

Exposure of a herbivorous fish to ^{134}Cs and ^{137}Cs from the riverbed following the Fukushima disaster



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ABSTRACT

Ayu *Plecoglossus altivelis*, a herbivorous fish, is an important fishery resource and key component of the foodweb in many Japanese streams. Radionuclide contamination of this species is likely transferred to higher trophic levels, include humans, in the food chain. After the Fukushima accident in March 2011, ayu were exposed to highly contaminated silt while feeding on algae attached to the riverbed stones. To understand the route by which herbivorous fish are exposed to radionuclides, the activity concentrations of sum of ^{134}Cs and ^{137}Cs (radiocesium) were analyzed in riverbed samples (algae and silt) and in the internal organs and the muscle of ayu in five river systems in the Fukushima Prefecture between summer 2011 and autumn 2013. Although there was a positive correlation between the radiocesium activity concentrations in the muscle and the internal organs of ayu, the median activity concentration in the muscle was much lower than those in the internal organs. The activity concentrations of radiocesium in the riverbed samples and the internal organs and the muscle of ayu were correlated with contamination levels in soil samples taken from the watershed upstream of the sample sites. The results of the generalized linear mixed models suggest that the activity concentrations in both the internal organs and the muscle of ayu declined over time. Additionally, the activity concentrations in the internal organs were correlated with those in the riverbed samples that were collected around the same time as the ayu. The activity concentrations in the muscle were correlated with ayu body size. Our results suggest that ayu ingest ^{134}Cs and ^{137}Cs while grazing silt and algae from the riverbed, and a part of the ^{134}Cs and ^{137}Cs is assimilated into the muscle of the fish.

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1. Introduction

A magnitude 9.0 earthquake and subsequent tsunami on 11 March 2011 in Northern Japan resulted in the atmospheric release of a large amount of radionuclides from the nuclear reactors at the Fukushima Dai-ichi Nuclear Power Plant (FDNPP) (Kinoshita et al., 2011; Morino et al., 2011). The radionuclides subsequently spread over central and northern Honshu, Japan. The rivers in Honshu typically have steep gradients and are subject to erosion during

snowmelt and typhoons (Yoshimura et al., 2005). As a result, the radionuclides in the sediment deposits and soils were transported from the mountains to the rivers in the Fukushima Prefecture (Evrard et al., 2013). An understanding accumulation processes of radionuclides in freshwater fish is essential to enable stakeholders to quantify and predict the concentrations of radionuclides in the fish.

Radioactive ^{134}Cs and ^{137}Cs is accumulated in the internal organs of freshwater fish primarily as a result of food intake (Hewett and Jefferies, 1976; Forseth et al., 1991; Rowan and Rasmussen, 1994; Ugedal et al., 1995; Yankovich et al., 2010). Therefore, spatiotemporal monitoring of the ^{134}Cs and ^{137}Cs throughout the food chain provides information on the route of contamination and the degree

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of bioaccumulation at different trophic levels. Body size and trophic level of lake fish had a significant influence on the activity concentrations of muscle ^{137}Cs following the Chernobyl disaster; piscivores accumulated ^{137}Cs more than planktivores (Elliott et al., 1992; Sundbom et al., 2003). The change in activity concentration in fish typically lagged behind the pulse of ^{137}Cs in the water, with maximum activity concentrations in fish occurring months to years after the fallout (Ugedal et al., 1995; Saxén and Koskelainen, 2001). However, ^{134}Cs and ^{137}Cs was detected in stream fishes, including both herbivores and piscivores, soon after the Fukushima disaster in 2011 (Iguchi et al., 2013; Mizuno and Kubo, 2013). Herbivores are at a lower trophic level than piscivores and planktivores. Unfortunately, little is known about the routes of radioactive uptake in herbivorous fish, though they may become contaminated more rapidly after a radionuclide pulse in the freshwater environment.

Ayu *Plecoglossus altivelis* is a herbivorous fish species that are commonly distributed throughout the Japanese Archipelago (Iguchi et al., 1999). The species exhibits an amphidromous and annual life history. After completing the marine part of its lifecycle during the juvenile stage in winter, young ayu migrate into rivers to graze on benthic algae attached to the riverbed (Iguchi and Hino, 1996). Ayu are also an important resource for humans and avian species, such as the great cormorant *Phalacrocorax carbo* (Takahashi et al., 2006). Thus, the radionuclide contamination of ayu may have a result in radionuclide exposure to both humans and non-human biota in aquatic and terrestrial ecosystems. In the Fukushima Prefecture, Iguchi et al. (2013) reported high levels of contamination in the riverbed sediments. Ayu ingest silt while grazing on algae attached to the stones in riverbeds, thereby resulting in exposure to the radionuclides from contaminated sediments, including the silt component.

Aerosol-bound Cs was transported from the atmosphere to the surface soil by dry or wet deposition within 2 months after the Fukushima disaster (Masson et al., 2011; Hirose, 2012; Yasunari et al., 2011). Subsequently, the rivers in the Fukushima Prefecture received radionuclides-contaminated soil originating from inland mountain ranges (Evrard et al., 2013). To understand the route by which herbivorous fish are exposed to radionuclides, we measured ^{134}Cs and ^{137}Cs activity concentrations in the riverbed samples (algae and silt), and in the internal organs and the muscle of ayu in Fukushima Prefecture.

2. Materials and methods

2.1. Study area and sampling

Ayu ($n = 166$, fork length of 68–206 mm) were collected by casting nets (16 m periphery, 9 mm mesh) in five rivers in the Fukushima Prefecture between 9 July 2011 and 14 October 2013 (Fig. 1, Table S1 in supplementary materials), with the exception of the Niida and Kido Rivers, which were not sampled prior to May 2012 because of concerns about radiation safety in those areas. The nets were casted for fish sampling ca. ten times per site per day. Water depths in all sampling sites were less than 1m. Additionally, riverbed samples, consisting primarily of benthic algae and silt were collected at each site, except during flood events. The riverbed samples were collected from the surface of stones using a toothbrush and placed in 500 mL bottles. Fish and riverbed samples were stored frozen at $-20\text{ }^{\circ}\text{C}$ until later analysis.

2.2. Radiocesium analyses

Each fish was dissected to separate the internal organs (i.e., stomach contents, stomach, intestine contents, intestine, liver, spleen, and gonad) and the muscle. The riverbed samples were filtered to separate water and particulates using a filter paper with 8 μm pore size. Each sample was placed into a polypropylene cylindrical container (55 mm diameter, 64 mm height) and measured for ^{134}Cs and ^{137}Cs activity concentrations using a high-purity germanium detector (ORTEC, Oak Ridge, USA). The energy-dependent counting efficiency of detector was calibrated using a set of volume standard sources in the same type containers used for the samples (MX033U8PP, Japan Radioisotope Association, Tokyo). The high-purity germanium detector has resolution of 1.44 keV at a peak of 662.15 keV. Coincidence summing effects of ^{134}Cs were corrected using ^{134}Cs standard solutions purchased from Japan Radioisotope Association. Measurement time for each sample was differed from 7200 to 57,949 s depending on intensity of gamma-ray in the measured sample. The activity concentrations of ^{134}Cs and ^{137}Cs for all samples were decay-corrected to the sampling date. The activity concentration of three times of the standard deviation from counting statistics was defined as the limit of detection.

2.3. Statistical analyses

Both ^{134}Cs and ^{137}Cs were detected in 119 internal organ samples and 98 muscle samples from 166 fish, and in 34 of 36 riverbed samples (i.e., fish dietary items). The activity concentrations of sum of ^{134}Cs and ^{137}Cs (hereafter radiocesium) were used for statistical analyses. To evaluate the relationships between the activity concentrations of radiocesium in the internal organs and the muscle of ayu, a generalized linear model (GLM) was fitted with a Gaussian distribution of errors. The data sets of 84 individuals were used, which were detected the activity concentrations both in the internal organs and the muscle. The activity concentrations of radiocesium in the muscle of ayu were treated as response variables; those in the internal organs were used as explanatory variable. Subsequently, to compare the median activity concentrations of radiocesium in the internal organs and the muscle, Wilcoxon signed-rank test was conducted.

To evaluate the spatial trend in the activity concentrations of radiocesium in the riverbed samples and the internal organs and muscle in ayu amongst the five rivers, a GLM was fitted with a Gaussian distribution of errors. Because the data for all five rivers were only available in 2012 and 2013, data describing the activity concentrations of radiocesium in 28 riverbed, 101 internal organ,

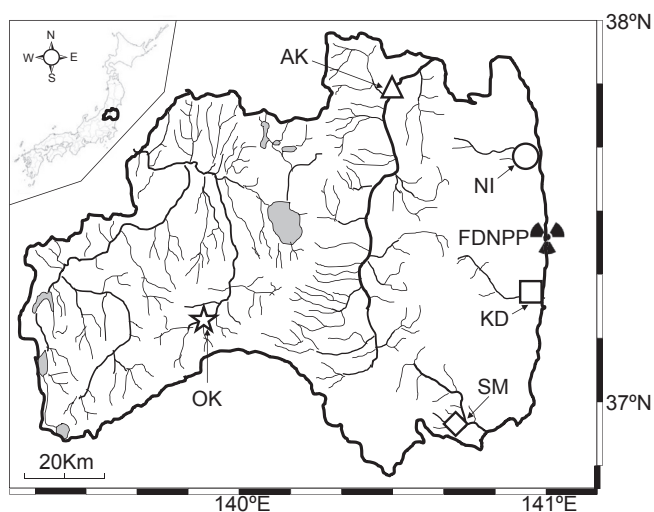


Fig. 1. Location of collection of ayu and riverbed samples (NI, Niida River; KD, Kido River; AK, Abukuma River; SM, Same River; OK, Okawa River). The symbol for each site corresponds to those in Figs. 2 and 3. FDNPP shows the Fukushima Dai-ichi Nuclear Power Plant.

and 77 muscle samples collected during these 2 y were used for analysis. The radiocesium activity concentrations in tissues were treated as response variables, while the mean deposition density of radiocesium in terrestrial surface soil in the watershed upstream of each site was used as explanatory variable. The mean deposition density of radiocesium in terrestrial soil were calculated from the results of the soil survey project by the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT), which were decay-corrected to June or July 2011 (MEXT, 2011; Saito et al., 2015). The deposition density of ^{134}Cs and ^{137}Cs for terrestrial soil were decay-corrected to each sampling date of the riverbed, and the internal organs and muscle of ayu.

To evaluate the temporal changes in radiocesium activity concentrations in the internal organs and muscle of ayu, a generalized linear mixed model (GLMM) was fitted with a Gaussian distribution of errors. The activity concentrations of radiocesium in the internal organs or muscle of ayu were treated as response variables; elapsed days since 11 March 2011, sampling season (spring, summer, autumn), fork length of ayu, and the activity concentrations of radiocesium in the riverbed samples were used as explanatory variables, and river ID was included as a random factor. To eliminate the effect of radioactive decay of ^{134}Cs , GLMM were also conducted using time-series data of ^{137}Cs . To identify subsets of significant variables, I used the Akaike information criterion (AIC). The regression model with the lowest AIC among the all-possible 16 models was selected as the best model. Because the activity concentrations of radiocesium in the riverbed sample in the Kido River on 30 May 2013 and the Okawa River on 10 October 2013 were below the limit of detection, the samples of fish collected on these dates were also excluded from the analysis. Radiocesium activity concentration data were measured in 114 internal organ and 90 muscle samples and compared to the activity concentration data from the riverbed collected around the same time as the ayu.

3. Results

All data from the analyses of ^{134}Cs and ^{137}Cs are listed in Table S2 in the supplementary materials. In 2013, the median of the $^{134}\text{Cs}/^{137}\text{Cs}$ ratio was 0.46 in all data for fish and riverbed samples, which is identical to the value 2 y after the fallout from the FDNPP. Radiocesium was detected both in the internal organs and the muscle of 84 individuals. Although there was a positive correlation

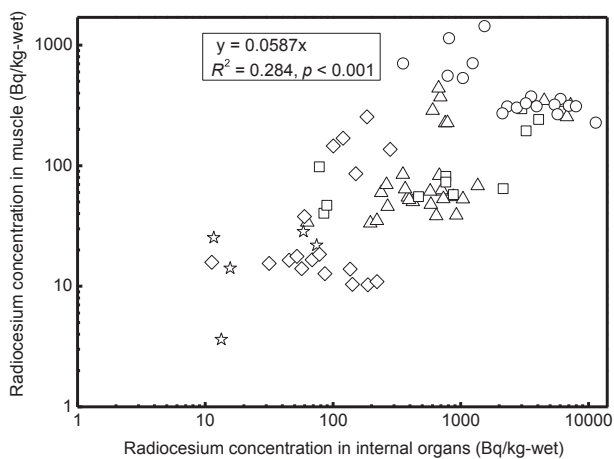


Fig. 2. Relationships between the concentrations of radiocesium (sum of ^{134}Cs and ^{137}Cs) in the internal organs (i.e., stomach contents, stomach, intestine contents, intestine, liver, spleen, and gonad) and muscle of 84 ayu individuals collected in five rivers between 2011 and 2013. The symbols correspond to the collection sites in Fig. 1.

between the activity concentrations of radiocesium in the internal organs and the muscle of ayu ($n = 84$, $R^2 = 0.284$, $p < 0.001$), the median activity concentration in the muscle was 14.5% that of the median activity concentration in the internal organs ($n = 84$, $p < 0.001$, Fig. 2). The activity concentrations of radiocesium in all three sample types were highly correlated with mean deposition density of radiocesium in the terrestrial soil samples from each watershed (Fig. 3). The results of the GLMM suggest that the radiocesium activity concentrations have declined through time in both the internal organs and the muscle of ayu (Table 1). Additionally, the activity concentrations in the internal organs of ayu were positively correlated with those in the riverbed samples (i.e., fish dietary items) that were collected around the same time as the ayu, but there was no correlation between the activity concentrations in the muscle and the riverbed samples, treating river ID as a random effect (Table 1, Fig. 4). A positive correlation was observed in the activity concentrations of radiocesium between the muscle, but not internal organs, and fork length (i.e., body size) of ayu (Table 1). The activity concentrations of ^{137}Cs have also declined through time in both the internal organs and muscle of ayu, and the same trends were exhibited for radiocesium (sum of ^{134}Cs and ^{137}Cs) (Table 2).

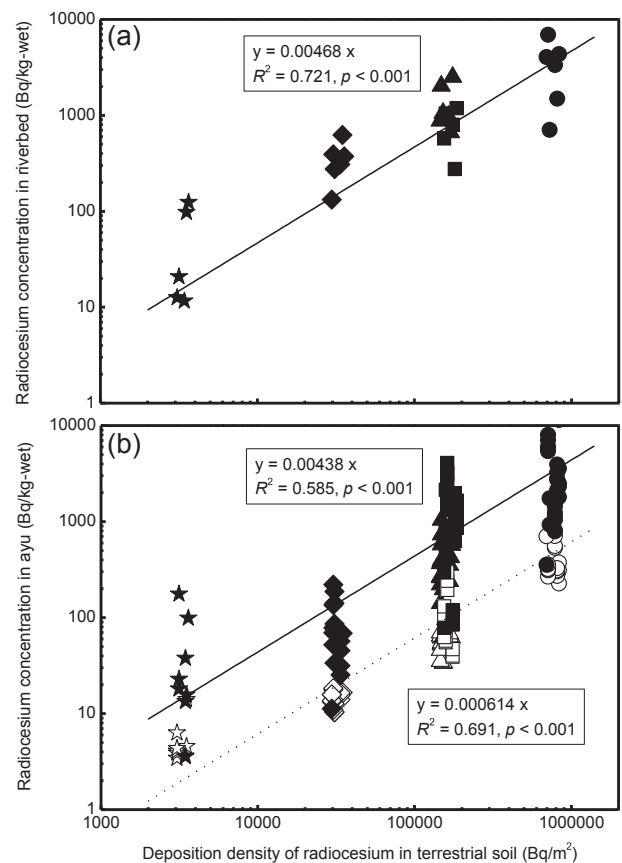


Fig. 3. Relationships between the activity concentrations of radiocesium (sum of ^{134}Cs and ^{137}Cs) in (a) the riverbed samples (i.e., fish dietary items) or (b) the internal organs (i.e., stomach contents, stomach, intestine contents, intestine, liver, spleen, and gonad; solid symbols and solid line) and the muscle (open symbols and dotted line) of ayu collected in five rivers in 2012 and 2013, and the mean deposition density of radiocesium in terrestrial surface soil from each watershed in June and July 2011, based on the results of the soil survey project by the Japanese Ministry of Education, Culture, Sports, Science and Technology. The deposition density of ^{134}Cs and ^{137}Cs for terrestrial soil were decay-corrected to each sampling date of the riverbed, and the internal organs and muscle of ayu. Error bar represents a standard deviation. The symbols correspond to the collection sites in Fig. 1.

Table 1

The best model of a generalized linear mixed model selected by Akaike Information Criteria for the effects of sampling date (elapsed days since 11 March 2011), sampling season, fork length (i.e., body size) of ayu, the activity concentrations of radiocesium (sum of ¹³⁴Cs and ¹³⁷Cs) in riverbed samples (i.e., fish dietary items) collected around the same time as the ayu, and river ID (as a random factor) on the radiocesium activity concentrations in the internal organs and the muscle of ayu.

Variable	Internal organs		Muscle	
	Coefficient	p	Coefficient	p
Sampling date	-1.42	0.013	-0.212	<0.001
Sampling season	-299	0.257	23.6	0.397
Fork length of ayu	10.6	0.056	2.84	<0.001
Radiocesium in riverbed samples	0.723	<0.001		

Table 2

The best model of a generalized linear mixed model selected by Akaike Information Criteria for the effects of sampling date (elapsed days since 11 March 2011), sampling season, fork length (i.e., body size) of ayu, the activity concentrations of ¹³⁷Cs in riverbed samples (i.e., fish dietary items) collected around the same time as with ayu, and river ID (as a random factor) on the activity concentrations of ¹³⁷Cs in the internal organs and the muscle of ayu.

Variable	Internal organs		Muscle	
	Coefficient	p	Coefficient	p
Sampling date	-0.650	0.048	-0.102	0.005
Sampling season	-69.3	0.640	17.1	0.326
Fork length of ayu	3.83	0.179	1.75	<0.001
¹³⁷ Cs in riverbed samples	0.865	<0.001		

4. Discussion

4.1. Exposure of ayu to radiation from the riverbed

The activity concentrations of radiocesium in the riverbed samples (algae and silt), and the internal organs and the muscle of ayu were correlated with the deposition density in the terrestrial soil samples collected from the upgradient watershed immediately after the Fukushima accident. There was a positive correlation between the radiocesium activity concentrations in the muscle and the internal organs of ayu. However, the radiocesium activity concentrations in the muscle were much lower than those in internal organs, mainly because the content of digestive tract was included in the internal organs. In addition, a positive correlation was observed in the activity concentrations of radiocesium between internal organs, but not muscle, and the riverbed samples. These results suggest that herbivorous fish ingested the ¹³⁴Cs and ¹³⁷Cs from the algae and silt on the riverbed stones as they graze, but not all of ¹³⁴Cs and ¹³⁷Cs was assimilated into the muscle. Unfortunately we have no data of the dissolved radiocesium activity concentrations in river water, the dissolved ¹³⁴Cs and ¹³⁷Cs may control the radiocesium activity concentration in muscle. Alternatively, a part of the ¹³⁴Cs and ¹³⁷Cs ingested from the riverbed appears to be transferred to the muscle through the digestive process. The riverbed samples were not separated into the composite algae and silt in this study. Cesium strongly interacts with clay minerals, especially vermiculite and illite (Comans and Hockley, 1992). Furthermore, leaching experiments have demonstrated that ¹³⁷Cs is relatively insoluble in the river suspended sediment in Fukushima, and the adsorption of ¹³⁷Cs to the suspended sediment is irreversible (Tanaka et al., 2013). Therefore, most of the ¹³⁴Cs and ¹³⁷Cs in the silt is also likely to be undigested in ayu, and instead, subsequently excreted. Culture experiments may provide useful insight into the detailed rate of ¹³⁴Cs and ¹³⁷Cs transfer from algae and silt into fish muscle. Aquatic invertebrates, such as Ephemeroptera and Trichoptera larva, may also be influenced by the contaminated soils

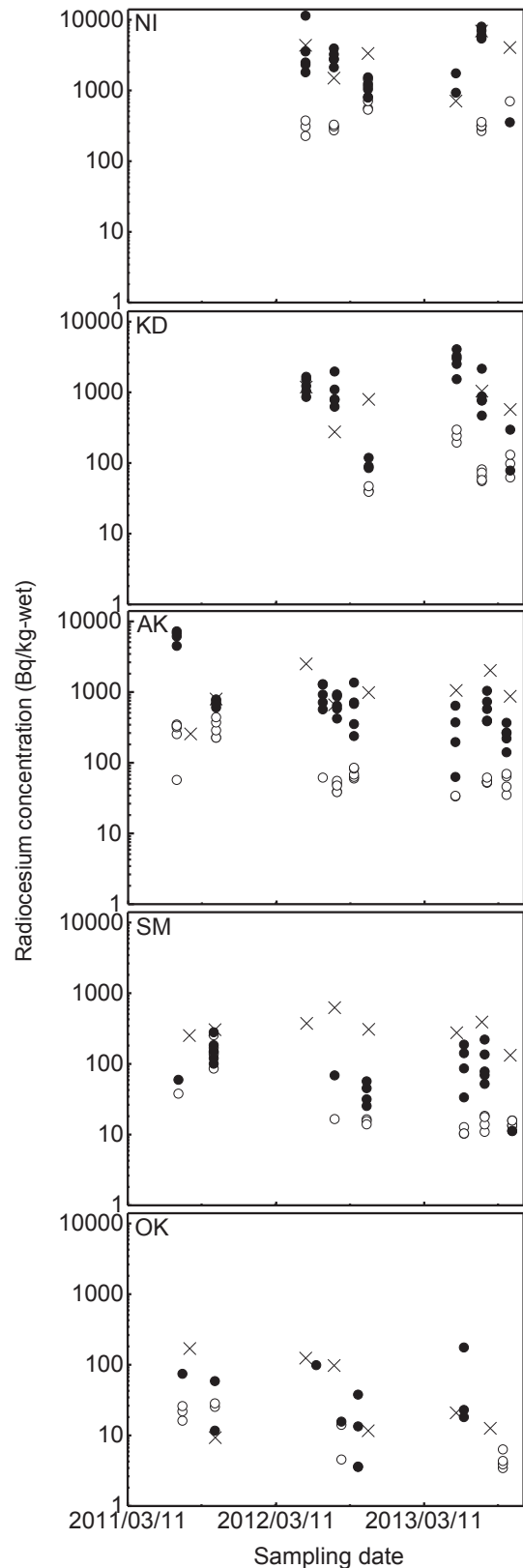


Fig. 4. Time series of the activity concentrations of radiocesium (sum of ¹³⁴Cs and ¹³⁷Cs) in the riverbed samples (i.e., fish dietary items, cross symbols), the internal organs (i.e., stomach contents, stomach, intestine contents, intestine, liver, spleen, and gonad; solid symbols) and the muscle (open symbols) samples from ayu collected in five rivers (NI, Niida River; KD, Kido River; AK, Abukuma River; SM, Same River; OK, Okawa River) between 2011 and 2013.

in the proximity of them. The aquatic invertebrates are key components of foodwebs in mountain streams and riparian forests (Nakano and Murakami, 2001; Sato et al., 2012). In order to determine the pathways of radionuclide contamination in inland ecosystems, the radioactive contamination from the riverbed would need to be taken into account.

4.2. Relationship between biological accumulation and body size

The activity concentration of ^{137}Cs in fish increases with fish size, according to a power function relationship because of changes in feeding patterns (Rowan and Rasmussen, 1994; Smith et al., 2002). For instance, the activity concentration in northern pike *Esox lucius* increased as the trophic level of the prey increased from plankton to invertebrates and then to small fish. Indeed, the level of radiocesium contamination in fish increased in order from herbivores (i.e., ayu) < omnivores < piscivores at Fukushima (Mizuno and Kubo, 2013). A positive correlation was observed in body size relative to the activity concentration of radiocesium in the muscle of ayu, a herbivorous fish that has an annual life history. Thus, in this case the effect of body size could not be explained by a change in feeding patterns, the prey size and the prey items do not change as ayu grows. In addition, the time (season) of collection had no effect on the activity concentrations of radiocesium in ayu muscle. The activity concentration of radiocesium in fish is a function of uptake and elimination rates. In hatchery-reared ayu, assimilation efficiency decreases as they grow (Akutsu et al., 2001). Thus, larger sized ayu need more food per unit weight gain than smaller individuals. At our study site, if a part of the ^{134}Cs and ^{137}Cs ingested from the riverbed are transferred to the muscle through the digestive process, larger sized ayu may have a greater potential to accumulate ^{134}Cs and ^{137}Cs from algae on the riverbed stones than smaller sized ayu. To understand the mechanisms causing the size-effect in ayu, it would be interesting to evaluate the relationship between growth rate of ayu size and the activity concentration of radiocesium in ayu muscle.

4.3. Temporal pattern of radiocesium contamination

Between 2011 and 2013, the activity concentration of radiocesium declined in the internal organs and the muscle of ayu, mainly due to the physical half-life of ^{134}Cs (2.07 y), which is considerably shorter than the 30.1 y for ^{137}Cs . The activity concentrations of ^{137}Cs also declined in the internal organs and the muscle of ayu. Furthermore, the activity concentration of ^{137}Cs tended to decrease in whole body of ayu during 2011 (Iguchi et al., 2013). Therefore, the decrease of the activity concentration of ^{137}Cs in the internal organs and muscle of ayu cannot be explained only by the physical half-life of ^{134}Cs . The activity concentration of ^{137}Cs in the internal organs, which represented the majority of the ^{137}Cs in whole body of ayu, was correlated with that in the riverbed samples. Therefore, the decrease of ^{137}Cs in the riverbed, which may have been caused by transport of the contaminated soil from the mountains, would explain the decrease of ^{137}Cs in the internal organs of ayu. In European lakes, the rate of decrease in muscle ^{137}Cs activity concentrations in fish was initially rapid following the Chernobyl disaster, but later slowed (Jonsson et al., 1999; Smith et al., 2000). Conversely, in Fukushima's rivers, the radiocesium contamination levels peaked in the muscle of ayu immediately after the Fukushima disaster, and then decreased slowly. Long-term monitoring of the radiocesium concentrations in dissolved water, algae, and ayu muscle may enable to understand the contamination route and predict the radiocesium concentrations in the muscle of ayu.

5. Conclusions

The overall radiocesium activity concentrations have declined through time in both the internal organs and the muscle of ayu. However, in some of rivers that were surveyed, the radiocesium activity concentrations in the whole body (i.e., internal organ and muscle) of ayu exceeded the Japanese standard limit for radiocesium in foods (100 Bq/kg-wet), as regulated by the Ministry of Health, Labour, and Welfare, Japan. Thus, fishing activities have been banned in three of the five rivers during the sampling periods in this study (Niida, Kido, and Abukuma Rivers). In addition, Japanese usually eat the whole roast ayu, including muscle and internal organ parts. Therefore, the whole ayu body including in the internal organs and muscle is measured in the inspection by the authorities. Our results suggest that most radiocesium in the internal organs of ayu was derived from the silt, indicating that the bans are likely to stay in place for some time in rivers, such as the Niida, Kido, and Abukuma, which have exceeding 100 kBq/m² in the terrestrial soil. Spatiotemporal monitoring of the levels of radiocesium in freshwater ecosystems, in areas close to human centers, should continue to increase understanding of the long-term dynamics of radionuclide contamination, and the effects of the biological and environmental characteristics of each ecosystem.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jenvrad.2014.11.012>.

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