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SEASONAL BLOOD LEAD CONCENTRATIONS IN MARSH HARRIERS *Circus aeruginosus* FROM CHARENTE-MARITIME, FRANCE: RELATIONSHIP WITH THE HUNTING SEASON

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Abstract

This paper reports on lead contamination in marsh harriers Circus aeruginosus throughout the year. Blood samples were taken from puni, fledged (first year) birds and adults. The results show very low blood lead (PbB) concentrations in pulli (median 0.8 µgldl PbB). A marked seasonal différence in the distribution of PbB concentrations in first yearladult birds was round. Significantly more birds had an elevated (> 30 µgldl or > 60 µgldl) PbB concentrations within than outside the hunting season, reflecting different levels of exposure to lead shot during these periods. In addition, a high proportion of regurgitated marsh harrier pellets contained lead shot during the hunting season (autumnlwinters 1994 and 1995-25% and 15.6%, respectively), whilst only 1.4% of pellets contained shot during spring 1994, after the hunting season. These results provide compelling evidence that spent lead shot carried in the carcasses ofprey is the source of lead contamination in marsh harriers in Charente-Maritime. ©1997 Published by Elsevier Science Ltd.

Keywords: lead, blood, marsh harrier, hunting and France.

INTRODUCTION

Lead poisoning in waterfowl has been well documented worldwide (see Pain, 1992). It occurs when spent lead gunshot are ingested along with grit or food. Shot may be retained in the muscular gizzard, eroded by the grinding action of gizzard grit and dissolved by stomach acids. The resulting lead salts are then absorbed into the bloodstream and poisoning ensues. Blood lead concentrations are the most frequently used indicator of lead exposure. In the USA, before a nationwide ban on the use of lead shot for waterfowl hunting, it was estimated that lead poisoning resulted in the deaths of 1-6-2.4 million waterfowl annually (Bellrose, 1959; USFWS, 1986). However, although a greater number of waterfowl than other species appear to be affected, lead poisoning is by no means confined to waterfowl. To date, lead poisoning mortality has occurred in over 30 species of birds other than waterfowl (Franson, 1996). After waterfowl, more is known about lead poisoning in Falconiformes than any other group of wild birds. Most information comes from the USA, where several hundred bald eagles *Haliaeetus leucocephalus* have died from lead poisoning (Franson, 1996). Several Californian condors *Gymnogyps californianus*, a globally threatened species, died from lead poisoning (Wiemeyer *et al.*, 1986, 1988) and the remaining free-ranging birds were taken into captivity in the 1980s mainly because of this threat (Locke & Friend, 1992). Less is known of the incidence of lead poisoning in free-ranging raptors in Europe (Pain & Amiard-Triquet, 1993; Pain *et al.*, 1993), although research has been stimulated over the last few years, as many countries have started to consider changes to legislation covering the use of lead shot (Fawcett & van Vessem, 1995). Lead poisoning in raptors results from the ingestion by predatory or scavenging species of shot or bullet fragments along with flesh or body parts of game species. Although raptors do not have the muscular gizzard of waterfowl, the pH in their stomach is very low (pH 1- 1.4) and lead is more soluble at lower pH values. Shot are frequently regurgitated in pellets by raptors, but more than 1 day can elapse between food ingestion and pellet regurgitation (up to 48 h in the marsh harrier; Bavoux *et al.*, 1990), and a proportion of the ingested lead will have been absorbed during this period. Hoffman *et al.* (1981) found that blood lead concentrations could become elevated within 24 h of shot ingestion by bald eagles. The one previous study of lead poisoning in marsh harriers (Pain *et al.*, 1993) found high blood lead concentrations in birds caught during the hunting season. Here, we present the results of a study on pulli, first-year and older birds in Charente-Maritime (France) throughout the year, i.e. within and outside the hunting season.

METHODS

Marsh harriers were caught at Charente-Maritime between 2 February 1994 and 4 February 1995 using clap-traps and by mist-netting at night roosts. Claptraps were baited with shot-free material, usually lung tissue. Marsh harriers are trapped, ringed and wingtagged annually at Charente-Maritime as part of a longterm population study (Bavoux *et al.,* 1988, 1990). Blood samples (c. 1 ml) were taken (under licence from the French Environment Ministry) via brachial venipuncture using 23-gauge needles into metal-free plastic tubes using EDTA (ethylene diamine tetraacetic acid) as an anticoagulant. Blood samples were agitated to prevent coagulation and frozen prior to analysis. Samples were taken from pulli at the nest. The birds were ringed, wing-tagged and weighed to the nearest 5 g. Bill length (tip of cere to tip of bill) was measured according to Spencer (1984). Birds were classified as < 1 or > 1 year old according to plumage characteristics (feather abrasion, uniformite of colour, moult, etc.), and sexed according to weight and bill length (Bavoux *et al.,* 1988). They were released 1-2h after capture during daylight, and released at dawn the next morning when captured in the evening. Local (or resident) birds were defined as those known from their rings to have been born or be breeding in Charente-Maritime (Bavoux et *al.,* 1992, 1994); wintering birds were birds of unknown origin captured between the beginning of November and the end of February; migratory birds were those of unknown origin captured during March, April, September and October.

Collection of regurgitated pellets

A total of 309 regurgitated pellets were collected from below night roosting areas during the winter 1993-94, spring 1994 and winter 1994-95. X-rays were taken of all pellets to check for the presence of regurgitated lead shot.

Blood lead analyses and interpretation of concentrations

Whole blood samples were analysed for lead using flame atomic absorption spectrometry (AAS) using the method of Delves (1970). Samples were run in duplicata. Internal quality control standards were used to check for consistency of results. For blood samples with PbB concentrations < 1 00 µg/dI, standard deviations ranged from 0 to 0.7 µg/dl (with a mean of 0.27). On the basis of a wide range of studies (Hoffman *et al.,* 1981; Pattee & Hennes, 1983; Janssen & Anderson, 1986; Wiemeyer *et al.,* 1988; Redig, 1984; Bloom *et al.,* 1989) investigating blood lead concentrations in both captive and wild raptors, Pain *et al.* (1993) chose the following ranges of blood lead concentration to indicate different levels of exposure in marsh harriers: PbB < 30, µg/dl for background exposure; 30-60 µg/dl as suggestive of elevated exposure to lead and sublethal effects of lead poisoning; 60-100, µg/dl to indicate clinical poisoning; and values > 1 00 µg/dl to indicate acute poisoning. We have used these ranges in this paper.

Statistical analyses

Analyses were carried out using a SYSTAT statistical package. Non-parametric statistics were used for PbB comparisons (Kruskal-Wallace) and geometric means have been presented to illustrate seasonal differences.

RESULTS

Table 1 shows the ranges of blood lead concentrations in all birds sampled. Overall, 36% of fledged birds and no pulli had elevated (> 30 µgldl) blood lead concentrations.

Sex and age differences

Blood samples were taken from a total of 34 pulli from 16 nests between 30 May and 22 June 1994. Samples were also taken from 25 first-year birds and 63 older birds between March 1994 and February 1995.

Table 1. Blood lead concentrations from marsh harriers trapped at Charente-Maritime

Group	Sample size	Blood lead concentration µg/dl				
		< 30	30-60	60-100	100-500	> 500
Pulli	34	34(100)[a]				0
1994-95						
First-year males	14	7	6	0	0	1
Adult males	25	21	1	0	2	1
First-year females	11	6	0	1	4	0
Adult females	38	22	8	2	5	1
Total 1994-95 (First-year/adult)	88	56 (64)	15 (17)	3 (3)	11(12-5)	3 (3-4)
First-year and adult birds 1990-91[b]	45	30 (67)	10 (22)	1 (2)	4 (9)[c]	

[a]Number of individuals (percentage in parentheses).
[b]Data from Pain *et al.* (1993).
[c]Four birds had > 1 00 µg/dl PbB (upper limit not specified).

First-year birds comprised 14 males and 11 females. Older birds comprised 25 males and 38 females. The sex of pulli was not- determined. A lower proportion of adult males had elevated blood lead levels (> 30 µg/dl) than was found in either adult females or juvenile males (respectively $X^2 = 5.96$ and $X^2 = 5.12$, $p < 0.05$, 1 d.f.). However, when a Kruskal-Wallace test was applied no significant differences were found between groups (p=0.199, 3 d.f.). Larger sample sizes would give a better indication of whether sex/age differences in susceptibility to elevated PbB concentrations are real. PbB concentrations in pulli were significantly lower than in other birds combined (Mann-Whitney, U= 37.5, $p<0.001$, 1 d.f.). The median concentration was 0.8 µg/dI, and only one bird had a PbB concentration in excess of 10 µg/dl (13.5). The lowest PbB concentration recorded in a first-year bird was 3.7 µg/dI. Pulli also had significantly lower PbB concentrations than other birds (n=12, median PbB=10.5µg/dl) caught during the same period (June 1994, Mann-Whitney, U=11-0, $p<0.001$, 1 d.f.). As values from siblings may not be independent, the above analyses were also run using one (median) blood lead concentration per nest. Results were similar with pulli having lower PbB than other birds, and than birds caught in June (Mann-Whitney, U=1298, $p<0.001$, *1* d.f.; U=6.0, $p<0.001$, 1 d.f., respectively).

Seasonal differences

In France, the hunting season opened on 14 July 1994 over coastal areas with public shooting rights. However, most marsh harriers feed over marshes and agricultural areas, where the hunting season opened in mid-September and closed on 28 February 1995. Figure 1 illustrates a substantiel increase in geometric mean PbB concentrations after September through to the end of February. Figure 2 details the ranges and distributions of blood lead concentrations found in birds during and outside the hunting season, along with those found in pulli. Significantly more birds (excluding pulli) had elevated PbB concentrations (>30 µg/dl) during the hunting season (mid September-end February) than outside the season (X^2 = 15-46, p < 0.001, 1 d.f.). Pulli had by far the lowest blood lead concentrations. Table 2 illustrates a more than tenfold increase in the number of regurgitated marsh harrier pellets containing shot during the two winters of the study period (during the hunting season) compared with the summer period (outside the hunting season).

DISCUSSION

Sex and age differences

Food items taken by marsh harriers in the CharenteMaritime marshes have been recorded by Bavoux *et al.* (1990). They recorded that 60% of prey items taken were mammals and 30% birds, 20% of all prey were rabbits *Oryctolagus cuniculus* or coypu *Myocastor coypus*, which were probably scavenged, and are frequently shot. Thirteen percent of the birds taken were waders, which are heavily hunted in Charente-Maritime, and again may have been carrying shot in their flesh. The presence of shot in a high proportion of regurgitated pellets (Table 2) confirms their presence in some food items.

As in the present study, Pain *et al.* (1993) found that adult female marsh harriers trapped in the Camargue in 1991/92 had higher PbB concentrations than males. This could reflect differences in feeding behaviour between the sexes. Like other sexually dimorphic birds

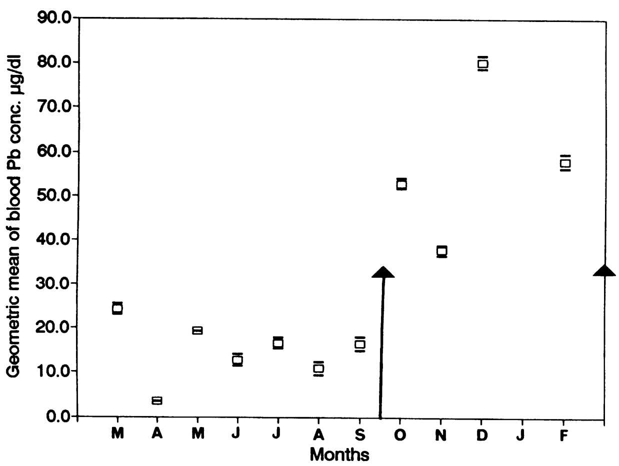

Fig. 1. Relationship between the timing of the hunting season and blood lead concentrations of marsh harriers (first-year and adult birds) trapped throughout the year. Arrows indicate the start and end of the hunting season. Symbols indicate geometric mean blood lead concentrations and standard errors. Only one sample was collected during each of April and May 1994.

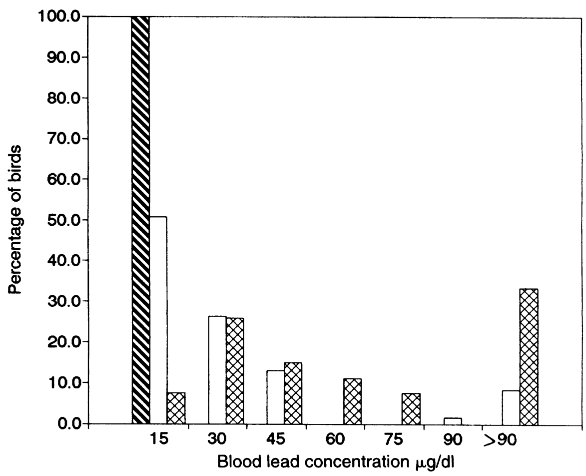

Fig. 2. Distribution of blood lead concentrations found in pulli and first-year/adult marsh harriers within and outside the hunting season. pulli (n = 34), , first-year and adult birds outside the hunting season (March to mid-September 1994, n = 52); indicate first-year and adult birds within the hunting season (mid-September 1994 to 28 February 1995, n = 36).

Table 2. Number and percentage of regurgitated marsh harrier pellets found to contain lead shot

Date of collection	Number of pellets	Number of shot				Total%
		1	2	3	> 3	
Winter 1993-94	72	14	1	2	1	25
May and June 1994	71	1	0	0	0	1.4
Winter 1994-95	116	16	8	2	0	15.6
Winter 1991-92[a]	200	20	3	0	0	11.5

[a]Data from Pain *et al.* (1993).
Winter = December-February.

of prey, female marsh harriers often take larger prey than males (Schipper, 1973; Opdam, 1975; Newton & Marquis, 1982; Underhill-Day, 1985). This may increase their susceptibility to shot ingestion, as larger game such as duck, rabbits or coypu are more likely to have shot (and larger quantifies of shot) in their flesh.

The apparent increased PbB levels in adult females over males may have some ecological significance. Increased female mortality could accelerate the rate of a population decline. Even if females do not suffer a higher rate of lead poisoning mortalite than males, elevated PbB concentrations may result in reduced reproductive success, or increased susceptibility to diseases due to the immuno-suppressive effects of lead (Elder, 1954; Edens *et al.,* 1976; Franson, 1986; Wobester, 1986). A similar proportion of juvenile male and female birds, and more juvenile than adult males, had elevated PbB levels. This could be explained if juvenile birds scavenge more than adult males. The fact that a higher proportion of juveniles than adults were caught in baited clap-traps relative to mist nets in Charente-Maritime in 1991/92 lends support to this idea (Bavoux *et al.,* 1990; Pain *et al.,* 1993).

Blood lead concentrations have not previously been measured in pulli. If the hypothesis that elevated blood lead concentrations result from the ingestion of shot in the flesh of prey is correct, we would predict very low PbB levels in the blood of pulli. Pulli are being fed well outside the hunting season, primarily with relatively small or young prey items that are less likely to have been scavenged and unlikely to contain any shot. In Charente-Maritime, pulli are fed mainly upon young mammals and small or young birds (Passeriformes), young gamebirds and waterbirds (Anatidae, Rallidae, Limnicolae), frogs, snakes, lizards, fish and invertebrates, but no obviously scavenged items were found amongst the prey (authors' own data). However, adults wili regularly scavenge in an opportunistic fashion to feed themselves during the breeding season (authors' observations).

The very low blood lead concentrations found in pulli support our hypothesis and indicate very low levels of exposure to lead. In addition, PbB concentrations in pulli were significantly lower than the already low (median 10.5 µg/dl) PbB levels in adult birds sampled over the same period (June 1994). This may be explained by the fact that PbB concentrations are in a fairly mobile equilibrium with concentrations in the liver and kidney, which are medium-term deposition sites for lead. Adults that have been exposed to lead, even at «background» levels, will have a certain amount already deposited in these tissues, thus influencing the blood lead equilibrium. This, and differences in the diets of pulli and adult birds during the breeding season, probably accounts for the small, but significant, differences in PbB concentrations.

Seasonal differences and source of elevated PbB concentrations

Figures 1 and 2 clearly illustrate the relationship between blood lead concentrations and the hunting season, with higher concentrations during than outside the season. The low PbB concentrations in pulli, the seasonal differences in PbB concentrations, the presence of shot in regurgitated pellets, and the more than tenfold increase in pellet shot incidence during than outside the hunting season, confirm that the source of elevated PbB is ingested shot.

The slightly higher geometric mean PbB concentration just after the 1994 hunting season (in March, Fig. 1) presumably reflects either a few birds that have ingested contaminated prey just after the season, or still elevated PbB concentrations in birds that had been exposed during the hunting season. Similarly, Fig. 2 shows that during the hunting season even birds with PbBs below the threshold of 30 1 µg/dl have a distribution skewed towards higher concentrations.

Following shot ingestion, blood lead concentrations may remain elevated for several weeks (and several months in the case of prolongea exposure to lead) (e.g. Pain & Rattner, 1988; Pain, 1996). Consequently, it could be argued that birds with elevated PbB concentrations in the autumn/winter, when both resident and migratory/wintering birds are present in Charente-Maritime, could have picked up shot elsewhere. However, whilst this may bc true for a small proportion of individuals, exposure to shot within the Charente-Maritime area must be considerable, as high proportions of regurgitated pellets contained shot during the winters of 1993/94 and 1994/95, and most pellets are regurgitated within a couple of days of food ingestion in the marsh harrier (Bavoux *et al.,* 1990). In addition, 40% of resident birds (four out of 10 birds known to have bred locally or seen during the previous summer) and 33% of wintering birds (five out of 15 birds observed or caught during the wintering period November-February) had PbB concentrations in excess of 60, µg/dl during the winter (November-February-migration usually takes place during September/October and March/April). This confirms a local source of contamination.

Significance of elevated blood lead concentrations

It is very difficult to relate a single measurement of blood lead concentration to a possible effect, as this is trelated to a range of factors. These include duration of exposure, previous history of exposure, i.e. tissue lead concentrations at the time the birds were sampled, and the overall health of the individuel. In addition, lead may have immunosuppressive effects, and interact with other disease agents (Franson, 1986-, Wobester, 1986). Consequently, a given blood lead concentration may be associated with mortalite in one situation, but not in another. Although it is not possible accurately to predict the effect at an individuel level, it is possible to give an indication of the effects of various different tissue concentrations, based upon tissue concentrations associated with lead poisoning lesions, clinical effects and death, as reported in the literature. In a recent review, Franson (1996) provided some guidelines with which to interpret tissue lead residues in birds other than waterfowl. The following were suggested for Falconiformes: birds with PbB concentrations of 20-150µg/dl may suffer physiological injury from which they would bc likely to recover if exposure to lead were terminated; birds with > 100, µg/dl may suffer severe physiological effects that could lead to death should lead exposure continue; PbB concentrations > 500 µg/dl are consistent with lead poisoning mortalite. Following these guidelines, three of the birds sampled (3-4%) had potentially lethal PbB concentrations, and a further Il (12-5%) had concentrations that could lead to death should exposure continue. Although shot frequently appear to be regurgitated by raptors, thus limiting the duration of exposure, birds that have absorbed lead are more susceptible to lead poisoning after subsequent shot ingestion; Pattee *et al.* (1981) reported mortalite in bald eagles resulting from the cumulative effect of ingesting, regurgitating and reingesting lead shot.

In the current study, the likelihood of repeated shot ingestion appears high, as 11-25% of the pellets sampled were found to contain at least one lead shot during the winters of 1991/92, 1993/94 and 1994/95. The potentiel impact of repeated shot ingestion is especially high for resident populations of marsh harriers at the study site. This is especially significant as the breeding population of marsh harriers at l'Île d'Oleron (Charente-Maritime) bas declined by more than 60% over the last decade. Although the cause(s) of this decline are currently unknown, the results of this paper suggest that lead poisoning may have been a contributing factor.

Elsewhere in Europe, the extent of lead poisoning in marsh harriers is unknown, but they are likely to be equally susceptible to shot ingestion in similar situations. The marsh harrier is not currently considered to have an unfavourable conservation status in Europe (Tucker & Heath, 1994). However, lead poisoning bas also been reported in golden eagle *Aquila chrysaetos,* which is a Species of Conservation Concern in Europe (e.g. Pain, 1992; Tucker & Heath, 1994).

It is not only scavenging raptors that are susceptible to shot ingestion. Predators taking heavily hunted game species, like pigeons or waterfowl, may also be exposed to shot in the flesh or prey that have been weakened, but not killed. For example, elevated tissue lead concentrations, suggesting lead poisoning as the likely cause of death, have been reported in goshawk *Accipiter gentilis,* and high concentrations have been reported in several other species (Pain & Amiard-Triquet, 1993). Other raptors in Europe that may be at risk from lead poisoning in wetlands include the globally threatened greater spotted eagle *Aquila clanga* and the white-tailed *eagle Haliaeetus albicilla,* a Species of Conservation Concern in Europe (Tucker & Heath, 1994). Both species hunt over wetlands and their diets include waterfowl. In the USA, several hundred bald eagles and several globally threatened Californian condors have died from lead poisoning, and a range of other raptor species are known to be affected (Franson, 1996, Wiemeyer *et al.,* 1986, 1988). The relatively scant records of lead poisoning in free-ranging raptors in Europe are likely to result from lack of research into this problem.

Conservation measures

The problem of lead poisoning is not new, and action, in the form of voluntary or statewide bans on the use of lead shot for waterfowl hunting, was taken in the USA over a decade ago (USFWS 1985, 1986; Morehouse, 1992). This legislation was aimed at the protection of waterfowl and the bald eagle, and large scale die-offs of waterfowl were instrumental in increasing awareness of the problem. Although other countries have acted more slowly, there bas been a considerable increase in awareness and response to this problem over the last five years (Table 3).

Many conventions and directives (e.g. the RAMSAR Convention on Wetlands of International Importance; the 79/409/EEC Directive on the Conservation of Wild Birds) require, in general terms, that natural resources be used in a sustainable or judicious way (a concept commonly called «Wise Use»), and, assuming that acceptable alternatives to lead shot are available, the use of lead and consequent poisoning of waterfowl and other birds cannot be considered as Wise Use of this (i.e. the avian) resource (Lecocq, 1992).

Within the remit of the Bonn Convention (Convention on the Conservation of Migratory Species of Wild Animals), a specific Agreement on the Conservation of African-Eurasian Migratory Waterbirds bas been developed for the Western Palearctic region and Africa (agreement concluded in June 1995 and final act to adopt the Agreement signed by 54 States). Within the Action Plan included in the agreement is the following statement: «4.4-1. Parties shall endeavour to phase out the use of lead shot for hunting in wetlands by the year 2000». Once the Agreement comes into force, Range States that sign it are committed to implementing this action. Such commitments are important statements of intent. Whilst aimed at waterfowl conservation, the implementation of this agreement would considerably reduce the exposure of marsh harriers and other birds to

Table 3. Countries that have taken action to limit the use of lead shot for hunting waterfowl (or over wetlands). Information from Fawcett and van Vessem (1995)

Countries that have implemented or announced legislative bans on the use of lead shot for hunting waterbirds (or for hunting over wetlands)	Canada: announced for 1997, all migratory birds. Denmark; 1996, all hunting except for forests. Finland: 1996, waterfowl. The Netherlands: 1993, all hunting. Norway: 1991, waterfowl. USA: 1991, waterfowl.
Countries that have implemented or announced bans on the use of lead shot in certain parts of their territory	Australia: South Australia, 1994, Northern Territory (planned). Mexico: Yucatan. Sweden: Ramsar sites, 1994. Switzerland: Thurgau.
Countries where voluntary bans on the use of lead shot for hunting waterfowl (or for hunting over wetlands) are being strongly encouraged, implemented or have been announced	Germany: 1993. Japan. United Kingdom: 1995.

lead shot within wetlands. The risk of lead poisoning posed to raptors, waterfowl and other species is unnecessary. Alternatives to lead have been used for many years in some countries (e.g. those with legislative bans in place-Table 3), and considerable new research and/ or development of non-toxic shot alternatives was reported in a recent international update document (Fawcett & van Vessem, 1995).

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