez-Vilalta, A.; Guitart, R. Lead shot pellets in the Ebro delta, Spain: Densities in sediments and prevalence of exposure in waterfowl. *Environ. Pollut.* **1997**, *96*, 335–341.
- (24) Mateo, R.; Green, A. J.; Lefranc, H.; Baos, R.; Figuerola, J. Lead poisoning in wild birds from southern Spain: A comparative study of wetland areas and species affected, and trends over time. *Ecotox. Environ. Saf.* **2007**, *66*, 119–126.
- (25) Holdner, J.; Vainman, B.; Jayasinghe, R.; Van Spronsen, E.; Karagatzides, J. D.; Nieboer, E.; Tsuji, L. J. S. Soil and plant lead of upland habitat used extensively for recreational shooting and game bird hunting in Southern Ontario, Canada. *Bull. Environ. Cont. Toxicol.* **2004**, *73*, 568–574.
- (26) Frederickson, L. H.; Baskett, T. S.; Brakhage, G. K.; Cravens, V. C. Evaluating cultivation near blinds to reduce lead poisoning hazard. *J. Wildl. Manage.* **1977**, *41*, 624–631.
- (27) Pain, D. J. Lead shot densities and settlement rates in Camargue marshes, France. *Biol. Conserv.* **1991**, *57*, 273–286.
- (28) Mudge, G. P. Densities and settlement rates of spent shotgun pellets in British wetland soils. *Environ. Pollut.* **1984**, *8*, 299– 318.

- (29) Bonet, A.; Olivares, C.; Picó, M. L.; Sales, S. L'acumulació de perdigons de plom al Parc Natural del Fondó d'Elx (Alacant): distribució espacial i propostes d'actuació. *Bull. Inst. Cat. Hist. Nat.* 1995, 63, 149–166.
- (30) Merchant, M. E.; Shukla, S. S.; Akers, H. A. Lead concentration in wing bones of the mottled duck. *Environ. Toxicol. Chem.* 1991, 10, 1503–1507.
- (31) DeStefano, S.; Brand, C. J.; Rusch, D. H. Prevalence of lead exposure among age and sex cohorts of Canada geese. *Can. J. Zool* **1992**, *70*, 901–906.
- (32) Franson, J. C. Interpretation of tissue lead residues in birds other than waterfowl. In Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations; Beyer, W. N., Heinz, G. H., Redmon-Norwood, A. W., Eds.; Lewis Publishers: Boca Raton, FL, 1996.
- (33) Gionfrido, J. P.; Best, L. B. Grit use by birds: A review. Curr. Ornitholoy 1999, 15, 89–148.
- (34) Trost, R. E. Dynamics of grit selection and retention in captive mallards. J. Wildl. Manage. 1981, 45, 64–73.

ES800215Y