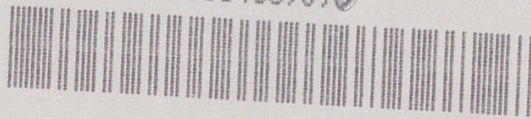


Lead Toxicity in *Mute Swans*
Cygnus olor (Gmelin)

John O'Halloran

0004067570



2358.

DP1987 OHAL
Lead toxicity in m



DP 1987 OHAL
406757

LEAD TOXICITY IN MUTE SWANS

Cygnus olor (Gmelin).

By

JOHN O'HALLORAN

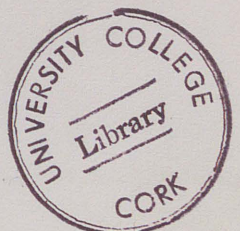
A thesis submitted to the National University of Ireland
in candidature for the degree of Doctor of Philosophy

September 1987

The study was conducted at the
Department of Zoology, University College, Cork.

Head of Department: Professor M.F. Mulcahy

Supervisor: Professor A.A. Myers



This thesis is dedicated to
my mother and late father
without whom it would never
have been possible.

'The tame swan is frequently met with near gentlemen's seats on
their ponds and reservoirs' Smith (1749) History of Cork.

CONTENTS

	Page No.
ABSTRACT	1
INTRODUCTION	2
CHAPTER ONE	
DETERMINATION OF HAEMOGLOBIN IN BIRDS BY A MODIFIED ALKALINE HAEMATIN (D-575) METHOD	7
CHAPTER TWO	
BLOOD LEAD LEVELS AND FREE RED BLOOD CELL PROTOPORPHYRIN AS A MEASURE OF LEAD EXPOSURE IN MUTE SWANS <u>Cygnus olor</u>	12
CHAPTER THREE	
LEAD POISONING IN SWANS AND SOURCES OF CONTAMINATION IN IRELAND	43
CHAPTER FOUR	
SOME BIOCHEMICAL AND HAEMATOLOGICAL REFERENCE VALUES IN BLOOD CHEMISTRY IN MUTE SWANS <u>Cygnus</u> <u>olor</u> WITH ACUTE LEAD POISONING	85
CHAPTER FIVE	
TISSUE LEAD LEVELS AND HAEMATOLOGICAL CHANGES IN MUTE SWANS <u>Cygnus olor</u> WITH ELEVATED BLOOD LEAD	112
ACKNOWLEDGEMENTS	131
APPENDICES.....	133
APPENDIX 1.....	134
APPENDIX 2.....	149

ABSTRACT

Lead toxicity in Mute swans Cygnus olor (Gmelin) was investigated. Two methods for the assessment of lead exposure were used: (1) blood lead level and (2) free red blood cell protoporphyrin. An accurate estimation of haemoglobin was found to be a prerequisite to determining lead exposure. A measurement of haemoglobin based on converting all haem species to alkaline haematin was found to give accurate and reproducible results. Variation in blood lead during the diel cycle in caged birds was investigated. Blood lead levels in a flock of Mute swans at a coarse-fish angling site were examined over a two year period. Forty-two percent of blood samples ($n = 870$) from this site were shown to have elevated lead. X-ray examination of swans revealed the source of contamination to be ingested lead pellets. Post mortem examination showed that 68% ($n = 101$) of all Mute swans examined died from lead poisoning. Two sources of lead were identified: spent gunshot and lost or discarded anglers' weights.

Biochemical and haematological aspects of swan blood were also investigated. Reference haematological and biochemical values were established from 'normal' healthy Mute swans. These reference values were used as a baseline against which changes in lead poisoned birds could be measured. Moulting and immaturity were identified as causing natural variation, while acute lead poisoning was found to increase protoporphyrin, cholesterol and two serum enzymes: lactate dehydrogenase and aspartate amino transferase. Hypochromic anaemia was noted in swans suffering from acute lead poisoning. The possible role of lead in causing other sub-lethal effects, for example collisions, is also discussed.

INTRODUCTION

Lead is an ubiquitous element in the environment and background levels are often exceeded because of the use of organo-lead additives to petrol. In the urban environment, lead levels may lead to chronic lead poisoning. Ohi, et al., (1974) and Hutton and Goodman (1980), for example, have reported chronic lead poisoning in urban pigeons Columba livia (L). Acute lead poisoning due to the ingestion and retention of shotgun pellets is now recognised as a cause of mortality, particularly in waterfowl. Large 'die-offs' due to acute lead poisoning have been recorded in the U.S. and Europe. Anders et al., (1982), carried out experimental studies to examine the dynamic behaviour of lead in birds and extrapolated the results to the field situation. Such extrapolations have been questioned due to differences in the levels and duration of exposure (Hutton, 1980).

Acute lead poisoning has been reported in swans feeding on vegetation contaminated by lead from mining (see Benson, et al., 1976), but the most important source of lead contamination in Mute swans Cygnus olor (Gmelin) is the ingestion of discarded anglers' lead weights. Simpson et al., (1979), were the first to identify discarded anglers' lead weights as a cause of mortality in Mute swans in the United Kingdom. The most recent data suggests that between 3,370-4,190 Mute swans die from lead poisoning each year in the U.K. (see Thomas et al., 1987). O'Halloran and Duggan (1984) identified ingested anglers' lead weights as a cause of elevated blood lead and mortality of Mute swans in Ireland. This study raised a number of important questions:

- what are the best methods for the assessment of lead exposure?
- what are the sources of lead contamination in swans in Ireland?
- what are the normal haematological and biochemical values for Mute swan?
- does lead cause any identifiable sub-lethal effects?

The present study set out to attempt to answer these questions.

There are two main methods for the assessment of lead exposure; (1) blood lead estimation and (2) measurement of free red blood cell protoporphyrin. Birkhead (1983) was one of the few workers to employ in 'the field' assessment. The need to correct blood lead levels and protoporphyrin IX for haemoglobin has been recognised for decades, (see Chisolm, 1973), but many workers have ignored it's importance in birds probably due to the inaccuracy of techniques for estimating haemoglobin. Problems in estimating haemoglobin in birds have arisen from the fact that most workers have directly applied mammalian techniques. Chapter one adopts a recently developed technique for estimating haemoglobin in humans (Zander et al., 1984) which, unlike previous methods, also gives accurate and reproducible results in birds. Chapter two outlines variations in blood lead levels during the diel cycle in captive birds and over a two year period from uniquely ringed swans in the field. The use of protoporphyrin IX in assessing the level of lead exposure is also discussed.

Chapter three examines the role of angling and shooting in causing elevated lead in swans. The contribution of general environmental lead is also discussed.

Lead is known to be a potent toxicant of the haemopoietic system,

inhibiting the synthesis of haemoglobin and causing raised protoporphyrin IX values (Lee, 1981). However, in common with other studies on toxic metals, understanding the effects of lead depends on a thorough knowledge of the swans' normal physiology and biochemistry. Some workers, for example Simpson et al., (1979), have reported haemoglobin values for Mute swans and Janssen et al., (1986) have examined blood chemistry in California condors [Gymnogyps californicus (Shaw)] suffering from lead poisoning. In general, however, few reference values for blood parameters in birds are available. Such knowledge of 'normal' haematological, biochemical and physiological values and function is necessary if we wish to make an attempt to assess the importance of an effect of a toxic metal such as lead on Mute swans. Thus, environmental and/or ecological assessments cannot be made without a good knowledge of biochemical and physiological factors. Chapter four reports haematological and biochemical reference values for Mute swans and compares these values with those of six swans that died from acute lead poisoning.

Chapter five reports on tissue and blood lead levels of Mute swans that were involved in collisions. The possible role of elevated lead in causing these collisions is investigated.

REFERENCES

- Anders, E., Diet, D.D., Bagnell, C.R., Gaynor, J., Krigman, M.R., Ross, D.W., Leander, J.D. and Mushak, D. (1982). Morphological, pharmacokinetic and hematological studies of lead exposed pigeons. Environmental Research. 28: 344-363.

- Benson, W. W., Brock, O.W., Gabrica, J. and Loomis, M. (1976). Swan mortality due to certain heavy metals in the Mission Lake area. Idaho Bulletin of Environmental Contamination and Toxicology. 15: 171-174.
- Birkhead, M. (1983). Blood lead levels in Mute swans Cygnus olor on the River Thames. Journal Zoology (London). 198: 15-25
- Chisolm, J.J. (1973). Management of increased lead absorption and lead poisoning in children. New England Journal of Medicine. 289: 1016-1024.
- Hutton, M. (1980). Metal contamination of Feral pigeons, Columba livia from the London area: Part 2. Biological effects of lead exposure Environmental Pollution. Series (A): 22: 281-293.
- Hutton, M. and Goodman, G.T. (1980). Metal contamination of Feral pigeons Columba livia from the London area: Part 1 - Tissue accumulation of lead, cadmium and zinc. Environmental Pollution Series. (A): 207-217.
- Janssen, D.L., Oosterhuis, J.E., Allen, J.L., Anderson, M.P., Kelts, D.G. and Wiemeyer S.N. (1986). Lead poisoning in free ranging California condors. Journal of American Veterinary and Medical Association. 189: 1115-1117.
- Lee, W.R. (1981). What happens in lead poisoning ? Journal of the Royal College of Physicians of London. 15: 48-54.
- O'Halloran, J. and Duggan, P.F. (1984). Lead levels in Mute swans in Cork. Irish Birds: 2: 501-514.
- Ohi, G., Seki, H., Akiyama, K, Yagyu, H. (1974). The pigeon as a sensor of lead pollution. Bulletin of Environmental Contamination and Toxicology. 12: 92-98.

- Simpson, V.R., Hunt, A.E. and French, M.C. (1979). Chronic lead poisoning in a herd of Mute swan. *Environmental Pollution*. 18: 187-202.
- Thomas, G.J., Perrins, C.M. and Sears, J. (1987). Lead poisoning and waterfowl. In: *Angling and Wildlife in freshwaters*. Ed by P.S. Maitland and A.K. Turner, 5-6. (Institute of terrestrial Ecology, Symposium No. 19) Abbots-Ripton, Institute of terrestrial Ecology.
- Zander, R., Lang, W. and Wolf, U.H. (1984). Alkaline haematin 575-AHD a new tool for the determination of haemoglobin as an alternative to the cyanhaemoglobin method 1: Description of the method. *Clinical Chemica Acta*: 136: 83-93.

CHAPTER 1

DETERMINATION OF HAEMOGLOBIN IN BIRDS BY A MODIFIED ALKALINE HAEMATIN
(D-575) METHOD.

This chapter is presented in the form of a paper published in
Comparative Biochemistry and Physiology.

DETERMINATION OF HAEMOGLOBIN IN BIRDS BY A MODIFIED ALKALINE HAEMATIN (D-575) METHOD

(Received 3 June 1986)

Abstract—1. A recently published method for measuring human haemoglobin based on alkaline haematin (Zander *et al.*, *Clin. chem. Acta* 136, 83-93, 1984) has been adopted for bird samples.

2. The new method yields comparable haemoglobin values with that of a previously used alkaline haematin method.

3. Levels of haemoglobin estimated using alkaline haematin were higher than for cyanhaemoglobin, the reference method for human haemoglobin. This difference is due to the loss of haemoglobin in the cyanhaemoglobin procedure due to insolubility.

4. The values for haemoglobin found by the alkaline haematin method did not vary significantly between a range of bird species.

5. The method overcomes some important deficiencies of the cyanhaemoglobin method, in particular, problems of turbidity and quality control assessment.

INTRODUCTION

Haematology plays an important diagnostic role in human and veterinary medicine and modern haematological methods are now being increasingly used in avian research and wildlife investigations. The direct application of methods developed for mammalian studies is, however, problematical in birds due to the presence of red blood cell nuclei, in addition to other cell debris on haemolysis. This debris is liable to lead to substantial error due to turbidity of the specimen. The accuracy and precision of the various methods for estimating haemoglobin differs markedly (Van Assendelft, 1972; Rick, 1976; Bankowski, 1942). The spectrophotometric method using cyanhaemoglobin has been the most widely accepted (Spaander, 1964; Saundermann, 1956) and was recommended by the International Committee for Standardisation in Haematology (ICSH) in 1965 and in 1967. Since cyanhaemoglobin is considered the only stable derivative (Richterich, 1971) all other methods were rejected (Rick, 1976). Thus the cyanhaemoglobin method is considered the reference method against which all others must be tested.

While the use of cyanhaemoglobin has many significant advantages it also has a number of deficiencies which were recently restated by Zander *et al.* (1984). These can be summarised as follows:

(1) The cyanhaemoglobin reagent contains cyanide, is toxic and thus hazardous.

(2) The reaction solution is light labile.

(3) The concentration of the reaction components, especially that of cyanide and the buffer have to be carefully chosen and kept constant.

(4) Standardization of the method is based on purified cyanhaemoglobin solutions, the quality of which is controlled only indirectly by spectrophotometry.

(5) The reaction times of the different haemoglobin species and derivatives differ markedly.

While the above disadvantages are important for mammalian studies their significance becomes even greater for animals with nucleated red blood cells. Thus the following disadvantage can be added to the above:

(6) The reaction end-product is turbid and requires centrifugation before reading spectrophotometrically. With centrifugation, the supernatant becomes clear and a residue forms as a pellet at the bottom of the tube.

A further disadvantage of the cyanhaemoglobin method which is important for the field biologist is the fact that the reaction end-product is light labile which imposes time constraints on field biologists, in remote areas wishing to study blood parameters. Information on the precise haemoglobin levels in healthy birds and birds contaminated by environmental toxins, such as lead, is needed but to what extent cyanhaemoglobin may assay actual avian haemoglobin is not known (Archer, 1977).

This investigation therefore examined a new method described for estimating haemoglobin in humans (Zander *et al.*, 1984) to see if current difficulties in estimating non-mammalian haemoglobin could be resolved. This method is based on the conversion of all haeme, haemoglobin, and haemoglobin species into stable end-products by an alkaline solution of a non-ionic detergent. The reaction product, designated as alkaline haematin D-575, is extremely stable and shows an absorption peak at 575 nm. This paper describes the results obtained from using this modified alkaline haematin method for avian blood. These results were also compared with the reference method (cyanhaemoglobin), and a previously used alkaline haematin method of Bell *et al.* (1965).

MATERIALS AND METHODS

Blood was obtained from an urban flock of Mute Swans *Cygnus olor* (Gmelin) which were known to have elevated lead levels (O'Halloran and Duggan, 1984). In addition, blood samples were collected from Chilean flamingos *Phoenicopterus andinus* (Philippi); Black-necked swans *Cygnus melanocoryphus* (Molina); Emperor Geese *Anser canagicus* (Sewastianov); a Canada Goose *Branta canadensis* (Linnaeus) and Cape Barren Geese *Cereopsis novaehollandiae* Latham, from Fota Wildlife Park, County Cork. Eight Warren Studler chickens, *Gallus domesticus* (L), were sampled from a private poultry farm in Cork City. In each case, blood was collected from the brachial vein using a 23 gauge 1 in. needle and placed in potassium EDTA tubes to prevent clotting. Haemoglobin concentration was estimated by the following methods:

(a) Measurement of total haemoglobin as cyanhaemoglobin

A sample of 20 μ l blood was mixed thoroughly with 5 ml of Drabkin's solution (i.e. 200 mg potassium ferricyanide, 50 mg potassium cyanide, 140 mg potassium dihydrogen phosphate and distilled water made up to a litre) and spun in a Mason centrifuge at 2500 g (determined stroboscopically). The absorbance values of the decanted supernatant were read immediately or within 12 hr using a flow-through cuvette with 1 cm light path in a C.E.393 digital spectrophotometer at 540 nm. The haemoglobin concentration of the blood sample in g/100 ml blood was calculated from a standard curve prepared from known standards (Merz and Dade, Hi.Cn Standard, Hergestell, Dundingen, Switzerland). The pellet, following centrifugation, was retained for later extraction with alkaline-haem detergent (ADH).

(b) Measurement of total haemoglobin as alkaline haematin

1. This method followed that of Bell *et al.* (1965): 0.06 ml of well mixed blood was pipetted into 20 ml of 0.1 N NaOH solution. After mixing, the solution was kept at 37°C for 1 hr, then cooled rapidly to room temperature and, within 30 min, the absorption at 705 nm measured and compared with standards prepared from alkaline haematin (99% pure from Serva, Heidelberg, FRG).

2. The procedure for haemoglobin determination by the alkaline haematin D-575 method followed Zander *et al.* (1984). Twenty microlitres of blood were mixed with 3 ml of a solution of 25 g scintillation grade Triton X-100 in 1 l of 0.1 mol/l NaOH (termed ADH). The absorbance values were read, immediately or within 12 hr of preparation, at 575 nm. These results were then converted to haemoglobin concentrations. The absorbance readings were compared with those of known concentration prepared from purified alkaline haematin (99% pure from Serva, Heidelberg, FRG) and haemoglobin concentrations calculated.

(c) Treatment of pellet

Following centrifugation and reading of the supernatant, ADH was added to the wet pellet and vortex mixed for

10 sec and the absorbance measured. Values of haemoglobin were calculated from standards as above. All concentrations of haemoglobin are presented in g/100 ml.

Statistics

Statistical analyses (calculation of means, standard errors and linear regression analyses) was carried out using a Minitab (Pennsylvania State University) statistical package.

RESULTS

A comparison of chicken haemoglobin values using the three methods of estimation is given in Table 1. Each sample was analysed 10 times and the mean and standard error calculated. The quantity of haemoglobin estimated from the pellet for all bird species studied is presented (Tables 1 and 2) and is added to the level of haemoglobin estimated from reference method to yield the total haemoglobin (Tables 1 and 2). It can be seen there was good agreement between the level of haemoglobin estimated using the alkaline haematin method of Zander *et al.* (1984) and the sum of the soluble and sedimentable cyanhaemoglobin by the reference method. Note also that the precision of this modified reagent is much better than that of Bell *et al.* (1965). Values of haemoglobin using the reference method and ADH-575 for different species of birds is presented in Table 2, again each sample was analysed 10 times and the mean and standard error calculated. Figure 1 shows a scattergram of levels of haemoglobin from mute swans. The linear relationship can be described by the following regression equation $y = 1.19x + 0.686$ (y = alkaline haematin, x = cyanhaemoglobin).

DISCUSSION

As a general test for health in animals an accurate estimate of haemoglobin is very useful. In all animals except mammals the presence of nuclei in red blood cells poses problems for the cyanhaemoglobin procedure. Because this method utilises a secondary biological standard comparisons between laboratories is difficult and so is not useful for external quality control assessment schemes. While the cyanhaemoglobin method is useful for obtaining reproducible results in birds, this study has shown that a modified alkaline haematin method (ADH-575 after Zander *et al.*, 1984) is more appropriate for an accurate and reproducible assessment of avian haemoglobin.

Table 1. Chicken haemoglobin levels estimated using three different methods ($\bar{X} \pm$ SE of 10 replicates, g/100 ml)

Specimen no.	Cyanhaemoglobin	Pellet haemoglobin	Total haemoglobin	Alkaline haematin	
				ADH-575 after Zander <i>et al.</i> (1984)	Bell <i>et al.</i> (1965)
1	7.74 $\pm$ 0.08	2.15 $\pm$ 0.33	9.90 $\pm$ 0.20	9.79 $\pm$ 0.13	9.30 $\pm$ 0.20
2	8.49 $\pm$ 0.13	3.12 $\pm$ 0.40	11.62 $\pm$ 0.40	10.85 $\pm$ 0.18	10.73 $\pm$ 0.68
3	7.33 $\pm$ 0.06	2.27 $\pm$ 0.08	9.61 $\pm$ 0.12	9.63 $\pm$ 0.13	10.51 $\pm$ 0.60
4	8.20 $\pm$ 0.11	1.96 $\pm$ 0.12	10.15 $\pm$ 0.17	9.67 $\pm$ 0.15	9.87 $\pm$ 0.34
5	8.53 $\pm$ 0.13	2.40 $\pm$ 0.09	10.94 $\pm$ 0.16	10.75 $\pm$ 0.15	11.46 $\pm$ 0.83
6	9.90 $\pm$ 0.27	2.64 $\pm$ 0.13	12.54 $\pm$ 0.24	12.50 $\pm$ 0.20	14.14 $\pm$ 0.60
7	9.12 $\pm$ 0.25	2.66 $\pm$ 0.15	11.24 $\pm$ 0.22	11.24 $\pm$ 0.22	11.13 $\pm$ 1.00
8	8.23 $\pm$ 0.09	2.40 $\pm$ 0.11	10.63 $\pm$ 0.12	10.63 $\pm$ 0.12	13.50 $\pm$ 0.77
Total	8.44 $\pm$ 0.28	2.45 $\pm$ 0.12	10.82 $\pm$ 0.34	10.63 $\pm$ 0.34	11.13 $\pm$ 0.60

Determination of haemoglobin in birds

Table 2. Haemoglobin levels in different species of birds ($\bar{X} \pm \text{SE g/100 ml}$) using cyanhaemoglobin and ADH-575 methods

Specimen no.	No. of replicates	Cyanhaemoglobin	Pellet haemoglobin	Total haemoglobin	Alkaline haematin ADH-575
Chilean Flamingoes					
1	10	12.19 $\pm$ 0.42	4.07 $\pm$ 0.20	16.26 $\pm$ 0.48	15.58 $\pm$ 0.63
2	10	13.84 $\pm$ 0.32	3.75 $\pm$ 0.40	17.60 $\pm$ 0.61	16.85 $\pm$ 0.17
3	10	12.88 $\pm$ 0.16	3.02 $\pm$ 0.09	15.90 $\pm$ 0.20	15.18 $\pm$ 0.22
4	10	13.16 $\pm$ 0.13	3.20 $\pm$ 0.10	16.35 $\pm$ 0.17	16.03 $\pm$ 0.25
5	10	12.82 $\pm$ 0.25	3.62 $\pm$ 0.09	16.44 $\pm$ 0.29	16.16 $\pm$ 0.42
6	10	12.39 $\pm$ 0.27	3.05 $\pm$ 0.09	15.45 $\pm$ 0.34	14.97 $\pm$ 0.15
7	10	12.55 $\pm$ 0.19	3.52 $\pm$ 0.09	16.07 $\pm$ 0.25	15.23 $\pm$ 0.22
8	10	13.62 $\pm$ 0.28	3.44 $\pm$ 0.08	17.06 $\pm$ 0.27	16.25 $\pm$ 0.19
Black-necked Swan					
9	3	11.36 $\pm$ 0.64	2.72 $\pm$ 0.20	14.20 $\pm$ 0.91	14.33 $\pm$ 0.11
10	3	14.33 $\pm$ 1.00	3.44 $\pm$ 0.26	17.78 $\pm$ 1.30	17.18 $\pm$ 0.26
Emperor Goose					
11	3	11.78 $\pm$ 0.25	2.94 $\pm$ 0.09	14.92 $\pm$ 0.33	15.37 $\pm$ 0.15
12	3	11.41 $\pm$ 0.24	2.94 $\pm$ 0.07	14.34 $\pm$ 0.26	14.77 $\pm$ 0.46
Cape Barren Goose					
13	3	14.45 $\pm$ 0.63	3.00 $\pm$ 0.60	17.70 $\pm$ 0.73	17.29 $\pm$ 0.40
14	3	14.06 $\pm$ 0.44	3.88 $\pm$ 0.36	17.10 $\pm$ 0.65	17.95 $\pm$ 0.11
Canada Goose					
15	3	14.77 $\pm$ 0.46	3.80 $\pm$ 0.12	18.57 $\pm$ 0.50	18.67 $\pm$ 0.56

One of the most significant aspects of this study is the difference in haemoglobin levels which results from the ADH-575 method and the reference method. In all cases the difference between the two methods was constant and proportional to the level of haemoglobin in the specimen (Table 2). The difference in haemoglobin levels is attributed to the proportion which is lost during incomplete haemolysis using Drabkin's solution in the cyanhaemoglobin procedure. Following centrifugation the supernatant appeared as a clear solution of cyanhaemoglobin; the cells and debris (pellet), on the other hand, were bright red in colour. A similar situation was found by Hunter *et al.* (1940) using acid haematin with chicken blood. In his study he found partial retention of haemoglobin in a pellet following addition of acid and centrifugation.

In this study, on adding ADH reagent to the wet pellet the solution quickly turned green as haemoglobin was extracted. The sum of the pellet haemoglobin and supernatant cyanhaemoglobin was

equivalent to the haemoglobin level obtained by the new method (Tables 1 and 2).

In mute swans which were known to have elevated lead levels (O'Halloran and Duggan, 1984), the reference method gave lower haemoglobin values than the alkaline haematin procedure. This is a result of the formation of insoluble lead-haemoglobin complexes (Clarkson and Kench, 1958; Ong and Lee, 1980) which the Drabkin's solution was unable to breakdown. In the D-575 method Triton X, a potent detergent, probably broke down the complex, yielding the true haemoglobin value for the birds. The linear regression equation ($y = 1.19x + 0.686$) can therefore be used retrospectively to calculate true values of haemoglobin estimated in mute swans, when the cyanhaemoglobin method has been used.

In all cases alkaline haematin gave higher values than the cyanhaemoglobin reference method. While Ponder (1942), working with human blood suggested that lipids and pigments influence the dispersion of haemoglobin derivatives and thus colour absorption in alkaline haematin (compared with the reference method) Zander *et al.* (1984) found no such difference in human blood. Comparing ADH-575 with the reference method the correlation coefficient varied from 0.989–0.996 (Zander *et al.*, 1984). In this present work the results of ADH-575 compare favourably with those obtained from the method devised by Bell *et al.* (1965). Note however, that the standard errors of Bell *et al.*'s method are much higher. From the practical point of view the new method is simpler and does not require centrifugation or any other time consuming processes.

The alkaline haematin 575 method provides the biologist with a tool to investigate blood parameters for non-mammalian animals. It is non-toxic and therefore safe to use, no centrifugation is required and more importantly for the field biologist is provides an end-product which is insensitive to light (Zander *et al.*, 1984) and thus specimens can be transported without deterioration. Since commercial

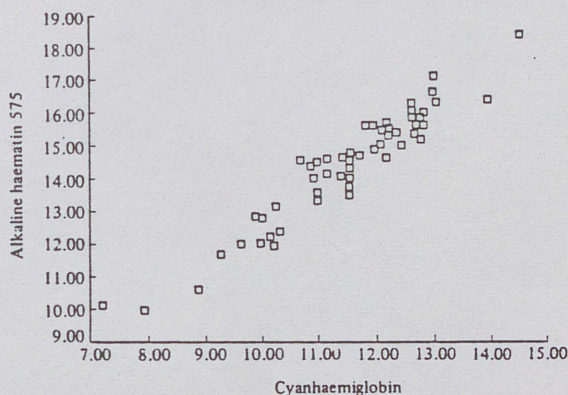


Fig. 1. A scattergram of mean haemoglobin values from mute swans using the cyanhaemoglobin (reference method) plotted against alkaline haematin D-575. The line is described by the linear regression equation $y = 1.19x + 0.686$. ($R = 0.9586$, $S = 0.490$, $df = 53$, $P < 0.001$).

standards are now available, it is possible to standardize analyses, using primary standard material which cancels out an important disadvantage of the cyanhaemoglobin method. ADH-575 therefore provides an efficient tool to carry out detailed investigation of the comparative haematology and haemopoietic sensitivity of all animals to environmental toxicants.

Acknowledgements—We are grateful to Mr S. McKeown of Fota Wildlife Park for assistance with sampling, Mr D. O'Leary for allowing access to his poultry farm, and Drs T. F. Cross, T. C. Kelly and Y. Okasha for helpful discussion.

REFERENCES

- Archer R. K. (1977) Technical methods. In *Comparative Clinical Haematology* (Edited by Archer R. K. and Jeffcott L. B.). Blackwell Scientific, Oxford.
- Bankowski R. A. (1942) Studies of the haemoglobin content of chicken blood and evaluation of methods for its determination. *Am. J. Vet. Res.* (October) 373–381.
- Bell D. J., Bird T. P. and McIndoe W. M. (1965) Changes in erythrocyte levels and the mean corpuscular haemoglobin concentration in hens during the laying cycle. *Comp. Biochem. Physiol.* 14, 83–100.
- Clarkson T. W. and Kench J. W. (1958) Uptake of lead by human erythrocytes *in vitro*. *Biochem. J.* 69, 432–439.
- Hunter F. R., Stringer L. D. and Weiss H. D. (1940) Partial retention of haemoglobin by chicken erythrocytes. *J. Cell. comp. Physiol.* 16, 123–129.
- International Committee for Standardisation in Haematology of the European Society of Haematology (1965) Recommendations and requirements for haemoglobinometry in human blood. *J. Clin. Pathol.* 18, 353–355.
- International Committee for the Standardisation in Haematology (1967) Recommendations for haemoglobinometry in human blood. *Br. J. Haematol.* 13 (Suppl.), 71–75.
- O'Halloran J. and Duggan P. F. (1984) Lead levels in Mute Swans in Cork. *Irish Birds* 2, 501–514.
- Ong C. N. and Lee W. R. (1980) Interactions of calcium and lead in human erythrocytes. *Br. J. Med.* 37, 70–77.
- Ponder E. (1942) Errors affecting the acid and alkaline haematin methods of determining hemoglobin. *J. Biol. Chem.* 144, 339–342.
- Richterich R. (1971) Hamoglobin als Hamoglobincyanid. In *Klinische Chemie, Theorie und Praxis*, 3rd edn, pp. 380–382. Karger, Basel.
- Rick W. (1976) Hamoglobinbestimmung in Vollblut. In *Klinische Chemie und Mikroskopie*, 4th edn, pp. 47–49. Springer-Verlag, Berlin.
- Saundersmann F. W. (1956) Status of clinical hemoglobinometry in the United States. *Am. J. clin. Pathol.* 43, 9–15.
- Spaander J. (1964) Die Hamoglobinbestimmung, *Strahlensch Fürst Prax* 4, 122–127.
- Van Assendelft O. W. (1972) The measurement of haemoglobin. In *Modern Concepts in Haematology* (Edited by Izak G. and Lewis S. M.), Symposia of the International Committee for Standardisation in Haematology, pp. 14–25. Academic Press, New York.
- Zander R., Lang W. and Wolf U. H. (1984) Alkaline haematin 575-ADH, a new tool for the determination of haemoglobin as an alternative to the cyanhaemoglobin method 1: description of the method. *Clin. chem. Acta* 136, 83–93.

CHAPTER 2

BLOOD LEAD LEVELS AND FREE RED BLOOD CELL PROTOPORPHYRIN AS A MEASURE
OF LEAD EXPOSURE IN MUTE SWANS.

This chapter is in the form of a manuscript which was recently
submitted for publication to Environmental Pollution Series (A).

ABSTRACT

The use of blood lead levels in assessing lead exposure in Mute swans Cygnus olor (Gmelin) is investigated. 823 blood samples were taken from 456 uniquely ringed Mute swans at a coarse-fish angling site over a period of 24 months. Blood lead values in individual swans monitored over several weeks were shown to conform to a recently reported kinetic model for blood lead values in birds. The highest median lead level for flock birds were recorded in the winter and spring and the lowest during the summer moulting period. The use of Free Red Blood Cell Protoporphyrin for detecting lead exposure was examined. Whole blood lead detected 44% (n=357) with elevated lead, while free red blood cell protoporphyrin detected 34.50%. In a large number of cases levels of protoporphyrin were above the normal level while the corresponding lead levels were low. The value of both methods and the need to correct each value for haemoglobin is discussed.

INTRODUCTION

Lead poisoning due to ingestion and retention of shotgun pellets has been well documented in waterfowl. In the United States of America, it is a serious problem, with losses of up to two million ducks and geese each year from lead poisoning (Roscoe et al., 1979). During feeding, spent gunshot is ingested from the marshy bottoms of shallow waters in heavily hunted areas. Lead poisoning has also been recorded in upland game birds (Reiser and Temple, 1981) and birds of prey (Wilcove and May, 1986). In Denmark and Ireland lead poisoning has been reported from areas of clay-pigeon shooting (Clausen and Wolstrup; 1979 and O'Halloran et al., 1987a). Lead 'split shot' and 'ledger weights' used for fishing have caused mortality in Mute swans, Cygnus olor (Gm) [O'Halloran et al., (a) in press, Birkhead, 1982].

Post-mortem examinations may reveal lead poisoning in wildbird populations, but there is an increasing need to monitor the situation in live wild birds. Most prior investigations have involved the feeding of lead to live birds in the laboratory, but the validity of extrapolating these results to the natural environment has been questioned because of the contrasting pattern and duration of exposure to pollutants in the two situations (Hutton, 1980). Birkhead (1983) was one of the few workers to employ routine live bird assessment 'in the field'.

Two methods are commonly employed in assessing the extent of lead poisoning in live birds: (1) measurement of whole blood lead and (2) measurement of free red blood cell protoporphyrin IX (FRBCP); birds have nucleated red blood cells, thus the term erythrocyte should not be used. The rationale for blood lead assessment is clear: (a) blood lead is easily accessible for biopsy, (b) methods of analysis are well

developed and (c) information on blood lead levels in other species is readily available. FRBCP is routinely used in screening for lead exposure in birds. Protoporphyrin IX is a precursor of haemoglobin, the concentration of which is known to increase in lead poisoning (Van Den Bergh, 1933; Lamola et al., 1975). Though there is a correlation between blood lead and FRBCP, these two parameters reflect different types of exposure. Blood lead gives an indication of exposure of up to 60 days in humans (Moore, 1986) and is estimated to be 20 days in birds (Anders et al., 1982). FRBCP gives a measure of metabolic damage caused by lead at erythropoiesis, thus any damage caused by lead at this stage can be assessed over the life span of the red blood cell. The life span of avian red blood cells is short (c.35-40 days) (Hodges, 1977) and not c.120 days as in humans (Dacie and Lewis, 1975; Eccleston, 1977).

The level of blood lead and FRBCP reported in birds following lead exposure is very variable and much of the available data is from experimental studies on captive birds (see Lumeij, 1985). This raises, in particular, two questions about the methods for determining lead exposure in birds. Is the large variation in blood lead levels explained in any way by the variation in haemoglobin or haematocrit? And how representative are values obtained during dosing experiments to the field situation?

Blood lead must be considered in the context of its distribution in the blood cells. In humans, 80% of lead is bound to haemoglobin in the erythrocytes (Ong and Lee, 1980) and this appears to be true also in birds (Anders et al., 1982). Blood lead values should therefore, be corrected for haematocrit or haemoglobin (Chamberlain, 1985; Landsdowne and Yule, 1986). FRBCP is, to an even greater extent than lead, associated predominantly with erythrocytes, therefore the whole blood

fluorescence intensity should strictly be corrected for haematocrit or haemoglobin values' (Chisolm et al., 1974; Chisolm, 1973). No investigations using corrected lead levels have been carried out in birds.

This study examined variations in blood lead levels in Mute swans over time, for different sexes, ages and physiological states and examined the value of corrected blood lead. The use of FRBCP for screening lead poisoning in swans was also investigated.

MATERIALS AND METHODS

Study sites.

The main sampling site was at Cork Lough, in the western suburbs of Cork city (Fig.1). This location was selected because of the relatively large flock of Mute swans present there. The number of swans varies seasonally with a maximum of 160 birds in winter and a minimum of 60 in summer. The birds, a heterogenous assemblage of non-breeders, immatures and breeding birds, congregate there as a result of artificial feeding. Cork Lough includes two areas of open water and an island where the birds roost. The maximum depth at Cork Lough is 1.5m in the northern basin and the substrate is soft and silty throughout, with very little grit. The area is an internationally renowned coarse angling site (Anon, 1986). A second site was selected at the Gearagh, Macroom, Co.Cork where a number (c.100) of swans annually congregate for moulting (Fig.1).

Collection of blood samples.

Over 823 blood samples were taken from 456 uniquely ringed Mute swans between December 1984 and November 1986. Groups of birds were caught each week by hand; each bird was fitted with a numbered metal ring and a unique yellow 'Darvic' ring for individual recognition. Blood samples (5ml) were obtained using a 23 gauge 1 inch needle either from the brachial vein or , in the case of cygnets, from the tarsal vein. Blood was immediately placed in a low lead sodium heparin tube and placed in a polystyrene ice box for transportation and then stored at -20°C before analysis. An aliquot was also placed in dipotassium ethylene diamine tetra-acetate (EDTA) tube for haemoglobin estimation.

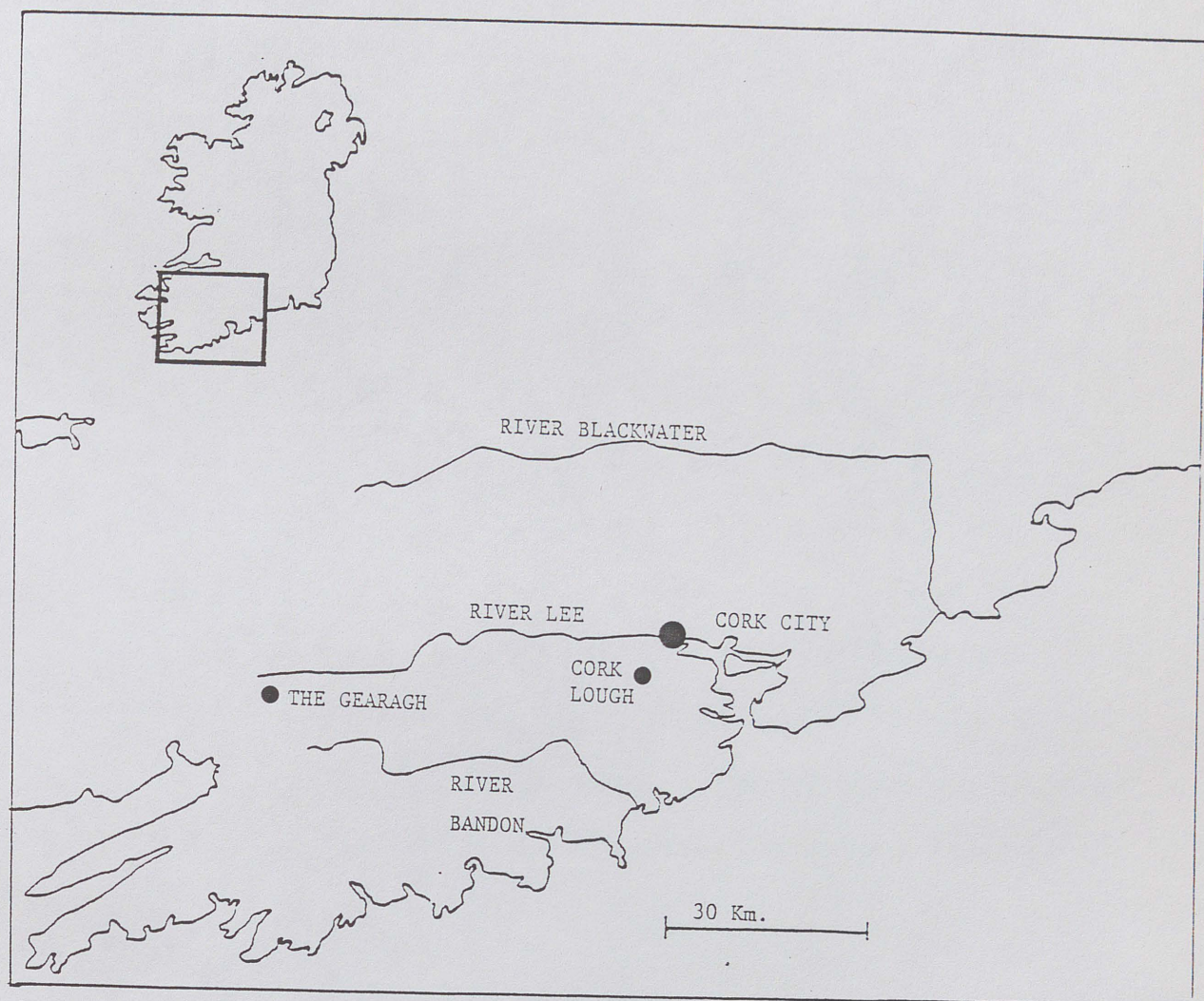


Fig. 1. Map indicating locations of sampling sites.

Swans were sexed by cloacal examination (Hochbaum, 1942) and age was determined by plumage characteristics and beak colour.

Moulting birds were caught by herding the swans into a large netted area.

Caging regime.

To determine if diel changes occurred in haemoglobin and blood lead it was necessary to cage the swans for blood sampling. Swans were caged for 15hrs in separate pens, each measuring 3m X 4m, with peat moss on the floor. Crushed turkey feed, green vegetables and water were freely available to the birds.

The sampling times for the eight swans sampled during the day was as follows: 10.00, 12.00, and 16.30 hrs and at night from 16.00/16.30, 24.00, 02.00, 05.00 and 07.00 hrs. Two regimes were set up, depending on the amount of light, (1) natural darkness from 16.00-07.00 hrs in winter together with an artificial dark period 23.00-03.30 hrs. (2) natural light cycle with short night period from 23.30-04.00 and an artificial dark period from 16.30-07.00. 1.5ml of blood was taken for haematocrit, haemoglobin and lead analyses. Birds were released following the sampling period.

Blood lead analyses.

Blood lead was estimated following a wet acid digestion. 400ul of whole blood were digested using 1.6mls of 10% nitric acid (Aristar grade). Specimens were spun in a Beckman bench centrifuge at 2,500 x g (determined stroboscopically) and 10ul of the supernatant was removed and injected into graphite tube for analysis. Standard material was prepared by adding known amounts of lead to a human blood pool. The

human pool was used to account for the matrix effect of blood; pooled swan blood was not used because it gave excessively high readings.

Lead estimations were carried out on a PYE Unicam SP192 atomic absorption spectrophotometer with a flameless atomiser attachment, and at a wavelength of 217nm. Estimations were carried out at least twice. If the range of duplicates exceeded 10% of the mean, further aliquots were analysed. Appropriate dilutions were made if specimen values exceeded those of the standards. Lead concentrations were calculated by reading directly from a standard curve or calculated using the regression equation from the line of best fit.

Control specimens of human blood (from Guilford, U.K.), with known consensus mean values, were run every third swan specimen to ensure stability of the instrument.

Haemoglobin analyses.

Haemoglobin concentration and haematocrit (packed cell volume) were estimated for all blood samples. Haemoglobin concentration was estimated by converting all haem species to alkaline haematin using a non-ionic detergent. 20ul of fresh, well mixed whole blood was added to 3ml of a solution of 25g scintillation grade Triton X 100 in 1 litre of 0.1 molar NaOH (see O'Halloran et al., 1987b). Three replicates were made and read in a spectrophotometer at 575nm. The haemoglobin concentration was calculated from a standard curve, prepared from pure alkaline haematin (99% pure from Serva, Heidelberg, FRG). Packed cell volume was estimated by spinning three replicates of each sample in a Hawksley microcentrifuge at 12,000 x g (determined stroboscopically) for 30 mins and values of haematocrit calculated.

Red blood cell protoporphyrin.

The extraction method followed Peter et al., (1978): 25ul of whole blood was added to 250ul of normal saline (0.90%) and then 2.5ml of a mixture of ethyl acetate/acetic acid [(4/1 vol) Aristar grade] was added and the solution vortex-mixed for 15s. A second extraction transferred protoporphyrin from the ethyl-acetate/acetic acid mixture into 2.5ml (1.5 molar) hydrochloric acid. The lower aqueous phase was removed and spun at 2,500 x g to clear turbidity, protected from light and read fluorometrically. All readings were made within four hours of extraction.

Fluorescence was measured on a Farrand Mark 1 Spectrofluoremeter with a primary filter of 405nm and secondary filter of 600nm using a Xenon lamp and 10nm slit widths. Duplicate samples were run and if the range exceeded 10% of the mean, further aliquots were analysed. Protoporphyrin concentration was calculated from a standard curve prepared from purified protoporphyrin material (Porphyrin Products, Logan Utah 84321, U.S.A.). Human blood pools were run as controls to ensure reproducibility of the instrument.

Selecting a Maximum Acceptable Limit.

A maximum acceptable limit (M.A.L.) of 40 ugPb/100mls of whole blood was adopted to identify swans with excess lead following Birkhead (1982). 40ug/100mls is equivalent to 2.00umoles/ L. Since few estimations of lead are accurate to 5% (Chamberlain, 1985), a conservative value of 2.20umoles/L is used here.

For corrected lead values, a mean value of haemoglobin for Mute swans of 142g/L was used (after O'Halloran et al., (b) in press) and

the maximum tolerable limit for lead was calculated as follows:

$$\frac{2.20 \text{umoles/L} \times 207^1}{142} \equiv 3.00 \text{ ug Pb/ g Hb.}$$

¹=atomic weight of lead.

thus, values of whole blood lead > 3.00ug Pb/g Hb is considered elevated. Similarly values of greater than 1.07 umoles/L (60.00ug/100mls) protoporphyrin have been adopted as indicative of excess lead exposure after Bush et al., (1982). On the same basis as lead, the limit can be calculated as follows:

$$\frac{1.07 \text{umoles} \times 561^2}{142} \equiv 4.00 \text{ ug PPIX/ gHb}$$

²= molecular weight of PPIX.

thus samples with in excess of 4.00 ugPPIX/ gHb are considered to have been exposed to elevated lead. Age and physiological state were taken into consideration for values of protoporphyrin IX and haemoglobin after O'Halloran et al., (b, in press).

Statistics

All statistical calculations were carried out using a Minitab statistical package (Pennsylvania University, U.S.A.) on a Vax VMS. A B.M.D.P. PLR stepwise logistic regression analysis was used to examine the observed pattern of the proportion of birds of each class and age with elevated lead at Cork Lough. Medians and 25% percentiles were calculated to describe the distribution of blood lead levels over the two year sampling period.

RESULTS

Blood Lead Levels

Diel variation:

Caged birds: During the day (10.00-16.00 hrs), samples taken from caged birds showed variation in haematocrit, haemoglobin and blood lead both in individual birds and between birds (Fig.2A and 2B). Haemoglobin varied less than haematocrit, so blood lead values were corrected for haemoglobin. Corrected lead values were less variable than the uncorrected values (Fig. 2A, 2B).

At night (16.00-07.00), haematocrit, haemoglobin and blood lead varied considerably with no pattern emerging irrespective of the degree of light exposure (false day or false night) (Fig.2C and Fig.2D). Blood lead levels were corrected for as above (Fig 2C, 2D).

Wild birds: Two birds sampled at intervals of 6 hours in the field showed similar patterns to caged birds. There were no differences in the values of blood lead corrected for haemoglobin for the two birds examined.

One swan (specimen A, Table 1) showed very little variation in the blood lead level over the period sampled. A second swan (specimen B Table 1) ingested a lead weight on or about day 7 and the blood lead level did not reach its maximum concentration until 28 days after ingestion. The blood lead level dropped within 14 days of this maximum value. A third swan, (specimen C, Table 1) had ingested at least one lead weight on or before the first sampling date and had a maximum concentration on day 21. Although the blood lead concentration (umoles/L) on day 147 is less than on day 84 the opposite is the case

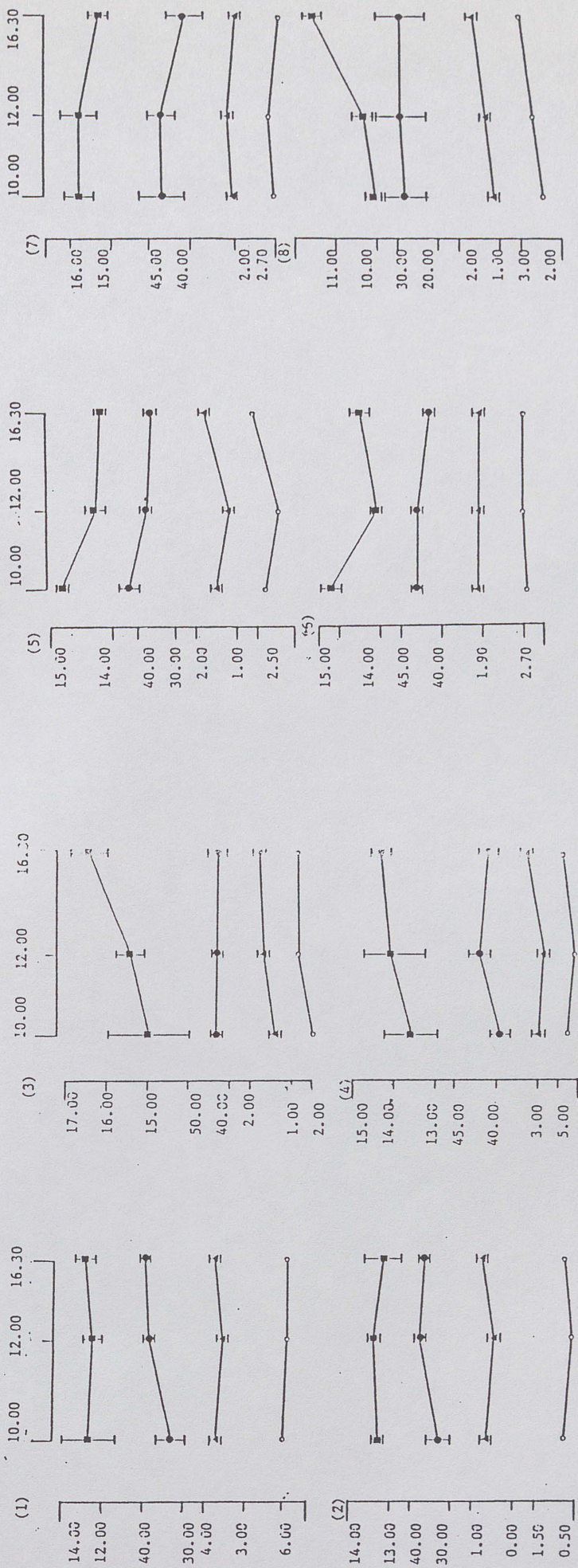


Fig. 2A

Fig. 2B

Fig. 2. (A-D). Changes in haematocrit $\blacksquare$, haemoglobin $\bullet$, lead (umoles/L) $\blacktriangle$ and lead corrected for haemoglobin (ug Pb/ gHb) $\circ$ in eight caged Mute swans during the day (2A and 2B). 2C and 2D (overleaf) changes in haematocrit, haemoglobin, lead and corrected lead in eight caged swans during the night. For C, specimens 9 and 10, the black shading indicates false night and for specimens 11 and 12 true night. For specimens 13 and 14 the black line indicates false night and true night for specimens 15 and 16.

Figure 2. Continued.

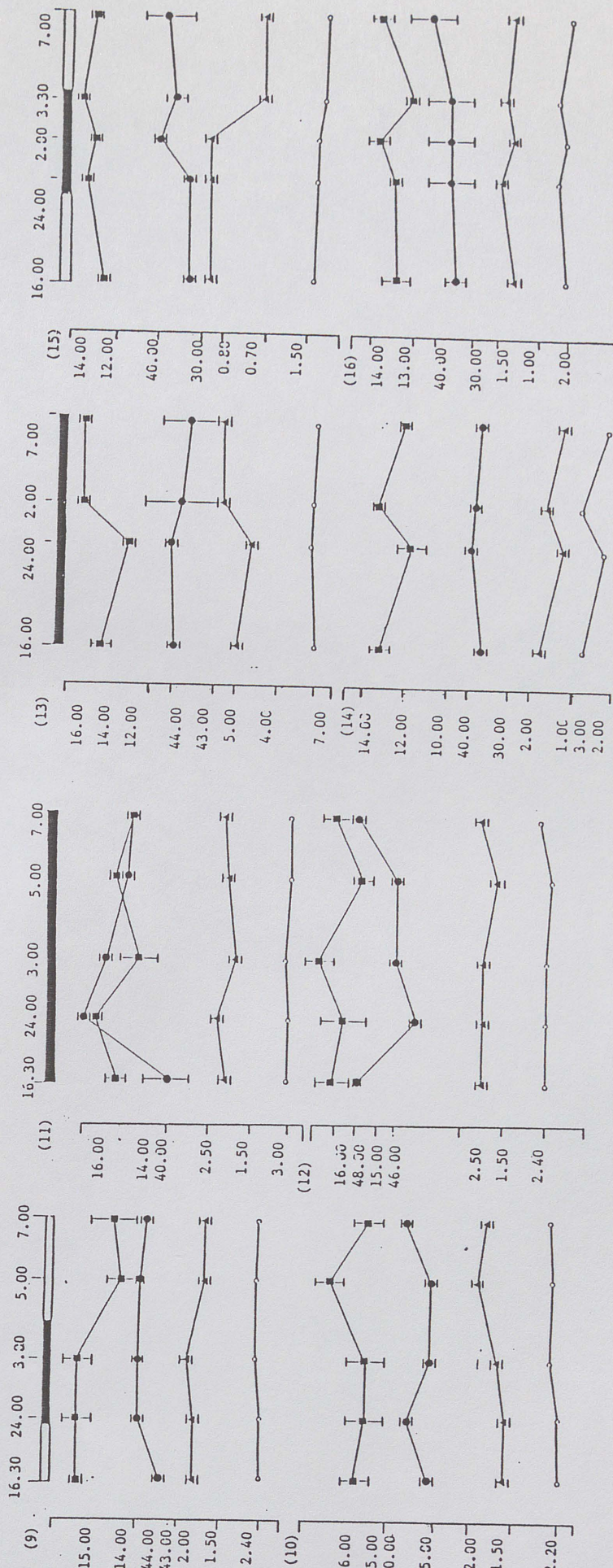


Fig. 2C

Fig. 2D

Table 1. Blood lead levels of Mute swans sampled more than once and details of time between each sample.

A.								
<u>Time (days)</u>	<u>0</u>	<u>28</u>	<u>49</u>	<u>56</u>	<u>91</u>	<u>105</u>	<u>175</u>	<u>189</u>
Pb (uM/L)	1.20	0.90	1.00	1.40	1.00	0.80	1.10	0.60
Pb (ug/g Hb)	2.40	1.60	1.80	2.50	2.10	1.45	2.00	1.20
B.								
<u>Time (days)</u>	<u>0</u>	<u>7</u>	<u>14</u>	<u>21</u>	<u>35</u>	<u>49</u>	<u>280</u>	
Pb (uM/L)	1.30	2.30	2.50	2.25	16.00	3.00	0.85	
Pb (ug/g Hb)	2.40	3.60	4.60	3.84	28.64	5.20	1.40	
C.								
<u>Time (days)</u>	<u>0</u>	<u>21</u>	<u>84</u>	<u>147</u>	<u>189</u>			
Pb (uM/L)	3.40	4.70	2.80	2.50	2.00			
Pb (ug/g Hb)	4.90	6.60	4.00	4.50	3.50			
D.								
<u>Time (days)</u>	<u>0</u>	<u>7</u>	<u>14</u>	<u>21</u>				
Pb (uM/L)	0.40	0.80	1.65	2.30				
Pb (ug/g Hb)	0.70	1.30	2.60	3.70				
E.								
<u>Time (days)</u>	<u>0</u>	<u>28</u>	<u>63</u>					
Pb (uM/L)	1.20	0.70	1.10					
Pb (ug/ gHb)	1.90	1.05	1.70					

for corrected lead values (ug /g Hb). Specimen D (Table 1) was a two month old cygnet. Blood lead level was low and gradually increased with age until at some time between day 14 and 21, the cygnet ingested a lead weight after which it dramatically increased. Specimen E sampled three times in 63 days had low lead levels.

The range of blood lead values from Mute swans at Cork Lough was considerable, being from 0.46 to 106.30 ugPb/ gHb (0.30 - 66.00 umoles/L n = 823) with a seasonal trend in the pattern of elevated lead levels.

Seasonal Variation

Blood lead levels corrected for haemoglobin in swans from Cork Lough is presented in Fig. 3. There was no difference in the proportion of birds with elevated lead for the individual sex or age class, thus the pattern for the total population is presented. The proportion of birds with elevated blood lead levels (3.00 ug/ gHb) was highest in winter (63% and 66%) and spring (43% and 73.50%) and lower in summer (31% and 31%) and autumn (48% and 22%). The median lead levels were highest in the winter and spring months (Fig. 3). There was no change in the observed pattern for corrected or uncorrected blood lead levels in the flock birds.

Moulting birds.

A number of birds were sampled before and after moulting and lead levels are presented in Table 2. Blood lead levels were always lower during primary moult than at any other time. In some instances (e.g. specimen E) the blood lead value returned to its former elevated level.

Moulting birds sampled at Macroom Co.Cork had very low blood levels (median = 0.84ug Pb/ gHb, range = 0.25 - 2.00ug Pb/ gHb, n = 24) with no birds showing elevated lead levels. Similarly, at Cork Lough, adult

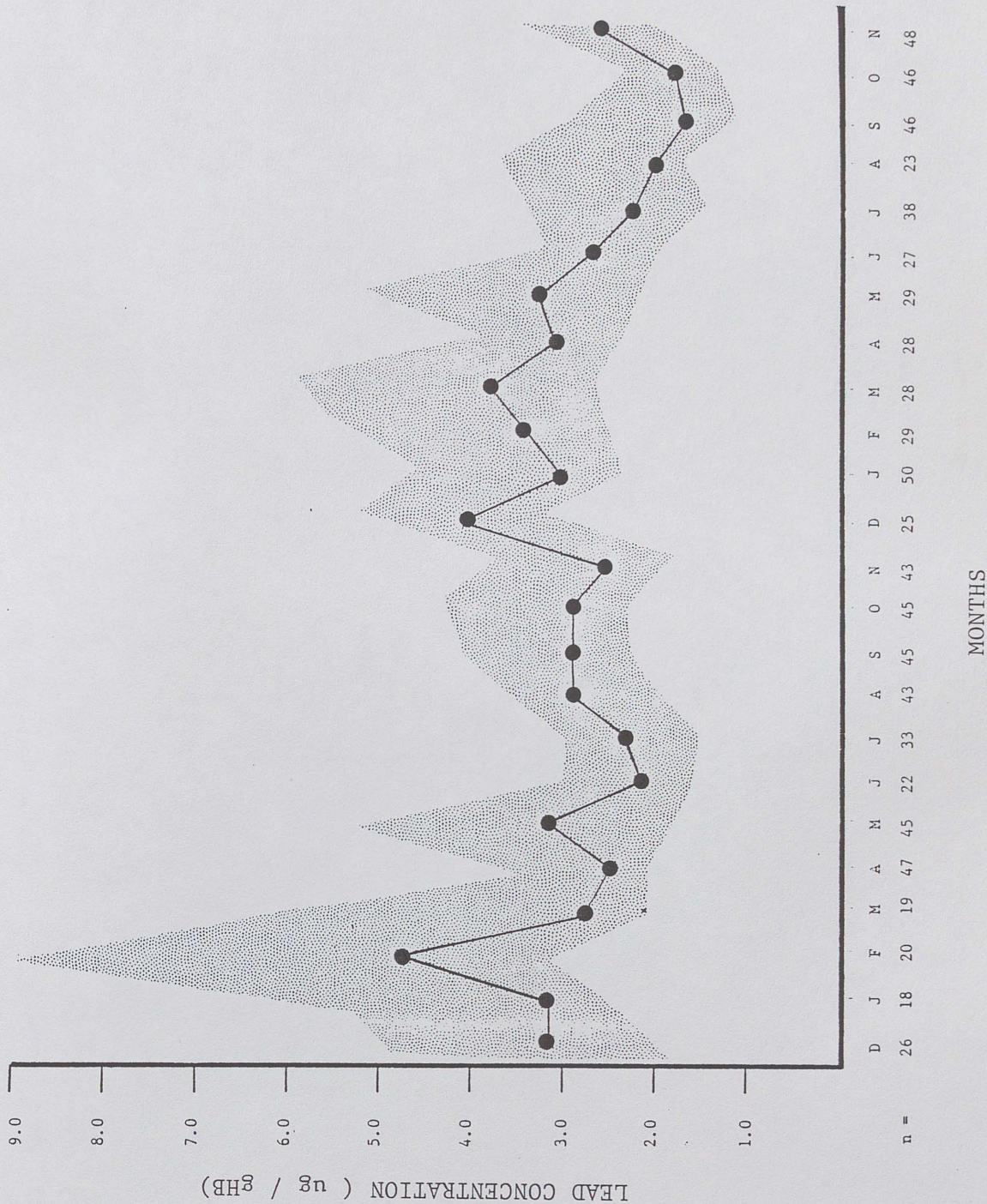


Fig. 3. Median blood lead levels in Mute swan at Cork Lough over a two year period. Shaded area indicates the 25% percentile above and below the median line.

Table 2. Blood lead levels of individual moulting Mute swans and interval between samples. * = time of moulting.

A.			
<u>Time (days)</u>	<u>0</u>	<u>42</u> *	<u>180</u>
Pb (uM/L)	2.30	0.90	1.60
Pb (ug/ gHb)	3.40	1.50	2.20
B.			
<u>Time (days)</u>	<u>0</u>	<u>56</u> *	<u>140</u>
Pb (uM/L)	2.70	0.70	1.25
Pb (ug/ gHb)	3.50	1.00	2.00
C.			
<u>Time (days)</u>	<u>0</u>	<u>28</u>	<u>120</u> *
Pb (uM/L)	2.10	1.20	0.80
Pb (ug/ gHb)	3.30	1.60	1.20
D.			
<u>Time (days)</u>	<u>0</u>	<u>7</u>	<u>119</u> *
Pb (uM/L)	1.70	1.90	0.85
Pb (ug/ gHb)	2.70	3.00	1.32
E.			
<u>Time (days)</u>	<u>0</u>	<u>182</u> *	<u>238</u>
Pb (uM/L)	5.00	0.75	5.25
Pb (ug/ gHb)	7.45	1.00	9.70

birds had the lowest median level during the moulting period, June-August (Fig. 3).

Blood Lead Level and Protoporphyrin as measures of exposure.

There was a considerable range of protoporphyrin values from a maximum of 40.00 ug PPIX/ gHb in acutely lead poisoned birds to 1.90 ug PPIX/ gHb in normal healthy birds (6.0 umoles - 0.10 umoles, n = 357).

In both caged and field individuals, there was no difference in the protoporphyrin level either during the day or night. To determine whether FRBCP alone would confidently predict lead poisoning in swans, a sample of 357 specimens with different lead levels were run 'blindly' for FRBCP. The distribution of blood lead and FRBCP are given in Fig.4. The percentage of birds detected with elevated lead levels, using whole blood lead as a measure, was 44.00% (Fig.4) being higher than the proportion detected using FRBCP, which was 34.50% (Fig. 4a, 4b). However, of the 158 birds detected as having elevated lead, using whole blood lead, only 43% showed elevated FRBCP. Similarly of the samples with elevated FRBCP, 50% had low lead levels and were thus false positives by this criterion. These false positives were eliminated from the analysis and the distribution of PP IX values is presented in Fig. 4C. The details of the birds eliminated are as follows: 10 birds were moulting, five birds had just completed moult, nine were cygnets of less than 6 months old, one was anaemic, 10 birds had high blood lead levels from two months to one year before being sampled for protoporphyrin and in some cases the blood lead levels in the interval were low and 25 were anomalous on the basis that no other sample was available prior to the PP IX reading.

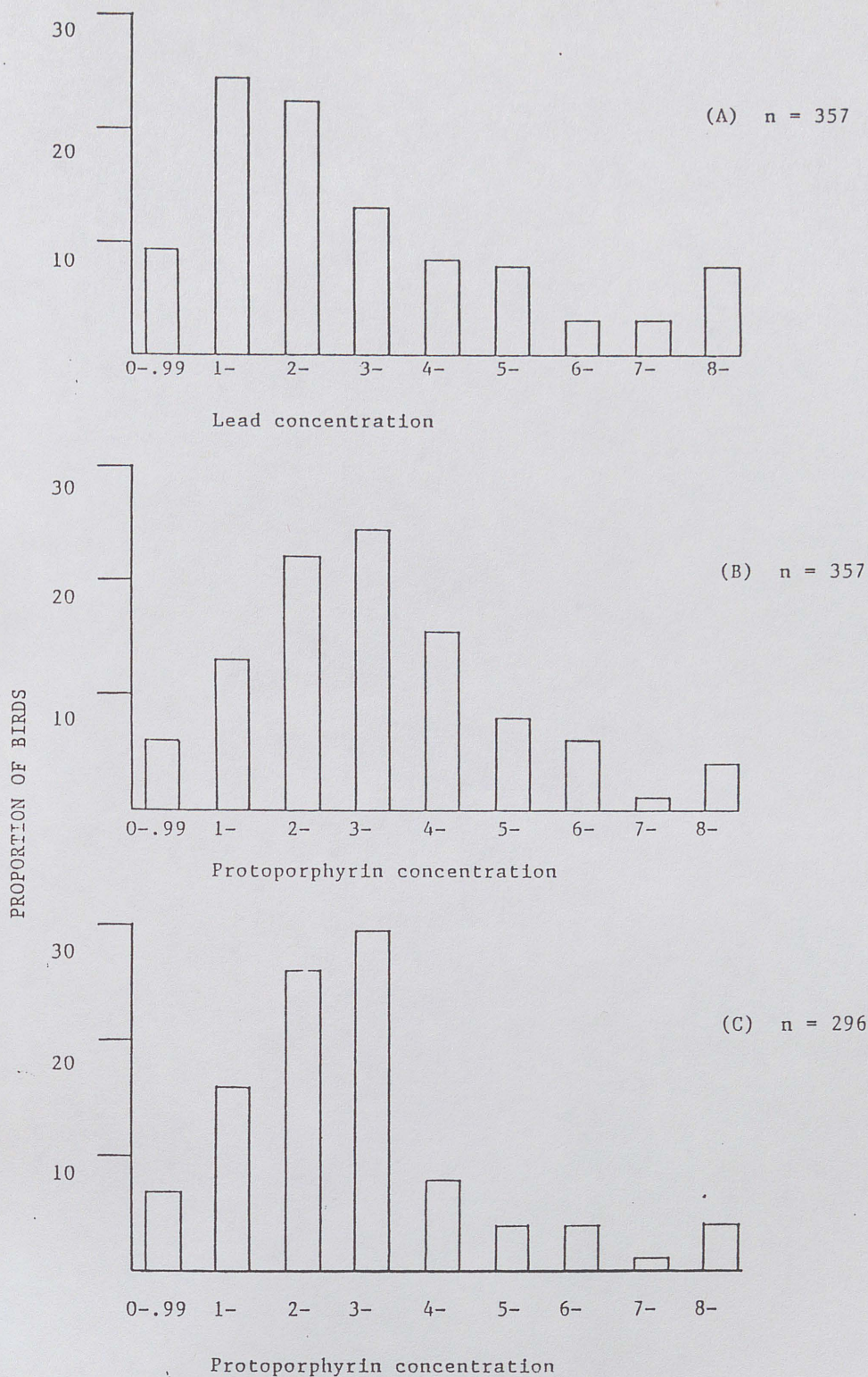


Fig. 4. Distribution of (A) blood lead levels ($\mu\text{g Pb/gHb}$) for a sample of swans ($n = 357$), (B) free red blood cell protoporphyrin ($\mu\text{g PPIX/gHb}$) for the same sample and (C) the distribution of protoporphyrin with false positives eliminated, see text for details.

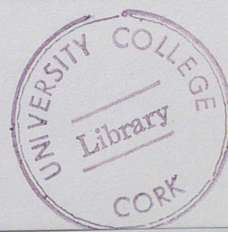
DISCUSSION

Blood lead levels as a measure of exposure to lead have been used by many workers. Ohi et al., (1974) and Hutton (1980) for example identified birds exposed to lead in urban and rural sites. In addition to anthropogenically produced lead from organo-lead emissions, lead pellets from shooting and angling weights also pose a hazard to wildfowl. Experiments based on the dosing of captive birds with lead have been carried out with a view to understanding the high and variable blood lead levels in birds (Anders et al., 1982). These results have then been extrapolated to the field situation. In the present study, some of the variation in blood lead in caged birds was due to changes in haemoglobin concentration during the diel cycle (Fig. 2). Since the variation of haematocrit was considerable and in view of the large reference range for haematocrit reported by O'Halloran et al., (b) (in press), all blood lead values were corrected for haemoglobin. This reduced the variation (Fig. 2) and the reduction was not exclusive to caged birds, but was also evident in two birds sampled in the field at six hour intervals.

The correction of lead levels for haemoglobin becomes more important in chronically exposed animals (Peter et al., 1978) and this can clearly be seen from specimen C (Table 1). When the blood level is expressed per litre of blood, on day 147 the concentration appears less than that of day 84, however in the corrected value the reverse is true. This is due to anaemia as a result of lead poisoning, since the proportion of red blood cells (which contains most of the lead) was low and as a consequence the amount of lead in the sample was low (Table 1, specimen C).

Blood lead is very labile (Chamberlain, 1985) and a great deal of flux occurs before it reaches a steady state. Many workers (e.g. Rabinowits et al., 1974) have attempted to describe the kinetic behaviour of blood lead in humans with a view to understanding this variation. Similarly, Anders et al., (1982) proposed a kinetic model to describe blood lead values in experimentally exposed pigeons. Anders et al.'s model predicts that blood lead values during chronic exposure reach a maximum concentration of 16.90 umoles three to four weeks from the day of first dosing and then maintain a steady state of 7.2 umoles following the maximum value. In the present study, some of the swans sampled on more than one occasion in the field gave results generally in agreement with this model. Specimen B, (Table 1) for example, reached a maximum concentration of 16.00 umoles (28.60 ugPb/gHb) in 28 days and reached a steady state of 3.00 umoles (5.20 ugPb/gHb) within two weeks. Similarly specimen C (Table 1) reached maximum blood lead concentration by day 21 and reached a steady state between 21 and 84 days. Therefore, the kinetic model proposed by Anders et al., (1982) in the experimental situation seems to be quite close to that reported here for the field results. The time of maximum lead concentration in the blood is equivalent to that of the model, while the steady state concentration is lower.

Although some of the swans sampled were suffering from acute lead poisoning, others e.g. specimen A (Table 1), had a low but variable blood lead level. Individual variations are due to different rates of physiological processes and different susceptibility of individuals to background urban lead levels (Chamberlain, 1985; Haust et al., 1985). In specimen D (Table 1), a cygnet, as it matured its lead load gradually increased. On or about day 21, however, it ingested at least



one lead weight and it's blood lead increased dramatically. This cygnet was later found dead with three lead weights in it's gizzard. Thus, while a great deal of variation occurs in blood lead values in swans, on ingestion of a lead weight or pellet, a dramatic increase in concentration occurs.

Acute lead poisoning resulting from the ingestion of lead pellets has resulted in the deaths of 41 per cent of Mute swans found dead at Cork Lough [O'Halloran et al., (a) in press)]. Most of the lead poisoned birds died in winter and spring. This pattern of mortality coincides with the time of high median blood lead levels recorded here (Fig. 3). This is in contrast to the findings of Birkhead (1983) for Mute swans on the River Thames. There, the median blood lead level was highest in July, August and September, which coincided with the peak coarse-angling season. The peak angling period at Cork Lough is the same as on the Thames and the cause of increased lead levels at Cork Lough is due to ingestion of lead weights as shown by radiology [O'Halloran et al., (a) in press)]. A number of factors may be responsible for the different pattern of blood lead levels at Cork Lough; firstly, as mentioned earlier, the number of birds present is greater in winter, secondly, poor natural feeding may force the birds to forage more and thus ingest lead weights; and thirdly, because of the poor diet in winter, when the birds feed almost exclusively on bread, their susceptibility to absorption of lead is greater. Morton et al., (1985) has shown that after a human has eaten, only 3 per cent of lead administered is absorbed by the small intestine, but this increases to 60 per cent when the human has fasted. Trost (1981) also noted a different pattern of lead absorption in ducks exposed to lead on different experimental diets and grit types. At Cork Lough, the

median value of lead in April is similiar to that found by Birkhead (1983) on the River Thames. It is suggested that increased movement of birds, particularly adults in search of breeding sites, is responsible for the lower median value at this time.

The level of lead in moulting birds was particularly low. At The Gearagh, the median lead level was 0.84 ugPb/ gHb and no birds were suffering from lead poisoning. The Gearagh (Fig. 1) is a country site with low organo-lead input and although wildfowling is carried out during the winter, the swans do not seem to ingest lead pellets during the moult. The lowest median levels at Cork Lough were also during the moulting period. It is not known whether lead is mobilised from body tissues into the plumage of the Mute swan as are other metals in some birds (see Furness et al., 1986).

Protoporphyrin as a measure of lead exposure

Protoporphyrin IX (FRBCP) has been used for decades as a measure of lead exposure in humans (Chisolm and Brown, 1975). Barrett and Karstad (1971) were the first to adopt this method qualitatively for waterfowl. Roscoe (1979) pioneered the use of the direct method of FRBCP in waterfowl using a haematofluoremeter. Birkhead (1983) also employed a haematofluoremeter to detect increased blood lead exposure in Mute swans on the River Thames. The 'normal' values of FRBCP in Mute swans vary greatly and may depend on physiological state (O'Halloran et al., (B) in press). The reference distribution varies from 0.40 - 3.90 ug PPIX/ gHb (0.16 - 1.20 umoles) and values of up to 13.40 ug/ gHb may be recorded in healthy cygnets. Because of the variation in FRBCP and because the direct method of estimating FRBCP may detect other disorders in protoporphyrin, this study employed the

extraction method. The extraction method is reported to be more specific in its selection of protoporphyrin species (Bush et al., 1982; Chisolm, 1973). Despite the fact that the extraction method was employed, variable levels of FRBCP were detected. The levels recorded (0.10 - 6.00 umoles) were similar to those reported by Roscoe (1979) for lead poisoned Mallard Anas platyrhynchos (L), but many orders of magnitude lower than those found by Franson et al., (1986) for Canvas back ducks Aythya valisineria (Wilson) or by Janssen et al., (1986) for California Condors Gymnogyps californicus (Shaw). The ability of protoporphyrin IX to detect elevated lead levels in humans has also been investigated. Bush et al., (1982) found that the ability of protoporphyrin analysis to predict lead levels was poor. Up to 30% of urban children with lead concentrations of ≥ 1.93 umoles (40ug/ 100mls) and 60% of children with ≥ 1.45 umoles (30ug/100mls) would remain undetected if protoporphyrin analysis alone was used as a screening method (Bush et al., 1982). In the present work, 43% of the swans sampled (n = 357) had elevated lead (> 3.00 ug/ gHb), while FRBCP detected only 34.50% (Fig. 4). However only 43% of the swans with elevated lead had elevated FRBCP, so the number of false negatives was high. In addition, 61 swans, which had elevated FRBCP, had low lead levels, although a single swan had had an elevated lead two months earlier. Fifteen birds had completed or were in the process of moulting.

According to Voitkevich (1966), during moulting the plasma volume changes and erythropoietic activity is increased. There may therefore have been a number of immature red blood cells circulating during moulting. Smith and Engelbert (1969) reported undifferentiated immature red blood cells circulating in the blood of young chickens.

O'Halloran et al., (b) (in press) reported high circulating protoporphyrin levels in young swans, probably due to raised protoporphyrin IX in the immature red blood cells. A high level of FRBCP was also found in this study in nine cygnets with low lead levels. One bird suffering from anaemia and 25 FRBCP specimens were eliminated from the analyses as their blood lead levels were low and only one blood sample had been taken. They were not included in the positive group as they may have been false positives. This point illustrates that caution should be used in interpreting protoporphyrin values for Mute swans and the full physiological state should be considered before incriminating lead as the cause of elevated readings.

The need to correct FRBCP for haemoglobin has been recognised for over a decade (Chisolm, 1974; Chisolm and Browne, 1975; Roscoe, 1979) and most workers now express FRBCP in haemoglobin equivalents. If haemoglobin is not considered when screening lead poisoned birds, many may go undetected both for blood lead levels in the chronic state and for FRBCP in the anaemic state.

The rationale for using FRBCP is based on the ability to detect metabolic damage which can be measured during the life span of the red blood cell. This is justified in the case of humans on the basis that the erythrocyte has a long life span c.120 days but in birds the red cell life span is much shorter. In the present study, blood lead values reached a peak between 21 - 28 days. This is equivalent to that reported by Anders et al., (1985) and is close to the known life span of bird red blood cells.

In conclusion, the following points should be considered when screening birds for lead exposure: (1) Two blood lead samples taken at intervals of three weeks from swans should be sufficient to detect

elevated lead levels. (2) Haemoglobin level should also be considered and (3) although FRBCP is a good indicator of the metabolic damage caused by lead, until a greater knowledge of FRBCP and its metabolism in birds is known, caution in the use and interpretation should be observed. Only then may it be possible to use FRBCP as an exclusive method for screening lead exposure in swans.

REFERENCES

- Anon. (1986). Central Fisheries Board of Ireland Report. 'Inland Fisheries Strategies for Management and Development'. 195pp
- Anders, E., Dietz, D.D., Bagnell, C.R., Gaynor, J., Krigman, M.R., Ross, D.W., Leander, J.D. & Mushak, D. (1982). Morphological, Pharmacokinetic and Hematological studies of lead exposed pigeons. Environmental Research. 28: 344-363.
- Barrett, M.W. & Karstad, L.H. (1971). A fluorescent erythrocyte test for lead poisoning in waterfowl. Journal of Wildlife Management. 35: 109-118.
- Birkhead, M. (1982). Causes of mortality in the Mute swan Cygnus olor on the River Thames. Journal of Zoology (London). 198: 15-20.
- Birkhead, M. (1983). Lead levels in the blood of Mute swans Cygnus olor on the River Thames. Journal of Zoology (London). 199: 59-73.
- Bush, B., Doran, D.R. & Jackson, K.W. (1982). Evaluation of erythrocyte protoporphyrin and zinc protoporphyrin as micro-screening procedures for lead poisoning detection. Annals of Clinical Biochemistry. 19: 71-76.
- Chamberlain, A.C. (1985). Prediction of response of blood lead to airborne and dietary lead from volunteer experiments with lead isotopes. Proceedings of Royal Society (London). (B) 224: 149-182.
- Chisolm, J.J., Jr. (1973). Management of increased lead absorption and lead poisoning in children. New England Journal of Medicine. 289: 1016-1024.
- Chisolm, J.J., Mellitis, E.D., Keil, J.E. & Barret, M.B. (1974). A simple microhaematocrit procedure as a screening technique for increased absorption in young children. Journal of Paediatrics. 84: 490-498.

- Chisolm, J.J. & Browne, D.H. (1975). Micro-scale photofluorometric determination of 'free erythrocyte porphyrin' (Protoporphyrin IX). *Clinical Chemistry* 21: 1669-1682.
- Clausen, B. & Wolstrup, C. (1979). Lead poisoning in game from Denmark. *Danish Review of Game Biology*. 11: 22pp.
- Dacie, J.V. & Lewis, S.M. (1975). *Practical Haematology* 5th Edition. Churchill Livingstone. London.
- Eccleston, E. (1977). Normal haematological values in rats, mice and marmosets. In: *Comparative Clinical Haematology*. Ed. R.K. Archer and L.B. Jeffcott. Blackwell. Oxford. 611-619.
- Franson, J.C., Haramis, G.M., Perry, M.C. & Moore, J.F. (1986). Blood protoporphyrin for detecting lead exposure in wild waterfowl. A Workshop. Ed. J.S. Feierabend and A.B. Russell. National Wildlife Federation, Washington D.C. 139pp.
- Furness, R.W., Muirhead, S.J., Woodburn, M. & Fitzpatrick, P. (1986). Using bird feathers to measure mercury in the environment: Relationship between mercury content and moult. *Marine Pollution Bulletin*. 17: 27-30.
- Haust, H.L., Poon, H.C., Inwood, M., O'Hea, E.K., Haines, D.S.M., Forret, C.J. & Keys, D.S. (1985). Occupational Lead Exposure: Studies in two brothers showing differential susceptibility to lead. *Clinical Biochemistry*. 18: 102-108.
- Hodges, R.D. (1977). Avian haematology. In: *Comparative Clinical Haematology*. Eds. R.K. Archer and L.B. Jeffcott. Blackwell. Oxford. p483-517.
- Hochbaum, H.A. (1942). Sex and age determination of waterfowl by cloacal examination. *North American Wildlife Conference Transactions*. 7: 294-307.

- Hutton, M. (1980). Metal contamination of feral pigeons Columba livia from the London area: Part 2. Biological effects of lead exposure. Environmental Pollution. 22: (A) 281-293.
- Janssen, D.L., Oosterhuis, J.E., Allen, J.L., Anderson, M.P., Kelts, D.G. & Wiemeyer, S.N. (1986). Lead poisoning in free ranging California condors. Journal of American Veterinary and Medical Association. 189: 1115-1117.
- Lamola, A.A., Joselow, M. & Yamane, T. (1975). Zinc protoporphyrin (ZPP): A simple, sensitive fluorometric screening test for lead poisoning. Clinical Chemistry. 21: 93-97.
- Landsdowne, R. & Yule, W. (1986). The Lead Debate: The Environment, Toxicology and Child Health. Croom Helm. London.
- Lumeij, J.T. (1985). Clinicopathologic aspects of lead poisoning in birds: A review. The Veterinary Quarterly. 7: 133-136.
- Morton, A.P., Partridge, S. & Blair, J.A. (1985). The intestinal uptake of lead. Chemistry in Britain. October 923-927.
- O'Halloran, J., Myers, A.A. & Duggan, P.F. (1987 a). Lead poisoning in Mute swans and fishing practice in Ireland. In: Biological Indicators of Pollution. Ed. D.H. Richardson, 183-191. Royal Irish Academy.
- O'Halloran, J., Duggan, P.F. & Myers, A.A. (1987 b). Determination of haemoglobin in birds by a modified alkaline haematin (D-575) method. Comparative Biochemistry and Physiology. 86 (B): 701-704.
- O'Halloran, J., Myers, A.A. & Duggan, P.F. (a, in press). Lead poisoning in swans and sources of contamination in Ireland. Journal of Zoology. (London).
- O'Halloran, J., Duggan, P.F. & Myers, A.A. (b, in press). Some biochemical reference values and changes in blood chemistry in Mute swans Cygnus olor with acute lead poisoning. Avian Pathology.

- Ohi, G., Seki, H., Akiyama, K. & Yagu, H. (1974). The pigeon as a sensor of lead pollution. *Bulletin of Environmental Contamination and Toxicology*. 12: 92-98
- Ong, C.N. & Lee, W.R. (1980). Interactions of calcium and lead in human erythrocytes. *British Journal of Medicine*. 37: 70-77.
- Peter, F, Growcock, G. & Strunc, G. (1978). Fluorometric determination of erythrocyte protoporphyrin in blood, a comparison between direct (hematofluorometric) and indirect (extraction) methods. *Clinical Chemistry*. 24: 1515-1517.
- Rabinowits, M.D., Wetherill, G.W., & Kopple, J.D. (1974). Studies of human lead metabolism by stable isotope tracer. *Environmental Health Perspect.* 7: 145-153.
- Reiser, M.H. and Temple, S.A. (1981). Effects of chronic lead ingestion on birds of prey. In: *Recent advances in the Study of Raptor Diseases*. Ed J.E. Cooper and A.G. Greenwood. Chiron Publications.
- Roscoe, D.E., Nielsen, S.W., Lamola, A.A. & Zuckerman, D. (1979). A simple quantitative erythrocytic protoporphyrin in lead poisoned ducks. *Journal of Wildlife Diseases*. 15: 127-136.
- Smith, N, & Engelbert, O.E. (1969). Erythropoiesis in chickens' peripheral blood. *Canadian Journal of Zoology*. 47: 1269-1273.
- Trost, R.E. (1981). Dynamics of grit selection and retention in captive mallards. *Journal of Wildlife Management*. 45: 64-73.
- Van Den Bergh, A.A.H. & Grotepas, W. (1933). Porphyrinamie ohne Porphyrinurie. *Klin Wochenschr*. 12: 586-592.
- Voitkevich, A.A.(1966). The feathers and plumage of birds. Sidgwick and Jackson. London.
- Wilcove, D.S. & May, R.M. (1986). The fate of the California condor. *Nature*. (London). 319: 16.

CHAPTER 3

This chapter is presented in two sections:

Section 1. Is in the form of a paper published in Biological Indicators of Pollution. Royal Irish Academy.

Section 2. Is in the form of a manuscript recently submitted for publication to the Journal of Zoology. (London).

CHAPTER 3: SECTION 1

LEAD POISONING IN MUTE SWANS AND FISHING PRACTICE IN IRELAND.

LEAD POISONING IN MUTE SWANS AND FISHING PRACTICE IN IRELAND

ABSTRACT

Lead poisoning in waterfowl resulting from the ingestion of spent gunshot pellets was first recognised in the USA in 1874. Since then, research in fourteen European countries has shown spent gunshot to be the cause of considerable bird mortality. In Britain in particular, 'angling litter' has caused the deaths of thousands of mute swans, *Cygnus olor* (Gmelin). The incidence of lead poisoning in Irish mute swans has been investigated, and blood samples and post-mortem examination have revealed local elevated lead levels. Blood samples from swans at Cork City Lough have revealed that 50% of live birds have elevated lead levels. Although legislation banning the use of lead weights has been drafted in Britain, no such statute has been considered in the Republic of Ireland. Six alternatives to lead shot are now commercially available. Few scientific data are available on accumulation of 'angling litter' in Ireland; however, if angling practices resemble those in Britain, it is essential that available information be collated.

INTRODUCTION

The importance of lead shot from hunters' cartridges as a source of environmental contamination has been widely discussed, especially in N. America (Philipps and Lincoln 1930), since first discovered in 1874. Ingested lead shot affects waterfowl in particular, though other species are not exempt. In the western United States, the Californian condor, *Gymnogyps californicus* (Shaw), for example, is becoming extinct with only six condors remaining in the world (Wilcove and May 1986). 'Lead has been implicated in this decline, with at least two birds having died in the last three years from dining on animals that have been shot by hunters.' The lead levels in the remaining wild condors are alarmingly high (Wilcove and May 1986). 'Waterfowl appear to be at least twice as sensitive to the bio-chemical effects of lead as are man and other

mammals' (Finley *et al.* 1976). This relates to the use of the gizzard which breaks down the food by abrasion with grit and small stones which have been collected by the bird. Lead shot is dissolved in the gizzard by digestive enzymes, leading to the absorption of lead salts across the gizzard wall and the intestine. In North America there have been many 'die-offs' of waterfowl attributed to lead poisoning (Bellrose 1959; Hawkins 1965; Anderson 1975), and lead pellets have been found in 19 species of waterfowl. The large numbers of waterfowl dying in each of the major migratory flyways (4% in the Mississippi flyway and 2-3% in the other flyways (Bellrose 1959) clearly highlight the importance of lead as a mortality factor. In 1973, 21 out of 47 states in the USA responding to a questionnaire indicated that poisoning from ingested lead occurred in birds in their state. Fifteen states reported acute poisoning from lead shot (Thomas 1980). Research in at least eight countries (Canada, Denmark, Federal Republic of Germany, the Netherlands, Sweden, Switzerland, United States and the United Kingdom) has shown that lead poisoning results from wildfowl or clay-pigeon shooting. In Britain, Owen and Cadbury (1975) have shown that at least 37 (29%) of 128 swans [*Cygnus olor* (Gmelin), *C.c. cygnus* (Linnaeus) and *C.c. bewickii* Yarrell] found dead at the Ouse Washes between 1969 and 1975 died from lead poisoning. Hunt (1977) reported that lead poisoning was responsible for the deaths of 107 (52%) of 206 *C. olor* from rivers, lakes and gravel pits in the English midlands. The birds died from ingesting anglers' split lead shot and fishing weights. In 1981, the Nature Conservancy Council reported that between 3000-4000 *C. olor* were dying annually from ingestion of angling lead (N C C 1981).

In Ireland, the first published fatality due to lead in mute swans was in 1983 at Cork City Lough (O'Halloran and Duggan 1984). Since then, further records have been made available to us. Two shelduck, *Tadorna tadorna* (L.), died at the Cork City Lough in 1962. Veterinary records of this incident revealed ingested lead as the cause of mortality (D. Twomey, pers. comm). In 1980, 21 mute swans died of lead poisoning on the southern shore of Lough Neagh, Co. Down, the location of a clay pigeon shooting site (R. Davidson, pers. comm.).

This paper reports on current Irish work on lead poisoning of swans, examines relevant fishing practice and considers possible legislation.

CURRENT RESEARCH

Current research aims to establish the occurrence and extent of elevated lead levels and causes of mortality in Irish mute swans. Blood samples are collected from swans and methods of sampling and analysis follow those published elsewhere (O'Halloran and Duggan 1984). In

addition, birds are fitted with coded rings for individual recognition and to determine the extent of movement within the country. Knowledge of movements should prove to be of considerable use in evaluating birds as biological indicators of pollution, and in determining a focus of contamination. Where possible, live birds have been X-rayed to determine if ingested lead shot is present.

Lead is a ubiquitous element and although lead shot, either from anglers' weights (Birkhead 1982) or from hunters' gunshot cartridges, is commonly regarded as a direct contaminant, it should be realised that background levels of lead vary considerably and probably contribute to elevated levels in birds. Environmental lead, particularly from vehicle exhaust, undoubtedly increases blood lead levels in urban populations of birds. Fifty per cent of birds sampled at Cork City Lough, for example, had between 0–2 μ moles Pb/L, equivalent to 40 μ g/100mL (cf. levels in humans: see paper by M. Murphy, this volume) (Fig. 1), which probably reflects background levels of lead in the environment. It should be borne in mind, however, that the swan community of the lough is composed, on the one hand, of a mainly urban group and, on the other hand, of an immigrant component originating in a wide range of rural sites. Many rural birds have less than 1 μ mole Pb/L of blood, while urban birds range from 1–2 μ moles Pb/L. Whilst the number of samples taken elsewhere (Dublin, Shannon and Killarney) is small, a trend of low values of lead is apparent (Fig. 1). These examples illustrate that environmental lead is present in varying degrees and the total amount varies from location to location.

In the present work, Cork City Lough was chosen as the main sampling site. The lough is a small shallow freshwater lake located in the western suburbs of Cork city about 7.5 ha (15 acres) in size. It is one of the most famous coarse fishing areas in the country and the risk from discarded anglers' weights is considerable. In addition, spent gunshot, from shooting during the early part of this century, must also be considered as a possible source of lead contamination. Owing to artificial feeding by man, a large flock of mute swans (up to 200) visit and feed in the area. These birds are at high risk from lead poisoning.

It is generally considered that birds with greater than 2.0 μ moles of Pb/L blood have been contaminated by excess lead (Birkhead 1983), and this criterion is used here. On the same basis, more than 50% of birds sampled at the lough have elevated blood lead levels. However, because of the mobility of the lough's swan flock, a focus of contamination is difficult to determine. O'Halloran and Collins (1985), for example, have shown that 30% of ringed swans travel over 30km. Current results show that birds of the lough move a considerable distance and frequently

commute from areas up to 20km away (Fig. 2). The recovery of a bird at the lough which had been ringed in Dublin 10 months earlier demonstrates this.

The problem of determining a source of contamination is compounded by the fact that regular benthic sampling techniques are inadequate for surveying areas like the lough. A standard sampling grab will sample mud

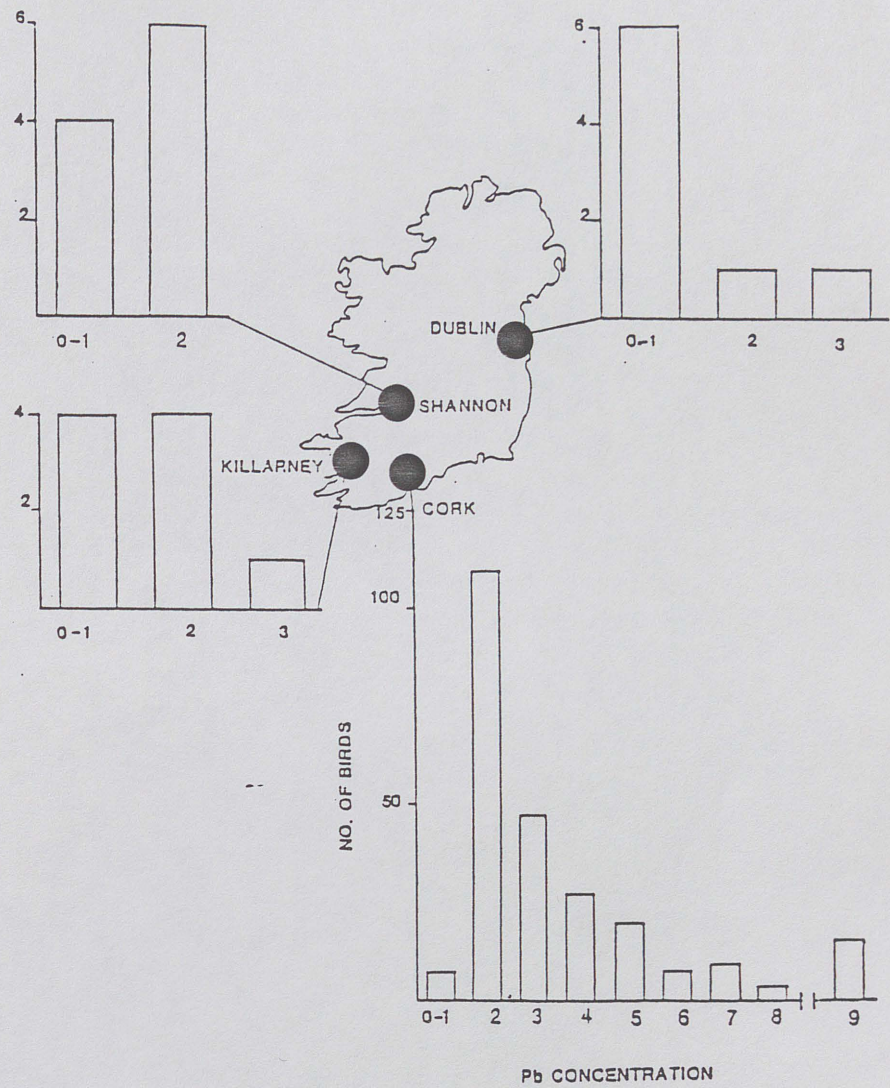


FIGURE 1: The recorded blood lead levels in Irish mute swans from various locations. The blood lead levels are in $\mu\text{moles per litre}$.

effectively, but may not pick up lead weights or line snagged in weed or stones. Nevertheless, the death of a 2-month-old, flightless cygnet at the lough, which was shown to have elevated lead levels and contained four angling shot at *post-mortem*, implicates the lough as one of the main sources of contamination.

Radiological examination of birds has confirmed that elevated levels of blood lead are due to ingested lead. Seven of the thirty-eight swans X-rayed showed lead pellets to be present. Three of these birds had ingested lead and four had been sprayed with gunshot. Interestingly, 'shot-in' lead caused no increase in blood lead level, while ingested lead caused a considerable elevation. One individual which had one anglers' weight in its gizzard, as revealed by X-raying, and a blood lead level of over $4\mu\text{moles Pb/L}$, had suffered considerable loss of body weight and metabolic damage (anaemia). Subsequent radiological examination of this individual (8 weeks later) revealed the lead weight had been worn away. The bird's condition, however (as indicated by plumage condition and haemoglobin level), remained poor. Although lead levels such as those mentioned above may not be sufficient to cause death, the birds' reproductive capacity (Birkhead 1983) and dispersal ability (Bellrose 1959) may be affected.

Although the levels of lead in the blood of swans in the lough are high, the incidence of death as a result of lead poisoning is unknown. While the presence of anglers' lead in the gizzards of dead birds is presumptive evidence of lead poisoning, only chemical analysis of body tissues can

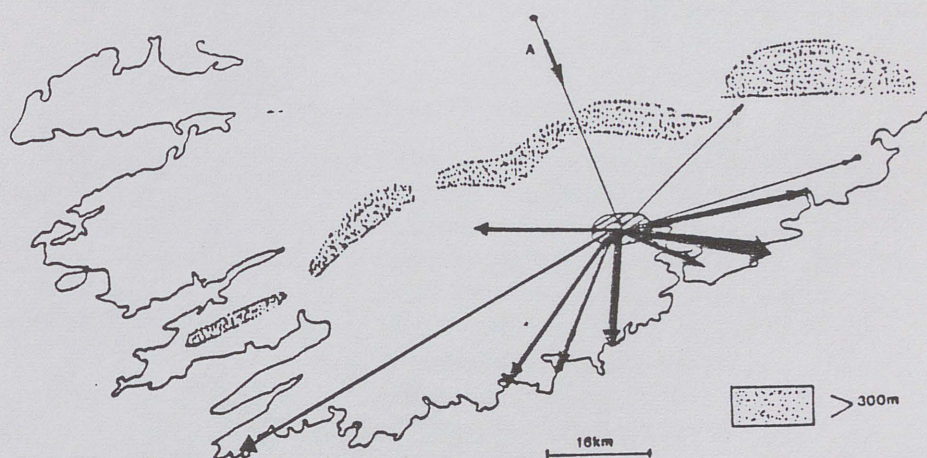


FIGURE 2: Recorded movements of swans ringed at Cork City Lough. A = one bird transported to Charleville which later flew back to the lough.

TABLE 1: Causes of death in Irish swans found at post-mortem. Five in the 'unknown' category had lead shot present in their gizzards.

Location	hypo- thermia	collis- ions	oil	Tuberc- ulosis	choked	Unknown	Total
Cork	2	7	0	1	4	19	33
Dublin	0	2	2	0	0	1	5
Clare	3	0	0	0	0	0	3
Limerick	1	0	0	0	0	0	1
Waterford	0	0	0	0	0	1	1
Mayo	0	0	0	0	0	1	1
Total	6	9	2	1	4	22	45

confirm acute lead poisoning as the cause of death. Chemical analyses have yet to be carried out on the tissues of dead birds examined. Causes of mortality in a sample of Irish swans are presented in Table 1.

Despite the lack of confirmatory chemical evidence, coarse angling activity, both past and present, at Cork City Lough appears to be implicated in causing the elevated blood lead levels recorded in 50% of mute swans sampled. At present, however, no mass mortalities have resulted from the ingestion of discarded anglers' lead weights. Because of the potential damage to swan populations through effects on metabolic pathways as well as through mortalities, it is appropriate to review angling practices in Ireland.

ANGLING PRACTICES AND ACTIVITIES

One of the noteworthy aspects of lead poisoning in waterfowl is its emergence as a problem only recently. Anglers have been using lead shot for over a century, but no mortalities were recorded prior to the 1970s. It is now considered that a change in angling practices in the 1960s has caused this environmental hazard. Prior to the early 1960s, anglers used cotton lines with hooks and lead weights attached. In the early 1960s, with an increase in fishing as a recreational activity and the construction of many gravel pits and reservoirs, particularly in Britain, coarse and game fishing became popular. With this change, a revolution in fishing accessories occurred. Nylon lines were introduced and hooks and lead weights were frequently stripped off and thrown away at the end of the day's angling. The availability of different types of nylon lines with different breaking strains also increased the possibility of more lead weights being lost when lines became snagged. Thus, enhanced fishing

activity and a change in angling accessories initiated the problem of lead poisoning in mute swans.

In Ireland, there are approximately 1000 coarse anglers (E. Parkes, pers. comm.). In addition, many coarse anglers visit Ireland to participate in international competition. However, as in England, little information is available on 'angling litter' discarded by anglers in this country. Recently, Bell *et al.* (1985) have researched the problem in south Wales. Angling litter was compared from two sites, one a game fishery and the other a coarse fishery. Considerable quantities of nylon monofilament line were recovered from the game fishery, where anglers were found to drop, on average, 2-3m of line per fishing trip. At the coarse fishery, less nylon line was recovered. There were, however, high densities of lead shot in the mud, with up to 125 shot m⁻² surface area in front of the fishing pegs. It was estimated that more than 15,000 lead shot (total weight 5kg) are deposited at the fishery (area 1 ha) each year. This represents 2-3 shot dropped per fishing trip (Bell *et al.* 1985). Shot weights were in the range 0.03 to 0.10g (this corresponds to shot size 8 and SSG), and represent the entire range of commercially available split shot. It is also interesting to note that, on average, 75% of discarded shot which was recovered in the sampling area was within 1m of the bank. It is clear that such a density of lead shot in the mud could pose a serious threat to waterfowl using the site. The coarse angling density in Ireland is considerably lower, except during infrequent international meetings, but it is essential that threats to our waterfowl be minimised. In view of the fact that coarse angling is the fastest growing aspect of the sport (E. Parkes, pers. comm.), it is essential that alternatives to lead weights be used to protect our environment and wildlife.--

To date, there are six non-toxic products on the market as alternatives to lead weights. The criteria required are high density and cheapness; details of alternatives are as follows:

1. Sandvik Safeweight: split weights and ledgers made from a tungsten-based polymer.
2. Anglers' Weight by Evode: a tacky, rubber-based, powdered steel mixture in convenient strips.
3. Saturn-shot: reusable zinc-plated steel weight, of various sizes, coloured to resemble lead.

4. Coiled shot: malleable stainless steel coils of various sizes.
5. Thamesly Sure Shot: a tough durable alloy that locks firmly and duplicates lead shot in colour, size and weight.
6. Blu-tack: a pliable polymer which can be shaped and attached to a line.

Although alternatives to lead shot are difficult to obtain in Ireland, it is hoped that they will soon become widely available. Alarminglly, despite widespread availability in Britain (the Royal Society for the Protection of Birds found in 1984 that alternatives were available in over half of the tackle shops visited in major coarse fishing areas) they were not being purchased by anglers. A survey of anglers, carried out by Cambridge Division of Anglian Water Authority, reported that in 1984 only 3% were using alternative weights. While the British government would like to see a voluntary withdrawal of lead weights by the end of 1986, it does not propose to introduce legislation until 1987. The introduction of legislation in Britain could result in the dumping of large quantities of lead weights on the Irish market. Thus legislation in Ireland may become necessary soon. The first attempts to introduce such legislation for the banning of lead angling weights was made by Irish members of the European Parliament in May 1985. In their motion for a resolution, a call was made on the EEC Commission to examine possible ways of imposing a total ban on the use of lead weights. In Britain, legislation is to be introduced from 1 January 1987. if the voluntary approach, supported by angling organisations, has not worked by the end of the 1986 fishing season. At present, the British government has banned the use of lead weights for those applying for new licences to fish in royal parks. Although existing licences will not be affected, steps are being taken to encourage all anglers to use the alternative weights. The government has instructed the English and Welsh Water Authorities to introduce regulations under Water Authority by-laws to ban lead weights.

In Ireland, angling interests have taken steps to encourage their members to use alternatives to lead shot. However, with proposed legislation in Britain, there is a considerable risk of surplus lead shot being brought into Ireland. In addition, if no legislation is enforced here, visiting anglers will still be using lead, causing a hazard to our wildlife. The banning of the sale and use of lead weights in Ireland would prevent such an environmental problem.

ACKNOWLEDGEMENTS

We are grateful to Mr T. Carruthers, Mr R. Collins and Mr P. Brennan for help in the fieldwork, and Dr T. Kelly for helpful discussion.

REFERENCES

- ANDERSON, W.L. 1975 Lead poisoning in waterfowl at Rice Lake, Illinois. *Journal of Wildlife Management* 39, 264-270.
- BELL, D.V., ODIN, N. and TORNES, E. 1985 Accumulation of angling litter at game and coarse fisheries in south Wales, U.K. *Biological Conservation* 34, 1-11.
- BELLROSE, F.C. 1959 Lead poisoning as a mortality factor in waterfowl populations. *Illinois Natural History Survey Bulletin* 27, 235-288.
- BIRKHEAD, M. 1982 Causes of mortality in the mute swan *Cygnus olor* on the River Thames. *Journal of Zoology* (London) 198, 15-25.
- BIRKHEAD, M. 1983 Blood lead levels in mute swans *Cygnus olor* on the Thames. *Journal of Zoology* (London) 199, 59-73.
- FINLEY, M.T., DIETER, M.P. and LOCKE, L.N. 1976 Lead in tissues of Mallard Duck dosed two types of lead shot. *Bulletin of Environmental Contamination and Toxicology* 16, 261-269.
- HAWKINS, A.S. 1965 The lead poisoning problem in the four flyways. In M.A. Cox (chairman), *Wasted Waterfowl*, 21-60. Report by Mississippi Flyway Central Planning Committee.
- HUNT, A.E. 1977 Lead poisoning in swans. *British Trust of Ornithology News*, No. 90, 1-2.
- NATURE CONSERVANCY COUNCIL 1981 *Lead poisoning in swans*. Nature Conservancy Council, London.
- OWEN, M. and CADBURY, C.J. 1975 Winter feeding ecology and mortality of swans at the Ouse Washe, England. *Wildfowl* 26, 31-42.
- O'HALLORAN, J. and DUGGAN, P.F. 1984 Lead levels in mute swans in Cork. *Irish Birds* 2, 501-514.
- O'HALLORAN, J. and COLLINS, R. 1985 Preliminary analysis of ringing mute swans. *Irish Birds* 4, 85-89.
- PHILIPS, J.C. and LINCOLN, F.C. 1930 Cited in Bellrose, F.C. 1959, in *Illinois Natural History Survey Bulletin* 27, 235-288.
- THOMAS, G.J. 1980 Review of ingested lead poisoning in waterfowl. *International Waterfowl Research Bureau Bulletin* 46, 40-43.
- WILCOVE, D.S. and MAY, R.M. 1986 The fate of the California Condor. *Nature* (London) 319, 16.

CHAPTER 3: SECTION 2

LEAD POISONING IN SWANS AND SOURCES OF CONTAMINATION IN IRELAND.

ABSTRACT

A study of blood lead levels in Mute swans Cygnus olor in Ireland has revealed that ingested lead pellets are responsible for acute lead poisoning. Forty two percent of blood samples from 870 live birds at one site showed elevated lead levels. X-ray examination of live birds revealed the source of contamination to be ingested lead pellets. Urban birds were shown to have higher ($P < 0.001$) lead levels than rural birds, the blood lead levels of which were presumed to reflect natural background levels. Urban grass was shown to have elevated lead but this did not cause lead poisoning in Canada geese Branta canadensis. Post-mortem examination has shown that 68% (n=101) of all Mute swans examined from a number of sites died from lead poisoning. Two sources of poisoning were identified; spent gunshot from a claypigeon shooting site at Lough Neagh Northern Ireland and lost or discarded anglers' weights at Cork Lough and at a fishing pond in Belfast, N.I. The first known case of lead poisoning in Whooper swans Cygnus cygnus in Ireland is recorded which resulted from the ingestion of gunshot used almost two decades earlier. Aspects of the pathology of lead poisoned swans is discussed.

INTRODUCTION

The importance of lead shot from shotgun cartridges as a source of environmental contamination has been widely investigated in North America since first recognised in 1874 (Philips and Lincoln, 1930; Jordan and Bellrose, 1951; Bellrose, 1959; Rosen and Bankowski, 1960; Trainer and Hunt, 1965; Bagley et al., 1967). The significance of this source of contamination has also been investigated in Europe, especially in England (see Olney, 1960; Thomas, 1975; Mudge, 1983). Other countries in Europe where investigations have been carried out include: Scotland (Owen, 1987), Italy (Del Bono, 1970), Sweden (Danell and Anderson, 1975), Denmark (Clausen and Wolstrup, 1979), France (Hovette, 1974), Switzerland (Bouvier and Horning, 1965), Federal Republic of Germany (Borkenhagen, 1979), Norway (Holt et al., 1978) and the Netherlands (Th.Smit pers. comm.1986). In many of the above studies workers have simply looked at the prevalence of ingested pellets in the gizzard which, though useful, is only presumptive evidence of lead poisoning. Quantitative chemical analyses of tissues for lead is the only diagnostic test of lead poisoning in dead birds.

Lead is an ubiquitous element in the environment, principally from the use of organo-lead additives in petrol. Thus it is useful to define the categories of lead exposure. Two categories are recognised, namely background and acute exposure. Background exposure is a function of organo-lead input and thus is variable but can be roughly divided into two groups depending on traffic density, namely rural and suburban/urban exposure (Johnson et al., 1982). Exposure to high urban background lead for considerable periods of time may lead to chronic lead poisoning, this has recently been reported in Feral pigeons

[Columbia livia (Gmelin); Ohi et al., 1974; Johnson et al. 1982].

Acute poisoning occurs from excessive exposure to lead over a short period of time, through for example, the ingestion of lead weights.

In 1973, 18 Mute swans [Cygnus olor (Gm)] were found dead or dying on the River Trent, England. Post-mortem examination implicated lead poisoning from fishermen's weights rather than gunshot (Simpson et al., 1979). At least (29%) of 128 swans [C. olor ; C. c. cygnus (L) and C. c. bewickii (Yarell)] found dead at the Ouse Washes between 1969 and 1975 (Owen and Cadbury, 1975) and 52% of C. olor from rivers, lakes and gravel pits in the English Midlands (Hunt, 1977) died from acute lead poisoning following ingestion of anglers' split lead shot and fishing weights. The most recent data suggests that between 3,370 - 4,190 Mute swans die in England each year from lead poisoning following the ingestion of anglers' lead weights (Thomas et al., 1987). As a consequence regulations and legislation have been implemented banning the use of certain angling accessories to protect birds from lead poisoning.

Despite the fact that pieces of lead have been found in swans' gizzards, many anglers maintain that sources of lead other than angling weights have caused lead poisoning in swans (Haines, 1984). One such case has been reported by Benson et al., (1976), who reported the deaths of 13 Whistling swans Olor columbianus (Ord) from the ingestion of vegetation contaminated from nearby mines and smelters.

In Ireland there are approximately 90,500 people with game endorsements to their shot-gun licences (Dept Justice, pers. comm.). The quantity of lead discharged into the environment is therefore considerable. Of 122,000 anglers resident in the State, it is estimated that 9,000 (7.1%) are coarse-fish anglers. In addition

42,000 anglers visit the State each year, of whom, 16,380 (39%) are said to be coarse-fish anglers (Anon, 1986). O'Halloran and Duggan (1984) reported the death of a Mute swan from lead poisoning resulting from the ingestion of anglers' weights in Co.Cork. Because of the seriousness of the problem in England it is essential to know the prevalence of lead poisoning in swans in Ireland, but to date very little research has been carried out on Mute swans. There is a great need to establish baseline information on the species, one of the most susceptible to lead poisoning.

Little is known about what contribution contaminated grass in an urban environment makes to acute lead poisoning in swans. In the present work, blood lead levels in a bird [Canada Geese Branta canadensis (L)] which habitually grazes grass, were examined to see if ingestion of lead contaminated grass resulted in an increased blood lead levels and caused acute poisoning.

Some workers, (e.g. Birkhead, 1982), have suggested that boating activities, particularly those which 'churn up' sediments, may cause lead weights to be made more readily available to birds. Careful selection of sample sites is necessary to determine what contribution, if any, urban lead makes to cause lead poisoning in swans.

Two methods are commonly employed to evaluate the level of lead poisoning in animals (a) measurement of whole blood lead, which has been useful in measuring lead levels in humans (Chamberlain, 1985) and other animals including Mute swans (Birkhead, 1983) and (b) examination of dead birds, which can provide conclusive evidence of lead poisoning.

In the present study, blood lead levels were examined in live Mute swans from different regions and these data were coupled with

examination of all available dead birds to determine the extent and causes of lead poisoning in Irish swans. Only with this type of information can appropriate conservation policy of swans in Ireland be formulated.

MATERIALS AND METHODS

Study sites

Blood samples were collected from Mute swans at six sites in Cork, Galway, Belfast, Dublin, Clare and Kerry, corpses were collected from Kilcolman Wildfowl Refuge and Lough Neagh and as many other sites (Table I). The principle sampling site was at a Lough in the western suburbs of Cork city (Fig.1). This freshwater lake (c.15 acres) is a refuge for a number of wildfowl including 60 Canada geese and up to 160 Mute swans. The maximum depth is 1.5 M in the northern basin and no boating is permitted. It is an internationally renowned coarse angling site (Anon, 1986). Two further urban sites were selected namely, the Antrim Road Waterworks, Belfast and Galway Harbour. It was hoped that these samples would reflect levels of organo-lead contamination at an urban site (Table I). The other three sites, Broad Meadows estuary, Malahide; Shannon Lagoon, and Lough Leane (Fig.1) were selected to represent areas with low and presumably background levels of organo-lead (Table I).

Collection and analyses of blood samples

Blood samples were taken from uniquely ringed birds between December 1984 and November 1986. Samples (5ml) were taken from the brachial vein or, in the case of cygnets, from the tarsal vein (O'Halloran et al., 1987a). Blood samples were also taken from Canada geese throughout the period of sampling at Cork Lough. Samples were immediately placed in low lead lithium heparin tubes and stored at -20°C. An aliquot was also placed in dipotassium ethylene diamine tetra acetic acid (EDTA) tubes for haemoglobin (Hb) analysis.

Table.I. Location and number of live birds blood sampled and corpses collected from October 1984 to April 1987.

Location	Irish Grid Reference	No. Live birds Blood sampled	No. Corpses examined	Sources of lead contamination
Cork Lough	W665704	870	41	High organo-lead* and angling lead Shooting 100 years ago.
Lough Neagh	H901661	0	49	Low organo-lead claypigeon shooting.
Antrim Road Belfast	J325775	16	3	High organo-lead angling in the catchment of swans
Galway	M297249	13	0	High organo-lead
Shannon Lagoon	R375601	10	2	Low organo-lead
Malahide	O195475	53	4	Low organo-Lead
Lough Leane	V930880	9	0	Low organo-Lead
Kilcolman Wildfowl Refuge	R581111	0	4	Low organo-Lead Shooting 18 years ago.
Others		0	2	-

* Organo-lead estimates based on traffic density (after Cabot, 1985).



Fig.1 Map of Ireland indicating the main areas of sampling.

1 = Cork Lough, 2 = Malahide, Co.Dublin,
3 = The Waterworks, Belfast, 4 = Lough Neagh,
5 = Galway Harbour, 6 = Shannon Lagoon, Co.Clare, 7 = Lough
Leane, Co.Kerry and 8 = Kilcolman Wildfowl Refuge, Co.Cork.

Blood lead concentration was determined on a Pye Unicam SP192 atomic absorption spectrophotometer with a flameless atomiser attachment and at a wavelength of 217nm. 10% nitric acid (Aristar grade) was used for digestion. Control specimens (United Kingdom Quality Assurance scheme for trace elements with known consensus means) were run routinely. Haemoglobin concentration was estimated for all blood samples based on a method converting all haem species to alkaline haematin using a non-ionic detergent and the end-product was read spectrophotometrically at 575nm (for detailed account see O'Halloran et al., 1987b).

Post mortem examination

Corpses were examined at the Department of Zoology, Cork, or specimens from Northern Ireland, at the Veterinary Research Laboratories at the Department of Agriculture, Northern Ireland by personnel from the pathology laboratory. Where possible the following parameters were recorded: month of death, locality of corpse, age, sex, weight, presence and number of lead shot in the gizzard, lead levels (in ug/g W.M.= wet matter) in liver and kidney. Lead was determined following extraction in 10% nitric acid (Aristar Grade) at 115°C for 12 hours and estimated on a Pye Unicam SP192 graphite furnace. The upper alimentary canal (from beak to gizzard) was examined for fishing tackle and/or lead shot. Ingested angling lead was distinguished from ingested gun-shot lead by the grey colour and flattened appearance as opposed to the black and round appearance of shot-gun pellets. The contents of the proventriculus were washed into a white dish and rinsed so that any lead weights or fragments could be identified. Following examination the contents were further dried at 100°C and examined for

lead. They were subsequently stored for later re-examination using X-ray. Birds were weighed on a 20Kg Salter Spring balance, but if the plumage was wet or any part of the body was missing, no weight was taken. Age was determined by plumage characteristics and beak colour.

X-ray of live birds

Forty one live swans were X-rayed on a C.G.R. Unimax 500 X-ray machine. Two X-rays (17cm X 14cm) were taken of each bird on a 'potter buckey' set at 300 mA, 60Kv for 0.60 sec. X-rays were stored and subsequently examined for lead signatures, identified as white spots on the X-ray film.

Environmental lead

In order to estimate the quantity of lead (other than pellets) in the environment, grass and water samples were taken at Cork Lough from January 1985 to January 1986. Grass samples were dried to a constant weight at 70°C for 24 hours and then digested using 10% nitric acid as for tissues. Lead levels were determined using a Pye Unicam graphite furnace as mentioned above.

At Cork Lough, eleven randomly selected stations on the bed were sampled for discarded lead weights. A total of 200 grab samples (0.02m^2 each) were taken and returned to the laboratory, sieved through 2.8mm and 1.4mm sieves (to aid easy examination) and then examined for lead shot. At Lough Neagh, grab and core samples (depth =40cm) of the bed were taken close to where dead birds had been recovered to establish the distribution and density of lead shot. At Kilcolman Wildfowl Refuge, eight replicate quadrats (0.25m^2) were taken and examined for lead shot. The number of anglers using lead weights and the time of peak angling was also noted at Cork Lough.

Classification of lead poisoned swans

The following criteria were used to classify live lead poisoned birds: birds with a blood lead level in excess of 3.00ug Pb/gHb were considered to have exceeded the maximum tolerable limit of lead [equivalent to 2.00 umoles/L or 40ug/ 100ml following Simpson et al., (1979) and Birkhead (1982)]. This criteria is used since 80% of lead in whole blood is bound to haemoglobin (Ong and Lee, 1980) and it was found (O'Halloran et al., in press) to be a more precise criterion for classifying birds with elevated lead. In dead birds, values of lead greater than 31.25ug/g (W.M.) in kidney and 12.50ug/g (W.M.) in the liver were considered diagnostic of lead poisoning following Clarke and Clarke (1975).

Distributions were compared using a Mann-Whitney U test. Chi squared tests were used to measure observed versus expected ratios of sex and age in dead lead poisoned birds versus birds dying from other causes. All analyses were carried out using a Minitab (Pennsylvania University) statistical package.

RESULTS

Blood levels

Blood lead levels varied depending on the degree of exposure to lead, and were significantly lower ($P < 0.001$) at 'country' sites (Malahide, Killarney and Shannon), reflecting the expected lower levels of lead (Table.1). At these sites, only 4.5% of samples showed elevated lead levels (i.e. $> 3.00\text{ugPb/ gHb}$) with 82% of samples less than 2.00ugPb/ gHb (Fig.2). A significant increase in blood lead level was found in birds sampled in urban areas (Galway and Belfast) where 17% of birds had elevated lead. There was a noticeable increase in the class $2.00 - 3.00\text{ugPb/ gHb}$ from 13.50% at the country sites to 27.50% at the urban sites (Fig.2). It is presumed that at least one of these birds had ingested lead weights since, when it was found dead a few months later with ingested anglers' weights in it's gizzard, it had a whole blood lead value of 62.00ugPb/ gHb .

At Cork Lough, values of blood lead differed significantly from samples from other sites ($P < 0.001$), the proportion of samples with high lead levels being much greater (see Fig.2). Forty two percent of birds sampled, were suffering from acute lead poisoning (i.e. $> 3.00\text{ug Pb/ gHb}$). This sample constituted over 90% of the birds at the Cork Lough. The range of the values was wide ($0.46 - 106.30\text{ugPb/ gHb}$).

Post-mortem results

A total of 101 dead or moribund Mute swans, mostly from Co.Cork and Lough Neagh, and four Whooper swans from Kilcolman Wildfowl Refuge, Co.Cork, were examined (Table II). Four categories of birds could be identified at post-mortem:

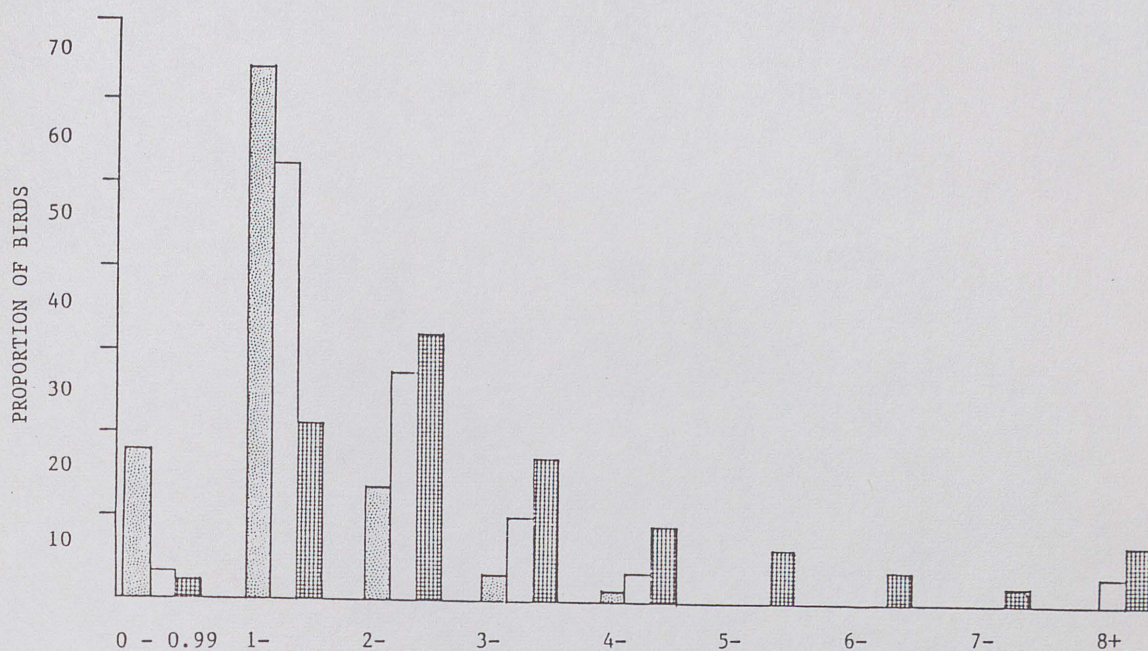


Fig.2. Blood lead levels (ugPb/ gHb) of swans in country sites (stippled) (n=72), Urban sites (white), (n=29) and The Lough (cross-hatched), (n=870).

Table.II Proportion of lead poisoned swans from those examined from October 1984 to April 1987, with details of other causes of mortality.

	LOCATION					OTHERS ¹	TOTAL
	CORK	DUBLIN	BELFAST	LOUGH	NEAGH		
NO.EXAMINED	41	4	3	29	(20) ²	4	101
NO. LEAD POISONED	17	0	3	29	(20)	0	69

¹ Others = Co.Clare (2), Co.Limerick (1), Co.Mayo (1). ² Prior to this date 20 Mute swans were lead poisoned at Lough Neagh in 1980 which are also included. Other causes of mortality include hypothermia (6), Collisions (9), Oiled (3), Shot (2), Infection (1), Choked (1), killed by another swan (1) and Unknown (9).

Group (A) ten Mute swans with ingested anglers' pellets and with kidney lead levels (range = 40.00-305.00 ug/g W.M.) and liver lead levels (range = 170.50-450.00 ug/g W.M.) diagnostic of acute lead poisoning.

Group (B) ten Mute swans and two Whooper swans with liver lead (range = 18.00-550.00 ug/g W.M.) and kidney lead levels (range = 12.50-145.00 W.M.) exceeding the critical value diagnostic of lead poisoning, which had no lead pellets in their gizzards, but tissue lead levels and pathological findings which incriminated lead poisoning as the cause of death.

Group (C) twenty Mute swans with low lead levels in kidney (range = 0.40-19.00 W.M.) and liver (range = 1.00-14.00 W.M.) which had died from causes other than lead poisoning (Table II).

Group (D) 49 Mute swans with shotgun pellets in their gizzards and with high tissue lead levels. kidney lead levels exceeded the criteria for diagnosis of lead poisoning (range = 142.00-350.00ug/g W.M.) and numbers of pellets varied from five to over 100 per bird (P.Cush, pers. comm.). In addition two Whooper swans that fell into this category had kidney and liver lead levels that exceeded the values for diagnosis of lead poisoning, being from 46.50 and 360.00ug/g W.M. and 30.00 and 800.00ug/g W.M. respectively. Both birds had ingested gunshot in their gizzards (one with seven and one with 25 pellets).

On the basis of diagnostic tissue lead levels, 68% of all Mute swans examined were shown to have died from lead poisoning which was thus the single largest cause of mortality. Other causes of mortality are shown in Table II. The pathological characteristics of lead poisoned birds in this study agree with those of other workers (e.g. Simpson, et al., 1979). These included gross emaciation, impaction

of the gizzard and oesophagus and distended gallbladder. In the case of the Whooper swans, two of the birds showed impaction of food from the beak to and beyond the gizzard, with a further 130g of grass and food impacting the duodenum. A number of moribund birds with symptoms of lead poisoning were observed prior to death. Symptoms included abnormal carriage of neck, lethargy, inability to fly for four to five weeks prior to death and poorly orientated movements.

There was no significant difference (Mann Whitney U) between the weights of lead poisoned and other dead swans. The observed ratio of sexes in lead poisoned swans did not differ (X^2 , $P > 0.05$) from the normal population ratio of 54% males:46% females. More adults than juveniles died from lead poisoning than expected (X^2 , $P < 0.001$).

Most lead poisoned swans died in winter. In the Cork Lough catchment, 14 birds died from lead poisoning between November and March, but only six birds died of lead during the other months, though the large sample from Lough Neagh ($n=49$) greatly influences the overall data (Table III).

Of birds X-rayed, 27% (of 41) had lead in their bodies (Table IV). Three had been sprayed with shotgun pellets but this shot-in lead did not cause any elevation in blood lead level (Table IV). Ingested pellets in contrast, caused an increase in whole blood lead. Nevertheless two birds, (Table IV) though positive for lead, showed no increase in blood lead. Eight birds, which had no pellets in their gizzards, had elevated blood lead values ranging from 3.10 ugPb/ gHb to 5.20 ugPb/ gHb. Five of these birds had histories of elevated lead (as recorded from a number of previous blood samples). One bird when X-rayed was shown to have seven pellets in it's gizzard, but after death a week later, post-mortem revealed 11 pellets. X-ray of gizzard

Table III Seasonal pattern of dead Mute swans submitted for examination.

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Number	12	3	7	5	4	2	2	1	3	4	52	4
No. Lead Poisoned	5	1	4	2	1	1	1	0	0	1	51	2

Table IV. 11 (of 41) X-rayed swans with
lead pellets in their bodies.

Shot-in		Ingested	
No.	Blood lead	No	Blood Lead
4	2.60	1	2.50
5	2.00	1	3.60
6	1.45	1	4.84
		1	5.00
		1	9.50
		3	2.60
		7	40.00
		11	80.73

contents, performed as part of the post-mortem routine, did not reveal any additional pellets to those found during manual examination.

Environmental lead

Grass lead levels varied from month to month with the lowest values in summer (Table V). Water lead levels in some instances were so low as to be almost undetectable by the instrument. Median blood lead levels (2.00 ugPb/ gHb, n=23) of Canada Geese, which spend a considerable period of time grazing on the grass around Cork Lough, varied from 0.94 ugPb/ gHb to 2.50 ugPb/ gHb throughout the year but no values exceeded 3.00 ugPb/gHb. In addition, blood lead levels did not show any seasonal pattern or any relationship to grass lead levels (Table V).

Discarded lead weights were only found at one sampling station at the Cork Lough where five lead pellets were found ($/0.02\text{m}^2$). At Lough Neagh Co. Antrim, spent gunshot pellets were found along 100m of shore in front of a clay pigeon shooting site and on the lake bed up to 60m from the shore. The pellet density in the top 5cm was 2,400 pellets/ m^2 . Lead pellets were also found in the mud at Kilcolman Wildfowl Refuge, 14 shotgun pellets were recovered from 2 m^2 . Numbers of coarse-fish anglers at Cork Lough using lead weights varied with peak angling in summer (July/August) with a maximum of 12 rods per day.

Table V. Median water lead levels (umoles/L), grass lead levels (ug/g dry weight) from Cork Lough, January 1985 to January 1986.
 Range of values in parentheses.

	JANUARY	APRIL	JUNE	AUGUST	OCTOBER	JANUARY
WATER	0.10	0.01	0.10	0.70	0.45	0.50
GRASS	90.00	20.70	8.50	12.00	58.00	105.84
	(64.00 - 294.00)	(13.60 - 39.00)	(6.80 -107.00)	(11.86 -18.76)	(44.00 - 208.00)	(10.10 --182.00)
	n = 6	n =6	n =6	n =6	n =6	n = 6

DISCUSSION

Blood lead levels provide a very useful measure of the degree of contamination of environmental lead in swans. The level of lead found in birds from country sites was low ($<2.00 \text{ ugPb/gHb}$) and can be considered the background level of lead for Mute swans. However, Mute swan flocks are not sedentary, except at times of full primary moult, and one must consider the catchment area of the flock when interpreting blood lead levels. It has been shown (O'Halloran and Collins, 1985) that, in Ireland, ringed Mute swans travel up to 100Km. It is not surprising therefore that three (4.5%) rural birds show elevated lead. These birds probably ingested lead at some shooting or angling pond. At the 'urban' sites (Belfast and Galway), there was a noticeable increase in the proportion of birds with higher lead (Fig.2). This is also reflected in blood lead levels of swans at Cork Lough. Ohi et al. (1974) noted a similiar increase in blood lead levels in pigeons in urban areas of Tokyo.

In excess of 90% of the Mute swans in the catchment area of the Cork Lough were blood sampled. Of these, 42% were suffering from acute lead poisoning. Because of the nature of the flock status (i.e. a mixture of breeding birds, family parties and non-breeding birds), a very heterogenous assemblage of birds occurs at Cork Lough. This heterogeneity explains the variation in the blood lead levels found. Low lead levels indicate swans which had recently arrived, as shown from observations of uniquely ringed birds, from rural areas, whilst increased lead levels indicate those birds which were exposed to different levels of urban contamination. This usually depends on the duration of stay at Cork Lough of the birds and whether ingestion

of pellets had occurred. What is significant is the proportion (42%) of birds sampled which were suffering from acute poisoning following the ingestion of lead pellets. Since a largely non-breeding flock composed of sub-adults and non-breeding adults occurs at Cork Lough, it may have implications for the population as a whole in the south-west catchment. Birkhead and Perrins (1986) consider that non-breeding flocks are the reservoir of future breeding stock, so any sub-lethal effects or debilitation caused by lead poisoning at Cork Lough may have a serious effect on recruitment.

Of the dead birds examined in the present study, 68% had died from lead poisoning. This figure has to be treated with caution however, in that it is based on 1) three incidences in Northern Ireland, two involving a large number of birds and one involving four birds; 2) an intensive study of the Lough/ River Lee catchment in south-west Ireland and 3) a few birds sent to us for examination from other parts of Ireland (Table II). At Cork Lough and Willis' Pond, Belfast, ingestion of anglers' weights was the source of lead poisoning in swans. Similarly Birkhead (1982) working on the River Thames in the U.K. found that lead poisoning was almost exclusively attributable to ingestion of anglers' weights. By contrast, at Lough Neagh, lead poisoning in swans resulted exclusively from the ingestion of gunshot at a clay-pigeon shooting site. Deaths of Mute swans at clay-pigeon shooting sites have also been reported from Denmark (see Clausen and Wolsrup, 1979). Gun-shot pellets from wildfowling which had occurred more than 18 years earlier was responsible for the deaths of four Whooper swans at Kilcolman wildfowl refuge in Co.Cork.

Most birds affected by lead showed impaction of the oesophagus and gizzard, two Whooper swans also showed impaction of the duodenum. A

similiar pathology was described by Beer and Stanley (1965) in ducks but not by Simpson et al., (1979) in Mute swans. In a study on pigeons Cory-Slechta et al., (1980) no food particles were found beyond the crop. The exact mechanism by which lead toxicity affects the gastrointestinal tract is uncertain though crop dysfunction was induced in pigeons by feeding them lead (Cory-Slechta et al., 1980). Histo-pathological evidence available does not indicate degeneration in the neural innervation and the mechanism may be at the cholinesterase level, as suggested by Cory-Slechta et al., (1980). In the present work, there is some support for this suggestion since in Whooper swans the duodenum as well as the gizzard was impacted.

Lead poisoned birds did not differ significantly in weight from birds which died from other causes. This is important since Bacon and Coleman (1986) suggested that weight may be a useful indicator of lead poisoning in birds. Weight loss however, may be useful in identifying sick birds which could be further examined for evidence of lead poisoning.

Most lead poisoned birds died in winter. This pattern differs from the summer peak of lead related mortality on the river Thames reported by Birkhead (1982), but agrees with the pattern found by French (1984) in his study in East Anglia. Although there is no formal fishing season at Cork Lough, most fishing occurs in summer (July-August). It is suggested that the high density of birds and poor feeding (including some birds which have an almost exclusive diet of bread) are responsible for the lead related mortality in the winter.

Eleven of the X-rayed birds (27%) had lead pellets present of which three (7%) had been sprayed with gunshot (Table IV). The gunshot in this study caused no elevation of blood lead. Fluoroscopy of live

birds by Bellrose (1959) in the U.S. revealed 10.4% of live birds were carrying lead in their gizzards. In this study, 17% of Mute swans X-rayed had ingested lead present in their gizzards. The presence of at least one lead pellet caused an increase in blood lead in four out of five birds carrying lead. Blood lead varied considerably from 3.60-4.84 ugPb/ gHb (Table IV), presumably depending on the degree of pellet erosion. However, it is possible that some of the pellets were well eroded and/or masked by larger pieces of lead and thus not detected. Montalbano and Hines (1984) found that comparing whole gizzard X-ray with X-ray of gizzard contents, 7.8% of lead pellets went undetected by the former method. This may account for the discrepancy in the present work between numbers of pellets detected and blood lead levels recorded. This is further shown by one specimen in this study which had seven pellets when X-rayed, but eleven (some well worn), pellets at post-mortem a week later. This may also explain some of the eight birds found in this study with elevated lead - but no pellet signatures on X-ray examination. The other possible explanation is that these birds which had a history of lead poisoning had lost or had worn down their pellets while the blood lead level remained high. In view of investigations on lead poisoning elsewhere (see for example Janssen, et al., 1986), and from this current work it is suggested that in the case of the two specimens (with one and three ingested pellets and low blood lead levels Table IV), lead pellets may have been ingested just prior to X-raying and thus the blood lead level may not yet have increased. X-ray of gizzard contents revealed no extra lead pellets to those discovered during manual examination. This illustrates that careful examination of wet gizzard contents followed by drying and then further examination is adequate for detecting

ingested lead pellets in gizzards.

Grass lead levels at Cork Lough were variable with lowest values in summer. Furthermore, in Canada geese which are largely grazers, there was no relationship to the blood lead level despite a seasonal pattern of lead levels in grass. No large increase in blood lead or no acute lead poisoning occurred in the geese. Infact, the median lead level for Canada geese at Cork Lough only once exceeded that of background levels in Mute swans. It is probable that fresh grass growth after mowing is responsible for the lower grass lead levels in summer. No boating is carried out at Cork Lough, so this activity did not have any influence on the birds or their susceptibility to picking up lead pellets. The sampling methods employed for the recovery of lead pellets and their patchy distribution in the mud may have been responsible for the low recovery of angling lead pellets from Cork Lough. Nevertheless, the death through lead poisoning from ingesting anglers' weights of a flightless two month old cygnet in 1985 implicates the Lough as one of the contaminating sites. It is unlikely that the birds picked up angling lead from the river Lee. This river, which is part of the Lough catchment, is relatively fast flowing and this, coupled with tidal action in locations of high swan density and angling, is likely to 'wash away' any angling lead pellets. However, as mentioned earlier, because the Lough is only part of a catchment area within which the swans are resident (O'Halloran et al., 1987a), the possibility of Mute swans picking up lead pellets from other ponds cannot be dismissed. The concentration of lead pellets at Lough Neagh ($2,500/\text{m}^2$) was due to the presence of a clay pigeon shooting site adjacent to a swan feeding area, which lead to the Mute swans ingesting

the pellets as they moved in shore to feed on macrophytes. Clay-pigeon shooting has ceased since the deaths of 25 swans in 1985 (Table II). However, metallic lead persists in the environment, as illustrated by the fact that four more Mute swans died at the same location in 1987, two years after the last clay-pigeon shoot. At Kilcolman wildfowl refuge Co.Cork, the first known case of fatal plumbism in Whooper swans in Ireland occurred following the ingestion of gun-shot pellets scattered there at least 18 years previously. Beer and Stanley (1965) reported a similar situation in a study of lead poisoning at Slimbridge where there had been no shooting for almost two decades. Only two detectable differences were noticed at Kilcolman in the winter of 1986/87 from those of previous years. Firstly, there were more Whooper swans than previous years, 90 compared with 50 in 1981 (O'Halloran, 1981) and secondly, the birds spent more time feeding on potatoes. Whether it was a change in bird density and/or a change of feeding behaviour and hence the need for more grit which caused this incident to occur is unknown.

In Ireland, there are two main sources of acute lead poisoning in swans: a) shooting pellets (past and present) and b) discarded anglers' weights. While urban lead contributes to blood lead in urban birds it does not cause acute lead poisoning. Any policy to reduce lead poisoning in birds in Ireland should consider both past and present inputs of lead into the environment before any decision is made on the sighting of shooting sites or the holding of large scale angling competitions.

REFERENCES

- Anon (1986). Central Fisheries Board of Ireland Report. 'Inland fisheries strategies for management and development'. 195pp Dublin.
- Bacon, P.J. & Coleman, A.E. (1986). An analysis of weight changes in Mute swan Cygnus olor. Bird Study. 33: 145-158.
- Bagley, G.E., Locke, L.N. & Nightingale, G.T. (1967). Lead poisoning in Canada geese in Delaware. Avian.Dis. 11: 601-608.
- Beer, J.V. & Stanley, P. (1965). Lead poisoning in Slimbridge Wildfowl collection. Wildfowl.Trust.Ann.Rep. 16: 30-34.
- Bellrose, F.C. (1959). Lead poisoning as a mortality factor in waterfowl populations. Ill.Nat.Hist.Surv.Bull. 27: 235-288.
- Benson, W.W., Brock, O.W., Gabrica, J. & Loomis, M. (1976). Swan mortality due to certain heavy metals in the Mission Lake area. Idaho. Bull. Environ. Contam. and Toxic. 15: 171-174.
- Bouvier, G. & Horning, B. (1965). La pathologie du cygne tubercule (Cygnus olor) en Suisse. Mem.Soc.Vand.Sci.Nat. 14: 1-36.
- Borkenhagen, P. (1979). Schrotblei- und Bleivergiftungen der Wasserwild. Zeitschrift für Jagdwissenschaften. 3: 18-197.
- Birkhead, M. (1982). Causes of mortality in the Mute swan Cygnus olor on the River Thames. J.Zool. (Lond) 198: 15-25.
- Birkhead, M. (1983). Blood lead levels in Mute swans Cygnus olor on the River Thames. J. Zool. (Lond). 199: 59-73
- Birkhead, M. & Perrins, C.M. (1986). The Mute Swan. 157pp Croom Helm, London.
- Cabot, D. (1985). Editor, The State of the Environment. 206pp An Foras Forbartha. Dublin.

- Chamberlain, A.C. (1985). Prediction of response of blood lead to airborne and dietary lead from volunteer experiments with lead isotopes. *Proc.R.Soc.Lond.(B)* 224: 149-182.
- Clarke, E.G.C. & Clarke, M.L. (1975). *Veterinary Toxicology*. Bailliere Tindall. London.
- Clausen, B. & Wolstrup, C. (1979). Lead poisoning in game from Denmark. *Danish Rev. of Game Biol.* 11: 22pp.
- Cory-Slechta, D., Garman, R.H., & Seidman, D. (1980). Lead induced crop dysfunction in the pigeon. *Toxicol. and Applied.Pharm.* 52: 462-467.
- Danell, K. & Anderson, A. (1975). Lead shot in gizzards of ducks. *Statens Naturvardsverk, PM.583*: 23pp
- Del Bono, G. (1970). Il saturnismo degli uccelli acquatici. *Annali.Fac.Med.Vet.Univ. Pisa* 23: 102-151.
- French, M.C., (1984). Lead poisoning in Mute swans - an East Anglian Survey. In: *Metals in animals*, edited by D.Osborn, 25-29, (ITE Symposium no.12.) Abbots Ripton: Institute of Terrestrial Ecology.
- Haines, A. (1984). Angling paper bans lead-free advert. *New.Sci.* 104: 8.
- Holt, G., Froslic, A., & Norheim, G. (1978). Lead poisoning in Norwegian waterfowl. *Nod.Vet - Med.* 30: 380-386.
- Hovette, C. (1974). Le saturnisme des anatides sauvages. *L'Institut Technique de l'Aviculture*. July. 1974.
- Hunt, A.E. (1977). Lead poisoning in swans. *British Trust of Ornithology News*. 90: 1-2.
- Janssen, D.L., Oosterhuis, J.E., Allen, J.L., Anderson, M.P., Kelts, D.G. & Wiemeyer, S.N. (1986). Lead poisoning in free ranging California condors. *Amer.Vet.Med.Assoc.* 189: 1115-1117.

- Johnson, M.S., Pluck, H., Hutton, M., & Moore, G. (1982) Accumulation and Renal effects of lead in urban populations of Feral Pigeons Columbia livia. Arch. Environm.Contam.Toxicol. 11, 761-767
- Jordan, J.S. & Bellrose, F.C. (1951). Lead poisoning in wild waterfowl. Ill.Nat.Hist.Surv.Biol.Notes 26. 27pp.
- Motalbano, F. & Hines, T.C. (1984). An improved X-ray technique for investigating ingestion of lead by waterfowl. Proc.Ann.Conf.S.E.Assoc. Fish and Wildl.Agencies. 32: 364-368.
- Mudge, G. (1983). The incidence and significance of ingested lead pellet poisoning in British wildfowl. Biol.Conserv. 27: 333-372.
- O'Halloran, J. (1981) Cork Bird Report 33pp Irish Wildbird Conservancy.
- O'Halloran, J. & Duggan, P.F. (1984). Lead levels in Mute swans in Cork. Irish Birds. 2: 501-514.
- O'Halloran, J. & Collins, R. (1985). Preliminary analysis of ringing Mute swans in Ireland. Irish Birds. 3: 85-89.
- O'Halloran, J., Myers, A.A. & Duggan, P.F. (1987a). Lead poisoning in Mute swans and fishing practice in Ireland. In: Biological Indicators of Pollution, edited by D.H.Richardson, 183-191. Royal Irish Academy. Dublin.
- O'Halloran, J., Duggan, P.F. & Myers, A.A. (1987b). Determination of haemoglobin in birds by a modified alkaline haematin (D-575) method. Comp.Biochem.Physiol. 86B 701-704.
- O'Halloran, J., Duggan, P.F. & Myers, A.A. (in press). Blood lead levels and Free red blood cell protoporphyrin as a measure of lead exposure in Mute swans. Environmental Pollution Series (A)
- Ohi, G., Seki, H., Akiyama, K. & Yagyu, H. (1974). The pigeon, as a sensor of lead pollution. Bull.Environ.Contam. and Toxic. 12: 92-98.
- Olney, P.J.S. (1960). Lead poisoning in wildfowl. Wildfowl Trust Ann. Rep. 11: 123-134.

- Ong, C.N. & Lee, W.R. (1980). Interactions of calcium and lead in human erythrocytes. *Br.J.Med.* 37: 70-77.
- Owen, M. (1987). Wetland Newsdesk. *Wildfowl World*. 96: 28.
- Owen, M. & Cadbury, C.J. (1975). Winter feeding ecology and mortality of swans at the Ouse Washes, England. *Wildfowl*: 26 31-42.
- Philips, J.C. & Lincoln, F.C. (1930). Cited in Bellrose, F.C. 1959, *Illinois Natural History Survey Bulletin* 27, 235-288.
- Rosen, M.N. & Bankowski, R.A. (1960). A diagnostic technique and treatment for lead poisoning in swans. *Calif.Fish and Game*. 46: 81-90.
- Simpson, V.R., Hunt, A.E. & French, M.C. (1979). Chronic lead poisoning in a herd of Mute swans. *Environ.Pollut.* 18: 187-202.
- Thomas, G.J. (1975). Ingested lead pellets in waterfowl at the Ouse Washes, England 1968-73. *Wildfowl*. 26: 43-48.
- Thomas, G.J., Perrins, C.M. & Sears, J. (1987). Lead poisoning and waterfowl. In: *Angling and Wildlife in fresh waters*, edited by P.S.Maitland & A.K.Turner, 5-6, (ITE Symposium No.19) Abbots Ripton: Institute of Terrestrial Ecology.
- Trainer, D.O. & Hunt, R.A. (1965). Lead poisoning of Whistling swans in Wisconsin. *Avian Dis.* 4: 252-263.

CHAPTER 4

SOME BIOCHEMICAL AND HAEMATOLOGICAL REFERENCE VALUES AND CHANGES IN BLOOD CHEMISTRY IN MUTE SWANS Cygnus olor WITH ACUTE LEAD POISONING.

This chapter is presented in the form of a manuscript recently submitted for publication in Avian Pathology.

SUMMARY

Reference levels of some biochemical and haematological parameters were determined for Mute swans (Cygnus olor Gmelin). Two physiological states, moulting and immaturity, were identified as factors which broadened the reference levels. Blood parameters of six acutely lead-poisoned swans were determined and compared with the reference distribution. Protoporphyrin IX, cholesterol and two serum enzymes, lactate dehydrogenase (LDH) and aspartate aminotransferase (AST), were found to increase in lead poisoned birds. Concentrations of haemoglobin, mean cell haemoglobin concentration and haematocrit indicated severe hypochromic anaemia in birds with acute lead poisoning. The diagnostic value and interpretation of reference values is discussed.

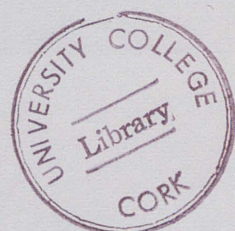
INTRODUCTION

Biochemistry plays an important diagnostic role in human and veterinary medicine. Clinical biochemistry is now increasingly being used in avian research and wildlife investigations. Unfortunately, the interpretation of findings is often difficult, partly because normal blood chemistry reference values for many species are not available, and partly because the response of the blood to toxic substances, diseases and infections has not been determined (Hawkey et al., 1983).

The veterinary diagnosis of lead poisoning in birds is increasingly being studied throughout the world. In the United States, Janssen et al., (1986), cited values for blood parameters of the California condor [Gymnogyps californicus (Shaw)], but the significance of these values is not clear, as no normal reference values were given. Similarly, Simpson et al., (1979), in the United Kingdom, examined haemoglobin and haematocrit values in Cygnus olor (Gmelin), but again these were not compared with 'normal' blood values. Roscoe et al., (1979) and Birkhead (1983) used free red blood cell protoporphyrin to measure lead exposure in Mute swans without knowing the normal range of values. While the blood chemistry of many species has been well studied e.g. domestic fowl (Freeman, 1984), and reference haematological values are available for some other species, e.g. cranes and flamingoes (see Hawkey et al. 1983, 1984), no reference values for Mute swan blood chemistry exist.

This paper presents some biochemical and haematological reference values, derived from Mute swans which were known to have low lead levels (O'Halloran et al., 1987a). Normal values were compared between

adults, immatures, females and males. Some biochemical and haematological changes in acutely lead poisoned birds were identified by comparison with reference values and related, where possible, to post-mortem findings.



MATERIALS AND METHODS

Collection of samples

Blood samples (5ml) were collected as part of a major study on lead poisoning in Mute swans at Cork Lough, Co.Cork, SW Ireland (see O'Halloran et al., in press). Manually restrained (unanaesthetised) birds were blood sampled from the brachial vein, or in the case of cygnets from the tarsal vein, using a 21 gauge 1 inch needle. All samples were taken each day between 10.00-11.30. Blood was placed in lithium heparin tubes. An aliquot was also placed in tubes containing dipotassium salt of ethylene diamine tetra-acetic acid (EDTA). For serial sampling, four live birds were caged for 15 hours (16.00-07.00) in pens, 4m X 1m, for the blood sampling procedure and subsequently released. Food and water were freely available at all time. Sex was determined by cloacal examination (after Hochbaum, 1942) and age was determined by plumage characteristics and beak colour. Cygnets and any birds with brown feathers were designated as immatures. Details of moulting status were noted.

In six lead poisoned birds, four Mute and two Whooper swans Cygnus cygnus (L), blood samples were obtained prior to death. Tissue samples (liver, kidney, pancreas, breast muscle, heart muscle and gizzard muscle) were taken at post-mortem from lead poisoned swans for lead analyses.

Blood chemistry and tissue analyses.

Haemoglobin (Hb) content was determined by converting all haem species to alkaline haematin by techniques described elsewhere (see O'Halloran, et al., 1987b). Haematocrit was measured following

centrifugation in microhaematocrit tubes for 30 mins at 12,000 X G (determined stroboscopically) in a Hawksley microcentrifuge.

Other blood chemical parameters were estimated as follows:

Aliquots of blood specimens collected in lithium heparin tubes were spun in a microcentrifuge (10,000 X G) for 5 mins. The plasma was removed and placed in cuvettes for analysis. Analysis was carried out in a selective multichannel autoanalyser (Hitachi Model, 737). A scanning wavelength of 340 to 700 nm was set, using a Halogen lamp source and a 0.6 cm light path. Calibration was based on Serva validated reference serum and normal saline (0.9% W/V), was used as a blank.

Protoporphyrin (free red blood cell protoporphyrin) was determined following a double phase extraction technique (after Peter et al., 1978). Protoporphyrin IX standards were obtained from Porphyrin Products (Logan, Utah, U.S.). Fluorescence was measured on a Farrand Mark 1 Spectrofluorometer with a primary filter of 405nm and a secondary filter of 600nm using a Xenon lamp and 10nm slit widths. Lead and protoporphyrin values were related to haemoglobin content (after O'Halloran et al., in press).

Lead levels in blood and tissues were estimated following acid digestion, using an SP192 Flameless Atomic Absorption Spectrophotometer (for details see O'Halloran and Duggan, 1984). Liver and kidney lead levels in excess of 12.50 ug/g and 31.25 ug/g wet matter were considered diagnostic of lead poisoning after Clarke and Clarke (1975).

Statistics.

Blood chemistry and haematological reference distributions were calculated using a reference value statistical package based on the

estimation of fractiles of 95% of the distribution, following guidelines of the International Federation of Clinical Chemists, (I.F.C.C.) [after Solberg, (1983), (1987)]. Student t-tests were carried out to compare means as the distributions were Gaussian.

RESULTS

All blood samples included in the reference range had blood lead values less than 3.00ug Pb/ gHb (equivalent to 2.00 umoles/L or 40 ug/100mls, assuming a mean haemoglobin concentration of 14.20g/100mls after O'Halloran et al., in press) at which level there was no sign of lead toxicity.

The range of values of blood parameters is presented in Tables 1-2. Reference distributions (95% of the distributions) were calculated to describe the data.

Reference Distributions

The reference haemoglobin concentrations, mean cell haemoglobin concentration and haematocrit values for the total number of samples (including birds of each age and sex) are shown in Table 1. There was a significant difference ($P < 0.001$) between male (all male) haemoglobin and female (all female) haemoglobin concentrations. There was no significant difference (Student-t test) between adults or immatures of either sex, although immatures had lower reference ranges than adults (Table 1). Adult males had significantly higher ($P < 0.001$) haemoglobin concentrations than adult females or immature females ($P < 0.01$), with females having lower reference levels than males, the numerical difference however, between the lower and the upper fractiles were almost the same (Table 1). Immature males had higher ($P < 0.01$) levels of haemoglobin than immature females. Mean cell haemoglobin concentration varied very little. There was no difference in mean cell haemoglobin concentration though females had higher upper reference limits for MCHC than males, while immature males had slightly lower values (Table 1). There were no differences between ages or sexes for

the haematocrit values (Table 1). Adults had smaller reference ranges than immatures (Table 1).

Reference limits for the other blood parameters based on a sample of about 100 Mute swans are shown in Table 2. While the range for most parameters is small, the range for urates is large (Table 2). Two reference ranges are given for protoporphyrin IX, uncorrected values and values related to haemoglobin content.

Blood chemistry values for caged swans are shown in Table 3. There was little change in the levels of blood parameters studied throughout the period (16.30 - 07.00hrs), except in the case of three compounds. The concentration of urate was variable between individuals but all values dropped from the initial time of caging. In contrast, the levels of cholesterol increased from the time of caging until after release, with one bird twice exceeding the upper reference limit (Table 3). In almost all cases, sodium exceeded the upper reference range throughout the period of night sampling.

Protoporphyrin IX and haemoglobin showed values outside the reference distribution in some apparently healthy birds, though the values were within the maximum/minimum limit (Table 2). Some cygnets (immatures less than four months old) had high levels of circulating Protoporphyrin IX (Table 4). Haemoglobin, haematocrit and mean cell haemoglobin values for eight Mute swans with apparent anaemia are given in Table 5. All values were outside the reference distribution, though they approached and equalled the minimum values recorded (Table 1). Mean cell haemoglobin concentration values were low in all the females (Table 5), indicating hypochromic anaemia. Haematocrit values in moulting birds, however, were variable (Table 5). These birds were at an advanced stage of plumage replacement, following primary moult.

Table 1. Mean ($\pm$ s.e.) maximum and minimum values and reference limits of haemoglobin concentration (Hb), mean cell haemoglobin concentration (MCHC) and haematocrit (HAEM) for normal Mute swans (Cygnus olor) of different ages and sex.

Class		n	X $\pm$ s.e.	Min - Max	Reference range
Total	Hb	530	14.20 $\pm$ 0.10	9.25 - 19.55	10.50 - 17.80
	MCHC	429	35.70 $\pm$ 0.18	23.30 - 55.00	29.70 - 43.00
	HAEM	560	41.30 $\pm$ 0.20	27.50 - 61.00	30.50 - 57.30
All Males	Hb	270	14.20 $\pm$ 0.10	10.40 - 19.55	11.20 - 16.90
	MCHC	241	35.90 $\pm$ 0.20	25.55 - 47.60	29.60 - 40.75
	HAEM	319	41.00 $\pm$ 0.30	27.50 - 61.00	31.00 - 54.00
Adult males	Hb	161	14.30 $\pm$ 0.14	10.40 - 19.55	11.20 - 17.80
	MCHC	150	36.00 $\pm$ 0.30	25.55 - 47.60	29.80 - 41.40
	HAEM	195	41.00 $\pm$ 0.40	27.50 - 55.00	30.00 - 53.00
Imm. males	Hb	100	14.10 $\pm$ 0.14	10.60 - 16.30	10.90 - 16.20
	MCHC	91	35.66 $\pm$ 0.35	26.60 - 47.25	30.80 - 40.70
	HAEM	120	41.60 $\pm$ 0.60	32.00 - 61.00	31.20 - 57.30
All Females	Hb	196	13.65 $\pm$ 0.10	9.25 - 18.50	10.20 - 16.90
	MCHC	188	35.46 $\pm$ 0.30	23.30 - 55.00	29.30 - 41.35
	HAEM	228	40.20 $\pm$ 0.40	28.00 - 61.00	31.00 - 54.00
Adult females	Hb	120	13.60 $\pm$ 0.15	9.25 - 18.50	10.40 - 17.00
	MCHC	115	35.20 $\pm$ 0.40	23.30 - 55.00	29.50 - 42.80
	HAEM	138	40.00 $\pm$ 0.50	28.00 - 54.50	29.60 - 53.00
Imm females	Hb	76	13.70 $\pm$ 0.20	9.80 - 16.00	10.00 - 16.50
	MCHC	73	35.90 $\pm$ 0.45	25.00 - 47.00	32.10 - 41.35
	HAEM	95	40.00 $\pm$ 0.60	29.00 - 60.00	30.00 - 55.00

Table 2. Mean ($\pm$ s.e.), maximum and minimum values and reference values of some biochemical parameters for normal healthy Mute swans (Cygnus olor).

Compound ¹	n	X	s.e.	Min	-	Max	Reference range	Units
T.P.	128	50.45 $\pm$	0.50	32.50	-	61.00	41.00 - 61.00	g/L
Ca.	128	2.52 $\pm$	0.05	2.10	-	2.90	2.21 - 2.81	mM/L
Crea.	128	31.10 $\pm$	0.60	17.00	-	91.00	21.00 - 45.20	uM/L
Ur.	128	286.32 $\pm$	12.60	66.00	-	820.00	80.60 - 617.35	uM/L
Na.	121	142.00 $\pm$	0.30	135.80	-	159.00	136.00 - 149.30	mM/L
K.	101	4.10 $\pm$	0.05	3.00	-	5.60	3.15 - 5.30	mM/L
Cl.	93	102.20 $\pm$	0.40	90.20	-	113.00	93.00 - 110.00	mM/L
Alt.	107	0.52 $\pm$	0.23	0.24	-	1.51	0.20 - 1.35	uKat/L
Alk.Phos.	104	1.54 $\pm$	0.80	0.33	-	5.50	0.50 - 3.70	uKat/L
Ast.	104	0.60 $\pm$	0.20	0.26	-	1.94	0.40 - 1.05	ukat/L
LDH.	104	8.70 $\pm$	3.00	4.52	-	17.45	4.64 - 17.00	uKat/L
Glu.	108	9.14 $\pm$	1.40	6.50	-	14.00	7.00 - 12.76	mM/L
Chol.	128	4.28 $\pm$	0.80	2.66	-	6.90	2.78 - 6.00	mM/L
Bili.	120	0.90 $\pm$	0.04	0.00	-	1.00	0.00 - 1.00	uM/L
PP IX	181	0.60 $\pm$	0.02	0.10	-	1.60	0.16 - 1.20	uM/L
PP IX	181	2.40 $\pm$	0.10	0.35	-	13.40	0.40 - 3.90	ug/g Hb

¹ = T.P.= Total Protein, Ca.= Calcium, Crea. = Creatinine, Ur. = Urate, Na. = Sodium, K.= Potassium, Cl. = Chloride, Alt.= Alanine Amino Transferase, Alk.Phos = Alkaline Phosphatase, Ast. = Aspartate Amino Transferase, LDH. = Lactate Dehydrogenase, Glu. = Glucose, Chol. = Cholesterol, Bili = Bilirubin and PPIX = Protoporphyrin IX, or Free Red Blood Cell Protoporphyrin (uncorrected and corrected for haemoglobin).

Table 3. Haematological and biochemical values for four Mute swans (Cygnus olor) blood sampled over 15 hours.

No.	Time	T.P.	Ca.	CRE.	UR.	NA.	K.	ALT.	ALK. PHOS.	LDH.	GLU.	CHOL.	Hb	HAEM	MCHC
1	16.30	45.00	NA.	25	337	147	NA.	0.47	NA.	11.00	12.30	5.34	14.20	39.60	35.85
	24.00	50.00	2.79	32	250	146	3.64	0.56	0.74	11.84	9.10	6.70	15.40	44.00	35.00
	03.00	50.00	2.71	30	180	150	4.10	0.56	0.73	8.86	9.80	6.77	11.40	42.30	26.95
2	16.30	47.00	NA.	32	379	151	NA.	0.33	NA.	10.25	14.30	5.00	16.00	47.30	33.80
	24.00	50.00	2.72	36	194	150	4.10	0.27	1.10	6.24	11.90	5.48	15.85	45.40	34.90
	03.00	51.00	2.92	37	191	154	3.84	0.31	1.11	5.30	12.00	5.82	16.40	46.30	35.40
	07.00	49.00	2.75	33	151	154	3.50	0.23	1.03	5.97	11.30	6.00	15.90	48.00	33.00
3	16.30	41.00	NA.	25	314	159	NA.	0.39	NA.	7.95	12.40	4.26	14.50	43.80	33.00
	24.00	47.00	2.63	27	296	155	4.16	0.44	NA.	8.42	10.00	5.22	15.40	43.60	35.30
	03.00	50.00	2.76	33	245	153	3.60	0.44	0.82	6.95	10.20	5.60	15.50	44.10	35.10
	07.00	47.00	2.68	30	159	158	3.70	0.40	0.78	4.70	10.60	5.78	14.60	43.80	33.30
4	16.30	52.00	NA.	23	431	154	NA.	0.34	NA.	6.44	13.50	4.70	15.70	44.90	35.00
	24.00	56.00	2.96	36	298	152	3.97	0.41	0.89	10.50	9.70	5.63	15.50	47.50	32.60
	03.00	56.00	2.96	40	271	157	3.86	0.40	0.89	8.93	9.70	5.82	15.40	43.40	35.50
	07.00	54.00	2.96	40	226	153	3.73	0.36	0.83	7.78	10.30	5.86	15.50	48.00	32.30

NA = not analysed. Other abbreviations as in Tables 1-2.

Table 4. Uncorrected and corrected protoporphyrin IX values for six young Mute swans (Cygnus olor).

Specimen No.	Protoporphyrin IX (umoles/L)	Protoporphyrin IX (ug/gHb)
1	1.60	13.40
2	1.50	6.95
3	1.40	5.60
4	1.30	5.00
5	1.10	5.20
6	1.00	4.00

Table 5. Haemoglobin, haematocrit and mean cell haemoglobin concentration (MCHC) values for healthy moulting Mute swans (Cygnus olor) with apparent anaemia. (see text for details).

Specimen No.	Age	Sex	Haemoglobin (g/100mls)	Haematocrit %	MCHC (g/100mls)
1	Adult	F	10.20	34.35	29.70
2	Adult	F	10.60	40.00	26.50
3	Adult	F	10.60	40.40	26.20
4	Adult	F	10.20	30.00	34.00
5	Imm.	F	9.80	31.00	31.60
6	Adult	M	10.30	31.70	32.50
7	Adult	M	10.80	33.00	32.70
8	Adult	M	10.70	33.50	31.90

Lead poisoned swans

Tests were carried out on six birds: four Mute swans, (specimen numbers 978^a and 978^b are from the same individual with an interval of one week between collection of specimens), and two Whooper swans were suffering from acute lead poisoning following the ingestion of anglers' weights or spent shot-gun pellets. They had kidney and liver lead levels diagnostic of lead poisoning (Table 7) (see O'Halloran et al., in press). Biochemical abnormalities were identified by comparison with the reference distributions given in Tables 1-2 and are listed in Table 6. The level of lead in the pancreas was high, while the levels of lead in the other tissues were variable (Table 7). All birds had high blood lead levels and had lost weight, (up to 40% in one case). The Mute swans were clearly suffering from hypochromic anaemia, as values of haemoglobin concentration and mean cell haemoglobin concentration values approached or were less than the lower reference limits. The two Whooper swans also had very low haemoglobin concentrations (Table 6). On the basis of haematocrit however, some birds would not be classified as anaemic since three adult males suffering from lead poisoning had haematocrit values greater than the lower reference range limit.

Changes in blood chemistry were variable in lead poisoned birds, with eight biochemical parameters showing deviations from the reference distribution (Table 6). Levels of total protein (TP), aspartate amino transferase (AST), and lactate dehydrogenase (LDH) showed a consistent pattern of change, with low total protein values and high levels of plasma enzymes. Levels of protoporphyrin (PPIX) were high and exceeded the values of the reference range and the maximum level in 'normal' swans. Other parameters ranged above and below the reference distribution (Table 6).

Table 6. Age, sex, blood lead level, haematological and biochemical changes in acutely lead poisoned Mute swans (Cygnus olor).

NO.	AGE	SEX	PB ¹	PB	WT.	Hb	MCHC	HAEM	TP.	CA.	UR.	AST.	LDH.	GLU.	CHOL.	PPIX
978 ^a	Imm.	F	7.0	18.30	7.40	7.90	31.00	25.20	31	2.10	236	1.94	17.40	9.50	3.55	NA.
978 ^b	Imm.	F	4.20	34.30	6.00	7.00	33.65	20.80	32	2.10	266	1.04	NA	9.70	3.64	5.64
1007	Ad.	M	21.00	48.00	7.20	9.00	30.00	30.00	42	2.45	1278	3.90	19.00	7.60	10.15	37.40
1008	Ad.	M	36.00	74.80	7.80	10.00	30.00	33.20	42	2.80	555	1.65	11.45	10.00	7.00	16.80
1009	Ad.	M	4.70	9.50	6.80	10.20	32.70	31.20	37	2.32	165	2.25	21.00	9.70	4.00	6.00
1010	Ad.	F	4.20	14.50	4.60	6.00	29.60	20.30	31	2.28	169	1.40	34.25	20.10	1.84	46.75
1011	Ad.	M	18.00	40.00	6.50	9.30	27.20	34.25	35	2.53	170	0.85	17.50	12.30	10.00	33.80

¹ = lead. NA. Not analysed. Other abbreviations as in Tables 1-2.

Table 7. Number of lead pellets and tissue lead levels (ug/g) of acutely lead poisoned Mute swans (Cygnus olor). Specimen numbers correspond to those in Table 6.

Specimen No.	978 ^b	1007	1008	1009	1010	1011
Number of lead pellets	0	7	14	0	0	7
Kidney	18.40	230.00	334.00	268.00	360.00	47.00
Liver	70.90	42.00	130.70	145.00	NA	47.00
Pancreas	10.10	68.00	50.00	240.00	400.00	450.00
Heart Muscle	1.20	14.50	160.00	NA	NA	NA
Breast Muscle	1.20	46.00	10.30	NA	NA	NA
Gizzard Muscle	5.60	28.00	6.40	NA	NA	NA

NA = Not analysed.

DISCUSSION

The normal range of haemoglobin, mean cell haemoglobin concentration and haematocrit values reported here for Mute swans (Table 1) do not differ greatly from those reported elsewhere for other species of birds (Hodges, 1977) or mammals (Eccleston, 1977). Methodological deficiencies in the estimation of haemoglobin concentration have however resulted in a great deal of variation in reported values (Hodges, 1977). What is noticeable is the variation in the degree of sensitivity of different tests. Haematocrit has such a large reference range and is a crude method for the assessment of health or the effects of disease. This is further shown by the fact that no detectable differences were found in haematocrit values for age and sex. This may explain why Custer et al., (1984) found no difference in the haematocrit values of American kestrels Falco sparverius (L) which were exposed to high lead. On this basis, the use of haematocrit without knowledge of haemoglobin level as a measure of health or as measure of change in sick birds must be treated with caution. The level of haemoglobin was less variable and significant differences were found (Table 1). However, any differences between sexes should be viewed with caution, since levels of haemoglobin varied greatly between individuals. While there were apparent differences on the gross scale, these differences may not be significant in individual birds. This can be seen from the large overlap in the reference distributions for the different classes (Table 1). Values of mean cell haemoglobin, provide a very useful measure of haemoglobin status and no differences were found between sexes. In most mammals and birds a MCHC value of less than 30.00 g/100mls is considered abnormal, (C. Hawkey pers. comm.).

Though lower values were recorded in this study, the lower reference limits did not drop below 29.00 g/ 100mls (Table 1). While in lead poisoned birds (Table 6), and moulting birds the values of MCHC are lower. Thus when considering MCHC, the physiological state must be noted before any diagnosis is made.

Fifteen other blood parameters were measured to establish reference distributions (Table 2). The details of the data were not broken down to individual sexes since the number of specimens would not allow sufficient precision in the estimates of percentiles (Solberg, 1983). The decision to include all the specimens in the reference distribution is substantiated by the fact that many of the blood parameters conformed to a Gaussian distribution. The recorded values for the various blood constituents are close to those reported for other species (Freeman, 1984), though levels of cholesterol are higher than in chickens, while levels of glucose and sodium are lower (Sturkie, 1965; Panigahy et al., 1986)

There was a wide range of values for protoporphyrin. Bush et al., 1982 suggest that values of protoporphyrin greater than 1.07 uM/l are indicative of lead poisoning in humans. Roscoe (1979) selected a more conservative value of 0.70 uM/L (40ug/100mls) in ducks, to distinguish lead poisoned birds. In the present study, values of protoporphyrin ranged from 0.10-1.20 uM/L (Table 2) for 95% of the normal values, with a maximum value of 1.60 uM/L in one healthy cygnet (Table 2). The importance of relating protoporphyrin to haemoglobin content has been neglected by many workers. Chisolm, 1973 and Chisolm et al. (1974) reports that 'strictly speaking the whole blood fluorescence should be corrected for haematocrit or haemoglobin'. Thus, the corrected reference distribution for protoporphyrin IX (Table 2) should be

considered when using protoporphyrin to measure lead exposure in Mute swans.

In the swans sampled during the night, little variation was recorded in the blood parameters and all remained within the reference range, with the exception of sodium. The sodium values in the birds sampled during the night were the highest recorded in this study. Though the exact reasons for the change in sodium levels are not known, it is suggested that stress due to caging and/or abnormal conditions were responsible for the change. The only other two parameters to show noticeable changes in caged swans were urate and cholesterol. This was not unexpected, in that the birds did not feed very much during the period, and urate is the breakdown product of protein (Sturkie, 1965). Protein levels did not vary during the study, the lower urate levels were presumably due to the slowing down of the digestive process. While food was freely available to the birds, they were not seen to feed and this may explain the increase in circulating plasma cholesterol as the birds mobilised lipid to maintain body functions.

The level of protoporphyrin IX was high for six cygnets (less than 4 months old) and all values were above the upper reference range and the values set the maximum level recorded for the normal swans (Table 4). While the exact reason for this is unknown, Lucas and Jamroz, (1961) reported occasional early developmental stages of red blood cells in the blood of chickens. Similarly, Smith and Engelbert (1969), reported peripheral erythropoiesis in the blood due to mother cells releasing 'clone' cells which are relatively undifferentiated, immature red blood cells. The frequency of such 'clone' cells in the peripheral blood of young hatched chicks averages 5.2 percent (Smith and Engelbert, 1969). The values obtained from the young birds in this study are probably due to the presence of a proportion of red blood

cells which have not completed erythropoiesis and thus have high protoporphyrin concentrations. Clearly it is important that these factors be taken into account when measuring protoporphyrin IX in birds exposed to lead; otherwise birds screened exclusively for raised protoporphyrin would be considered positive for lead exposure, when they may not, in fact, have high blood lead levels.

In some healthy moulting birds, low levels of haemoglobin and mean cell haemoglobin were recorded and were indicative of hypochromic anaemia. They were close to, or equal to, the minimum concentration of haemoglobin recorded for normal birds (Table 1). Low haemoglobin and MCHC values were not detected in all moulting birds, but occurred in some apparently healthy birds at an advanced stage of primary feather replacement. At this time the thyroid is extremely active (Voitkevich, 1966) and the condition could be described as hyperthyroidism.

Hyperthyroidism, among other pathological conditions, is known to change the volume of blood plasma (Bushby, 1970). The plasma volume increases while the other constituent components remain the same, causing an apparent anaemia. It is believed that the thyroid activity in the moulting swans may have contributed to the low haemoglobin and mean cell haemoglobin values reported in this study (Table 5).

Reference distributions are a useful diagnostic tool, but it is important to consider all aspects of the animals' physiological states before drawing any conclusions.

Using the reference values (Tables 1-2), abnormalities in the blood were detected in each of the clinical cases. All birds had typical symptoms of lead poisoning (see Simpson et al., 1979; Lumeij, 1985). The main features were anorexia, impaction of the gastro-intestinal tract and lethargy. These features resulted in a variety of changes in

the blood constituents, reflecting an overall deterioration in condition. Typically, the birds were suffering from hypochromic anaemia, with haemoglobin values below the reference range and MCHC values below or equal to lower reference limit. While haematocrit values did not always drop in tandem with the haemoglobin concentration, the low mean cell haemoglobin values reflected abnormalities in haemoglobin synthesis. It is clear from the reference values (Tables 1-2) that the values of haemoglobin (9g/100mls) reported by Simpson et al., (1979) were indicative of anaemia. Similarly, the values of haematocrit (25%) reported by Bates et al., (1968), for lead poisoned Mallard Anas platyrhynchos (L) are similar to the values reported here for lead poisoned swans. However, as mentioned earlier, because of the large reference range for haematocrit these values should be looked at critically before interpretation. But mean cell haemoglobin will detect this difference as it expressed in haemoglobin concentration per the number of red cells. The rate and degree of decrease of haemoglobin can be seen in specimens 978^{a-b} (Table 7). This demonstrates the toxicity of lead to the haemopoietic system.

In lead poisoned birds, the minimal (and apparently critical) total protein level was 31g/L (Table 7). Janssen et al., (1986) noted a drop in total protein in lead poisoned Condors to a level similar to that reported here. The increase of some plasma enzymes, lactate dehydrogenase (LDH) and aspartate aminotransferase (AST), are typical of animals with large tissue destruction and muscle damage. This increase is due to muscle atrophy and damage resulting from acute lead poisoning. A rise in aspartate aminotransferase has also been reported in lead poisoned ducks (Eastin et al., 1983). The raised cholesterol levels are probably due to mobilisation of lipids due to physiological

stress. The concentration of protoporphyrin IX is elevated and is indicative of lead poisoning, following disruption of haeme synthesis (Lee, 1981). Glucose levels were variable in the lead poisoned birds, but if the high levels of lead in the pancreas caused this elevation, it is not known.

Since no reference values were available for Whooper swans, the reference levels for Mute swans (Tables 1-2) were used to detect changes in blood chemistry examined. However, only with further study of blood chemistry for Whooper swans will it be possible to detect accurately biochemical changes due to lead poisoning.

From the reference distributions established in this study, changes occurring in the blood of Mute swans following lead ingestion can be detected. While values for acutely lead poisoned birds are informative, small changes can be detected in chronically poisoned birds, using reference values reported here. Caution in the interpretation of levels of haemoglobin and protoporphyrin should, however, be exercised. Further study is required to determine sub-lethal effects of lead in Mute swans.

REFERENCES

- Bates, F.Y., Barnes, D.M. and Highbee, J.M. (1968). Lead toxicosis in Mallard ducks. Bulletin of Wildlife Diseases Association. 4: 116-125.
- Birkhead, M. (1983). Lead levels in the blood of Mute swans Cygnus olor on the River Thames. Journal of Zoology (London). 199: 59-73.
- Bush, B., Doran, D.R. and Jackson, K.W. (1982). Evaluation of erythrocyte protoporphyrin and zinc protoporphyrin as micro screening procedures for lead poisoning detection. Annals of Clinical Biochemistry. 19: 71-76.
- Bushby, S.R.M. (1970). Haematological studies during toxicity tests. In: Methods in toxicology. Ed. G.E. Payet. Blackwell: Oxford. p 338-371.
- Chisholm. J.J., Jr., (1973). Management of increased lead absorption and lead poisoning in children. New England Journal of Medicine. 289: 1016-1024.
- Chisholm. J.J. Mellitis, E.D., Keil, J.E. and Barret, M.B. (1974). A simple microhaematocrit procedure as a screening technique for increased lead absorption in young children. Journal of Paediatrics. 84: 490-498.
- Clarke, E.G.C and Clarke, M.L. (1975). Veterinary Toxicology. Bailliere. London.
- Custer, T.W., Franson, J.C. and Pattee. O. (1984). Tissue lead distribution and haematological effects in American kestrels (Falco sparverius) fed biologically incorporated lead. Journal of Wildlife diseases. 20: 39-43.

- Eastin, W.C., Jr., Hoffman, D.J. and O'Leary, T. (1983). Lead accumulation and depression of delta aminolaevulinic acid dehydratase (ALAD) in young birds fed automotive waste oil. Archives of Environmental contamination and Toxicology. 12: 31-35.
- Eccleston, E. (1977). Normal haematological values in rats, mice and marmosets. In: Comparative Clinical Haematology. Ed. R.K.Archer and L.B.Jeffcott. Blackwell. Oxford. 611-619.
- Freeman, B.M. (1984). Ed. Physiology and Biochemistry of the domestic fowl. Academic Press. London.
- Hawkey, C., Samour, J.H., Ashton, D.G., Hart, M.G., Cindery, R.N., Ffinch, J.M., and Jones, D.M. (1983). Normal and clinical haematology of captive cranes (Gruiformes). Avian Pathology 12: 73-84.
- Hawkey, C., Hart, M.G., Samour, J.H., Knight, J.A. and Hutton, R.E. (1984). Haematological findings in healthy and sick captive rosy flamingos (Phoenicopterus ruber ruber).
- Hochbaum, H.A. (1942). Sex and age determination of waterfowl by cloacal examination. North American Wildlife Conference Transactions. 7: 294-307.
- Hodges, R.D. (1977). Avian haematology. In: Comparative Clinical Haematology. Eds R.K.Archer and L.B.Jeffcott. Blackwell. Oxford. p483-517.
- Janssen, D.L., Oosterhuis, J.E., Allen, J.L., Anderson, M.P., Kelts, D.G. and Wiemeyer, S.N. (1986). Lead poisoning in free ranging California condors. Journal of the American Veterinary and Medical Association. 189: 1115-1117.
- Lee, W.R. (1981). What happens in lead poisoning?. Journal of the Royal College of Physicians of London. 15: 48-54.

- Lucas, A.M. and Jamroz, C. (1961). Atlas of avian haematology. Agricultural Monograph No.25. Washington, U.S. Departmenty of Agriculture.
- Lumeij, J.T. (1985). Clinicopathologic aspects of lead poisoning in birds: A review. The Veterinary Quarterly. 7: 133-136.
- O'Halloran, J. and Duggan, P.F. (1984). Lead levels in Mute swans in Cork. Irish Birds. 2: 501-514.
- O'Halloran, J., Myers, A.A. and Duggan, P.F. (1987a). Lead poisoning in Mute swans and fishing practice in Ireland. In: Biological Indicators of Pollution. Ed. D.H.Richardson. Royal Irish Academy. Dublin. p183-191.
- O'Halloran, J., Duggan, P.F. and Myers, A.A. (1987b). Determination of haemoglobin in birds by a modified alkaline haematin (D-575) method. Comparative Biochemistry and Physiology. 86(B): 701-704.
- O'Halloran, J., Myers, A.A. and Duggan, P.F. (in press). Lead poisoning in swans and sources of contamination in Ireland. Journal of Zoology (London).
- Panigahy, B., Rowe, L.D. and Corrier, D.E. (1986). Haematological values and changes in blood chemistry in chickens with infectious bursal disease. Research in Veterinary Science. 40: 86-88.
- Peter, F., Growcock, G. and Strunc, G. (1978). Fluorometric determination of erythrocyte protoporphyrin in blood, a comparison between direct (hematofluorometric) and indirect (extraction) methods. Clinical Chemistry. 24: 1515-1517.
- Roscoe, D.E., Nielsen, S.W., Lamola, A.A. and Zuckerman, D. (1979). A simple, quantitative test for erythrocytic protoporphyrin in lead poisoned ducks. Journal of Wildlife Diseases. 15: 127-136.

- Simpson, V.R., Hunt, A.E. and French, M.C. (1979). Chronic lead poisoning in a herd of Mute swans. *Environmental Pollution*. 18:187-202.
- Smith, N. and Engelbert, V.E. (1969). Erythropoiesis in chicken peripheral blood. *Canadian Journal of Zoology*. 47: 1269-1273.
- Solberg, H.E. (1983). The theory of reference values. Part.5. Statistical treatment of collected reference values, determination of reference limits. *Journal of Clinical Chemistry and Clinical Biochemistry*. 21: 749-760.
- Solberg, H.E. (1987). Approved recommendations (1986) on the theory of reference values. Part 1. The concepts of reference values. *Clinica Chimica Acta*. 165: 111-118.
- Sturkie, P.D, (1965). *Avian Physiology*. 2nd Edition. Bailliere, Tindall and Cassell. London.
- Voitkevich, A.A. (1966). *The feathers and plumage of birds*. Sidgwick and Jackson. London.

CHAPTER 5

TISSUE LEAD LEVELS AND HAEMATOLOGICAL CHANGES IN MUTE SWANS

Cygnus olor WITH ELEVATED BLOOD LEAD.

This chapter is presented in the form of a manuscript recently submitted for publication in Avian Pathology.

SUMMARY

Tissue lead levels in Mute swans Cygnus olor (Gmelin) were investigated. The selection of tissues for diagnosis of acute lead poisoning is discussed. Three categories of swan mortality were defined: (1) acute lead poisoning; (2) collisions and (3) other reasons. In most tissues, lead concentrations were highest according to the cause of death in order: lead poisoning > collisions > other causes. Elevated blood lead levels and haematological disorders were detected in swans which had collided with objects. The possible role of elevated lead in causing collisions in Mute swans is discussed.

INTRODUCTION

Lead poisoning through the ingestion of discarded anglers' weights or spent gunshot is known to be a major cause of mortality in Mute swans [Cygnus olor, (Gmelin), Birkhead, 1982; O'Halloran et al., 1987a)]. Conclusive evidence that death is due to lead poisoning, however, can only be achieved through chemical analyses of tissues. The most widely used tissue for the diagnosis of lead poisoning in birds is the liver and frequently only a single tissue sample is taken. Roscoe et al., (1979); and Anders et al., (1982) attempted to determine the flux of lead in the bodies of caged birds by feeding them lead. They then extrapolated the results to the field situation. The validity of extrapolating experimental results to the field situation has been questioned, because of the contrasting pattern and duration of exposure to pollutants in the two situations (Hutton, 1980).

While high tissue lead levels are diagnostic of birds which have died from lead poisoning, there is a need to investigate sub-lethal effects. Some workers have demonstrated the immuno-suppressive effects of lead in birds (Franson, 1986), while others have shown a change in reproductive performance (e.g. Elder, 1954). Hunter and Wobesser (1980) found encephalopathy and peripheral neuropathy in lead poisoned Mallard Anas platyrhynchos (L). These changes preceded anaemia which was induced following the feeding of lead pellets.

Acute lead poisoning is considered to be responsible for the deaths of a large proportion of Mute swans, but an additional cause of mortality is collision with over-head power cables. Birkhead and Perrins (1986) suggest that sub-lethal doses of lead may have an effect on high degree co-ordination in Mute swans. Sub-lethal levels

of lead may therefore result in collisions with objects. Hypochromic anaemia has been recorded in Mute swans dying from lead poisoning (O'Halloran et al., (A) in press). O'Halloran et al., (A) (in press) have recently reported haematological reference levels in healthy Mute swans, which allow changes in blood parameters to be measured to demonstrate some sub-lethal effects of lead, particularly on the haemopoietic system.

This study reports the results of tissue lead levels in Mute swans found dead or moribund and on haematological changes in live mute swans with elevated blood lead levels.

MATERIALS AND METHODS

Collection and analyses of samples

Mute swan corpses were collected as part of a major study on the sources of lead contamination in this species in Ireland (see O'Halloran et al., (B) in press). Cause of death was determined, birds were weighed, and samples of liver, kidney, pancreas, heart, breast and gizzard muscle were taken for analysis. Blood and feather samples were taken from live uniquely ringed Mute swans at Cork Lough in S.W. Ireland and from live birds which had been involved in collisions.

Blood and tissue lead levels were determined following acid digestion with 10% nitric acid (Aristar grade). Lead estimations were carried out using an SP 192 atomic absorption spectrophotometer with a flameless atomiser attachment and a wavelength of 217 nm (O'Halloran and Duggan, 1984). Primary covert feathers were removed from live swans and the age of feathers (i.e. whether the bird was at a moult or premoult stage) were noted. Each feather was washed three times in acetone and then in de-ionised water to remove surface contamination (Hiderbrand and White, 1974). Specimens were then dried to constant weight, reweighed and digested as for tissues.

Haemoglobin (Hb) was determined by converting all haem species to alkaline haematin using a non-ionic detergent (O'Halloran et al., 1987b). Haematocrit (Haem) was calculated following micro-centrifugation at 12 000 X G. Mean cell haemoglobin concentration (MCHC) was calculated following the method of Dacie and Lewis (1984) and lead levels were corrected for haemoglobin after

O'Halloran et al., (C) in press. Birds were X-rayed on a Unimax 500 X-ray machine on a 'potter buckey' as described elsewhere [O'Halloran et al., (B) in press]. All tissue lead levels are presented in ug/g wet matter. Tissue lead levels in excess of 12.50 ug/g (liver) and 31.50 (kidney) were considered diagnostic of lead poisoning (Clarke and Clarke, 1975). Blood lead levels in excess of 3.00 ug Pb/ gHb (2.20 un/L) were considered elevated (O'Halloran et al., (C) in press). Haematological values were compared with reference haemoglobin (Hb), mean cell haemoglobin concentration (MCHC) and haematocrit reference values for healthy Mute swans (O'Halloran et al., (A) in press).



RESULTS

Tissue lead levels

Tissue lead levels were determined in six tissues (Tables 1-2). Tissue lead levels in five swans acutely poisoned as a result of the ingestion of lead weights are presented in Table 1. Blood lead levels were very high (36.00 - 39.00 ug/gHb). The kidney lead levels were the highest recorded for tissues (Table 1). The level of lead in the pancreas was also high in four birds. The level of lead found in a juvenile bird was lower than that of the adults. Lead levels in dead swans are given in Table 2: group A includes swans which had ingested lead weights; in lead poisoned swans the median lead level was higher in the liver than in the kidney (Table 2); group B, those which died from collision with electric/telephone wires, and group C, swans which died from other causes. The high lead levels in group A (Table 2) are consistent with similar levels reported in acutely lead poisoned birds (Table 1). Muscle lead levels (heart, breast and gizzard) were generally within the range 6.00 - 270.00 ug/g but one specimen of heart muscle specimen was very high, (730.00 ug/g). Birds which died from collisions (group B) had high lead levels in the liver, kidney and particularly in the breast muscle (Table 2). Median lead levels in the tissues of group B were intermediate between levels found in the other two groups. Group C includes both urban and rural swans. In these, the lead levels were low and did not exceed the values diagnostic of lead poisoning. The level of lead in the pancreas for this group was also high, with a maximum value of 48.00 ug/g (Table 2).

Feather lead levels were variable and no clear pattern emerged. The median lead level in the feathers of birds with a history of

elevated lead was 11.00 (3.00 - 85.00, n = 13) for new coverts and 27.35 (4.40-90.00, n = 11) for old primary coverts . In birds with no history of elevated lead values, levels were lower in the new feathers (median = 6.50, 3.80-160.00, n = 12) and higher in old feathers (median = 47.00, 38.00 - 67.40, n = 6).

Blood lead levels and changes

Lead levels, haemoglobin concentration, mean cell haemoglobin concentration and haematocrit of three swans sampled before they were involved in collisions are presented in Table 3. Changes in haemoglobin, haematocrit, mean cell haemoglobin and lead levels in repeatedly sampled birds with elevated lead are given in Table 4. X-ray examination of three birds revealed that elevated lead was due to the ingestion of lead weights (Table 4). One swan (specimen 1, Table 4) had a low blood lead level (2.80 ug/ gHb) though X-ray revealed a lead weight to be present. The blood lead level increased until day 116 when it began to drop. When subsequently X-rayed on day 130, the lead weight was absent and the blood had returned to normal (Table 4). Swans with high blood lead levels had low haemoglobin concentrations. In specimen 2, the haemoglobin level did not drop for a time after the highest blood lead value. In many cases, the haemoglobin level dropped below the reference limit for healthy swans, but only in two cases did the mean cell haemoglobin drop outside the reference limit (specimens 2 and 6). As the lead level declined, the haemoglobin level began to rise back to normal levels until about day 50, when it fully recovered (Table 4).

Table 1. Tissue lead levels (ug/ g), sex and number of lead weights in Mute swans Cygnus olor with acute lead poisoning.

NO.	SEX	NO. LEAD PELLETS	WEIGHT (Kg)	BLOOD (uM/L)	LIVER	KIDNEY	PANCREAS	HEART MUSCLE	BREAST MUSCLE	GIZZARD MUSCLE
1	F	11	5.80	39.00	135.00	182.00	72.30	8.20	49.00	14.85
2	M	14	7.80	36.00	130.76	333.20	50.00	163.00	10.20	6.35
3	M	4	8.20	NA	223.00	346.00	18.00	11.70	18.95	NA
4	M	10	6.80	37.00	256.70	374.00	155.45	5.70	7.86	5.00
5	X	3	1.00	NA	93.15	172.00	77.85	23.90	NA	16.15

NA = not analysed.

Table 2. Numbers of ingested lead weights and median lead levels (ug/ g) for three categories of Mute swans, Cygnus olor. Group A: Mute swans which died from acute lead poisoning; Group B: Mute swans which died from collisions and Group C: Mute swans which died from other causes or collisions. Parentheses include range of values.

CATEGORY	n	A	n	B	n	C
NO. PELLETS		7		0		0
Kidney	10	113.00 (40.00-305.00)	6	28.24 (20.50-60.00)	20	8.70 (0.40-19.00)
Liver	8	315.00 (93.15-450.00)	7	33.00 (12.50-194.00)	17	8.00 (1.00-14.00)
Pancreas	7	67.00 (20.00-155.00)	6	21.00 (9.20-97.00)	18	11.00 (1.20-48.00)
Heart Muscle	7	14.50 (6.00-730.00)	4	12.10 (5.20-41.30)	17	6.50 (1.70-17.00)
Gizzard Muscle	9	13.00 (6.00-85.00)	6	12.80 (5.60-43.00)	20	6.80 (0.50-19.00)
Breast Muscle	7	19.00 (8.00-273.00)	5	18.20 (16.00-188.00)	13	9.00 (0.50-15.00)

Table 3 Age, sex, blood lead level and haematological changes in live Mute swans Cygnus olor which were involved in collisions.

No.	SEX/AGE	T days	Pb ug/gHb	Pb um/1	Hb g/100mls	MCHC g/100mls	PCV %
1	M Adult	0*	2.20	1.60	14.90	43.00	34.60
		21	5.20	2.00	7.95	25.90	30.70
2	M Adult	0	4.00	3.00	15.44	36.00	42.85
		63 ¹	3.25	2.10	13.15	30.80	42.70
3	M Adult	0*	6.80	4.00	12.20	29.75	41.00

* denotes time of collision.
¹ this bird was reported to have collided eight days after this blood sample was taken.

Table 4. Changes in levels of haemoglobin (Hb), mean cell haemoglobin concentration (MCHC) and haematocrit (Haem) in Mute swans Cygnus olor with elevated lead levels.

No.	SEX/AGE	T days	Pb ug/gHb	Pb um/L	Hb g/100mls	MCHC g/100mls	PCV %
1	M Adult	0*	2.80	1.80	13.30	35.00	38.00
		60	4.80	4.80	10.30	35.50	29.20
		116*	3.65	2.20	12.40	32.80	37.80
		130	2.90	1.90	13.20	35.30	37.40
2	M Adult	0	3.50	2.30	13.70	35.80	38.30
		7	7.90	5.00	13.14	34.40	38.15
		21	5.30	2.50	9.70	29.35	33.00
		35	2.90	1.45	10.30	32.50	31.70
3	M Adult	0	1.00	0.75	15.50	37.95	41.00
		63	9.70	5.25	11.20	34.10	32.80
		119	4.55	3.20	14.55	33.30	43.60
4	M Adult	0	3.76	2.40	13.20	34.00	38.90
		56	6.10	3.20	10.80	31.00	34.70
		105	1.84	1.20	13.50	34.20	39.50
5	M Adult	0*	2.10	1.40	13.70	37.30	36.70
		021	16.55	9.20	11.50	32.00	36.00
		105	1.70	1.30	15.75	36.00	43.70
6	F Adult	0	6.60	3.60	11.30	26.52	42.60
		98	8.60	4.15	10.00	25.50	39.25
		158	14.50	6.30	9.00	27.42	32.80
7	M Adult	0	7.80	5.20	13.80	36.30	38.00
		7	7.50	4.30	11.85	28.40	41.60
		175	5.32	3.20	12.45	29.80	41.80
8	F Adult	0	6.40	3.80	12.25	27.70	44.20
		14	4.70	2.50	11.00	25.40	43.30
9	M Imm.	0	0.46	0.30	13.30	34.40	38.65
		42	13.25	6.60	10.30	30.00	34.35
10	M Adult	0*	8.70	4.40	10.50	30.00	35.00
		91	2.60	1.45	11.60	32.00	36.00

* denotes when swans were X-rayed.

DISCUSSION

Tissue lead levels have been used by many workers as an indication of levels of lead exposure in birds. Johnson et al., (1982) and Hutton and Goodman (1980) for example, have recorded increased lead levels in Feral pigeons Columba livia (L) exposed to different lead levels. Levels of lead in tissues were correlated with increased urbanisation, (Johnson, et al., 1982). Tissue lead levels have also been used as a diagnostic tool for lead poisoning in birds. The liver is the most widely used organ in diagnosing lead poisoning. It should be borne in mind that lead in the body is not static in most tissues and levels are in flux. Rabinwits et al., (1974) and Anders et al., (1982) have proposed models for predicting lead levels in humans and birds exposed to lead with a view to interpreting lead levels in experimental studies. O'Halloran et al., [(B) in press] found good agreement between blood lead levels in Mute swans in the field and experimental models. To test the model in wild birds is more difficult since usually tissues from dead birds only are examined. In the present study, six tissues were examined (Tables 1-2). Kidney, liver and pancreas lead levels were generally high in lead poisoned birds. In lead poisoned swans, however, the median liver lead level was higher than in the kidney. This is not unexpected, based on the model of Anders et al., (1982), since liver lead is very labile. In pigeons exposed to lead, the concentration of lead in the liver reaches its maximum in seven weeks and reaches a lower steady-state level in a about 18 weeks (Anders et al., 1982). In contrast, levels of lead in the kidney were found to rise to a value greater than that for other tissues (Anders et al., 1982). In the present study, maximum lead

values were generally found in the liver of lead poisoned swans which had ingested lead weights, though high levels were also found in the kidney. The significance of these findings is that, if only a single tissue is taken for analysis, the lead status may not be clearly determined. Barry (1975) found raised pancreatic lead levels in humans exposed to lead. In the present study, pancreatic lead levels were high and variable but lower than those of the kidney or liver (Tables 1-2). No pancreatic lead values have been recorded for birds.

O'Halloran et al., [(A) in press] found changes in circulating plasma glucose levels in swans suffering from acute lead poisoning. Whether lead has a high affinity for pancreatic tissue and causes sub-lethal effects is not known. It is interesting to note that some birds that died from reasons other than lead poisoning or collisions, had high levels of lead in the pancreas (Table 2). Lead may have a high affinity for pancreatic tissues and the levels present may be indicative of past exposure.

The way in which lead exerts its toxic effect on the nervous system is poorly understood, but it may be mediated through primary vascular damage (Christian and Tryphonas, 1971); direct action of lead on neurons (Bouldin et al., 1975) or alterations in porphyrin metabolism (Pentschew and Garro, 1966). Hunter and Wobeser (1980) found encephalopathy and nervous disorder in Mallard ducks exposed to lead in an experimental situation. These pathological findings were found to precede anaemia. In the present study, levels of lead in the tissues of swans which died from collisions were elevated (Table 2). Some tissue lead levels would be considered diagnostic of acute lead poisoning yet the birds were alive. No lead weights were found in the birds which had had collisions, but they appear to have been exposed to excess lead at some time in the past. It is noteworthy that swans

which had collided had raised blood lead levels (>3.00 ug/gHb) and two were anaemic. Values of MCHC and haemoglobin were close to or below the lower reference limits for healthy Mute swans (see O'Halloran et al., (A) in press). One swan collided with a wire eight days after a high blood lead level and reduced haemoglobin concentration had been recorded. Elevated lead levels may have been responsible for the collisions as a result of reduced co-ordination as suggested by Birkhead and Perrins (1986).

The levels of lead found in feathers were variable being higher in older feathers. The range of values of lead in old feathers was almost the same irrespective of known lead history (as measured from blood samples). In birds with a known history of lead poisoning, median values of lead were lower, though in one case, the maximum concentration exceeded the levels found in the old feathers. Because of the small sample size in the present study, and because of the various endogenous sources of lead (e.g. salt gland excretion, see Howarth et al., 1981, 1982) as well as exogenous sources, it is difficult to assess whether feathers give an accurate measure of lead status.

Lead is known to affect the synthesis of haemoglobin (Lee, 1981). It interferes with the binding of iron to the porphyrin IX ring at the final step of haemoglobin synthesis. Thus, excess lead causes raised protoporphyrin levels and anaemia. O'Halloran et al., (A) in press, have recently reported hypochromic anaemia and increased free red blood cell protoporphyrin in Mute swans dying from lead poisoning, based on comparisons with reference values determined from healthy normal swans. In general, healthy swans have a haemoglobin value of $10.50 - 17.80$ g/100mls; MCHC of $29.70 - 43.00$ and haematocrit values between $30.50 - 57.30$; though sex and physiological conditions cause some

differences [see O'Halloran et al., (A) in press]. In the present study, changes in haematological parameters were detected in live swans with elevated lead. X-ray examination of birds revealed that the elevated lead was due to the ingestion of lead weights. O'Halloran et al., (B) [in press] reported that 22% of swans x-rayed at Cork Lough in S.W. Ireland had ingested lead weights in their gizzards. In this study, in two of the x-rayed birds, a lead weight was present in the gizzard and elevated blood lead levels. Swan no. 1 (Table 4), x-rayed a few weeks later, had lost it's lead weight and blood parameters had returned to normal. A third bird, (specimen no 9, Table 4) had no lead weights and a low blood lead level, but at some time between then and day 21, at least one lead weight was ingested. The elevated lead levels resulted in a decrease in circulating haemoglobin in the cell (hypochromic anaemia, specimens 6,7,8, Table 4), while in other swans, there was a reduction in the number of circulating red blood cells. Despite the drop in haemoglobin levels, most birds recovered. In one swan, (specimen 1), the plumage remained poor for a year and the over-all condition of the bird was poor. Though haematological damage was found in these birds, it is likely that other metabolic damage had also been caused by elevated lead. Hunter and Wobesser (1980) reported that in a lead poisoned Mallard, anaemia was preceded by damage to the nervous system and this is likely to be true also for Mute swans. Location of waterways, numbers of pylons/wires and degree of urbanisation may be contributing factors in causing collisions. However, this study does suggest that sub-lethal effects of lead cause anaemia and other pathological damage which may lead to increased risk of colliding with objects.

REFERENCES

- Anders, E., Dietz, D.D., Bagnell, C.R., Gaynor, J., Krigman, M.R., Ross, D.W., Leander, J.D. and Mushak, D. (1982). Morphological, pharmacokinetic and hematological studies of lead exposed pigeons. *Environmental Research*. 28: 344-363.
- Barry, P.S.I. (1975). A comparison of concentrations of lead in human tissues. *British Journal of Industrial Medicine*. 32: 119-139.
- Birkhead, M. (1982). Causes of mortality in the Mute swan Cygnus olor on the River Thames. *Journal of Zoology (London)*. 198: 15-20.
- Birkhead, M. and Perrins, C. (1986). The Mute swan. Croom Helm. London.
- Bouldin, T.W., Muschak, P., O'Tuama, A. and Krigman, M.R. (1975). Blood brain barrier dysfunction in acute lead encephalopathy: a reappraisal. *Environmental Health Perspective*. 12: 81-88.
- Christian, R.G. and Tryphonas, L. (1971). Lead poisoning in cattle: brain lesions and hematologic changes. *American Journal of Veterinary Research*. 32: 203-216.
- Clarke, E.G.C. and Clarke, M.L. (1975). *Veterinary Toxicology*. Bailliere. London.
- Dacie, J.V. and Lewis, S.M. (1984). *Practical Haematology*. 6th Edition. Churchill and Livinstone. London.
- Elder, W.H. (1954). The effects of lead poisoning on the fertility and fecundity of domestic Mallard ducks. *Journal of Wildlife Management* 18: 315-323.
- Franson, J.C. (1986). Immunosuppressive effects of lead. In Freierabend, J.S. and Russell, A.B. Eds. *Lead poisoning in wildfowl- A Workshop*; Natioanl Wildlife Federation. Washington, D.C. 139pp.

- Howarth, D.M., Hulbert, A.J. and Horning, D. (1981). A comparative study of heavy metal accumulation in tissues of crested tern, Sterna bergii, breeding near industrialised and non-industrialised areas. Australian Wildlife Research. 8: 665-670.
- Howarth, D.M., Grant, T.R. and Hulbert, A.J. (1982). A comparative study of heavy metal accumulation in tissues of crested tern Sterna bergii, breeding near an industrialised port before and after harbour dredging and ocean dumping. Australian Wildlife Research. 9: 571-579.
- Hiderbrand, P.C. and White, D.H. (1974). Trace element analysis in hair: An Evaluation. Clinical Chemistry: 148-151.
- Hunter, B. and Wobesser, G. (1980). Encephalopathy and peripheral neuropathy in lead poisoned Mallard ducks. Avian Diseases. 24: 169-178.
- Hutton, M. (1980). Metal contamination of Feral pigeons Columba livia from the London area: Part 2- Biological effects of lead exposure. Environmental Pollution. Series (A): 22: 281-293.
- Hutton, M. and Goodman, G.T. (1980). Metal contamination of Feral pigeons Columba livia from the London area: Part 1- Tissue accumulation of lead cadmium and zinc. Environmental Pollution. Series (A): 22: 207-217.
- Johnson, M.S., Pluck, H. and Moore, G. (1982). Accumulation and renal effects of lead in urban population of Feral pigeons Columba livia. Archives of Environmental Contamination and Toxicology. 11: 761-767.
- Lee, W.R. (1981). What happens in lead poisoning? Journal of the Royal College of Physicians of London. 15: 48-54.
- O'Halloran, J., Duggan, P.F. (1984). Lead levels in Mute swans in Cork. Irish Birds. 2: 501-514.

- O'Halloran, J., Myers, A.A. and Duggan, P.F. (1987a). Lead poisoning in Mute swans and fishing practice in Ireland. In: Biological Indicators of Pollution. Ed. D.H. Richardson, 183-191. Royal Irish Academy.
- O'Halloran, J., Duggan, P.F. and Myers A.A. (1987b). Determination of haemoglobin in birds by a modified alkaline haematin (D-575) method. Comparative Biochemistry and Physiology. 86: 701-704.
- O'Halloran, J., Duggan, P.F. and Myers, A.A. (A) (in press). Some biochemical reference values and changes in blood chemistry in Mute swans Cygnus olor with acute lead poisoning. Avian Pathology.
- O'Halloran, J., Myers, A.A. (B) (in press). Lead poisoning in swans and sources of contamination in Ireland. Journal of Zoology (London).
- O'Halloran, J., Myers, A.A. and Duggan, P.F. (C) (in press). Blood lead levels and free red blood cell protoporphyrin as a measure of lead exposure in Mute swans. Environmental Pollution Series (A).
- Pentscew, A. and Garro, F. (1966). Lead encephalopathy of the suckling rat and its complications of porphyrino-pathic nervous system. Acta Neuropath. 6: 266-278.
- Rabinowits, M.D., Wetherill, G.W., and Kopple, J.D. (1974). Studies of human lead metabolism by stable isotope tracer. Environmental Health Perspect. 7: 145-153.
- Roscoe, D.E., Nielsen, S.W., Lamola, A.A. and Zuckerman, D. (1979). A simple quantitative erythrocytic protoporphyrin in lead poisoned ducks. Journal of Wildlife Diseases 15: 127-136.

ACKNOWLEDGEMENTS

Firstly, I thank my parents and family who always encouraged my interests in birds, and supported my education

I would like to express my sincere thanks to Professor Alan Myers for his help and guidance, as my supervisor, throughout the entire period of my research.

I am grateful to Professor Barry Duggan, Biochemistry Laboratory, Cork Regional Hospital for his friendly advice and for provision of facilities at his laboratory.

I would like to thank Professor Maire F. Mulcahy, Head of Department of Zoology, University College Cork for providing research facilities and guidance throughout the study and Mr. Joe Philpott for technical facilities.

I would like to say thank you to the following people;
Dr. D.E.A. Murray, Dr. P.S. Giller, Dr. T.F. Cross, Dr. T.C. Kelly and Dr. C.H. Hawkey for helpful discussion. Mr. R. McNamara, Ms. S.O'Driscoll, Ms. N. Buttimer and Ms. E.O'Reardan for laboratory and field assistance. Ms. I.O'Sullivan for patience and secretarial advice; Mr. B. Crowley, Mr. N.O'Leary, Mr. N.O'Sullivan, Mr. T. Hurley, Ms. M.O'Connell, Ms. M. Morrison for helpful discussion and advice for biochemical laboratory work; Ms. J. Morrison and Ms. M.Honohan and Dr. P Barret for radiological facilities; Mr. P. Smiddy, Mr. D. McMahon and the staff of the forestry and wildlife service; Mr. T. O'Connor CSPCA for his help with swan corpse collection; Mr. D. Fitzgerald for bags of bread.

I acknowledge Ms. M. Cole, Dr. D. Barry and Mr. M O'Halloran for statistical and computing advice. Mr. R. Collins, Mr. I. Forsyth, Mr. T. Carrauthers, Mr. P. Brennan, Mr. B. O'Mahony, Mr. F. Buckley and

many others for helping to catch swans and for reporting sightings of ringed swans. To my sisters, particularly Anne and Eleanore for their help and to my brother Jer for a bucket of letraset. To Paul, Anne, Tricia, Donal and Edith for their help.

I acknowledge maintaince awards received from Cork Corporation and Department of Education.

I thank my colleagues, Jervis, Chutima, Anne, Neil, Susan, Catherine and fellow post-graduate students for their friendly co-operation and company over the past few years. I thank Mr. D. Murray for photocopying facilities and finally to Deirdre Murray for encouragement and help throughout.

APPENDICES

OTHER RELEVANT PUBLICATIONS

APPENDIX 1

LEAD LEVELS IN MUTE SWANS IN CORK

Lead Levels in Mute Swans in Cork

By John O'Halloran and P.F. Duggan

Introduction

Lead poisoning, apparently resulting mainly from the ingestion of spent lead gun shot pellets and discarded anglers' weights, has been shown to be an increasing hazard to waterfowl throughout the Holarctic. (Rosen and Bankwoski 1960, Evans *et al* 1973, Birkhead 1983). In the United States of America, where the condition was first recognised in 1874, the situation is now considered to be widespread (see Philips and Lincoln 1930). However, though researched in a total of 14 other countries, information on lead poisoning in waterfowl has never been addressed in Ireland. In an effort to remedy this situation this present research* was carried out (and is continuing).

Mute Swans *Cygnus olor* were selected for study in Co. Cork at a fishing area with a resident swan population of up to 200 birds and, for comparison, at areas where there was little coarse fishing. The Lough, Cork City (Plate 70) is fished throughout the year, often with

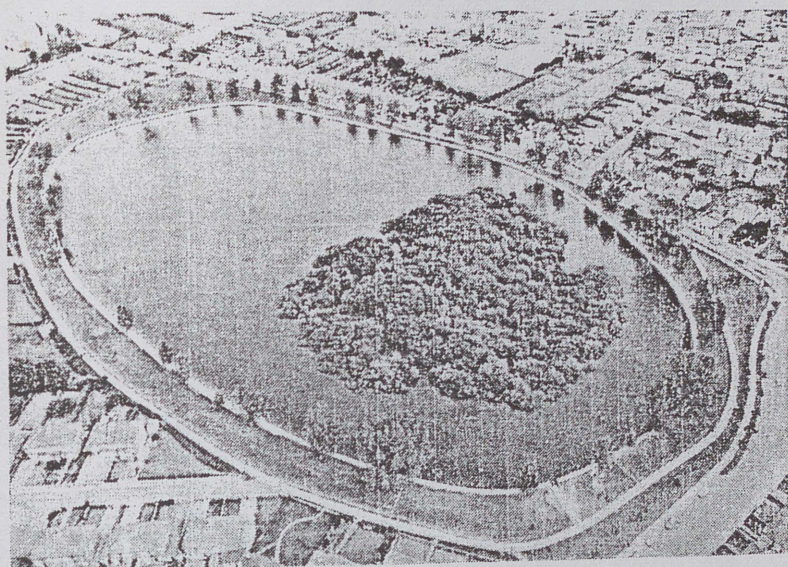


Plate 70. The Lough, Cork

Photo: Richard T. Miller

*This work formed part of a thesis for the Honours Degree in Zoology of the National University of Ireland, at University College, Cork. (O'Halloran 1984).

Irish Birds 2:501-514, 1984.

intense fishing in summer and large flocks of swans congregate there. For comparison, sites on the River Lee, at Ballycotton, Castlemartyr, Lough Aderry, and Kanturk were selected (Fig. 1). (Fly fishing is carried out at Lough Aderry but lead weights are usually not used).

The primary objects of the study were:

1. To determine blood lead levels of Mute Swans at The Lough, Cork City.
2. To determine blood lead levels of Mute Swans at areas of little fishing activity.
3. To examine the relationship between blood lead levels and age.
4. To determine the source of increased lead contamination (i.e. discarded anglers' weights or other sources of lead).
5. To carry out post-mortem examinations of available dead swans, to establish tissue lead levels and to examine for any pathological changes.

Materials and Methods

A. Blood Sampling

Blood samples were obtained from 104 Mute Swans at The Lough and 18 birds at other sites. Birds were caught by hand or with a hook when lured close enough with bread.

Once caught, birds were placed in an open ended nylon bag. This bag was attached to a Salter spring balance and the weight recorded.

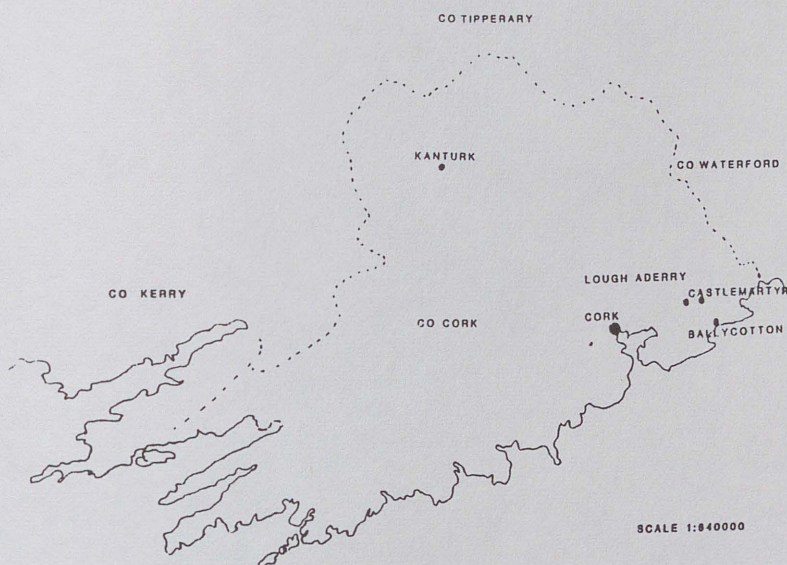


Fig. 1. The location of the blood sampling sites mentioned in the text. Broken lines indicate county boundaries.

Each sampled bird was dye marked using three dyes; Malachite Green, Eosin and Gentian Blue. The purpose of dye marking the birds was two-fold, firstly to prevent resampling of the same individual and secondly to see if movement of birds occurred between sampling sites. The dyes were applied with a brush to the plumage of the birds before release.

Age was determined by plumage characteristics and beak colour. Two-millimetre blood samples were taken from the brachial vein using a 23g 1" syringe and stored in low lead heparinised tubes. Blood lead concentrations were determined on a Pye unicam SP192 atomic absorption spectrophotometer with a flameless atomiser attachment and with a wavelength of 217nm. 10% nitric acid (proanalar grade) was used for digestion. All blood lead levels are presented in $\mu\text{g}/100\text{mls}$ of blood.

B. Post Mortem Examination

Two corpses were available for post mortem, one of a moribund bird and the other of a cygnet killed in a collision with a high tension electric cable.

The birds were dissected and their pathological condition recorded. Blood samples were removed from the heart using the above mentioned syringes. The kidney was removed from the moribund bird and prepared for electron-microscope examination using standard methods. Sections of breast muscle, kidney, liver and pancreas were removed, weighed and stored at -30°C for tissue analysis. The liver was weighed without gall bladder. The oesophagus, proventriculus and gizzard were removed for examination.

Since the cygnet suffered multiple fractures in the accident, the heart was the only tissue to be weighed. Nevertheless, sections of liver and breast muscle were taken for tissue analysis. The oesophagus, proventriculus and gizzard were removed for examination. The contents of the alimentary canal were washed into a glass dish and sieved through 2.8mm and 1.4mm sieves. The sieves were then examined for spent lead shot. The linings of the gizzard were examined and both were weighed.

C. Lead in the Environment

In order to try and establish the source of increased lead contamination, the bed of the main sampling site was examined for discarded lead weights and the water of The Lough was examined for elevated lead levels.

The bed of The Lough was examined using two methods.

- (1) A bottom grab sampler was dropped from a boat at 11 randomly selected stations. A total of 200 grab samples, covering a surface

- area of 4m², were taken. The samples were bagged and returned to the laboratory, sieved through 2.8mm and 1.4mm sieves respectively and subsequently examined for lead shot.
- (2) A 1m dredge was dragged 50m along the bed of The Lough, the contents of the dredge were sieved as previously described and examined for lead shot.

Five 10ml water samples were taken from The Lough, Cork City and lead content was estimated on a Perkin Elmer 2380 atomic absorption spectrophotometer in the flameless atomising mode with a wavelength of 217nm.

Standard statistical analysis was applied to the data. Means and standard deviations were calculated on a desk top calculator. For comparison of blood lead levels and data was logarithmically transformed to compare using a Students-T-test.

Results

Weight of swans sampled

At the various sites the mean weight of the live birds varied little. (Table 1, Fig. 2). The weights of the two dead birds were lower with the weight of the lead poisoned bird particularly low.

Movements of Birds during Study Period

No movement of birds from the River Lee to The Lough was recorded. However, dye marked birds at The Lough were recorded on the Lee. The number of birds marked at The Lough and observed on the Lee varied daily, with a maximum of eight on one occasion. However, on most occasions only single birds were recorded. No movement of dyed birds from other sites was recorded.

Table 1
Mute Swan Body Wgt. (kg)

Sample Location	ALIVE			DEAD
	Lough	Lee	Others	
Number	104	11	7	2
Range	4.5-12	7-12	7.5-13.5	(5) & (7)
Mean	9.23	9.27	10.2	—
S.D.	±1.4	±1.4	±1.97	—

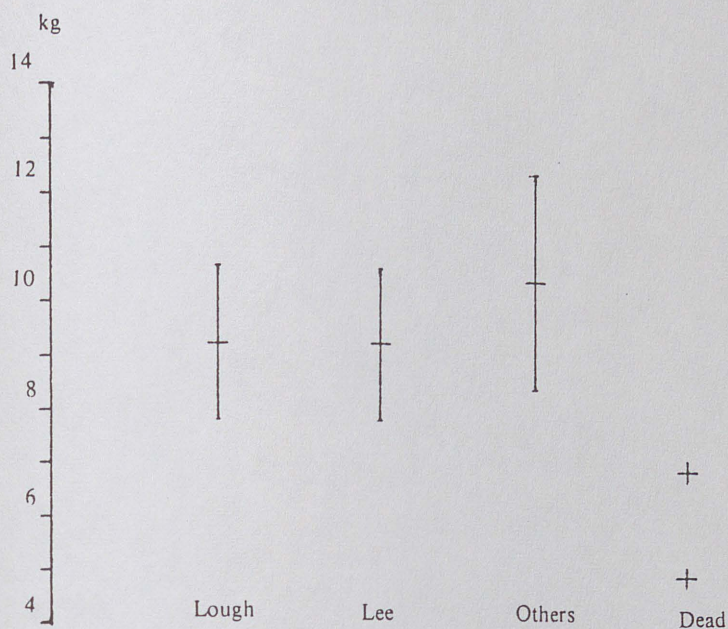


Fig. 2

The Mean and Range (Vertical Column) of Swan Body Weight of Each Group.

Blood Lead Levels

Table 2 gives details of lead levels of blood for the different age categories of birds at The Lough. Table 3 summarises the situation on the River Lee and Table 4 summarises the situation at the country sites.

The mean blood levels of The Lough birds were significantly different from those sampled on the Lee (Student T-test $P < 0.05$) and also from the country sites (Student T-test $P < 0.01$). The blood lead

Table 2
Blood Lead levels ($\mu\text{g}/100\text{mls}$)
for different age groups at
THE LOUGH

AGE	ADULT	SUB-ADULT	CYGNET	TOTAL
MEAN	43.0	200.0	21.04	87.68
S.D.	± 43.13	± 420.0	± 9.11	± 224.90
RANGE	4.14-354	4.14-1477.0	8.28-29.80	4.14-1477.0
n	71	30	3	104

levels of the birds on the Lee also differed from the country sites (Student T-test $P < 0.05$). The highest blood lead levels were recorded at The Lough, with medium levels on the River Lee and low levels at the country sites.

Ten of the birds sampled on the Lee had blood lead levels between 10-40 $\mu\text{g}/100\text{mls}$ (Fig. 3B). All the birds sampled at the country sites had blood lead levels less than 30 $\mu\text{g}/100\text{mls}$ (Fig. 3A).

The range of blood lead levels at The Lough was extensive (Fig. 3C). The majority of the birds had a blood lead level between 20 and 50 $\mu\text{g}/100\text{mls}$ (Fig. 3C). However, three birds had exceptionally high blood lead levels, in excess of 1400 $\mu\text{g}/100\text{mls}$ (1434, 1434, 1478 $\mu\text{g}/100\text{mls}$ respectively).

The first bird to be sampled at The Lough had been ringed and could, therefore, be recognised when recaptured. The blood lead level of a sample taken from this individual on 4th October 1983 was 354 $\mu\text{g}/100\text{mls}$ while on 31st October 1983 the blood lead level was 104 $\mu\text{g}/100\text{mls}$.

Post Mortem Examination Results

(a) Moribund Bird

On the water the moribund bird was easily recognised by the abnormal carriage of its neck (Plate 71). The bird always remained on its own away from other members of the flock. Over a four day period it was only observed to feed once, on soaked bread. The bird seemed to drift aimlessly around on the water. On one occasion it drifted into a channel near the Lee Maltings and remained alone there all night. Though appearing obviously weak the eyes remained open and alert throughout the period of observation. When captured the bird was noticeably light, and the breast bone (keel) could be felt through the plumage.

Table 3
Blood Lead levels ($\mu\text{g}/100\text{mls}$)
for different age groups at
THE LEE

AGE	ADULT	SUB-ADULT	CYGNET	TOTAL
MEAN	28.04	—	24.84	27.75
S.D.	± 15.43	—	—	± 15.46
RANGE	14.5-70.40	—	—	14.5-70.38
n	10	—	1	11

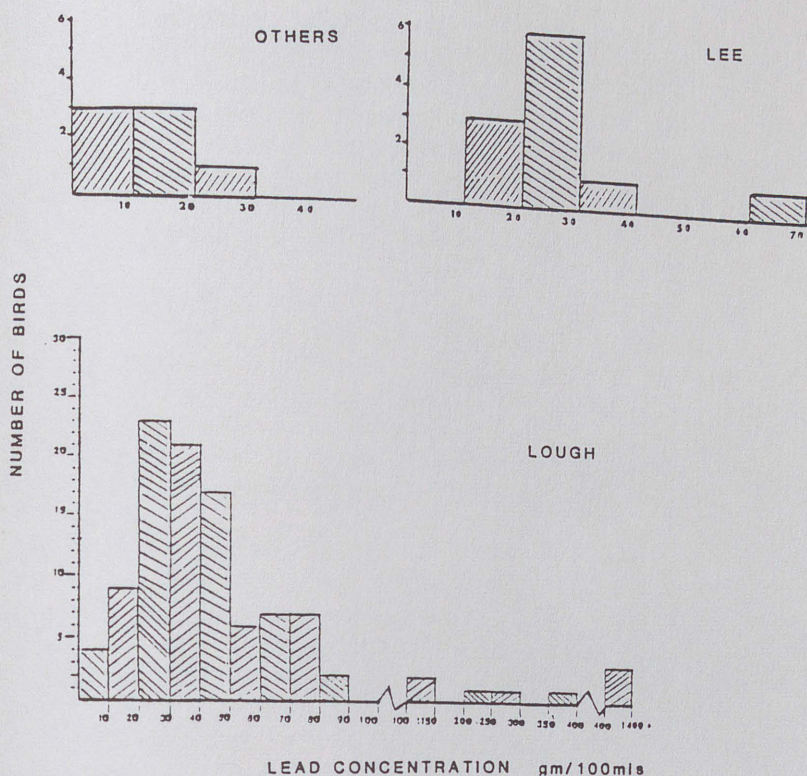


Fig. 3

Lead concentration $\mu\text{gm}/100\text{mls}$ vs. number of birds sampled from the country sites (a), from the River Lee (b) and from The Lough (c).

The carcass was noticeably light (Table 1), and atrophy of the pectoral muscles was a striking feature of *post mortem*. The liver appeared noticeably darker than the non-poisoned bird and the gall-bladder was greatly distended. The heart was flaccid. The

Table 4
Blood Lead levels ($\mu\text{g}/100\text{mls}$)
for different age groups at
OTHERS

AGE	ADULT	SUB-ADULT	CYGNET	TOTAL
MEAN	13.04	—	5.7	10.94
S.D.	± 6.13	—	± 0.5	± 6.64
RANGE	4.14-22.7	—	5.2-6.2	4.14-22.77
n	5	—	2	7

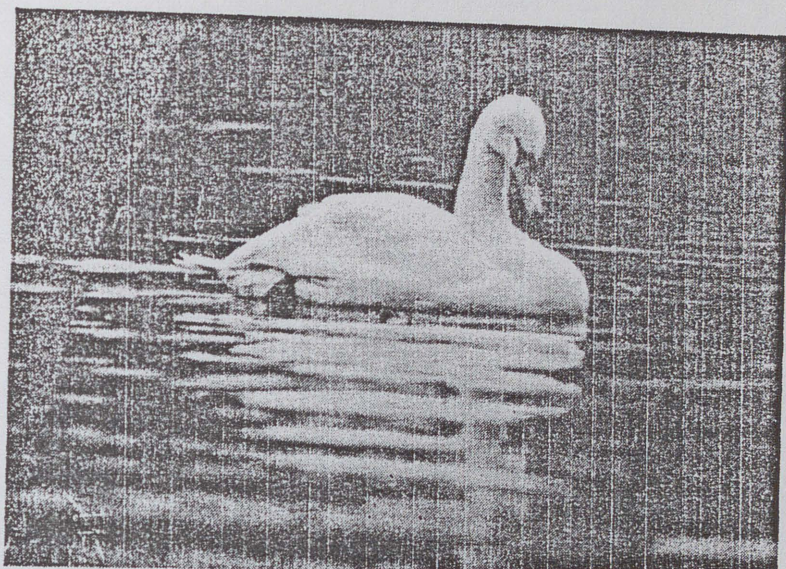
oesophagus, proventriculus and gizzard were impacted with food (150 gms consisting mostly of grass and a small quantity of bread). The horny epithelium of the gizzard was yellow in colour and broken away from the basement membrane. Four split shot lead weights were found in the gizzard. By comparison with those of the non-poisoned cygnet, the remaining internal organs appeared normal. However, in the lead poisoned bird the weight of the heart and gizzard were noticeably lighter (Table 5).

(b) The Cygnet

The cygnet had suffered severe fractures and many of the tissues were damaged due to the accident. The weight of the bird was normal for a bird of its age (Table 1). The pectoral muscle was normal, and the oesophagus, proventriculus and gizzard showed no sign of being impacted. The gizzard contained no lead weights, its weight and that of the heart were heavier than those of the poisoned bird (Table 5).

Histological Results

Plate 72 reveals a large intra-nuclear inclusion body within the nucleus of a proximal tubule cell from the kidney of the poisoned swan. Electron microscope examination revealed that these inclusion bodies were present throughout the kidney tissue. The inclusion bodies were not only found in the nucleus but were also found in the mitochondria and cytoplasm. These bodies were not confined to the



*Plate 71. Abnormal camage of neck of lead poisoned Mute Swan at Cork city*Photo: J. H. Daly

Table 5
Details of Tissue Weight (gms, wet weights)
of Dead Swans

TISSUE	SWAN	CYGNET
Liver	125.37	—
Heart	43.1	70.13
Pancreas	11.32	—
Kidney	21.4	—
Gizzard	186.58	232.66
Gall Bladder	30.48	—
Contents of Oesophagus	148.64	—

proximal tubule but were also found in those of the distal tubules. The number of intra-nuclear inclusion bodies in each nucleus varied from one to three.

Tissue Lead Levels

Six tissues of the poisoned bird and four from the cygnet were analysed. Table 5 gives details of the swans tissue lead levels, age, sex and number of lead shot found in the gizzard.

Lead in the Environment

Lead Shot

Of 200 grabs samples taken only four 'split shot' lead weights and one ledger weight was found. Fragments of fishing line were also collected from two other stations. No lead weights were found along the dredge transect.

Water Lead

No trace lead was found in the samples taken.

Discussion

This study examined for the first time the problem of lead poisoning of Mute Swans in Ireland. The measurement of lead contamination used was total blood lead. From previous work, both in dosing captive birds and examining the blood lead levels of wild individuals, it has been possible to delineate maximum tolerable levels

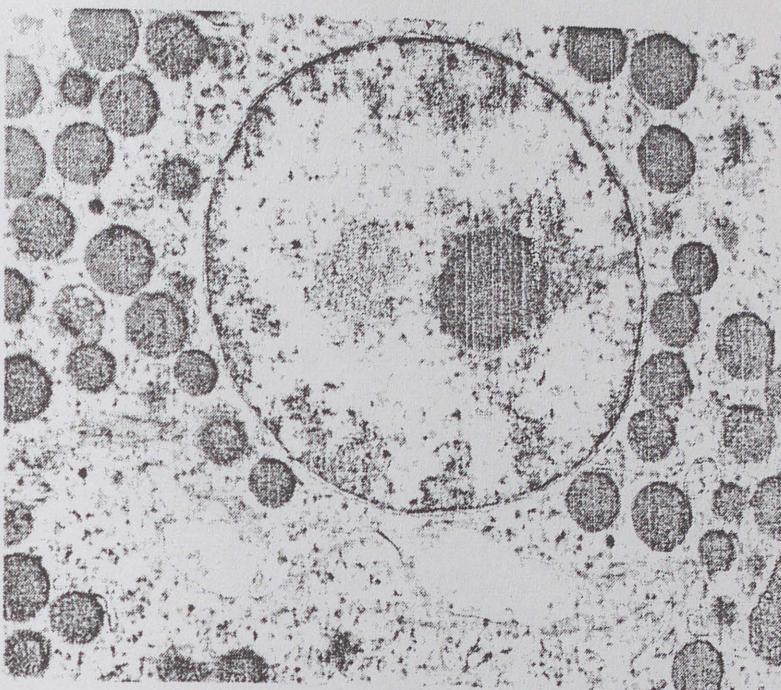


Plate 72. Electron micrograph of kidney tubule cell nucleus showing a dense compact granular region termed intra-nuclear inclusion body from poisoned swan. X 20,000.

in blood. In general, healthy birds have blood lead levels below $40\mu\text{g}/100\text{mls}$. (Simpson *et al* 1979 and Birkhead 1981). Forty-one percent of the birds sampled at The Lough had blood lead levels above this limit. By contrast the level at the country sites was well below maximum tolerable limits.

The estimated values in the blood of the birds sampled on the Lee must be treated with caution for two reasons. Firstly, less than 12% of the birds present were sampled and these results may not be representative of the whole flock. Secondly, as mentioned, the Lee population is heterogenous in that an as yet unquantified proportion utilise the Lough from time to time. Nevertheless, the blood lead levels of the River Lee sample were consistently lower than those of population at The Lough.

It is clear from the sample at The Lough, and perhaps somewhat unexpected, that adults have lower blood lead levels than sub-adults (Table 2). One possible explanation for this might be that there are differences in the extent to which both age-classes utilize The Lough. It might be surmised therefore, that because of the artificial food supply sub-adults (i.e. non-breeders) congregate and occupy The Lough continuously for prolonged periods of time. In contrast and

for obvious reasons, the visits of the adult birds are necessarily brief. This would mean, in consequence, that exposure of sub-adult birds, to the admittedly as yet undefined lead source at The Lough is relatively greater and is thus reflected in the blood picture.

Although it is unlikely that the source of increased lead was the River Lee — since it would be washed away by the swift river flow — grab sampling and dredging has failed to confirm that the birds obtained lead at The Lough. The few lead weights found there and zero trace lead levels in the water are perplexing and may reflect the inadequacy of the sampling techniques. Further research is obviously necessary in this area. Nevertheless, preliminary results from grab samples at The Lough indicate that the sub-littoral is silty and has very little grit. Thus any spent lead or lead shot having fallen into the water or been caught in the vegetation would soon be picked up by birds searching for grit.

The cygnets at The Lough, as expected, had low levels in their blood (8.28-29.80 μ g/100mls) compared to adult or sub-adult birds. However, cygnets contaminated at The Lough had demonstratively higher levels than those of adults at 'country sites'. The only cygnet value lower (8.28 μ g/100mls) was from a very young bird — introduced into The Lough from Kanturk — where its parents had been killed. Note that the other cygnet in the sample had hatched at The Lough.

However, blood lead levels are known to fluctuate and it has been suggested that this may be due to temporal variations in exposure. (Birkhead 1983). Indeed the degree of exposure as indicated by the blood picture is not now regarded as a totally reliable correlation as the elevated blood lead levels following an acute exposure are not necessarily maintained for more than seven to ten days.

A good example of this problem was noted in The Lough sample. The very first bird examined on 4th October 1983 had a blood lead level of 354 μ g/100mls. Some three weeks later this elevated level had more than halved (31st October 1983 the blood lead level was 104 μ g/100mls). Comparable variation in blood lead levels has also been noted by Birkhead (1983). Because of the interpretation difficulties which these fluctuations cause, it has been recommended that further assessment of the damage caused by high lead levels be obtained by measuring zinc protoporphyrin in the erythrocyte (Bush *et al* 1982). This is because one of the susceptible enzymes is ferrochelatase which catalyses the conversion of protoporphyrin into haem (Lee 1981). This inhibition of protoporphyrin synthesis causes anaemia, a characteristic of lead poisoning.

Although only two corpses were available for *post-mortem* examination, they proved sufficient to illustrate the difference between a lead poisoned bird and one with very low, presumably non-

toxic lead levels. The *post-mortem* examinations in this study revealed the first incidence of a Mute Swan fatality caused by lead poisoning in Ireland. The symptoms were consistent with the findings of previous workers. Both Simpson *et al* (1979) and Birkhead (1981) refer to the abnormal carriage of the neck as a pronounced symptom of lead poisoning. This clinical feature is caused by partial paralysis of the alimentary track that inhibits peristalsis with a resultant clogging of the gizzard, proventriculus and eventually the oesophagus.

Jordan and Bellrose (1951) found impacted gizzards in 44% of lead poisoned ducks and a distinct starvation syndrome, which resulted in an enlarged gallbladder, a symptom also found in this study.

Quantitative chemical analysis of soft tissues employed in this investigation provided sufficient evidence to incriminate lead as the cause of fatality. The level of lead in the liver of the poisoned bird ($44.3\mu\text{g/g}$ wet matter) exceeds the level ($50\mu\text{g/g}$ dry matter or $12.5\mu\text{g/g}$ wet matter) widely recognised as lethal in plumbism. Similarly the kidney lead level ($111.78\mu\text{g/g w.m.}$) exceed the limit ($125\mu\text{g/g d.m.}$ or $31.25\mu\text{g/g w.m.}$) required to confirm death due to lead poisoning. (Clarke and Clarke 1975, Birkhead 1982). The blood lead was also greatly elevated. Little data on the diagnostic value of the remaining tissues is published. Nevertheless, the levels found in kidney, liver and other tissues were greater than that of the cygnet by many orders of magnitude and were presumably toxic (Table 6).

The toxic effect of lead on the kidneys of many organisms including swans is under intensive investigation (Birkhead 1982 and Chisholm 1971). Much of the excess lead is concentrated in the form of dense inclusion bodies in the nuclei of the proximal and distal tubule cells (Locke, Bagley and Irby 1966).

The significance of the inclusion bodies found in this study were that they were located in the nucleus, mitochondria and cytoplasm. Although the size and number of inclusion bodies is known to vary

Table 6
Details of two swans taken for post mortem

Sex	Age	Nr. of Lead shot in Gizzard	LEAD					
			$\mu\text{g}/100\text{mls}$		$\mu\text{g}/\text{gm}$ (wet matter)			
			Blood	Breast & Heart Muscle	Kidney	Liver	Pancreas	
Male	Sub-Adult	4	652	4.24	57.34	111.78	44.43	43.78
Male	Cygnet	0	18.6	—	1.86	2.4	5.11	—

(Locke, Bagley and Irby 1966), those discovered in this study lacked the characteristic fibrillar margin found by Hutton (1980) to be present after acute exposure in mammalian and avian species. (Goyer *et al* 1971, Simpson *et al* 1979). However the number of inclusion bodies in the nuclei is consistent with that found by Locke, Bagley and Irby (1966) where two, three or four inclusions per nucleus were present in Mallard *Anas platyrhynchos* kidneys.

The effects of lead on birds varies according to the level of exposure. Unfortunately most of the information collected to date has been confined to fatal plumbism. However, the need to investigate sub-lethal levels is urgently required. It is also desirable to strengthen the monitoring technique, particularly by using zinc protoporphyrin levels more widely. As far as Ireland is concerned it is now essential to map the extent of the problem throughout the country. Only then can the full implications of apparently rising levels of lead in our environment and their potential effects on our wildlife be assessed.

Summary

Lead poisoning in Mute Swans *Cygnus olor* was studied for the first time in Ireland. 104 swans were sampled at a coarse fishing site and 18 at other sites. Forty one percent of birds at the coarse fishing site had blood lead levels greater than the maximum tolerable limit of 40ug/100mls. Only two birds at the other sites exceeded this value. The first recorded fatality of an Irish Mute Swan due to lead poisoning is described. The bird showed all the typical characteristics of the lead poisoning syndrome.

Acknowledgements

We should like to thank Professor M. F. Mulcahy and Dr. Alan Myers for facilities, assistance and supervision throughout this work; Dr. Tom Kelly for assistance and critically reading an earlier manuscript; Clive Hutchinson and Ann-Marie Ryle for their help; Mr. Joe Philpott and Robert McNamara for technical assistance.

References

- Birkhead, M. 1981 How Fishermen Kill Swans, *New Sci* 90:14-15.
 — 1982 Causes of Mortality in the Mute Swan *Cygnus olor* on the River Thames, *J. Zool, London* 198-25.
 — 1983 Lead Levels in the Blood of Mute Swan *Cygnus olor* on the River Thames, *J. Zool, London* 199:59-73.
 Bush, B., D. P. Doran and J. W. Jackson 1982 Evaluation of Erythrocyte Protoporphyrin and Zinc Photoporphyrin as Micro Screening Procedures for Lead Poisoning Detection, *Ann. Clin. Biochem.* 19:71-76.
 Chisholm, J. J. 1971 Lead Poisoning, *Sci American* 224:15-23.
 Clarke, E. G. and M. Clarke 1975 *Veterinary Toxicology*, 3rd Ed. London Beamillere.
 Evans, M. E., R. A. Wood and J. Kear 1973 Lead Shot in Bewick's Swans Wildfowl 24:56-60.
 Goyer, R. A. 1971. Lead toxicity. A problem of Environmental Pathology. *Ann J. Pathol.* 64:167-182.
 Hutton, M. 1971 Metal Contamination of Feral Pigeons *Columbia livia* from the

- London area. Part 2 *Biological Effects of Lead Exposure Enviro. Poll. Series (A)* 22:281-293.
- Jordan, J. S. and F. C. Bellrose** 1951 Lead Poisoning in Wild Waterfowl III *Nat. His. Surv. Biol. Notes*:26-27.
- Lee, W. R.** 1981 What Happens in Lead Poisoning, *Journal of Royal College of Physicians of London* 15 (1) 48-54.
- Locke, L. W., E. E. Bagley and H. D. Irby** 1966 Acid Fast Intra-Nuclear Inclusion Bodies in the Kidney of Mallards Fed Lead Shot. *Bull Wildlife Disease Assoc.* 2 127-131.
- O'Halloran, J.** 1984 A Study of Lead Levels in Mute Swan, *Cygnus olor*, in Cork. (unpublished thesis).
- Phillips, J. C. and F. C. Lincoln** 1930 Cited in Bellrose, F.C. 1959, *In Nat. His. Sur. Bull.* 27 235-288.
- Rosen M. N. and R. W. Bankowski** 1960 Diagnostic Technic and Treatment for Lead Poisoning in Swans *Calf. Fish and Game* 46.

*John O'Halloran, Department of Zoology, University College,
Cork.*
*Dr. P. F. Duggan, Biochemistry Laboratory, Regional Hospital,
Cork.*

APPENDIX 2

PRELIMINARY RESULTS OF RINGING MUTE SWANS
IN IRELAND.

Preliminary results of ringing Mute Swans in Ireland

John O'Halloran

Department of Zoology, University College Cork

Richard Collins

10 Biscayne, Malahide, Co. Dublin

Recoveries and controls of Mute Swans ringed in Ireland are analysed. The longest movement within the country was 155km. Most deaths occurred in spring. The longest known lifespan for an Irish swan is at least 12 years 9 months.

Introduction

Detailed studies of Mute Swan *Cygnus olor* populations in Dublin and Cork commenced in 1983 (Collins 1985b, O'Halloran et al 1985). These studies involve intensive colour-ringing of birds, whereas all Mute Swans ringed in Ireland before 1983 were given only standard metal rings. Studies on gulls (*Laridae*) have shown that colour-ringed birds are much more likely to be reported than birds bearing only a metal ring (Shedden et al 1985). It is useful therefore to summarise this earlier work as its results will not be directly comparable with those of the Dublin and Cork studies.

The present data have been extracted from the British Trust for Ornithology's ringing data base. This paper examines the data available on recovery rates, movements and life-expectancies of birds.

Ringing Recoveries

The number of Mute Swans ringed in Britain and Ireland up to the end of 1982 was 37,130 (Mead and Hudson 1983). The number recovered or controlled by that date was 12,688 (a "control" is a ringed bird recaptured away from its place of ringing and released again). This represents an overall recovery and control rate for Britain and Ireland of 34%. From 1975 to 1982, 258 Mute Swans were ringed in Ireland and during those eight years 31 were recovered or controlled. This suggests that the Irish recovery and control rate is of the order of 12%, much lower than the rate for Britain and Ireland as a whole.

Sixty-one Mute Swans ringed in Ireland before 1983 were recovered or controlled. No Irish Mute Swan bearing a metal ring only has been recorded abroad, though a colour-ringed swan was recently recovered in Wales (Collins 1985a).

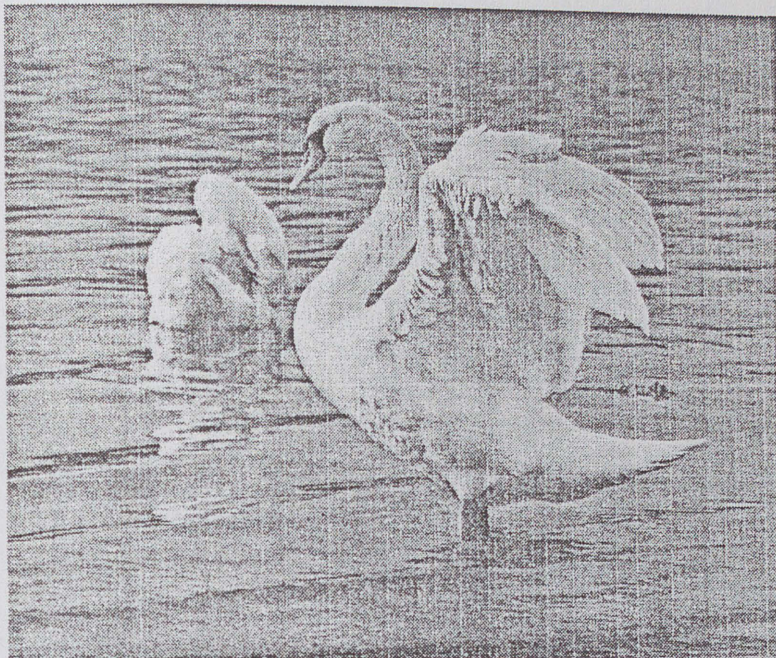


Plate 7. Mute Swans (*Ken Preston*)

A Swan ringed in Coventry, Warwickshire, was recovered in Rosscarbery, Co. Cork (Spencer and Hudson 1978). The distance between the ringing and recovery locations was 527km. Five birds colour-ringed in the Scottish Western Isles have been sighted in counties Donegal, Antrim and Derry (Spray 1981, Forsyth 1983), at distances of 284, 282 and 244km respectively from their ringing locations.

Movements of Ringed Birds

The distances of the recovery locations from their respective ringing locations varied from zero to 155km. These distances are grouped in 16km zones in Table 1. Thirty percent of the birds moved more than 32km.

Table 1. Distances moved by Irish Mute Swans: number of birds recovered or controlled in each 16km zone.

Distance	0-16	17-32	33-48	49-64	65-112	113+	km
Number of swans	22 (36%)	21 (34%)	5 (8%)	6 (10%)	3 (5%)	4 (7%)	

Ringed Mute Swans

Of the 61 Irish-ringed birds recovered or controlled, 19 were ringed as juveniles. Five of the juvenile birds were recovered or controlled more than 50km from their ringing locations. The six British birds recorded here were all ringed as juveniles. (A "juvenile" is a bird known to be less than two years old. A swan's age is reckoned from 1 July since most are hatched by then).

No swan could be confidently identified as an adult (at least three years old) when ringed. However, nine birds were known to be at least two years old at ringing. Of these, six were recovered or controlled within 20km of their ringing locations and the greatest recovery distance was 31km. Minton (1971) found that most large movements, among a population studied by him in Britain, took place during the first two years of a swan's life.

The recovery of a swan in Portarlinton in April 1963, 155km away from Lurgan where it had been ringed in January 1961, represents the largest distance known to have been travelled by an Irish-ringed Mute Swan within Ireland.

Life Expectancy

Forty ringed birds were found dead. Twelve of these (all juveniles) were of known age when ringed and hence their approximate life-spans are known. These are given in Table 2. Five had reached adulthood. The oldest died at an age of approximately 4 years 9 months.

Table 2. Survival of Irish Mute Swans of known age.

Age at death	0 to 1	1 to 2	2 to 3	3 to 4	4 to 5 years
Number of swans	4	3	0	4	1

For the 28 birds of unknown age recovered dead and for the 21 controlled birds (including seven of known age subsequently controlled), only minimum lifespan data can be given. These data are summarised in Table 3.

Table 3. Minimum lifespans for Irish Mute Swans of unknown age found dead and birds of known age subsequently controlled.

Lifespan: at least	½	1	2	3	4	5	6	7	8	11	12	years
Number of swans	1	9	9	11	4	2	5	2	1	3	2	

J. O'Halloran and R. Collins

Of the 61 birds recovered or controlled, at least 35 are known to have attained adulthood. Fifteen birds reached at least the age of five. The longest lifespan recorded is that of a bird ringed at Dundrum, Co. Down in August 1973 and recovered at the same location in April 1984. The natal year of this bird was 1971 or earlier, its lifespan, therefore, being 12 years 9 months at least. This is the longest documented lifespan of an Irish Mute Swan. The longest lifespan on record for the species is 19 years 5 months (Cramp and Simmons 1977).

The monthly pattern of recoveries is shown in Table 4. Twenty-four birds (60% of the total recovered) died during the period from February to April. In his analysis of data for Britain, Ogilvie (1967) found a similar seasonal pattern of mortality there.

Table 4. Number of recoveries in each calendar month.

Month	J	F	M	A	M	J	J	A	S	O	N	D
Recoveries	2	9	7	8	4	0	0	1	2	1	4	2

Discussion

The number of ringing recoveries and controls here to date is small. Although the sample size is small some trends appear to be emerging. It appears, for example, that Mute Swans in Ireland, as elsewhere, are more mobile during their first two years of life than in adulthood.

While Irish swans appear to travel greater distances than those found by Ogilvie (1967) for British swans, this may not be the case. The proportion of juvenile birds ringed in Britain may have been lower than the proportion of juveniles (31%) ringed here. A higher proportion of the more mobile juvenile birds will give an impression of greater overall mobility.

The patterns of seasonal mortality in Ireland and in Britain are similar. Most swans die in the spring. This may be due to the increased mobility of swans at this time of the year, with pairs seeking out territories and the last remaining young of the preceding year being displaced from their natal territories. Swans are prone to collisions with man-made obstacles, particularly power and telephone cables. In eight (13%) of the reports of Irish recoveries and controls, members of the public reported the cause of death or injury to have been a collision with a man-made structure.

No firm conclusions on patterns and causes of mortality can yet be drawn from Irish ringing data. These observations serve rather to indicate topics for further study and it is hoped that current studies will help to describe more fully the biology of the Mute Swan in Ireland.

Ringed Mute Swans

Acknowledgments

This paper is based on the work of ringers in Ireland, particularly P. Brennan, P. Cummins, D. B. McMahon, O. J. Merne, T. F. O'Mahony, P. J. Smiddy and J. A. Whatmough. Out thanks are due to the BTO who supplied the recovery data and to Dr. T. F. Cross and Dr. T. C. Kelly for reading earlier drafts of this paper.

References

- Collins, R. 1985a. Movement of a Mute Swan from Ireland to Britain. *Irish Birds* 3: 98-99.
- Collins, R. 1985b. Mute Swan population study. Abstract. First National Ornithological Research Conference presentation. *Irish Birds* 3: 144.
- Cramp, S., Simmons, K. E. L. 1977. *The Birds of the Western Palearctic, Vol 1*. Oxford University Press, Oxford.
- Forsyth, I. 1983. Irish ringing report 1982. *Irish Birds* 2: 411-429.
- Mead, C. J., Hudson, R. 1983. Report on bird ringing for 1982. *Ringing and Migration* 4: 281-320.
- Minton, C. D. T. 1971. Mute Swan flocks. *Wildfowl* 22: 71-88.
- Ogilvie, M. A. 1967. Population changes and mortality of the Mute Swan in Britain. *The Wildfowl Trust 18th Annual Report*: 64-75.
- O'Halloran, J., Myers, A. A., Duggan, P. F. 1985. The occurrence and effects of elevated lead levels in the Mute Swan. Abstract. First National Ornithological Research Conference presentation. *Irish Birds* 3: 156.
- Shedden, C. B., Monaghan, P., Ensor, K., Metcalfe, N. B. 1985. The influence of colour-rings on recovery rates of Herring and Lesser Black-backed Gulls. *Ringing and Migration* 6: 52-54.
- Spencer, R., Hudson, R. 1978. Report on bird ringing for 1976. *Ringing and Migration* 1: 189-252.
- Spray, C. J. 1981. Movements of Mute Swans from Scotland to Ireland. *Irish Birds* 2: 82-84.