

# Incidence of lead ingestion in managed goose populations and the efficacy of imposed restrictions on the use of lead shot

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Lead is a toxic heavy metal that when ingested can cause death or sub-lethal fitness effects. Despite its toxicity, it is still widely used in recreational and management shooting globally. To reduce the impacts of lead on wildfowl, recent European Union legislation has banned the use of lead shot in and around wetlands from 2023. Understanding the effectiveness of such mitigation is vital to inform future policy. On Islay, Scotland, the licensed shooting of Barnacle Geese Branta leucopsis to reduce agricultural damage has adhered to the ban on use of lead shot over Ramsar-designated wetlands legislated in Scotland in 2004. On average 2380 lead cartridges were fired annually between 2005 and 2020 outside designated wetlands, where Barnacle Geese and other wildfowl forage. From faecal samples, it is possible to infer whether birds have ingested lead and are therefore potentially suffering from lead poisoning. After sampling faeces from Barnacle Geese (n =193) and Greenland White-fronted Geese Anser albifrons flavirostris (n = 150) we found only four (1.2%) faecal samples with elevated lead levels that may be indicative of lead shot ingestion. Further post-mortem examinations (n = 102 Barnacle Geese only) and Xray of live birds (n = 293) revealed similarly low levels of shot ingestion in both species (post-mortem < 4%, and X-ray < 2%), corroborating findings from faecal sample analysis. When subsequently accounting for limited shot retention time within individuals, the proportion of each population ingesting a single lead shot over a winter was estimated at a maximum of 9.4% (Barnacle Geese) and 16.8% (White-fronted Geese). We propose that high compliance with the ban on using lead shot over wetlands because of carefully controlled shooting management on Islay has led to relatively low instantaneous ingestion rates, probably resulting in minimal lead poisoning mortality. However, ingestion was not eliminated and the potential fitness effect of chronic lead poisoning in both goose populations therefore persists, although use of lead shot in organized shooting has subsequently been discontinued. Recent European Union bans on lead shot use over wetlands may reduce lead ingestion in waterfowl if compliance rates are high, but as foraging often occurs outside wetlands (as in this study), further restrictions including use on other key foraging sites may help to further mitigate the risk of lead poisoning in waterfowl.

Keywords: Barnacle Goose, faecal sampling, lead poisoning, shooting management, White-fronted Goose.

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Lead negatively affects most physiological systems in animals and is a well-documented cause of morbidity and mortality in numerous wildfowl species (Mateo et al. 2007, Mateo 2009, Newth et al. 2012). At high doses, lead poisoning causes death, but chronic, low-level exposure has numerous sub-lethal physiological and behavioural impacts including reductions in productivity and migratory performance (Pain et al. 2019, Green & Pain 2020). In addition, lead poisoning can cause death indirectly by increasing vulnerability to powerline collision (Kelly & Kelly 2004, Ecke et al. 2017) and future shooting mortality because of neural impairment (Bellrose 1959, Fisher et al. 2006, Green & Pain 2016). In the UK, an estimated 50 000-100 000 (Pain et al. 2015) wildfowl die annually because of ingestion of spent lead gunshot, and fitness effects are evident in a further 74 000-353 000 individuals every winter (Cromie et al. 2015).

In wildfowl, direct ingestion is the primary source of lead poisoning as individuals inadvertently swallow shot when foraging for food or grit (Mateo 2009, Pain et al. 2015, Romano et al. 2016). Globally, thousands of tonnes of spent lead gunshots are irretrievably deposited in the environment each year and between 8000 and 13 000 tonnes are used in the UK annually (Cromie et al. 2015). Lead pellets degrade very slowly, allowing lead to persist in the environment for decades after deposition (Takamatsu et al. 2010, Binkowski 2017, Kanstrup et al. 2020) and the risk of lead poisoning is greater in areas with intense, regular shooting (Aloupi et al. 2015, Mateo et al. 2016, Pain et al. 2019). To reduce the impact of future lead poisoning on wild populations, various restrictions have been put in place. This includes legislation to control and ban the use and trade of lead in specific habitats/regions, as well as increasing pressure for community-led voluntary transitions to non-lead alternatives (Fisher et al. 2006, Avery & Watson 2009, Mateo & Kanstrup 2019, Green et al. 2021). In the UK, the use of lead shot was banned over the foreshore and specified wetland SSSIs (Sites of Special Scientific Interest) for hunting wildfowl (swans, geese and ducks), Coot Fulica atra and Moorhen Gallinula chloropus in England in 1999 and Wales in 2002 (HMSO 1999, 2002a, 2002b). In Scotland and Ireland (bans introduced in 2004 and 2009.

respectively), the ban refers to lead in specified wetlands (as defined by the Ramsar Convention) and does not refer to specific species (HMSO 2004, 2009). Although these restrictions can prevent further lead contamination of protected areas, they fail to cover all habitats used by waterfowl, compliance is often low, with little enforcement (Mateo & Kanstrup 2019, Green *et al.* 2021, 2022, Stroud *et al.* 2021, Widemo 2021) and historically deposited lead persists in the environment, meaning that many species are still at risk of lead ingestion, long after bans have been introduced (O'Connell *et al.* 2008, Newth *et al.* 2012, Haig *et al.* 2014, Binkowski 2017).

Where lead shot continues to be used and in areas of historical high-intensity shooting, understanding the extent of lead ingestion is vital for species protection and to inform future policy and practice (Romano et al. 2016, Green & Pain 2020). In wildfowl, ingested lead shot is broken down in the gizzard and subsequently absorbed into the bloodstream and deposited in soft tissues such as the liver and kidneys (short-term), and into bone (long-term; Tsipoura *et al.* 2011, Pineau et al. 2017). Previous work has established threshold values of lead concentrations that are indicative of toxicity in waterfowl and provides information as to short-term and long-term exposure using blood, tissue and bone samples (Binkowski et al. 2013, Markowski et al. 2013, French et al. 2017). The presence and number of ingested metal shot, not necessarily lead, can also be determined by examining the gizzards of dead birds or by X-raying live birds and looking for the presence of radiodense metal in the gizzard. Both methods can detect up to 75% of shot pellets or fragments present in the gizzard (Montalbano & Hines 1978). Faecal sampling also provides an assessment of current and recent lead exposure non-invasively by delineating between ingestion of lead shot and other shot types such as steel, and has been used in a variety of goose species including Red-breasted Goose Branta ruficollis (Mateo et al. 2016); Greylag Goose Anser anser (Martinez-Haro et al. 2013); and Lesser White-fronted Goose Anser erythropus and Greater White-fronted Goose Anser albifrons (Aloupi et al. 2015). Geese ingest soil or sediment that contains trace amounts of aluminium and lead when actively collecting grit or accidentally when ingesting vegetation. The amount of aluminium in

faeces is indicative of the level of soil or sediment ingestion and if that is the primary source of faecal lead then concentrations of lead and aluminium will tend to be closely correlated across individuals (Martínez-Haro *et al.* 2010, Aloupi *et al.* 2015, Mateo *et al.* 2016). Any intake of additional lead, such as ingested lead shot, would cause a significant deviation from the normally strong correlation between aluminium and lead (Elliott *et al.* 2008, Yin *et al.* 2008, Martínez-Haro *et al.* 2010, Martinez-Haro *et al.* 2011a).

Islay, Scotland, is an important wintering site for the Greenland Barnacle Goose Branta leucopsis (Barnacle Goose hereafter) and Greenland Whitefronted Goose Anser albifrons flavirostris (Whitefronted Goose hereafter). The Barnacle Goose population has increased considerably since the 1980s to a peak of c. 50 000 individuals in 2005, resulting in intensifying conflict with local agricultural stakeholders because of goose grazing pressure on farmland (Mason et al. 2017, McKenzie & Shaw 2017). This led to the implementation of successive goose management schemes, first set up in 1992, which have included scaring, monetary subsidies to compensate for damage, divisionary feeding and, more recently, shooting (McKenzie & Shaw 2017). Since the 2000s. Barnacle Geese have been subject to licensed shooting on Islay, with licensing controlled by NatureScot (formally Scottish Natural Heritage: McKenzie & Shaw 2017). Management plans have adapted to changing population sizes, by altering shooting intensity annually (Fig. 1). Before 2000, Barnacle Goose shooting levels were very low because shooting was not used as a management approach. Since the implementation of the first shooting management schemes in 2000, lead shot has been used on sites across Islay, except for wetlands within Ramsar sites since 2004 (HMSO 2004). We estimate that 21% of agricultural fields have been shot over with lead on Islay since 2000 as part of co-ordinated goose management (see Methods section).

Barnacle Geese and White-fronted Geese preferentially feed on agricultural grasslands, especially recently reseeded pasture (Griffin *et al.* 2016), which are often outside protected Ramsar sites. The Islay Sustainable Goose Management Strategy (ISGMS), implemented in 2014 by NatureScot (and delivered by the goose scheme), aims to reduce damage to agricultural pasture by several means, including by reducing the Barnacle Goose population. The White-fronted Goose population declined from *c*. 13 500 birds in 1999 to *c*. 4500 birds in 2012 (Fox *et al.* 2018), mirroring their global population decline. Since 2012, the population has grown and stabilized, numbering *c*. 6000 in 2020. Given the prevailing conservation concerns for White-fronted Geese, they are not shot as part of the ISGMS. However, they use many of the same foraging sites as Barnacle Geese and, as a result, both species are at risk of ingesting lead shot and subsequent poisoning. For Barnacle Geese this is of concern because of unobserved mortality not being accounted for in the annual shooting totals, whereas for White-fronted Geese any additional mortality could jeopardize population recovery (Griffin *et al.* 2020).

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In this study we collected faecal samples from Barnacle Geese and White-fronted Geese during the winters of 2017/18 and 2018/19 to determine lead shot ingestion rates. We combined this with assessments of shot ingestion from post-mortems of shot Barnacle Geese and X-ray screening of live captured birds. All approaches sampled individuals from sites across Islay to assess the effectiveness of mitigation strategies in preventing additional mortality due to lead poisoning from the ISGMS and provide an Islay-wide assessment of shot ingestion rates.

# **METHODS**

# **Study species and site**

Barnacle Geese and White-fronted Geese use the northern and western regions of Scotland and Ireland as non-breeding grounds, and the southern and western regions of Iceland as staging grounds, with White-fronted Geese breeding in Western Greenland and Barnacle Geese breeding in Eastern Greenland. Barnacle Geese arrive in the UK and Ireland around mid-October and depart by mid- to late April. White-fronted Geese have a shorter wintering period arriving back around 2 weeks later and departing around 1 week earlier. Islay is a southerly island in the Inner Hebrides archipelago (Scotland) located around 30 km off the west coast of the Scottish mainland. On Islay the two taxa differ in their selection of roosting sites. Barnacle Geese use three to five large roosting sites that are coastal tidal areas with large expanses of sand flats and saltmarsh (RPS Ecology 2016). White-fronted Geese use a larger number of roosting sites (hundreds) found mainly in mire/quaking bog habitats

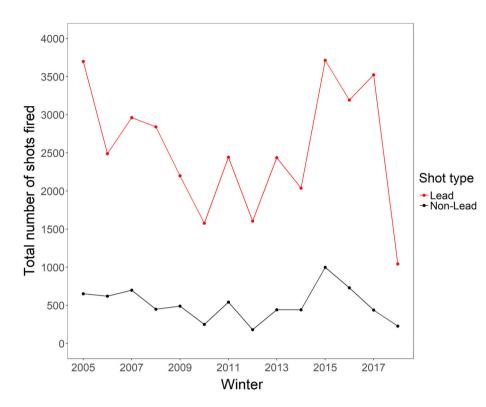


Figure 1. The total number of lead and non-lead shotgun cartridges fired by NatureScot-licensed marksmen as part of the goose management programme on Islay, Scotland, between winter 2005/06 and 2018/19. This does not include shots fired by farmers and therefore represents the minimum number of shots fired as part of the goose scheme. [Colour figure can be viewed at wileyonlinelibrary.com]

in unenclosed upland (Griffin *et al.* 2016). The two populations therefore forage in sympatry but roost in allopatry.

#### **Faecal sample collection**

Single species flocks were located during the day and faecal samples were collected in those locations the subsequent night. This avoided disturbing geese while foraging during collection and prevented the species origin of the sample being ambiguous. For each flock, two to four samples were collected at least 10 m apart and from relatively large flock sizes (Barnacle Goose sampled mean flock size, n = 540, White-fronted Goose sampled mean flock size n = 90) to minimize the chances of repeat sampling the same individual. Samples were stored in a -10 °C freezer before analysis. Samples were collected opportunistically to maximize the number available for analysis but all large flocks on Islay were sampled at least once. Sampling was conducted in February 2018 for Barnacle Geese and in January-April 2019 for Barnacle Geese and White-fronted Geese. This was at least 2 months after birds had returned from staging in Iceland to ensure that any elevated lead levels were from ingestion on Islay (as opposed to Iceland). For Barnacle Geese 193 faecal samples were collected and for White-fronted Geese 150 samples were collected (Fig. 2).

#### Metal analysis of faecal samples

Metal analysis was adapted after Martín-Vélez et al. (2021). Briefly, faecal samples were dried at 105 °C and then between 0.20 and 0.25 g of each sample was weighed accurately into pre-cleaned low-pressure PTFE microwave digest vessels (50mL vessels, placed in a 24-vessel carousel). To the samples, 4.5 mL of trace metal grade concentrated nitric acid (> 67%; Fisher Scientific, Hampton, NH, USA) was added and vessels were left overnight to pre-digest. The following day, 1.5 mL of trace metal grade hydrogen peroxide (> 30%; Fisher Scientific, Loughborough, UK) was added, vessels were sealedand the samples were digested

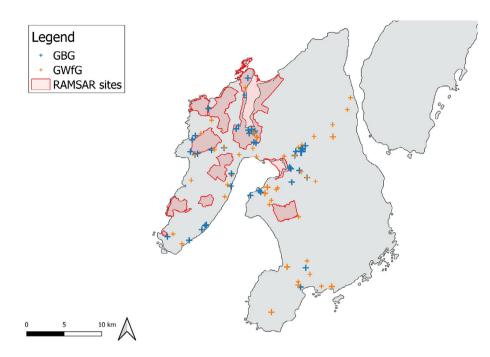


Figure 2. Map of the Ramsar designated wetlands on Islay, Scotland. The locations where faecal samples were collected are marked for Barnacle Geese (GBG, blue) and White-fronted Geese (GWfG, orange). The larger cross sizes indicate a greater number of samples collected at that particular site, which range from two to four. [Colour figure can be viewed at wileyonlinelibrary.com]

in a microwave system (Multiwave PRO; Anton Paar, Graz, Austria), using a programme with a maximum temperature of 180 °C. Final digests were then decanted into polypropylene sample tubes and made up to a final volume of 16 mL with Milli-Q Type I ultrapure water. Inductively coupled plasma – optical emission spectrometry was performed on a Varian 720-OES (Agilent Technologies, Santa Clara, CA, USA) instrument to determine metal concentrations. A Certified Reference Material (CRM) was used to assess the recovery of elements during the digestion and analytical process. The CRM used was Domestic Sewage Sludge (CRM031-40G; Sigma-Aldrich, St Louis, MO, USA), which had mean ( $\pm$  standard deviation (sd)) certified lead and aluminium levels of  $105 \pm 7$  mg/kg and  $15.4 \pm 1.5$  g/kg, respectively. Mean  $(\pm sd)$  lead and aluminium levels attained during analysis of this CRM were  $89.4 \pm 2$  and  $18.5 \pm 0.5$  g/kg (n = 5), respectively, and the limit of detection (LOD) in faeces determined for lead and aluminium were 0.45 and 5.26 mg/kg, respectively (n=31 procedural blanks). All samples, CRMs and procedural blanks were digested using the same procedure. All data points that fell below the LOD were replaced by 0.5\*LOD (0.23 mg/kg for lead and 2.53 mg/kg for aluminium) in the dataset used for further analysis, following the approach in Martínez-Haro *et al.* (2010).

#### **Calculating historical shooting intensity**

As part of the goose scheme on Islay, every agricultural field has been assigned its own unique code. Therefore, each shooting event and the number of shotgun cartridges fired by marksmen employed by NatureScot have been recorded at the individual field level since 2005. As this is one of the main sources of anthropogenic lead on Islay, we use the shooting data to calculate a measure of anthropogenic lead deposition at each sampling site. Shooting is also carried out by farmers but constitutes less than 20% of goose shooting annually (Fig. 3) and lacks locational data, and therefore it was not incorporated into this analysis. Recreational shooting does not generally take place on the agricultural land (improved grassland fields) targeted by the goose scheme shooting; for this reason, we feel that recreational shooting would not significantly contribute to lead deposition around our faecal sampling sites. We assume that sampled individuals used the area around the sampling site for feeding

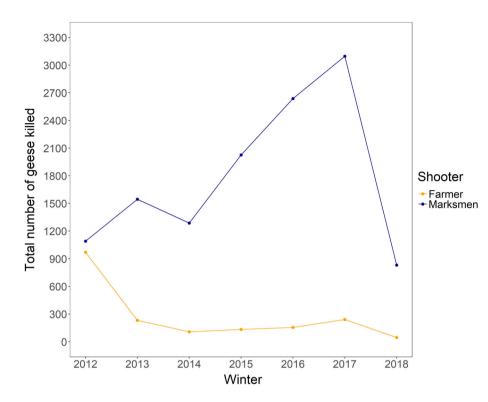


Figure 3. The number of Barnacle Geese killed by NatureScot-contracted marksmen versus farmers on Islay, as part of the goose scheme, between winters 2012/13 and 2018/19. [Colour figure can be viewed at wileyonlinelibrary.com]

before sample collection. This seems reasonable as White-fronted Geese are known to be site-faithful within winters (Wilson et al. 1991) with less than 1% of birds switching sites, and Barnacle Geese also appear to have high within-winter site fidelity (Percival 1988). To calculate anthropogenic lead deposition, we first created a 1-km-radius buffer around the centroid of the fields where faecal samples were collected. We then identified any fields that fell within or overlapped the 1-km buffer and summed the total number of lead shotgun cartridges fired in those fields since 2005 (Fig. 4). We trialled different buffer sizes around the sampling field (2, 3, 4 and 5 km) but this did not alter the sub-model that was retained during model selection, and therefore our inference remained the same.

We used shooting records to calculate the percentage of fields shot over with lead and the average density of lead in those fields as part of the goose scheme. A field was shot over with lead if at least a single lead cartridge had been fired over a field from 2005 to 2020. We then calculated the total number of pellets in all the lead cartridges fired, assuming 175 pellets in a 50-g cartridge of size two shot, which was the most used cartridge in the goose scheme. The total number of pellets was then divided by the area of all fields shot over with lead, in  $\text{km}^2$ , to give a density estimate. It should be noted that these are minimum estimates as additional shooting with lead will have been carried out by farmers in the goose scheme.

#### Statistical analysis of faecal lead levels

Lead and aluminium levels in mg/kg dry weight of faeces were log transformed to better fit a normal distribution (following the approach of Martínez-Haro *et al.* 2010). To understand what might explain variation in faecal lead, we used a linear mixed model with log(lead) as the response variable, shooting intensity and log(aluminium) as continuous explanatory variables, species as a fixed two-level explanatory factor (i.e. Barnacle Goose and White-fronted Goose) and interactions between species as the continuous variables. This allowed the intercept and the slope of the regression line between log(lead) and log(aluminium) to vary between the two species. Individual field code nested withinfarm assignment (a collection of fields

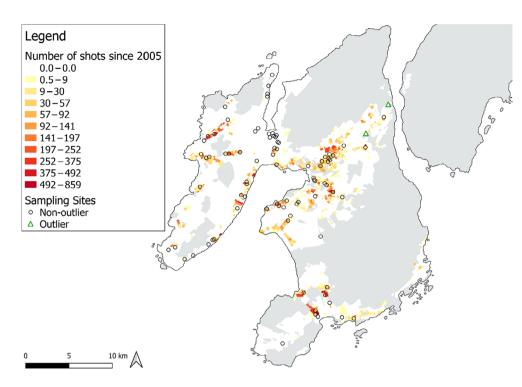


Figure 4. The minimum number of lead shotgun cartridges fired in each field on Islay, Scotland, as part of the goose management scheme between 2005 and 2019. The faecal sampling sites for Barnacle Goose and White-fronted Goose are also depicted. Grey areas depict non-agricultural habitats, mainly forestry and moorland. The two sampling sites where the four White-fronted Goose samples with elevated lead levels were collected are depicted (green triangles). [Colour figure can be viewed at wileyonlinelibrary.com]

grouped in space but owned by the same farm) was used as a random intercept term to control for the non-independence of sampled individuals from the same flock and from flocks that used similar areas for foraging. All potential sub-models, containing all possible combinations of explanatory variables and their interactions were derived from the maximal model and run using the dredge function in the R package MuMin (Bartoń 2020) and compared using Akaike's information criterion (AIC). We used the nesting rule to eliminate models that were more complex versions of nested models that had lower AIC values; for example, if model 'A + B' has the lowest AIC then model 'A + B + C' is removed but not model 'A + C + D'. This prevents the selection of overly complex models (Burnham et al. 2011). Only models within six AIC points of the top model were retained in our 'top model set' (Burnham et al. 2011, Harrison et al. 2018). Parameter estimates presented are those from the 'top model set' following multimodel inference and are not back-transformed as the response and aluminium explanatory variable were both log transformed. Coefficients for continuous predictors should be interpreted as the percentage increase in the dependent variable for every 1% increase in the independent variables. All analysis was carried out using R version 3.6.3 (R Core Team 2020). For data manipulation we used the packages within the *tidyverse* (Wickham *et al.* 2019) and for plotting model results we used the *effects* package (Fox & Weisberg 2019).

#### **Identifying elevated lead levels**

We used the amount of aluminium in faeces as an assessment of the amount of soil/sediment ingestion and assumed that co-ingestion of lead from the same soil/sediment is normally closely correlated with aluminium. Large deviations from this relationship are then indicative of lead ingestion from an additional source. Individual birds with elevated lead levels in faeces have been detected previously (Martínez-Haro *et al.* 2010) by visually identifying data points that are large positive outliers from the regression line between log(lead) and log(aluminium). We used a more robust method to classify outliers, by considering whether

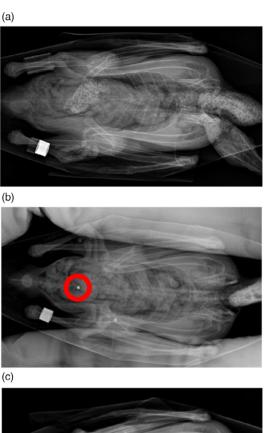
the data point fell outside the 95% prediction interval. A 95% prediction interval was used as the probability that a future observation will fall within this interval is 95%, given the values already observed. We further verified this finding by considering the aluminium:lead ratios in Scottish soil samples from habitats and soil types similar to the areas used by geese on Islay (see Figs S1-S3; Soil Survey of Scotland Staff 1981). Outliers were detected by identifying samples that differed from the normal variation of aluminium: lead in soil samples and the findings corroborated those from the 95% prediction interval approach above. We used a Fisher's exact test to test if there was a statistically significant association between the number of samples above the 95% prediction interval plus the number of birds with lead ingestion from X-rays and post-mortems (see below) and the species those samples were from.

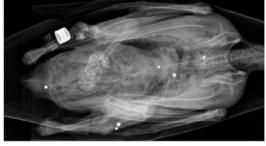
# **Post-mortems of Barnacle Geese**

Post-mortems were carried out on 106 Barnacle Geese shot as part of the goose scheme during the winters of 2018/19 and 2019/20. Each winter, geese were killed between 1 November and 29 March across Islay. Carcasses were classified as adult or juvenile according to plumage characteristics (Percival *et al.* 1997, Inger *et al.* 2006). Any gizzards with apparent shooting-induced damage were excluded from further post-mortem examination (n = 4) as any detected metal could be from the shooting optimate examinations of the gizzard contents to identify the presence of ingested metal shot. Pellets were tested for magnetism and malleability to distinguish between lead and other non-lead shot.

## X-ray procedure and analysis

Mobile X-ray scanning was used to assess the presence of ingested and embedded shot in live Barnacle Geese and White-fronted Geese caught using cannon nets at baited sites across Islay, with capture and handling performed under licence from the British Trust for Ornithology's Special Methods Technical Panel of the UK ringing scheme. Each bird was restrained in a Velcro jacket during X-ray screening. Two images were taken initially, of the dorsal and lateral sides. When an ingested or embedded shot was preliminarily identified, a third image was often taken of





**Figure 5.** Example X-rays of live-caught Barnacle Geese wintering on Islay. Ingested and embedded shot pellets appear as bright white spots. (a) An example of a clean X-ray with no ingested or embedded shot, (b) an example of ingested shot in the gizzard (circled in red), and (c) an example of an individual with numerous embedded pellets throughout the body. [Colour figure can be viewed at wileyonlinelibrary.com]

the ventral side to assist with determining the shot's position. Embedded metal shot was frequently present in both goose species (25% of Barnacle Geese and 11% of White-fronted Geese Xrayed), typically in tissue such as the breast, or bone such as in the wing (Fig. 5). Images with suspected shot ingestion (within the gizzard or intestine) were verified by a member of the Wildfowl and Wetlands Trust veterinary team, although it was not possible to determine the type of shot (lead or non-lead). In total, 110 White-fronted Geese were X-rayed during the winters of 2016/17 and 2019/20 and 183 Barnacle Geese between 2016/17 and 2019/20.

# Accounting for retention time of lead shot in the gizzard

Lead shot is retained in the gizzard for a limited amount of time as the metal is eroded by other gizzard contents (i.e. grit), the shot will undergo dissolution and the gizzard contents are replenished (Plouzeau et al. 2011). When calculating the number of individuals that will ingest lead over a certain time, e.g. an entire winter, a cross-sectional sample produce an underestimate (Destefano will et al. 1995). This is because the retention time of shot is generally shorter than the time period of interest and therefore not all ingestion events will be captured by a cross-sectional sample. The retention time of lead shot has been estimated to be c. 20 days in Mallards Anas platyrhynchos (Sanderson & Bellrose 1986) but there is uncertainty in this estimate between individuals and species; for example, in Canada Geese Branta canadensis retention times of up to 48 days have been recorded (Cook & Trainer 1966). Therefore, we randomly drew 1000 retention times from a Poisson distribution where  $\lambda$ = 20. A Poisson distribution was chosen because studies that measure retention time record time as a discrete variable and it enabled us to draw retention times similar to those recorded in closely related Canada Geese (Cook & Trainer 1966). For each of the 1000 random retention times, R, we calculated the percentage of individuals that would ingest shots over the course of a winter, *E*, using estimates of the proportion of individuals that ingested lead in our cross-sectional sample, I, and the length of the winter, T, which was 150 days in this instance. We then calculated E with the following equation (Destefano et al. 1995):

$$E = \left(1 - (1 - (I))^{T_{R}}\right) * 100$$

#### RESULTS

#### Metal analysis of faecal samples

The arithmetic mean  $\pm$  sd (range) of untransformed faecal lead levels was  $3.03 \pm 2.09$  mg/kg

(< 0.45 to 10.7 mg/kg) in dry faeces for Barnacle Geese (n = 193) and  $3.30 \pm 3.41$  mg/kg (< 0.45 to 22.5 mg/kg) in dry faeces for White-fronted Geese (n = 150).

#### Statistical analysis of faecal lead levels

After applying the nesting rule and removing models with a  $\Delta AIC > 6$  we were left with a single model in our 'top model set'. This top model (AIC = 337.6,  $R^2$  (marginal) = 0.317) contained one fixed effect, log(aluminium), and one random intercept term, individual field code nested within farm assignment. The null model had an AIC score of 761.6 ( $\Delta AIC = 424.1$ ). Any models containing species and shooting intensity were dropped during model selection because they did not lower the AIC when added to the top model reported here. The estimate of the regression slope (Fig. 6) for log(aluminium) in the top model was  $0.800 \pm 0.026$  sd, the variance of the nested random intercept term was 0.331 and the residual variance was 1.141.

#### **Identifying elevated lead levels**

The data points plotted with the 95% prediction interval from our top model are presented in Figure 6. Four White-fronted Goose samples (marked with their lead values in mg/kg) had large positive residuals, outside the 95% prediction interval, which is potentially indicative of lead shot ingestion. If we assume that these four highlighted White-fronted Goose samples were displaying signs of lead shot ingestion and that no Barnacle Goose samples were, then this equates to 2.7% of White-fronted Geese and 0% of Barnacle Geese. The results of a Fisher's exact test suggested a significant difference in lead ingestion rates between the two species (P = 0.036).

#### **Post-mortems of Barnacle Geese**

We identified two adult birds and one juvenile with a single pellet of lead shot, and one adult with a single pellet of non-lead shot in the gizzard (steel). Combined, this equates to 2.8% of individuals examined having ingested lead shot.

#### X-ray procedure and analysis

For White-fronted Geese we identified one individual with a single metal shot in the gizzard and

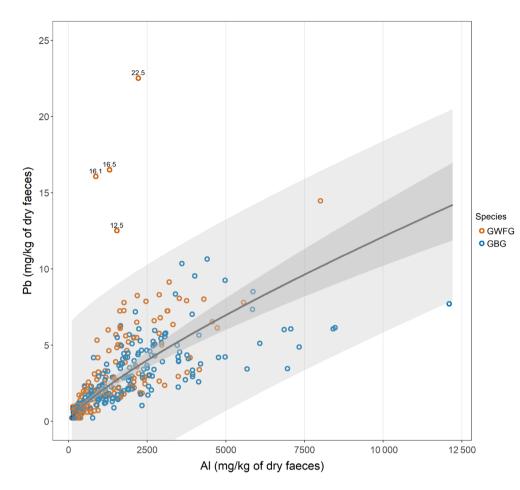


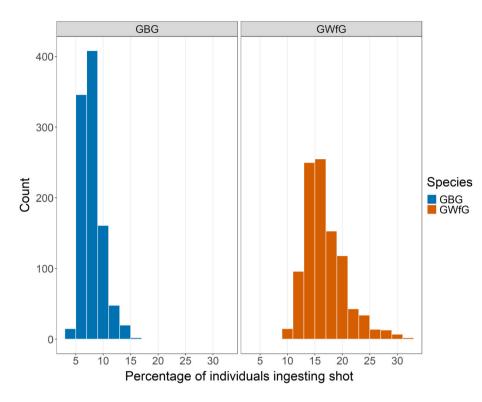
Figure 6. The relationship between the amount of lead and aluminium in faecal samples of Barnacle Goose (GBG, blue) and Whitefronted Goose (GWfG, orange) collected from Islay, Scotland, in the winters of 2018/19 and 2019/20. The regression line of lead against aluminium is shown with the 95% confidence interval (dark grey shading) and 95% prediction interval (light grey band). Four outlier points with large positive residuals, outside the 95% prediction interval, are labelled with their lead values in mg/kg. [Colour figure can be viewed at wileyonlinelibrary.com]

one potentially with a single metal shot in the intestines (see Fig. S4). Combined, this equates to 1.8% of the individuals X-rayed. For Barnacle Geese we identified two individuals with a single metal shot in the gizzard (see Fig. S5) equating to 1.09% of all individuals X-rayed.

# Accounting for retention time of lead shot in the gizzard

Across all three methodologies we found that 1.0% of Barnacle Geese (n = 478) had ingested shot in the gizzard and 2.3% of White-fronted Geese (n = 260); these figures were used as our estimates of I in the Destefano *et al.* (1995) equation (see Methods). From the 1000 randomly

drawn values of shot retention time, R, we calculated distributions of the percentage of individuals that would ingest shot at least once over the course of a winter (Fig. 7). This was done separately for Barnacle Geese and White-fronted Geese because they had different baseline ingestion rates calculated from the faecal sampling, Xrays and post-mortems. For Barnacle Geese over the course of a winter  $9.4 \pm 2.2\%$  of individuals would ingest shot at least once over the course of a winter and  $16.8 \pm 3.70\%$  for White-fronted Geese (mean  $\pm$  sd). We highlight here that these results are likely to be maximum estimates because we could not ascertain whether the shot identified through X-rays was lead or another metal, such as steel.



**Figure 7.** The percentage of individual geese that ingested at least one metal shot over the course of a winter (November to March) on Islay. The distributions for Barnacle Geese (GBG, blue) and White-fronted Geese (GWfG, orange) are shown separately. One thousand values were randomly drawn from a Poisson distribution where  $\lambda = 20$  for the length of shot retention time. [Colour figure can be viewed at wileyonlinelibrary.com]

# DISCUSSION

We found no Barnacle Geese with elevated faecal lead concentrations and only low levels of metal shot ingestion in X-ray screening and post-mortem examinations, suggesting that at any point in time only a small proportion of sampled individuals had ingested lead shot. Similarly, we found evidence of low metal shot ingestion in White-fronted Geese through X-ray screening. However, four Whitefronted Goose samples had elevated faecal lead concentrations (indicative of lead shot ingestion; Mateo et al. 2006, Martinez-Haro et al. 2011b, 2013) but variation in lead concentration did not appear to be explained by goose scheme shooting intensity (which was dropped during model selection), suggesting that goose management may not be the source of raised lead levels in these individuals, although the low number of individuals with elevated lead concentrations provides poor statistical power to detect a relationship. Despite extensive deposition of lead gunshot annually as part of the goose scheme, neither species appears to be

experiencing high instantaneous rates of lead shot ingestion compared with similar studies at hunting hotspots. We found the instantaneous presence of lead shot in the gizzard was 1.0% (Barnacle Geese) and 2.3% (White-fronted Geese) across all sampling methods, which is substantially lower than comparable averages of 10.4% in Argentinian waterfowl (Ferreyra et al. 2014) and highs of 70% in Northern Pintail Anas acuta and Common Pochard Aythya ferina from Spain (Mateo et al. 1997). Our ingestion rates are similar to those found by Mudge (1983) of 3.8% for geese across the UK at hunted and non-hunted sites. When accounting for shot retention time in the gizzard this scaled up to 9.4% of Barnacle Geese and 16.8% of White-fronted Geese likely to be ingesting at least a single shot over an entire winter. There are a number of explanations as to why we observe lower levels of lead ingestion on Islav compared with other hunting hotspots and we discuss these below.

First, our results may be a consequence of a spatially restrictive ban on lead shot use over

Ramsar-designated wetlands where both species collect at least some of their gizzard contents. Shooting with lead shot as part of the goose scheme was only permitted on designated improved agricultural grassland fields, mainly fields reseeded in the previous 2 years (McKenzie et al. 2014, Trinder 2014, McKenzie & Shaw 2017). We therefore expected high intake rates of shot on these areas given that both species preferentially forage on improved grassland. However, post-mortem examination of Barnacle Goose gizzard contents found that they mostly comprised sand, meaning that much of their gizzard contents were probably ingested at Ramsar protected roost sites on coastal areas and saltmarsh where lead shot use has been prohibited as part of the goose scheme (McKenzie et al. 2014, McKenzie & Shaw 2017). This thereby minimizes the liklihood that Barnacle Geese ingest lead shot when collecting grit. Several Whitefronted Goose roost sites are also protected but many are located in areas not protected within Ramsar sites where recreational game shooting may cause additional lead deposition. Therefore, Whitefronted Geese could ingest lead when collecting grit or foraging at unprotected roosts. This could explain the higher rates of elevated faecal lead levels in White-fronted Geese and, although they preferentially forage on improved grassland fields, they also feed on and uproot the stem bases of sedges, which may expose them to more historical lead pellets than Barnacle Geese (Hartikainen & Kerko 2009). All spatial restrictions on lead shot have high compliance rates since goose scheme associated shooting is predominantly conducted by employed shooters (Fig. 3), whose activity and cartridge use are closely monitored by NatureScot. This high-compliance spatial ban on the use of lead shot perhaps prevents ingestion at roost sites, particularly for Barnacle Geese, and other wetland areas where the risk of lead ingestion is often higher compared with other habitats (Pain & Handrinos 1990, Green & Pain 2016).

Secondly, Barnacle Geese and White-fronted Geese are predominantly grassland grazers (Trinder 2014, Griffin *et al.* 2020), so there is extensive overlap between goose foraging ranges and sites where lead shot was deposited as part of the goose scheme. However, grazing on grassland has been associated with lower shot ingestion rates than other habitat types because of lower soil ingestion rates when pecking uniform grass swards (selected for in both species; Pain & Handrinos 1990, Mateo et al. 2000, Mateo 2009, Green & Pain 2016), compared with digging through soil or wetland areas to access grit or stem bases of grasses and sedges (Mateo 2009, Green & Pain 2016).

Thirdly, shooting is often focused at popular hunting sites where waterfowl, and consequently hunters, congregate. Deposition of lead shot is therefore concentrated, resulting in a high prevalence of lead shot ingestion in waterfowl populations (Kanstrup *et al.* 2020). By contrast, shooting management on Islay is dispersed across the entire island, with shooters moving between sites to provide scaring protection across numerous farms. Additionally, the agricultural fields where licensed shooting is permitted by NatureScot change on an annual basis, and consequently shot deposition, and therefore lead density, is probably lower than that observed in focused recreational hunting areas.

Finally, geese forage over wide areas, encompassing Ramsar sites (where the use of lead shot is banned) and non-shooting fields, meaning that not all of their foraging sites have been shot over with lead. Foraging site selection is strongest for recently reseeded agricultural fields where shooting with lead has occurred (Griffin *et al.* 2020). However, recently reseeded fields have been ploughed at some point in the previous 2 years, and consequently much historical lead could be underground, reducing the risk of ingestion.

Spatial variation in lead pellet density within and between sampling locations has been shown to impact lead shot ingestion rates (Rocke et al. 1997, Mateo et al. 1998, 2016). We sampled flocks from across the entire island to account for spatial variability in shooting intensity across Islay. However, this resulted in the collection of few samples from fields where the number of shots fired was above the 90th quantile for all shooting fields (n = 21 and n = 16 for Barnacle Goose andWhite-fronted Goose, respectively). Hence, our sampling method may have limited our ability to detect high lead ingestion at the few high-intensity shooting areas on Islay, and if this were the case, there could still be lead poisoning impacts in a small proportion of the population.

Four individual White-fronted Geese (2.6% of White-fronted Goose samples) were outliers in the regression of lead versus aluminium (Fig. 6). All other faecal samples contained aluminium:lead ratios that fell within the natural variation of matched Scottish soil samples (see Fig. S3). Faecal

samples did contain slightly higher aluminium:lead ratios than the average for matched soil samples, which may reflect the proportions of soil types on Islay being different from those within the matched samples used. Nevertheless, the average aluminium:lead ratio of all faecal samples fell well within the variation observed in the soil sample data. Although these four samples showed elevated lead levels compared with other samples, lead levels were relatively low compared with those found in other studies with individuals known to have ingested lead shot (Martínez-Haro et al. 2010, Aloupi et al. 2015). In Martínez-Haro et al. (2010) the lowest level of faecal lead detected in a Mallard with lead confirmed in the gizzard was 33.8 mg/kg. By contrast, the highest lead level detected on Islay was 22.5 mg/kg. This disparity may be explained by differences in feeding rates and retention times between species and individuals, with higher feeding rates and shorter retention times leading to lower faecal lead levels (Coburn et al. 1951, Bellrose 1959, Figuerola et al. 2005). Alternatively, these four Whitefronted Geese could have recently ingested or expelled lead shot from the gizzard resulting in lower faecal lead concentrations. The source of raised lead levels may also not be the result of lead shot ingestion and interestingly all four elevated samples were collected from an area that had been mined for lead up until c. 1900 (Cressey 1996). Nevertheless, we believe that the most likely explanation for the elevated lead level in these four individuals is ingestion of an additional lead source, such as lead shot, and not just the result of soil ingestion. Faecal lead has been shown to correlate with blood and liver concentrations and therefore faecal lead levels can be an indicator of lead poisoning (Mateo et al. 2006, Berglund 2018). Although the minimum blood lead levels that give rise to negative fitness effects are known (Newth et al. 2016), the conversion between blood and faecal lead levels for geese is unknown and therefore it is difficult to speculate what fitness effects the four elevated faecal lead levels here would have.

Ingested lead shot can be retained in the avian gizzard for 20–44 days while being dissolved and absorbed into the blood and deposited in tissues (Cook & Trainer 1966, Clemens *et al.* 1975, Roscoe *et al.* 1979, Ochiai *et al.* 1993). Faecal sampling provides an instantaneous measurement of lead exposure, and elevated faecal lead levels may

only persist while lead shot is present in the gizzard. Retention times can vary by species from a few weeks to over a month (Sanderson & Irwin 1978, Beyer et al. 1998). Therefore, over an entire winter a much larger number of individuals may ingest lead than our findings suggest. Once the retention time of lead shot in the gizzard was accounted for, we found that  $9.41 \pm 2.18\%$  of Barnacle Geese and  $16.78 \pm 3.70\%$  of White-fronted Geese would ingest shot each winter at least once (mean  $\pm$  sd). These are probably maximum average estimates because not all the identified metal shot in the X-rays is likely to be lead and the average retention time of 20 days used is conservative because longer retention times of up to 48 days have been recorded in closely related Canada Geese (Cook & Trainer 1966). Previous studies have shown that the likelihood of mortality due to ingestion of lead shot is associated with the number of pellets ingested in the gizzard (approximately four to eight; Ochiai et al. 1993). Given that our X-ray scans and post-mortems only identified ingestion of a single pellet at a time, and that we found relatively low faecal lead concentrations. significant direct mortality due to lead shot poisoning seems unlikely on Islay. However, chronic lead ingestion could have implications for population viability due to fitness costs and greater likelihood of mortality due to hunting and collision with power lines (Kelly & Kelly 2004, Fisher et al. 2006, Green & Pain 2016, Ecke et al. 2017). The Islay White-fronted Goose population has declined and any additional fitness costs due to lead ingestion that lower adult survival could affect long-term population viability.

Between 1500 and 3800 lead shotgun cartridges were fired annually on designated shooting sites across Islay since 2005, as part of the goose scheme (Fig. 1). Considering this intensity of shooting, the apparent low level of lead shot ingestion suggests that current policies and restrictions on the use of lead shot have minimized, although not eliminated, lead shot ingestion by geese on Islay. NatureScot ceased using lead ammunition on Islay in November 2020, which will limit future lead deposition and further reduce the risk of lead shot ingestion and subsequent poisoning in these two goose species. However, we only examined two species with a specific foraging ecology and the potential impact on other species is unknown. The (now historical) lead deposition from the goose management scheme, combined with other sources of lead such as game shooting and fishing gear, may be a risk for other species through direct ingestion from the soil or propagation up the food chain to predators and scavengers. These additional effects warrant further investigation to fully assess the risk of lead poisoning to other species on Islay.

In the UK, the risk of lead poisoning in wildfowl due to ingestion of spent lead shot persists (Newth et al. 2012), with lead shot ingestion estimated to kill 50 000-100 000 wildfowl in the UK every winter (Mateo 2009, Pain et al. 2015, Green & Pain 2016). Phasing out the use of lead shot, specifically in wetlands, is widely recognized as a critical solution in protecting numerous species and has recently been brought into practice by the European Union, with many countries also implementing further legislation and regulations to limit its use (Avery & Watson 2009, Mateo 2009, Pain et al. 2019). The results of this study demonstrate how banning the use of lead shot in Ramsar wetlands that encompass key roosting sites with high compliance and enforcement can minimize but not eliminate lead shot ingestion. Therefore, the recent European Union ban on lead shot use over wetlands may reduce lead ingestion in waterfowl but, because foraging occurs outside wetlands for many waterfowl species, further restrictions covering additional key foraging sites may help to mitigate the future risk of lead poisoning. The enforcement of such lead shot bans is also key, with a voluntary phasing out of lead-based ammunition for wild-shot game birds (e.g. Common Pheasants Phasianus colchicus) so far proving ineffective because of very low compliance (Green et al. 2021, 2022). Going forward, safeguarding wildfowl from lead could benefit from greater spatial restrictions on the use of lead shot and further monitoring of shooting and hunting practices to ensure high compliance rates with legislation.

We thank Rae McKenzie and Morven Laurie (NatureScot) for their help with this project and would like to thank James Howe (RSPB) and all the farmers on Islay who provided access to their land for sampling. We also thank Dr David Braidwood and Dr Yuan Li for their assistance with the analysis of faecal samples for lead and aluminium and Larry Griffin and Ed Burrell for their assistance in collecting X-rays. This work was funded by The Wildfowl and Wetlands Trust, NatureScot and the Natural Environment Research Council (Grant reference number: NE/P010210/1).

# AUTHOR CONTRIBUTIONS

Aimée Louise Stuart McIntosh: Data curation; formal analysis; investigation; methodology; writing – original draft; writing – review and editing. Luke Ozsanlav-Harris: Data curation; formal analysis; investigation; methodology; writing – original draft; writing – review and editing. Mark A. Taggart: Methodology; writing – review and editing. Jessica M. Shaw: Writing – review and editing. Geoff M. Hilton: Conceptualization; writing – review and editing. Stuart Bearhop: Supervision; writing – review and editing.

# CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

# FUNDING

Natural Environment Research Council, (Grant/ Award Number: 'NE/P010210/1'), Wildfowl and Wetlands Trust and NatureScot.

# ETHICAL NOTE

None.

# **Data Availability Statement**

R code, data and metadata used for this study are available at: https://github.com/LukeOzsanlav/ Islay\_LeadGeese.

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Received 25 March 2022; Revision 27 February 2023; revision accepted 16 March 2023. Associate Editor: Lei Cao.

# SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Variations in the amounts of lead present in soil samples (n = 721) from across Scotland.

Figure S2. Map depicting locations of soil samples (n = 164) from the National Soil Inventory of Scotland that were collected from agricultural land and were of the same soil type as the fields where faecal samples were collected on Islay.

**Figure S3.** The concentrations of aluminium and lead in faecal samples from Greenland Barnacle Geese *Branta leucopsis* and Greenland Whitefronted Geese *Anser albifrons flavirostris*.

**Figure S4.** X-ray scans of live-caught Greenland White-fronted geese *Anser albifrons flavirostris* caught on Islay.

Figure S5. X-ray scans of live-caught Greenland Barnacle Geese *Branta leucopsis* caught on Islay.