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Expanding the use of portable XRF to monitor lead exposure in an Australian duck species two decades after a ban on lead shot

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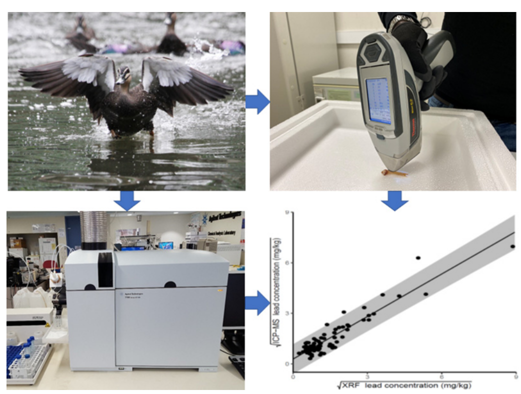
HIGHLIGHTS

- Lead exposure threatens waterbirds globally, but is under-recognized in Australia.
- Bone lead was measured in Pacific black ducks ~20 years after a ban on lead shot.
- Portable X-ray fluorescence was validated for measuring bone lead levels.
- Lead levels were reduced since the last published study >30 years ago.
- XRF data were strongly correlation with digestion-based lead concentration data.

GRAPHICAL ABSTRACT



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ABSTRACT

There is growing worldwide recognition of the threat posed by toxic lead for wildlife and humans. Lead toxicity from ammunition has been shown to be a threat to waterbirds across the globe. Lead shot was banned for all waterfowl hunting in Victoria, Australia, in 2002. However, no assessments of lead exposure in Australian waterfowl have been published since the 1990s. Our aim was to estimate contemporary lead exposure via measuring bone lead concentrations in a harvested dabbling duck, the Pacific black duck (*Anas superciliosa*). We collected wings from 77 Pacific black ducks, spanning 2018 (n = 30) and 2021 (n = 47), from nine sites with long-term histories of regular waterfowl hunting. We sought to validate portable X-ray fluorescence (XRF) for this purpose by taking a piece of humerus bone from each bird, and measuring lead concentration (mg/kg), first via non-destructive XRF and then via destructive inductively coupled plasma mass spectrometry (ICP-MS) and validated the relationship via regression analysis. Portable XRF bone lead measurement demonstrated a strong correlation with ICP-MS results using root-transformed regression (R^2 = 0.85). Greater than 92 % of ducks had only background lead exposure (<10 mg/kg). When compared to historical studies in the same species at similar field sites from the 1990s, lead exposure levels were considerably lower, with mean lead concentrations ~2-fold lower (3.7 c.f. 7.7 mg/kg), and the frequency of birds with severe lead exposure (>20 mg/kg) ~3-fold lower (2.6 c.f. 7.5 %). Our results confirm that portable XRF is a useful option for measurement

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of bone lead in Australasian waterbird species. Our findings also demonstrate that a ban on the use of lead shot around 20 years ago has been associated with a substantial reduction in lead exposure in at least one species of waterfowl.

1. Introduction

Waterbirds face an imperilled future in Australia. Anthropogenic threats to their populations include climate change, habitat loss and degradation, competition with humans for water use, introduced (invasive) species, hunting, infectious diseases from domestic animals and harmful chemical pollutants (Kingsford, 2013). The role that pollutants play for many Australian waterbirds is unclear, with the field of ecotoxicology having been under-recognized in Australia until recently (Death et al., 2019). Aside from the harm that contaminants may cause to birds themselves, waterbird species are considered excellent bioindicators of environmental change (Amat and Green, 2010), especially of wetland heavy metal pollution (Zhang and Ma, 2011).

Lead (Pb) is a toxic heavy metal that negatively affects multiple body systems in vertebrates, especially the nervous system. Lead exposure can cause harm to animals through lethal effects (mortality) and nonlethal effects (morbidity) such as inhibited spatial movements (Kelly and Kelly, 2005), immunotoxic impacts (Vallverdú-Coll et al., 2019) and reduced breeding success (Pain et al., 2019). Evidence is accruing that no level of lead exposure is safe for vertebrates (Ecke et al., 2017; Singh et al., 2021). Lead from gunshot poses particular health risks to wild birds including waterfowl that ingest grit (gastroliths). This effect has been known for more than a century (Wetmore, 1919), and continues to be of global conservation significance (Pain et al., 2019).

Despite the global nature of this threat, relatively little attention has been devoted to this issue in Australia (Death et al., 2019; Hampton et al., 2018). In Australian ecosystems, the ingestion of lead shot affects mainly diving ducks such as hardhead (*Aythya australis*), blue-billed ducks (*Oxyura australis*), musk ducks (*Biziura lobata*) and dabbling ducks such as Pacific black ducks (*Anas superciliosa*) (Wickson et al., 1992). Studies conducted between the 1950s and the 1990s examined lead exposure in multiple Australian waterfowl species from a range of geographical sites and using a variety of analytical techniques (Table 1). These techniques included gizzard inspection for ingested shot (Norman and Brown, 1985) and measurement of lead concentrations in tissues including blood, liver and bone (Wickson et al., 1992), using traditional spectroscopic methods that require acid digestion such as atomic absorption spectrophotometry (Table 1). In the 1980s, there were also mass mortality events affecting magpie geese (*Anseranas semipalmata*) (Harper and Hindmarsh, 1990) and black swans (*Cygnus atratus*) (Koh and Harper, 1988) at the same site in the state of South Australia attributed to lead poisoning.

Much has changed in ecotoxicology since lead exposure studies focused on Australian duck species were last published in the 1990s, and recently developed technology has made the measurement of heavy metal contamination quicker and cheaper. Newer analytical methods such as inductively coupled plasma mass spectrometry (ICP-MS) have been developed, but remain consumptive (causing damage to archived specimens), time-consuming, and expensive. Other emerging methods avoid some of these limitations. For example, hand-held LeadCare® Plus analyzers use a technique called anodic stripping voltammetry (ASV) to allow real-time blood lead levels to be measured in the field (Boesen et al., 2019). More recently, X-ray fluorescence (XRF) has been applied to the measurement of bone lead concentrations in wildlife studies after its initial development for human medicine (Specht et al., 2019a).

XRF devices use a low energy x-ray tube (50 kV) source to quantify elemental content of samples through the fluorescence produced from X-ray interactions. A recent technological advance has seen the wide availability of commercially available portable XRF units. The portable XRF has the advantage of being handheld and battery-powered, with only a 3-minute measurement time for most applications, making it inexpensive and convenient for measurements in the field, as well as allowing non-destructive sampling of archived specimens. Portable XRF has been validated for use in measuring bone lead in vivo in humans (Specht et al., 2016) and in vitro for bone lead measurement in California condors (Specht et al., 2018b), common loons (*Gavia immer*) (Specht et al., 2019b), and wedge-tailed eagles (*Aquila audax fleayi*) (Hampton et al., 2021), all of which demonstrated strong correlations with ICP-MS results.

In recognition of the threat posed by lead shot to Australian waterbird species, their ecosystems, and human consumers of hunted duck meat, lead shot has been prohibited for use in most recreational hunting of waterfowl in states and territories of Australia where such hunting is permitted: Victoria, Tasmania, South Australia and the Northern Territory (Avery and Watson, 2009). In all four jurisdictions, lead shot was banned for recreational hunting of waterfowl between 1998 and 2004 (Avery and Watson, 2009). However, lead shot continues to be legally used for culling (shooting for damage mitigation on farmland) of ducks in other Australian states, including New South Wales (McLeod, 2022). Lead shot use is also legally allowed for waterfowl hunting by Indigenous people in the Northern

Table 1
Details of historical published studies to quantify lead exposure in Australian Pacific black ducks (*Anas superciliosa*). AAS = Atomic absorption spectrophotometry. ASV = anodic stripping voltammetry. NR = Not reported.

State or territory of Australia	Year of collection	*n*	Tissue	Analytical method	Analysis type	Lead concentration: mean ± SD (mg/kg)	Citation
New South Wales	1978	8	Wing	AAS	Wet weight	2.81 ± NR	(Olsen, 1984)
New South Wales	1978	30	Liver	AAS	Wet weight	0.42 ± 0.89	(Kingsford et al., 1989)
New South Wales	1978	53	Liver	AAS	Wet weight	0.37 ± 1.1	(Kingsford et al., 1989)
New South Wales	1978	6	Liver	AAS	Wet weight	0.19 ± 0.07	(Kingsford et al., 1989)
New South Wales	1978	7	Liver	AAS	Wet weight	0.08 ± 0.02	(Kingsford et al., 1989)
New South Wales	1979	19	Muscle	AAS	Wet weight	0.26 ± NR	(Olsen, 1984)
Northern Territory	1981–1984	9	Feather	AAS	Dry weight	1.5 ± 1.5	(Brennan et al., 1992)
Northern Territory	1981–1984	8	Muscle	AAS	Dry weight	0.053 ± 0.048	(Brennan et al., 1992)
Northern Territory	1981–1984	7	Liver	AAS	Dry weight	0.037 ± 0.15	(Brennan et al., 1992)
Victoria	1990	281	Bone	ASV	Dry weight	7.7 ± 28.2	(Wickson et al., 1992)
Victoria	1990	194	Liver	AAS	Wet weight	0.53 ± 1.29	(Wickson et al., 1992)
Victoria	1990	190	Feather	AAS	Dry weight	6.0 ± 14.3	(Wickson et al., 1992)
New South Wales	1992	98	Liver	AAS	Wet weight	0.27 ± 1.68	(Kingsford et al., 1994)
New South Wales	1992	67	Liver	AAS	Wet weight	0.08 ± 0.16	(Kingsford et al., 1994)
New South Wales	1992	93	Liver	AAS	Wet weight	0.19 ± 0.29	(Kingsford et al., 1994)
New South Wales	1992	11	Liver	AAS	Wet weight	0.13 ± 0.17	(Kingsford et al., 1994)

Territory (Skov et al., 2016). It is unknown how effective these bans have been in reducing lead exposure in susceptible waterfowl species.

In this study, we validated the use of portable XRF to measure lead concentrations in bone samples from Pacific black ducks sampled in Victoria in 2018–2021, and compared the values to those obtained using ICP-MS. Our rationale for monitoring lead exposure in this species at this time point, despite the existence of a ban on lead shot for duck hunting in the state of Victoria, was that: 1) no lead exposure data have been published for this species in Australia in over 30 years (Wickson et al., 1992), 2) compliance with lead shot bans is often imperfect (Stroud et al., 2021; Widemo, 2021), 3) 'legacy' lead sources may persist in the environment for >30 years (Kanstrup et al., 2020), 4) lead shot is allowed for waterfowl shooting ('culling') in a neighbouring Australian state, New South Wales (McLeod, 2022), and 5) multiple lead sources may impact wild birds (Finkelstein et al., 2003). Our hypotheses were that: 1) bone lead concentrations measured via portable XRF in Pacific black ducks were comparable to ICP-MS results, 2) Pacific black ducks currently show negligible long-term lead exposure two decades after the ban on lead shot use in their habitat.

2. Materials and methods

2.1. Study species

Pacific black ducks (Fig. 1) are one of the most common duck species in Australasia, inhabiting coastal wetlands year-round and seasonally using ephemeral inland wetlands (McEvoy et al., 2017). They are the hunted waterbird species most commonly observed to ingest lead shot in Victoria (Norman et al., 1993; Wickson et al., 1992) and have shown the highest evidence of lead exposure in past studies (Table 1) due to their feeding habits. For context, Wickson et al., 1992 measured bone lead concentrations in 12 species of waterbirds in Victoria and Pacific black ducks showed the highest levels of bone lead, with the exception of musk ducks (*Biziura lobat*) ($n = 2$).

2.2. Sample collection

We tested bone lead from hunter-donated wing samples collected during the opening weekend of duck hunting seasons as per Wickson et al., 1992. Similar methods have been used by the United States Fish and Wildlife Service (USFWS) to collect data on American woodcocks (*Scolopax minor*) (Myatt and Krementz, 2007). Lead stored in bones is considered the best indicator for evaluating lead exposure over the total lifetime (Franson and Pain, 2011), and has been used for this purpose in several avian groups, including waterbirds (Merendino et al., 2005) and raptors (Wiemeyer et al., 2017). Wings were collected from a total of 77 ducks from nine sites across Victoria (Fig. 2) by government recreational hunting enforcement officers in 2018 ($n = 30$) and 2021 ($n = 47$). A single wing was collected from each duck. To remove the possibility of contamination with shot or bullet fragments containing lead, any wings with visible shooting damage were excluded from the study. The humerus bone was then dissected from each wing, dried, and stored in labeled plastic containers (Fig. 3).

2.3. Portable XRF bone lead measurement system

The portable XRF used for the bone lead measurements in this study was the Niton XL3t GOLDD+ (Thermo-Fisher Scientific, Omaha, USA), as per past studies of bone lead measurement in wild birds (Hampton et al., 2021; Specht et al., 2019b; Specht et al., 2018b). The device uses a thermoelectric cooled silicon drift detector with 25 mm^2 area and 1 mm thickness. The commercial device allowed for customization, which we used to optimize X-ray tube settings and filtration to be used for in vivo measurements. An optimized setting of 50 kV, 40 μA, and filter combination of silver and iron were selected for bone lead measurements as per previous studies (Hampton et al., 2021; Specht et al., 2019b; Specht et al., 2018b). The device was not calibrated using lead-doped bone phantoms as per previous work (Specht et al., 2019b; Specht et al., 2018b) due to the unavailability of these products in Australia at the time, but instead the device was calibrated against the known ICP-MS measures from the same bones. Each sample was analysed using a 3-minute (180 s) read.

Each humerus was laid flat on a horizontal surface with the XRF device held directly above it, so that the samples were as close as possible to the beam aperture and detector (Fig. 3). The measurements were analysed using the standard procedure of previous studies, namely, peak fitting, through MATLAB was used to identify the net counts of the lead peak and corresponding Compton scattering peak associated with the silver X-ray tube anode. The silver Compton Scattering peak was used to normalize

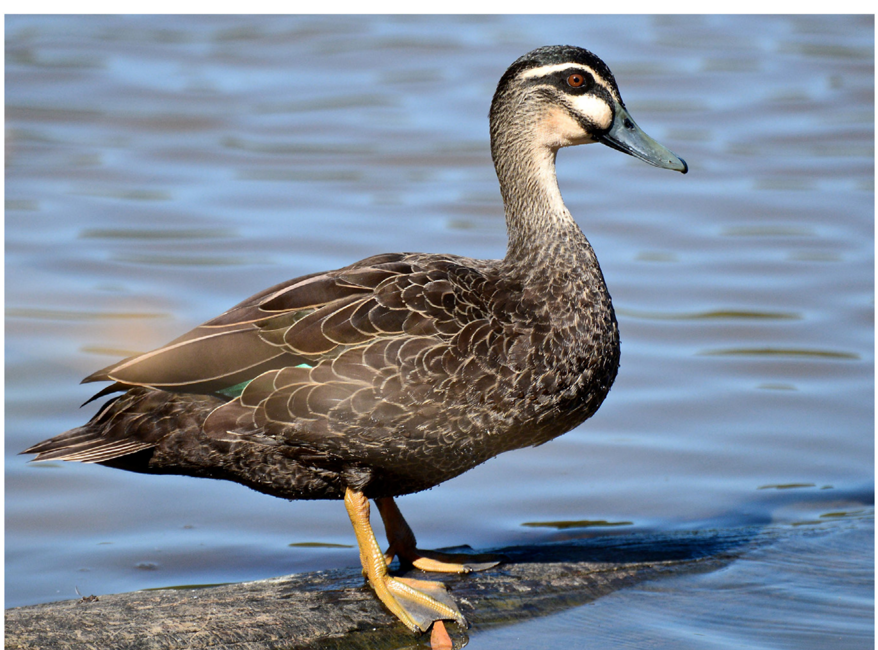

Fig. 1. Our study species, the Pacific black duck (*Anas superciliosa*) in its habitat in south-eastern mainland Australia. Photo: Victorian Game Management Authority.

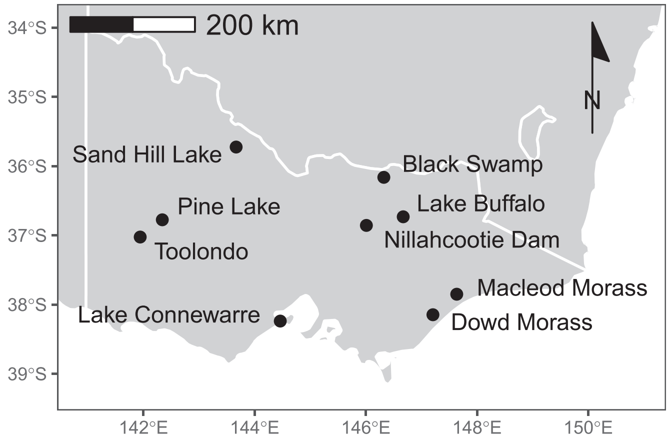

Fig. 2. Our nine field sites, where we collected wings from harvested Pacific black ducks (*Anas superciliosa*) in Victoria, south-eastern mainland Australia, in 2018 and 2021.

the mass, density, and geometry differences between samples from different birds, which has been shown to be an effective process for abnormal sample geometries and compositions (Specht et al., 2018a). Lead concentrations were measured as mg/kg dry weight.

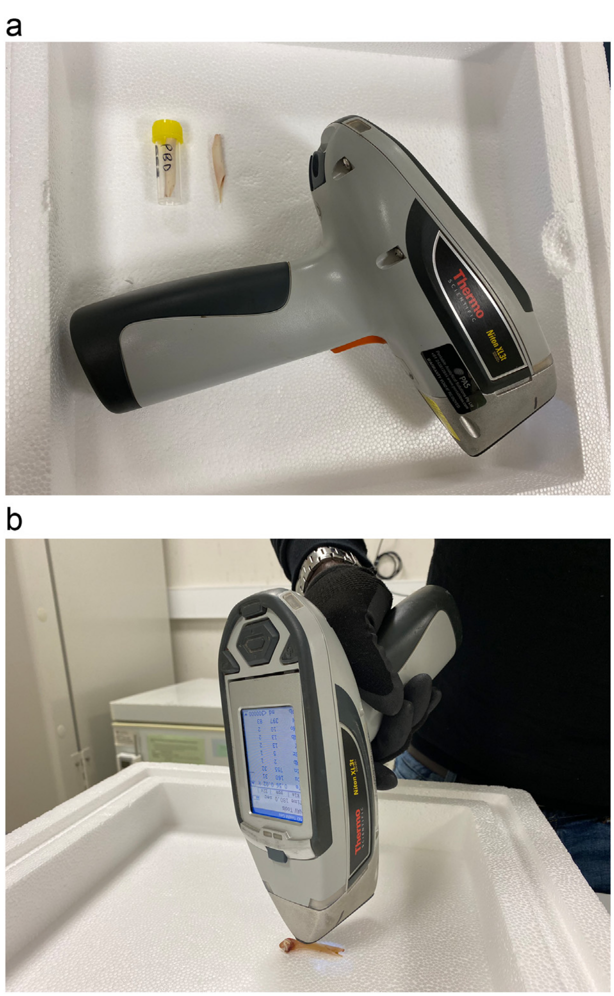

Fig. 3. Equipment used to measure bone lead concentration (a) and the process of measuring a single bone (b) from a Pacific black duck (*Anas superciliosa*) from south-eastern mainland Australia via portable X-ray fluorescence (XRF).

2.4. ICP-MS measurements

Bones were cleaned of attached tissues with distilled water and dried to a constant weight in the oven for 48 h at 40 °C (Ishii et al., 2018). After drying, diaphysis without bone marrow was weighed and homogenized using a ring mill grinder (Ferreyra et al., 2014). Approximately 50 mg of homogenized samples were weighed and placed into acid-washed labeled test tubes. In a fume hood, 0.5 mL of 70 % nitric acid was carefully added to each test tube and covered with glass beads on top of the test tubes to prevent sublimation and evaporation. Samples were kept in the tubes overnight until all frothing has stopped. The pre-digested solutions were placed on heating blocks at 70 °C for at least 24 h until the solution was completely digested and shows a light-yellow color. After the reaction was completed, the blocks were turned off and the solution was allowed to cool to room temperature before dilution (2 % v/v of 70 % HNO3). We then analysed samples in a 7700® Series ICP-MS unit (Agilent Technologies, Santa Clara, USA). For the quality control, trace elements in human hair ERM-DB001 and Mussel-2976 standard reference materials (National Institute of Standards and Technology (NIST), Gaithersburg, USA) were both triplicated and used to determine the experimental recovery efficiency. Their mean recovery was 98–101 %. We did not run duplicate samples. Lead concentrations were measured as mg/kg dry weight.

2.5. Statistical analysis

We calculated the proportion of ducks displaying evidence of different categories of lead exposure. As per convention (reviewed in Franson and Pain (2011)), we used an exposure threshold of bone lead concentrations <10 mg/kg as indicative of low exposure, 10–20 mg/kg as elevated, and concentrations >20 mg/kg as severe.

We used ordinary least squares regression to estimate the relationship between bone lead concentrations estimated using ICP-MS and portable XRF. To reduce heteroscedasticity, both variables were square root-transformed prior to analysis:

$$\sqrt{ICP_i} = \beta_0 + \beta_1\left(\sqrt{XRF_i}\right) + \epsilon_i$$

where β_0 is the intercept, β_1 describes the slope of the relationship and ϵ_i represents the residual error. The uncertainty values for the portable XRF measurements were determined as in previous studies (conducted using this same equipment), using counting statistics in the fitted area for lead. The uncertainty (σ) of each measurement was calculated using the equation:

$$\sigma = \frac{c \times \sqrt{\frac{BKG}{t}}}{NET}$$

where c is the concentration, BKG is the background counts as estimated by our fitting, t is measurement time, and NET is the net lead counts from the Gaussian function in our fitting (Zhang et al., 2021). This produced two negative XRF point estimates (-0.19, -1.24) for two samples which were excluded from the regression analysis because they could not be square root-transformed. The predictive ability of the regression model was assessed using 10-fold cross validation repeated three times. Models were fitted and evaluated using the caret package (Kuhn et al., 2020) in the R statistical environment (R Core Team, 2022).

3. Results

3.1. Validation of portable XRF

Table 2 and Fig. 4 show the correlation between portable XRF and ICP-MS bone lead measurements ($n = 75$). At low values (<0.43 mg/kg), portable XRF estimates of lead concentration in the bones of Pacific black ducks were lower than corresponding values estimated using ICP-MS (Fig. 4). The expected square root ICP-MS estimate corresponding to a

Table 2
Bone lead measurements and uncertainties for ICP-MS and portable XRF measurements from the bare humeri of 77 Pacific black ducks (*Anas superciliosa*) collected from a range of wetlands in Victoria, Australia, in 2018 and 2021. NR = not reported.

	n	Mean	Maximum	Minimum	Standard deviation	% <10 mg/kg	% 10–20 mg/kg	% >20 mg/kg
ICP-MS (mg/kg)	77	3.7	48.5	0.1	7.6	92.2	5.2	2.6
Portable XRF (mg/kg)	77	3.9	78.6	−1.2	10.0	92.2	3.9	3.9
Portable XRF uncertainty (mg/kg)	77	0.8	3.1	0.2	0.5	NA	NA	NA
Historical ICP-MS data (mg/kg) (Wickson et al., 1992)	281	7.7	360.6	<0.5	28.2	NR	NR	7.5

portable XRF estimate of 0 mg/kg was 0.286 mg/kg (SE = 0.037). However, the expected square root of ICP-MS lead concentration increased by only 0.841 mg/kg (SE = 0.037) for every unit increase in the square root of lead concentration estimated by portable XRF (R^2 = 0.85, t_{73} = 22.525, $p < 0.001$). Hence, expected ICP-MS values could be predicted using the equation:

$$ICP_i = \left(\beta_0 + \beta_1\left(\sqrt{XRF_i}\right)\right)^2$$

where $\beta_0 = 0.286$ and $\beta_1 = 0.841$. The cross-validation estimate of root mean-squared error was 0.44.

3.2. Lead concentrations in Pacific black duck bones

Table 2 shows the bone lead measurements and uncertainties derived from portable XRF and ICP-MS. Using ICP-MS as the gold standard (n = 77), lead levels were generally low, with 92.2 % of samples showing background lead exposure (<10 mg/kg), 5.2 % elevated exposure (10–20 mg/kg), and 2.6 % showing severe lead exposure (>20 mg/kg). The mean lead concentration was 3.7 ± 7.6 mg/kg (Table 2).

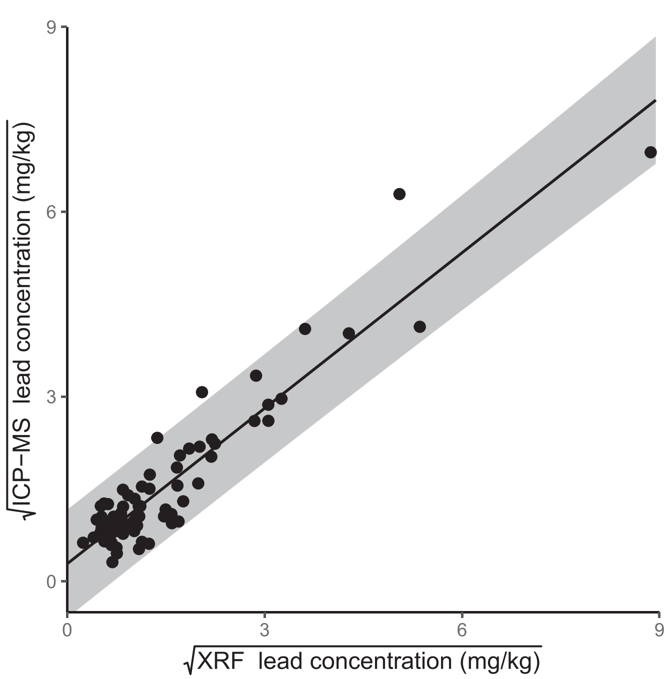

Fig. 4. Correlation between portable XRF and ICP-MS bone lead concentration measurements (mg/kg) in bare humerus bone samples from 75 Pacific black ducks (*Anas superciliosa*) from south-eastern mainland Australia. The shaded polygon represents the 95 % prediction interval. Values from two samples were excluded due to negative XRF estimates.

4. Discussion

To our knowledge, this is the first trial of portable XRF for bone lead measurement in a waterbird species outside of North America and the first study to report lead exposure levels in an Australian waterbird species since the 1990s. Our results confirmed that lead exposure levels in this species have decreased over the past three decades. Our data also showed strong agreement between portable XRF and the traditional gold standard for bone lead measurement, ICP-MS.

Lead exposure levels were considerably lower than historical assessments, with mean lead concentrations ~2-fold lower (3.7 c.f. 7.7 mg/kg), and the frequency of birds with severe lead exposure (>20 mg/kg) ~3-fold lower (2.6 c.f. 7.5 %) than those reported from similar sites in the 1990s (Wickson et al., 1992) (Table 1). The substantial decline in lead exposure levels suggests that the ban on the use of lead shot for recreational duck hunting enacted in Victoria has been successful in reducing lead contamination of wetlands. Interestingly, the study of Lewis et al. (2021) reported an even greater reduction in lead exposure in North American ducks over a similar time period. They found that the prevalence of American black ducks (*Anas rubripes*) in coastal north-eastern USA (New Jersey) with elevated blood lead levels (BLLs) (>1.0 ppm), declined from 19 % in 1978 to 1 % in 2017.

In our study, 7.8 % of ducks still showed evidence of elevated lead exposure. We did not perform isotope analysis to permit source attribution for this lead (Finkelstein et al., 2003; Finkelstein et al., 2014), so we can only speculate as to the likely source(s). There are at least three alternatives that warrant consideration.

First, it may suggest that lead shot is continuing to be used illegally by a minority of duck hunters in Victoria. Compliance with lead shot bans is often imperfect (Stroud et al., 2021; Widemo, 2021), and there has been recent media concern regarding this possibility in the context of duck hunting in Victoria (Rollason, 2022). A government report from 2017 examined lead concentrations in nine wetlands in Victoria and reported generally low levels in water and sediment but found that water lead levels at several sites slightly exceeded an ecosystem protection guideline value designated for 95 % species protection (Environment Protection Authority Victoria, 2017).

Second, 'legacy' lead shot deposited in lake beds during recreational hunting prior to 2002 in Victoria may still be available to dabbling ducks, given that lead shot may persist in such environments for >30 years (Kanstrup et al., 2020). This process may be particularly relevant for our study given that drought conditions are believed to precipitate cases of lead poisoning in waterfowl, whereby low water levels allow access to lead shot (Degernes, 2008). For example, on the west coast of the US, a large number of wild swans (*Cygnus buccinator* and *Cygnus columbianus columbianus*) died of lead poisoning >10 years after lead was banned for waterfowl hunting in that area (Degernes et al., 2006). Prolonged drought conditions were recently experienced in southeastern Australia and peaked in 2019 (this was the hottest and driest year on record in Australia) (Wintle et al., 2020).

Third, sampled ducks could have ingested lead shot in wetlands in New South Wales, the state that borders Victoria to the north, and where the use of lead shot remains legally allowed for culling nine native duck species for the purpose of damage mitigation to promote sustainable agricultural management in the rice growing region of the Riverina region (NSW

Department of Primary Industries, 2021). This route is plausible, given that Pacific black ducks are a semi-nomadic species, capable of spatial movements >200 km (McEvoy et al., 2017; McEvoy et al., 2015; Norman, 1971).

There were important limitations to our ability to extrapolate from our results. First, we didn't use standard lead-doped bone phantoms to calibrate the portable XRF as per previous work (Specht et al., 2019b). However, we were able to account for this by using comparisons to known ICP-MS results to arrive at concentration data, as per Hampton et al. (2021). Second, the sampling methodology for these Pacific black ducks was not random, but they were birds harvested by recreational hunters at a limited number of geographical sites. Third, our sample size was relatively small.

There has been very little investigation of heavy metal exposure in Australian waterbirds over the past three decades (Hampton et al., 2018). We encourage further investigations in this field and our results indicate that this is still a contemporary One Health issue (Arnemo et al., 2022; Pokras and Kneeland, 2008), with current contamination levels in a wildlife species commonly eaten by humans far from negligible.

Our results support the viability of monitoring bone lead levels in other Australian waterbird species and taxa using portable XRF. We feel that this recently developed technology could facilitate efficient and cost-effective ongoing monitoring of contaminant exposure in waterbirds. The average uncertainty of 0.8 ± 0.5 mg/kg for portable XRF measurements demonstrate that this technique could be used to effectively quantify bone lead level for Australian ducks and distinguish highly exposed individuals from normal environmental exposures. The portable XRF unit we used cost USD$1484 for one-week hire, and we analysed a total of 77 specimens for an average of USD$19 per sample. For purchasing a system, this cost could be further reduced, as there are no real running costs associated with the measurements. Given the inexpensive and non-time consuming nature of portable XRF use, we suggest that this method could be used to generate real-time data on heavy metal exposure in hunted waterbirds during wing collection surveys (Myatt and Krementz, 2007).

5. Conclusions

Our study suggests that the ban on the use of lead shot over 20 years ago in Victoria has resulted in lower bone lead levels in dabbling ducks. Notwithstanding, we still found some evidence of elevated lead exposure in Pacific black ducks, which warrants further investigation. XRF analysis was effective for this species and tissue type and is a quick and inexpensive alternative to traditional methods for determining bone lead concentrations that require acid digestion.

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CRediT authorship contribution statement

Damien Nzabanita: Conceptualization, Methodology, Validation, Data Analysis, Investigation, Data Curation, Writing – Original Draft, Review and Editing. **Jordan O. Hampton:** Supervision, Sample Acquisition, Methodology, Data Curation, Project Administration, Funding Acquisition, Writing – Review and Editing. **Simon D. Toop:** Sample Acquisition, Funding Acquisition, Writing – Review and Editing. **Andrew J. Bengsen:** Data Analysis, Validation, Writing – Review and Editing. **Aaron J. Specht:** Methodology, Validation, Data Analysis, Writing – Review and Editing. **Jason S. Flesch:** Sample Acquisition. **Jasmin Hufschmid:** Supervision, Writing – Review and Editing. **Dayanthi Nugegoda:** Supervision, Project Administration, Funding Acquisition, Writing – Review and Editing.

Data availability

Data will be made available on request.

Declaration of competing interest

Two authors, Simon D. Toop and Jason S. Flesch, are employees of a government agency, the Victorian Game Management Authority, involved with the management of duck hunting activities in the state of Victoria, Australia. Their employment could not reasonably have interfered with the full and objective presentation of this research.

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References

Amat, J.A., Green, A.J., 2010. Waterbirds as bioindicators of environmental conditions. In: Hurford, C., Schneider, M., Cowx, I. (Eds.), Conservation Monitoring in Freshwater Habitats. Springer, New York City, USA, pp. 45–52.

Arnemo, J.M., Fuchs, B., Sonne, C., Stokke, S., 2022. Hunting with lead ammunition: a One Health perspective. In: Tryland, M. (Ed.), Arctic One Health. Springer, Cham, Switzerland, pp. 439–468.

Avery, D., Watson, R.T., 2009. Regulation of lead-based ammunition around the world. In: Watson, R.T., Fuller, M., Pokras, M., Hunt, W.G. (Eds.), Ingestions of Lead From Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, USA, pp. 161–168.

Boesen, A.H., Thiel, A., Fuchs, B., Evans, A.L., Bertelsen, M.F., Rodushkin, I., et al., 2019. Assessment of the LeadCare® plus for use on Scandinavian brown bears (Ursus arctos). Front.Vet.Sci. 6, 285.

Brennan, K., Noller, B., Morton, S.R., Dostine, P., LeGras, C., 1992. Heavy Metals in Waterbirds From the Magela Creek Flood Plain, Alligator Rivers Region, Northern Territory, Australia. Australian Government Publishing Service, Canberra, Australia.

Death, C.E., Griffiths, S.R., Story, P.G., 2019. Terrestrial vertebrate toxicology in Australia: an overview of wildlife research. Curr.Opin.Environ.Sci.Health 11, 43–52.

Degernes, L.A., 2008. Waterfowl toxicology: a review. Vet.Clin.N.Am.Exotic Anim.Pract. 11, 283–300.

Degernes, L., Heilman, S., Trogdon, M., Jordan, M., Davison, M., Kraege, D., et al., 2006. Epidemiologic investigation of lead poisoning in trumpeter and tundra swans in Washington state, USA, 2000–2002. J. Wildl. Dis. 42, 345–358.

Ecke, F., Singh, N.J., Arnemo, J.M., Bignert, A., Helander, B., Berglund, Å.M.M., et al., 2017. Sublethal lead exposure alters movement behavior in free-ranging golden eagles. Environ. Sci. Technol. 51, 5729–5736.

Environment Protection Authority Victoria, 2017. Lead in Victorian Wetlands Study: Preliminary Investigation. Environment Protection Authority Victoria, Melbourne, Australia.

Ferreyra, H., Romano, M., Beldomenico, P., Caselli, A., Correa, A., Uhart, M., 2014. Lead gunshot pellet ingestion and tissue lead levels in wild ducks from argentine hunting hotspots. Ecotoxicol. Environ. Saf. 103, 74–81.

Finkelstein, M.E., Gwiazda, R.H., Smith, D.R., 2003. Lead poisoning of seabirds: environmental risks from leaded paint at a decommissioned military base. Environ. Sci. Technol. 37, 3256–3260.

Finkelstein, M.E., Kuspa, Z.E., Welch, A., Eng, C., Clark, M., Burnett, J., et al., 2014. Linking cases of illegal shootings of the endangered California condor using stable lead isotope analysis. Environ. Res. 134, 270–279.

Franson, J.C., Pain, D.J., 2011. Lead in birds. In: Beyer, W.N., Meador, J.P. (Eds.), Environmental Contaminants in Biota: Interpreting Tissue Concentrations. CRC Press, Boca Raton, USA, pp. 563–593.

Hampton, J.O., Laidlaw, M., Buenz, E., Arnemo, J.M., 2018. Heads in the sand: public health and ecological risks of lead-based bullets for wildlife shooting in Australia. Wildl. Res. 45, 287–306.

Hampton, J.O., Specht, A.J., Pay, J.M., Pokras, M., Bengsen, A.J., 2021. Portable X-ray fluorescence for bone lead measurements of Australian eagles. Sci. Total Environ. 789, 147998.

Harper, M., Hindmarsh, M., 1990. Lead poisoning in magpie geese Anseranas semipalmata from ingested lead pellet at Bool Lagoon Game Reserve (South Australia). Wildl. Res. 17, 141–145.
Ishii, C., Nakayama, S.M., Kataba, A., Ikenaka, Y., Saito, K., Watanabe, Y., et al., 2018. Characterization and imaging of lead distribution in bones of lead-exposed birds by ICP-MS and LA-ICP-MS. Chemosphere 212, 994–1001.
Kanstrup, N., Fox, A.D., Balsby, T.J.S., 2020. Toxic lead gunshot persists accessible to waterbirds after a 33-year ban on their use. Sci. Total Environ. 714, 136876.
Kelly, A., Kelly, S., 2005. Are mute swans with elevated blood lead levels more likely to collide with overhead power lines? Waterbirds 28, 331–334.
Kingsford, R., 2013. Conservation of waterbirds in Australia. Pac. Conserv. Biol. 19, 366–378.
Kingsford, R., Flanjack, J., Black, S., 1989. Lead shot and ducks on Lake Cowal. Wildl. Res. 16, 167–172.
Kingsford, R., Kacprzak, J., Ziaziaris, J., 1994. Lead in livers and gizzards of waterfowl shot in New South Wales,Australia. Environ. Pollut. 85, 329–335.
Koh, T., Harper, M., 1988. Lead poisoning in black swans, Cygnus atratus, exposed to spent lead shot at Bool Lagoon Game Reserve,South Australia. Wildl. Res. 15, 395–403.
Kuhn, M., Wing, J., Weston, S., Williams, A., Keefer, C., Engelhardt, A., et al., 2020. Caret: Classification and Regression Training.
Lewis, N.L., Nichols, T.C., Lilley, C., Roscoe, D.E., Lovy, J., 2021. Blood lead declines in wintering American black ducks in New Jersey following the lead shot ban. J.Fish Wildl. Manag. 12, 174–182.
McEvoy, J.F., Roshier, D.A., Ribot, R.F., Bennett, A.T., 2015. Proximate cues to phases of movement in a highly dispersive waterfowl,Anas superciliosa. Mov. Ecol. 3, 21.
McEvoy, J., Ribot, R., Wingfield, J., Bennett, A., 2017. Heavy rainfall triggers increased nocturnal flight in desert populations of the Pacific black duck (Anas superciliosa). Sci. Rep. 7, 17557.
McLeod, S.R., 2022. 2022–2023 Annual Waterfowl Quota Report to NSW DPI Hunting, NSW Department of Primary Industries. NSW Department of Primary Industries, Orange, Australia.
Merendino, M.T., Lobpries, D.S., Neaville, J.E., Ortego, J.D., Johnson, W.P., 2005. Regional differences and long-term trends in lead exposure in mottled ducks. Wildl. Soc. Bull. 33, 1002–1008.
Myatt, N.A., Krementz, D.G., 2007. American woodcock fall migration using central region band-recovery and wing-collection survey data. J. Wildl. Manag. 71, 336–344.
Norman, F., 1971. Movement and mortality of black duck, mountain duck and grey teal banded in South Australia 1953–1963. Trans. R. Soc. S. Aust. 95, 1–7.
Norman, F., Brown, R., 1985. Gizzard grit in some Australian waterfowl. Wildfowl 36, 77–80.
Norman, F., Garnham, J., Lowe, K., 1993. Further notes on lead concentrations in tissues of waterfowl in Victoria. Wildl. Res. 20, 621–624.
NSW Department of Primary Industries, 2021. 2021–2022 Annual Waterfowl Quota Report to DPI Hunting. NSW Department of Primary Industries, Sydney, Australia.
Olsen, P.D., 1984. Heavy metal residues in Pacific black duck Anas superciliosa from Griffith, New South Wales. Emu-Austral Ornithol. 84, 44–46.
Pain, D.J., Mateo, R., Green, R.E., 2019. Effects of lead from ammunition on birds and other wildlife: a review and update. Ambio 48, 935–953.
Pokras, M.A., Kneeland, M.R., 2008. Lead poisoning: using transdisciplinary approaches to solve an ancient problem. EcoHealth 5, 379–385.
R Core Team, 2022. R: A Language and Environment for Statistical Computing, Vienna, Austria.
Rollason, B., 2022. Environmental Watchdog Investigating 'Elevated' Lead Levels in Ducks From Victorian Wetlands. ABC News. Australian Broadcasting Corporation, Sydney, Australia.
Singh, N.J., Ecke, F., Katzner, T., Bagchi, S., Sandström, P., Hörnfeldt, B., 2021. Consequences of migratory coupling of predators and prey when mediated by human actions. Divers. Distrib. 27, 1848–1860.
Skov, S., Hardie, K., Barkly, S., Manchikanti, P., 2016. Get the lead out! Lead shot shotgun shot and elevated lead levels in children and adults in 3 remote top end aboriginal communities. North.Territory Dis.Control Bull. 23, 1–6.
Specht, A.J., Lin, Y., Weisskopf, M., Yan, C., Hu, H., Xu, J., et al., 2016. XRF-measured bone lead (Pb) as a biomarker for Pb exposure and toxicity among children diagnosed with Pb poisoning. Biomarkers 21, 347–352.
Specht, A.J., Kponee, K., Nkpaa, K.W., Balcom, P.H., Weuve, J., Nie, L.H., et al., 2018a. Validation of x-ray fluorescence measurements of metals in toenail clippings against inductively coupled plasma mass spectrometry in a nigerian population. Physiol. Meas. 39, 085007.
Specht, A.J., Parish, C.N., Wallens, E.K., Watson, R.T., Nie, L.H., Weisskopf, M.G., 2018b. Feasibility of a portable X-ray fluorescence device for bone lead measurements of condor bones. Sci. Total Environ. 615, 398–403.
Specht, A.J., Dickerson, A.S., Weisskopf, M.G., 2019a. Comparison of bone lead measured via portable x-ray fluorescence across and within bones. Environ. Res. 172, 273–278.
Specht, A.J., Kirchner, K.E., Weisskopf, M.G., Pokras, M.A., 2019b. Lead exposure biomarkers in the common loon. Sci. Total Environ. 647, 639–644.
Stroud, D., Pain, D.J., Green, R.E., 2021. Evidence of widespread illegal hunting of waterfowl in England despite partial regulation of the use of lead shotgun ammunition. Conserv. Evid.J. 18, 18–24.
Vallverdú-Coll, N., Mateo, R., Mougeot, F., Ortiz-Santaliestra, M.E., 2019. Immunotoxic effects of lead on birds. Sci. Total Environ. 689, 505–515.
Wetmore, A., 1919. Lead Poisoning in Waterfowl. US Department of Agriculture, Washington, D.C., USA.
Wickson, R., Norman, F., Bacher, G., Garnham, J., 1992. Concentrations of lead in bone and other tissues of Victorian waterfowl. Wildl. Res. 19, 221–231.
Widemo, F., 2021. Shooting habits and habitats-effects of education and legislation on the phasing out of lead shot. Environ. Sci. Policy 118, 56–62.
Wiemeyer, G.M., Pérez, M.A., Bianchini, L.T., Sampietro, L., Bravo, G.F., Jácome, N.L., et al., 2017. Repeated conservation threats across the Americas: high levels of blood and bone lead in the Andean Condor widen the problem to a continental scale. Environ. Pollut. 220, 672–679.
Wintle, B.A., Legge, S., Woinarski, J.C.Z., 2020. After the megafires: what next for Australian wildlife? Trends Ecol.Evol. 35, 753–757.
Zhang, W.W., Ma, J.Z., 2011. Waterbirds as bioindicators of wetland heavy metal pollution. Procedia Environ. Sci. 10, 2769–2774.
Zhang, X., Specht, A.J., Wells, E., Weisskopf, M.G., Weuve, J., Nie, L.H., 2021. Evaluation of a portable XRF device for in vivo quantification of lead in bone among a US population. Sci. Total Environ. 753, 142351.