LEAD POISONING IN MUTE SWANS (*CYGNUS OLOR*) IN IRELAND: RECENT CHANGES

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ABSTRACT

Studies over two decades have shown a marked trend towards a decrease in blood lead levels in the mute swan (*Cygnus olor*) in parts of Ireland. However, this study shows that a small percentage of some populations of mute swans in Co. Cork still have significantly elevated lead levels, causing some potentially sublethal effects to the health status of the swans. Overall, at both urban and rural sites the median blood lead levels in adults and cygnets of both sexes were below a threshold level of 1.21μmol l^{-1}, which is indicative of elevated lead. However, 27% of the population at Cork Lough had elevated lead levels, with the highest blood lead level being 27.66μmol l^{-1}, indicating that acute poisoning is still a problem in some individuals. Mute swans from rural sites generally showed no variation, and median blood lead levels were generally low. Lead levels reached a peak in October 2004 and again during the spring months of 2005. Swans may be more susceptible to lead poisoning during spring, as it follows the winter months when natural food is scarce and lead is easily absorbed across the gut after ingestion. Autumn peaks may be explained by an increase in fishing activity over the summer months. A positive association was found between elevated lead levels and packed cell volume—an indicator of health status. Overall, a decline in acute lead poisoning at urban and rural sites is apparent from this study. However, due to its continued and persistent use in the environment, lead still poses a threat at a sublethal level.

INTRODUCTION

Although blood lead levels in mute swans (*Cygnus olor*) have dropped significantly in both urban and rural sites in Ireland since the 1980s (O'Halloran *et al.* 2002), elevated lead levels still occur in some areas due to the continued use and persistence of lead in the environment; for example, at Cork Lough and the Wexford Slobs (O'Connell *et al.* 2008). Recent data have revealed that some swans still have high blood lead levels that are causing at least some sublethal effects (O'Halloran *et al.* 2002). A survey of blood lead levels in mute swans in the UK, undertaken by Perrins *et al.* (2003), revealed a sharp reduction in the number of swans dying or being chronically lead poisoned in most areas, but elevated lead levels were found in some parts of the national population. Blood lead levels ranged from 0.01 to 114μmol l^{-1} despite a ban on lead weights ranging in size between 0.06 and 28.36g since 1987. Another study, by Kelly and Kelly (2004), found that mortality due to lead is still occurring in the UK. Mateo *et al.* (2007) have also reported elevated lead levels in waterbirds in the internationally important wetland areas of Andalusia in southern Spain; species such as the greater flamingo (*Phoenicopterus ruber*) and glossy ibis (*Plegadis falcinellus*) are at risk despite a ban on the use of lead shot in this area since 2002.

Little work has been undertaken on the sublethal effects of lead, although Franson (1984) has demonstrated immunosuppressive effects, while others, such as Burger (1995), have detected some changes in reproductive performance. The latter is particularly the case at high doses, causing reduced shell thickness (Dauwe *et al.* 2004), lowered clutch sizes (Eeva and Lehikoinen 1995), an increased egg-laying interval (Burger and Gochfeld 1993) and decreased embryo weights. All these factors can lower reproductive success and thus could potentially affect recruitment into the next generation. Other effects of lead on health indices include reduced haemoglobin (Hb) and haematocrit levels in mute swans (O'Halloran *et al.* 1988c) and also in nestling pied and collared flycatchers (*Ficedula hypoleuca* and *Ficedula albicollis*) (Nyholm 1998). O'Halloran *et al.* (1989) described an additional cause of mortality in swans with medium to high lead levels: collisions with overhead cables. It is believed that sublethal doses affect flight coordination in swans. Snoeijs *et al.* (2005) also described the effects of increased lead deposition in the bones of zebra finches (*Taeniopygia guttata*) as a result of calcium deficiency in the diet.

Work undertaken in the US relating to lead gunshot suggests that wildfowl are more likely than other birds to find more recently used shot;

presumably, shot slowly sinks into the ground, and thus recent shot is more easily found by foraging birds (Birkhead and Perrins 1986). This may account for some of the seasonality of lead poisoning reported in mute swans, especially during summer months when fishing and angling activities are most prevalent (O'Connell 2007). In contrast to studies on the River Thames by Birkhead (1983), Sears (1988) and O'Halloran *et al.* (1991), peaks in blood lead levels were found during the winter season. A number of factors may be responsible for this, including the number of birds present in waterways being greater during the winter months. Poor natural-food availability at this time of year may also force swans to forage more intensely, thereby increasing the chances of ingesting lead. Also, due to poor diet, many swans at Cork Lough feed almost exclusively on bread provided by the public, and this can increase susceptibility and toxicity to lead due to greater absorbance of lead in these swans compared to swans on a natural diet (O'Halloran *et al.* 1991). This can be due to a lack of nutrients such as calcium, iron, zinc and vitamin D (Levander 1979; Pattee *et al.* 2006). In birds, blood parameters seem to be the most direct indicators of physiological condition, including health status (Nadolski *et al.* 2006), and in this study Hb and packed cell volume (PCV) are examined.

This study aims to determine recent patterns in blood lead levels in mute swans in Co. Cork. The susceptibility of individuals to background lead levels is assessed, and the sublethal effects of chronic exposure over the years are explored. Finally, trends of blood lead levels over twenty years in some parts of Ireland are examined; in particular, findings from Cork Lough are compared with past winter data collected by O'Halloran *et al.* (2002). This study also sets out to test the hypothesis that swans with elevated lead levels have a reduced health status and may be at a higher risk of infection.

MATERIALS AND METHODS

STUDY AREA

Blood samples were collected from mute swans at four different locations in Cork city and county. The urban site was Cork Lough in the western suburbs of Cork city (51°53′ N/8°29′ W). Cork Lough, a freshwater lake (6.07ha), is a refuge to a number of wildfowl, including approximately 60 Canada geese (*Branta canadensis*) and 120 mute swans. The maximum depth is 1.5m, in the northern basin. The area is an internationally renowned coarse-angling site, where lead weights and sinkers continue to be used (O'Halloran *et al.* 2002). The rural sites sampled in Co. Cork were Lough Aderry in east Cork (51°54′ N/8°05′ W) and Ring (51°36′ N/8°50′ W) and Rosscarbery (51°34′ N/9°02′ W) in west Cork. Lough Aderry is a freshwater lake with approximately 60 mute swans, and Ring and Rosscarbery are coastal marine sites where the numbers of mute swans vary across the seasons. The historical data for Cork Lough were taken from O'Halloran *et al.* (2002) and compared with data from this study.

SAMPLE COLLECTION

A sample of approximately 2ml of blood was taken from the brachial veins of swans using a 23-gauge hypodermic needle. The sample was placed into a 5-ml lithium heparin blood tube, which was laid on ice and transported to the laboratory for haematological analysis. A portion of the sample was frozen (−18°C) for lead analysis. All swans were weighed (kg), aged and sexed by cloacal examination, as described by Baker (1993). Cloacal swabs of 104 mute swans were randomly taken during sampling and placed in viral transport medium for analysis.

BLOOD AND TISSUE ANALYSIS

Haematological analyses were carried out on fresh samples following standard methodology whereby all samples must be analysed within twelve hours to avoid lysis. Hb concentration was determined using cyanmethaemoglobin solutions as standards and was measured using a spectrophotometer at a wavelength of 540nm. PCV was estimated by spinning three replicates of each sample in a Hawksley microcentrifuge at 10,000rpm for 5min. The PCV value was then calculated using a microhaematocrit reader, and the mean value of the three replicates for each sample was obtained (O'Halloran *et al.* 1988a). Blood lead levels were determined using a Varian SpectrAA-300 Zeeman atomic absorption spectrophotometer, with a flameless atomiser attachment, at a wavelength of 217nm. Whole blood samples were initially digested in 10% nitric acid (Aristar grade) and spun in a centrifuge for 10min at 2,500rpm. The clear supernatant on top of the sample was removed for analysis. Samples were prepared using a standard addition method with routine inter-calibration and quality control (for details see O'Halloran *et al.* 1988a).

Swans with blood lead levels exceeding 1.21μmol l^{-1} were considered to have elevated lead levels (Perrins *et al.* 2003) that are above background levels and indicative of lead ingestion (O'Halloran *et al.* 1988b). Blood lead has recently been documented in μmol l^{-1} by Perrins *et al.* (2003), with levels of 1.21μmol l^{-1} and above indicating elevated lead levels. This is a lower level than that used by previous workers when threshold levels of blood lead were >40μg per 100ml

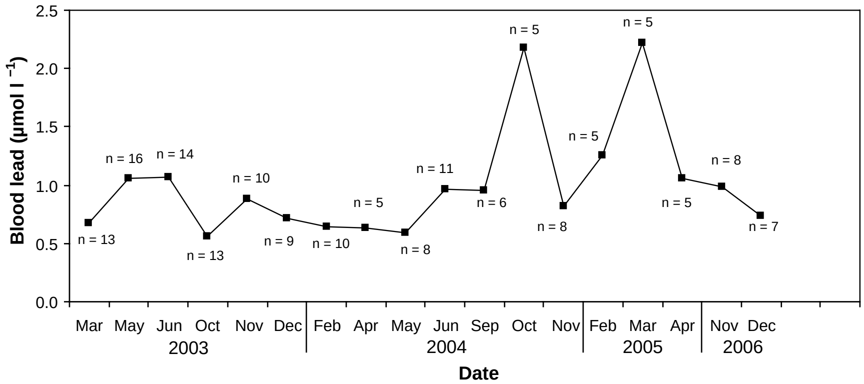

Fig. 1—Median blood lead levels in mute swans over all months (2003–6) at Cork Lough.

(1.93µmol l^{-1}) (O'Connell 2007; O'Connell *et al.* 2008). A simple conversion to enable comparison with earlier studies is to multiply the µmol l^{-1} unit by 20.7, giving the equivalent value in the µg per 100ml unit. Acute lead poisoning occurs when a bird ingests a significant amount of lead (two to three lead pellets) over a short period of time, and the symptoms are almost immediate.

The cloacal swabs were screened for a range of infections, including duck virus enteritis (duck plague), *Riemerella anatipestifer* infection (New duck disease) and diseases caused by orthomyxovirus (avian influenza virus), paramyxovirus (Newcastle disease) and *Aspergillus*. All viral isolation was performed according to the protocol described in Council Directive 92/40/EEC (Council of the European Union 1992). For a full description of the methods please refer to Raleigh *et al.* (2008).

DATA ANALYSIS

All statistical calculations were carried out using Minitab statistical package (Pennsylvania University) and SPSS version 11.0. As the data were not normally distributed, non-parametric Mann–Whitney U and Kruskal–Wallis tests were used throughout. Data were grouped into a 'winter' category when comparing with past data from O'Halloran *et al.* (2002), which were collected only in the winter months.

RESULTS

BLOOD LEAD

Blood from 191 mute swans in both urban and rural sites throughout Co. Cork was collected and analysed between February 2003 and December

Table 1—Median blood lead levels in mute swans at Cork Lough and rural sites in Co. Cork during 2003–6.

	All months				*Winter (Oct–Jan)*			
	N	*Median lead (µmol l^{-1})*	*Range*	*Interquartile*	*N*	*Median lead (µmol l^{-1})*	*Range*	*Interquartile*
Cork Lough								
Males	82	0.88	0.27–7.39	0.58	26	0.85	0.34–7.39	0.40
Females	44	1.02	0.16–27.66	1.52	20	0.90	0.23–27.66	1.96
Cygnets	30	0.80	0.04–1.59	0.59	14	0.60	0.45–1.57	0.35
Total	**156**	**0.88**	**0.04–27.66**	**0.66**	**60**	**0.79**	**0.23–27.66**	**0.57**
Rural sites								
Males	19	0.56	0.06–1.21	0.43	16	0.55	0.21–1.21	0.42
Females	8	0.85	0.51–1.36	0.59	5	0.76	0.51–1.07	0.45
Cygnets	8	0.79	0.24–1.69	0.87	6	0.72	0.24–1.37	0.73
Total	**35**	**0.65**	**0.06–1.69**	**0.53**	**27**	**0.64**	**0.21–1.37**	**0.42**

2006; 35 individuals were sampled more than once, mostly at intervals of less than 30 days. There was significant variation (Kruskal–Wallis, $H = 9.16$, $P < 0.05$, d.f. $= 3$) in median blood lead levels (μmol l^{-1}) of swans sampled over 2003 (n = 75), 2004 (n = 52), 2005 (n = 14) and 2006 (n = 15) at Cork Lough. Swans sampled in 2005 had the highest median lead level of 1.4μmol l^{-1}. There was, however, no significant variation (Kruskal–Wallis, $H = 1.86$, $P > 0.05$, d.f. $= 1$) in median blood lead levels of swans sampled over 2003 (n = 21) and 2004 (n = 14) at rural sites in Co. Cork. Median blood lead levels for males, females and cygnets are summarised across urban and rural sites and over the winter months and all months (Table 1). There was no significant difference between blood lead levels in males and females or in adults and cygnets (Mann–Whitney $U = 1579$, $N_1 = 82$, $N_2 = 44$, $P > 0.05$; $U = 1582$, $N_1 = 126$, $N_2 = 30$, $P > 0.05$, respectively) at Cork Lough. At rural sites males and females varied significantly in blood lead levels (Mann–Whitney $U = 34$, $N_1 = 19$, $N_2 = 8$, $P < 0.05$), with females having higher levels, while adults and cygnets did not vary in this respect (Mann–Whitney $U = 89$, $N_1 = 27$, $N_2 = 8$, $P > 0.05$).

There was significant variation across the seasons (Kruskal–Wallis, $H = 8.29$, $P < 0.05$, d.f. $= 9$). This was particularly evident during spring 2005, when the median blood lead level was 1.43μmol l^{-1}. Trends in blood lead levels over all months from 2003 to 2006 show peaks in late 2004 and spring 2005, with levels apparently dropping after this period (Fig. 1).

GENERAL HEALTH STATUS

General health parameters (weight, PCV, Hb and blood lead levels) were examined across all sites in a proportion (81%) of the swans sampled, and the results are summarised in Table 2. There was significant variation between urban and rural sites in blood lead levels and Hb values (Mann–Whitney $U = 1088$, $N_1 = 127$, $N_2 = 28$, $P < 0.05$; $U = 1059$, $N_1 = 127$, $N_2 = 28$, $P < 0.05$, respectively), but no significant association was found between PCV levels in swans from urban and rural sites (Mann–Whitney $U = 1284$, $N_1 = 127$, $N_2 = 28$, $P > 0.05$). Lead was also correlated against the health parameters, revealing that there was no relationship between lead and Hb or weight ($r_s = 0.09$, $p > 0.05$; $r_s = -0.14$, $p > 0.05$, respectively).

There was no significant difference in weight, PCV, Hb and blood lead levels between females in urban and rural sites (Kruskal–Wallis, $H = 0.41$, $P > 0.05$, d.f. $= 1$; $H = 0.35$, $P > 0.05$, d.f. $= 1$; $H = 0.07$, $P > 0.05$, d.f. $= 1$; $H = 0.93$, $P > 0.05$, d.f. $= 1$, respectively). However, significant differences were found in PCV, Hb and blood lead levels between males in urban and rural sites (Kruskal–Wallis, $H = 7.04$, $P < 0.05$, d.f. $= 1$; $H = 12.47$, $P = 0$, d.f. $= 1$; $H = 10.47$, $P < 0.05$, d.f. $= 1$, respectively). Blood lead and Hb levels were particularly variable between urban and rural males, both values being higher in rural males.

All 104 cloacal swab samples, which were analysed for infections, were negative.

TRENDS IN LEAD OVER TIME (1983–2006)

There was significant variation (Kruskal–Wallis, $H = 228.38$, $P = 0$, d.f. $= 8$) and a significant decline ($r = -0.83$, $P < 0.01$) in median winter blood lead levels of swans sampled during the winter at Cork Lough between 1983/4 and 2006 (Fig. 2). The median blood lead level declined from a peak of 2.25μmol l^{-1} during 1985/6 to 0.93μmol l^{-1} in 2006. There was also a significant decline ($r = -0.84$, $P < 0.01$) in the proportion of swans suffering from elevated lead levels (Fig. 3). This percentage peaked at 95% during the winter of 1984/5, fell dramatically to just 7% during the winter of 1994/5, increased to 31% in 2005 and dropped slightly to 27% in 2006.

DISCUSSION

Blood lead is a widely used and very useful tool for assessing the lead status of birds (Anders *et al.* 1982). Birkhead (1983), Sears (1988), O'Halloran *et al.* (1988b) and Perrins *et al.* (2003) have all documented lead poisoning in relation to blood lead. Despite an overall decline in blood lead levels in Co. Cork and in parts of the UK (O'Halloran *et al.* 2002; Perrins *et al.* 2003), elevated lead levels are still occurring in some swan populations. In this study blood lead levels in the mute swan were investigated between February 2003 and December 2006 at various sites in Co. Cork. Overall, median blood lead levels at both urban and rural sites, across both sexes and in adults and cygnets were below the threshold level of 1.21μmol l^{-1} (Table 1). However, some individuals were found to have elevated levels; one female swan at Cork Lough had an acute blood lead level of 27.66μmol l^{-1}.

No significant difference was found in median blood lead levels between males and females or between adults and cygnets in the urban site. However, in rural sites females were found to have higher blood lead levels compared to males, despite a low sample size of females (N = 8). Birkhead (1983) found similar results and related this difference to the mobilisation of calcium from the bones for egg formation in breeding females. Lead follows a similar metabolic path to calcium, therefore, during egg laying lead may become mobilised from the bones, thereby contributing to

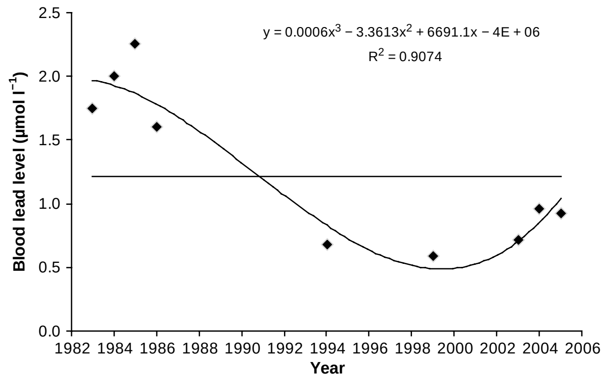

Fig. 2—Median winter blood lead levels (µmol l^{-1}) in mute swans over time (1983–2006) at Cork Lough. The 1.21µmol l^{-1} blood lead threshold (indicative of elevated lead; Perrins *et al*. 2001) is indicated by the horizontal line.

elevated blood lead levels in females. This might be the case in this study since some females were sampled at rural sites during the breeding season. These lead levels in females may reflect chronic exposure. Most of the birds at Cork Lough consist of non-breeders, so the seasonality of lead levels in females at this site is unlikely to be due to lead mobilisation, in comparison to breeding females at rural sites.

There was some variation in median blood lead levels at Cork Lough (2003–6), with swans sampled in 2005 having the highest median level over the four-year study. However, at rural sites no significant variation was found, with median blood lead levels generally quite low and constant. These results were not unexpected and are similar to levels found by O'Halloran *et al.* (2002).

Seasonally, there was some disparity, particularly during the spring of 2005 when median blood lead levels were elevated to 1.43µmol l^{-1}. O'Halloran *et al.* (1988a) also found a peak during the winter/spring months at Cork Lough. This is in contrast to findings by Birkhead (1983) on the River Thames, where the highest levels occurred during July, September and November. However, angling activity at Cork Lough starts to increase in

Table 2—Mean (± SE) weight, blood lead level, PCV and Hb values for mute swans sampled at Cork Lough and rural sites in Co. Cork during 2003–6.

	N	*Weight (kg)*	*Lead (µmol l^{-1})*	*PCV*	*Hb (g dl^{-1})*
All Sites					
Males	82	12.00 ± 0.14	1.07 ± 0.12	41.26 ± 0.58	14.28 ± 0.25
Females	40	9.43 ± 0.18	2.34 ± 0.72	40.17 ± 0.65	13.53 ± 0.38
Cygnets	33	9.42 ± 0.29	0.80 ± 0.07	39.48 ± 0.87	13.77 ± 0.36
Total	**155**	**10.78 ± 0.15**	**1.34 ± 0.20**	**40.60 ± 0.40**	**13.98 ± 0.18**
Cork Lough					
Males	65	11.94 ± 0.17	1.20 ± 0.15	42.22 ± 0.50	14.72 ± 0.26
Females	34	9.44 ± 0.21	2.60 ± 0.84	40.35 ± 0.71	13.57 ± 0.42
Cygnets	28	9.46 ± 0.34	0.81 ± 0.07	39.55 ± 0.98	14.04 ± 0.39
Total	**127**	**10.73 ± 0.17**	**1.49 ± 0.24**	**41.13 ± 0.40**	**14.26 ± 0.20**
Rural Sites					
Males	17	12.19 ± 0.27	0.58 ± 0.06	37.57 ± 1.83	12.63 ± 0.52
Females	6	9.36 ± 0.31	0.87 ± 0.13	39.17 ± 1.60	13.31 ± 0.89
Cygnets	5	9.20 ± 0.54	0.72 ± 0.21	39.13 ± 1.82	12.28 ± 0.76
Total	**28**	**11.05 ± 0.33**	**0.67 ± 0.62**	**38.19 ± 1.19**	**12.72 ± 0.39**

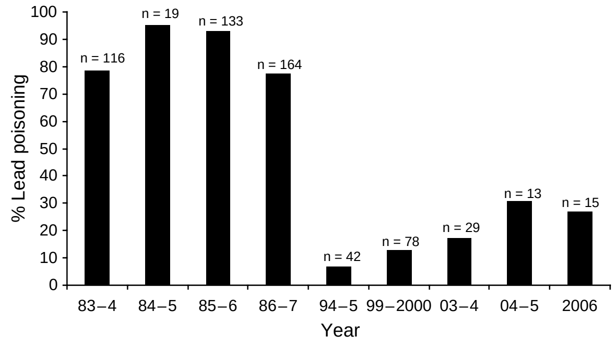

Fig. 3—Percentage of elevated lead levels (> 1.21µmol l^{-1}) in mute swans during winters (1984–2006) at Cork Lough.

the spring, and while many lead alternatives for fishing are now available, most fishermen still use lead weights (pers. obs.). Birds may also be particularly susceptible to poisoning following food scarcity during the winter months, forcing them to forage deeper for natural sources of food, thereby increasing the possibility of ingesting lead weights discarded on the lough bed. This is despite the fact that several hundred tonnes of grit has been added to Cork Lough since 1997—50 tonnes each year from 1997 to 2001 and an additional 150 tonnes from 2001 to 2005 (L. Casey, Cork City Council, Environment Department, pers. comm.). Perrins *et al.* (2003) also found instances of high lead on the Thames and based this on the fact that swans were finding 'long-lost leads' in their search for grit. Lead is highly persistent in the environment, potentially lasting 100–300 years in the sediment (Kendall *et al.* 1996), so it is quite possible that the swans at Cork Lough were picking up 'old' lost lead with the new deposits of grit, depending on the time of year and water level.

In this study long-term trends in blood lead levels from 1983 to 2006 were found, and, as expected, there was significant variation in winter blood lead levels over the years. During the winter of 1984/5 blood lead levels peaked, with 95% of the Cork Lough population above the threshold level of 1.21µmol l^{-1} (Fig. 3). Sears (1988) also documented a high percentage of lead poisoning in the Thames region around this time (1982–5), with 62.5% of the population in the Windsor area and 93.5% of the population in Richmond having elevated levels. These percentages would be even higher if the data were recalculated using the now accepted critical level of 1.21µmol l^{-1} considered indicative of elevated lead levels. Background levels around this time were particularly high, and seasonal fishing would have resulted in a large accumulation of lead weights being accessible to swans. This was also the case at Cork Lough (O'Halloran *et al.* 1987), with reports of considerable amounts of discarded anglers' weights. In addition, spent gunshot from shooting during the early part of the century added to the lead load. Blood sampling of the swans remained regular through to the winter of 1986/7, with 77% of the population still poisoned (Fig. 3). However, a break in sampling until the winter of 1994/5 revealed a dramatic decrease, with only 7% of the population having elevated levels. This is most likely explained by the provision of grit supplements at Cork Lough (Fig. 3).

Although lead levels generally remain low and have significantly reduced over the years in Ireland, the sublethal effects of low lead levels remain largely under-researched (O'Halloran *et al.* 1989). Little information is available on the effects of swans' sublethal exposure to lead. The effect of lead on the immune system has been well documented in some avian species (Trust *et al.* 1990; Lee *et al.* 2001; Fairbrother *et al.* 2004), such as mallards (*Anas platyrhynchos*) and chickens (*Gallus gallus*), but not in any swan species. Other factors, including weather conditions, nutrition (calcium deficiency) and disease, may influence the toxicity of this metal. Scheuhammer (1996) demonstrated a 400% increase in the accumulation of lead and increased inhibition of δ-aminolevulinic acid dehydratase in zebra finches (*T. guttata*) when fed a diet low (0.3%) in calcium compared to a group fed a diet containing 3% calcium. The immunosuppressive effects of lead may lead to increased incidences of viral and bacterial infections; for example, in 2001 duck virus enteritis killed over 40 swans at Cork Lough (unpublished data).

Other haematological parameters, including PCV and Hb levels, are also used as indicators of health status in birds (Hauptmanova *et al.* 2006), and some of these were examined in this study. O'Halloran *et al.* (1988c) developed reference values for these parameters from similar sites to those in this study. Generally, Hb and PCV levels found in this study were within the healthy reference values, except for rural males, which appeared to have lower Hb and PCV levels compared to O'Halloran's study and also to males in urban sites in this study. The reason for this is not clear as it might be expected that these populations would have a better health status due to abundant natural-food supplies and less exposure to lead. Elevated blood lead levels, particularly in urban females, did not appear to affect haematological parameters. These swans have only slightly elevated lead levels in comparison to the acutely poisoned swans sampled by O'Halloran *et al.* (1988c), which displayed very low Hb levels and weight (male: 10.20g dl^{-1} Hb, 6.8kg) (female: 6.0g dl^{-1} Hb, 4.6kg). Other factors that may influence blood parameters include time of year in relation to breeding and moulting, both of which lower PCV and Hb levels due to physiological demands (Kasprzak *et al.* 2006).

This study demonstrates that, due to its continued and persistent use in the environment, lead still poses a threat to waterfowl and that elevated levels are still occurring in some mute swan populations. The use of lead shot and lead fishing weights in Ireland needs to be addressed in order to bring legislation in line with other EU countries such as the UK, Denmark, Norway, Sweden, Finland and the Netherlands. The EU has called for the phasing out of the use of lead shot over wetlands as soon as possible, ultimately by 2009. However, within the Republic of Ireland there are no indications of any proposed restrictions as yet.

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