Isotopic Diet Analysis of the Japanese Water Shrew Chimarrogale platycephala to Estimate Their Feeding Habits and the Usefulness of Body Hair Samples

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Isotopic diet analysis of the Japanese water shrew *Chimarrogale platycephala* to estimate their feeding habits and the usefulness of body hair samples

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Abstract.   The water shrew *Chimarrogale platycephala* is an endangered species in Japan. Although immediate conservation actions are necessary, detailed information on this species is inadequate. We compared dietary trends obtained via *C. platycephala* digestive contents analysis with those through stable isotope analysis (δ^{13}C, δ^{15}N, ‰) of non-invasive body hair and invasive muscles to elucidate their diet and evaluate usefulness of the dietary stable isotope analysis. We captured 20 shrews from three streams in Aomori Prefecture from 2013 to 2016 barring snow accumulation seasons. The digestive contents analysis showed that water shrews mainly fed on aquatic insects, whereas freshwater crabs, fishes, and terrestrial insects were also observed as diets. δ^{13}C values from the stable isotope analysis were not significantly different between muscles and body hair and indicated a primary diet of aquatic invertebrates and fishes. δ^{15}N values were significantly lower in muscles and indicated a similar trophic position of water shrews to fishes. In the isotope mixing model, the contribution of terrestrial invertebrates was less than 0.35 except for two individuals that showed the highest terrestrial invertebrate ratio (> 0.5) and the lowest aquatic invertebrate ratio. This study also demonstrated that body hair from any part of the back was sufficiently useful for dietary stable isotope analysis.

Key words: digestive content, endangered species, isotope mixing model, non-damaged sample, stable isotope analysis.

The Japanese water shrew *Chimarrogale platycephala* (Soricidae, Soricomorpha) is a small mammalian species endemic to Japan that has adapted to stream ecosystems. This species is mainly nocturnal (Hidaka 1996) and forages underwater while diving, using woody debris and rocks as shelter and breeding grounds. These behaviors render the water shrew difficult to observe, and consequently, little of its ecological information, including its diet, has been obtained. The water shrew has already been declared extinct in Shikoku Island (Abe 2003) and has been categorized as extinct, critically endangered, endangered, vulnerable, and nearly threatened species in one,

three, 14, 13, and five prefectures in Japan, respectively (Wildlife Research and EnVision, The Search System of Japanese Red Data, http://jpnrdb.com/, Accessed 13 June 2022), thus making its immediate conservation crucial. Therefore, it is essential to acquire its fundamental ecological information, especially on its diet and foraging behaviors (Yokohata et al. 2008), for effective conservation and management.

The diet of the Japanese water shrew consists of mainly aquatic insects and fishes but also includes terrestrial resources, including amphibious crabs, amphibians, and terrestrial invertebrates (Abe 2011). Its aquatic and terres-
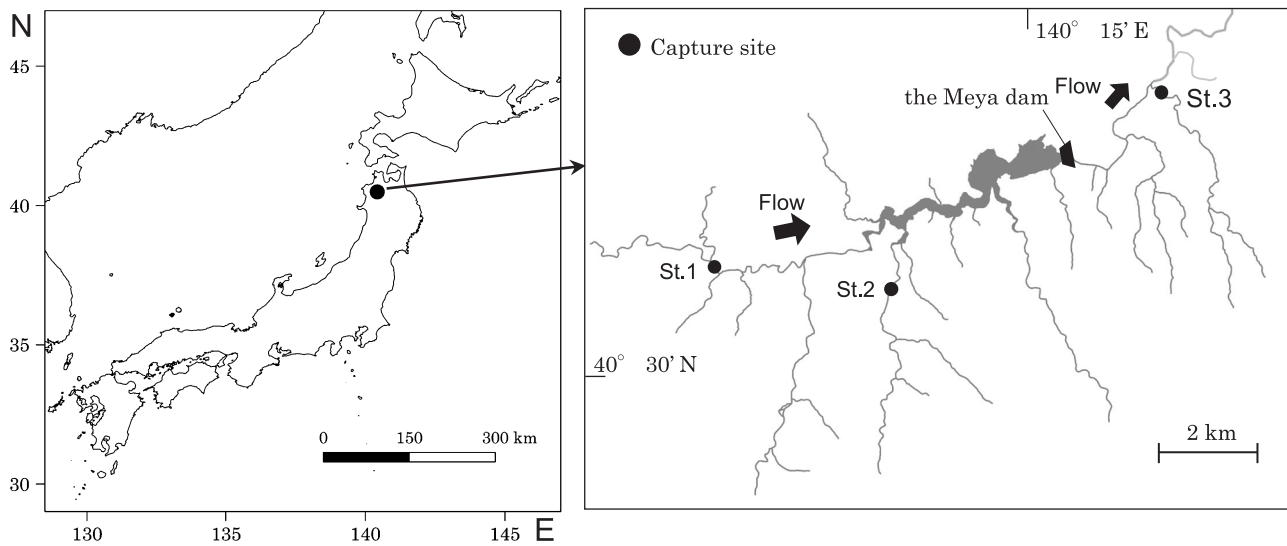

Fig. 1. A map of study sites in Aomori prefecture, Japan. The map was prepared with data from the National Land Information (Ministry of Land, Infrastructure, Transport and Tourism) (https://nlftp.mlit.go.jp/ksj/jpgis/jpgis_datalist.html, Accessed 12 May 2022).

trial predation plays an important role in linking aquatic-terrestrial food webs and material cycling (Murakami and Nakano 2001). On the other hand, the diet of the water shrew has been difficult to clarify because the conventional gastrointestinal analysis requires invasive handling of an endangered species and even if the gastro-intestinal analysis was possible, prey morphology would have been lost due to chewing and crushing. Therefore, developing a method from a different perspective for non-invasive dietary analysis is an urgent task to clarify the diet of the water shrews.

Recently, body hair for the stable isotope ratio (SIR) analysis has been demonstrated as an effective non-invasive technique for mammals (Roswag et al. 2018; Pulkkinen et al. 2020; Rogers et al. 2020). The SIR analysis can clarify the varying dependencies of the Japanese water shrew on aquatic or terrestrial resources, which is essential for conservation management efforts. However, it is still unclear whether the body hair of our target species could be used for the isotopic diet analysis.

To address these concerns described above, first, this study determined the feeding habits of water shrews by the gastrointestinal tract content analysis and by the SIR analysis using shrew muscle. Second, we determined the usefulness of body hairs for the SIR analysis, as a non-invasive method, in comparison with the muscle SIR. We used the carbon and nitrogen SIRs ($\delta^{13}C$ and $\delta^{15}N$) to infer the origins of prey based on the isotopic signatures of prey and producers obtained from the field. The heavier stable isotopes of an element were more concentrated at

the upper trophic level. $\delta^{13}C$ was used to infer resources with an isotopic enrichment rate (i.e., the rate of heavier isotope enrichment between trophic levels) of 0–0.1‰, representing mean isotopic differences between the consumer and resources.

Materials and methods

Study sites

This study was conducted along the Anmon stream (St.1; 40°31'18"N, 140°10'36"E), Ohsawa stream (St.2; 40°30'50"N, 140°13'13"E), and Hirasawa stream (St.3; 40°32'59"N, 140°16'58"E) of the Iwaki River system in Aomori Prefecture, northern Japan (Fig. 1). These streams originate from the Shirakami Mountains. St.1 and St.2 flow into the Miyama reservoir of the Meya dam, and St.3 flows directly into the Iwaki River. Fishes (e.g., char, dace, and sculpin), crustaceans (freshwater crabs), and aquatic insects also inhabit these waterways. The study sites have characteristics of typical mountain streams—the stream bottoms are covered mainly with boulders and rocks submerged in gravel, and the stream sides are intermittently covered by riparian forest. There are few rocks protruding above the water surface, therefore providing limited space in which the water shrews could hide.

Collection of shrews, preys, and producers

We sampled Japanese water shrews, shrew prey (fishes, aquatic macroinvertebrates, and terrestrial invertebrates),

Table 1. Body measurements of water shrews

Site	No.	Sex	LTv (mm)	HB (mm)	TL (mm)	BW (g)	Captured date
St.1	1-1	♂	110.0	123.0	233.0	50.1	2014/7/29
	1-2	♂	113.5	122.5	236.0	51.6	2014/8/31
	1-3	♂	117.0	108.0	225.0	39.3	2014/9/15
	1-4	♀	106.0	124.0	230.0	52.9	2015/6/19
	1-5	♂	107.0	125.5	232.5	49.0	2015/6/19
	1-6	♂	115.5	126.0	241.5	53.3	2016/7/5
	1-7	♂	122.0	123.0	245.0	52.1	2016/8/12
	1-8	♀	112.0	118.0	230.0	37.0	2016/8/12
St.2	2-1	♀	111.0	120.0	231.0	46.8	2013/10/3
	2-2	♂	122.5	125.0	247.5	46.6	2013/10/23
	2-3	♂	116.0	136.0	252.0	58.4	2014/6/25
	2-4	♀	101.5	120.0	221.5	41.3	2014/6/25
	2-5	♂	115.0	115.0	230.0	46.0	2014/7/29
	2-6	♂	114.0	128.0	242.0	53.9	2015/9/23
	2-7	♀	106.5	113.5	220.0	39.2	2015/9/29
St.3	3-1	♂	111.0	117.0	228.0	57.5	2014/7/1
	3-2	♂	110.0	131.0	241.0	51.0	2014/9/25
	3-3	♂	107.0	112.0	219.0	49.4	2015/9/29
	3-4	♂	105.0	138.0	243.0	55.0	2016/7/5
	3-5	♂	110.0	119.0	229.0	53.6	2016/8/12

LTv: length of tail vertebrae, HB: head and body length, TL: total length, and BW: body weight.

and primary producers (periphytic algae and terrestrial plants) for digestive contents and carbon and nitrogen stable isotope analyses.

We captured water shrews at Sts.1, 2, and 3 once or twice a month from October 2013 to October 2016, except winter (November to May) due to snow accumulation (Table 1). Water shrews were captured using reticular cylindrical Spring-Mondori traps (φ300 mm in diameter and 650 mm in length) with fish bait (mainly capelin, *Mallotus villosus*), and some cobbles as weight. Ten traps were set overnight underwater at each site during the investigation. The distance between traps was approximately 10–30 m. Throughout the investigation period, the total number of traps was 110, 150, and 140 in Sts.1, 2, and 3, respectively. All trappings were performed with permission from the Aomori Prefectural Government (mandate numbers: 4049, 4060, 4047, and 4069, in 2013, 2014, 2015, and 2016, respectively). We also followed the guidelines of Hirosaki University regarding animal ethical treatment. Although we made an effort to keep shrews alive via frequent observations during capture, all individuals unfortunately died. The body weight and length (total, head and body, and tail lengths) of the captured shrews were measured, and then the specimens

were brought back to the laboratory in a cooler box. The samples were stored in a freezer at –30°C until analysis.

For the prey, we captured fishes using a D-frame net (4 mm mesh, 32 cm in length, and 36 cm in height) and an electro shocker with the voltage set to 400 V. The sampling was conducted in May, July, and October 2014, and June and August 2015. The captured fishes were returned to the laboratory in a cooler box. The samples were stored in a freezer at –30°C until analysis.

Aquatic macroinvertebrates were collected from Sts. 1–3 in May, July, and October 2014, and in June and August 2015. During collection, we set four quadrats (20 cm × 20 cm) on the streambed at the center of a riffle. We disturbed the streambed in the quadrats and collected macroinvertebrates in each quadrat using a D-frame net (φ4 mm mesh, 32 cm in length, and 36 cm in height). Four samples were subsequently pooled into a zip bag, brought back to the laboratory in a cooler box, and kept in a freezer at –30°C until analysis.

To collect terrestrial invertebrates, we conducted pitfall trapping and hand capture at the streambanks of St.1 and 2 in August 2015 and July 2016. However, we could not collect terrestrial invertebrates at St.3, where the surface of the streambank was occupied by immobile

boulders and rocks, which were unsuitable for setting the pitfall traps and performing hand captures. For pitfall trapping, ten plastic cups (7 cm diameter × 9 cm height) with 70% EtOH were deployed overnight in holes at the streambank. For hand capture, we randomly collected invertebrates creeping on the ground at the streambank. The terrestrial invertebrate samples that fell into the cups were collected in one jar and brought back to the laboratory, and kept in a freezer at –30°C until analysis.

Primary producer samples were also collected from Sts. 1–3 in October 2016. For autochthonous producers, periphyton in a quadrat with 25 cm^2 of the upper surface of the cobble at the center of a riffle was scrubbed off with a brush, rinsed with distilled water, and placed into a bottle as a sample. For allochthonous producers, drifted fallen leaf litter near the streambanks (approximately every 20 leaves) was collected into a zip bag. The samples were then brought back to the laboratory in a cooler box.

Laboratory analyses

Individual water shrews were thawed in a refrigerator at 4°C prior to analysis. Before dissection, body hair was collected from the individual's back. The back of the head to the buttocks was divided equally into four sections each approximately 3 cm apart (I, II, III, and IV), and the hairs at the center of each section were cut as our samples. The hairs were observed to confirm the state of molting and were washed three times with 70% EtOH. The samples were placed in zip bags and stored at room temperature until the stable isotope analysis was performed.

We dissected the individuals, and their sex was identified by observing the reproductive organ. The stomachs, intestines, and muscles of the hind limbs were then obtained. The stomach and intestines were used for the digestive contents analysis. The digested samples were moved into a petri dish, and the undigested residue (e.g., exoskeleton of invertebrates and bone and scale of fish) was examined with a 40× microscope while referencing sources for taxon identification (Kawamura and Ueno 1973; Kurosawa et al. 1985; Kawai and Tanida 2005). The muscle specimens were immediately dried at 90°C for 24 h in an oven for the stable isotope analysis (presented in detail below).

For the prey, we identified the fish to the species level according to Nakabou (2013) and classified it as pelagic or benthic fish. The invertebrates were identified mainly to the order level and classified as aquatic and terrestrial ones under a 40× microscope as described above. For the

primary producers, we labeled leaf litter to the lowest taxon possible according to The Plant Data around Mountains of Shirakami (The Shirakami Research Center for Environmental Sciences, http://www.shirakami-database.jp/, Accessed 31 January 2017). A periphyton sample of the well-mixed contents from each bottle was filtered through a glass fiber filter (GF/C; GE Healthcare). The filter was then dried in an oven at 60°C for 24 h and used for the stable isotope analysis.

Stable isotope analysis and mixing model

We measured the $\delta^{13}C$ and $\delta^{15}N$ of the shrews, their prey (fishes, and aquatic and terrestrial macroinvertebrates), and the producers (periphyton and terrestrial plants). To obtain the isotopic values of the water shrews, their dried muscle samples were crushed into powder using a homogenizer (MicroSmash MS-100; TOMY SEIKO CO.) and then enfolded at 0.2–0.3 mg within tin-foils for the stable isotope analysis. The hairs were dried at 60°C for 24 h in convection ovens, and a few randomly selected hairs were enfolded at 0.2–0.3 mg within tin-foils. For the prey, the aquatic and terrestrial invertebrates and drifted leaf litter were dried at 60°C for 24 h in an oven, crushed using a homogenizer, and enfolded at 0.3–0.5 mg within tin-foils. We removed the muscles near the dorsal fin of the fish and treated them as described above for the stable isotope analysis. Periphyton was removed from the filter and treated as described above. Supplementary Information presents all the samples for Sts.1, 2, and 3.

We determined the $\delta^{13}C$ and $\delta^{15}N$ values of each sample using a conventional method of elemental analyzer/isotope ratio mass spectrometry (EA-IRMS, Finnigan Delta V Advantage interfaced with Flash 2000 Elemental Analyzer, Thermo Electron Corporation) in the Forest Research and Management Organization of Japan. Stable isotope abundances were expressed in δ notation as the deviation from standards in parts per thousand (‰), according to the following equation:

$$\delta^{13}C \text{ or } \delta^{15}N \text{ (‰)} = (R_{sample}/R_{standard} - 1) \times 1000,$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$, R_{sample} is the isotope ratio of the sample, and $R_{standard}$ is the isotope ratio of the Vienna Pee Dee Belemnite international standard for carbon (${}^{13}C$) and air N_2 (${}^{15}N$). We measured L-Histidine ($\delta^{13}C$ = –11.4‰, $\delta^{15}N$ = –7.6‰), Glycine (–33.8‰, 1.3‰), L-Alanine (–19.6‰, 1.54‰), L-Alanine (–19.6‰, 5.0‰), and L-Alanine (–19.6‰, 10.1‰) as working

Table 2. Digestive contents of water shrews by the digestive contents analysis

Site	Individual No.	Nymph of Ephemeroptera spp.	Larva of Plecoptera spp.	Larva of Trichoptera spp.	Japanese Freshwater Crab	Stream pelagic fish	Bentic fish	Unidentified fish	Other
St.1	1-1	○	○	–	–	–	–	–	
	1-2	○	○	○	–	–	–	–	
	1-3	–	–	○	○	–	–	–	Terrestrial beetle
	1-4	○	○	–	–	–	–	○	
	1-5	–	○	–	–	○	–	–	
	1-6	○	○	○	–	–	–	–	Terrestrial beetle
	1-7	–	○	○	–	–	–	–	
	1-8	–	–	○	–	–	–	–	
St.2	2-1	–	○	○	–	–	○	–	Adult of Neuroptera sp., Larva of Lepidoptera sp.
	2-2	○	–	○	–	–	–	–	
	2-3	○	○	–	○	–	–	–	Terrestrial beetle
	2-4	–	○	○	–	○	–	–	
	2-5	–	–	–	–	–	–	○	
	2-6	○	–	–	–	–	–	○	
	2-7	○	○	○	–	○	–	–	
St.3	3-1	○	○	○	–	○	–	–	
	3-2	–	○	○	–	–	–	○	Larva of Lepidoptera sp.
	3-3	–	–	○	–	–	–	○	
	3-4	○	○	–	–	–	–	–	Larva of Lepidoptera sp.
	3-5	○	–	–	–	–	○	–	

Fish species were divided into stream pelagic fish and benthic fish.

standards to fix each sample value. $\delta^{13}C$ and $\delta^{15}N$ can normally be measured with an analytical error range of ± 0.2‰.

We then evaluated the proportional contribution of each food resource to the diets of the individual water shrews using the Bayesian isotopic mixing model (Parnell et al. 2010) available in the SIAR package ver. 4.2 in R ver. 3.3.2 (R Core Team 2016). *SIAR* uses a Dirichlet prior to a probability distribution. Using two stable isotopes and considering the uncertainty of all parameters, *SIAR* generated probable source contributions to the water shrews' diets. The model was run using the siarsolomcmcv4 command, which is for single-organism models with default parameters (iterations = 500 000, burnin = 50 000, thinby = 15). We assigned an elemental concentration (e.g., % C and % N; Phillips and Koch 2002) and standard deviation (*SD*) for each parameter of the model. Since different tissues incorporate isotopes at different rates (Reich et al. 2008), we applied a correction factor to the muscle to incorporate the isotopic discrimination between consumers and prey before generating the model.

If a water shrew individual had been primarily feeding on terrestrial organisms, their $\delta^{13}C$ would be expected to be similar to that of terrestrial plants (e.g., 0–1‰ in general, DeNiro and Epstein 1978). The $\delta^{15}N$ values were used to infer the trophic position with an enrichment rate of 3.4 ± 1.1‰ (DeNiro and Epstein 1978, 1981; Minagawa and Wada 1984; Zanden and Rasmussen 2001; Post 2002). In the mixing model, we set the $\delta^{13}C$ enrichment rate ($\Delta^{13}C = 1.3 \pm 0.3$‰) for muscle tissues of all animals and the $\delta^{15}N$ enrichment rate ($\Delta^{15}N = 3.3 \pm 0.26$‰ for fishes and 1.4 ± 0.2‰ for aquatic/terrestrial invertebrates) from a meta-analysis result (McCutchan et al. 2003), because the fractionation values of shrews were unknown. We then used the individual values of shrews and the mean and *SD* of each food resource (fishes and aquatic and terrestrial invertebrates) for the mixing model analysis. In the case of St.3, we could not evaluate the proportional contribution because the data on terrestrial invertebrates were not obtained.

Statistical analysis

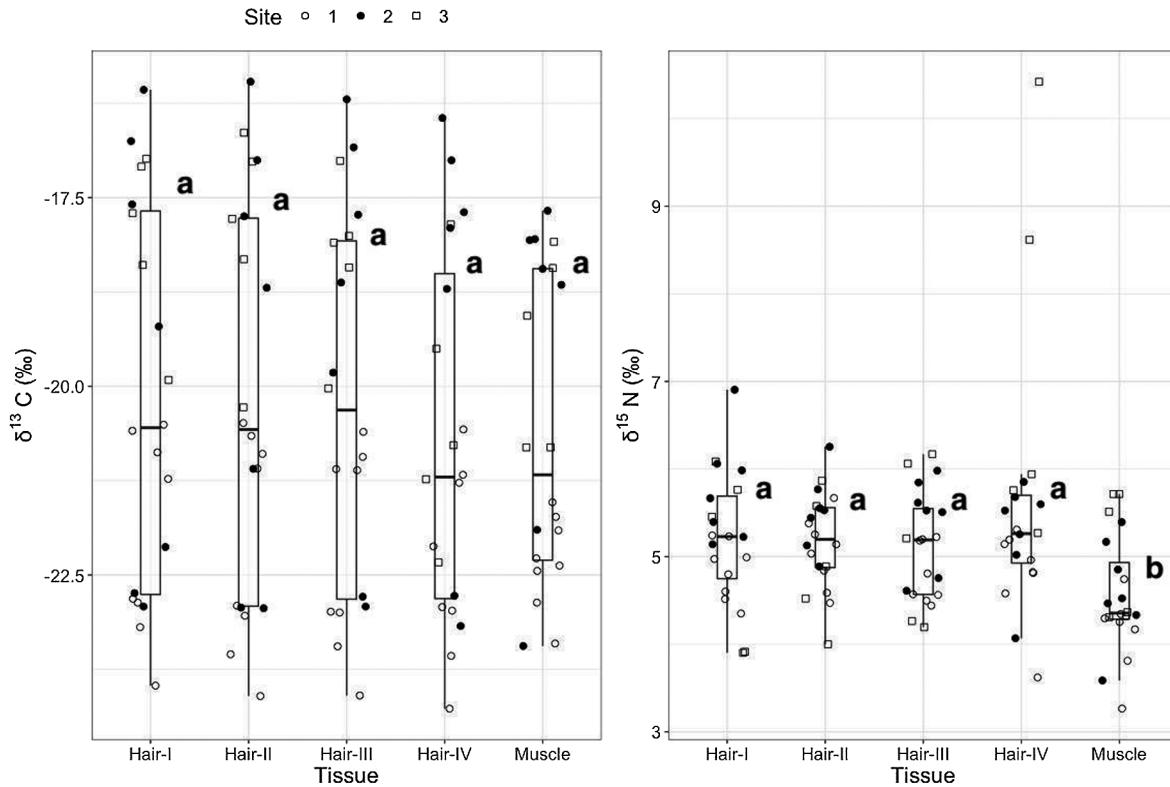All statistical analyses were performed using the R ver.

Fig. 2. Comparison of carbon and nitrogen stable isotope ratios (δ^{13}C and δ^{15}N) among the hairs I–IV and muscle of water shrews. Results of general linear mixed model (GLMM) are also shown, values of the tissue labeled with the same letters are not significantly different.

3.3.2 (R Core Team 2016). A chi-squared test was used to compare the sex ratios of the captured shrews. The total length and body weight of the captured shrews in each stream were compared using a one-way analysis of variance (ANOVA). We compared the individual δ^{13}C and δ^{15}N values of the shrews' muscles and hairs from sections I–IV via general linear mixed models (GLMMs) using *lmer* function in *lme4* package ver. 1.1.27.1. The fixed effect was the tissue type, which included the two categories; muscles and hairs, and the random-intercept factors were the IDs of the individuals and the study sites, as the nested category of data.

Results

The number of captured Japanese water shrews was eight, seven, and five in Sts.1, 2, and 3, respectively, and the captured data of sex, tail length, head and body length, total length, and body weight are shown in Table 1. Out of the total 20 individuals, the number of females was significantly lower than that of males ($\chi^2 = 5.00$, $df = 1$, $P = 0.025$), with 15 males to five females. No significant differences in TL and BW were observed among sites

($P > 0.05$).

Three orders of aquatic insects were identified from the digestive contents analysis, as well as Japanese freshwater crabs, stream pelagic, benthic and unidentified fishes, and terrestrial insects (Table 2). Some organs of aquatic insects (legs or head) were observed in all shrew individuals, while fish organs (muscles, bones, and scales) were observed in about half of the individuals. Terrestrial invertebrates were observed in only one-third of the individuals. All invertebrate sizes estimated from these undigested materials were less than 1 cm, which was small in comparison with some of the sizes of invertebrates observed in the field. In addition, non-animal detection, such as leaf litter and inorganic matter (sand and gravel) were observed.

The stable isotope analysis showed no significant difference in δ^{13}C between the body hairs of all sections and the muscles of water shrews at all the sites (Fig. 2, $P > 0.05$ in all the tissues). In contrast, the δ^{15}N was significantly lower in the muscles than in the body hairs across body sections I–IV ($P = 0.0145$ in muscle), although no significant effects of random factors were observed ($SD = 0.056$ and 0.376). There were two outliers in section IV;
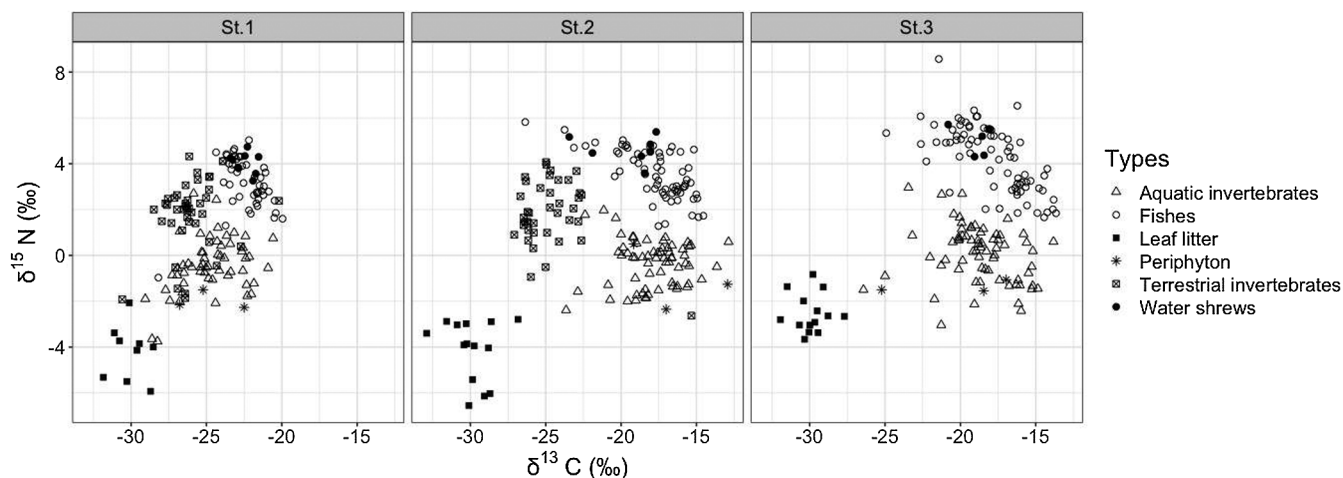
Fig. 3. Carbon and nitrogen stable isotope ratios (δ^{13}C and δ^{15}N) of water shrews (muscle), preys (aquatic invertebrates, fishes, and terrestrial invertebrates), and primary producers (periphyton and terrestrial leaf litter) collected in each study site, Sts.1, 2, and 3. The raw data are shown in Supplementary Table S1.
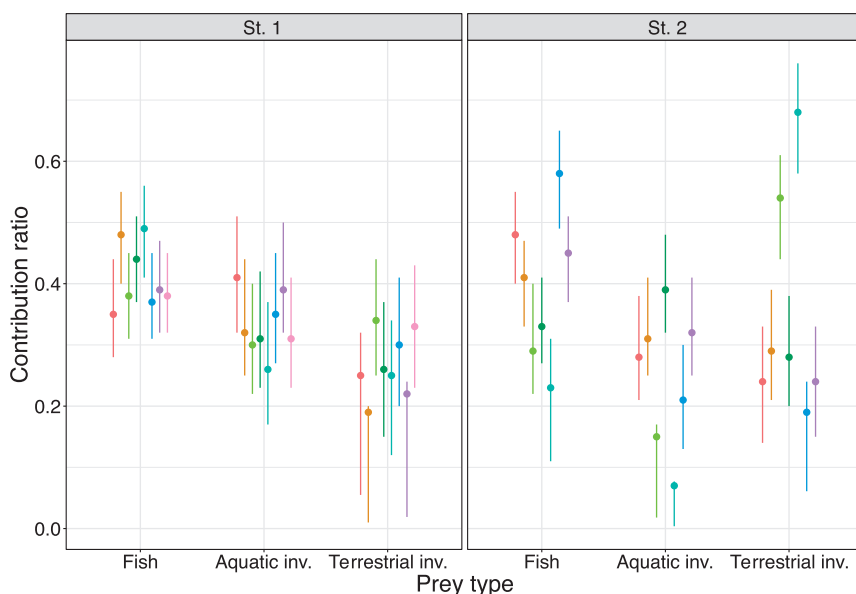

Fig. 4. Results of stable isotope analysis in R (SIAR) in Sts.1 and 2. Contribution ratio (median, ± 95% range) of preys; fishes, aquatic invertebrates, and terrestrial invertebrates for water shrews were shown. Plots with different colors indicate different individuals. In St.3, SIAR were not performed because of lack of data (no terrestrial invertebrates).

however, after removing them, the statistical results remained unchanged. The difference in δ^{15}N between the body hair and muscle was calculated to be 0.8 on average.

Two-dimensional plots of δ^{15}N and δ^{13}C values for water shrews (muscle), prey, and primary producers are shown in Fig. 3. The δ^{13}C values of the water shrews were similar to those of fishes and aquatic invertebrates. The δ^{15}N of shrews was similar to that of fishes and higher than that of aquatic invertebrates, terrestrial invertebrates

(Sts.1 and 2), and aquatic invertebrates (St.3).

The results of SIAR in St.1 and 2 are shown in Fig. 4. At St.1, the contribution ratios of each prey were dispersed to a smaller range than those at St.2, revealing small differences among individuals. Furthermore, the contribution ratios of each prey tended to vary, that is, it decreased in the following order: fishes > aquatic invertebrates > terrestrial invertebrates. On the contrary, at St.2, the contribution ratio of each prey species varied greatly

among individuals. The contribution ratio of terrestrial invertebrates in the five individuals in St.2 was less than 0.35, which was the highest ratio in St.1, and it was lower than that of fishes and aquatic invertebrates. Two individuals had the highest contribution from terrestrial invertebrates (> 0.5), which generally contributed the least at St. 1, and one individual had the highest contribution from fish. The three abovementioned individuals had the lowest contribution of aquatic invertebrates.

Discussion

Diet of C. platycephala

The digestive contents analysis indicated that the water shrews fed on a wide variety of prey, including aquatic insects, crustaceans, fishes, and terrestrial insects. Aquatic insects were the most abundant prey throughout the study period, from spring to autumn, which is consistent with the findings of $\delta^{13}C$ values. Fishes and terrestrial invertebrates were found only in half to one-third of the individuals' guts, for which the results of stable isotopes were supported. No individuals had higher $\delta^{15}N$ than that of fishes. Although there is a limitation that the results obtained in the present study could not be extrapolated to the wide-ranged water shrew populations, as we focused only on one relatively small water system, which the northmost population of the water shrew inhabited, our results are consistent with the recent findings on digestive contents analysis in individuals collected from all over Japan (Abe 2011), which showed that water shrews fed mainly on aquatic insects and occasionally on fishes. This study revealed that water shrews regularly depended on aquatic insects rather than fishes for a stable diet, therefore indicating that the trophic position of the water shrew is similar to that of fishes that feed on aquatic insects.

It is not clear whether the water shrews positively selected aquatic insects for their diets and, if so, what the benefits of such selection are. Although mammals have not been reported to feed on insects because they are not particularly nutritious (Redfords and Dorea 1984), it has more recently been reported that insects are nutritious and valuable, which allows mammals to meet their copper and phosphorus requirements (Barker et al. 1998). A recent study revealed that the habitat preference of water shrews was not dependent on the abundance of aquatic insects in their diets (Iwasa 2019). However, there have been reports that aquatic insects are mainly preyed on by *C. placentula* and *C. hantu* in the Malay Peninsula (Liat et al. 2013) and *Galemys pyrenaicus* (Pyrenean desman;

Esnaola et al. 2021). The diet of the desman is diverse, including Ephemeroptera, Plecoptera, Trichoptera, and Diptera (Oehm et al. 2011; Divoll et al. 2018; Alberdi et al. 2019). These diverse aquatic insects also represent diverse life cycles, allowing their constant presence in streams (Hershey and Lamberti 1998) from which shrews may benefit.

The aquatic insects in the digestive contents of the Japanese water shrews were estimated to be less than 1 cm in length. Although there are larger species, such as Plecoptera (~ 5 cm in length) and over 7 cm Megaloptera (Kawai and Tanida 2005), these species were not found in the water shrew guts. These large insects move quickly and are more abundant in the hyporheic zone than on the streambed surface (Lancaster and Downes 2013). Similarly, fishes and crustaceans moving faster than small insects were not found in their digestive contents. Nevertheless, these large organisms might be suitable prey due to their high biomass if they were successfully preyed upon. The water shrews have a preference to occupy fish farms and feed on fish in the aquariums, probably because they are easy to collect (Iwasa 2019). This circumstantial evidence suggests that the water shrews are not good at food acquisition and that they might feed on small aquatic insects that can be easily captured.

Importance of terrestrial food for C. platycephala

Most shrew individuals in our study had $\delta^{13}C$ data that indicated prey of aquatic origins, but only two individuals from St. 2 showed data similar to those of terrestrial insects. These were male individuals 2-3 and 2-5, collected on 25 June and 29 July 2014, respectively. In addition, the digestive analysis revealed that terrestrial insects were detected in six individuals' guts throughout the study period, which is one-third of the total individuals. However, the $\delta^{13}C$ of these individuals was not similar to that of terrestrial insects. This suggests that, especially during the period when the canopy covered a stream with leaves, the water shrews also feed on terrestrial insects which drop from the canopy, and some individuals would primarily feed on them.

For fishes with similar trophic positions to the water shrews in this study, terrestrial invertebrates are considered an important food resource during summers when aquatic insects are scarce in streams (Murakami and Nakano 2001). However, such resource subsidization does not seem to be as important for the water shrews considering that only two individuals fed predominantly on terrestrial insects. Nevertheless, anthropogenic dis-

turbances disconnecting streams and forests might have an influence on some water shrews. Therefore, maintaining linkages between streams and forests should be kept in mind for the conservation of Japanese water shrews.

Usefulness of body hairs for SIR analysis

To clarify dietary trends for an endangered mammal species, it is important to use body hairs for SIR analysis because these samples can be collected through non-invasive sampling. Herein, we showed that body-hair $\delta^{13}C$ remained the same as that of the muscle. On the other hand, $\delta^{15}N$ tended to be higher in the body hair than in the muscle at all sites, which might be due to two main reasons: one is the time lag between the tissues and the other is the isotopic fractionation difference between the tissues. The time lag occurred because body hair reflected the food consumed during the growth of the individual, whereas the muscle reflected the food that an individual had consumed over a few days or weeks. For instance, Miller et al. (2008) have shown that the C and N isotopic half-life of muscles of deer mice were 18.7 and 24.7 days, respectively. The hair isotope has been reported to have a longer isotopic half-life (Crawford et al. 2008; Wada et al. 2013; Balčiauskas et al. 2016). There have been no shrew studies on isotope turnover time, including our study. Meanwhile, studies on the hair isotope on deer mice have shown non-turnover during the feeding experiment (Miller et al. 2008, 2011) or a few-months half-life in the field (Tabacaru et al. 2010). Therefore, we presumed that the turnover time of body hair is long, similar to that of deer mice reflecting long-term diet assimilation. Consequently, the body hair contains information about the diet consumed at any time point, ranging from a day to several months.

Hair replacement, in which the hairs with long-term information are lost and replaced by new hairs with current dietary information, would need to be carefully considered. For example, seals, which molt several times a year, have hairs that contain isotopic signals of short periods that allow for short-term dietary reconstructions (Pulkkinen et al. 2020), while most Soricidae species including the water shrews molt twice a year, once in spring and once in autumn (Ohdachi et al. 2015; Ivanter 2021). The isotope values of new hairs can reflect a relatively recent diet, while those of old hairs reflect a diet consumed almost half a year ago. In this study, although the hair samples collected from different body parts of the water shrew yielded no information on how old the exam-

ined hair had been maintained, none of the individuals were obviously in the process of molting. The lack of significant differences among the hair samples from body sections I–IV collected from June to October suggested that the time since molting was relatively long, thereby indicating a relatively long period of information. It has recently been shown that between the molting and the non-molting periods, the $\delta^{13}C$ of small rodent species hairs were not significantly different but the $\delta^{15}N$ tended to vary based on the individual (deer mice: Miller et al. 2008), season (common hamster: Roswag et al. 2018), and environmental factors such as drought and land-use patterns (California vole: Crumsey et al. 2019). These might account for the $\delta^{15}N$ outliers in this study, and more importantly, might also signify the usefulness of body hairs for the SIR analysis, regardless of the concerns on the molting period.

Another significant factor that needs to be considered is the difference in isotopic fractionation difference between hair and muscle (Caut et al. 2009) due to the different metabolic rates between body tissues (Tieszen et al 1983). In mammals, not many studies have simultaneously examined the stable isotope ratios in hair and other tissues such as muscles, which are more metabolically active (Young and Ferguson 2014), but a few studies have examined whether body hair reflects signals stored in muscle (e.g., cattle, Osorio et al. 2011; seal, Pulkkinen et al. 2020). Among seals, although the detailed mechanism was not investigated, the isotope ratios between muscle and hair can be predicted by linear regression (Pulkkinen et al. 2020). This is similar to our observations from the stable isotope analysis in water shrews, in which the $\delta^{15}N$ of muscle can be estimated by subtracting 0.8 on average from that of hairs.

Unlike the findings of Miller et al. (2008), the present study provides limited information for the effectiveness of small mammalian species hair on the stable isotope analyses because we only used wild individuals whose SIR of the food actually consumed was unknown. However, at least, our study demonstrated that body hair could be used instead of muscles, with some correction for N. The hair for the SIR analysis could be collected from any part of the back, as long as areas with signs of clear molting are avoided. As the collection of hairs from endangered species must be performed quickly so as not to affect the individuals adversely, the present results, which showed that hair from any part of the back yields the same isotopic results, may add value to body hair usefulness for the stable isotopic analysis.

Supplementary data

Supplementary data are available at *Mammal Study* online.
Supplementary Table S1. Stable isotope ratio analysis results for water shrews, fishes, terrestrial and aquatic invertebrates, terrestrial plants, and periphyton. Sampling date and tissues used for analysis were also noted.

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