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Lead isotopes and lead shot ingestion in the globally threatened marbled teal (*Marmaronetta angustirostris*) and white-headed duck (*Oxyura leucocephala*)

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Abstract

Lead isotope ratios ($^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$) and concentrations in the livers and bones of marbled teal and white-headed duck found dead or moribund were determined in order to establish the main lead source in these waterfowl species. Lead concentrations in bone (dry weight) and liver (wet weight) were found to be very high in many of the white-headed ducks (bone: geometric mean = 88.9 ppm, maximum = 419 ppm; liver: geometric mean = 16.8 ppm, maximum = 57.0 ppm). Some of the marbled teal had high lead levels in the bones but liver lead levels were all low (bone: geometric mean = 6.13 ppm, maximum = 112 ppm; liver: geometric mean = 0.581 ppm, maximum = 4.77 ppm). Ingested lead shot were found in 71% of the white-headed duck and 20% of the marbled teal. The $^{206}Pb/^{207}Pb$ ratio in livers and bones of white-headed ducks and marbled teals showed no significant differences compared to the ratios obtained from lead shot. The $^{206}Pb/^{207}Pb$ ratio in bones of marbled teal ducklings with the highest lead concentrations tended to resemble the ratios of lead shot, which supports our hypothesis that the lead was derived from the hens. We also found that the lead ratios of lead shot and lead ratios described for soils in the area overlapped, but also that the isotopic ratio $^{206}Pb/^{207}Pb$ in lead shot used in Spain has a narrow range compared with those used in North America. The principal source of lead in many of these birds was, however, most likely lead shot, as supported by the similar isotopic ratios, high lead concentrations in tissues and evidence of ingested shot.

Keywords: Lead shot; Lead isotope ratio; Lead poisoning; Marbled teal; White-headed duck

1. Introduction

Lead poisoning in waterfowl due to lead shot ingestion has been recognized for more than 100 years (Mateo et al., 1998; Sanderson and Bellrose, 1986). Waterfowl may consume lead shot when they ingest it with grit or mistake it for seeds in wetlands where lead shot has accumulated after years of hunting (Mateo et al., 2000). The coastal wetlands of Mediterranean Europe, and particularly in the Valencian community in Eastern Spain, have some of the highest recorded lead shot densities in the world (Mateo et al., 1998). El Hondo is one such wetland (Fig. 1) and

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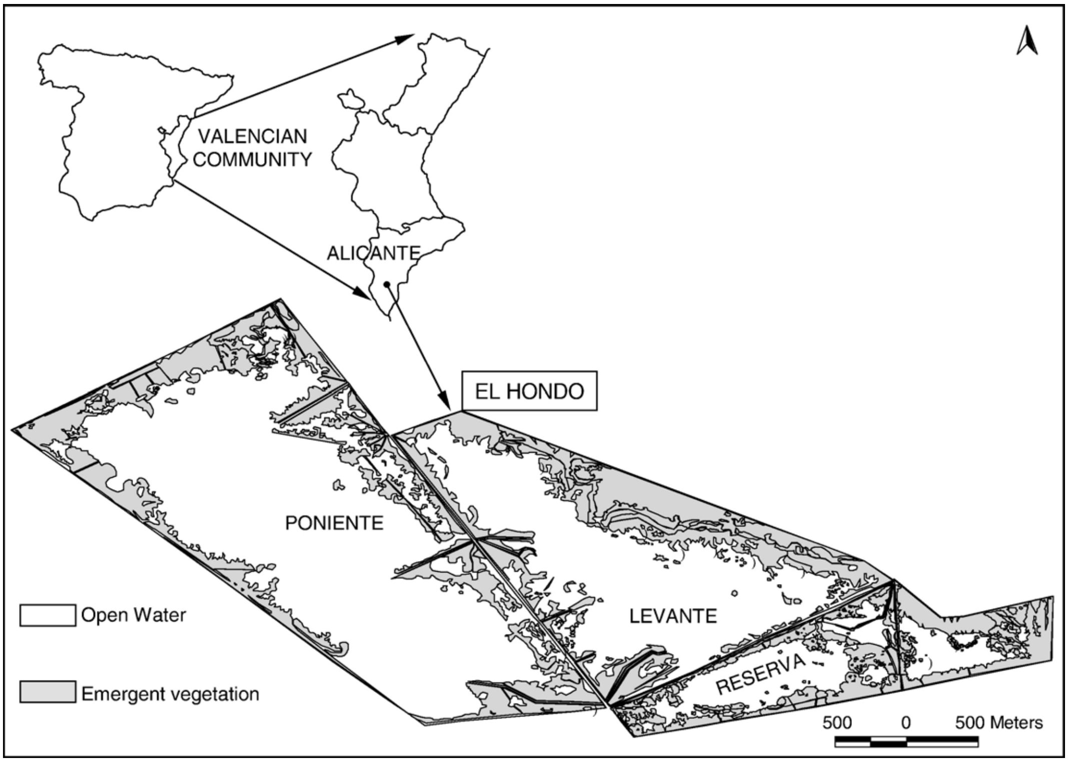

Fig. 1. El Hondo (38°11′N, 00°45′W; 1650 ha); a wetland in the Valencian community, Spain. Marbled teal and white-headed duck found dead or moribund were collected from this area between the years 1996 and 2001.

an estimated density of 163 lead shot/m^2 have been recorded in the sediments (Mateo et al., 1998). El Hondo is also one of the most important European wetlands for the globally threatened marbled teal (*Marmaronetta angustirostris*) and the white-headed duck (*Oxyura leucocephala*), and often holds most of the European population of one or both species at a given moment in time (Green and Navarro, 1997; Torres and Moreno-Arroyo, 2000; Madroño et al., 2004).

The marbled teal has a world population of 14 000 to 26 000 birds (Wetlands International, 2002) but the numbers are decreasing and the species is classified as Vulnerable by IUCN (BirdLife-International, 2000). The marbled teal has a fragmented distribution in the Mediterranean and western and southern Asia. It is recorded in wetlands in Spain all year round but most birds breeding there winter in north-west Africa (Green and Navarro, 1997; Madroño et al., 2004). The white-headed duck has a world population estimated at just 8000 to 13 000 birds (Wetlands International, 2002). The population is decreasing, and the species is classified as Endangered by IUCN (BirdLife-International, 2000). The white-headed duck has a fragmented distribution in the Palaearctic, with a resident population in Spain (Green and Hughes, 2001; Madroño et al., 2004).

Lead poisoning is a major cause of mortality in ducks at El Hondo, and waterfowl at this and other Valencian wetlands are exposed to higher lead shot densities and ingest more lead shot than birds at most other wetlands in Spain (Mateo et al., 1998, 2001). Lead shot is probably the major source of lead poisoning in waterfowl in the area, but industries polluting the water supply (mainly via the Segura River that supplies water to El Hondo), leaded petrol or the natural lead contents of soils may also act as secondary sources. Mateo et al. (2001) found elevated lead levels in the bones of young marbled teal ducklings. As no lead shot were found in the gizzards of these ducklings, they suggested that lead was transferred from hens to eggs and then became deposited in the chicks during bone formation.

The ratios of stable lead isotopes can be used to distinguish between different sources of lead in the environment and in wildlife (Flament et al., 2002; Hopper et al., 1991; Meharg et al., 2002; Scheuhammer et al., 2003; Scheuhammer and Templeton, 1998). However, different lead sources may sometimes be similar in ratio, which can make it difficult to distinguish between them conclusively.

Herein, we analyse isotope ratios ($^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$) in combination with tissue concentrations and report the presence of ingested shot to assess whether lead shot were indeed the major source of lead in marbled teal and white-headed duck from El Hondo, Spain. Isotopic ratios were further used to investigate whether lead found in marbled teal chicks was derived from hens that had ingested lead shot.

2. Materials and methods

Birds were collected dead or moribund in El Hondo (38°11′N, 00°45′W; 1 650 ha), Spain, between 1996 and 2001 (Table 1, Fig. 1). The birds were not the same individuals as those reported in Mateo et al. (2001). Twenty-five of the marbled teals and all (35) of the white-headed ducks studied were fully-grown individuals. Marbled teal chicks studied (42) were divided into three age groups: small chicks, <8 days old; medium chicks, 8–39 days old; and large chicks or newly fledged juveniles, 40–100 days old (see Green, 1998 for details of age classes). Twenty-seven of the chicks were classed as small, 11 were medium sized and 4 were large chicks or newly fledged juveniles.

All birds were frozen shortly after collection and sent to the Centre for the Study and Protection of Nature, El Saler, Valencia, for necropsy. Presence of lead shot in the gizzard was determined by X-ray. Samples of liver and/or bone (femur and/or humerus) were removed and stored in metal free plastic vials. The diagnosis of lead poisoning was based on necropsy findings and liver lead analyses.

The cause of death for most fully-grown marbled teal and medium and large chicks, was traumatism due to illegal shooting or unknown causes (Table 1). Several showed clinical signs of lead poisoning. Since most were found within hours or days of death, many were partially decomposed; hence livers could not be sampled from some of the birds. The small marbled teal chicks studied died (probably from stress) within hours of being rescued from a concrete lined irrigation channel that runs though El Hondo (Fuentes et al., 2004). The cause of death for the majority of the white-headed ducks studied was lead poisoning. Other birds died after being illegally shot or from an unknown cause (Table 1). As sampling was not random, comparisons of lead exposure levels between species are preferably referred to a previous study (Mateo et al., 2001).

All birds were analysed for total lead concentrations and isotopes of lead in the livers and bones (femur and/or humerus). Lead shot analysed for lead isotope ratios were collected from the gizzards of marbled teals and white-headed ducks from El Hondo during a previous study (Mateo et al., 2001). The birds analysed in this study were collected in El Hondo over a similar time period as the birds used in the previous study, and we therefore expect the lead shot to be representative of that available to these species within this area.

In total, 67 marbled teal bone samples were analysed, 25 of which were from fully-grown birds and 42 were from chicks. Thirty-four bone samples were also analysed from fully-grown white-headed ducks. A central section of the fully-grown duck bone was cut from the bulk bone using stainless steel bone cutters, and weighed to an accuracy of ±0.001 g. Where enough bone was available, approximately 0.25 g of bone was used. For the chicks, the whole bone was used. The bones were placed in thoroughly cleaned glass digestion tubes and 2.5 ml of concentrated HNO_3 was added to the tubes; the tubes were covered and left overnight to digest at room temperature (~20 °C). The following day the digests were further treated with the addition of 2.5 ml of concentrated (30%) H_2O_2 and then slowly heated to 100 °C. The samples were digested for 1 h, the temperature was then increased to 120 °C and digestion continued for at least another hour. Complete digestion was considered to be reached when the digests formed a clear liquid. Exactly the same procedure was performed for three blank samples (replacing tissue with 1 ml of deionised water), three spiked samples (using 1 ml of 100 µg Pb/L standard solution — diluted from certified 1000 mg/l stock solution provided by Fisher Chemicals) and for three samples

Table 1
Number of birds collected each year and causes of death (C. Gerique, personal communication) for the marbled teals and white-headed ducks

	Year	No. of birds collected	Cause(s) of death
Fully-grown marbled teals	2000	22	Traumatism due to illegal shooting, lead poisoning, unknown causes.
	2001	3	
Marbled teal small chicks	1996	5	Died after being trapped in the concrete lined irrigation channels that run though El Hondo.
	1997	9	
	1998	16	
Marbled teal medium chicks	1998	3	Possible traumatism of being illegally shot, unknown causes.
	2000	8	
Marbled teal large chicks	2000	4	Traumatism from being illegally shot, lead poisoning, unknown causes.
Fully-grown white-headed ducks	2000	35	Lead poisoning, traumatism from being illegally shot, unknown causes.

All birds were collected in El Hondo, Spain. See Fuentes et al. (2004) for more details regarding the collection of marbled teals.

of bone reference material (National Institute of Standards and Technology (NIST), Standard Reference Material (SRM) 1486 bone meal).

In total, 24 marbled teal liver samples were analysed, of which 12 were from fully-grown birds and 12 were from chicks. Thirty-five fully-grown white-headed duck liver samples were also analysed. Lead shot and liver samples were digested using the same methodology as described for bones, using whole lead shot and approximately 0.25 g of liver (wet weight). The digests were washed into 10 ml polypropylene centrifuge tubes using deionised water and made up to 10 ml. The diluted samples were initially analysed for total lead in a graphite furnace atomic absorption spectrometer (GF-AAS, Perkin-Elmer 3300) using appropriate matrix modification. These values were then used to facilitate further dilution of the digests in 2% nitric acid, the aim being to create solutions ≈ 10 μg kg^{-1} in lead concentration in order that all stable lead isotopes (^{206}Pb, ^{207}Pb, ^{208}Pb) could then be analysed on an ICP-MS system (Inductively Coupled Plasma with Mass Spectrometer; Agilent 7500 series) within the pulse mode of the detector. The limit of detection (LOD) on the GF-AAS (back calculated to within tissue concentrations) was 0.20 mg kg^{-1}; spike recovery was 106% (±2%; arithmetic mean±S.E.) and bone meal CRM recovery was 82% (±6%).

On the ICP-MS, a calibration with a lead isotope standard solution of different concentrations was derived at the start of every set of measurements. Standard solutions were then analysed after every fourth sample to correct samples for instrumental mass bias. The standard solutions were made from a standard reference material (SRM 981). The SRM 981 consisted of lead of 99.9% purity, and has a certified isotopic composition of, ^{206}Pb: 24.1442±0.0057%; ^{207}Pb: 22.0833±0.0027%; ^{208}Pb: 52.3470±0.0086% (NIST). Isotope values were corrected for mass bias by applying an identical correction to that required to achieve the certified values for SRM 981. Isotope ratios determined for SRM 981 were consistently within ±2% of the certified value.

The isotope ratios for the liver and bone samples were compared with isotope ratios of lead shot and consideration was also given to the lead concentrations in these tissues. Liver isotope ratios were compared between birds with and without ingested lead shot. Lead isotope ratios of lead shot, and of lead in liver and bone samples were further compared to ratios of lead in the air and in soils (i.e. mineral deposits) in the area. Since no samples from soil or air were taken at the time we have used data on ratios taken from the literature (Arribas and Tosdal, 1994; Bollhöfer and Rosman, 2002). Total lead concentrations were compared between males and females and between fully-grown marbled teals and marbled teal chicks. The level of lead poisoning in these birds was determined with help from suggested lead threshold levels for waterfowl provided by Pain (1996). Levels below 10 ppm DW in bone were considered as background, but above this level, waterfowl can start to show signs of clinical poisoning. The equivalent threshold level for lead in livers is 2 ppm WW for waterfowl. Birds with 2–6 ppm WW were considered sub-clinically poisoned by lead and those with >6 ppm WW were considered clinically poisoned.

Lead isotope ratios and total lead concentrations were analysed statistically with Kruskal–Wallis one-way ANOVA and Dunn's tests, two sample *t*-tests, Mann–Whitney *U*-tests and the Spearman rank correlation test. All sample values were tested for normality and data were log transformed if necessary. The non-parametric tests were used when samples did not contain enough values needed for a normality test or when a sample was not normally distributed, even after a log transformation.

3. Results

3.1. Total lead concentrations

There was considerable variation in bone lead concentrations and many of the white-headed ducks and some of the marbled teals had extremely high concentrations. Liver concentrations were also extremely high for many of the white-headed ducks but not for the marbled teals. Prevalence of ingested lead-shot was higher in white-headed ducks than in marbled teals but differences between species must be taken with a degree of caution since sampling birds found dead is biased by the cause of mortality (Table 2).

For the white-headed duck, 31 of 34 (91%) had bone lead concentrations higher than 10 ppm DW, and 31 of 35 (89%) had liver lead concentrations above 2 ppm WW. Liver lead concentrations indicative of clinical lead poisoning (>6 ppm WW) were found in 86% of the white-headed ducks. Of the fully-grown marbled teals, 6 of 25 (24%) had bone lead concentrations higher than 10 ppm DW. Only 1 of 12 (8%) of the fully-grown marbled teals had a liver concentration above 2 ppm WW. That bird had a liver concentration of 4.78 ppm WW, which can be interpreted as sub-clinical poisoning.

There was no significant difference related to sex in total lead concentration for the marbled teals, neither in liver (Mann–Whitney $U=12.00$, $P>0.05$) nor in bone (Unpaired *t*-test — $t=0.1508$, $df=22$, $P>0.05$). Female white-headed ducks, however, had significantly higher bone lead levels than males (Unpaired *t*-test — $t=3.465$, $df=32$, $P<0.001$). Liver concentrations were not

Table 2
Liver and bone lead concentrations and prevalence of ingested lead shot (%) for fully-grown male and female marbled teals and white-headed ducks

	Liver Pb (ppm of wet weight)		Bone Pb (ppm of dry weight)		Prevalence of birds with ingested Pb shot (%)
	N	Geometric mean with 95% CI	*N*	Geometric mean with 95% CI	
Marbled teal[a]	12	0.5807 (0.3020–1.117)	25[b]	6.133 (3.410–11.03)	20% (4 of 20)
Males[a]	7	0.7156 (0.2398–2.135)	13	5.304 (2.234–12.59)	30% (3 of 10)
Females[a]	5	0.4335 (0.1614–1.64)	11	5.774 (2.396–13.92)	11% (1 of 9)
Small chicks	12	0.3126 (0.1915–0.5104)	27	1.288 (0.7904–1.908)	0
Medium chicks	4	0.3242 (0.05824–1.804)	11	0.9212 (0.5687–1.492)	0
Large chicks	3	0.4866 (0.03352–7.056)	4	1.791 (0.3049–10.53)	25% (1 of 4)
White-headed duck[a]	35	16.81 (10.32–27.39)	34	88.87 (56.33–140.2)	71% (24 of 34)
Males[a]	9	5.462 (0.9878–30.21)	9	23.30 (6.465–83.96)	44% (4 of 9)
Females[a, c]	26	24.81 (18.41–33.43)	25	143.9 (107.3–192.9)	80% (20 of 25)

Liver and bone lead concentrations and prevalence of ingested shots are also shown for small, medium and large marbled teal chicks. *N*=no. of samples.
[a] Fully-grown birds.
[b] Including one bird of unknown sex.
[c] This group may contain some immature birds confused with females due to similar plumage characteristics (see Green and Hughes, 2001).

significantly different between male and female white-headed ducks (Mann–Whitney $U=70.00$, $P>0.05$).

Medians for bone lead concentrations varied significantly between the fully-grown marbled teals and the different classes of chicks (Kruskal–Wallis test=21.17, $P<0.0001$). Fully-grown marbled teals had higher levels and a wider range of lead concentrations in bone than the small and medium chicks ($P<0.001$ Dunn's test). However, lead levels were not significantly higher in fully-grown teal compared to the large chicks ($P>0.05$ Dunn's test). There was no significant difference in liver lead concentrations between fully-grown marbled teal livers and the different classes of chicks (Kruskal–Wallis test=2.997, $P>0.05$).

3.2. Lead isotopes

The isotope ratio of $^{206}Pb/^{207}Pb$ found in livers and bones of fully-grown white-headed ducks did not differ significantly from the ratios observed in the lead shot (Fig. 2; Mann–Whitney $U=454.0$, $p>0.05$ and, Mann–Whitney $U=456.5$, $P>0.05$ respectively). The $^{208}Pb/^{207}Pb$ ratios, however, were significantly different between liver and lead shot, but no difference was found between bone and lead shot (Mann–Whitney $U=277.0$, $p<0.01$ and Mann–Whitney $U=348.0$, $P>0.05$ respectively). A few of the white-headed ducks had bone ratios that were very different when compared to the ratios of the majority of samples. The four outliers for the $^{206}Pb/^{207}Pb$ ratio correspond to four of the five outliers for the $^{208}Pb/^{207}Pb$ ratio as seen in Figs. 2 and 3.

The ratios $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ in liver of fully-grown marbled teal and lead shot did not show any significant difference (Unpaired *t*-test with Welch's correction — $t=2.061$, $df=14$, $P>0.05$ and Unpaired *t*-test — $t=1.274$, $df=37$, $P>0.05$ respectively). The ratio $^{208}Pb/^{207}Pb$ in bones of marbled teals differed

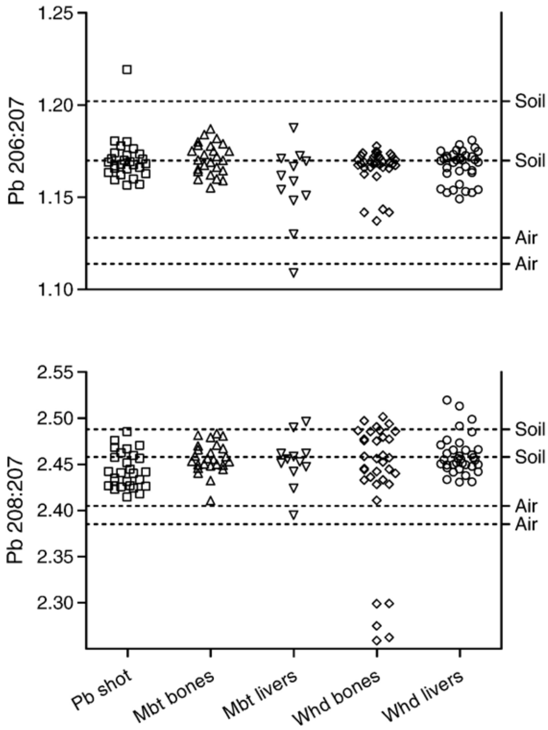

Fig. 2. Lead isotopic ratios ($^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$) in lead shot, bones and livers from marbled teals and white headed ducks collected in El Hondo, Spain. The horizontal dotted lines show the ranges for the lead ratios in soils (mineral deposits) and lead deposition from the air in the area (Arribas and Tosdal, 1994; Bollhöfer and Rosman, 2002).

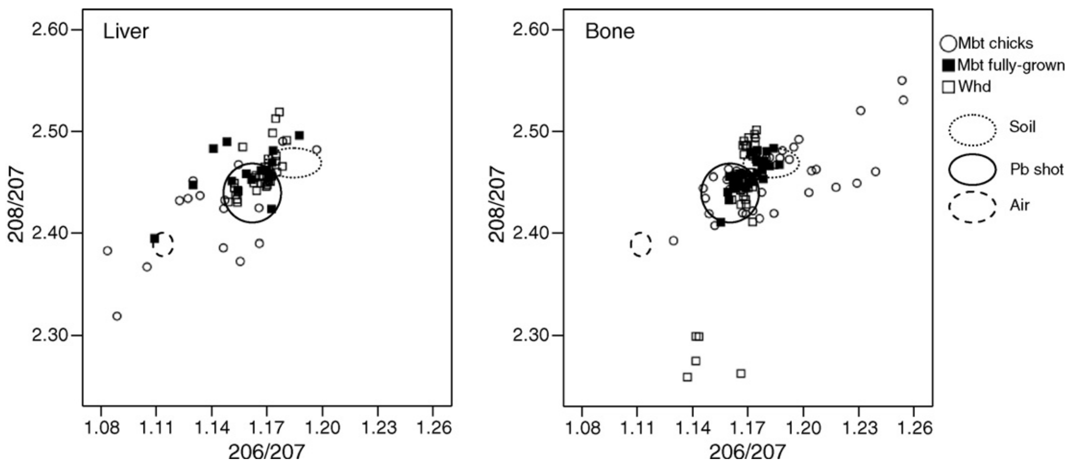

Fig. 3. Relationship between the $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ ratios in the liver and bone of chicks and fully-grown marbled teal (Mbt) and white-headed ducks (Whd). Most of the birds show a signature similar to lead shot.

significantly from the ratios of the lead shot, but the ratio $^{206}Pb/^{207}Pb$ did not show any significant difference (Unpaired t-test — $t=2.658$, $df=50$, $P<0.05$; and Mann–Whitney $U=290.0$, $P>0.05$ respectively).

The use of both ratios together permits to detect a high number of birds with the lead shot signature (Fig. 3) although some of the white-headed ducks and marbled teal chicks differed largely in bone or liver.

When birds with ingested lead shot ($n=28$) and without ($n=37$) were compared, they showed significantly different $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ liver ratios (Fig. 4; Unpaired t-test with Welch's correction — $t=3.048$, $p<01$ and $t=2.284$, $p<0.05$ respectively).

The $^{206}Pb/^{207}Pb$ ratio in the bones of the small chicks analysed varied greatly, and in a few individuals, the ratio seems to diverge away from the possible lead sources discussed here, especially when the bones contained very low total lead concentrations (Fig. 5). However, as the lead concentration increased, the ratios converged more on the mean ratio of lead shot and the correlation between concentration and ratio was found to be significant with the Spearman rank correlation test ($P<0.0001$, $r=-0.6944$). This trend is observed also for the complete sample of animals (Fig. 5).

The isotope ratios tended to be more similar to lead shot in birds with liver lead concentrations indicative of sub-clinical (2–6 ppm, $n=3$) or clinical poisoning (>6 ppm, $n=30$) than in birds with background levels (<2 ppm, $n=35$). The percentage of birds with an isotope ratio within the mean±2S.D. of the lead shot was 100% in those with sub-clinical or clinical exposures and 62.9% in birds with background exposures. Similar results can be observed for isotope ratios found in bone, were lead concentrations of >10 ppm is associated with sub-clinical–clinical lead poisoning. The percentages of bones within the mean±2S.D. of the lead shot were 80% and 89.2% for those with background ($n=65$) and sub-clinical–clinical ($n=37$) exposures, respectively.

4. Discussion

Lead poisoning was the cause of death for many of the birds collected, especially for the white-headed ducks, and the necropsy findings and the high lead levels

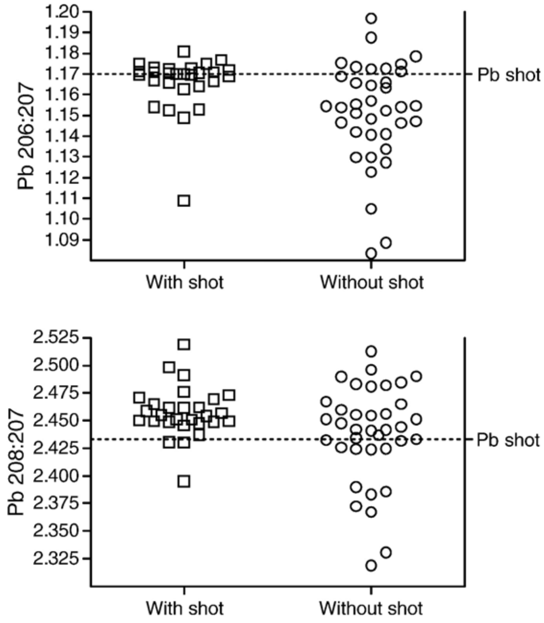

Fig. 4. $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ liver ratios for white-headed ducks and marbled teal with and without ingested lead shot indicated by squares and circles. The dashed lines show the mean ratios of lead shot.

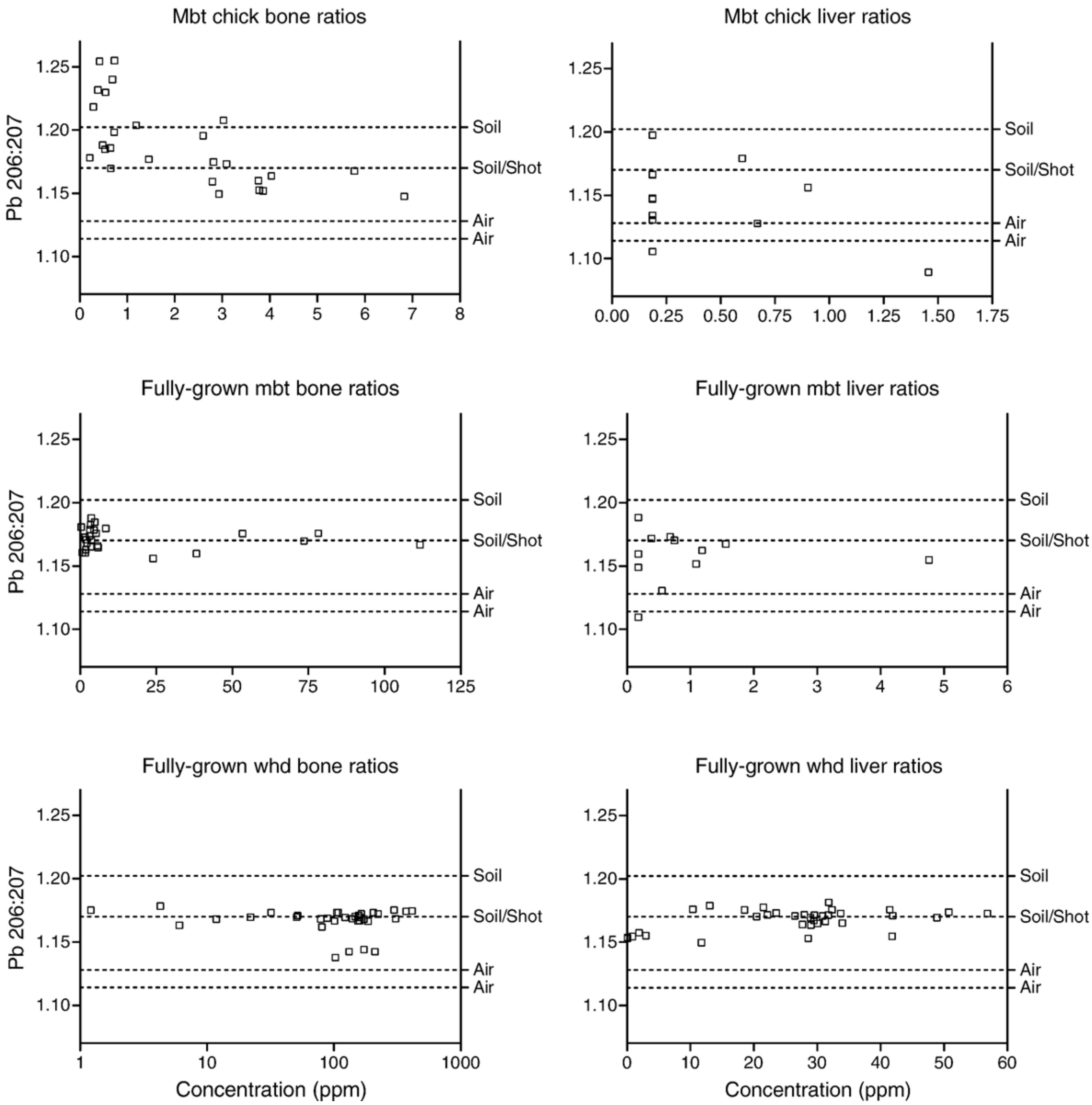

Fig. 5. The graphs illustrate the relationship between lead tissue concentrations and the ^{206}Pb/^{207}Pb ratios. In liver and bone with low lead concentrations, the ratios have a greater variation than in high concentrations. As the concentrations increase in tissues, the ratios seem to converge more on that of lead shot. The horizontal lines show the mean ^{206}Pb/^{207}Pb ratio for lead shot, and the ranges for lead in soils (mineral deposits) and in air lead deposition for the area (Arribas and Tosdal, 1994; Bollhöfer and Rosman, 2002).

reported support this. The concentration levels found are, however, obviously likely to represent an overestimate with regard to the overall populations of these species in El Hondo.

Lead poisoning seems to be more severe in white-headed duck at El Hondo than in marbled teal (Mateo et al., 2001). The very high liver levels in many of the white-headed ducks and in a few of the marbled teal probably indicate a relatively recent ingestion of one or more lead shot pellets. Lead absorbed into the blood and soft tissues remains elevated for several weeks to months after the initial exposure (Pain, 1996). Likewise, bone levels were also very high for many of the white-headed ducks and marbled teal studied, and the lead deposited in these bones tends to be relatively immobile and accumulates with age in exposed waterfowl (Pain, 1996). The extremely high lead bone concentration in many of the white-headed ducks and in some of the marbled teal indicates that these individuals have undergone long-term exposure to lead. A few of the white-headed ducks had bone ratios that are completely different compared to the ratios of the majority (Fig. 2). They also had very high bone lead concentrations (Fig. 5) and a possible explanation for this is that they might have ingested shot produced from ore of a completely different origin, e.g. from a different wetland where other brands of lead shot are more commonly used.

Female white-headed ducks had higher bone lead levels than males, as was observed in a previous study (Mateo et al., 2001) and this may be explained by increased lead absorption in laying hens as a consequence of higher calcium requirements. Moreover, Gómez-Serrano and Rioja (2004) found that with a larger sample size, females had actually ingested more lead shot and had significantly higher liver lead levels than males.

The isotopic $^{206}Pb/^{207}Pb$ ratios in soils in the surrounding regions of Spain range from 1.170 to 1.202 (Arribas and Tosdal, 1994) and appear to be slightly higher than ratios observed in lead shot and ducks (lead shot mean with 95% CI: 1.170, 1.165–1.175; ducks mean with 95% CI: 1.167, 1.164–1.170). Soil and lead shot ratios overlap in part, thus it is not possible to discern the origin of lead in one bird without using other evidence, i.e., of lead shot ingestion, or of elevated lead levels existing naturally in the soil or present due to anthropogenic activity in the study area. Lead shot ingestion has been confirmed as a very significant source of lead exposure, and a cause of death in these two threatened waterfowl. Elevated industrial lead sources such as mines or smelters are not present. Air samples from Valencia (E Spain) in 1998–1999 had $^{206}Pb/^{207}Pb$ ratios of 1.114–1.128 (Bollhöfer and Rosman, 2002). Other countries in Southern and Western Europe exhibited similar ratios at the time, indicating a common source for the alkyl lead used in petrol. These ratios are lower than the ratios found in the lead shot used for waterfowl hunting in Spain.

In a similar study to this, Scheuhammer et al. (2003) used stable lead isotope ratios to identify the origin of the high lead values found in American woodcock (*Scolopax minor*) from Eastern Canada. In the habitats of the American woodcock, lead shot have been used for hunting and were still being deposited in those areas at the time of the study. Like the marbled teal and especially the white-headed duck the American woodcock has feeding behaviour that makes it possible for it to ingest lead shot if available in their habitats (Scheuhammer et al., 2003). Scheuhammer et al. (2003) concluded that the range of $^{206}Pb/^{207}Pb$ ratios in wing bones of woodcocks with elevated lead exposure was not consistent with the exposure to environmental lead from past gasoline combustion or mining waste, but was consistent with ingestion of spent lead shotgun pellets. However, on the contrary to our findings, the American woodcocks with high lead concentrations in wing bones had a high dispersion of the $^{206}Pb/^{207}Pb$ ratios. This may be only explained by the use in Canada of lead shot pellets with a more diverse signature of stable isotopes ($^{206}Pb/^{207}Pb$ ratio: 1.07–1.27) among the different manufacturers (Scheuhammer and Templeton, 1998) than in Spain (Fig. 2). In fact, Canadian waterfowl also show a wide range in the $^{206}Pb/^{207}Pb$ (1.09–1.26; Scheuhammer and Templeton, 1998) compared to our results. In another study, Meharg et al. (2002) used lead isotopes to identify the sources of lead contamination found in white storks (*Ciconia ciconia*) from a marshland in Doñana, Spain. In this case, storks were less at risk to ingest lead shot and the observed $^{206}Pb/^{207}Pb$ ratio corresponded to the pyrite sludge released from the accidental spill of the Aznalcóllar mine in SW Spain.

Lead shot were not found in the gizzard in any of the small chicks in this or the previous study of Mateo et al. (2001), so it is highly likely that the lead found in chick bones was either transferred from the mother, or from contaminated food, water, air or sediment. As the bone lead concentration for the small marbled teal chicks increased, the $^{206}Pb/^{207}Pb$ bone ratio converged on the mean lead shot $^{206}Pb/^{207}Pb$ ratio. This observation may be explained by a process of hen to duckling lead transfer via lead deposited in the egg. Several studies have shown that lead exposure in the ovum can affect immune systems, brain development and hatchability (Birge and Roberts, 1976; Hui, 2002). Early exposure to lead in chicks of herring gull (*Larus argentatus*) produces neurobehavioral deficits like slower learning and improvement of motor skills, (Burger and Gochfeld, 2005).

5. Conclusions

Herein we provide significant evidence to show that lead shot are the principal cause of lead contamination in the fully-grown white-headed duck and the marbled teal populations in El Hondo. The $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ isotopic ratios indicate lead shot as the most probable source in the white-headed ducks, and this data is supported by the high tissue concentrations and high prevalence of lead shot ingestion. The $^{206}Pb/^{207}Pb$ isotope ratios provide the strongest indication that lead shot is the source of recent lead exposure in these species, but such information should ideally be accompanied by additional evidence of a high prevalence of ingested shot in the gizzards. Otherwise, without such supporting evidence, it is difficult to conclusively separate lead isotope signatures where there is a significant overlap between ratios in lead shot and in local sediments. The $^{206}Pb/^{207}Pb$ ratio can also be used to help to explain why high lead bone concentrations have been found in marbled teal ducklings here and in a previous study (Mateo et al., 2001). The effects of high lead exposures on female reproduction and duckling development should be studied further in the future.

The Spanish government banned lead shot use in internationally important wetlands (Ramsar sites) such as El Hondo in October 2001 (Mateo and Guitart, 2003). Although the Valencian government postponed implementation of this ban until 2005. Unfortunately, lead shot can remain at high densities in sediments even when lead shot has been banned for many years. In Tablas de Damiel National Park, in Central Spain, lead shot has been banned since 1965, but lead shot densities are similar to those of wetlands where lead shot has been in use in recent years (Mateo et al., 1998). In order to conserve threatened species such as the white-headed duck and the marbled teal, it is necessary to clean up wetlands such as El Hondo with high lead shot densities, so as to reduce future lead poisoning. Work to identify and clean the parts of El Hondo most contaminated by lead shot is already underway (Bonet et al., 2004).

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