

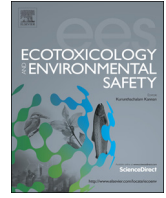


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## Review

### 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.

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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  $-20^{\circ}\text{C}$  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, Bahía 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).

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