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Title: Unraveling the Dietary Ecology and Traditional Entomophagy of *Vespula shidai* in Central Japan: Insights from DNA Metabarcoding and Local Practices

Short title: Cultural Ecology of *Vespula shidai*

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Abstract

Vespula shidai, a yellowjacket species, has long been reared for edible larvae and pupae in Central Japan; however, limited scientific knowledge exists regarding dietary differences between wild and reared nests. In this study, DNA metabarcoding was applied to larval gut contents from 12 nests (five wild, seven reared) in Central Japan to examine their dietary composition. Additionally, a questionnaire survey (n = 58) was conducted on taste perceptions of "*hachinoko*" (edible wasp brood) and on awareness of *V. shidai*'s feeding habits. DNA metabarcoding identified 324 prey species, including insects, arachnids, birds, mammals, reptiles, amphibians, and fish. Compared with reared nests, wild nests presented significantly greater diversity of wild vertebrate prey, suggesting that provisioning with human-supplied meats (e.g., chickens and deer) can partially replace natural foraging. Respondents who had wasp-rearing experience more frequently perceived taste differences between wild and reared *hachinoko*, often attributing these differences to the feed provided. They also recognised *V. shidai*'s predation on vertebrates, implying that practical engagement shapes ecological understanding and consumer preferences. These findings highlight *V. shidai*'s broad dietary flexibility under varying conditions of rearing and foraging. Future research could refine feeding strategies and cultural practices to sustain *hachinoko* as a valuable food resource while minimising its ecological impacts.

Introduction

Insects have long been integral to human diets, as evidenced by archaeological records spanning from the Palaeolithic era to the present (Pager, 1976; Backwell and d'Errico, 2001; Rothman *et al.*, 2014). Traditional entomophagy—practised for centuries in various regions—reflects deep-rooted cultural heritages and sustainable interactions between humans and local ecosystems (Ramos-Elorduy, 2009; Halloran *et al.*, 2015). Among these traditions, the consumption of Vespinae wasp larvae and pupae (broods) is notable throughout Southeast Asia and East Asia, particularly in mountainous areas ranging from Yunnan Province in China to Thailand (Matsuura, 2002; Ying *et al.*, 2010; Workshop recommendations: summary, 2010; Mitsuhashi, 2016; Feng *et al.*, 2018). In Japan, China, and India, hornet products have long been prized both as high-value delicacies and for their medicinal properties (Matsuura *et al.*, 1999; Nonaka, 2010; Kiewhuo *et al.*, 2022; Nonaka and Zhao, 2022). However, owing to their limited natural availability, these insects generally remain luxury items rather than dietary staples (Nonaka, 2010). Understanding such indigenous practices thus offers key insights into sustainable food production and consumption, contributing to ongoing discussions on environmentally responsible diets.

Japan's rural woodlands and farmland, known as satoyama, are areas of high biodiversity and sustain a unique food culture that includes entomophagy (Washitani, 2001; Indrawan *et al.*, 2014). In the mountainous regions of Central Japan, the larvae and pupae of social wasps such as *Vespula* (yellowjacket) and *Vespa* (hornet) are consumed as "*hachinoko*", a Japanese term for a delicacy of autumn (Nonaka, 2009; Payne, 2015). *Vespula shidai* is one of the most commonly consumed wasp species in Central Japan. Like other *Vespula* and *Vespa* species, each spring, the overwintered queen of *V. shidai* emerges and begins producing workers, thereby establishing a new colony (Spradbery, 1973; Matsuura and Yamane, 1990; Matsuura, 1995; Saga *et al.*, 2017). In autumn, colonies produce new queens and males for the next generation, and the queen mates with several males during this period (Matsuura, 1995; Saga *et al.*, 2020). By early winter (approximately November in central Japan), only new queens enter hibernation, whereas workers and males die (Matsuura and Yamane, 1990). As the colony reaches its maximum size in autumn, local people harvest the wasp colonies for consumption (Matsuura, 1995).

The practices of collecting, rearing, and cooking these insects have continued to develop (Nonaka, 2009; Payne and Evans, 2017; Van Itterbeeck *et al.*, 2021).

Enthusiasts not only cultivate wasps for consumption but also allow the next generation of queens to mate and hibernate and release them back into nature in the spring (Nonaka, 2009; Saga *et al*., 2024). *Hachinoko* consumption is thus firmly embedded in Japan's rural lifestyle and environment (Payne and Evans, 2017). Moreover, wasps in the subfamily Vespinae serve as both distinctive food resources and biological control agents that suppress agricultural and sanitary pest populations (Ono, 1997; Brock *et al*., 2021).

In Central Japan, wasp enthusiasts harvest *V. shidai* nests for consumption using distinctive baiting techniques and subsequently rear them by providing various meats (Saga, 2019; Van Itterbeeck *et al*., 2021). Although this practice has existed for decades, minimal information is available regarding what these wasps naturally consume in the wild. Previous surveys on the diet of *Vespula* wasps in satoyama near Tokyo were conducted more than 50 years ago (Shida, 1963; Iwata, 1971). They identified 54 prey species across 26 families and 7 orders, but subsequent reports provide only fragmentary updates, mentioning the consumption of frogs, snakes, and fish meat (Matsuura, 1995).

Recent advancements in genetic methods, including DNA metabarcoding, have enabled the identification of numerous prey species from fragments found in larval digestive tracts or faecal residues within nests (Takahashi *et al*., 2016; Lefort *et al*., 2020; Howse *et al*., 2022). These high-throughput techniques offer unprecedented insights into the dietary habits of social wasps. *Vespula* and *Vespa,* for example, retain faeces in their digestive tracts and excrete them at the prepupal stage, depositing faecal material at the bottom of each cell (Spradbery, 1973; Saga, 2019). In this study, we used DNA metabarcoding to examine the prey species found in the digestive tracts of *V. shidai* larvae. In Central Japan, November marks the peak period of reproduction (Saga *et al*., 2024), and workers presumably bring back a broad range of prey during this period. We therefore focused our sampling on November (the period during which wasp are harvested for consumption) to facilitate effective comparisons of prey diversity and composition.

The reared wasps are fed various foods, such as chicken, frog, deer meat, and fish by wasp keepers. However, how wasp keepers determine the feed and whether it reflects the natural prey diversity of *V. shidai* remain unclear. The purpose of supplementing feed in rearing nests is to promote colony growth and enhance taste, but little is known about whether these feeding practices influence consumer perceptions of taste. Understanding whether feeding primarily meat shifts the foraging patterns of wasps is also critical for optimising feeding strategies and mitigating ecological impacts. From a sociocultural perspective, insights into how keepers choose feed can further inform the sustainable use of wasp broods. We therefore conducted a short questionnaire survey targeting both experienced and inexperienced *Vespula* wasp keepers to assess their awareness of *V. shidai*'s wild prey and perceived taste differences.

This study has three main objectives. First, we used DNA metabarcoding to identify the broad range of prey items consumed by *V. shidai* and compared wild and reared nests to investigate how local rearing practices may affect natural foraging. Second, we examined whether such rearing experience translates into greater awareness of *V. shidai*'s feeding ecology. Third, we evaluated whether consumers perceive taste differences between *hachinoko* from wild nests and those from reared nests. Although our questionnaire was concise, it provides an initial overview of sensory impressions and feeding decisions among both keepers and nonkeepers. Based on the questionnaire results, we discuss potential gaps in local knowledge that could inform sustainable management and cultural practices. By integrating a molecular approach to diet analysis with a preliminary social survey, we provide a starting point for understanding how ecological data and local perceptions intersect, setting the stage for future, more detailed investigations into *V. shidai* biology, cultural significance, and sustainable utilisation.

Materials and Methods

1. **Sampling and Rearing Methods**

In Central Japan, wasp enthusiasts use a distinctive baiting method to locate and harvest *V. shidai* nests for consumption (Figure 1). By placing small, flagged meat balls (e.g., chicken or squid) in the field, they track foraging workers back to their colony (Saga, 2019; Figure 1ab). Once collected, nests are kept in semi-natural conditions, where the wasps are fed various foods such as chicken, frog, deer meat, and fish (Figure 1cde). The choice of feed often depends on the keeper's experience and wasp feeding preferences, although workers also remain free to forage in the surrounding environment.

This study collected five wild nests in Tsukechi Town and Takayama City, Gifu Prefecture, Japan. Additionally, seven nests were reared by local enthusiasts in Tsukechi Town, Ena City, Kushihara Town (Gifu Prefecture), and Anan Town (Nagano Prefecture; Figure 2). The wild nests were baited with Japanese dace (*Tribolodon hakonensis*) and chicken. From each nest, we obtained 1 to 6 final-stage larvae for dietary analysis. In

Tsukechi town, the appointed enthusiast fed reared nests with chicken, quail, deer, and wild boar meat, while in Ena City and Kushihara, only chicken was provided, and in Anan Town, chicken had been provided until two weeks prior to collection. The reared nests in Tsukechi Town were randomly selected each year from the same site containing around 20 nests, and the wild nests were collected from the same mountain slope within a 2.5 km radius of that site. Table 1 summarises the timing of nest collection (and sample collection for dietary analysis from reared nests), as well as the number of nests and individuals used. We followed Takahashi *et al*. (2016) to collect undigested gut content, obtaining approximately 2mm^3 of material from the mouth-side of the intestine.

Table 1. Number of worker larvae used for feed analysis

Location	Wild Nests	Reared Nests	Feed	Collection Dates
Tsukechi town	16 (4 nests)	20 (4 nests)	chicken, quail, deer, and wild boar meat	**Wild nests**: Sep. 2020 (1), Oct. 2021 (1), Nov. 2021 (2) **Reared Nests**: Nov. 2020, Nov. 2022.
Takayama city	4 (1 nest)	—	—	Nov. 2021
Anan town	—	4 (1 nest)	chicken	Nov. 2020
Ena city	—	4 (1 nest)	chicken	Nov. 2021
Kushihara town	—	4 (1 nest)	chicken	Nov. 2021
Total	20 (5 nests)	32 (7 nests)		

2. **DNA Experiments**

(1) DNA Extraction and Amplification

We extracted genomic DNA using the NucleoSpin® Tissue kit (MACHEREY–NAGEL, Germany) following standard procedures.

(2) 1st PCR

We used 1st-IntF (Leray *et al.* 2013) and 1st-HCOmR (Folmer *et al.* 1994) primers to amplify the mitochondrial DNA COI region. In each DNA extraction and PCR run, we included a negative control (sterile water) to monitor potential contamination and verify amplification success. The blocking primer specific to *V. shidai* mitochondrial COI (5'- CCA CCT TTA TCA TCT ATT ACA GGA CAT GAC TCT CCT TCT GTT G/3spC3/-3') was designed using the Primer-BLAST tool (NCBI) to specifically inhibit *V. shidai* COI amplification. We optimised the blocking primer concentration (0.2–0.5 µM) by running gradient PCR tests to minimise amplification of host DNA while maintaining robust amplification of prey DNA. We performed the PCR with TaKaRa Ex Taq Hot Start Version (Takara Bio Inc., Japan). To prevent amplification of the insect's own DNA from intestinal prey, we used a blocking primer specifically for V. shidai. The PCR conditions were an initial 2-minute reaction at 94°C, followed by 35 cycles of 30 seconds at 94°C, 15 seconds at 67°C, 30 seconds at 52°C, 30 seconds at 72°C, and a final extension for 5 minutes at 72°C.

(3) 2nd PCR

We performed tailed PCR using 2nd primers that include index sequences and KOD FX Neo (TOYOBO) at 0.20uL (1.0 U/uL). The PCR conditions started with a 2-minute reaction at 94°C, followed by 12 cycles of 10 seconds at 98°C, 30 seconds at 60°C, 30 seconds at 68°C, and a final 2-minute extension at 68°C. After the PCR, we added VAHTS DNA Clean Beads (Vazyme) in an amount equal to the PCR volume and purified the PCR products.

(4) Sequencing

We sequenced using the MiSeq system and MiSeq Reagent Kit v3 (Illumina) under 2x300 bp conditions. We used Qiime2 (ver. 2020.8) with the dada2 plugin to remove chimera and noise sequences, then created representative sequences and Operational Taxonomic Units (OTUs). We conducted BLASTN (ver.2.13.0) for phylogenetic estimation and compared sequences with those in Gen Bank (National Center for Biotechnology Information, USA) to identify species.

(5) Dilution

We removed low-frequency OTUs with fewer than 5 reads. We then used the rrarefy function in R to resample from each sample according to the obtained read counts, normalising the variation in total read counts of OTUs obtained from each wasp larva.

(6) Species identification

We identified sequences as belonging to the same species as the top-ranked reference in the GenBank database if the sequence similarity was 97% or higher, based on bit scores. If different species showed the same sequence similarity but different bit scores, we did not specify the species and instead considered it as belonging to the same genus, family, or order. We considered sequences with less than 97% similarity as unidentified. We also removed sequences of non-target species, the wasp themselves, mites, and humans due to the inability to distinguish them from contaminants.

3. **Questionnaire Survey**

A questionnaire survey was conducted on 3 November 2023, during the *V. shidai Nest Size Contest and Sales Event* in Kushihara, Ena City, Gifu Prefecture. The purpose of the survey was to assess whether individuals who consume *V. shidai* are aware of what the wasps feed on in the wild. Participants were recruited from visitors, including local residents and wasp enthusiasts. Of roughly 60 attendees, 58 chose to participate (response rate ~96%), suggesting responses may reflect a more informed subset of the population. To address possible self-selection bias, we note that our findings are most representative of this community rather than the broader public. Participants were informed that their responses would remain anonymous, and all provided verbal consent before completing the questionnaire. All questionnaires were administered in Japanese. The survey included the following items:

1. Age
2. Gender
3. Residence (up to the municipality level)
4. Questions about the consumption of *hachinoko* (larvae and pupae of *V. shidai*):
   (1) Have you ever eaten *hachinoko*?
   (2) If yes, when did you start consuming them?
   (3) Do you perceive a difference in taste between nests from wild and reared wasps?
   (4) If yes to (3), what kind of differences do you perceive?
5. Please select the organisms you think they feed on in the wild.
6. Please select the organisms you have observed them feeding on in the wild.
7. Please select the organisms you have been told by others that they feed on in the wild.
8. What do you think is the most suitable food for rearing these wasps?
9. Questions about rearing practices:
   (1) Have you ever reared *V. shidai*?
   (2) If yes, how many years of rearing experience do you have?
   (3) What food do you provide for them? Why did you choose that food?

For question 4(3), respondents were asked to select from the options "Yes," "No," or "Don't know." For questions 5–7, respondents could choose multiple answers from the following categories: "Insects and arthropods," "Mammals," "Birds," "Reptiles," "Amphibians," "Fish," or "None." This questionnaire survey was conducted with the approval of the Research Ethics Committee of the Graduate School of Human Development and Environment, Kobe University (Approval No. 692).

4. **Statistical Analysis**

(1) Feed of *Vespula shidai*

We conducted a t-test to compare the number of prey species between wild and reared *V. shidai* nests. To examine prey composition per larva, we calculated the dissimilarity using the Bray-Curtis index and applied non-metric multidimensional scaling (NMDS). We then conducted PERMANOVA (999 permutations in the adonis 2 function, vegan package in R), with nest type (wild or reared) and region as factors. Where necessary, we applied the Holm-Bonferroni method to correct for multiple comparisons. After detecting a regional effect, we focused on Tsukechi Town, which provided both reared and wild data, and conducted a PERMANOVA comparing reared versus wild nests.

We also employed an indicator species analysis (ISA) to identify species significantly associated with either wild or reared nests. This analysis was conducted using the indval function in R. A species-by-sample matrix was created from the metabarcoding data, and each sample was categorized into one of two groups: wild or reared nest. The IndVal (Indicator Value) for each species was calculated based on its relative frequency and relative abundance in each group, yielding a measure of how strongly that species is associated with a given group.

(2) Questionnaire Survey Analysis

Respondents who had no rearing experience and no *hachinoko* consumption experience were excluded. We first tested whether rearing experience influenced understanding about *V. shidai*'s wild diet and affected the feeding behaviours that were observed and reported. Fisher's exact tests were used for the analysis. In these analyses, the answer choices included broad taxonomic groups (e.g., insects/arthropods, mammals, birds, reptiles, amphibians, and fish). Because of limited sample size, mammals, birds, reptiles, amphibians, and fish were combined into a single "vertebrates" category for the statistical analyses. Where Fisher's exact test indicated a significant difference, a residual analysis was conducted as a post-hoc test to identify which specific answer choices differed according to wasp rearing experience. Next, to determine whether rearing experience was related to perceived taste differences between wild and reared *hachinoko* using a chi-squared test. All statistical analyses were conducted using R (R Core Team 2023).

Results

Diet of reared and wild nests of *Vespula shidai*

We analysed the gut contents of *Vespula shidai* larvae from 12 nests, identifying 324 prey species across 108 families and 26 orders (Figure 3; Table S1, S2, S3). The orders with significant diversity included Lepidoptera (163 species), Diptera (39 species), Hemiptera (27 species), Araneae (22 species), Hymenoptera (13 species), Orthoptera (9 species), Coleoptera (8 species), Psocodea (6 species), Odonata (4 species), and other insects/arachnids (10 species). Additionally, we identified 10 bird species and 13 other vertebrate species (including fish, reptiles, amphibians, and mammals).

Multiple vertebrate species were detected, including birds, mammals, amphibians, reptiles, and fish (Table S2). Bird DNA was detected in all nests (Table 2), including Japanese quail (*Coturnix japonica*), chicken (*Gallus gallus domesticus*), Eurasian jay (*Garrulus glandarius*), large-billed crow (*Corvus macrorhynchos*), and carrion crow (*Corvus corone*). Mammals such as sika deer (*Cervus nippon*) and wild boar (*Sus scrofa*) also appeared in multiple nests. Furthermore, three amphibian species (e.g., Schlegel's green tree frog, *Rhacophorus schlegelii*), three reptiles (e.g., Japanese grass lizard, *Takydromus tachydromoides*), and one fish (Japanese dace *Tribolodon hakonensis*) were detected.

The wild nests contained an average of 48.4 ± 34.9 prey species per nest (mean ± SD, n = 5), whereas 45.6 ± 19.9 prey species were present in the reared nests (n = 7), with no significant difference between the groups (t test, $p = 0.874$; see Table 2 for taxonomic details). The total number of vertebrate prey species did not differ significantly between the wild nests (5.0 ± 1.1) and reared nests (3.7 ± 1.4; t test, $p = 0.146$). However, when focusing specifically on wild vertebrate species, the number was significantly greater in wild nests (4.0 ± 1.6) than in reared nests (1.7 ± 1.2; t test, $p = 0.023$).

Bird DNA was detected in all the examined nests (Table 2). Mammalian DNA was absent in one wild nest in Tsukechi and one reared nest in Anan town (Table 2). The other ten nests exhibited mammalian predation, indicating broad feeding patterns. Deer DNA was detected in a reared nest that was intentionally fed deer in Tsukechi town. Additionally, deer and wild boar DNA were also found in a wild nest (Tsukechi town) and a reared nest (Ena town), neither of which were purposefully fed to the nest (see Table S2 for details).

We illustrated the prey species composition according to the collection region of *V. shidai* (stress value = 0.251, n = 44; Figure 4). Significant differences in prey species similarity were detected between reared nests and wild nests and across collection/breeding regions (PERMANOVA; n = 44; reared or wild: $R^2 = 0.08$, $p < 0.01$; region: $R^2 = 0.162$, $p < 0.01$; nest differences: $R^2 = 0.05$, $p < 0.01$). Further analysis restricted to Tsukechi town, where both reared and wild data were available, revealed distinct differences in prey composition on the basis of nest type (stress value = 0.214, PERMANOVA; n = 36; reared or wild: $R^2 = 0.13$, $p < 0.01$; nest differences: $R^2 = 0.340$ $P < 0.01$; Year: $R^2 = 0.08$, $p < 0.01$; Figure 5).

The indicator species analysis revealed that five species significantly associated with wild nests. Specifically, fruit-piercing moth (*Eudocima tyrannus*), *Ptilodon hoegei*, *G. glandarius*, *C. macrorhynchos*, and *C. corone* had significantly greater indicator values in wild nests ($P < 0.05$). Five species were significantly associated with reared nests, including giant hoverfly (*Phytomia* sp.), *Pyraustinae* sp., *C. japonica*, *G. gallus domesticus*, and *C. nippon* ($P < 0.05$). These indicator species showed the strongest specificity and fidelity for their respective nest types, thus distinguishing wild colonies from reared colonies within our dataset.

Table 2. Taxonomic composition of prey species from wild and reared nests of *Vespula shidai*. Data are presented for major taxonomic groups, with detailed data provided in Supplemental Tables S1-S3.

		Wild				Reared							
		Tsukechi				Takayama	Tsukechi				Kushihara	Ena	Anan
		a	b	c	d		e	f	g	h			
Insecta	Lepidoptera	17	19	18	16	70	25	13	31	19	28	41	10
	Diptera	0	6	1	0	14	2	1	7	1	10	9	3
	Hemiptera	3	4	2	1	6	0	2	1	2	3	5	3
	Hymenoptera	0	2	1	0	8	0	1	1	0	3	6	0
	Others[1]	0	6	0	0	9	5	1	3	3	10	8	1
Arachnida	Araneae	3	0	4	3	4	7	4	6	0	4	7	6
Malacostraca	Decapoda	0	0	0	0	0	0	0	1	0	0	0	0
Aves[†]	Galliformes	1	1	0	0	0	2	1	1	2	1	1	0
	Columbiformes	0	0	1	1	0	0	0	0	0	0	0	0
	Passeriformes	1	2	2	2	1	0	0	1	0	0	2	1
Mammalia	Cetartiodactyla	0	2	0	0	1	1	1	1	2	0	1	0
	Others[2]	0	0	1	1	0	0	1	0	0	1	0	0
Amphibia	Anura	0	0	0	1	3	1	1	1	0	0	0	0
Reptila	Squamata	0	0	1	0	0	0	0	0	0	1	2	0
Actinopterygii	Cypriniformes	1	1	0	0	1	0	0	0	0	0	0	0
	total	26	43	31	25	117	43	26	54	29	61	82	24

Note: Colony IDs (a-h) are provided only for Tsukechi town as multiple colonies were sampled in this region. Other regions had only one colony, so no IDs are assigned. [1] includes Blattodea, Coleoptera, Ephemeroptera, Mantodea, Neuroptera, Odonata, Orthoptera, Plecoptera, Psocoptera, Trichoptera.[2] includes Carnivora, Eulipotyphla, Rodentia. [†] Bird DNA was detected in all examined nests (see

Table S2 for detailed species list).

Diet of *Vespula shidai*: observations and implications

Significant differences were observed in participants' perceptions of *V. shidai* diets in the wild between the experienced and nonexperienced rearing groups (see "Believed" in Table 3; Fisher's exact test, $P = 0.024$). However, residual analysis revealed no significant differences ($P > 0.05$). Direct observations of *V. shidai* feeding behaviours revealed stronger group differences (see "Observed" in Table 3; Fisher's exact test, $P < 0.001$). Residual analysis revealed that among those without rearing experience, the proportion selecting "vertebrate only" was marginally lower than expected (Holm-adjusted $P = 0.077$). However, marginally fewer than expected participants with rearing experience selected the "none" category (Holm-adjusted $P = 0.077$), those without rearing experience selected this category more often than expected (Holm-adjusted $P = 0.018$). No significant differences were observed for the "insects only" or "insects and vertebrates" categories. Finally, concerning whether the participants had heard of *V. shidai* feeding on particular organisms in the wild, a marginally significant difference was found between those with and without rearing experience (Fisher's exact test, $P = 0.052$).

Table 3. Survey results on prey items of *Vespula shidai* for individuals with rearing experience (keepers) and those without rearing experience but with consumption experience (nonkeepers).

Question	Response	Keepers (n = 29)	Nonkeepers (n = 20)
Believed ("What do you think they feed on in the wild?")	Only insects/arthropods	5	2
	Only vertebrates	0	5
	Both insects/arthropods and vertebrates	22	11
	Unknown	2	2
Observed ("What have you actually seen them feed on?")	Only insects/arthropods	4	3
	Only vertebrates	4	1
	Both insects/arthropods and vertebrates	19	1
	None	2	15
Heard ("Have you heard they feed on specific organisms?")	Only insects/arthropods	3	0
	Only vertebrates	3	4
	Both insects/arthropods and vertebrates	14	4

None	9	12

Taste Differences Between Wild and Reared Hachinoko

A total of 58 individuals (49 men and 9 women) participated in the survey (mean age: 58.4 ± 21.9 years; range: 20–90 years). Among these participants, 29 had both rearing and consumption experience, whereas 29 had never reared wasps. Among those without rearing experience (n = 29), 20 had consumed *hachinoko*, and 9 had not.

Of the 29 participants who had reared *V. shidai*, 17 reported perceiving a difference in taste between *hachinoko* from wild nests and those from reared nests. In contrast, 3 reported no difference, and 9 were unsure. In contrast, among the 20 individuals who consumed *hachinoko* but had no rearing experience, 3 noted a difference, 1 reported no difference, and 16 were unsure. Responses regarding whether a taste difference exists between wild and reared *hachinoko* differed significantly between those with and without rearing experience (Fisher's exact test, $P = 0.002$). However, subsequent residual analysis indicated no significant differences ($P > 0.05$). With respect to the nature of the taste differences, the responses included comments on flavour (n = 3), including "wild *hachinoko* tastes better" (n = 3), "reared *hachinoko* is sweeter," "wild *hachinoko* is less sweet and tastes blander," "the reared *hachinoko* may reflect the taste of its provided feed," "there is a subtle difference," "there is a difference in taste," and "wild ones taste more natural." In addition, differences in aroma were noted. With respect to the specific feeds offered by those who reared *V. shidai*, 12 reported chicken liver, 10 reported chicken meat, 8 reported squid, 7 reported fish, 4 reported frog, 2 reported horse mackerel, 2 reported birds, 2 reported Japanese deer, and 2 reported crayfish. In addition, 1 participant each reported using chicken hearts, beef fillets, quail, minnows, rainbow trout, shrimp, apples, or pears. Most participants did not answer questions about their reasons for choosing these feeds; however, those who did respond noted that their choices were informed by information exchange during wasp nest competitions, affordability and availability, wasp-feeding preferences, and ease of handling.

Discussion

This study analysed 12 *V. shidai* nests (5 wild and 7 reared) using DNA metabarcoding, revealing 324 prey species across insects, arachnids, and vertebrates (Figure 3, Table 2). Among these species, 23 vertebrate species were detected, with greater diversity in wild nests than in reared nests (Table 2). These findings build on previous records of *V. shidai*'s diet, offering an expanded perspective on its prey spectrum. The differences in prey composition suggest that local feeding practices may partially replace or reduce natural foraging behaviours (Figure 4, 5). Moreover, the questionnaire survey provided insights into consumers' perceptions of *hachinoko* (Table 3). A comparison of the molecular evidence of diverse prey items with local recognition and experiences suggests that community knowledge may reflect aspects of *V. shidai*'s natural ecology. Below, we discuss how these results compare with those of global studies on social wasps' feeding ecology and examine the implications for sustainable management and cultural practices.

Dietary Diversity, Interspecies Comparisons, and Foraging Strategies

In this study, *V. shidai* displayed a broad prey spectrum, as evidenced by the detection of 324 prey species across 108 families and 26 orders from just twelve nests (see Figure 3 and Table 2 for details). All of the nests were collected from satoyama areas in Central Japan, which are known for their high biodiversity (Washitani, 2001; Kobori and Primack, 2003). This diverse ecological environment likely contributed to the large number of prey taxa observed. Notably, Lepidoptera accounted for the greatest number of species among the wasps' prey, which aligns with previous findings that Lepidoptera diversity is particularly high in satoyama habitats (Ohwaki *et al.*, 2007). Few comprehensive records of *V. shidai* prey items exist to date. In earlier surveys, *V. shidai* and *V. flaviceps* (sister species) were not yet distinguished, but Shida (1963) documented *V. flaviceps* prey species in satoyama near Tokyo, listing 27 Diptera, 10 Lepidoptera, 8 Hemiptera, 6 Araneae, and 1 species each of Orthoptera, Odonata, and Hymenoptera. These top four orders dominated in the present study (Figure 3), suggesting substantial biomass and easier hunting for *Vespula* wasps. However, newer taxonomic groups (e.g., Coleoptera and Psocodea) were detected for the first time using DNA metabarcoding (Figure 3; Table S1), indicating that *V. shidai* likely exploits a broader array of insects and arachnids than previously recognised.

Compared with those of other *Vespula* species, the prey spectrum of *V. shidai* is markedly broader. In Hawaii, workers of invasive *Vespula pensylvanica* from ten nests were shown, using DNA barcoding of retrieved meat samples, to prey on a total of 47 families across 14 orders, including pheasants (Phasianidae), perching birds

(Passeriformes), rodents (Muridae), and geckos (Gekkonidae) (Wilson *et al*., 2009). Similarly, on Ahuahu Island in New Zealand, DNA metabarcoding of nest faeces identified 33 prey species (including rodents) from three nests (9 individuals) of *Vespula germanica* and 68 prey species (including shorebirds) from 16 nests (48 individuals) of *Vespula vulgaris* (Schmack *et al*., 2021). More detailed interspecies comparisons, however, would require additional ecological data, such as colony densities. Although little is known about the population density of *V. shidai*, certain environments in New Zealand feature extremely high densities of *V. vulgaris* (Barlow et al., 2002). Adopting similar monitoring methods for *V. shidai* in Japan could facilitate robust cross-species comparisons and clarify how nest density might influence prey usage.

This study highlights the significant dietary behaviours of both reared and wild *V. shidai* nests and reveals that birds and mammals are common prey across almost all nests (Table 2). Recently, there has been a report of foragers of *Vespula germanica* preying on live bird chicks (Gorosito and Cueto, 2024), suggesting the possibility that *V. shidai* may also prey on chicks. However, it is generally considered that they primarily utilise vertebrate carcasses. In particular, wild nests that were not supplemented with vertebrate prey captured a significantly greater variety of vertebrate species than reared nests did (Table 2), suggesting that vertebrate carcasses, which are generally larger than arthropod carcasses and potentially rich in essential nutrients, contribute substantially to wasp development. These findings challenge the notion that wasps merely gather available prey near their nests, as nests reared at the same site display varied prey compositions. These findings suggest that individual nests may have distinct foraging preferences, which are influenced by the availability and nutritional value of prey.

The disparities in prey composition between reared and wild nests of *V. shidai* reveal the complexity of their dietary habits, which are influenced by both local environmental conditions and human intervention. This variation is crucial for understanding the survival, reproductive success, and broader ecological roles of these wasps. Hence, there is a need for species-specific feeding strategies, underscoring the importance of incorporating both natural and anthropogenic factors into conservation and management practices to ensure the sustainability of wasp populations and *hachinoko* food culture.

Despite these insights, our sampling was largely limited to nests collected in November and from only a few sites, primarily in Tsukechi town. This approach allowed us to focus on the peak reproductive phase, when the greatest variety of prey is likely returned to the nest. However, it also restricts our ability to draw robust conclusions about seasonal or regional variation. Future work could extend sampling to multiple seasons and localities, enabling researchers to discern how factors such as climate, local fauna availability, or habitat characteristics (e.g., suburbs and mountainous areas) may shape *V. shidai*'s dietary breadth.

Local Rearing Practices and Their Ecological Implications

The academic literature has provided only limited information on the diet of *V. shidai* beyond insects and arachnids. Occasional references to frogs and crayfish from Japanese sources (Matsuura, 1995) are among the few examples of nonarthropod prey. The present findings show that both wild and reared nests feed on a variety of vertebrates, including birds and mammals. In particular, reared nests provided with human-supplied meats, such as chicken or deer, consumed these items, suggesting the direct influence of local rearing practices on their diet. Notably, 65.5% (19/29) of the wasp keepers personally observed *V. shidai* preying on vertebrates in the wild. This aligns with the results of DNA metabarcoding, which detected numerous vertebrates in wild nests. Furthermore, reared nests contained fewer wild vertebrate species, indicating that once they obtain the minimum required amount of vertebrate prey, they might reduce active hunting. This pattern reflects their reliance on provided meats (chicken, deer, etc.) rather than foraging (see also Figures 4, 5). Although reared and wild nests differ significantly in their overall prey composition (Figure 5), the number of insect species they capture is similarly high in both groups. This finding implies that *V. shidai* may retain its potential role in insect population control under rearing conditions, despite relying less on wild vertebrate carrion. On the other hand, a shift away from carrion scavenging could modify the broader influence of wasps on food webs. For example, *Vespula germanica* disrupts carrion dynamics by outcompeting native vertebrate scavengers and altering decomposition processes (Spencer *et al*., 2020). These findings underscore the potential for wasps to influence energy flow in scavenging ecosystems, a dynamic that may also apply to *V. shidai* in Central Japan.

The indicator species analysis further revealed that avian DNA (e.g., quail, chicken, and crow) was strongly associated with both wild and reared nests, suggesting that birds are critical components of *V. shidai*'s diet. Notably, local wasp keepers recognised many of these same taxa as common prey, indicating a partial alignment between the DNA-based findings and lay observations. However, the simplified questionnaire did not allow us to fully explore how keepers' direct experiences match the diversity of prey revealed by metabarcoding. In the future, more detailed surveys or interviews could help clarify any discrepancies between actual prey usage and local knowledge, thus refining our understanding of how community-based insights intersect with molecular data.

Although the questionnaire did not explore the social or cultural mechanisms behind feed choices, it revealed a clear difference in observational knowledge between keepers and nonkeepers. Compared with 93.1% of keepers, only 25% of nonkeeper *hachinoko* consumers reported having observed what *V. shidai* feeds on in the wild—a difference that likely stems from the first-hand experience of rearing wasps. Leveraging keepers' knowledge could help accommodate regional differences in prey resources and guide novice keepers, with practical suggestions for sustaining the cultural tradition of *hachinoko* consumption.

From a cultural perspective, the rearing of *V. shidai* is a significant local tradition, with *hachinoko* serving as an autumn delicacy deeply ingrained in rural lifestyles. By bridging the gap between molecular data and community perceptions, this study underscores the potential for culturally informed management strategies that respect local food practices while promoting ecological sustainability. This integration of scientific insights and cultural traditions could ensure that wasp rearing remains both viable and ecologically mindful, particularly given the demand for *hachinoko*. Local knowledge emerges as a critical factor in enhancing our understanding of *V. shidai* diets and highlights opportunities for collaboration with citizen scientists. Further investigations into vertebrate predation by *V. shidai*, including its nutritional, ecological, and cultural dimensions, could inform wasp biology and guide sustainable management strategies.

Differences in Taste Perception between Wild and Reared Hachinoko

The questionnaire survey revealed that 58.6% of rearing practitioners perceived differences in taste between wild and reared *hachinoko*, with wild *hachinoko* is often described as having superior flavours and aromas. One intriguing response indicated that rearing-sourced *hachinoko* might reflect the flavour of the feed provided. Moreover, these perceptions align with our DNA metabarcoding findings, which revealed significant differences in prey composition between wild and reared nests. For example, wild nests presented greater vertebrate diversity, possibly contributing to flavour differences. Similar observations have been reported in crickets, where diet influences taste profiles (Ajdini *et al*., 2024; Umebara *et al*., 2024). These results suggest that dietary inputs may modulate the taste of *hachinoko*, warranting further investigation into flavour components and feeding practices.

These findings indicate that perceptions regarding potential taste differences between wild and reared *hachinoko* vary depending on whether individuals have rearing experience. The questionnaire data suggest that first-hand involvement in rearing can enhance one's ability to discern differences in flavour, whereas consumers without rearing experience may have limited opportunities for such comparisons. Furthermore, several keepers noted that *hachinoko* flavour varies on the basis of the type of feed provided (Saga, personal observation). Other factors, such as the duration of *hachinoko* consumption, rearing experience, and habitual consumption of wild foods, may also influence taste perception. However, further studies are needed to clarify these effects.

Conclusion

In conclusion, our study demonstrated that *Vespula shidai* has a broad prey spectrum, with DNA metabarcoding revealing 324 prey species across diverse taxonomic groups. Although reared nests rely less on wild vertebrate carrion due to supplementary feeding, the diversity of insect prey remains comparable to that of wild nests, suggesting that the capacity of wasps for insect population control is largely maintained. Furthermore, our preliminary questionnaire indicated that the direct rearing experience enhances awareness of *V. shidai*'s feeding ecology and influences taste perceptions of *hachinoko*. These integrated findings provide a valuable baseline for understanding the ecological and cultural dimensions of *V. shidai* utilisation. Future research should expand sampling across seasons and habitats and further investigate sociocultural factors affecting feed selection, ultimately guiding sustainable management of this unique food resource.

Author contributions

Tatsuya Saga conceived the ideas and designed the methodology; Tatsuya Saga and Haruna Fujioka collected the data; Tatsuya Saga analysed the data; Tatsuya Saga led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Competing interests

The authors declare that there are no competing interests.

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Figure legend

Figure1. Methods for collecting and rearing *Vespula shidai*. (a) A wasp lured by the feed is made to grasp a marked piece of meat. (b) A nest is excavated and placed inside a small wooden box to be transported back to the rearing site. (c) A collected nest is placed inside a wooden box for rearing. (d) Each keeper provides their chosen feed, such as chicken, deer meat, or frogs. (e) Workers swarming over chicken liver provided as feed. (f) A nest of *V. shidai* reared in a wooden box (positioned upside down).

Figure 2. Geographical distribution of collected and rearing sites for wild and reared nests in Gifu and Nagano Prefectures, Japan. Wild nests were collected in Tsukechi Town and Takayama City, Gifu Prefecture. Reared nests were raised by local enthusiasts in Tsukechi

Town, Ena City, Kushihara Town (Gifu Prefecture), and Anan Town (Nagano Prefecture).

Figure 3. Proportional representation of prey species identified in the gut contents of *Vespula shidai* larvae. This pie chart shows the distribution of prey species identified from the gut contents of *Vespula shidai* larvae, highlighting the diversity across 26 orders.

Figure 4. Similarity in diet composition between wild and reared nests of *Vespula shidai* in five municipalities (created using the Bray-Curtis index, stress value = 0.251, n = 44). Significant differences in the similarity of prey species were observed depending on whether the nests were reared or wild, and by the region where the wasps were collected and reared (PERMANOVA; n = 44; reared or wild: $R^2 = 0.08$, $p < 0.01$; collection region: $R^2 = 0.162$, $p < 0.01$; nest differences: $R^2 = 0.05$, $p < 0.01$).

Figure 5. Similarity in diet composition between wild and reared nests of *Vespula shidai* in Tsukechi town. Differences in prey composition were observed depending on whether the nests were reared or wild, and by the specific nest (PERMANOVA; stress value = 0.214, n = 36; reared or wild: $R^2 = 0.13$, $p < 0.01$; nest differences: $R^2 = 0.340$, $p < 0.01$; Year: $R^2 = 0.08$, $p < 0.01$).

Figure 1.

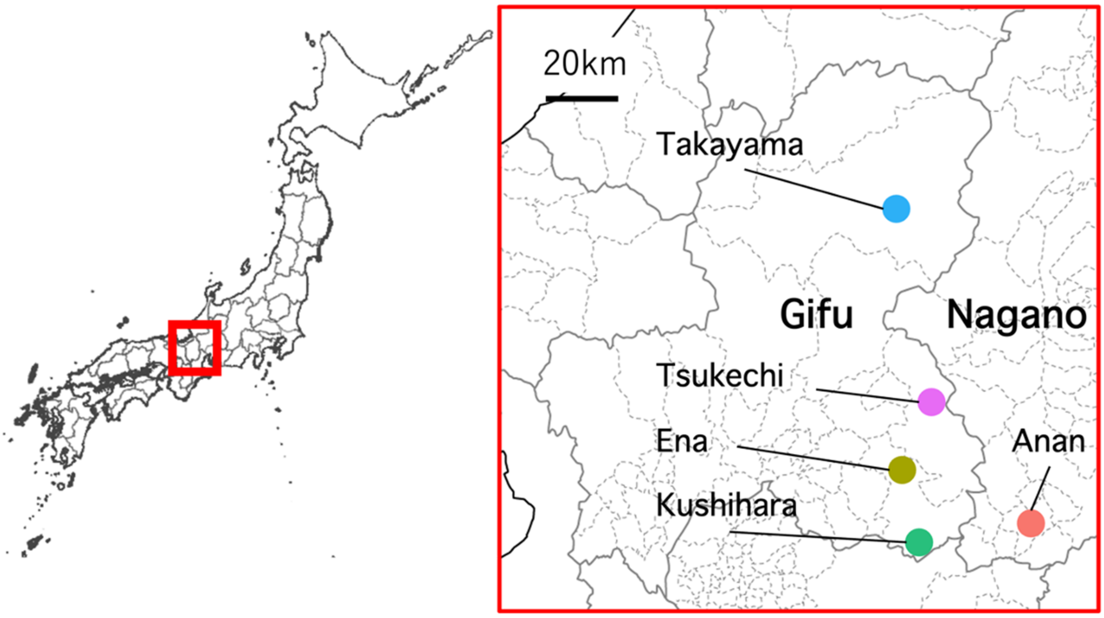


Figure 2.

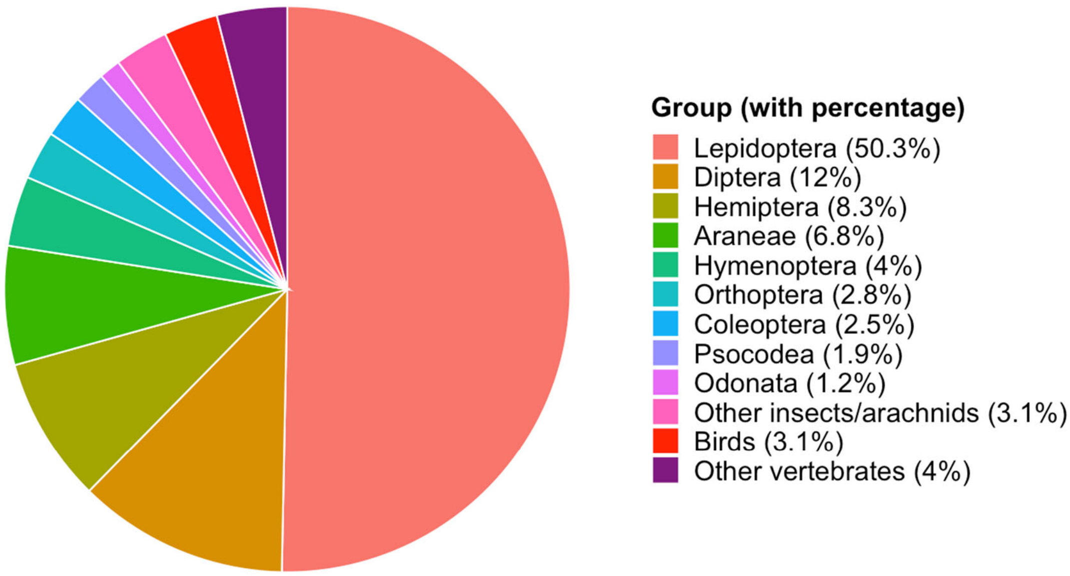
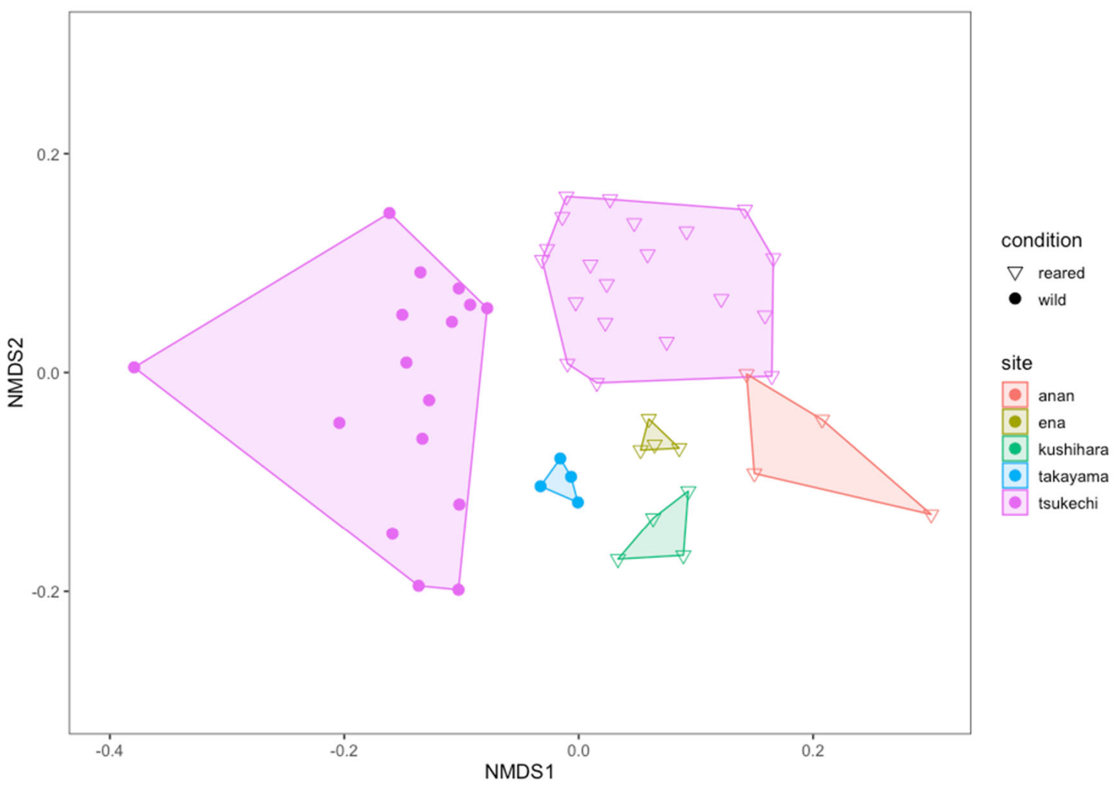


Figure 3.



NMDS1
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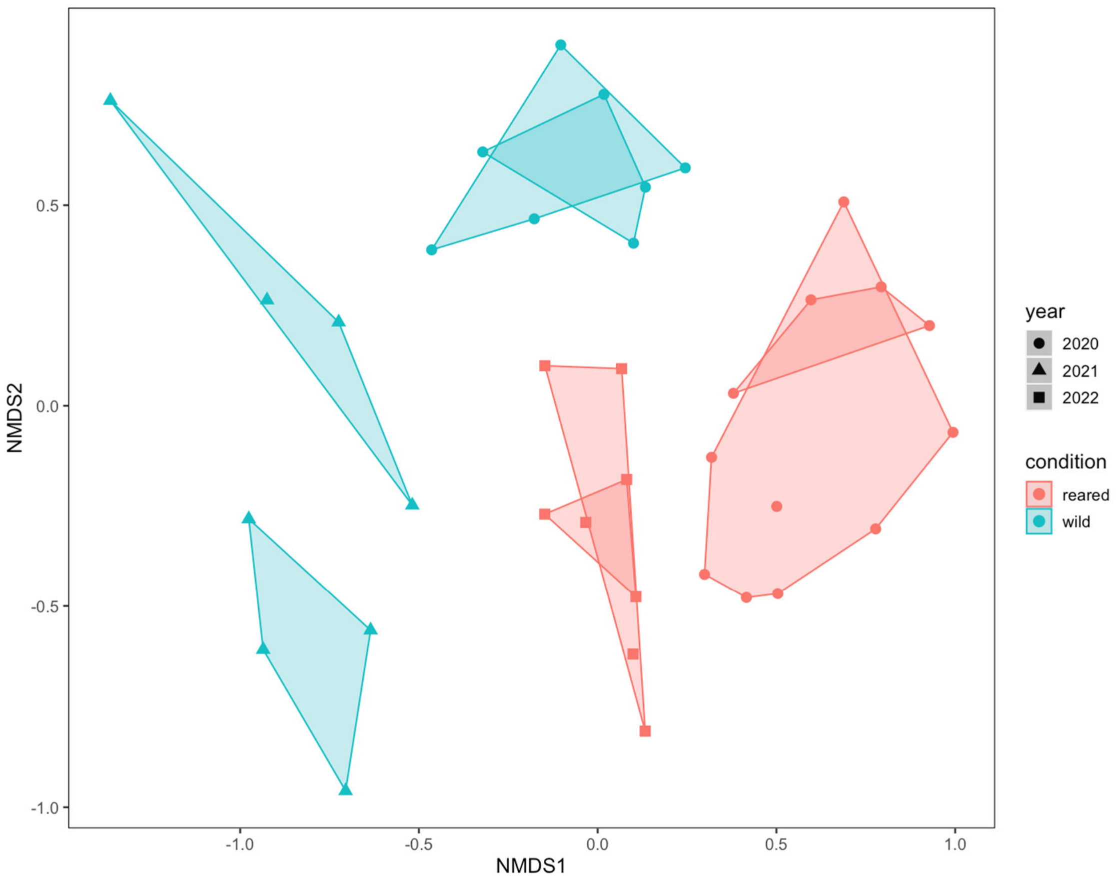
Figure 4.



NMDS1
NMDS2
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year
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2021
2022
condition
reared
wild

Figure 5.

Supplemental Material

Table S1. Taxonomic groups and number of prey species detected in the gut contents of Vespula shidai

Order	Family	Number of species detected
Neuroptera	Chrysopidae	1
	Hemerobiidae	1
Ephemeroptera	Berothidae	1
Psocodea	Trogiidae	1
	Liposcelididae	1
	Psocidae	4
Mantodea	Mantidae	1
Hemiptera	Flatidae	1
	Aphididae	7
	Tettigellidae	2
	Miridae	3
	Pentatomidae	2
	Acanthosomatidae	1
	Tingidae	1
	Reduviidae	1
	Cicadidae	1
	Coreidae	4
	Issidae	1
	Alydidae	1
	Rhopalidae	1
	Cicadellidae	1
Plecoptera	Nemouridae	2
Lepidoptera	Papilionidae	2
	Uraniidae	1
	Limacodidae	3
	Gelechiidae	7
	Lasiocampidae	3
	Gelechiidae	1
	Pantheidae	4
	Nolidae	4
	Geometridae	27
	Notodontidae	8
	Sphingidae	9
	Hesperiidae	2
	Nymphalidae	6
	Blastobasidae	1
	Pyralidae	17
	Epicopeiidae	1

	Lymantriidae	3
	Tortricidae	4
	Erebidae	5
	Scythrididae	1
	Psychidae	1
	Crambidae	3
	Noctuidae	45
	Saturniidae	5
Trichoptera	Limnephilidae	1
	Lepidostomatidae	1
Odonata	Lestidae	1
	Libellulidae	3
Coleoptera	Lucanidae	1
	Scarabaeidae	2
	Elateridae	4
	Silphidae	1
Blattodea	Blaberidae	1
Diptera	Fanniidae	1
	Muscidae	4
	Tipulidae	2
	Culicidae	1
	Calliphoridae	7
	Sarcophagidae	2
	Syrphidae	11
	Anthomyiidae	1
	Limoniidae	1
	Simuliidae	1
	Stratiomyidae	1
	Tachinidae	4
	Chironomidae	3
Hymenoptera	Formicidae	2
	Vespidae	5
	Trichogrammatidae	1
	Tenthredinidae	3
	Ichneumonidae	2
Orthoptera	Tettigoniidae	1
	Gryllotalpidae	1
	Gryllidae	1
	Stenopelmatidae	1
	Phaneropteridae	2
	Acrididae	3
Araneae	Tetragnathidae	3
	Uloboridae	1
	Clubionidae	2
	Araneidae	7
	Lycosidae	2

	Linyphiidae	1
	Nephilidae	2
	Salticidae	2
	Theridiidae	2
Decapoda	Potamidae	1
Cypriniformes (Osteichthyes)	Cyprinidae	1
Anura (Amphibian)	Rhacophoridae	2
	Hylidae	1
Squamata (Reptilla)	Lacertidae	1
	Scincidae	1
	Colubridae	1
Galliformes (Aves)	Phasianidae	2
Passeriformes (Aves)	Cettiidae	1
	Corvidae	3
	Leiothrichidae	1
	Muscicapidae	1
	Pycnonotidae	1
Columbiformes (Aves)	Columbidae	1
Carnivora (Mammalia)	Viverridae	1
Aetiodactyla (Mammalia)	Suidae	1
	Bovidae	1
	Cervidae	1
Eulipotyphla (Mammalia)	Talpidae	1
Rodentia (Mammalia)	Muridae	1

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Table S2. List of vertebrate preys detected from each nest.

		Wild nest					Reared nest						
		Tsukechi				Takayama	Tsukechi				Kushihara	Ena	Anan
		a	b	c	d		a	b	c	d			
Aves	Brown-eared bulbul (Hypsipetes amaurotis)	0	0	0	0	0	0	0	0	0	0	1	0
	Carrion crow (*Corvus corone*)	0	0	1	1	0	0	0	0	0	0	0	0
	Chicken (*Gallus gallus domesticus*)	1	1	0	0	0	1	1	1	1	1	1	0
	Eurasian jay (*Garrulus glandarius*)	1	1	0	0	1	0	0	0	0	0	0	1
	Japanese bush warbler (Horornis diphone)	0	0	0	0	0	0	0	1	0	0	0	0
	Japanese quail (*Coturnix japonica*)	0	0	0	0	0	1	0	0	1	0	0	0
	Kamchatka leaf warbler (Phylloscopus examinandus)	0	1	0	0	0	0	0	0	0	0	0	0
	Large-billed crow (*Corvus macrorhynchos*)	0	0	1	1	0	0	0	0	0	0	0	0
	Oriental turtle dove (*Streptopelia orientalis*)	0	0	1	1	0	0	0	0	0	0	0	0
	Red-billed leiothrix	0	0	0	0	0	0	0	0	0	0	1	0
Mammalia	Japanese grass vole (*Microtus montebelli*)	0	0	0	0	0	0	1	0	0	0	0	0

	Japanese serow (*Capricornis crispus*)	0	0	0	0	1	0	0	0	0	0	0	0
	Japanese shrew-mole (Urotrichus talpoides)	0	0	0	0	0	0	0	0	0	1	0	0
	Masked palm civet (*Paguma larvata*)	0	0	1	1	0	0	0	0	0	0	0	0
	Sika deer (*Cervus nippon*)	0	1	0	0	0	1	1	1	1	0	1	0
	Wild boar (Sus scrofa)	0	1	0	0	0	0	0	0	1	0	0	0
Amphibia	Forest green tree frog (Rhacophorus arboreus)	0	0	0	0	1	0	0	0	0	0	0	0
	Japanese tree frog (Hyla japonica)	0	0	0	1	1	1	1	1	0	0	0	0
	Schlegel's green tree frog (Rhacophorus schlegelii)	0	0	0	0	1	0	0	0	0	0	0	0
Reprila	Eastern Japanese common skink (Plestiodon finitimus)	0	0	0	0	0	0	0	0	0	0	1	0
	Japanese grass lizard (Takydromus tachydromoides)	0	0	0	0	0	0	0	0	0	1	1	0
	Japanese keelback (Hebius vibakari)	0	0	1	0	0	0	0	0	0	0	0	0
Actinopterygii	Japanese dace (Tribolodon hakonensis)	1	1	0	0	1	0	0	0	0	0	0	0
total		3	6	5	5	6	4	4	4	4	3	6	1

Note: Colony IDs (a-h) are provided only for Tsukechi town as multiple colonies were sampled in this region. Other regions had only one colony, so no IDs are assigned.

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Table S3. Taxonomic composition of prey species from wild and reared nests.

		wild				reared							
		Tsukechi				Takayama	Tsukechi				Kushihara	Ena	Anan
		a	b	c	d		e	f	g	h			
Insecta	Neuroptera	0	0	0	0	2	0	0	0	0	0	1	0
	Ephemeroptera	0	1	0	0	0	0	0	0	0	0	0	0
	Psocoptera	0	2	0	0	2	1	0	0	2	0	2	0
	Mantodea	0	0	0	0	1	0	0	1	0	0	1	0
	Hemiptera	3	4	2	1	6	0	2	1	2	3	5	3
	Plecoptera	0	1	0	0	1	1	0	0	0	0	0	0
	Coleoptera	0	1	0	0	1	0	0	0	0	6	0	0
	Blattodea	0	1	0	0	0	0	0	0	0	0	0	0
	Lepidoptera	17	19	18	16	70	25	13	31	19	28	41	10
	Trichoptera	0	0	0	0	0	1	0	0	0	0	1	0
	Odonata	0	0	0	0	1	1	1	0	0	0	1	1
	Diptera	0	6	1	0	14	2	1	7	1	10	9	3
	Hymenoptera	0	2	1	0	8	0	1	1	0	3	6	0
	Orthoptera	0	0	0	0	1	1	0	2	1	4	2	0
Arachnida	Araneae	3	0	4	3	4	7	4	6	0	4	7	6
Malacostraca	Decapoda	0	0	0	0	0	0	0	1	0	0	0	0
Aves	Galliformes	1	1	0	0	0	2	1	1	2	1	1	0
	Columbiformes	0	0	1	1	0	0	0	0	0	0	0	0
	Passeriformes	1	2	2	2	1	0	0	1	0	0	2	1

Mammalia	Cetartiodactyla	0	2	0	0	1	1	1	1	2	0	1	0
	Eulipotyphla	0	0	0	0	0	0	0	0	0	1	0	0
	Carnivora	0	0	1	1	0	0	0	0	0	0	0	0
	Rodentia	0	0	0	0	0	0	1	0	0	0	0	0
Amphibia	Anura	0	0	0	1	3	1	1	1	0	0	0	0
Reptila	Squamata	0	0	1	0	0	0	0	0	0	1	2	0
Actinopterygii	Cypriniformes	1	1	0	0	1	0	0	0	0	0	0	0
	total	**26**	**43**	**31**	**25**	**117**	**43**	**26**	**54**	**29**	**61**	**82**	**24**

Note: Colony IDs (a-h) are provided only for Tsukechi town as multiple colonies were sampled in this region. Other regions had only one colony, so no IDs are assigned.