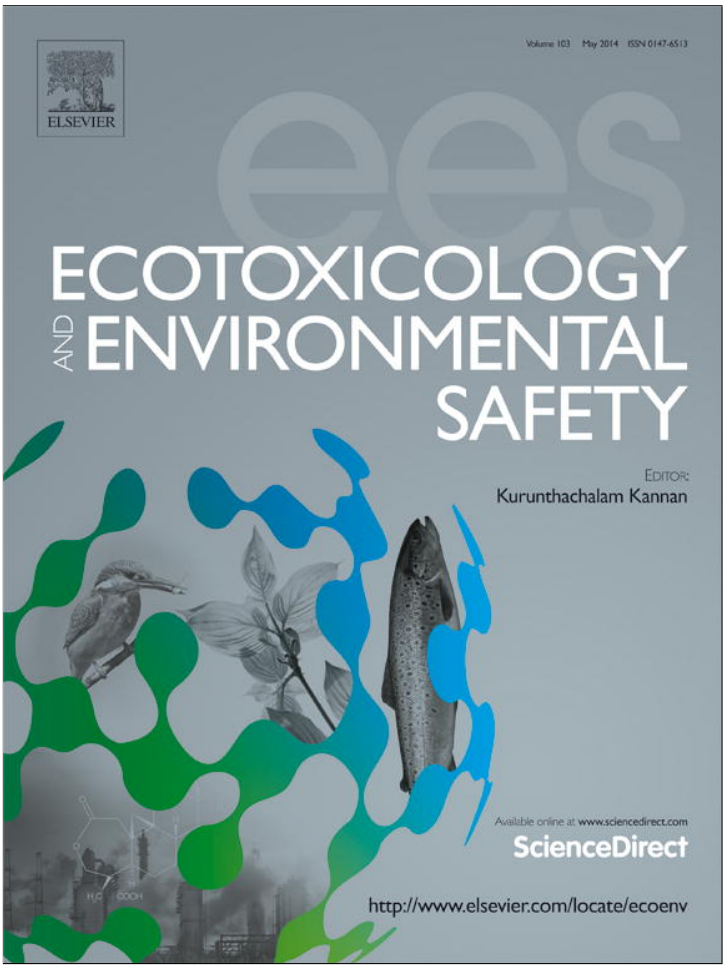

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Review

Lead gunshot pellet ingestion and tissue lead levels in wild ducks from Argentine hunting hotspots

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ABSTRACT

Lead poisoning in waterfowl due to ingestion of lead pellets is a long recognized worldwide problem but poorly studied in South America, particularly in Argentinean wetlands where duck hunting with lead gunshot is extensive. In 2008, we found high pellet ingestion rates in a small sample of hunted ducks. To expand our knowledge on the extent of lead exposure and to assess health risks from spent shot intake, during 2011 and 2012 we sampled 415 hunter-killed ducks and 96 live-trapped ducks. We determined the incidence of lead shot ingestion and lead concentrations in bone, liver and blood in five duck species: whistling duck (*Dendrocygna bicolor*), white-faced tree duck (*D. viduata*), black-bellied whistling-duck (*D. autumnalis*), rosy-billed pochard (*Netta peposaca*) and Brazilian duck (*Amazonetta brasiliensis*). The ingestion of lead shot was confirmed in 10.4% of the ducks examined (43/415), with a prevalence that varied by site and year, from 7.6% to 50%. All bone samples ($n=382$) and over 60% of liver samples (249/412) contained lead concentrations above the detection limit. The geometric mean lead concentration in tissues (mg/kg dry weight) was 0.31 (GSD=3.93) and 3.61 (GSD=4.02) for liver and bone, respectively, and 0.20 (GSD=2.55) in blood (mg/kg wet weight). Lead levels surpassed toxicity thresholds at which clinical poisoning is expected in 3.15% of liver samples, 23.8% of bones and 28% of blood samples. Ducks with ingested lead pellets were much more likely to have high levels of lead in their liver. Rosy-billed pochards were consistently more prone to ingesting lead shot than other duck species sampled. However, whistling ducks showed higher levels of lead in liver and bone. Our results suggest that lead from ammunition could become a substantial threat for the conservation of wild duck populations in Argentina. The replacement of lead by non-toxic shot would be a reasonable and effective solution to this problem.

Contents

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1. Introduction

Information on the negative impact of ingested lead gunshot on the health of aquatic birds has been accumulating for more than a century (Friend et al., 2009). Ducks are particularly susceptible as they commonly ingest lead shot as grit or while feeding on aquatic plants or invertebrates. Once ingested, lead pellets are dissolved by stomach acids whereas lead salts are absorbed into the bloodstream and rapidly deposited in tissues such as liver, kidneys, bones, and growing feathers (Clemens et al., 1975; Pain, 1996; Pain et al., 2009).

Lead toxicity can cause physiological, reproductive, behavioral, and immunological changes in animals, leading to poor fitness or death (Bates et al., 1968; Veit et al., 1983; Rocke and Samuel, 1991; Locke and Thomas, 1996). For this reason, twenty nine countries have implemented voluntary or legislative restrictions on the use of lead shot in wetlands, and Sweden and Denmark have banned all forms of lead ammunition (Avery and Watson, 2009).

Waterfowl hunting in northeastern Argentina is locally encouraged because some dominant duck species are considered agricultural pests (Zaccagnini, 2002; Blanco et al., 2006). Furthermore, duck hunting has become a profitable industry, and over the years Argentina has turned into an international hotspot that attracts hunters from all over the world (Zaccagnini, 2002). However, there is a paucity of information on registered outfitters and hunting licenses sold annually to allow for environmental impact estimates of this activity.

While lead shot is the only type of ammunition available in Argentina, lead toxicosis in waterfowl has been explored only recently. Preliminary assessments in ducks hunted in rice fields from Santa Fe province revealed that 31% of rosy-billed pochards (*Netta peposaca*) and 29% of whistling ducks (*Dendrocygna bicolor*) had lead shot in their gizzards. Furthermore, 47% of rosy-billed pochards and 15% of whistling ducks showed lead concentrations in their bones exceeding 10 mg/kg dry weight (Ferreyra et al., 2009).

Given the vast area where waterfowl hunting occurs and the limited information available, the main goal of this study was to expand our knowledge on the incidence of lead pellet ingestion in waterfowl at hunting hotspots in Argentina, and to measure lead in duck tissues to assess health risks from spent shot intake.

2. Materials and methods

2.1. Study site

This study took place in natural wetlands of Santa Fe and Corrientes provinces (Fig. 1). This region is an important waterfowl wintering area along the Paraná River flyway, which is one of the main waterfowl migratory routes in Argentina (Capllonch et al., 2008). The area is interspersed with rice farms which also attract ducks (Lesterhuis, 2011). Duck hunting is permitted for 3–4 months each year (May to July/August) across an extensive range.

In 2011, ducks were sampled in Santa Fe province. In 2012, high temperatures and drought delayed duck migration to Santa Fe and forced us to move to the neighboring province of Corrientes, where sampling was possible. The selected study sites were 40–80 km apart, one on the west margin of the Paraná river (Santa Fe), the other on the east side (Corrientes) (Fig. 2). At both sites, some samples were collected from the islands that are part of the Paraná river system (which we refer to as "island" wetlands), and others from landlocked water bodies (lagoons, marshes, artificial reservoirs) (which we call "inland" wetlands).

2.2. Sample collection from hunter-killed ducks

Over the hunting seasons (May–July) of 2011 and 2012, we collected digestive tracts, one wing bone and livers from a total 415 donated hunter-killed ducks in Santa Fe ($n=275$) and Corrientes ($n=140$). These included 134 (5 adult; 129 juvenile) white-faced tree ducks (*Dendrocygna viduata*), 103 whistling ducks (74 adult; 29 not determined), 103 rosy-billed pochards (97 adult; 6 juvenile), 57 Brazilian ducks (*Amazonetta brasiliensis*) (41 adult; 6 juvenile; 10 not determined), and 18 black-bellied whistling ducks (*Dendrocygna autumnalis*) (15 adult; 3 not determined).

We used liver and bone to measure lead exposure because they provide a robust assessment of recent and past exposure to lead, as have done other authors (Mateo et al., 2001; Franson and Pain, 2011). From each animal, we recorded body weight and sex (Table 1). All samples were frozen at −20 °C until processed in the laboratory. For bone lead determination, we used one humerus of each duck. In 14 ducks with fractured humerus we replaced them with a radius ($n=$one whistling duck), femur ($n=$one black-bellied whistling duck) or tibia ($n=12$, four rosy-billed pochards, three whistling ducks and five white-faced tree ducks); considering that similar lead concentrations are expected among skeletal bones of the same individual (Ethier et al., 2007).

2.3. Sample collection from live-captured ducks

The capture protocol used was approved by the Institutional Animal Care Use Committee (IACUC) of the Wildlife Conservation Society. Birds were captured using corral traps, baited with corn at night and checked for capture during the morning. Trapped ducks were handled with care and held in clean cotton fabric bags until processed within 99 min (range 20–220). All ducks were identified with a leg band and released at the site of capture immediately after sample collection.

We captured and sampled 97 ducks. These included 68 white-faced tree ducks (5 adults, 55 juveniles, 8 not determined), 6 (adult) Brazilian ducks, 1 whistling duck (age not determined), 4 rosy-billed pochards (3 adults, 1 juvenile), and 17 black-bellied whistling ducks (age not determined) (Table 3).

From each animal, we recorded body weight and sex (in species with sexual dimorphism) (Table 1). Heparinized blood samples (2.5–4 ml) were collected by venipuncture of the basilic vein of 23 ducks from Santa Fe and 73 from Corrientes (Table 3). An aliquot of 1 ml of each blood sample was snap-frozen in liquid nitrogen for lead determination.

2.4. Lead pellet ingestion assessment

The whole gastro-intestinal tract of each hunter-killed duck was X-rayed to identify radio-opaque silhouettes that resembled gunshot pellets. Digestive tracts showing images compatible with pellets were dissected for confirmation. A sensitive and a specific estimate of pellet ingestion are reported. For a sensitive estimate (lower false negative probability), we considered positive those ducks with X-ray evidence of at least one lead pellet in the gastro-intestinal tract. A more conservative estimate (less chance of a false positive) was calculated only counting as positive those in which ingestion was confirmed by recovering the lead pellets from the digestive tract lumen.

We examined the gastro-intestinal tract in detail to verify that it was not perforated by the shot received when the duck was hunted, to avoid inclusion of pellets not ingested during feeding.

2.5. Laboratory analysis for lead tissue levels

Lead concentration in frozen tissues (bone, liver and blood) was determined by inductively coupled plasma-atomic emission spectrometry (ICP-OES), following 200.7 EPA standards (U.S. Environmental Protection Agency), at the Chemical Analysis Laboratory-LANAQUI, Centro de Recursos Renovables de la Zona Semiárida, (CONICET, Universidad Nacional del Sur, Bahia Blanca, Argentina). The detection limit was 0.20 mg/kg dry weight (dw) for liver and bone, and 0.25 mg/kg wet weight (ww) for blood (Shimadzu 9000, Shimadzu Corporation, Kyoto, Japan).

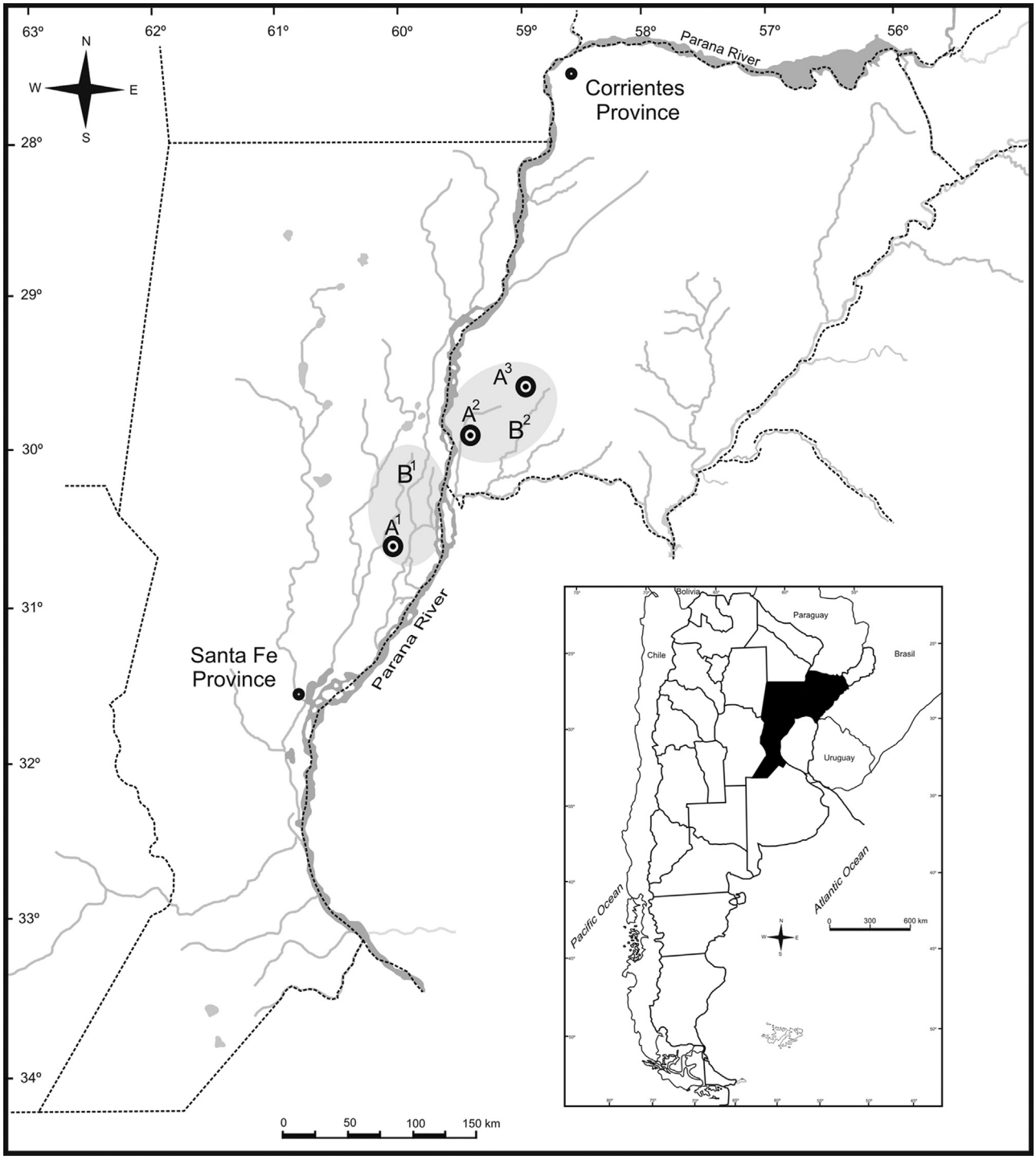

Fig. 1. Study sites, live duck sampling: corral trap locations A^1 (30° 38′37.94″ S/60° 07′48.47″ W), A^2 and A^3 (29° 57′47.14″ S/59° 26′53.39″ W and 29° 33′19.52″ S /59° 02′40.35″ W). Hunter-killed ducks were received from hunting areas B^1 and B^2, including inland and island wetlands.

Bone and liver samples were dried to a constant weight in a bench-top oven (48 h at 40 °C). Before lead determination, the product was homogenized by trituration. Standard Reference Materials were used to validate results from bone (NIST-1400 bone ash, certified value of lead: 9.07 ± 0.12 mg/kg), and blood (ERM-CE 195 bovine blood, certified value of lead: 0.416 ± 0.009 mg/kg). We were unable to use certified reference materials for liver (NIST 1577c, certified value of lead: 0.0628 ± 1 mg/kg), because the recoveries and standard deviations were not within the capabilities of the equipment used (detection limit = 0.02 mg/kg). SRM material NIST -1400 and ERM-CE 195 with a mean of lead concentration of 9.07 ± −0.12 and 0.416 ± 0.009, respectively, were analyzed with each batch of 45–50 samples. The mean (± S.E.) lead concentration determined in these reference materials ($n=5$) were 8.95 ± 0.16 and 0.386 ± 0.011, respectively. In addition, lead recoveries obtained with spiked blanks ($n=7$) and samples ($n=5$) were 101.1 and 96.3%, respectively.

2.6. Data analysis

The comparison of lead exposure (ingestion of pellets and concentrations in liver and bone) between species, age class, site/year and type of wetland (island/inland) was done using multivariable generalized linear models (GLM). The distribution of the dependent variables was either binomial (lead ingestion determined by X-rays) or negative-binomial (lead in liver and bone). This multivariable approach allowed us to adjust for the lack of independence of some groups of data (e.g., those coming from the same sites), and also to assess associations between lead exposure and some variables of interest while controlling for potential confounders. The software package used was R (The R Project for Statistical Computing; http://www.r-project.org/). Values below the detection limit

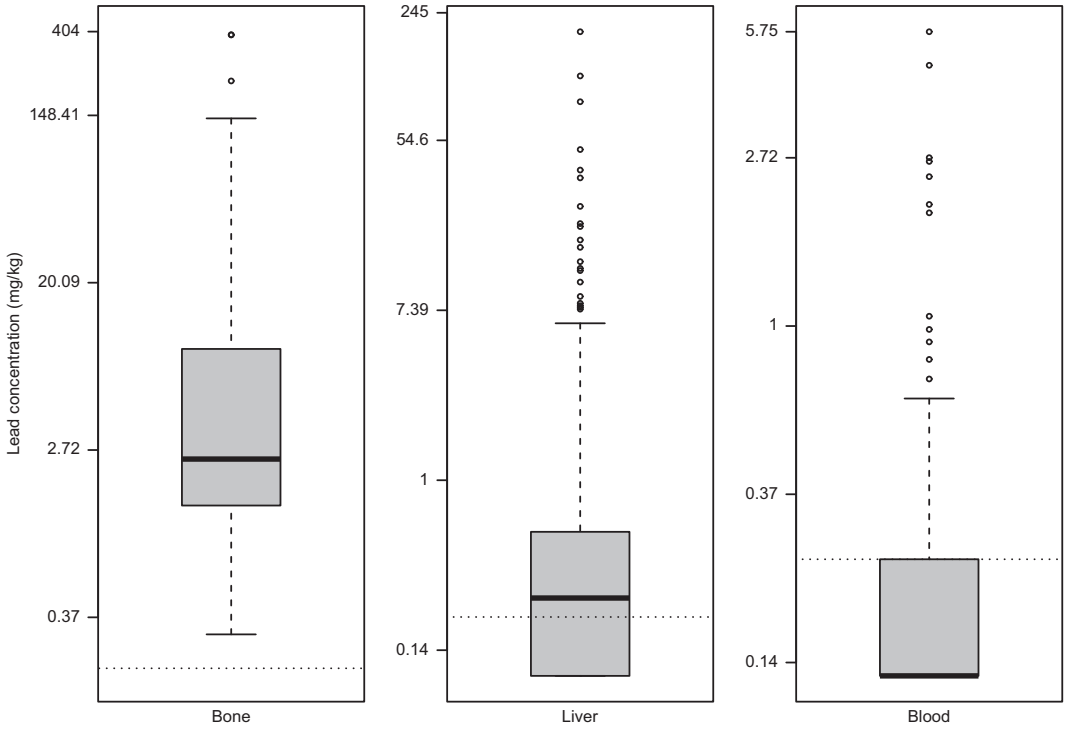

Fig. 2. Boxplots showing the distribution of the concentrations of lead found in bone, liver and blood of wild ducks from Argentina. The horizontal dotted lines represent the detection limit of the technique employed.

Table 1
Description of ducks sampled in Argentinean wetlands (both donated hunter-killed ducks and live trapped ducks), including information on location and year, species, sex and weight.

Year and location	Species	Female		Male		ND	
		N	Weight (g) mean (±SD)	*N*	Weight (g) mean (±SD)	*N*	Weight (g) mean (±SD)
2011—Santa Fe							
Live ducks	*Amazonetta brasiliensis*	3	483(±90)	3	606 (±59)	–	–
	Dendrocygna viduata	–	–	–	–	12	788 (±97)
	Dendrocygna bicolor	–	–	–	–	1	895
	Netta peposaca	4	1001 (±49)	–	–	–	–
	Callonetta leucophrys	–	–	1	–	–	480
	Total	**7**		**4**		**13**	
Hunted ducks	*Dendrocygna viduata*	35	787 (±79)	49	814 (±74)	–	–
	Dendrocygna bicolor	44	907 (±89)	55	932 (±117)	2	850 (±40)
	Netta peposaca	43	1099 (±96)	47	1147 (±98)	–	–
	Total	**122**		**151**		**2**	
2012—Corrientes							
Live ducks	*Dendrocygna viduata*	–	–	–	–	56	759 (±79)
	Dendrocygna autumnalis	–	–	–	–	17	752 (±71)
	Total					**73**	
Hunted ducks	*Amazonetta brasiliensis*	32	563 (±56)	25	596 (±49)	–	–
	Dendrocygna autumnalis	7	840 (±58)	11	849 (±109)	–	–
	Dendrocygna bicolor	1	860	1	960	–	–
	Dendrocygna viduata	20	758 (±86)	30	849 (±86)	–	–
	Netta peposaca	4	1160 (±136)	9	1269 (±102)	–	–
	Total	**64**		**76**			

ND—Sex was not determined in live-captured individuals of non-dimorphic species.

were established as the average between zero and the detection limit (0.1 for values <0.20 mg/kg in liver and bone, and 0.125 for values <0.25 mg/kg in blood).

3. Results

3.1. Hunter-killed ducks

A total of 415 digestive tracts, 382 bones and 412 livers from hunter-killed ducks were examined for lead shot ingestion and analyzed for lead concentration, respectively. Exposure by species, year/province and type of wetland (inland vs island) is summarized in Table 2 and 3.

Of the gastrointestinal tracts positive by X-ray (12.8%, 53/415), 81.13% (43/415) were confirmed to have lead pellets in the gastrointestinal tract. All of them had at least one pellet in the gizzard, of which 76.7% had only one pellet, 14% had two and 9.3%, three. In addition, four lead shot pellets were recovered from the esophagus and two from the intestine.

Table 2
Prevalence of ingested lead shot and lead concentrations (mg/kg dw) in liver and bone of hunter-killed ducks by wetland type (inland or island) and sampling year. Positives are defined as gastrointestinal tracts containing at least one pellet.

Year	Site	Species	Pellet ingestion			Lead concentration geometric mean ± GSD[a] (range)			
			N	X-ray[b] (%)	Confirmed[c] (%)	*N*	Liver	*N*	Bone
2011	Inland wetlands	*D. bicolor*	9	22.2	22.2	9	17.10 ± 5.33 (0.6–196)	9	42.40 ± 4.86 (1–224)
		D. viduata	4	75	75	4	2.5 ± 5.87 (0.91–35.10)	4	3.65 ± 4.72 (0.45–17.90)
		N. peposaca	7	71.45	71.45	7	2.20 ± 6.67 (0.24–38.50)	7	4.30 ± 6.70 (0.48–46.20)
		Total	**20**	**50**	**50**	**20**	**5.67 ± 7.14 (0.2–196)**	**20**	**11.64 ± 7.43 (0.45–224)**
	Island wetlands	*D. bicolor*	92	5.45	5.45	92	0.26 ± 3.63 (ND[d] -116.50)	77	4.32 ± 4.90 (0.33–388)
		D. viduata	80	8.75	6.25	80	0.25 ± 2.72 (ND-25.10)	71	3.1 ± 3.69 (0.36–89.70)
		N. peposaca	83	18	12	80	0.30 ± 3.66 (ND-7.65)	75	3.15 ± 4.25 (0.30–143)
		Total	**255**	**10.6**	**7.85**	**252**	**0.27 ± 3.34 (ND-116.5)**	**223**	**3.5 ± 4.29 (0.30–388)**
2012	Inland wetlands	*D. viduata*	34	14.7	14.7	34	0.31 ± 3.98 (ND-11.80)	34	3.30 ± 2.50 (0.90–43.20)
		D. autumnalis	14	7.2	7.2	14	0.21 ± 2.10 (ND-0.79)	14	3.68 ± 3.07 (1.30–34.40)
		Total	**48**	**12.5**	**12.5**	**48**	**0.28 (3.44) (ND-11.80)**	**48**	**3.40 ± 2.64 (0.90–43.20)**
	Island wetlands	*D. bicolor*	2	50	50	2	0.98 ± 3.31 (0.42–2.29)	2	5.8 ± 1.3 (4.8–7)
		D. viduata	16	6.25	6.25	16	0.24 ± 2.17 (ND-1.40)	16	5.45 ± 3.52 (1.10–38.60)
		N. peposaca	13	38.5	23	13	0.23 ± 2.26 (ND-1.04)	13	4.60 ± 4.42 (0.60–56.80)
		D. autumnalis	4	0	0	4	0.16 ± 2.42 (ND-059)	3	4.83 ± 3.62 (1.10–11.50)
		A. brasiliensis	57	17.6	3.5	57	0.28 ± 2.75 (ND-16.90)	57	2.35 ± 2.55 (0.30–389)
		Total	**92**	**10.9**	**7.6**	**92**	**0.27 ± 2.60 (ND-16.90)**	**91**	**3.13 ± 3.10 (0.30–389)**

[a] GSD: geometric standard deviation.
[b] Percentage of ingestion diagnosed by X-ray (positives/total examined).
[c] Percentage confirmed by gastrointestinal tract dissection.
[d] ND: below detectable limit.

Table 3
Lead concentration (mg/kg ww) in blood samples from live-captured ducks by species and sampling year.

Year	Species	N positives/ N total	Lead concentration geometric mean ± GSD (range)
2011	*A. brasiliensis*	0/6	ND
	D. bicolor	1/1	0.31
	D. viduata	5/12	0.26 ± 2.77 (ND-2.66)
	N. peposaca	0/4	ND
	Total	**6/23**	**0.19 ± 2.22 (ND-2.66)**
2012	*D. autumnalis*	4/17	0.19 ± 2.66 (ND-4.71)
	D. viduata	16/53	0.21 ± 2.70 (ND-5.75)
	Total	**20/70**	**0.20 ± 2.67 (ND-5.75)**

ND—below detectable limits. GSD: geometric standard deviation.

Lead was detected in all bones and in 60.4% of the livers analyzed. The geometric mean lead concentration for liver was 0.31 (GSD=3.93) mg/kg dw and 3.61 (GSD=4.02) mg/kg dw for bone.

Ducks that ingested lead pellets had a concentration of lead in liver much higher than those that were not found to have shot in their gastrointestinal tract (the geometric means were 1.134 and 0.272 (mg/kg dw), respectively; Mann-Whitney test $p < 0.001$).

The species that was consistently more prone to ingesting lead shot was the rosy-billed pochard, compared to all other species evaluated. According to the model estimates presented in Table 4, the lead intake in rosy-billed pochard was 3.6 times the odds of lead intake in white faced duck, 4.2 times the odds in whistling ducks, 4.7 times the odds in Brazilian ducks and 16 times the odds in black-bellied whistling duck.

However, whistling ducks showed the highest levels of lead in liver and bone (Table 4). Lead in liver of whistling ducks was 2.5 times greater than in rosy-billed pochards, 3.3 times greater than in white faced ducks, and 15.7 times greater than in black bellied ducks. Bone lead concentration in whistling ducks was greater than rosy-billed pochards (1.8 times), Brazilian ducks (1.9 times), white faced ducks (2.9 times) and black bellied ducks (3.4 times). The highest levels of lead in livers were found in 2011 (Santa Fe) (4.1 times higher than in 2012/Corrientes). The odds of ingesting lead pellets was 4.6 times greater in ducks from inland wetlands than from islands (Table 4). Similarly, the concentration of lead in liver was 10.7 times greater in inland sites than in the islands (Table 4).

3.2. Live-captured ducks

Lead was detected in the blood of 28% of 93 (26/93) wild ducks with a geometric mean of 0.20 (GSD=2.55) mg/kg ww, in three of the five species tested (Table 3).

4. Discussion

Lead toxicosis in birds due to the ingestion of discharged lead gunshot has been recognized for over a century, clearly establishing lead poisoning as a common and widely distributed problem in waterfowl worldwide (Friend et al., 2009). The amount of spent lead shot discharged into wetlands of Argentina yearly is unknown. However, the findings in this study strongly suggest that lead poisoning from spent shot is a risk for local waterfowl.

Because lead poisoning results from ingested lead pellets, the occurrence of lead shot in waterfowl gizzards provides an appropriate estimate of the severity of lead intoxication in waterfowl populations. The chances of death of an individual increase when more than one lead pellet is ingested (Cook and Trainer, 1966; Anderson and Havera, 1985; Kerr et al., 2010). We found a great variation in the total prevalence of ingestion in our study, which depending on the time of year and locality, ranged from 7.5% to 50% (Table 2).

Most individuals had only one pellet in their gizzards, but records of multiple pellet ingestions were not uncommon, as has been reported by Mudge (1983) and Sanderson and Bellorse (1986). According to the U.S. Department of Interior, ingestion rates above 5% are considered excessive and require ammunition replacement with non-toxic types (Anderson and Havera, 1989; Friend et al., 2009).

Table 4
Generalized lineal model with binominal responses describing the association between, species, locality/year and type of wetland, and exposure to lead (ingestion, lead concentration in liver and in bone).

Term	*Coefficients*	*Standard error*	*P-value*
Model=Lead ingestion (binomial)~species+province/year+wetland type			
Intercept	0.134	0.507	0.791
Species (*D. bicolor*)[a]	−1.437	0.449	0.001*
Species (*D. viduata*)[a]	−1.295	0.419	0.002*
Species (*A. brasiliensis*)[a]	−1.551	0.771	0.044*
Species (*D. autumnalis*)[a]	−2.771	1.145	0.016*
Province/year (Santa Fe/2011)	0.085	0.471	0.857
Wetland type (Island)	−1.474	0.436	<0.001*
Model=Lead in liver (negative binomial)~species+province/year+wetland type			
Intercept	1.179	0.333	<0.001*
Species (*D. viduata*)[b]	−1.195	0.243	<0.001*
Species (*N. peposaca*)[b]	−0.918	0.234	<0.001*
Species (*A. brasiliensis*)[b]	0.270	0.453	0.551
Species (*D. autumnalis*)[b]	−2.753	0.657	<0.001*
Province/year (Santa Fe/2011)	1.416	0.339	<0.001*
Wetland type (Island)	−2.372	0.285	<0.001*
Model=Lead in bone (negative binomial)~species+province/year+wetland type			
Intercept	3.262	0.275	<0.001*
Species (*D. viduata*)[b]	−1.059	0.206	<0.001*
Species (*N. peposaca*)[b]	−0.596	0.203	0.003*
Species (*A. brasiliensis*)[b]	−0.666	0.330	0.044*
Species (*D. autumnalis*)[b]	−1.222	0.426	0.004*
Province/year (Santa Fe/2011)	0.104	0.229	0.650
Wetland type (Island)	−0.349	0.227	0.125

[a] Simple contrasts—reference level: *Netta peposaca* (the coefficients reflect comparison with this group).
[b] Simple contrasts—reference level: *Dendrocygna bicolor* (the coefficients reflect comparison with this group).
* Statistically significant association.

Previous studies in inland wetlands from Santa Fe Province showed comparable high levels of ingestion for rosy-billed pochards (31%) and whistling ducks (28%), and similar levels of lead in tissue (Ferreyra et al., 2009). The incidence of ingested lead in ducks reported elsewhere varies greatly, even when samples were taken from populations without apparent clinical signs or mortality events. In Europe, lead pellet ingestion in mallards (*Anas platyrhynchos*) varied from 2% to 10% in wetlands of northern countries, and between 25% and 45% in Mediterranean deltas (Mateo, 2009). Similarly, values around 60–70% were recorded in the northern pintail (*Anas acuta*) and the common pochard (*Aythya ferina*) (Mateo, 2009). This wide lead ingestion range was also reported in ducks from USA, varying from 0.2% to 75% in wintering areas (Zwank et al., 1985; U.S. Department of the Interior (US. Fish and Wildlife Service), 1986).

Regarding lead levels in bone, our results were variable and comparable with those reported by Stendell et al. (1979) in hunting fields of USA, with levels in bone between <0.5 and 361 mg/kg dw.

We found that the prevalence of ingestion was greater in rosy-billed pochards than in the other species studied. The few studies that examined the diet of these species in Santa Fe report that the rosy-billed pochard and whistling ducks are essentially herbivorous and forage by dabbling, but they occasionally dive for food (Mosso and Beltzer, 1991; del Hoyo et al., 1992; Beltzer and Mosso, 1992). Rosy-billed pochards also include a proportion of animal items in their diet (Beltzer and Mosso, 1992).

Nonetheless, whistling ducks showed the highest concentration of lead in their liver and bones, even when they were found to ingest fewer lead pellets than rosy-billed pochards. We offer three non-exclusive plausible explanations to this unexpected finding. First, diet may differ among species, and diets high in protein and calcium (as those that include animal items; i.e. the rosy billed pochard's) tend to reduce the absorption of lead from the gastrointestinal tract, therefore lowering the general body burden of lead in the bird (Sanderson and Bellorse, 1986; Eeva and Lehikoinen, 2004; Martinez-Haro et al., 2009).

Second, the susceptibility to lead exposure may also vary among species. If rosy-billed pochards are less tolerant to lead exposure than whistling ducks, then at similar exposures to lead, the individuals of the first species would be more affected than those of the latter, and consequently would be less likely to be included in our sample (i.e. if whistling ducks are not as affected by lead as rosy-billed ducks, they would be able to fly – and thus be hunted – even if they have high lead concentrations in liver and bone). This would be an example of 'selection' bias (Dohoo et al., 2003). Lastly, natural grit and the characteristics of food ingested influence pellet erosion and the time lead shot remains in the bird's gastrointestinal tract (Stendell et al., 1979). Brewer et al. (2003) found that around 9–10% of the pellets ingested are not retained in the digestive system. Therefore, differences in the fraction of the pellets trapped in the gizzard may account for the differences observed between species. This differential lead exposure among duck species warrants further studies.

Despite the discrepancy in the pattern of exposure by species discussed above, lead levels in duck livers were highly correlated with the ingestion of lead pellets (even when the analysis was stratified by species; data not shown). This association was expected, as after acute exposure (recent pellet ingestion) lead salts from dissolved pellets are absorbed in the gut and stored in soft tissues like the liver and kidneys (Pain, 1996; Franson and Pain, 2011). However, we found no association between pellet ingestion and lead in bone. This might be because uptake of lead by bone tissue is quick but its loss is very slow, therefore reflecting both recent and cumulative lead exposure. Consequently, high levels of lead in bone might reflect the duck's lifetime exposure and not necessarily be linked to recent ingestion of lead shot (Stendell et al., 1979; Guitart et al., 1994; De Francisco et al., 2003).

For comparative purposes, we used the guidelines developed by Franson and Pain (2011), converting the values to dry weight

(1 mg/kg ww in liver is equivalent to 3.1 mg/kg dw) following their estimate of liver humidity (Franson and Pain, 2011). Waterfowl with no history of lead poisoning usually have lead concentrations in liver below 6 mg/kg dw (Franson and Pain 2011). Levels between 6 and 20 mg/kg dw of lead in liver have been associated with subclinical toxicity (levels that are not high enough to cause apparent clinical signs), 20–30 mg/kg dw with moderate clinical signs, and above 30 mg/kg dw with severe clinical poisoning (Pain, 1996; Svanberg et al., 2006). In our study, 3.15% of the ducks evaluated had liver lead levels above 6 mg/kg and 1.45% surpassed 30 mg/kg. Toxic ranges differ slightly for lead in bone, where values above 20 mg/kg dw can cause severe clinical signs (Franson and Pain, 2011). In our sample, 13.4% of ducks had bone lead levels above the 20 mg/kg threshold. Comparable to our results Stendell et al. (1980) and Guitart et al. (1994) reported relatively low levels of lead in liver from apparently healthy ducks, with geometric means between 0.072 and 2.86 mg/kg ww (approximately, 0.22 to 8.8 mg/kg dw), and <0.05 to 0.82 mg/kg ww (approximately, 0.15 to 2.54 mg/kg dw,) respectively.

Blood lead is a more sensitive method for measuring acute lead exposure in live waterfowl (Anderson and Havera, 1985). Blood lead concentration in unexposed waterfowl should be less than 0.2 mg/kg (ww) (Pain, 1996). In our study, 28% of sampled ducks exceeded that limit, and 8.6% were above 1 mg/kg, which is expected to cause severe poisoning (Franson and Pain, 2011). The species with detectable blood lead levels in our study were the white-faced tree duck and the black-bellied whistling-duck. Our small and species-biased sample of live-ducks precludes us from further interpreting these species-specific differences.

Despite the high lead levels found in our study, we did not observe sick or dead ducks during fieldwork sessions. Nevertheless, three live-captured white-faced tree ducks (with blood lead levels of 0.65, 0.73 and 5.75 mg/kg) showed difficulty in flying away after release, and remained standing still in the vegetation showing labored breathing. Furthermore, one of the ducks also presented stiffness in its limbs during handling. Our determinations of lead ingestion and liver and bone concentrations were obtained from hunter provided carcasses (i.e. non-probabilistic sampling). It has been argued that lead-intoxicated ducks are an easier target for hunters, and thus the levels of exposure measured in hunter-killed ducks may overestimate that of the population (Bellrose, 1959). However, with levels as high as the ones reported here, we might expect that all the ducks that were suffering from moderate to severe clinical disease due to lead poisoning were less able to fly and therefore little likely to be shot by hunters (Pain et al., 1992). In agreement with this notion Mudge (1983) found that the proportion of individuals with high levels of lead in liver was lower in specimens provided by hunters than in those sampled by other methods, indicating that the fraction of ducks exposed to high doses of lead are not available to the hunter. For all the above, we might be in the presence of 'selection' bias, as the individuals in the population which are moderately to severely affected by lead poisoning were less likely to be included in our sample, leading to under estimation of actual lead exposure in the population.

Lead shot ingestion and lead levels in liver were higher in inland wetlands than island environments. This difference may be due to a greater amount of lead shot in inland wetlands, which may be the result of the differential water flow between these two systems, with inland wetlands being more lentic and the insular more lotic. Moreover, pulses of periodical flooding in the islands of the Paraná river valley may cause a series of disturbances that can alter the bioavailability of the pellets, and thus their ingestion by ducks. Mudge (1983) also found that lead exposure depends on the type of environment, showing that waterfowl from inland areas tended to have higher lead exposure than those from coastal areas.

5. Conclusions

Our results indicate that ducks sampled in areas under heavy hunting pressure in Argentina are exposed to lead acutely and chronically, as evidenced by pellet ingestion rates and the levels of lead in their blood, liver and bones. From the data collected to date, we can assume that spent gunshot pellets are highly available in the region, and ingestion by ducks seems to be a common event. However, pellet intake by ducks depends on the feeding grounds used which, in turn, are related to the weather, food availability, the hydrological regime at the feeding sites, and land-use changes introduced by humans (i.e. crops, irrigation). Because our sampling method was non-probabilistic and there was potential for 'selection' bias, the actual levels of exposure may be underestimated, and lead toxicity risks for waterfowl might be worse than reported.

Long-term persistent pollution by lead threatens the sustainability and resilience of these wetland systems. Given the current magnitude of duck hunting in the Parana River floodplain, our results suggest that the replacement of lead pellets with non-toxic ammunition is both necessary and urgent. At present there are a variety of non-toxic alternative materials for the manufacture of ammunition, such as steel, nickel and bismuth–tin alloy, among others (Rattner et al., 2008). The substitution of lead by these materials has significantly reduced the detrimental effect of lead on wild birds in just 5–6 years post implementation (Moore et al., 1998; Anderson et al., 2000; Samuel and Bowers 2000).

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