The Journal of Veterinary Medical Science

FULL PAPER

Toxicology

Current situation regarding lead exposure in birds in Japan (2015–2018); lead exposure is still occurring

Chihiro ISHII[1)], Yoshinori IKENAKA[1,2)], Shouta M.M. NAKAYAMA[1)], Takeshi KURITANI[3)], Mitsuhiro NAKAGAWA[3)], Keisuke SAITO[4)], Yukiko WATANABE[4)], Kohei OGASAWARA[4)], Manabu ONUMA[5)], Atsushi HAGA[5)] and Mayumi ISHIZUKA[1)]*

[1)]Laboratory of Toxicology, Department of Environmental Veterinary Sciences, School of Veterinary Medicine, Hokkaido University, Kita18, Nishi9, Kita-ku, Sapporo, Hokkaido 060-0818, Japan
[2)]Water Research Group, Unit for Environmental Sciences and Management, North-West University, 11 Hoffman Street, Potchefstroom, 2531, South Africa
[3)]Graduate School of Science, Hokkaido University, Kita10, Nishi8, Kita-ku, Sapporo, Hokkaido 060-0810, Japan
[4)]Institute for Raptor Biomedicine Japan, 2-2101 Hokuto, Kushiro-shi, Hokkaido 084-0922, Japan
[5)]National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan

J. Vet. Med. Sci.
82(8): 1118–1123, 2020
doi: 10.1292/jvms.20-0104

Received: 28 February 2020
Accepted: 28 May 2020
Advanced Epub:
18 June 2020

ABSTRACT. Birds of a number of species have died as a result of lead (Pb) poisoning, including many Steller's sea eagles (*Haliaeetus pelagicus*) and white-tailed sea eagles (*Haliaeetus albicilla*) in Hokkaido, the northernmost island of Japan. To address this issue, the use of any type of Pb ammunition for hunting of large animals was prohibited in Hokkaido in 2004. However, Pb poisoning is still being reported in this area, and there are few regulations regarding the use of Pb ammunition in other parts of Japan, where it has been reported that eagles and water birds have been exposed to Pb. This study was performed to accurately determine the current level of Pb exposure of birds found dead in the field or dead in the wild bird centers in Japan (June 2015–May 2018) and to identify the sources of Pb. Pb exposure was found to still be occurring in raptors and water birds in various parts of Japan. Twenty-six point five % and 5.9% of the recorded deaths of Steller's sea eagles and white-tailed sea eagles, respectively, were found to have been poisoned by Pb. In addition, Pb isotope ratio analysis showed that both Pb rifle bullets and Pb shot pellets cause Pb exposure in birds, and these endangered eagles are also exposed to Pb in Hokkaido due to the illegal use of Pb ammunition. Changing to Pb-free ammunition, such as copper (Cu) rifle bullets, steel shot pellets, or bismuth shot pellets, will be essential for the conservation of avian species in Japan.

KEY WORDS: Pb ammunition, Pb exposure, Pb isotope ratios, raptor, water bird

Birds of a number of species have died as a result of lead (Pb) poisoning, including at least 33 raptors, 30 other terrestrial bird species, and water birds in the world [1, 23]. Furthermore, both the number of taxa affected and geographical spread of cases have increased [24]. Raptors can experience secondary Pb poisoning from feeding on animals with Pb ammunition embedded in their tissues, while water birds commonly suffer from Pb poisoning through ingestion of Pb shot pellets or sinkers lying in lakes and marshes, which they mistake for grit [2, 8, 27]. When raptors consume these water birds, they are also exposed to Pb.

Pb is an accumulative metabolic poison that produces toxic effects in a wide range of physiological and biochemical systems, including the hematopoietic, vascular, nervous, renal, immune, and reproductive systems, and elevated accumulation of Pb leads to high mortality rates [13, 24]. Sublethal doses of Pb alter movement behaviors [5] and affect sperm quality and reproductive success [29]. There is also a possibility that birds exposed to Pb cannot migrate. The ecological significance of mortalities and the effect on reproductive system associated with Pb exposure must be viewed within the context of cumulative risks to avian populations [13].

Although the use of Pb in gasoline, paints, and various household items is prohibited because of its toxicity, Pb ammunition is still widely used for hunting and shooting [1]. Non-Pb shot, such as copper (Cu) rifle bullets or iron-tungsten-nickel shot pellets, have been developed [3, 7] and their efficacy has been demonstrated [12]. However, there are few nationally regulated bans on the

*Correspondence to: Ishizuka, M.: ishizum@vetmed.hokudai.ac.jp
(Supplementary material: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2350/)

use of Pb ammunition despite the scientific evidence for the risks it poses and increasing policy imperatives [1].

In Hokkaido, the northernmost island of Japan, many Steller's sea eagles (*Haliaeetus pelagicus*) and white-tailed sea eagles (*Haliaeetus albicilla*) have died from Pb poisoning after ingesting Pb fragments from sika deer (*Cervus nippon*) carcasses. The number of sika deer is increasing in Hokkaido, and hunting is needed for management of their population. Due to the toxicity of Pb, it is required to use copper (Cu) rifle bullets or slugs instead of Pb bullets for hunting sika deer. A regulation to ban the use of Pb rifle bullets for hunting sika deer was introduced in 2000, and the use of Pb shot pellets for hunting sika deer was prohibited in 2001. In addition, an extended ban was initiated in 2004, which prohibited the use of any type of Pb-containing ammunition for hunting large animal species. This regulation was enforced in 2014, prohibiting the possession of Pb rifle bullets, slugs, or large shot pellets during hunting; prior to this regulation, it was not illegal to carry Pb ammunition when hunting, but hunters were punished if they were found to have used such ammunition. However, although more than 10 years have passed since the introduction of legislation regarding Pb ammunition, Pb poisoning is still being reported in Hokkaido [10]. In other parts of Japan, both regulations and surveillance were less extensive than in Hokkaido, however, eagles and water birds were exposed to Pb [9, 11, 20].

Various thresholds for Pb toxicity in birds have been reported in the literature [6, 13, 14, 18, 26]. The background level of Pb in the liver is generally <2 mg/kg wet weight (6–7 mg/kg dry weight) or <1 mg/kg wet weight (3 mg/kg dry weight) in raptors [21]. Some authors have reported that a liver Pb concentration of >6 mg/kg dry weight in raptors indicates abnormally high exposure to Pb, while a concentration of >20 mg/kg dry weight indicates acute exposure and absorption, resulting in Pb poisoning [21, 22]. The following categories are used in Japan based on the hepatic Pb concentration in wet weight: <0.2 mg/kg, normal range; 0.2−2 mg/kg, high level of Pb exposure; and >2 mg/kg, Pb poisoning [24].

The relative abundances of the naturally occurring isotopes of Pb (^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb) vary among industrial materials, allowing the isotope ratios in organisms to be used to determine the source of Pb exposure, as reported previously in raptors [4]. The following Pb isotope ratios (^{208}Pb/^{206}Pb, ^{207}Pb/^{206}Pb) were reported for various types of Pb ammunition obtained from stores in Japan: rifle bullets, 1.90−2.10 and 0.75−0.88; shot pellets, 2.07−2.14 and 0.85−0.87; and fishing sinkers, 2.08−2.20 and 0.84−0.90. These materials had been purchased or collected from the carcasses of birds up until 2001. We confirmed that the Pb isotope ratios of Pb rifle bullets, shot pellets, and fishing sinkers used by hunters and fishermen recently were comparable to these values and the results of Pb isotope ratio in the liver of bird would reflect that in the main source of exposure [10].

This study was performed to accurately determine the current levels of Pb exposure in wild birds carried to clinics or wild bird centers in Japan (June 2015−May 2018) and to identify the sources of Pb by analyzing the Pb isotope ratios.

MATERIALS AND METHODS

Sampling

Liver specimens from the carcasses of birds were obtained from the Institute for Raptor Biomedicine Japan, National Institute for Environmental Studies, or wild bird centers for analysis of their Pb concentrations and Pb isotope ratios. Samples were collected from June 2015 to May 2018 from the following locations and species: from Hokkaido, white-tailed sea eagle (*n*=51), Steller's sea eagle (*n*=34), Blakiston's fish owl (*Ketupa blakistoni*) (*n*=7), mountain hawk eagle (*Spizaetus nipalensis*) (*n*=3), northern goshawk (*Accipiter gentilis*) (*n*=3), Eurasian hobby (*Falco subbuteo*) (*n*=2), peregrine falcon (*Falco peregrinus*) (*n*=1), sparrow hawk (*Accipiter nisus*) (*n*=1), ural owl (*Strix uralensis japonica*) (*n*=1), jungle crow (*Corvus macrorhynchos*) (*n*=2), and whooper swan (*Cygnus cygnus*) (*n*=1); from Honshu (the main island) or Kyushu (southwestern island), peregrine falcon (*n*=6), northern goshawk (*n*=5), sparrow hawk (*n*=4), ural owl (*n*=4), mountain hawk eagle (*n*=2), whooper swan (*n*=2), and greater white-fronted goose (*Anser albifrons*) (*n*=2). Swans that had died in one lake in Honshu (see Introduction) were not included in the analysis to exclude any potential sampling bias around the ratio of Pb exposure in swans. The specimens were transported to the School of Veterinary Medicine, Hokkaido University, Sapporo, Japan, where they were stored at −20°C until analysis. The ages of raptors were estimated from their morphological characteristics, such as the development of the gonads and feathers, the color of their feathers and iris, and molting condition [19]. Sampling areas are shown in Supplementary Fig. 1 and individual information is shown in Supplementary Table 3. The map information for Supplementary Fig. 1 was downloaded from Geospatial Information Authority of Japan and depicted by sf package [25] and ggplot2 [30] on R software ver. 3.5.1 [28].

Four shot pellets from the stomach of a whooper swan and fragments of Pb ammunition from the stomachs of two Steller's sea eagles were also collected to analyze the Pb isotope ratios in Pb ammunition.

Pb concentration

Pb concentrations were analyzed according to the method of Yabe *et al*. [31]. Samples of 100−300 mg of soft tissues were digested with 5 m*l* of 30% nitric acid (Kanto Chemical Corp., Tokyo, Japan) and 1 m*l* of 30% hydrogen peroxide (Kanto Chemical Corp.) in a microwave digestion system (Speedwave Two; Berghof, Eningen, Germany), after which the volume was made up to 10 m*l* by adding 2% nitric acid. Digestion was performed under the following conditions: 180°C for 15 min, 200°C for 20 min, and 100°C for 20 min. The Pb concentration and isotope ratios were then measured using an inductively coupled plasma–mass spectrometer (ICP-MS) (7700 series; Agilent Technology, Tokyo, Japan) (see Supplementary Table 1 for detailed analytical conditions), which was calibrated using ICP-MS Calibration Standards (Agilent Technology) to establish standard curves before analysis. Standard solutions (0, 10, 50, 100, 250, and 500 μg/*l* were prepared with 2% nitric acid and the standard curves had r^2 values of 0.998. All chemicals and standard stock solutions were of analytical reagent grade (Wako Pure Chemicals Industries, Osaka, Japan). Distilled and deionized water was used (Milli-Q; Merck Millipore, Billerica, MA, USA), and analytical quality control was performed using DOLT-4 (dogfish liver) and DORM-3 (fish protein) certified reference materials (National Research Council of Canada, Ottawa, ON, Canada), which were shown to have good recoveries (95–105%) through replicate analysis. Thallium (^{205}Tl) was used as an internal standard for the Pb concentration analysis. The limit of detection for Pb was 0.001 μg/kg.

Pb isotope analysis

The chemical procedures were carried out in a clean room with a Class 1000. The sample dissolution procedure was similar to the method described by Kuritani and Nakamura [16]. The extracted solutions of liver were transferred into Teflon tubes after analysis of Pb levels. One drop (~30 μl) of 0.05 N phosphoric acid (orthophosphoric acid 85%; Merck KGaA, Darmstadt, Germany) was added to the tubes to avoid complete dryness prior to evaporation. The mixed solution was dried for 15 hr on a hotplate at 100°C. After evaporation, three drops (~90 μl) of 8 N hydrogen bromide (once distilled, Analytical Grade 48%; Kanto Chemical Corp.) was added to the tubes. The tubes were placed on a hotplate at 120°C for 1 hr for re-drying. At the final step of pre-treatment before applying to the column, 0.3 m*l* of 0.5 N hydrogen bromide was introduced into the tube to dissolve residues. A polyethylene column was charged with 0.1 m*l* of anion exchange resin (AG1-X8, analytical grade, 200–400 mesh, chloride form; Bio-Rad, Hercules, CA, USA). The resin bed was washed by flushing the column with 1.0 m*l* of 0.5 N nitric acid (EL Grade 61%; Kanto Chemical Corp.) followed by 1.0 m*l* of double distilled deionized water, at a rate of ~0.03 m*l*/min. The column was conditioned with 0.2 m*l* of 0.5 N hydrogen bromide. The sample dissolved in 0.3 m*l* of 0.5 N hydrogen bromide was loaded onto the column. To wash out residual organic compounds originally derived from liver samples, 0.3 m*l* of 0.5 N hydrogen bromide was introduced twice. Subsequently, 0.8 m*l* of 0.25 N hydrogen bromide–0.5 N nitric acid mixture was applied to the column to remove elements other than Pb. Finally, the column was washed again with 0.1 m*l* of double distilled deionized water. After all washing procedures, 1.3 m*l* of 3% nitric acid was introduced to drop Pb out from the resin. Then, 2 μl of 50 mg/*l* thallium (Tl) was added to Pb eluted solution as an external standard for the analytical procedure.

Pb isotopic ratios of ^{208}Pb/^{206}Pb and ^{207}Pb/^{206}Pb were determined on a multiple collector (MC)-ICP-MS (Neptune Plus; Thermo Finnigan, San Jose, CA, USA) in static mode with the Faraday cup configuration. Other general parameters are described in Supplementary Table 2. Mass fractionation factors for Pb were corrected using an external standard of Tl. In addition, mass-dependent inter-element fractionations were also corrected by applying a standard bracketing method using NIST SRM 981 (National Institute of Standards and Technology, Gaithersburg, MD, USA), and the data were finally normalized to ^{206}Pb/^{204}Pb=16.9424, ^{207}Pb/^{204}Pb=15.5003, and ^{208}Pb/^{204}Pb=36.7266 [17]. The 2SE values of ^{208}Pb/^{206}Pb or ^{207}Pb/^{206}Pb were <0.0002.

Statistical analysis

Differences in the Pb concentrations between Steller's sea eagles and white-tailed sea eagles were analyzed using the Mann–Whitney *U* test. Statistical analysis was performed with JMP Pro 14 (SAS Institute, Cary, NC, USA). In all analyses, $P<0.05$ was taken to indicate statistical significance.

RESULTS

In total, nine of 34 Steller's sea eagles (26.5%) and three of 51 white-tailed sea eagles (5.9%) from Hokkaido, and two whooper swans from Hokkaido or Honshu exceeded the background level of Pb poisoning (i.e., >2 mg/kg wet weight of Pb in the liver). In addition, two white-tailed sea eagles, three Steller's sea eagles, and one Eurasian hobby from Hokkaido, and one mountain hawk eagle and one greater white-fronted goose from Honshu showed high Pb exposure (0.2–2 mg/kg) (Tables 1 and 2, Supplementary Fig. 2).

As for the species difference between Steller's sea eagles and white-tailed sea eagles, statistical analysis showed that Steller's

Table 1. Hepatic Pb levels (mg/kg, wet weight, range) and the assesments of Pb exposure in birds from Hokkaido

Species	Sample size	Pb concentration in liver	Assessments		
		mg/kg, wet wt, median, (range)	Pb poisoning (>2.0 mg.kg)	High Pb exposure (0.2–2.0 mg/kg)	Non toxic (<0.2 mg/kg)
White-tailed sea eagle	51	0.068 (0.003–50.79)	3	2	46
Steller's sea eagle	34	0.082 (0.003–72.01)	9	3	22
Blakiston's fish-owl	7	0.036 (0.001–0.070)	0	0	7
Mountain hawk-eagle	3	0.095 (0.023–0.120)	0	0	3
Northern goshawk	3	0.025 (0.007–0.136)	0	0	3
Eurasian Hobby	2	(0.092, 0.278)	0	1	1
Jungle crow	2	(0.063, 0.107)	0	0	2
Peregrine falcon	1	0.093	0	0	1
Sparrow hawk	1	0.065	0	0	1
Ural owl	1	0.137	0	0	1
Whooper swan	1	19.56	1	0	0

Table 2. Hepatic Pb levels (mg/kg, wet weight, range) and the assesments of Pb exposure in birds from Honshu or Kyushu

Species	Sample size	Pb concentration in liver	Assessments		
			Pb poisoning (>2.0 mg.kg)	High Pb exposure (0.2–2.0 mg/kg)	Non toxic (<0.2 mg/kg)
Peregrine falcon	6	0.050 (0.023–0.095)	0	0	5
Northern goshawk	5	0.080 (0.032–0.157)	0	0	5
Sparrowhawk	4	0.047 (0.017–0.080)	0	0	3
Ural owl	4	0.031 (0.015–0.054)	0	0	3
Mountain hawk-eagle	2	(0.051, 0.705)	0	1	1
Whooper swan	2	(0.182, 19.58)	1	0	1
Greater white-fronted goose	2	(0.097, 0.719)	0	1	1

Table 3. Pb isotope ratios in liver and ammunition from the stomach of Steller's sea eagle and whooper swan

Sample		Pb isotope ratios	
		208/206 Pb	207/206 Pb
Steller's sea eagle	Liver	2.05	0.84
	Ammunition from the stomach	2.06	0.84
Steller's sea eagle	Liver	2.11	0.87
	Ammunition from the stomach_1	2.11	0.87
	Ammunition from the stomach_2	2.11	0.87
	Ammunition from the stomach_3	2.11	0.87
Whooper swan	Liver	2.13	0.88
	Ammunition from the stomach_1	2.16	0.90
	Ammunition from the stomach_2	2.12	0.87
	Ammunition from the stomach_3	2.10	0.86
	Ammunition from the stomach_4	2.10	0.86
	Avarage of the above ammunition	2.12	0.87

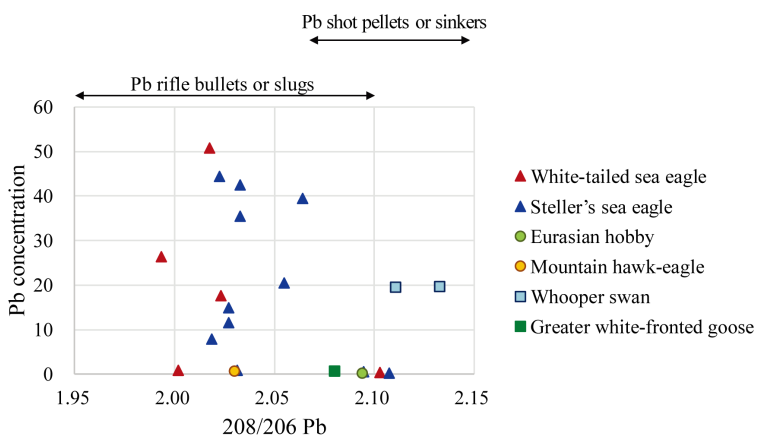

Fig. 1. Comparison of (a) Pb concentration and Pb isotope ratio ($^{208}Pb/^{206}Pb$) in the livers of birds that had high Pb concentrations (>0.2 mg/kg, wet weight).

sea eagles accumulated higher levels of Pb than white-tailed sea eagles, and there was a significant difference (P<0.03).

The fragments of Pb ammunition extracted from the stomachs of two Steller's sea eagles had the same isotope ratios as occurred in the livers of the same individuals. A whooper swan showed the same isotope ratios in its liver as the midrange of the isotope ratios of the four pieces of ammunition found in its stomach (Table 3).

The Pb isotope ratios of almost all of the white-tailed sea eagles and Steller's sea eagles, and one mountain hawk eagle were very similar to those of Pb rifle bullets or slugs (Fig. 1). While several sea eagles, one Eurasian hobby, whooper swans, and one greater white-fronted goose had similar Pb isotope ratios with Pb shot pellets or sinkers (Fig. 1). All data are shown in Supplementary Table 3.

DISCUSSION

Pb exposure in birds was shown to have occurred in both Hokkaido and Honshu. Despite prohibition of the use of Pb ammunition, endangered eagles were poisoned by Pb in Hokkaido. Pb poisoning has a large effect to the population of endangered eagles. Birds were also exposed to Pb in Honshu where regulation of the use of Pb ammunition is limited. As the sample number from Honshu was small, they may represent only a portion of the total number of cases.

As for the species difference between Steller's sea eagles and white-tailed sea eagles, Steller's sea eagles accumulated higher levels of Pb than white-tailed sea eagles ($P<0.03$). A previous study showed the same situation in Japan [10]. Steller's sea eagles have a higher risk of ingesting Pb from deer carcasses because their body size is larger than white-tailed sea eagles, and they regularly defeat white-tailed sea eagles to feed on deer carcasses. Furthermore, many sea eagles change their main food source from fish to deer during the hunting season. This tendency to change their diet is stronger in the Steller's sea eagle.

The Pb isotope ratios indicated that almost all of the white-tailed sea eagles and Steller's sea eagles examined were exposed to Pb from Pb rifle bullets or slugs, while several sea eagles had ingested Pb shot pellets or sinkers (Fig. 1). One mountain hawk eagle was found to contain Pb from a Pb rifle bullet, while one Eurasian hobby, whooper swans, and one greater white-fronted goose had ingested Pb shot pellets or sinkers (Fig. 1). Pb isotope ratio analysis showed that both Pb rifle bullets and Pb shot pellets cause Pb exposure in birds, and endangered eagles are also exposed to Pb in Hokkaido due to the illegal use of Pb ammunition. In addition, only limited numbers of birds were obtained from Honshu and Kyushu, but it was clear that birds here were also exposed to Pb from Pb shot pellets or sinkers. The observation that one swan contained four different Pb isotope ratios in four Pb shot pellets indicated that several types of Pb shot pellets had been used in the same area.

Previous study showed that 42% of Steller's sea eagles (18 of 43 cases) and 24% of white-tailed sea eagles (12 of 50 cases) exceeded the level of Pb poisoning (>2 mg/kg wet weight in liver) and one mountain hawk eagle exceeded the level of high Pb exposure (>0.1 mg/kg wet weight in blood) from 2004 to 2015 in Hokkaido [10]. In 2014, the regulation was enforced in Hokkaido that prohibited the possession of Pb rifle bullets, slugs, or large shot pellets for hunting. Prior to this regulation, it was not illegal for hunters to keep Pb ammunition, but they were punished if they were found to use such ammunition. This study, the ratio of Pb exposed birds in Hokkaido decreased. It would be because of the enforced regulation, however, Pb exposure still occurred in Hokkaido due to the illegal use of Pb ammunition. Previous study also showed that in Honshu and Shikoku (a southern island), threes golden eagles, one black kite, and one northern goshawk were exposed to Pb from 1993 to 2015 [10]. This study also indicated that several birds accumulated high levels of Pb in Honshu. Recent studies indicated that some partial bans on the use of lead ammunition do little to reduce lead poisoning mortality in raptors and scavengers [24]. Improvement to the regulation to prohibit the use of Pb ammunition across all parts of Japan is required to conserve avian species.

It has been demonstrated that Pb-free ammunition, such as copper (Cu) rifle bullets, steel shot pellets, and bismuth, shot pellets, have the same or even greater effectiveness as Pb ammunition for hunting, and are much less toxic to birds [12, 27]. For example, Cu toxicity was not confirmed in American kestrel (*Falco sparverius*) that had ingested Cu shot pellets [7]. However, Cu poisoning has sometimes been reported in the waterfowl [15]. Therefore, Cu rifle bullets or slugs would be useful as alternatives to the Pb ammunition that is the source of Pb poisoning in raptors. Steel shot pellets and bismuth pellets should be recommended as alternatives to Pb shot pellets to protect both raptors and waterfowl from Pb poisoning. In Hokkaido, the use of Cu rifle bullets is required instead of Pb rifle bullets and Cu rifle bullets are available at stores. Although many hunters in Hokkaido use Cu rifle bullets, some hunters prefer Pb rifle bullets to Cu ones. One of the reasons is Pb ammunition is inexpensive.

Although the fishing tackle was not found from the stomach of birds in this study, there is also a possibility that fishing sinker is one of the sources of Pb in birds. The use of all Pb fishing tackle is prohibited in Denmark, and there are some restrictions on the use of Pb fishing tackle in European countries, Canada, and the United States to conserve avian species from Pb exposure [8]. In Japan, anyone can obtain Pb-free fishing tackles at stores, however, information of Pb exposure in birds from the ingestion of Pb fishing tackles is not spread sufficiently. The consideration of the use of non-Pb fishing sinkers is also required in Japan.

Pb exposure is still occurring in raptors and water birds in various parts of Japan. In Hokkaido, both Pb rifle bullets and Pb shot pellets cause Pb exposure in birds, and endangered eagles are also exposed to Pb due to the illegal use of Pb ammunition. In Honshu and Kyushu, it was clear that birds were also exposed to Pb from Pb shot pellets or sinkers. It is clear that non-Pb ammunition needs to be used for hunting to reduce Pb exposure and conserve wild birds. In addition, continuous monitoring for Pb levels in wild birds to provide the accurate information about Pb exposure in birds would be important to improve the situation in Japan.

AKNOWLEDGMENTS. We would like to thank Yamashina Institute for Ornithology, Raptor Conservation Center, and The Miyagi Prefectural Izunuma-Uchinuma Environmental Foundation for providing samples. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan awarded to M. Ishizuka (No. 16H01779), Y. Ikenaka (No. 17K20038, 18H04132), S. Nakayama (No. 16K16197, 17KK0009), and C. Ishii (No. 18H06028). This research was also supported by the Inui Memorial Trust for Research on Animal Science, JST/JICA, SATREPS (Science and Technology Research Partnership for Sustainable Development) and the Environment Research and Technology Development Fund (SII-1/3-2, 4RF-1802/18949907) of the Environmental Restoration and Conservation Agency of Japan. We also acknowledge the financial support by the Nihon Seimei Foundation, the Soroptimist Japan Foundation, the foundations of Sumitomo. We are grateful to Takahiro Ichise, Mai Tamba and Nagisa Hirano (School of Veterinary Medicine, Hokkaido University) for their technical support.

REFERENCES

1. Arnemo, J. M., Andersen, O., Stokke, S., Thomas, V. G., Krone, O., Pain, D. J. and Mateo, R. 2016. Health and environmental risks from lead-based ammunition: science versus socio-politics. *EcoHealth* **13**: 618–622. [Medline] [CrossRef]
2. Bellrose, F. C. 1959. Lead poisoning as a mortality factor in waterfowl populations. *Bull. Ill. Nat. Hist. Surv.* **27**: 235–288. [CrossRef]
3. Brewer, L., Fairbrother, A., Clark, J. and Amick, D. 2003. Acute toxicity of lead, steel, and an iron-tungsten-nickel shot to mallard ducks (Anas platyrhynchos). *J. Wildl. Dis.* **39**: 638–648. [Medline] [CrossRef]
4. Church, M. E., Gwiazda, R., Risebrough, R. W., Sorenson, K., Chamberlain, C. P., Farry, S., Heinrich, W., Rideout, B. A. and Smith, D. R. 2006. Ammunition is the principal source of lead accumulated by California condors re-introduced to the wild. *Environ. Sci. Technol.* **40**: 6143–6150. [Medline] [CrossRef]
5. Ecke, F., Singh, N. J., Arnemo, J. M., Bignert, A., Helander, B., Berglund, Å. M. M., Borg, H., Bröjer, C., Holm, K., Lanzone, M., Miller, T., Nordström, Å., Räikkönen, J., Rodushkin, I., Ågren, E. and Hörnfeldt, B. 2017. Sublethal lead exposure alters movement behavior in free-ranging golden eagles. *Environ. Sci. Technol.* **51**: 5729–5736. [Medline] [CrossRef]
6. Fisher, I. J., Pain, D. J. and Thomas, V. G. 2006. A review of lead poisoning from ammunition sources in terrestrial birds. *Biol. Conserv.* **131**: 421–432. [CrossRef]
7. Franson, J. C., Lahner, L. L., Meteyer, C. U. and Rattner, B. A. 2012. Copper pellets simulating oral exposure to copper ammunition: absence of toxicity in American kestrels (Falco sparverius). *Arch. Environ. Contam. Toxicol.* **62**: 145–153. [Medline] [CrossRef]
8. Haig, S. M., D'Elia, J., Eagles-Smith, C., Fair, J. M., Gervais, J., Herring, G., Rivers, J. W. and Schulz, J. H. 2014. The persistent problem of lead poisoning in birds from ammunition and fishing tackle. *Condor* **116**: 408–428. [CrossRef]
9. Honda, K., Lee, D. P. and Tatsukawa, R. 1990. Lead poisoning in swans in Japan. *Environ. Pollut.* **65**: 209–218. [Medline] [CrossRef]
10. Ishii, C., Nakayama, S. M. M., Ikenaka, Y., Nakata, H., Saito, K., Watanabe, Y., Mizukawa, H., Tanabe, S., Nomiyama, K., Hayashi, T. and Ishizuka, M. 2017. Lead exposure in raptors from Japan and source identification using Pb stable isotope ratios. *Chemosphere* **186**: 367–373. [Medline] [CrossRef]
11. Ishii, C., Nakayama, S. M. M., Kataba, A., Ikenaka, Y., Saito, K., Watanabe, Y., Makino, Y., Matsukawa, T., Kubota, A., Yokoyama, K., Mizukawa, H., Hirata, T. and Ishizuka, M. 2018. Characterization and imaging of lead distribution in bones of lead-exposed birds by ICP-MS and LA-ICP-MS. *Chemosphere* **212**: 994–1001. [Medline] [CrossRef]
12. Kanstrup, N., Balsby, T. J. S. and Thomas, V. G. 2016. Efficacy of non-lead rifle ammunition for hunting in Denmark. *Eur. J. Wildl. Res.* **62**: 333–340. [CrossRef]
13. Kendall, R. J., Lacker, T. E., Bunck, C., Daniel, B., Driver, C., Grue, C. E., Leighton, F., Stansley, W., Watanabe, P. G. and Whitworth, M. 1996. An ecological risk assessment of lead shot exposure in non-waterfowl avian species: Upland game birds and raptors. *Environ. Toxicol. Chem.* **15**: 4–20. [CrossRef]
14. Kim, E.Y., Goto, R., Iwata, H., Masuda, Y., Tanabe, S. and Fujita, S. 1999. Preliminary survey of lead poisoning of Steller's sea eagle (Haliaeetus pelagicus) and white-tailed sea eagle (Haliaeetus albicilla) in Hokkaido, Japan. *Environ. Toxicol. Chem.* **18**: 448–451. [CrossRef]
15. Kobayashi, Y., Shimada, A., Umemura, T. and Nagai, T. 1992. An outbreak of copper poisoning in Mute swans (Cygnus olor). *J. Vet. Med. Sci.* **54**: 229–233. [Medline] [CrossRef]
16. Kuritani, T. and Nakamura, E. 2002. Precise isotope analysis of nanogram-level Pb for natural rock samples without use of double spikes. *Chem. Geol.* **186**: 31–43. [CrossRef]
17. Kuritani, T. and Nakamura, E. 2003. Highly precise and accurate isotopic analysis of small amounts of Pb using 205 Pb–204 Pb and 207 Pb-204 Pb, two double spikes. *J. Anal. At. Spectrom.* **18**: 1464–1470. [CrossRef]
18. Kurosawa, N. 2000. Lead poisoning in Steller's sea eagles and white-tailed sea eagles. First symposium on Stellar's and white-tailed sea eagles in east Asia. Wild Bird Society of Japan, Tokyo.
19. Lovette, I. J. and Fitzpatrick, J. W. 2016. Avian Anatomy. Handbook of Bird Biology, 3rd ed., John Wiley & Sons. Hoboken.
20. Ochiai, K., Hoshiko, K., Jin, K., Tsuzuki, T. and Itakura, C. 1993. A survey of lead poisoning in wild waterfowl in Japan. *J. Wildl. Dis.* **29**: 349–352. [Medline] [CrossRef]
21. Pain, D. J. and Amiard-Triquet, C. 1993. Lead poisoning of raptors in France and elsewhere. *Ecotoxicol. Environ. Saf.* **25**: 183–192. [Medline] [CrossRef]
22. Pain, D. J., Sears, J. and Newton, I. 1995. Lead concentrations in birds of prey in Britain. *Environ. Pollut.* **87**: 173–180. [Medline] [CrossRef]
23. Pain, D. J., Fisher, I. J. and Thomas, V. G. 2009. A global update of lead poisoning in terrestrial birds from ammunition sources. pp. 99–118. *In*: Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans, Peregrine Fund, Boise.
24. Pain, D. J., Mateo, R. and Green, R. E. 2019. Effects of lead from ammunition on birds and other wildlife: A review and update. *Ambio* **48**: 935–953. [Medline] [CrossRef]
25. Pebesma, E. 2018. Simple features for R: standardized support for spatial vector data. *R J.* **10**: 439–446. [CrossRef]
26. Saito, K. 2009. Lead poisoning of Steller's Sea-Eagle (*Haliaeetus pelagicus*) and Whitetailed Eagle (*Haliaeetus albicilla*) caused by the ingestion of lead bullets and slugs. Ingestion of Lead from spent Ammunition: Implications for Wildlife and Humans. pp. 302–309, Peregrine Fund, Boise.
27. Scheuhammer, A. M. and Norris, S. L. 1996. The ecotoxicology of lead shot and lead fishing weights. *Ecotoxicology* **5**: 279–295. [Medline] [CrossRef]
28. Team, R. C. R. 2013. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
29. Vallverdú-Coll, N., Mougeot, F., Ortiz-Santaliestra, M. E., Castaño, C., Santiago-Moreno, J. and Mateo, R. 2016. Effects of lead exposure on sperm quality and reproductive success in an avian model. *Environ. Sci. Technol.* **50**: 12484–12492. [Medline] [CrossRef]
30. Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.
31. Yabe, J., Nakayama, S. M. M., Ikenaka, Y., Yohannes, Y. B., Bortey-Sam, N., Oroszlany, B., Muzandu, K., Choongo, K., Kabalo, A. N., Ntapisha, J., Mweene, A., Umemura, T. and Ishizuka, M. 2015. Lead poisoning in children from townships in the vicinity of a lead-zinc mine in Kabwe, Zambia. *Chemosphere* **119**: 941–947. [Medline] [CrossRef]