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Widespread exposure to lead affects the body condition of free-living whooper swans *Cygnus cygnus* wintering in Britain

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ABSTRACT

Lead poisoning, through the ingestion of spent lead gunshot, is an established cause of morbidity and mortality in waterbirds globally, but the thresholds at which blood levels begin to affect the physiology of birds in the wild are less well known. Here we determine the prevalence of lead exposure in whooper swans and, for the first time, identify the level of blood lead associated with initial reductions in body condition. Blood lead elevated above background levels (i.e. >20 μg dL^{-1}) was found in 41.7% (125/300) of swans tested. Blood lead was significantly negatively associated with winter body condition when levels were ≥ 44 μg dL^{-1} (27/260 = 10%). Our findings indicating that sub-lethal impacts of lead on body condition occur at the lower end of previously established clinical thresholds and that a relatively high proportion of individuals in this population may be affected, reaffirm the importance of reducing contamination of the environment with lead shot.

1. Introduction

Lead is a highly toxic heavy metal that acts as a non-specific poison and is known to affect all physiological systems in animals (*e.g.* EFSA, 2010; Franson and Pain, 2011; Johnson et al., 2013; Pokras et al., 2009). Absorption of relatively large amounts of lead may cause rapid mortality from acute lead poisoning with few associated signs of poisoning at post-mortem examination. By contrast, the absorption of smaller amounts of lead, including through chronic, low level exposure, may result in a wide range of sub-lethal physiological, biochemical and behavioural impairments (*e.g.* Demayo et al., 1982; Franson and Pain, 2011; Pain et al., 2009; Scheuhammer, 1987; Vallverdú-Coll et al., 2015a).

Lead affects the function of the central and peripheral nervous systems and in birds may cause muscular paralysis resulting in impaction of the oesophagus, proventriculus, gizzard and the intestines, with subsequent weight loss, reduced body condition and an increased risk of starvation (Beyer et al., 1998; Pattee and Pain, 2003). Severe loss of body weight in birds therefore is widely identified as a characteristic sign of chronic lead poisoning and may cause extreme emaciation prior to death (Anderson, 1975; Beyer et al., 1988; Jordan and Bellrose, 1951; Pattee et al., 1981, 2006; Quortrup and Shillinger, 1941; Vallverdú-Coll et al., 2015a, b; Wobeser, 1997). Clinical changes related to body condition and associated with lead poisoning in birds include muscle atrophy, most notably breast muscle, and loss of subcutaneous or visceral fat (Beyer et al., 1998; Jordan, 1953). In a review of experimental studies spanning 35 years, Sanderson and Bellrose (1986) concluded that change in body mass was the most appropriate indicator for assessing levels of lead poisoning in birds. While the dose–response relationship can be affected by a wide range of biological and environmental factors in birds, field and experimental studies have shown that birds often die approximately 2–3 weeks after ingesting lead gunshot, often in an extremely emaciated condition (*e.g.* Barrett and Karstad, 1971; Beyer et al., 1998; De Francisco et al., 2003; Irby et al., 1967; Locke and Thomas, 1996; Sanderson and Bellrose, 1986; Szymczak and Adrian, 1978; USFWS, 1986; Wobeser, 1981). However, the effects of lead poisoning on body weight may vary considerably and absence of weight loss and other atypical signs of lead poisoning have been

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recorded in affected birds (Álvarez-Lloret et al., 2014; Martínez-Haro et al., 2011). For example, in cases of acute poisoning, birds may die rapidly in apparently good body condition (Scheuhammer and Norris, 1996).

Lead poisoning is a well documented cause of morbidity and mortality in waterbirds which ingest anglers' weights (UNEP-AEWA, 2011) or spent lead gunshot (Mateo, 2009; Pain et al., 2015), either inadvertently when such objects are mistaken for food particles or more actively as grit which is retained in the muscular gizzard to aid mechanical breakdown of food. Once in the gizzard, the shot are ground into smaller particles and lead salts absorbed into the bloodstream. Lead poisoning has been recorded in wild swans globally (*e.g.* Blus, 1994; Nam and Lee, 2011; Newth et al., 2012; Ochiai et al., 1992; O'Connell et al., 2008; O'Halloran et al., 2002; Perrins et al., 2003), with poisoning of mute swans (*Cygnus olor*) attributed to the ingestion of discarded lead fishing weights (Perrins et al., 2003), whereas migratory whooper swans (*Cygnus cygnus*) and Bewick's swans (*Cygnus columbianus bewickii*) more commonly ingest spent lead gunshot (Newth et al., 2012; O'Connell et al., 2008; Spray and Milne, 1988). Migratory swans feeding in areas shot-over with lead gunshot are thus particularly susceptible to lead exposure. In a study investigating the mortality of 2365 waterbirds recovered across Britain between 1971 and 2010, lead poisoning accounted for the deaths of 27.3% of whooper swans and 23% of Bewick's swans (Newth et al., 2012). Elevated blood lead levels (i.e. >20 μg dL^{-1}; Franson and Pain, 2011) were recorded in 43% of live whooper swans caught in Britain in the 2010/11 winter (Newth et al., 2012). Previously, O'Connell et al. (2008) recorded elevated lead levels (then defined as >25 μg dL^{-1}) in 44–70% of live whooper swans caught at wintering sites in Britain and Ireland.

Within the UK, the use of lead shot was banned over the foreshore and specified (wetland) Sites of Special Scientific Interest (SSSIs) for hunting wildfowl, coot (*Fulica atra*) and moorhen (*Gallinula chloropus*) in England in 1999 and Wales in 2002, and for hunting over wetlands (for any type of shooting activity) in Scotland in 2004 and Northern Ireland in 2009 (HMSO, 1999, 2002a, b, 2003, 2004, 2009). However, lead shot continues to be used legally in areas occupied by feeding swans and other waterbirds where restrictions do not currently apply (Newth et al., 2012) and compliance with restrictions in England has been poor (Cromie et al., 2002, 2010, 2015).

Field studies have shown reduced survival (Guillemain et al., 2007; Tavecchia et al., 2001) and a range of sub-lethal effects in wildfowl following ingestion of lead gunshot. Evidence also suggests that sub-lethal lead poisoning can increase the likelihood of mortality from other factors, such as flying accidents in wild mute swans (Kelly and Kelly, 2005) and the susceptibility to being hunted in a wide range of wildfowl (Bellrose, 1959; Demendi and Petrie, 2006; Heitmeyer et al., 1993). However, the relationship between blood lead levels and body condition in free-living wildfowl, including swans, has yet to be quantified. Identifying thresholds for tissue lead concentrations at which measurable physiological effects occur is important for determining the impacts of lead on individuals and populations. Franson and Pain (2011) considered that blood lead levels of >20 μg dL^{-1} in Anseriformes exceeded background levels. These concentrations have been considered as indicative of environmental lead ingestion (O'Halloran et al., 1988) and are consistent with adverse physiological effects. Anseriformes were found to have subclinical poisoning (whereby impairment of normal biological functioning occurs but is not sufficiently severe to develop apparent signs) at 20–<50 μg dL^{-1}, signs of clinical poisoning (including weight loss) at 50–100 μg dL^{-1} or of severe clinical poisoning at >100 μg dL^{-1} (Franson and Pain, 2011). However, these thresholds were largely determined from studies of captive birds that had been dosed with lead in experimental and controlled settings and included a limited number of studies of wild birds. Tissue lead residues associated with physiological injury, clinical signs and death due to lead poisoning may vary between individuals (Johnson et al., 2013; Pain, 1996; Pattee et al., 2006) and species (Franson and Pain, 2011; Pattee and Pain, 2003), for a range of ecological and biological reasons.

Body condition is an indicator of the energetic state of an animal, especially its energy reserves (fat and protein) relative to the skeletal body size of the animal (Gosler, 1996; Krebs and Singleton, 1993; Schulte-Hostedde et al., 2001), and is assumed to affect individual health and fitness (Peig and Green, 2009). Fat storage can greatly influence migration strategies (Pierce and McWilliams, 2004), over-winter survival (Rogers and Reed, 2003) and clutch size (Christians, 2000) in avian species. This study therefore aims to quantify the blood lead levels that have a significant influence on individuals' body condition in a population of free-living birds. The effects of age, sex, timing of blood sampling, wintering location and breeding status on susceptibility to lead poisoning are also considered. This analysis comes at a time when there is a global policy focus on the effects of lead ammunition on both wildlife and human health (Bellinger et al., 2013; Bernhoft et al., 2014; Stroud, 2015; UNEP-CMS, 2014a, b; Watson et al., 2009).

2. Methods

2.1. Study sites and sample collection

Whooper swans were caught in winters 2010/11, 2012/13 and 2013/14 in Britain; at Martin Mere, Lancashire (51° 58′ 98″ N, 2° 25′ 02″ W) and at Caerlaverock, Dumfriesshire (54° 58′ 02″ N, 3° 25′ 02″ W). Blood samples were taken from individual birds for lead level analysis; blood lead concentrations usually reflect recent exposure to lead, i.e. within the preceding 35–40 days (O'Halloran et al., 1988). A blood sample was taken under Home Office license from the medial metatarsal vein of each bird with a 2-mL syringe using a 23-gauge needle as part of on-going broader health studies. Sub-samples of any excess blood were then transferred into a 1.5-mL tube containing lithium heparin and chilled at 4 °C until analysis.

The swans were also sexed (by cloacal examination), ringed and aged (as either adults or first-winter juveniles by plumage characteristics). Body mass was determined (±0.1 kg) using a spring balance as described by Evans and Kear (1978). Skull and tarsus length were measured with a sliding calliper (±1.0 mm) for use in conjunction with body mass to determine the body condition of the birds; skull length was taken from the base of the skull to the tip of the bill and tarsus length from the notch of the intertarsal joint to the end of the tarsus bone (following Redfern and Clark, 2001).

2.2. Blood analysis

A sub-sample of 0.1 g was taken from each blood sample and 0.5 ml of concentrated nitric acid added. Samples were left overnight, after which 1.0 ml of hydrogen peroxide was added to each sample. The samples were then digested using a microwave digester (MARS, CEM), with a final incubation at 95 °C for 30 min. The digests were made up to 5 ml by mass with milliQ water. Samples were then analysed for lead using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500 series), and as an internal standard, a continuous concentration of 10 μg l^{-1} rhodium, prepared in 1% nitric acid, was introduced into the sample stream via a T-piece.

Several methods were used to ensure quality control. Blanks (digests without blood, processed using the methods outlined