Monitoring of Radioactive Cesium in Wild Boars Captured Inside the Difficult-To-Return Zone in Fukushima Prefecture Over a Five-Year Period

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**Monitoring of radioactive cesium in wild boars captured
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over a five-year period**

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Abstract

Following the Fukushima Daiichi Nuclear Power Plant accident in 2011, tissue samples from wild boar (*Sus scrofa*) outside the evacuation zone (difficult-to-return zone, DRZ) tended to show high concentrations of cesium-137 (^{137}Cs). Understanding the ^{137}Cs dynamics of wild boar populations inside the DRZ is necessary because they affect ^{137}Cs dynamics and wild boar management in areas outside the DRZ. Since few detailed, long-term studies have been conducted inside the DRZ, we measured ^{137}Cs concentrations in 221 wild boar muscle samples obtained from wild boar caught inside the DRZ and surrounding areas over a five-year period. Our results showed that the ^{137}Cs concentration in wild boar from inside the DRZ were higher than those in wild boar outside this zone. No significant difference was observed between muscle and soil ^{137}Cs levels, but significant correlations were observed between muscle ^{137}Cs concentrations and body length and body weight in the low-concentration season, but not between all seasons and the high-concentration seasons. It is considered that the size effects observed during the low-concentration season may be due to factors related to metabolism and changes in food habit. This is the first long-term survey of ^{137}Cs in wild boar inside the DRZ.

Introduction

The Tokyo Electric Power Company Fukushima Daiichi Nuclear Power Plant (hereafter, FDNPP) accident occurred as a result of the Great East Japan Earthquake in March 2011. Due to large amounts of artificial radioactive materials being released as a result of the FDNPP accident, the area within a 20-km radius around the FDNPP was immediately designated the Warning area to prevent radiation exposure [1]. In addition, areas with radiation dose rates above 20 mSv/yr were designated as Planned Evacuation Zones and access to these areas was restricted [1]. Subsequently, three evacuation zones—the difficult-to-return zone (hereafter, DRZ), the Restricted Residence Zone, and the Evacuation Order Cancellation Preparation Zone—were established based on predicted radiation doses to humans. Even though more than 10 years have passed since the accident, access to the DRZ is still restricted and previous residents of the DRZ have been prevented from returning to their homes due to the high radiation dose rates (>50 mSv/yr) [2].

Large amounts of radionuclides were released from the reactors at the time of the FDNPP accident [3]. Of these, concerns surrounding the long-term accumulation of cesium-137 (^{137}Cs) in wildlife have been raised due to its relatively long physical half-life (30.1 yr) and high bioavailability (i.e., absorption and transfer rates), as its chemical characteristics are similar to those of the monovalent cation potassium [4, 5]. After the FDNPP accident, numerous studies reported that ^{137}Cs was present in many wildlife species, and extensive studies have been conducted on the ^{137}Cs dynamics in organisms distributed in and around Fukushima (e.g., insects, [6]; amphibians, [7]; fishes, [8-10]; birds, [11]; and mammals, [12-18]).

Since the FDNPP accident in 2011, the government of Fukushima Prefecture has

conducted radioactive monitoring surveys of game meat, such as meat from wild boars (*Sus scrofa*) and Asian black bears (*Ursus thibetanus*). The results have shown that radiocesium contamination differs between these species, and that wild boars have higher radiocesium levels [14, 19, 20]. However, these studies did not examine the DRZ, which has high levels of radionuclide contamination. A previous study showed that there was a positive correlation between the ^{137}Cs concentration in wild boar muscle and ^{137}Cs deposition on soil at the sites where the wild boars were captured [14]. ^{137}Cs deposition on soil in the DRZ was high after the accident and most of the area has not yet been decontaminated. Therefore, the wild boars in the DRZ likely have high levels of ^{137}Cs contamination.

After the FDNPP accident, restrictions were placed on the shipment of foodstuffs throughout Fukushima Prefecture and on the consumption of wild boar meat in parts of the prefecture [19]. Removal of these restrictions is difficult because several wild boar meat samples have been found to contain radionuclide concentrations that exceed acceptable levels for consumption (i.e., samples that have total radionuclide (^{134}Cs and ^{137}Cs) levels that exceed the 100 Bq/kg limit prescribed by Japanese food standards).

Wild boars are an important game species in the region and they cause serious agricultural damage. In recent years, agricultural damage by wild boars has increased and become more widespread due to the marked increase in the wild boar population, which in turn is attributed to the decrease in human activity in rural areas, the increase in deserted arable land, and the decrease in hunting pressure as hunters age and decrease in number [21, 22]. In response to this increase in wild boar numbers, prefectures and municipalities have implemented control measures to capture wild boars and reduce the damage that they cause.

A unique problem for the management of wild boar in Fukushima Prefecture is that the motivation among hunters to hunt wild boar has decreased due to the restrictions imposed on the utilization of radionuclide-contaminated wild boar meat [23]. In addition, there is concern that wild boars in human settlements will transport high concentrations of radionuclides beyond the boundaries of the DRZ [23]. Of particular concern in the management of wild boar in the DRZ is that decrease in human activities, such as agricultural activities, in the zone will result in both range expansion and population increases of wild boars in the region [23]. Consequently, clarifying the ^{137}Cs concentration dynamics in wild boar inside the DRZ is considered necessary because this factor appears to be closely related to ^{137}Cs dynamics and wild boar management in areas outside the DRZ.

Few studies on ^{137}Cs concentrations in wild boars inside the DRZ and the surrounding areas have been conducted; these include the results of one-year trends in Tomioka town [17], and a comparison of methods used to examine aggregated transfer factors (T_{ag} : radionuclide concentration in muscle/radionuclide deposition in soil, m^2/kg) [24]. However, no detailed reports based on long-term or extensive sampling have been conducted inside the DRZ.

In this study, we conducted a monitoring survey of ^{137}Cs concentrations in muscle samples collected from wild boar inside the DRZ and surrounding areas (Fig. 1) over a five-year period.

Results

Comparison of ^{137}Cs concentrations in wild boar muscles collected from wild boars captured inside and outside the DRZ

The ^{137}Cs concentrations for all muscle samples from wild boars captured inside the DRZ exceeded detection limits, with levels greater than 100,000 Bq/ kg (FM) obtained for three samples collected in 2016 (Fig. 2, Table 1). The detection range was from 42 to 132,210 Bq/kg (FM), indicating that there was a large variation among individuals (Figure 2, Table 1, 2). Significance correlations were found between captured date and the ^{137}Cs concentration in in all four regions (i.e., inside the DRZ and three regions outside the DRZ (Hamadori, Nakadori, and Aizu)) (Fig. 2). The ^{137}Cs concentrations obtained from wild boar muscle samples collected inside the DRZ were tend to be higher than those collected outside the DRZ (Fig. 2). In addition, ^{137}Cs concentrations in meat samples varied significantly by sampling month (Fig. 3, Table 2, Kruskal-Wallis test, $\text{df} = 11$, $\chi^2 = 35.18$, $P < 0.001$). Specifically, significant differences were observed between February in the high-concentration season and August and September in the low-concentration season (Fig. 3, Table 2, Steel-Dwass' test, $P < 0.01$).

Relationships among ^{137}Cs concentration in muscle and ^{137}Cs deposition on soil, body length and body weight

From the results of the regression analysis in all seasons and the high-concentration season, no significant differences were observed among the ^{137}Cs concentration in muscle and ^{137}Cs deposition on soil, body length and body weight (Figs. 4, 5). Also, in the low-concentration season, no significant differences were observed between the ^{137}Cs concentration in muscle and ^{137}Cs deposition on soil, but significant correlations were observed among the ^{137}Cs concentration in muscle and body length and body weight (Fig. 6).

Discussion

The ^{137}Cs concentrations in wild boar muscle samples from inside the DRZ were higher than those from outside the DRZ. In addition, a large variation was observed among individuals, with differences of several orders of magnitude recorded (Fig. 2). In our study, the ^{137}Cs concentration in wild boar muscles tended to be low in August and September in the summer season and high in February in the winter season inside the DRZ, indicating that our results corroborate the findings of a previous study [14]. In Tomioka town, which includes areas inside the DRZ, the ^{137}Cs concentration in wild boar muscle was previously reported to vary significantly between different months [17].

No significant differences were observed between the ^{137}Cs concentrations in muscle and ^{137}Cs deposition on soil inside the DRZ in any of the time periods examined (i.e., all seasons, high-concentration and low concentration seasons) (Figs 4-6). ^{137}Cs deposition on soil tended to be higher in the DRZ than outside the DRZ; however, decontamination efforts have subsequently been initiated in areas close to and inside the DRZ (e.g., Specified Reconstruction and Revitalization Base). Therefore, the distribution of ^{137}Cs on soil is considered to be very heterogeneous in the region. This heterogeneity might account for the absence of any relationships between muscle ^{137}Cs concentrations and ^{137}Cs deposition on soil in this area. However, a previous study also reported that muscle ^{137}Cs concentrations in wild boars was highly variable, even when wild boars were captured in areas with similar ^{137}Cs soil deposition levels [14]. In addition, it is considered that the evaluation of the radiocesium contamination level in animal species based on soil deposition (i.e., T_{ag} values) is inappropriate for animal species with large home ranges such as wild boars [24]. Similar findings were reported in moose after the

Chernobyl nuclear power plant accident (hereafter, Chernobyl accident), where there was a weak correlation between ^{137}Cs soil deposition and the ^{137}Cs concentration in muscle [27]. In that study, the authors proposed that the annual fluctuations in the T_{ag} values observed in moose may have been attributed to various ecological factors, such as differences in food selection or habitat use [27]. In this study, we considered that the variations observed in the ^{137}Cs concentrations in the muscle of wild boars may be more strongly affected by ecological factors, such as food habits and migration than to ^{137}Cs soil deposition.

In some fish species, a size effect in which there is a tendency for radiocesium concentration in the body to accumulate as a function of increased body size and/or weight has been reported [9]. The factors affecting this size effect were considered to be related to ontogenetic changes in food habits and/or the physiological ability to retain radiocesium during growth [9, 28]. In our study, a significant positive relationship was observed between muscle ^{137}Cs concentration and both body length and weight of wild boars during the low-concentration season, but not during all seasons and the high-concentration season. Cui et al. [17] found no significant correlation between radiocesium concentration and the weight of the wild boars captured over the entire year in 2019-2020 in Tomioka town. A study on the mobility and home range size of wild boar reported that females with piglets have a smaller home range in the summer season than in the fall and parturitional season (i.e., spring) [29]. In France, the wild boar home ranges in the hunting season is larger than that in the summer season [30]. It is considered that the size effects observed during the low-concentration season in our study may be due to factors related to metabolism and changes in food habits, because the movements and habitat shifts in wild boars are not as great as they are during the

other seasons. However, since the activity, movement and home range of wild boars are influenced by numerous factors, such as food availability, population density and hunting pressure (e.g., [31, 32]), it is important to clarify the relationships between muscle ^{137}Cs concentration and seasonal mobility of wild boars in areas including DRZ in future studies.

Our study showed that the five-year trend in the ^{137}Cs concentration in the muscles of wild boar inside the DRZ was higher than that in wild boar from outside this area, and confirmed the existence of seasonal variation of these concentrations and the size effect of ^{137}Cs accumulation in the low-concentration season. After the Chernobyl accident, ^{137}Cs concentrations in wild boars were higher than those in other wild animals [33], with ^{137}Cs levels showing long-term accumulation [34-37]. Consequently, we consider that long-term monitoring of radiocesium dynamics should be undertaken in wild boars in areas affected by the FDNPP accident. Continued surveys focusing on the movement of wild boars inside the DRZ could therefore facilitate the management of wild boar populations and the monitoring of radiocesium dynamics in wild boars in areas outside the DRZ where high muscle ^{137}Cs concentrations have occasionally been observed.

Materials and methods

Sample collection

We collected muscle tissue samples from 221 culled wild boar individuals that were trapped as part of a survey conducted by the Ministry of the Environment in Japan; “The habitat survey and capture of the wild animals project in and around the former restricted areas (areas within 20-km radius from Fukushima Daiichi NPP (2015-2017))” and “The habitat survey and capture of the wild birds and mammals project in and

around the "difficult-to-return zone" in Fukushima (2018-2020)". The capture of wild boar and the collection of muscle samples were conducted by the Japan Wildlife Research Center, which was commissioned to conduct these surveys. For all wild boar samples, the data collected included the GPS coordinates of the capture location, body length, body weight and sex. We used muscle samples that were collected in five municipalities (Okuma, Katsurao, Tomioka, Namie, Futaba) from January in 2016 to November in 2020 (Fig. 1).

Measurement of muscle ^{137}Cs concentrations

The surface and any connective tissue were removed from the muscle block samples that were collected for analysis. Raw muscle samples were either minced or sliced, or freeze-dried for several days and then crushed using a bottle blender (Osaka Chemical Co., Ltd., Osaka, Japan). The samples were then placed in standard U-8 containers (100 ml, $\varnothing 56\text{ mm} \times 68\text{ mm}$) and gamma-ray emitting radionuclides were measured using a germanium semiconductor detector (Canberra GC3018, Meriden, USA). The ^{137}Cs concentration of freeze-dried muscle samples was calculated as fresh mass (hereinafter, "FM"). In addition, we used the ^{137}Cs concentration data for wild boar muscles that were collected from outside the DRZ area as part of a wild animal monitoring survey conducted by the Fukushima prefectural government [19]. The results obtained for the samples that were collected outside the DRZ were divided into three groups based on the region of collection, i.e., Hamadori excluding the DRZ (hereafter, Hamadori), Nakadori and Aizu (Fig. 2); the ^{137}Cs concentrations in these samples were compared against the results obtained for samples collected from wild boar inside the DRZ.

Calculation of ^{137}Cs deposited on soil

To examine whether there was any correlation between the ^{137}Cs concentrations in wild boar muscles and ^{137}Cs deposition on soil at the site of wild boar capture (Bq/m^2), ^{137}Cs concentrations were extracted from the ^{137}Cs ground deposition open data map compiled by the Japan Atomic Energy Agency's (JAEA) 5th Airborne Monitoring Survey (JAEA, 2012) using ArcGIS Pro (<https://pro.arcgis.com/en/pro-app/latest/get-started/install-and-sign-in-to-arcgis-pro.htm>). The ^{137}Cs deposited on soil at each capture site was then estimated considering the decay rate over the number of days between the soil ^{137}Cs measurements and the capture date of the wild boar using a physical half-life of ^{137}Cs (30.1 years).

Statistical analysis

To confirm the changes in the ^{137}Cs concentration in wild boar muscle samples collected in four regions (i.e., inside the DRZ and three regions outside the DRZ (Hamadori, Nakadori, and Aizu)) over time, we performed a regression analysis of the relationship between ^{137}Cs concentration in muscle and capture date (i.e., number of days to the capture date, with January 1, 1900 as the base date). Then, we excluded data that were below the detection limit [e.g., 26]. Differences in the ^{137}Cs concentration in wild boar muscle for each sampling month were evaluated using the Kruskal-Wallis test. Then, the Steel-Dwass test was performed to evaluate the difference in the mean values for each month. These statistical analyses were performed using R4.0.3 (<https://www.r-project.org/>).

We performed the regression analysis based on mixed linear models for wild boar datasets inside the DRZ. We used the ^{137}Cs concentration in wild boar muscle as the

response variable, and ^{137}Cs deposition on soil, body length and body weight as explanatory variables. The capture year was used for a random factor. These analyses were performed using the JMP 13.2.1 software package (SAS, Cary, NC, USA). Because the ^{137}Cs concentration in wild boar muscle exhibits seasonal variations [14], we divided the data into three seasons, i.e., all seasons, high-concentration season (December, January, February, March) and low-concentration season (July, August, September). For all statistical analysis, we used the ^{137}Cs concentration in muscle and ^{137}Cs deposition on soil with $\log_{10}$ transformation.

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Author contributions

R.S. conducted data analysis, wrote the main manuscript text and prepared all of the
figures. All authors conducted the experiments and reviewed the manuscript.

Competing interests

The authors declare no competing financial interests.

Data availability

The datasets generated during and/or analyzed during the current study are available

from the corresponding author on reasonable request.

Figure legends

Figure 1 Sites where wild boars were captured in the difficult-to-return zone (DRZ) (a) Fukushima Prefecture, (b) DRZ and surrounding area (including the Specified Reconstruction and Revitalization Base areas).

Symbols on the map indicate the sites where wild boars were captured. Differences in the shape and color of symbols indicate differences in the year of capture. Data for ^{137}Cs deposition on soil was obtained from the 5th airborne monitoring survey (<https://emdb.jaea.go.jp/emdb/en/portals/b1020201/>) by the Japan Atomic Energy Agency (JAEA). This figure was created using ArcGIS Pro 3.1.6 (<https://www.esri.com/en-us/arcgis/products/arcgis-pro/overview>). The map of Fukushima Prefecture was obtained from the Ministry of Land, Infrastructure, Transport and Tourism (MLIT) of Japan (<http://nlftp.mlit.go.jp/ksj/>).

Figure 2 Temporal changes in ^{137}Cs concentrations of wild boar muscle samples collected inside the difficult-to-return zone (DRZ) and three areas outside the DRZ (Hamadori, Nakadori and Aizu). The map shows each region using different colors. The DRZ (10 March in 2020) on the map includes the specific reconstruction areas in which restrictions on entry have been lifted. The simple linear regression equation (red line) and the 95% confidence interval about the regression line (grey shaded area) are shown in the figure.

Figure 3 Seasonal variation in ^{137}Cs concentration muscle samples from wild boar

inside the difficult-to-return-zone (DRZ).

The upper part of the box plot shows the 75th percentile and the lower part shows the 25th percentile. The horizontal lines in the box indicate the median value. The whiskers above and below the boxes indicate the maximum and minimum values, respectively. Black dots indicate outliers. The *P* values were calculated using the Steel-Dwass test (*P* < 0.01) between each month.

Figure 4 Relationships between ^{137}Cs concentration in wild boar muscle samples collected in all seasons and (a) ^{137}Cs deposition on soil ($\log_{10} \text{ Bq/m}^2$), (b) body length (cm), and (c) body weight (kg) inside the difficult-to-return zone (DRZ).

Results of the regression analysis are based on mixed linear models using years as random variables and the adjusted coefficient of determination (R^2) are shown in the figures.

Figure 5 Relationships between ^{137}Cs concentration of wild boar muscle samples collected in the high-concentration season (December to March) from inside the difficult-to-return zone (DRZ) and (a) ^{137}Cs deposition on soil ($\log_{10} \text{ Bq/m}^2$), (b) body length (cm), and (c) body weight (kg)

Results of the regression analysis are based on mixed linear models using years as random variables and the adjusted coefficient of determination (R^2) are shown in the figures.

Figure 6 Relationships between the ^{137}Cs concentration of wild boar muscle samples collected in the low-concentration season (July to September) from inside the

438 difficult-to-return zone (DRZ) and (a) ^{137}Cs deposition on soil ($\log_{10} \text{ Bq/m}^2$), (b) body
439 length (cm), and (c) body weight (kg).

440 Results of the regression analysis are based on mixed linear models using years as
441 random variables and the adjusted coefficient of determination (R^2) are shown in the
442 figures. The linear regression equation is shown only when a significant difference
443 between the two variables was observed.

Table 1 ¹³⁷Cs concentration in wild boars muscle of each year in difficult to return zone

Year	Mean (Bq/kg, FM)	Max (Bq/kg, FM)	Minimun (Bq/kg, FM)	Numver of individuals (N)
2016	15,028	132,210	608	56
2017	3,030	26,110	542	29
2018	8,303	38,385	427	30
2019	5,180	42,018	42	52
2020	3,508	20,010	213	54

Table 2 ¹³⁷Cs concentration in wild boars muscle of each month in difficult to return zone

Month	Mean (Bq/kg, FM)	Max (Bq/kg, FM)	Minimun (Bq/kg, FM)	Numver of individuals (N)
1	6,797	26,909	434	16
2	17,289	132,210	620	44
3	1,463	3,165	538	5
4	2,189	4,566	213	5
5	4,883	22,490	42	12
6	4,992	22,351	531	13
7	3,892	14,138	243	25
8	1,917	7,719	298	18
9	2,208	7,479	448	16
10	4,842	26,110	386	17
11	8,730	42,018	201	26
12	6,236	38,385	377	24

Figure 1 Sites where wild boars were captured in the difficult-to-return zone (DRZ) (a) Fukushima Prefecture, (b) DRZ and surrounding area (including the Specified Reconstruction and Revitalization Base areas).

Symbols on the map indicate the sites where wild boars were captured. Differences in the shape and color of symbols indicate differences in the year of capture. Data for ^{137}Cs

452 deposition on soil was obtained from the 5th airborne monitoring survey
453 (<https://emdb.jaea.go.jp/emdb/en/portals/b1020201/>) by the Japan Atomic Energy
454 Agency (JAEA). This figure was created using ArcGIS Pro 3.1.6
455 (<https://www.esri.com/en-us/arcgis/products/arcgis-pro/overview>). The map of
456 Fukushima Prefecture was obtained from the Ministry of Land, Infrastructure, Transport
457 and Tourism (MLIT) of Japan (<http://nlftp.mlit.go.jp/ksj/>).

460 Figure 2 Temporal changes in ^{137}Cs concentrations of wild boar muscle samples
461 collected inside the difficult-to-return zone (DRZ) and three areas outside the DRZ
462 (Hamadori, Nakadori and Aizu). The map shows each region using different colors. The
463 DRZ (10 March in 2020) on the map includes the specific reconstruction areas in which
464 restrictions on entry have been lifted. The simple linear regression equation (red line)
465 and the 95% confidence interval about the regression line (grey shaded area) are shown
466 in the figure.

467

468

469 Figure 3 Seasonal variation in ^{137}Cs concentration muscle samples from wild boar
470 inside the difficult-to-return-zone (DRZ).

471 The upper part of the box plot shows the 75th percentile and the lower part shows the
472 25th percentile. The horizontal lines in the box indicate the median value. The whiskers
473 above and below the boxes indicate the maximum and minimum values, respectively.
474 Black dots indicate outliers. The P values were calculated using the Steel-Dwass test (P
475 < 0.01) between each month.

476

Figure 4 Relationships between ^{137}Cs concentration in wild boar muscle samples collected in all seasons and (a) ^{137}Cs deposition on soil (log₁₀ Bq/m²), (b) body length (cm), and (c) body weight (kg) inside the difficult-to-return zone (DRZ). Results of the regression analysis are based on mixed linear models using years as random variables and the adjusted coefficient of determination (R^2) are shown in the figures.

Figure 5 Relationships between ^{137}Cs concentration of wild boar muscle samples collected in the high-concentration season (December to March) from inside the difficult-to-return zone (DRZ) and (a) ^{137}Cs deposition on soil (log₁₀ Bq/m²), (b) body length (cm), and (c) body weight (kg)

Results of the regression analysis are based on mixed linear models using years as random variables and the adjusted coefficient of determination (R^2) are shown in the figures.

Figure 6 Relationships between the ^{137}Cs concentration of wild boar muscle samples collected in the low-concentration season (July to September) from inside the difficult-to-return zone (DRZ) and (a) ^{137}Cs deposition on soil (log₁₀ Bq/m²), (b) body length (cm), and (c) body weight (kg).

Results of the regression analysis are based on mixed linear models using years as random variables and the adjusted coefficient of determination (R^2) are shown in the figures. The linear regression equation is shown only when a significant difference between the two variables was observed.