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A Preliminary Survey of Japanese Weasel (*Mustela itatsi*) Diet and Spatial Utilization in an Anthropogenically Modified Wetland

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ABSTRACT

The Japanese weasel (*Mustela itatsi*), endemic and widely distributed in Japan, is declining in numbers, and it may become endangered if its habitat conditions change. This study investigated the diet and spatial utilization of Japanese weasels at Lake Yanaka, within the Watarase Retarding Basin, an anthropogenically modified wetland, via faecal collection with metabarcoding analysis and camera trapping from April to November 2024. Dietary analysis of 13 faecal samples revealed that Japanese weasels primarily consumed animal matter, with rodents and shrews as the dominant food sources, appearing in eight samples. Other animals, such as insects, birds (e.g. *Yungipicus kizuki*) and reptiles, varied seasonally as food sources. Camera trap surveys (651 trap-nights) yielded only one Japanese weasel detection, suggesting a relatively small population, whereas other mammals, such as wild boars and raccoons, were abundant based on the number of detections. Its low detection rate is potentially due to anthropogenic water-level adjustments. The weasel was photographed in a reed field, highlighting these areas as crucial habitats that provide important food resources (rodents) and resting places. Despite being based on single-year and seasonal data, our findings offer valuable insights into the ecology of Japanese weasels in modified wetland ecosystems. We anticipate that these results will contribute to broader research, including meta-analyses of mesocarnivore ecology.

INTRODUCTION

The Japanese weasel (*Mustela itatsi*), endemic to Japan, is naturally and widely distributed across the country. However, its native populations are declining owing to habitat degradation caused by urbanisation and to the expanding distribution of invasive Siberian weasels (*M. sibirica*) (Suzuki, 2018). As of March 2025, the Japanese weasel was categorised as "Near Threatened" in the International Union for Conservation of Nature Red List, indicating that while its extinction risk is low it may become endangered if habitat conditions change (Kaneko *et al.*, 2016). Although the Japanese weasel is categorised as "Least Concern" in the Japanese Red List, it is categorized as "Vulnerable" or "Near Threatened" in the Red Lists of several local prefectures, emphasizing the local conservation challenges faced by this endemic species.

Foraging is essential for animal survival; thus, evaluating an animal's diet provides critical insights into its ecology and helps implement conservation measures (Elton, 1927). The Japanese weasel, an omnivorous species, consumes a diverse range of animals (including rodents, birds, insects, crustaceans and fish) as well as plant matter (e.g. fruits and seeds), although its primary food sources vary regionally (Furuya *et al.*, 1979; Fujii *et al.*, 1998; Kaneko *et al.*, 2009, 2013; Okawara *et al.*, 2014; Tsunoda *et al.*, 2024). This flexible foraging strategy, based on food resource availability, highlights the need for detailed and site-specific dietary studies to understand how local environmental factors shape this species' diet.

The Watarase Retarding Basin, a Ramsar Convention-registered wetland, is one of Japan's most biodiverse and valuable wetlands (Watarase Retarding Basin Acclimation Promotion Foundation, 2025), and the Japanese weasel is categorised as a "species to be considered for conservation, or a species of note due to its characteristic habitat" in Tochigi Prefecture, an area that includes part of the basin. Despite this, no prior studies have investigated its diet and spatial utilization in this wetland ecosystem. The area is known to have a high density of native rodent species such as Japanese field vole (*Alexandromys montebelli*), large Japanese wood mice (*Apodemus speciosus*), and harvest

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mice (*Micromys minutus*), a key prey item for weasels (Tone River Basin Residents' Council for the Protection of Watarase Retarding Basin, 2017) based on rodent burrow density. Nonetheless, quantitative data on the dietary importance for Japanese weasels of rodents, which are also consumed by raptors and various other omnivorous species, are lacking. We therefore investigated the diet and spatial utilization of Japanese weasels at Lake Yanaka, within the Watarase Retarding Basin, by collecting faeces and deploying camera traps.

MATERIALS AND METHODS

Study area

The Watarase Retarding Basin is a flood-control basin covering approximately 33 km² and spanning the borders of the Ibaraki, Tochigi, Gunma and Saitama Prefectures, Japan (Fig. 1). Lake Yanaka (36°12′51.3″N, 139°40′24.1″E), the study site, plays a vital role in supplying domestic water to surrounding areas and regulating river flow. Approximately half of the basin, covering 15 km², is covered by the largest reed field on Honshu, the main island of Japan. This environment supports over 1000 plant species and approximately 270 bird species (Ministry of Land, Infrastructure, Transport and Tourism, Kanto Regional Development Bureau, Tonegawa-Joryu River Office, 2024a). In recognition of its rich and diverse wetland environment, which meets one of the criteria for an internationally important wetlands, the Watarase Retarding Basin was registered as a Ramsar Convention wetland in 2012.

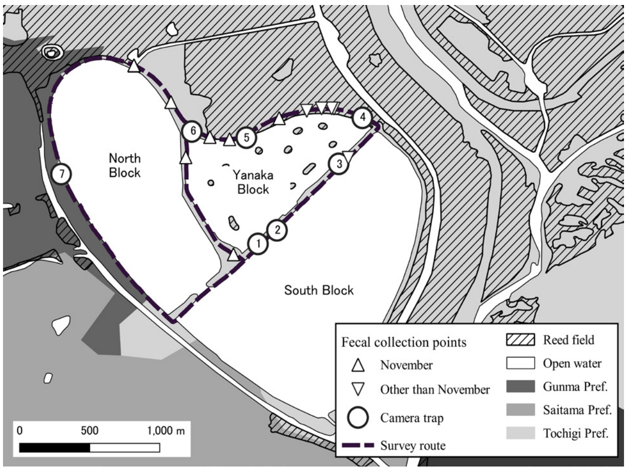

Fig. 1. Map of the study area. Lake Yanaka is the heart-shaped lake at the center of the map. Dashed lines: Survey routes for Japanese weasel (*Mustela itatsi*) faecal sample collection. Triangles: Faecal sample collection points. Circled number: Camera trap locations and numbers.

Dietary survey

Faecal samples were collected along a route of approximately 8.8 km, following the perimeter walkways of the North Block and Yanaka Block of Lake Yanaka (Fig. 1; access to the South Block was prohibited for safety reasons). Eleven surveys were conducted from April to November 2024 (excluding August), during which we searched for and collected faeces believed to be from Japanese weasels, while walking on opposite sides of the walkways.

We initially determined whether the collected faeces belonged to Japanese weasels based primarily on their size and shape. The typical size of the faeces was approximately 5–7 mm in diameter, which falls within the diameter range reported for Japanese weasel faeces in previous studies (Sekiguchi *et al*., 2002; Tsuji *et al*., 2011). However, given that many other animal species in the study area have similar characteristics, we immediately stored the samples in 99.5% ethanol in the field and stored them in −30 °C in the laboratory until processing. We subsequently identified the species in the collected samples by metabarcoding analysis with MiMammal primers (Ushio *et al*., 2017).

For metabarcoding analysis, we collected sample fragments by superficially scraping the surface of each faecal sample using forceps pretreated with sodium hypochlorite. The collected fragments were freeze-dried using a VD-250R Freeze Dryer (TAITEC, Saitama, Japan) and pulverised at 1500 rpm for 2 min using a multi-bead shocker (Yasui Kikai, Osaka, Japan). For DNA extraction, Lysis Solution F (Nippon Gene, Tokyo, Japan) was added to the pulverised samples, which were then incubated at 65 °C for 10 min. After centrifugation at 12000 ×*g* for 2 min, the supernatant was collected. DNA was purified from the solution using a Lab-Aid 824s DNA Extraction kit (Zeesan, Xiamen, China). Libraries were prepared from the purified DNA solution using a two-step tailed PCR method. The first PCR amplification mixture contained 5.0 µl of 2 × PCR Buffer for KOD FX Neo, 2.0 µl of dNTPs (each 0.2 µmol/L), 0.2 µl of 1st-MiMammal U/E/B mix F and R primers (Ushio *et al*., 2017), 1.0 µl of extracted DNA, 0.2 µl of KOD FX Neo (1.0 U/uL) (Toyobo, Japan), and 1.4 µl of nuclease-free water. The first amplification mixture was denatured at 94 °C for 2 min, followed by 35 cycles of 94 °C for 10 sec, 64 °C for 30 sec, 68 °C for 30 sec; and a final extension at 68 °C for 2 min. The second PCR amplification mixture contained 5.0 µl of 2 × PCR Buffer for KOD FX Neo, 2.0 µl of dNTPs (each 0.2 µmol/L), 0.5 µl of 2nd F and R primers, 1.0 µl of PCR product, 0.2 µl of KOD FX Neo (1.0 U/uL), and 0.8 µl of nuclease-free water. The second amplification mixture was denatured at 94 °C for 2 min, followed by 12 cycles of 98 °C for 10 sec, 60 °C for 30 sec, 68 °C for 30 sec; and a final extension at 68 °C for 2 min. After quantification and quality control, sequencing was performed using 2 × 300 bp paired-end reads using either the NextSeq 1000 system with the NextSeq 1000/2000 P1 Reagent Kit, or the MiSeq system with the MiSeq Reagent Kit v3 (Illumina, California, USA). After removing low-quality reads, species identification was performed using BLASTN 2.15.0 using the three merged sequences with the highest occurrence. As the difficulty of species identification using metabarcoding analysis is likely to increase with time after defaecation (Shimatani *et al*., 2008), only fresh faeces were collected and subjected to metabarcoding analysis. Freshness was judged based on being able to insert the forceps into the faeces with slight pressure. While this limited the sample size, it should yield more accurate results reflecting the diet at the time of defaecation.

Faecal samples identified as belonging to Japanese weasels via metabarcoding analysis were washed using a 0.5 mm mesh sieve, similar to the method of Tsunoda *et al*. (2024). The remaining residues were identified to the lowest possible taxonomic level using a stereomicroscope (maximum magnification: 45×). The washed faecal contents were dried at 80 °C for 24 h and classified into nine food item categories: rodents and shrews, birds, insects, reptiles, crustaceans, plant fragments, seeds, eggshells and others (including unidentified items). The dry weight of each category was determined to the nearest milligram using an electronic balance (MS603S, Zürich, Switzerland), and the dry weight ratio of each food item per faecal sample was calculated.

Camera trap survey

To investigate the spatial utilization of Japanese weasels in areas outside the range of the dietary survey, we deployed seven infrared sensor cameras (Ltl-6210 WMC PLUS; Ltl Acorn, Zhuhai, China) in the study area from 16 July 2024 to 4 December 2024 (Fig. 1). We mounted the cameras on trees along medium-sized mammal trails, and the installation height was set at 0.3–1.0 m from the ground based on the body size of the Japanese weasel. Video length was set to 30 sec (trigger speed, 0.8 sec). To prevent duplicate counts of the same individual, we considered consecutive videos of the same species captured within a 30 m interval as a single detection event. After retrieving the video data, we identified the recorded animal species to the lowest taxonomic level possible and counted both the number of detection events and number of individuals for each species. The data acquisition period varied among cameras owing to an increase in lake levels due to heavy rainfall and water gate adjustments (Table I).

RESULTS

Dietary survey

We collected 19 faecal samples presumably from Japanese weasels over the course of 11 surveys. Based on metabarcoding analysis, six samples showed the highest match rate for Japanese weasels. Another four of the samples produced high matches for the Japanese weasel, Japanese field vole, large Japanese wood mice and *Crocidura*. Furthermore, the other three samples showed the highest match rate for Japanese field voles. Given that these seven

Table I. Total detection frequency, number of individuals, species-specific detection frequency (%) and operational period of seven camera traps deployed at Lake Yanaka, Watarase Retarding Basin in 2024.

Camera No.	Numbers of detection frequency and individuals								Operational period
	Mustela itatsi	*Procyon lotor*	*Sus scrofa*	*Vulpes vulpes*	*Cervus nippon*	*Nyctereutes procyonoides*	*Paguma larvata*	Unknown	
1	0 (0) 0.0 %	9 (11) 21.4 %	14 (26) 33.3 %	0 (0) 0.0 %	0 (0) 0.0 %	4 (4) 9.5 %	1 (1) 2.4 %	14 (17) 33.3 %	16 July – 31 July, 5 September – 2 October (44 days)
2	0 (0) 0.0 %	56 (70) 40.0 %	49 (91) 35.0 %	1 (1) 0.7 %	0 (0) 0.0 %	26 (34) 18.6 %	0 (0) 0.0 %	8 (8) 15.7 %	16 July – 18 October, (95 days)
3	0 (0) 0.0 %	5 (5) 16.1 %	21 (34) 67.7 %	0 (0) 0.0 %	0 (0) 0.0 %	2 (2) 6.5 %	0 (0) 0.0 %	3 (3) 9.7 %	16 July – 30 July, September – 2 October (43 days)
4	1 (1) 0.9 %	36 (41) 33.3 %	43 (55) 39.8 %	0 (0) 0.0 %	0 (0) 0.0 %	20 (20) 18.5 %	0 (0) 0.0 %	8 (8) 7.4 %	16 July – 26 August, 5 September – 4 December (133 days)
5	0 (0) 0.0 %	4 (4) 14.8 %	16 (22) 59.3 %	0 (0) 0.0 %	0 (0) 0.0 %	5 (5) 18.5 %	0 (0) 0.0 %	2 (2) 7.4 %	16 July – 26 August, 5 September – 2 October, (70 days)
6	0 (0) 0.0 %	1 (1) 3.4 %	27 (35) 93.1 %	0 (0) 0.0 %	1 (1) 3.4 %	0 (0) 0.0 %	0 (0) 0.0 %	0 (0) 0.0 %	16 July – 26 August, 5 September – 4 December, (133 days)
7	0 (0) 0.0 %	35 (51) 63.6 %	19 (35) 34.5 %	0 (0) 0.0 %	0 (0) 0.0 %	0 (0) 0.0 %	0 (0) 0.0 %	1 (2) 1.8 %	16 July – 26 August, 5 September – 4 December, (133 days)

Table II. Collection dates and dry weight (g) of Japanese weasel (*Mustela itatsi*) faecal sample contents collected at Lake Yanaka, Watarase Retarding Basin, from April to November 2024.

Sample No.	Collection date	Dry weight (g)								
		Rodent and eulipotyphla	Bird	Insect	Reptile	Crus-tacean	Plant fragment	Seed	Eggshell	Other (including unidentified items)
1	26 April 2024	-	-	-	0.299	-	0.004	-	-	-
2	13 May 2024	0.438	-	-	-	-	-	-	-	-
3	13 May 2024	0.384	-	-	-	-	-	0.006	-	-
4	13 May 2024	0.206	-	-	-	-	-	-	-	-
5	16 July 2024	-	-	0.248	0.027	-	0.002	-	0.014	-
6	3 November 2024	-	0.009	0.243	-	0.798	-	-	-	-
7	3 November 2024	-	0.475	0.117	-	-	0.002	-	0.001	-
8	9 November 2024	0.065	-	0.170	-	0.353	0.048	0.003	-	0.005
9	10 November 2024	-	0.171	-	-	-	0.002	0.039	-	-
10	10 November 2024	0.130	-	-	-	-	-	-	-	-
11	10 November 2024	0.207	-	0.001	-	-	0.001	-	-	0.002
12	10 November 2024	0.465	-	-	-	-	0.001	-	-	-
13	10 November 2024	0.561	-	-	-	-	0.016	-	-	-

faecal samples were similar in size and shape to the six samples with the highest match rate for Japanese weasels, we concluded that these seven samples were derived from Japanese weasels that preyed on rodents or eulipotyphs.

The 13 faecal samples identified as originating from Japanese weasels were collected in April (Sample 1), May (Samples 2–4), July (Sample 5) and November (Samples 6–13). The collection sites differed slightly over the months (Fig. 1). Samples from April, May and July were predominantly found near the reed fields on the eastern side of the Yanaka Block, whereas those from November tended to be toward the reed fields on the northern side of the Yanaka and North Blocks.

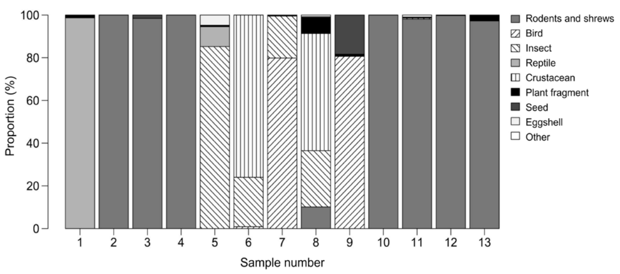

Fig. 2. Dry weight ratio (%) of Japanese weasel (*Mustela itatsi*) faecal sample contents. Samples were collected at Lake Yanaka, Watarase Retarding Basin, Japan, from April to November 2024. Table II provides the dry weight ratios for each food item category.

The sample collected in April was dominated by reptile remains (98.7%; Fig. 2; Table II). The three collected in May contained the remains of Japanese field voles, with one (Sample 3) containing seeds. Metabarcoding identified *Crocidura* in the sample collected in July, although no *Crocidura* hair or bones were found in this sample, which contained various other remains, including of Coleoptera, Hymenoptera, reptiles and eggshell. Of the faecal samples collected in November (Samples 10–13), half comprised >95% rodent remains (Table II), whereas the others (Samples 6–9) contained more diverse remains. Samples 6 and 7 contained Coleoptera and bird remains, and crustacean remains were predominant in sample 6 (76.0%). Sample 8 exhibited the greatest food item diversity among the samples, containing the remains of crustaceans, multiple insect species (coleopterans and grasshoppers), Japanese field voles and seeds. Sample 9 contained mostly Japanese pygmy woodpecker (*Yungipicus kizuki*) feathers and Polygonoideae seeds. Many of the samples also contained plant fragments, although these contributed minimally both in terms of proportion and dry weight.

Camera trap survey

During the 651 camera trap nights, mammals were detected 432 times (590 individuals). A Japanese weasel was detected only once (one individual) (Table I) by a camera installed in the northeastern part of the Yanaka Block (Fig. 1, Camera No. 4). This single detection occurred at 10:35 a.m. on 3 November 2024 showing that the weasel moved from the lakeside, across the walkway, toward the reed fields. The same camera also recorded 107 images of other mammals (124 individuals) throughout the study period.

Among the seven camera traps, the most frequently detected mammal was the wild boar (*Sus scrofa*), with 189 detections (298 individuals), accounting for approximately 44% of all records. This was followed by raccoons (*Procyon lotor*) with 146 detections (183 individuals) and raccoon dogs (*Nyctereutes procyonoides*) with 57 detections (65 individuals). Masked palm civets (*Paguma larvata*), red foxes (*Vulpes vulpes*) and sika deer (*Cervus nippon*) were each detected once (one individual of each). Other unidentified mammals were recorded 36 times (40 individuals).

DISCUSSION

This study examined the diet and spatial utilization of Japanese weasels at Lake Yanaka, within the Watarase Retarding Basin, based on faecal samples and camera trap surveys. This revealed that their diet comprised primarily animal remains (especially of rodents), with various other animal species being consumed depending on the season.

Rodent and shrew remains were found in more than half (eight) of the 13 samples; similarly, plant fragments were found in eight of the samples. When evaluating dietary proportions by dry weight, plant matter tends to be underestimated relative to animal matter; nonetheless, based on the very low dry weight of the plant matter in the samples, it is highly probable that the Japanese weasels ingested these plant fragments incidentally while feeding on other items (Fujii *et al*., 1998). Although Japanese weasels are omnivorous and consume various animals and plants (Kaneko *et al*., 2009, 2013), rodents often constitute their primary food resource (Fujii *et al*., 1998; Okawara *et al*., 2014). In forests or areas with limited agricultural land, especially during autumn and winter, rodent availability may decline, leading to a shift towards other food resources (Furuya *et al*., 1979; Kaneko *et al*., 2009; Okawara *et al*., 2014). Nevertheless, Japanese weasels were found to depend highly on rodents in the study area, where rodents may be abundant (based on their burrow density) (Tone River Basin Residents' Council for the Protection of Watarase Retarding Basin, 2017). Although we did not conduct field surveys during winter, over half of the late autumn (November) samples comprised >95% rodent remains. Although the species-level information is limited to secondarily findings revealed during the metabarcoding species identification of the faecal samples, the Japanese field vole was identified in Samples 10 and 12, and the large Japanese wood mouse in Samples 11 and 13. This confirms the predation of at least these native rodent species by Japanese weasels. Moreover, the other top predators at Lake Yanaka also prey on rodents during winter (Hirano *et al*., 2005). Rodents are therefore likely to be an important food resource for Japanese weasels at Lake Yanaka.

Many of the faecal samples also contained insect remains, bird feathers and eggshells. The insect species differed depending on the collection month; the samples collected in July contained the remains of Coleoptera and wasps, which are highly active in summer, while those collected in November contained the remains of Scarabaeidae larvae and leg fragments of Orthoptera. This suggest that insect consumption by Japanese weasels at Lake Yanaka is seasonal. Consumption of bird eggs and chick by weasels (*M. nivalis*) has been reported (Dunn, 1977; Takahashi *et al*., 2010), raising concerns about their impact on bird reproduction. Given that endangered grassland bird species such as the Japanese marsh warbler (*Locustella pryeri*) inhabit the Watarase Retarding Basin (Ministry of Land, Infrastructure, Transport and Tourism, Kanto Regional Development Bureau, Tonegawa-Joryu River Office, 2024a), the consumption of birds by Japanese weasels in this area warrants further investigation.

In this study, camera trap height and angle were adjusted based on Japanese weasel body size and traces. However, Japanese weasels were detected only once. In contrast, raccoons (*Procyon lotor*) and raccoon dogs (*Nyctereutes procyonoides*) were detected significantly more frequently. Despite the likely variation in detection probability among mammal species influenced by factors such as body size and movement speed in front of the camera traps our results implied that the population of Japanese weasels at Lake Yanaka is considerably smaller than that of other mammals in the area. The low detection frequency of the study species could also be related to water level adjustments at Lake Yanaka by the administration for water quality preservation (Ministry of Land, Infrastructure, Transport and Tourism, Kanto Regional Development Bureau, Tonegawa-Joryu River Office, 2024b). Fish and crustaceans are important food resources for Japanese weasels (Kaneko *et al*., 2009, 2013; Okawara *et al*., 2014), and many fish and crustacean species occur at Lake Yanaka (Watarase Retarding Basin Accretion Promotion Foundation, 2025). Remarkably, however, crustaceans were found in only two of the samples, and fish were not found in any. While a certain area of mixed forest and grassland extends along the shore of Lake Yanaka, the lakebed is entirely constructed of concrete (Fig. 3). Constructed areas that are exposed for longer periods throughout the year (such as areas closer to the shore) provide limited habitat for fish and crustaceans, even during temporary periods of rising water levels; this may in turn have reduced utilization of the lakeside area by Japanese weasels. Note that, despite fluctuating water levels, weasels can access the lake shore year-round. Indeed, rising water levels prevented mammal detection by cameras 1, 3 and 5 after 2 October 2024 and by camera 2 after 18 October 2024. Conversely, the only camera to detect a Japanese weasel was located in an area that is not submerged at any time of year, with reed fields that extend across the road. Japanese weasels sometimes use holes and crevices in rocks or soil as resting or breeding sites (Fujii, 2015); reed fields provide such sites in abundance and contain vegetation that is tall enough to offer protection from predators. Therefore, the reed fields around Lake Yanaka are a crucial component of the wetland ecosystem of the Watarase Retarding Basin, providing both resting places for the Japanese weasel and suitable habitat for rodents (Kuroda *et al*., 2009), one of their key food sources, thus helping to support the species. Moreover, Japanese weasel diet and spatial utilization at Lake Yanaka may be negatively affected by environmental factors other than food resources, such as anthropogenic water level adjustments.

Fig. 3. Lakeside view of the North Block, Lake Yanaka, Watarase Retarding Basin (photographed by the author on April 11, 2023). The photograph was taken when the water level was recovering from its lowest level (the bed-drying period during winter). The difference between the lowest water level and the non-flooded season maximum water level is approximately 5.5 m. While grasslands extend alongside the roadway (left of the photo), the entire lakebed within the survey area, including that of the grassland portion, is constructed from concrete.

In this study, we examined the diet and spatial utilization of the Japanese weasel, an endemic and declining mesocarnivore, within the Watarase Retarding Basin, a Ramsar Convention-registered wetland in Japan. This study was based on single-year seasonal survey data, and its sample size was limited due to the small population size of Japanese weasels in the study area and the cost associated with DNA metabarcoding. Nevertheless, it should provide valuable insights into the ecology of Japanese weasels in an anthropogenically modified wetland ecosystem. These findings therefore support future studies, including meta-analyses, of mesocarnivore ecology.

DECLARATIONS

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Ethical statement

The study was non-invasive, consisting of field observations of Japanese weasel diet and spatial utilization. No animals were captured or handled, and thus, no specific ethical approval for animal research was required.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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