Radioactive cesium contamination and its biological half-life in larvae of *Stenopsyche marmorata* (Trichoptera: Stenopsychidae)

Takeshi Fujino¹ · Shota Kobori¹ · Takeshi Nomoto² · Masaru Sakai³ · Takashi Gomi⁴

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Abstract

The total radioactive Cs concentration in final-instar *Stenopsyche marmorata* larvae from the Mano River, Fukushima Prefecture, in 2013 was found to be $>1000 \text{ Bq kg}^{-1}$ in dry weight. The concentration of radioactive Cs in the river water itself was on the order of 0.01 Bq kg^{-1} , while its concentration in drifting fine particulate organic matter (FPOM) was on the order of $10,000 \text{ Bq kg}^{-1}$ in dry weight. When excretion experiments were performed in the summer and winter, significant decreases in the Cs concentrations in the whole body of *S. marmorata* were observed in both seasons. The biological half-life of Cs-137 was 5.3 and 8.8 h for the nonwintering generation and the wintering generation, respectively. Another elimination test using a stable Cs solution was performed, and the Cs concentration was observed to decrease rapidly to 7.5% of its original level in the digestive tract of *S. marmorata* after 72 h, but stable Cs remained in muscle and extra-alimentary tissue of *S. marmorata*. As the total radioactive Cs in the water was quite low, its concentration in *S. marmorata* from the Mano River reflects the fact that it feeds on FPOM. The radioactive Cs concentration in the FPOM significantly declined in 2015, but a corresponding decline was not observed for *S. marmorata*. We therefore infer that ultrafine particulate matter such as suspended vermiculite clay is strongly contaminated with radioactive Cs, and that this radioactive Cs passes continuously through the digestive tract of *S. marmorata*.

Keywords Biological half-life · Cs contamination · FPOM · *S. marmorata*

Introduction

Stenopsyche spp. (Trichoptera: Stenopsychidae) are widely distributed in gravel bed rivers in Asian countries (Dudgeon 1999; Tanida 2002; Nozaki et al. 2016). Due to their high individual biomass and high productivity compared with other trichopteran species, they have been used in the

biomonitoring of various types of contamination, such as heavy metals released from industrial waste and microcystins from toxic algae (Tochimoto et al. 2000, 2003; Katagami et al. 2004; Aizawa et al. 2009; Matsuzaki 2011; Rainbow et al. 2012). Because the life cycle and variation in individual biomass of *S. marmorata* are well studied compared with those of other trichopteran species, it is common to investigate its fifth-instar larvae, which is the final and longest phase before the pupal stage (Gose 1970; Nishimura 1984; Aoya and Yokoyama 1987).

Since the Fukushima Dai-ichi Nuclear Power Plant (FDNPP) accident in March 2011, significant levels of radioactive cesium, Cs-134 and Cs-137, have been detected in (for example) freshwater fish, insects, leaves, sediment, and detritus in many Japanese rivers and streams. The Ministry of the Environment of Japan has published annual reports on radioactive-substance monitoring surveys for aquatic organisms. Among the various aquatic insects in river and lake waters investigated in this context, radioactive Cs was reported to be at its highest level in *S. marmorata* (Ministry

✉ Takeshi Fujino
fujino@mail.saitama-u.ac.jp

¹ Department of Environmental Science and Technology, Saitama University, Saitama 338-8570, Japan

² Civil Engineering and Eco-Technology Consultants Co., Ltd., Saitama 338-0851, Japan

³ Faculty of Science and Engineering, Chuo University, Tokyo 112-8551, Japan

⁴ Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan

of the Environment, Japan 2015). Although the level of radioactive Cs in river water has decreased to 0.01 Bq L^{-1} in most streams and rivers since 2012, a high level of radioactive Cs is still being detected in the larvae of *Stenopsyche* spp., which are sedentary and net-spinning filter feeders. Drifting fine particulate organic matter (FPOM), which has a particle size of less than 1.0 mm and comprises suspended solids such as periphyton and phytoplankton, is captured in the nets of these larvae, causing them to take in organic matter contaminated with radioactive Cs (Yoshimura and Akima 2014). In the study reported in the present paper, we evaluated the biological half-life of radioactive Cs in an excretion experiment and verified the residual ratio in the digestive tract, muscle, and extra-alimentary tissue of *S. marmorata* larvae.

Materials and methods

Study site and sample treatment

Between April 2013 and November 2015, larvae of *S. marmorata* and drifting FPOM were collected 1 km upstream (Mano-U) of the inlet and 5 km downstream (Mano-D) of the Hayama reservoir dam ($37^{\circ}43.06'\text{N}$, $140^{\circ}49.58'\text{E}$) on the Mano River, Fukushima Prefecture, Japan (Fig. 1). The annual precipitation at the dam site was 1248 mm in 2013, 1957 mm in 2014, and 1899 mm in 2015. The averages of the annual maximum and minimum temperatures of the river water for the last ten years were 22.7 ± 2.5 and 1.9 ± 1.1 °C, respectively. The head of the Mano River basin is in an area of dense conifer and deciduous forest. At each sampling site, the river valley is steep, leading to considerable inflow of leaf litter. The gravel bed river channel width is about 5–10 m, and emerged plants grow along the bank of the river. A bivoltine lifecycle with nonwintering and wintering generations was noted for *S. marmorata*.

Data were collected on April 23, May 21, July 2, August 30, and November 21 in 2013, January 9 in 2014, and May 2, July 29, and November 8 in 2015. During each collection, a lump of *S. marmorata* larvae was preserved in 95% ethanol and transported back to the laboratory in Saitama University. The head capsule lengths of the larvae were then measured and classified into final-instar groups under a dissecting microscope. After drying whole-body samples including the gut contents at 50 °C for 24 h, their individual dry weights were measured to the nearest 0.1 mg and confirmed using an electric balance (GR-120, A&D, Tokyo, Japan). Each dried lump of larvae was ground and homogenized.

Drifting FPOM was collected using a net sampler 30 cm in diameter and with a mesh size of 100 μm for 5 min to obtain five replicates (Ock and Takemon 2010). FPOM samples were classified into four size groups (64–125, 125–250,

Fig. 1 Map of the Mano River basin and sampling sites (Mano-U and Mano-D) in the present study, and the modified radioactive cesium (Cs-137) surface deposition distribution, based on data from the Fourth Airborne Monitoring Survey performed from October 22 to November 5, 2011 (MEXT 2011). FDNPP Fukushima Daiichi Nuclear Power Plant. **a** Nonwintering generation, **b** wintering generation

250–500, and 500–1000 μm) by wet sieve analysis and dried at 110 °C in an oven. The carbon content of the FPOM was analyzed using a CHN coder (MT-6, Yanaco, Kyoto, Japan).

Intensive larvae collection for excretion experiments

One lump of the final-instar larvae of the nonwintering generation (i.e., N_0) was collected and immediately preserved in 95% ethanol on August 26, 2013 at Mano-D at a temperature of 20 °C. Another 200 larvae were collected at the site and kept alive in a tank with river water including trace amounts of suspended solids, and were then transported to the laboratory. The larvae were moved to four cylindrical containers that were 30 cm in diameter and filled with river water, which was used as rearing water. Each cylindrical container was placed on a magnetic stirrer to create a circular flow. The water temperature was maintained at the temperature of the river using a cool-water circulating device (CA-1116A, EYELA, Tokyo, Japan), and oxygen was supplied by an aerator (SA-1200S, KOTOBUKI, Tokyo, Japan). Collected larvae were divided into five lumps (i.e., N_1 – N_5), and these lumps of larvae were preserved in 95% ethanol after 3, 6, 12, 24, and 48 h, respectively, in order to evaluate the decreasing trend in radioactive Cs contamination during fasting. The same handling procedure as described above was performed for 9 lumps of larvae for the wintering generation (i.e.,

W_0 , W_1 – W_8) on December 5, 2013, when the temperature was 7 °C. These lumps were fixed in cylindrical containers containing 95% ethanol after 3, 6, 12, 24, 48, 72, 96, and 120 h, respectively. Each lump contained between 18 and 21 individuals. The radioactive Cs concentrations in the lumps were analyzed and regression curves were then plotted and fitted by least-squares methods. The biological half-life of radioactive Cs in each season was determined using the SPSS ver.20 software package (IBM, Tokyo, Japan).

Radioactivity measurement

The radioactive Cs-134 and Cs-137 concentrations in *S. marmorata* larvae and drifting FPOM samples were measured using a germanium coaxial gamma-ray detector (EGC25-195-R, Canberra-Eurisys, Meriden, CT, USA) at Tokyo University of Agriculture and Technology. The energy and efficiency of this detector were calibrated using standard and blank (background) samples. For the analysis of radionuclide activity, each sample was measured until a 10% error count per net area count was reached (Sakai et al. 2015).

Verification of the stable Cs residual ratio in the digestive tract and other parts of *S. marmorata*

Other final-instar larvae of *S. marmorata*, drifting FPOM, and algae attached to the surfaces of stones were obtained from Arakawa River, Yorii Town, in Saitama Prefecture (36° 6.56'N, 139° 13.11'E), where stable Cs contamination was observed. These samples were taken to the laboratory, where they were placed in a cylindrical container 30 cm in diameter, and a 10 mg L⁻¹ stable Cs solution (Wako Chemicals Co. Ltd., Osaka, Japan) was added to the river water. The same system without the stable Cs was used as a blank. The FPOM was separated into 125-μm and 64-μm sizes, used in the test, and then kept for 72 h at 20 °C. Three larvae were removed and the digestive tract, muscle, and extra-alimentary tissue were carefully trimmed from each using small scissors. Then the scissors were inserted along the side of the body from the first abdominal section (near the posterior limb). Muscle and extra-alimentary tissue were discriminated by color and firmness. The other three individuals were moved to systems without contaminants and left to stand for a further 72 h. The same treatment was conducted, and the quantity of stable Cs remaining in each body part of the individuals and the FPOM was measured by ICP-MS (iCAP Qc, Thermo Fisher Scientific Inc., Waltham, MA, USA).

Results and discussion

Elimination of radioactive Cs in *S. marmorata* larvae

The radioactive Cs-134 and Cs-137 concentrations after each fasting time for each lump of *S. marmorata* larvae (both the nonwintering and wintering generations) are shown in Table 1. The gut contents were included in the assessments for both generations. Cs-134 was not detected after 12 h of fasting in the tests. Cs-137 was detected up to 48 h of fasting in the nonwintering generation, at which point 20% of the initial Cs-137 remained in the larvae. In the wintering generation, Cs-137 was detected up to 96 h of fasting, at which point 15% of the initial Cs-137 remained.

The two-compartment model used in a nickel concentration transfer investigation of *S. marmorata* by Tochimoto et al. (2003) was applied to highlight the attenuation trends in the Cs-137 values for each generation (Fig. 2). This modeling of the time course of toxicant disposition in the whole organism is to present the body as a system of plural compartments (Shen 2013). The larvae eliminated radioactive Cs rapidly during the first 6 h for the nonwintering generation and rapidly during the first 9 h for the wintering generation. The excretion of gut contents took longer in winter than in summer because of the lower temperature (Mizuno and Tanaka 1969; Tsuda 1975). The slow decrease in Cs-137 concentration after 12–24 h appears to be due to the excretion of gut contents, because the radioactive Cs was obtained from FPOM. The total for the two compartments is close to the gut-emptying time for each generation of *Stenopsyche* spp. larvae, which is over 24 h according to an analysis by Tanida (2002) using data from Gose (1959) and Nishimura (1978). These results imply that most of the Cs-137 is not assimilated instantly by *S. marmorata* larvae because it is bound not only to the organic matter but also to fine-grained minerals (Motokawa et al. 2014; Tanaka et al. 2014). The calculated biological half-life of Cs-137 was 5.3 h in the nonwintering generation. In contrast, it was 8.8 h in the wintering generation. These half-life values are smaller than the first biological half-life of nickel from food, which is 1.4 days at 14.6 °C, as reported by Tochimoto et al. (2003). The second biological half-life is 12.1 days, indicating that nickel remains in the body, and reflects the long time required to achieve a state of equilibrium. Our results from short interval tests show a clear trend for gut contents to be excreted over the course of a few days; however, a physiological interpretation of the difference between the two compartments is not readily apparent because they do not have exact correspondence to anatomical structure or physiological processes (Shen 2013).

Table 1 Radioactive Cs concentration after each fasting time for each lump of *Stenopsyche marmorata* larvae (including gut contents)

Lump	TDW ^a (gDW)	Fasting time (h)	Cs-134 (Bq kg ⁻¹)	Cs-137 (Bq kg ⁻¹)	Cs-134 + Cs-137 (Bq kg ⁻¹)
Nonwintering generation					
N_0	2.03	0	491	1170	1661
N_1	1.57	3	443	1030	1473
N_2	1.62	6	128	375	503
N_3	1.05	12	118	236	354
N_4	1.11	24	N.D.	210	210
N_5	1.04	48	N.D.	225	225
Wintering generation					
W_0	1.03	0	521	1172	1693
W_1	1.63	3	419	1012	1432
W_2	1.41	6	297	668	965
W_3	1.25	12	237	475	712
W_4	1.09	24	N.D.	264	264
W_5	1.53	48	N.D.	159	159
W_6	1.58	72	32	56	88
W_7	1.37	96	N.D.	48	48
W_8	1.84	120	N.D.	N.D.	N.D.

^a TDW is the total dry weight of the lump **Fig. 2** Results from the excretion experiments for radioactive Cs in each lump of *Stenopsyche marmorata* larvae (including gut contents)

Residual stable Cs in the digestive tract and in extra-alimentary tissue

The average stable Cs concentrations in the digestive tract,

muscle, and extra-alimentary tissue of the *S. marmorata* larval body are shown in Fig. 3. The wet weights of the digestive tract, muscle, and extra-alimentary tissues per individual before excretion (i.e., immediately after the samples

Fig. 3 The stable Cs concentrations in digestive tract, muscle, and extra-alimentary tissue of the fifth-instar larvae of *Stenopsysche marmorata*. Error bars refer to the S.E. ($n = 3$); *before excretion*: 72 h of immersion in a solution containing stable Cs; *after excretion*: 72 h of immersion in water without stable Cs after the immersion in a solution containing stable Cs

had been immersed in a solution containing stable Cs for 72 h) were 49.0 ± 20.9 , 2.1 ± 0.9 , and 237.2 ± 71.0 mg, respectively. Those per individual after excretion (i.e., after the samples had been moved to the system without stable Cs and immersed for another 72 h) were 12.1 ± 3.2 , 3.4 ± 1.2 , and 188.8 ± 53.9 mg, respectively.

The stable Cs concentration in the digestive tract was significantly higher ($105.4 \mu\text{g g}^{-1}$) than those in the other parts of the larvae. The stable Cs concentrations in the 125- μm and 64- μm suspended FPOM were 22.6 and $52.4 \mu\text{g g}^{-1}$, respectively. The stable Cs concentrations after excretion

were decreased to just 7.5% of the original in the digestive tract, 29.3% of the original in muscle, and 60.1% of the original in the extra-alimentary tissue. In this experiment, two uptake processes of stable Cs—feeding on FPOM and infiltration of water—were considered. The observed rapid elimination of stable Cs from the digestive tract supports the results of on-site experiments. The ratio of muscle to total weight for *S. marmorata* larvae is very low, and most of the remaining stable Cs was found to occur in the extra-alimentary tissue. It appears that these differences arise from the osmosis of dissolved stable Cs in the tissues of the larval body.

Relationship of the radioactive Cs concentration in *S. marmorata* larvae to that in FPOM

From 2013 to 2015, the annual peak value of the radioactive Cs concentration in drifting particulate organic matter of size 125–250 μm (FPOM) was recorded during the rainy season (i.e., July–September). There was no clear decrease in the radioactive Cs concentration in *S. marmorata* larvae (STENO) during the 2 years of observations (Table 2). The ratio STENO/FPOM was generally less than 0.1 in 2013; however, it increased to 0.39 on May 2, 2015 for Mano-U and to 0.89 on November 8, 2015 for Mano-D. The carbon content of FPOM decreased from greater than 15% in 2013 to less than 5% in 2015 at both sites. Upon performing excretion experiments in 2013, we found that most of the contaminants and food consumed passed through the digestive tract of the larvae without being assimilated into

Table 2 The radioactive Cs concentration in drifting fine particulate organic matter 125–250 μm in size (FPOM) and in lumps of *Stenopsysche marmorata* larvae (STENO), the radioactive Cs concentration ratio STENO/FPOM, and the carbon content of FPOM at various dates

Date	① Cs-134 + Cs-137 in FPOM (Bq kg^{-1})	② Cs-134 + Cs-137 in STENO (Bq kg^{-1})	③ = ①/② (i.e., STENO/FPOM)	Carbon content of FPOM (%)
Mano-U site				
April 23, 2013	22,160	3630	0.16	5.3
May 21, 2013	28,320	1467	0.05	17.5
July 2, 2013	29,500	1870	0.06	20.5
August 30, 2013	59,700	5450	0.09	16.6
November 21, 2013	39,500	2205	0.06	13.7
May 2, 2015	6,705	2611	0.39	3.6
July 29, 2015	10,450	2525	0.24	3.4
Mano-D site				
April 23, 2013	28,580	1267	0.04	14.7
May 21, 2013	20,620	1356	0.07	19.3
August 30, 2013	75,200	5580	0.07	23.3
November 21, 2013	34,240	1991	0.06	7.7
January 9, 2014	12,330	1068	0.09	9.2
May 2, 2015	6337	650	0.10	15.0
July 29, 2015	8099	1235	0.15	13.3
November 8, 2015	3270	2898	0.89	6.8

The number of individuals in each lump was between 20 and 30

their bodies. The ratio of total body weight to digestive tract weight was 10:1 on a dry weight basis. The radioactive Cs concentration found in the larvae of *S. marmorata* reflected the Cs concentration in FPOM. The uptake of radioactive Cs in 2015 is thought to derive from ultrafine particulate matter such as suspended vermiculite clay (Motokawa et al. 2014).

Tanaka et al. (2014) reported that particles smaller than 125 µm had higher radioactive Cs concentrations, reflecting their enhanced specific surface areas, but the contribution of the clay-size fraction (which had the highest Cs-137 concentration) was small because clay-size particles comprise only a very small fraction of the total volume of particles. The Forestry and Forest Products Research Institute (2014) reported that the proportion of radioactive Cs distributed in the soil surface layer (< 5.0 cm) has increased year on year, reaching 77–86% of the total radioactive Cs in 2014. Sakai et al. (2016) reported that, the concentration in Cs-137 in stream litter is significantly lower than in forest litter resulting of Cs-137 reaching from litter in stream water in the early stage of the accident. The high Cs-137 concentration in *S. marmorata* larvae in 2015 may have been due to particularly high contamination of the larvae with ultrafine (less than a few µm in diameter) particulate matter. A large amount of radioactive Cs deposition occurred; this correlates with the particulate Cs-137 concentrations in streams and rivers in Fukushima Prefecture (Tsuji et al. 2014). Further continuous monitoring and analyses of both *S. marmorata* and suspended solids should be carried out. In the field survey, we noted that the Hayama Reservoir tends to trap FPOM in the Mano River catchment. Much transported sediment has been trapped in the reservoir. However, radioactive Cs was deposited across the whole river catchment by the FDNPP accident, and the smaller size fraction of particulate matter still contains radioactive Cs at significant levels and worked as its carrier in the catchment.

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