

## Lead poisoning of Greater Flamingos *Phoenicopterus ruber*

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Lead poisoning is an important cause of waterfowl mortality. In the United States of America 1.4 to 2.6 million waterfowl were estimated to die annually (Bellrose 1959), and in Britain a conservative estimate of Mudge (1983) indicate that about 8000 Mallard *Anas platyrhynchos* die each winter. In some European wetlands such as the Camargue (Rhône river delta, France) (Pirrot & Taris 1987, Pain 1990, 1991) and the Evros Delta (Greece) (Pain & Handrinos 1990), recent research has revealed that the incidence of ingested lead shot in waterfowl is high. In this paper we report Greater Flamingo *Phoenicopterus ruber* mortality due to this cause in Doñana National Park, Southwest Spain.

Between 6 and 26 March 1991, 22 moribund or dead flamingos were observed at Mary López lagoon, and another 30 had difficulty flying. During this period about 5000 flamingos were present. The flamingos collected still alive produced bright green droppings, suggestive of lead poisoning (Pain 1989). These individuals were able to stand upright until just before death. Some of them had a tumefacted head but, if they remained alive for one or two days, this tumefaction decreased, or

even disappeared.

Two individuals (a and b, Table 1) were examined at the Laboratory of Animal Health (Córdoba, Spain), where 18 and 37 lead pellets respectively were found in their gizzards. Histological study showed chronic liver lesions; microbiological samples showed no pathogenic microorganism from the gastrointestinal tract. Another two individuals (c and d, Table 1), together with water and mud samples from Mary López lagoon, were examined for organochlorine and organophosphorus pesticides, and heavy metals at the laboratory of Toxicology (Sevilla, Spain). Results showed no pathological or pathogenic concentrations, except for levels of lead in liver; 93.2 and 72.1 ppm dry weight for each bird, respectively (Table 1). The methodology for liver lead analysis was as follows: 500 mg of liver were dried and 1 ml of nitric acid (14N) added, afterwards samples were heated in a microwave oven during 1-2 minutes and carried out to a volume of 25 ml with deionized water. Lead determinations were carried out on a Perkin-Elmer atomic absorption spectrophotometer in conjunction with a graphite furnace.

**Table 1. Results of the gizzard and liver analyses of dead Greater Flamingos (M = males, F = females).**

Date of death	Sex	Body mass(kg)	<i>n</i> seeds <i>S. litoralis</i>	<i>S. maritima</i>	Unidentified material	Greatest size of grit(mm)	<i>n</i>	Shot Size(mm)	Shot Mass(g)	Lead levels in liver(ppm)	
14 March	a	?	?	?	?	?	18	?	0.29	?	
13 March	b	?	?	?	?	?	37	?	1.09	?	
12 March	c	?	?	?	?	?	?	?	?	93.2 d.w.	
12 March	d	?	?	?	?	?	?	?	?	72.1 d.w.	
10 March	1	?	?	?	?	?	328	1-2	11.63	63.0 w.w.	
13 March	2	?	?	?	?	?	105	1.5-2.5	1.70	70.3 w.w.	
11 March	3	M	2.75	246	1	-	6 x 8	84	1-2	1.66	57.0 w.w.
6 March	4	M	2.80	30	0	+	4 x 3	243	1	2.56	36.1 w.w.
11 March	5	M	2.55	34	0	+	9 x 10	200	1-2	6.70	79.6 w.w.
13 March	6	M	2.68	1511	11	+	3 x 4.5	8	1-1.5	0.09	12.6 w.w.
26 March	7	M	2.60	5	0	+	8 x 8	92	0.25-1.5	0.42	81.5 w.w.
23 March	8	M	2.00	1139	0	+	4 x 4	49	1-2	0.25	93.5 w.w.
11 March	9	F	2.35	198	0	-	4 x 5	76	1-2	1.95	112.2 w.w.
13 March	10	M	2.90	7	1	-	6 x 7	41	1-2	0.34	57.5 w.w.
13 March	11	M	2.65	420	0	+	4 x 7	87	0.5-2	0.80	37.8 w.w.
13 March	12	M	2.60	160	0	-	6 x 6	133	1-2.5	3.55	56.5 w.w.

Subsequently, it was decided to proceed with an autopsy of the remaining 12 flamingo bodies, which had been frozen. The specimens were weighed and sexed, and analyses of gizzard contents were performed with the aid of a binocular microscope. Shot pellets were counted and weighed, and the smallest and largest ones measured. Lead concentrations in the liver were determined at the Institute of Organic Chemistry (Madrid, Spain). About 500 mg of tissue were accurately weighed into a teflon cup and 4 ml of nitric acid (8N) added. The cup was covered and put onto a hot plate at 90°C for 2 h. The final solution was filtered through Whatman paper. The filtering was carried out to a volume of 10 ml with distilled water and used for lead determination at 283.3 nm by atomic-absorption spectrophotometry (Haseltine *et al.* 1981, Lachica 1988). The determinations were carried out using a Perkin-Elmer model 2380 atomic-absorption spectrophotometer equipped with a lead hollow cathode lamp. The lower limit of reportable residues was 0.02 µg/g and the average recovery from fortified chicken livers was 91.7%. All residues were expressed as µg/g (ppm) wet weight.

Results of the analyses are shown in Table 1. All of the gizzards examined contained seeds of *Scirpus litoralis*, whereas *Scirpus maritimus* was found in only 30% of gizzards. Both plant species are common in Doñana marshes. We could not identify the remaining plant material. Grit,

although not present in the marshes, was abundant in all gizzards. All the gizzards contained shot, their number varying between eight and 328, and their mass between 0.09 and 11.63 g. Levels of lead in the liver varied between 12 and 112 ppm w.w.

In waterfowl the ingestion of one to three shotgun pellets (Kowalczyk 1986), or 16 mg of leadshot/kg (Hatch 1987) can be lethal. In our case the amount of ingested lead greatly exceeds these values. Taking into account the lead concentration in livers, it has been considered that levels above 6 ppm w.w. or above 20 ppm d.w., indicate recent exposure to large amounts of lead (Pain 1989). Flamingos could ingest gunshot in mistake for grit or seeds, since gunshot size is within the range of grit (0.25-9x10 mm) and seeds (1.25x1.50-2x3).

The fact that this incident occurred during a short period of time (only 0.44% of the flamingos present in the lagoon died within 20 days), and that large numbers of shot were ingested, suggest that a small group of flamingos were feeding in a place with a large accumulation of shot, such as a sport firing range. Perhaps the proportion of individuals affected by lead poisoning will be higher for species foraging in flocks rather than as solitary feeders. The conspicuousness and gregarious habits of the flamingos facilitated identification of dead bodies, but in the case of other species such as ducks, mortality could pass unnoticed.

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