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Multi-locus DNA metabarcoding reveals seasonality of foraging ecology of western spotted skunks in the Pacific Northwest

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Abstract

There are increasing concerns about the declining population trends of small mammalian carnivores around the world. Their conservation and management are often challenging due to limited knowledge about their ecology and natural history. To address one of these deficiencies for western spotted skunks (*Spilogale gracilis*), we investigated their diet in the Oregon Cascades of the Pacific Northwest during 2017–2019. We collected 130 spotted skunk scats opportunistically and with detection dog teams and identified prey items using DNA metabarcoding and mechanical sorting. Western spotted skunk diet consisted of invertebrates, such as wasps, millipedes, and gastropods; vertebrates, such as small mammals, amphibians, and birds; and plants, such as *Gaultheria*, *Rubus*, and *Vaccinium*. Diet also consisted of items, such as black-tailed deer, that were likely scavenged. Comparison in diet by season revealed that spotted skunks consumed more insects during the dry season (June–August), particularly wasps (75% of scats in the dry season), and marginally more mammals during the wet season (September–May). We observed a similar diet in areas with no record of human disturbance and areas with a history of logging at most spatial scales, but scats collected in areas with older forest within a skunk's home range (1-km buffer) were more likely to contain insects. Western spotted skunks provide food web linkages between aquatic, terrestrial, and arboreal systems and serve functional roles of seed dispersal and scavenging. Due to their diverse diet and prey switching, western spotted skunks may dampen the effects of irruptions of prey, such as wasps, during dry springs and summers. By studying the natural history of western spotted skunks in the Pacific Northwest forests, while they are still abundant, we provide key information necessary to achieve the conservation goal of keeping this common species common.

KEYWORDS
diet, DNA metabarcoding, generalist predator, land-use change, small carnivore, *Spilogale gracilis*

INTRODUCTION

Globally, many small mammalian carnivores (<16 kg) face decreasing population trends due to multiple threats, including land use change, disease, and overhunting (Belant et al., 2009; Marneweck et al., 2021). Even small carnivore species that were previously widely distributed and considered "least concern" by the International Union for Conservation of Nature (IUCN), such as weasels (*Mustela* spp. and *Neogale* spp.), have shown signs of significant population decline (Gompper, 2017; Jachowski et al., 2021). These declines are problematic because small carnivores can play important roles in ecosystem function, predator–prey dynamics, and disease transmission dynamics (Roemer et al., 2009). Despite their potentially important roles, many small carnivores remain neglected in ecological research, data deficient, or understudied (Marneweck et al., 2021; Proulx, 2010). Limited knowledge about small carnivore ecology and natural history continues to hinder management and conservation of declining populations.

In the Pacific Northwest, the western spotted skunk (*Spilogale gracilis*) is putatively a common forest carnivore, but little is known about their ecology due to their nocturnal nature (Doty & Dowler, 2006; Neiswenter et al., 2010; Neiswenter & Dowler, 2007; Verts et al., 2001). Most spotted skunk literature is derived from the island spotted skunk subspecies (*Spilogale gracilis amphialus*) (Crooks, 1994a, 1994b), the congeneric eastern spotted skunk (*Spilogale putorius*) (Kinlaw, 1995; Lesmeister et al., 2009; Lesmeister et al., 2010), and plains spotted skunk subspecies (*Spilogale putorius interrupta*) (Crabb, 1948), or other skunk species such as the pygmy skunk (*Spilogale pygmaea*) (Cantú-Salazar et al., 2005). These spotted skunks, however, inhabit markedly different ecosystems, such as on islands, prairie, or desert where there is limited forest vegetation. The Pacific Northwest, in comparison, is a temperate rainforest system that is dominated by large coniferous trees, and the functional role of western spotted skunks in this system is largely unknown. Due to their dietary plasticity, spotted skunks could vary from omnivorous generalist (e.g., eastern and plains spotted skunk) (Baker & Baker, 1975; Cheeseman et al., 2021; Crabb, 1941; Selko, 1937), insectivorous specialist (e.g., pygmy skunk) (Cantú-Salazar et al., 2005), or key carnivorous predator of small vertebrates (e.g., island spotted skunk) (Crooks & Van Vuren, 1995) in Pacific Northwest forests.

Eastern and plains spotted skunk populations are in severe decline, and, as a result, the eastern spotted skunk is now listed as vulnerable by the IUCN (Gompper & Jachowski, 2016), and the plains spotted skunk subspecies had been petitioned for listing under the US Endangered Species Act (US Fish and Wildlife Service, 2012). The mechanism for these declines is poorly understood, but multiple mechanisms have been proposed, including land-use change, disease outbreaks, and changes in predator communities (Gompper, 2017; Gompper & Mundy Hackett, 2005; Sasse, 2021). Although western spotted skunks are still relatively common and considered a species of least concern by the IUCN (Cuarón et al., 2016), western spotted skunks may be prone to future, rapid declines similar to those of eastern and plains spotted skunks. Studying western spotted skunks provides an opportunity to understand the functional roles of the species and amass basic ecological knowledge that may inform conservation and land management decisions.

Land use change is a potent disturbance in the Pacific Northwest given that it is an internationally important center of timber production (Simmons et al., 2016). Forest management can influence the structure and composition of forests with unknown consequences on the ecology of western spotted skunks. One way that land use change could cause declines in spotted skunk population densities is by causing declines in prey populations. For example, potential small mammal prey, such as Trowbridge's shrews (*Sorex trowbridgii*), shrew moles (*Neurotrichus gibbsii*), red tree voles (*Arborimus longicaudus*), and flying squirrels (*Glaucomys oregonensis*), are less abundant in young forest stands than in mature and old-growth forest stands (Carey, 1989, 1995; Gilbert & Allwine, 1991). Disturbances, such as commercial thinning, can reduce density of some small mammal prey, such as flying squirrels (Manning et al., 2012). In contrast, forest management can increase the abundance of flowers and fruits of understory plants through increased light penetration onto the forest floor (Wender et al., 2004). Thus, it remains important to investigate how western spotted skunk diets are impacted by forest management.

Characterizing the diet of small carnivores, however, has been difficult. New techniques, such as detection dogs and DNA metabarcoding, have improved our ability to find scat and identify prey items, respectively. Previously, diets of spotted skunks were difficult to study because scats were often deposited in rest sites (Lesmeister, Gompper, & Millspaugh, 2008; Selko, 1937), not on trails, and because spotted skunks exhibit an omnivorous diet consisting of insects, small vertebrates, and fruit (Baker & Baker, 1975; Crabb, 1941; Crooks & Van Vuren, 1995; Howell, 1906). These scats were typically collected opportunistically, mechanically sorted, and morphologically identified (Ewins et al., 1994; Sándor & Ionescu, 2009), but these processes had biases related to digestion that may render prey items unrecognizable (Galan et al., 2012; Symondson, 2002) and lead to misidentification of rare species

(Massey et al., 2021). This can be particularly problematic for small omnivorous predators that consume a wide breadth of prey items including plants, animals, and invertebrates because identifiers must have taxonomic expertise. Moreover, misidentification of carnivores from scat morphology has been problematic and has potentially led to biased results (Morin et al., 2016). Newer genetic approaches such as DNA metabarcoding (Eriksson et al., 2019; Monterroso et al., 2019; Roffler et al., 2021) can increase confidence in correctly identifying the carnivore (Morin et al., 2016), increase the number of prey items that can be correctly identified (Massey et al., 2021), and increase efficiency of identifying diet for a high volume of samples (Kartzinel et al., 2015), especially for omnivorous species (De Barba et al., 2014).

Here, we use DNA metabarcoding and mechanical sorting to provide the first comprehensive analysis of western spotted skunk diet in the Pacific Northwest and quantified seasonal variability in diet as a function of land use change. This improved understanding of western spotted skunk foraging ecology can elucidate their functional role as small vertebrate predators, insectivores, and frugivores.

METHODS

Study area

This study was centered around the H. J. Andrews Experimental Forest (HJA), which is located on the western slope of the Cascade Mountain Range near Blue River, Oregon (Figure 1). The area is surrounded by the McKenzie River Ranger District of the Willamette National Forest. Elevations range from 410 to 1630 m. The maritime climate consists of warm, dry summers and mild, wet winters. Mean monthly temperature range from 1°C in January to 18°C in July. Precipitation falls primarily as rain and is concentrated from November to March, and averages 230 cm at lower elevations and 355 cm at higher

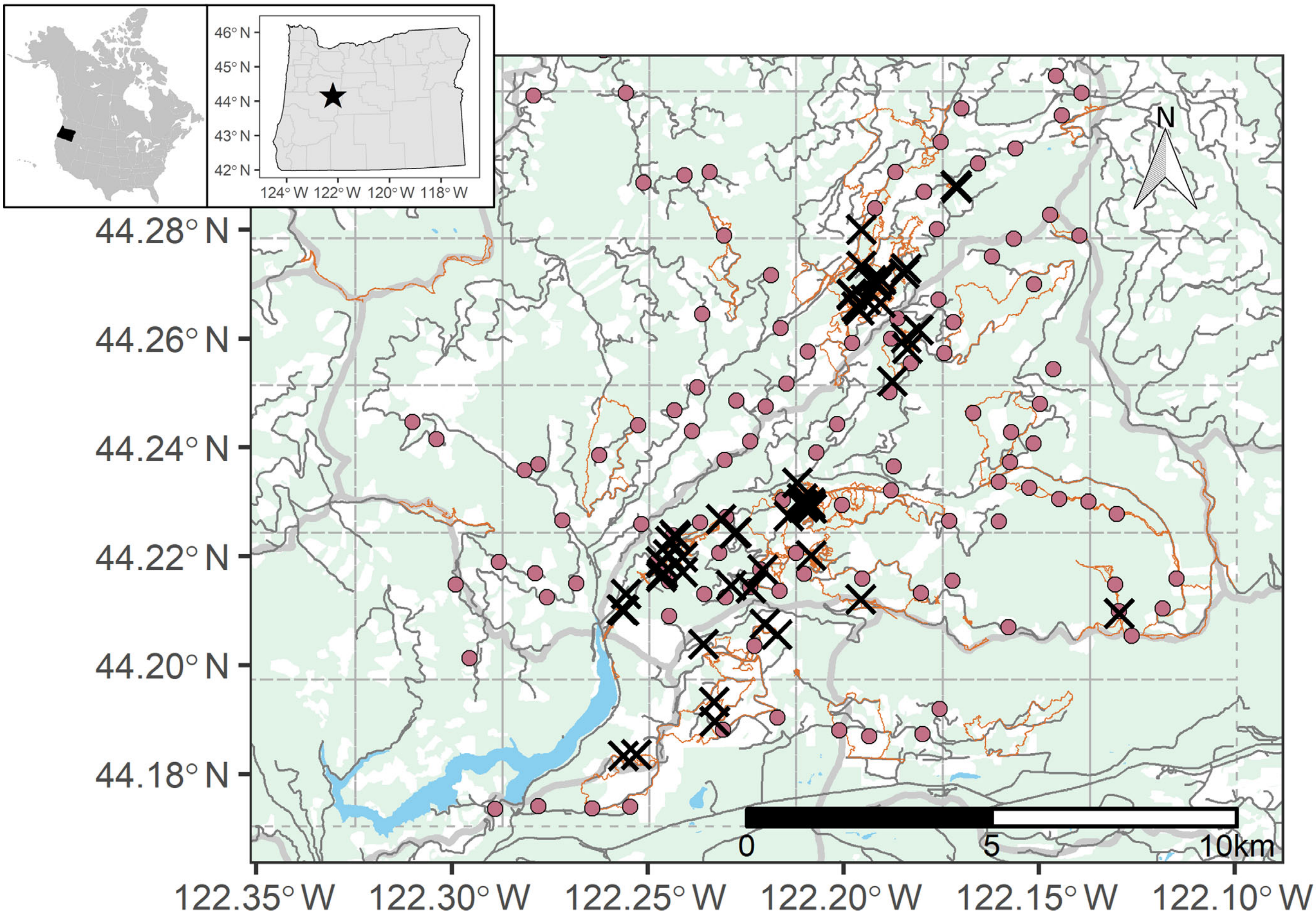

FIGURE 1 Study area within the Willamette National Forest in the Cascade Range of Oregon, USA, and locations of western spotted skunk (*Spilogale gracilis*) scats (black crosses). Detection dog tracks during the summer and fall of 2018 are shown in orange, 3 × 3 km survey grids are shown in gray dashed lines, and locations of camera traps are shown in maroon circles. Previously logged areas are shown in white. Roads are shown in dark gray lines, and outlines of watersheds are shown in thick, light gray lines.

elevations (Greenland, 1993; Swanson & Jones, 2002). During 2018–2019, western Oregon experienced an extreme drought (USDM, 2022). In Lane County, drought severity was greatest during August 2018–February 2019, but abnormally dry conditions began as early as January 2018 and moderate drought conditions began as early as June 2018 (Appendix S1: Figure S1).

Lower-elevation forests are dominated by Douglas-fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga hetemphylla*), and western red cedar (*Thuja plicata*). Upper-elevation forests are dominated by noble fir (*Abies procera*), Pacific silver fir (*Abies amabilis*), Douglas-fir, and western hemlock. The understory is variable and ranges from open to dense shrubs. Common shrubs included Oregon grape (*Mahonia aquifolium*), salal (*Gaultheria shallon*), sword fern (*Polystichum munitum*), vine maple (*Acer circinatum*), Pacific rhododendron (*Rhododendron macrophyllum*), huckleberry (*Vaccinium* spp.), and blackberry and salmonberry (*Rubus* spp.).

Before timber cutting in 1950, 65% of the HJA was covered in old-growth forest. Approximately 30% of the HJA was clear-cut or shelterwood cut to create plantation forests varying in tree composition, stocking level, and age. In 1980, the HJA became a charter member of the long-term ecological research network, and no logging has occurred since 1985. The Willamette National Forest immediately surrounding the HJA has a similar logging history, but logging continues to occur. Currently, the HJA consists of a higher percentage of old-growth forest than the surrounding Willamette National Forest (approximately 58% in the HJA vs. 37% in the study area) (Davis et al., 2022). Wildfires are the primary disturbance type, followed by windthrow, landslides, root rot infections, and lateral stream channel erosion. Mean fire return interval of partial or complete stand-replacing fires for this area is 166 years and ranges from 20 to 400 years (Morrison & Swanson, 1990; Teensma, 1987).

Field methods

Our western spotted diet study was part of a larger study on their spatial ecology in the temperate rainforest ecosystem of western Oregon that was conducted between April 2017 and September 2019. During this study, we set and maintained 112 baited trail cameras and captured and tracked western spotted skunks ($n_{\mathrm{F}} = 12$, $n_{\mathrm{M}} = 19$) using Tomahawk traps (Models 102 and 103, Tomahawk Live Trap, Hazelhurst, WI) and VHF radio collars (M1545, 16 g; Advanced Telemetry Systems, Isanti, MN). Cameras placed in the HJA were paired with previously established long-term songbird monitoring (Frey et al., 2016) and small mammal monitoring sites (Weldy et al., 2019). Cameras placed outside of the HJA were stratified based on elevation and old-growth structural index (Spies & Franklin, 1988) and chosen randomly within logistical constraints. Both cameras and live traps were baited with a frozen house mouse (*Mus musculus*), a can of sardines (*Culpidae*), and/or various carnivore scent lures. We located skunks using radiotelemetry triangulation and homing techniques daily, weather permitting. Homing techniques were mainly used to locate rest site locations during the day, whereas triangulation was used to locate skunks during the night when skunks were most active. All animal capture and handling were conducted in accordance with the guidelines set by the American Society of Mammalogists and were approved by the USDA Forest Service Institutional Animal Care and Use Committee (IACUC #2016-015) and the Oregon Department of Fish and Wildlife (Permit #107-17, 059-18, 081-19).

We collected western spotted skunk scat in multiple ways: (1) during western spotted skunk capture, (2) opportunistically while tracking western spotted skunks with radio collars and checking trail cameras, and (3) using detection dog teams (summer and fall of 2018). Detection dog teams either surveyed 3 × 3 km grids within the study area for a minimum of 6 h near camera trap locations where we detected western spotted skunk or focused their surveys around known spotted skunk rest sites. Focused surveys were necessary to increase skunk scat sample sizes and increase spotted skunk scat detection rates. Moreover, western spotted skunk scats were difficult to locate opportunistically because, typically, they were deposited after we tracked skunks to their rest sites and were in hard-to-search locations such as in hollow logs or a short distance from the rest site. We froze all scat samples until we processed them in the laboratory, and processed scats were dried for long-term storage.

Laboratory methods

In the lab, we identified the diet of western spotted skunks using DNA metabarcoding (Eriksson et al., 2019; Massey et al., 2021) and mechanical sorting. For DNA metabarcoding, we extracted DNA in a laboratory dedicated to processing degraded DNA using the DNeasy Blood and Tissue kit (Qiagen, Germantown, MD) or the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germantown, MD). We included an extraction blank with every batch of extractions as a negative control, where we used the same protocol but without a fecal sample (hereafter called extraction blanks). We kept extraction blanks throughout the DNA metabarcoding process.

Following DNA extraction, we amplified three regions of the mitochondrial and chloroplast DNA. First, we amplified a ~100-base pair DNA segment of the ribosomal mitochondrial 12S gene using universal vertebrate primers (12S-V5-F′: YAGAACAGGCTCCTCTAG and 12S-V5-R: TTAGATACCCCACTATGC) (Kocher et al., 2017; Riaz et al., 2011) and the chloroplast-encoded intron region of the trnL gene using universal plant primers (g-F: GGGCAATCCTGAGCCAA and h-R: CCATYGAGT CTCTGCACCTATC) (Taberlet et al., 2007) in a multiplex polymerase chain reaction (PCR). In a separate singleplex PCR reaction, we amplified the mitochondrial-encoded cytochrome oxidase subunit I (COI) gene using ANML universal arthropod primers (LCO1490-F: GGTCAACAAA TCATAAAGATATTGG and CO1-CFMRa-R: GGWACTA ATCAATTTCCAAATCC) (Jusino et al., 2019). We performed three PCR replicates per sample using the QIAGEN Multiplex PCR kit (Qiagen, Germantown, MD) (Appendix S1). To aid in identifying contamination, we performed PCR on a negative control on each plate (hereafter called PCR blanks) in addition to the extraction blanks. Each reaction was amplified with identical eight-base pair tags on the 5′ end of the forward and reverse primers that were unique to each sample to identify individual samples after pooling and to prevent misidentification of prey samples due to tag jumping (Schnell et al., 2015). We normalized and pooled the PCR products and used the NEBNext Ultra II Library Prep Kit (New England BioLabs, Ipswich, MA) to adapt the library pools into Illumina sequencing libraries (Illumina, San Diego, CA). We purified libraries using the Solid Phase Reversible Immobilization beads and sent libraries to the Center for Genome Research and Biocomputing at Oregon State University for 150 base pair paired-end sequencing on the Illumina HiSeq 3000.

We paired raw sequence reads using PEAR (Zhang et al., 2014) and demultiplexed samples based on the eight-base pair-index sequences using a custom shell script (Appendix S2). We counted unique reads from each sample replicate and assigned taxonomy using BLAST against the 12S, COI, and trnL sequences in a local database and GenBank (www.ncbi.nlm.nih.gov/blast). Scat amplification was considered successful if DNA sequencing produced over 100 total reads per replicate, and we limited the effects of contamination by retaining only species that consisted of more than 1% of the total reads. Furthermore, we used extraction and PCR negative controls to set additional filtering thresholds for species read counts. Species were only retained in the final species list if they were present in at least two of the three replicates and if their species distribution maps included our study area or were included on the species lists of the study area (https://andrewsforest.oregonstate.edu/about/species). We identified plants to genus since congeners are difficult to differentiate using these primers.

To mechanically sort scats, we placed dried scat contents in a petri dish and separated items using forceps. We identified remains macroscopically to the lowest taxonomic order possible (typically class or order). If we had used all fecal matter for DNA metabarcoding, we would have relied on notes on identifiable parts from when the scat was collected or processed samples for DNA extraction. Once mechanically sorted, we compared our findings to the DNA metabarcoding results for each scat. If the identified taxon was not included in the DNA metabarcoding results, we augmented the results with the missing taxon. We used mechanical sorting to augment results from DNA metabarcoding because of known biases introduced by mismatches in the universal invertebrate ANML primers we used, which are attributed to a lack of conserved regions across all invertebrates (Deagle et al., 2014).

We confirmed scats as defecated by western spotted skunks using the metabarcoding data following criteria: (1) western spotted skunk was the only carnivore (order: Carnivora) identified in the scat or (2) western spotted skunk was one of the carnivores identified in the scat, and the other carnivores consisted of less than 10% of the read count. We confirmed the predator in this way because predators are frequently misidentified through scat morphology (Lonsinger et al., 2015; Morin et al., 2016).

Data analysis

We conducted analyses and produced figures using the Program R (R Core Team, 2019). We quantified the importance of each taxonomic group by first calculating the frequency of occurrence of broader taxonomic group of prey (i.e., vertebrates, invertebrates, or plants). We calculated frequency of occurrence as a proportion of the number of scats in which each taxonomic group was present divided by the total number of scats. Due to the broad breadth of diet, we then calculated conditional frequency of occurrence of each species as the number of scats that contained the prey species divided by the total number of scats containing the broader taxonomic group. We also calculated the importance of each prey item by calculating the relative read abundance (RRA) for items identified through DNA metabarcoding by:

$$\mathrm{RRA}_i = \frac{1}{S}\sum_{k=1}^{S}\frac{n_{i,k}}{\sum_{i=1}^{T} n_{i,k}},$$

where $n_{i,k}$ is the number of sequences of prey species i in sample k, S is the number of scat samples, and T is the number of species. We produced figures relating taxonomy of prey items using the metacoder package

(Foster et al., 2017), and we produced rarefaction curves for each taxonomic group (species for vertebrates, genus for invertebrates and plants) using the iNEXT package (Hsieh et al., 2016) to estimate completeness and expected taxonomic richness of diet based on sample size.

To investigate the effect of season and disturbance history on western spotted skunk diet, we summarized the presence or absence (i.e., 1 for present, 0 for absent) of each taxonomic class (e.g., Mammalia, Insecta, and Gastropoda) per sample and fitted a binomial generalized linear model for multivariate data using the manyglm function in the mvabund package (Wang et al., 2012). We defined season as wet (1 for scat collected between October and May) or dry (0 for scat collected between June and September) and characterized past disturbance to the area at multiple scales: where the scat was collected (1 for previously logged, 0 for no record of logging) and within 0.1-, 0.5-, 1-, and 5-km buffer of where the scat was collected (0%–100% area within buffer that had been previously logged). We included season and past disturbance in additive models.

RESULTS

During October 2017–August 2019, we collected and genetically confirmed 130 western spotted skunk scats (summer, $n = 47$; fall, $n = 62$; opportunistic, $n = 21$). Fifty-eight scats (dry, $n = 32$; wet, $n = 26$) were collected from previously logged areas, and 72 scats (dry, $n = 25$; wet, $n = 47$) were collected from areas with no record of timber harvest.

We identified 27 vertebrate species, 43 plant genera, 15 arthropod species, and 3 mollusk species as prey using DNA metabarcoding (Figure 2). Atlantic herring (*Clupea harengus*), which we used to bait skunks to trail cameras and traps, was identified in 15 scats. Atlantic herring was the only prey item in two scats, so we removed these samples from the following analyses. After removing Atlantic herring, invertebrates were the most common prey items identified through metabarcoding and mechanical sorting (Figure 3). Invertebrates, especially insects, occurred in 85.2% of all scats ($n = 109$) (Figure 3). Vertebrates were the next most common prey item (58.6%, $n = 75$), and we detected mammals in 46.9% ($n = 60$), birds in 14.1% ($n = 18$), and amphibians in 13.3% ($n = 17$) of all scats (Figure 4). Finally, we detected plants in 28.9% of all scats ($n = 37$) (Figure 5).

Wasps (*Vespula* spp.) and millipedes (Diplopoda) were the top invertebrate prey items comprising 67.0% ($n = 73$) and 40.4% ($n = 44$) of scats containing invertebrates, respectively (Figure 3). The most frequent vertebrate naturally occurring prey items were the shrew mole (*N. gibbsii*),

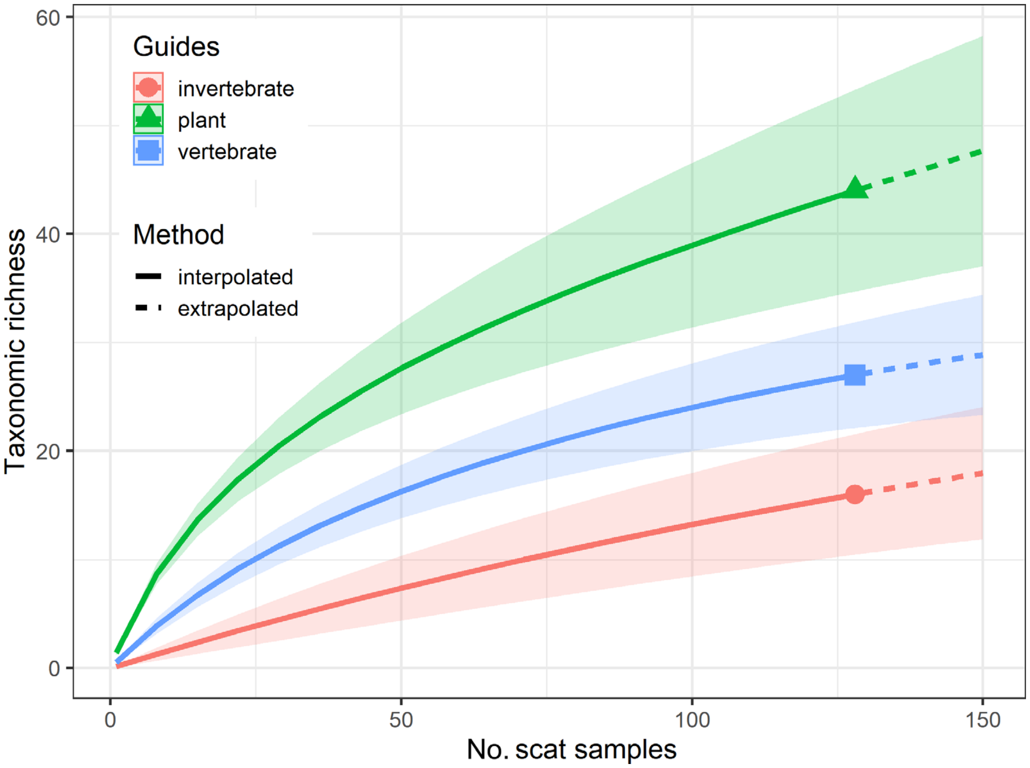

FIGURE 2 Estimation of prey taxonomic richness for western spotted skunks (*Spilogale gracilis*) in the Willamette National Forest. Vertebrate taxonomic richness (blue line) represents species richness. Invertebrate (red line) and plant (green line) richness represent genus richness.

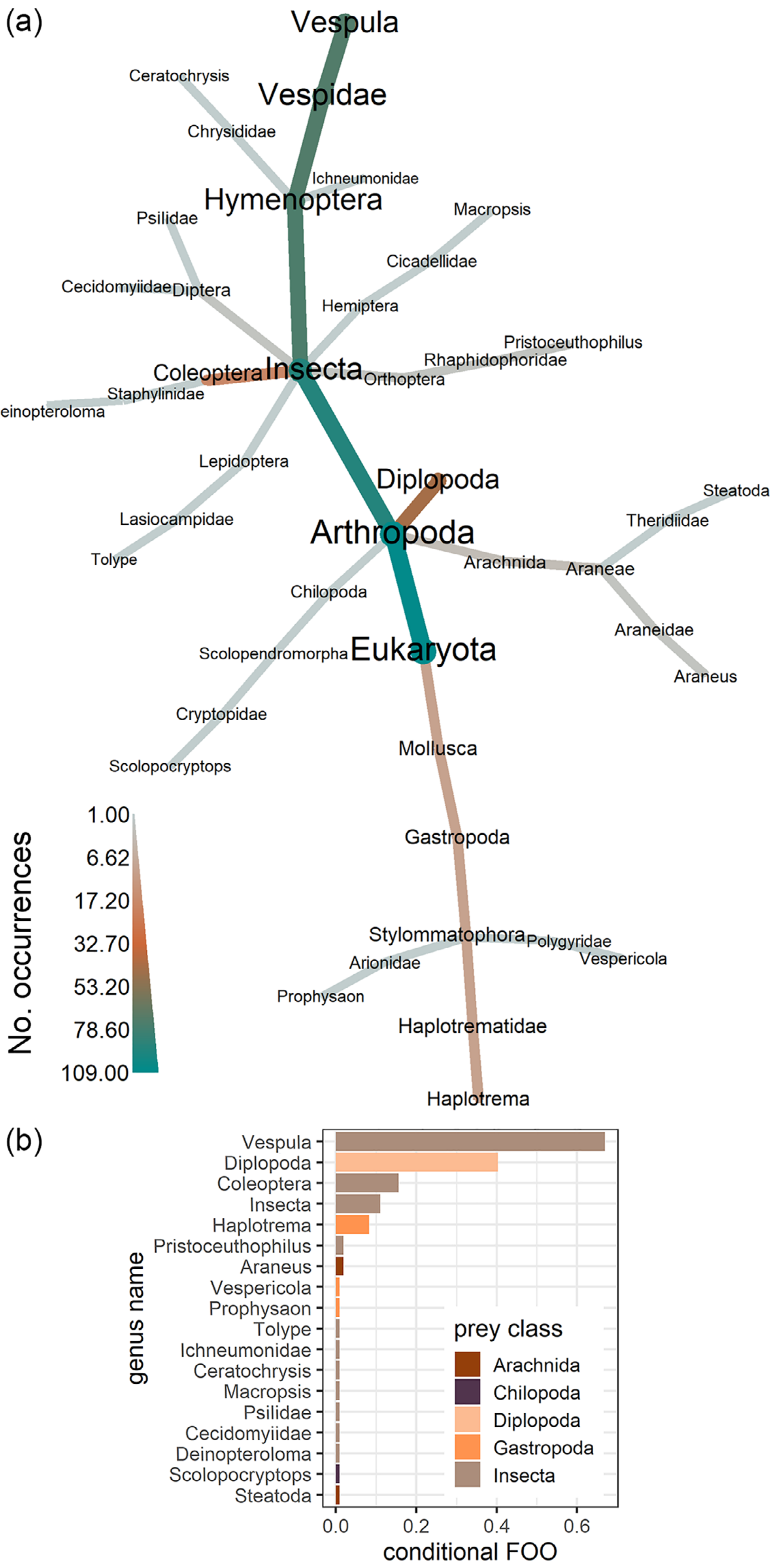

FIGURE 3 Invertebrate contents of western spotted skunk (*Spilogale gracilis*) scats ($n = 130$) collected from 2017 to 2019 in the Willamette National Forest were identified through DNA metabarcoding and mechanical sorting. (a) Taxonomic relationships of invertebrate diet items where color and size of nodes represent number of occurrences. (b) Frequency of occurrence (FOO) conditional on the presence of invertebrates in scat ($n = 109$).

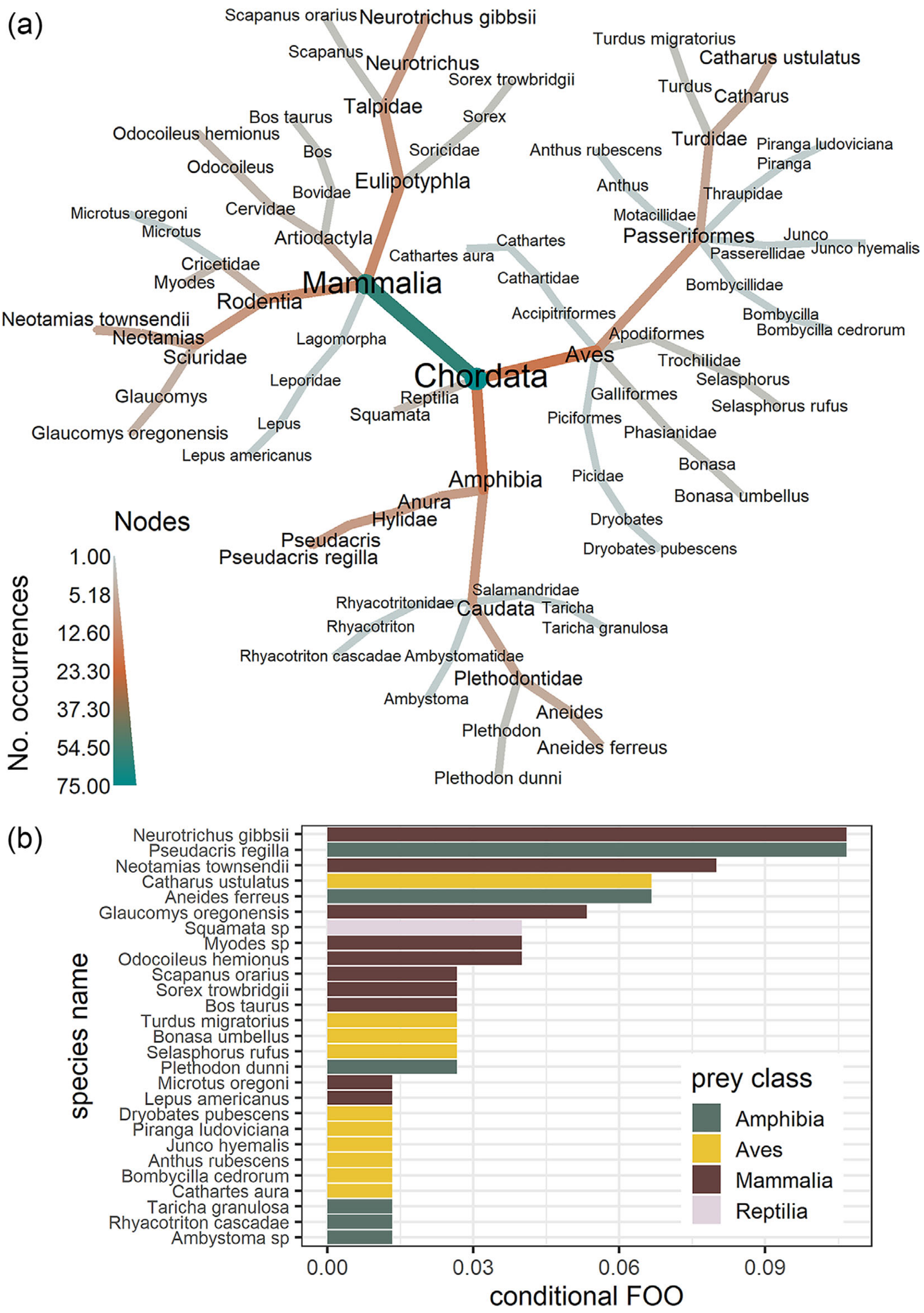

FIGURE 4 Vertebrate contents of western spotted skunk (*Spilogale gracilis*) scats ($n = 130$) collected from 2017 to 2019 in the Willamette National Forest were identified through DNA metabarcoding and mechanical sorting. (a) Taxonomic relationships of vertebrate diet items where color and size of nodes represent number of occurrences. (b) Frequency of occurrence (FOO) conditional on the presence of vertebrates in scat ($n = 75$).

Pacific tree frog (*Pseudacris regilla*), Townsend's chipmunk (*Neotamias townsendii*), Swainson's thrush (*Catharus ustulatus*), clouded salamander (*Aneides ferreus*), and Humboldt's flying squirrel (*G. oregonensis*) (Figure 4).

The most frequent plant items were Douglas fir (*Pseudotsuga*), maple (*Acer*), Hemlock (*Tsuga*), Gaultheria (*Gaultheria*), Alder (*Alnus*), and Rhododendron (*Rhododendron*) (Figure 5).

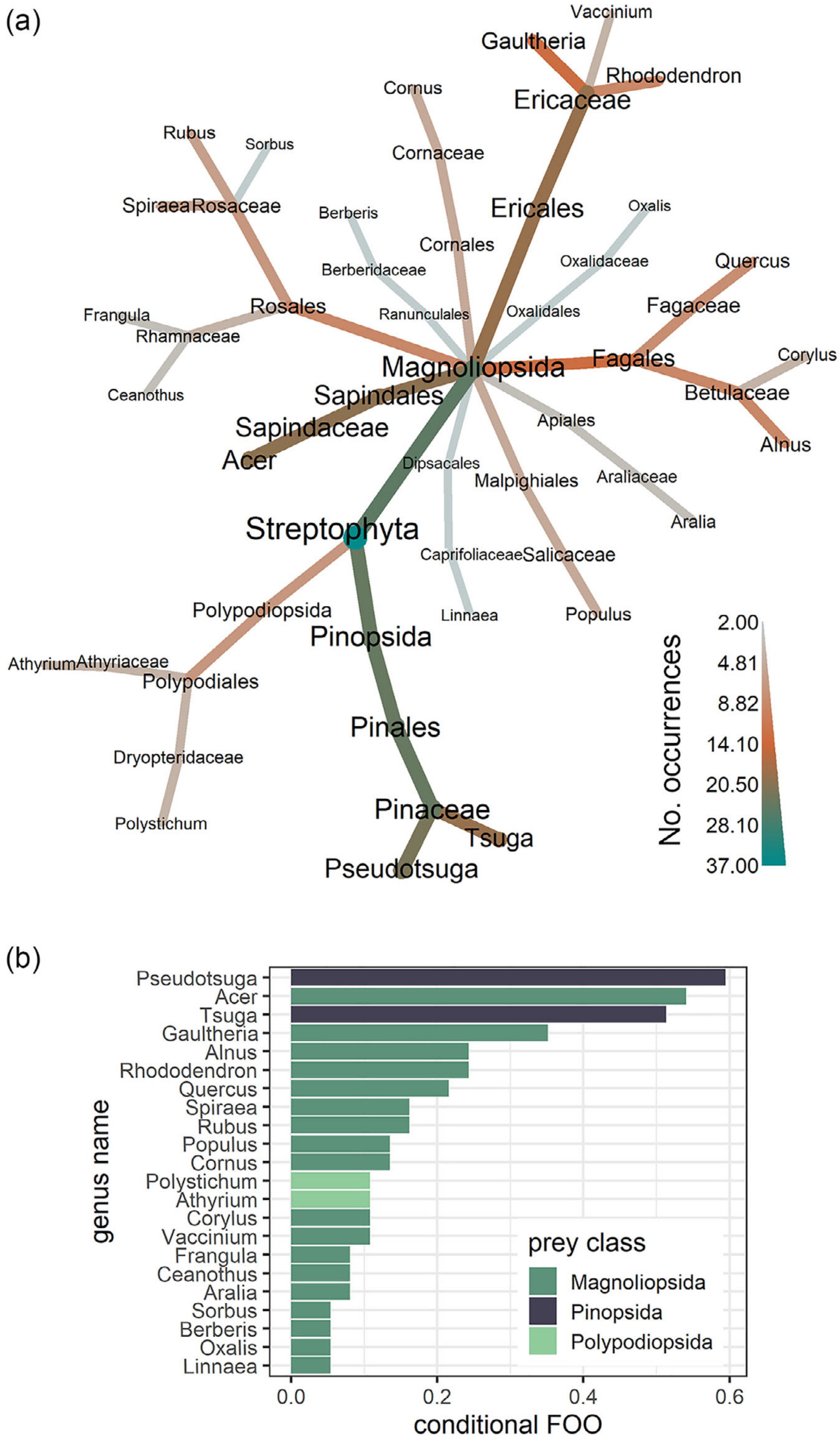

FIGURE 5 Plant contents of western spotted skunk (*Spilogale gracilis*) scats ($n = 130$) collected from 2017 to 2019 in the Willamette National Forest were identified through DNA metabarcoding and mechanical sorting. (a) Taxonomic relationships of plant diet items where color and size of nodes represent number of occurrences. (b) Frequency of occurrence (FOO) conditional on the presence of plants in scat ($n = 37$). Note only plants detected in more than one scat were shown.

Of the 130 scats, 51 scats (39.2%) only amplified western spotted skunk DNA, and 67 scats (51.5%) did not contain any vertebrate DNA other than Atlantic herring or western spotted skunk. Manual inspection of all scat samples revealed that the majority consisted of invertebrate body parts such as wasps (Vespidae) ($n = 71$), millipedes (Diplopoda) ($n = 43$), and snail shells (Gastropoda) ($n = 6$) that failed to amplify with ANML primers.

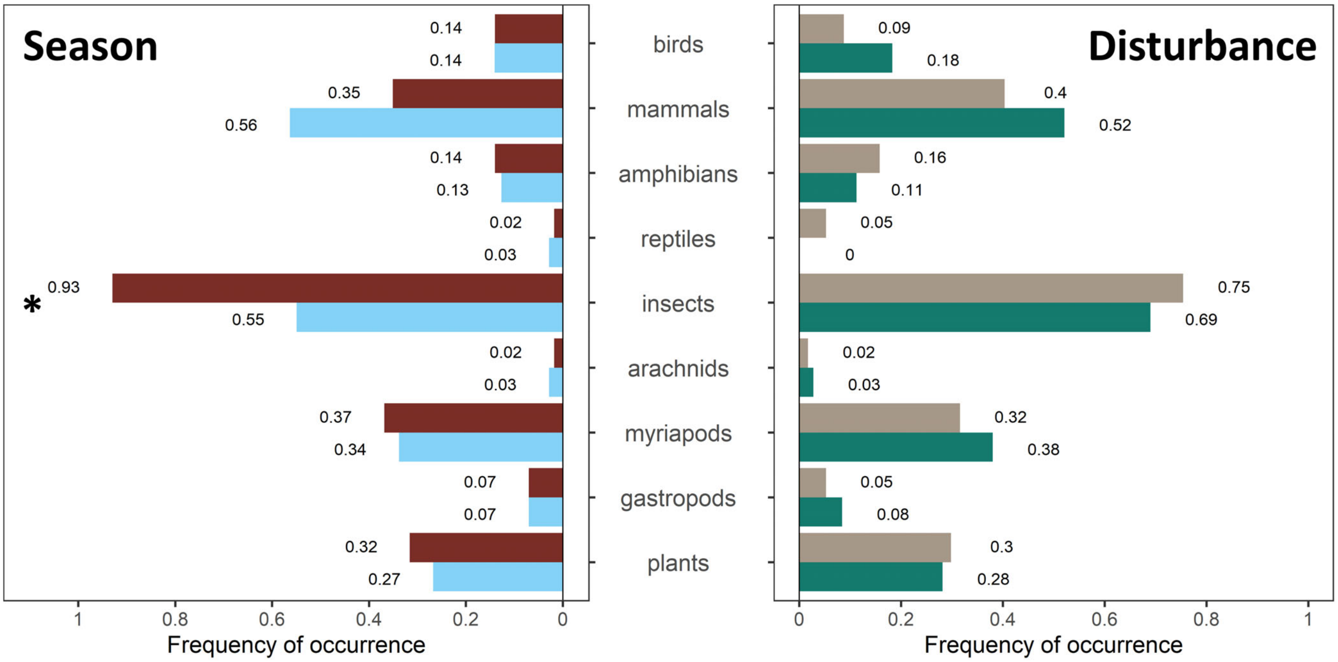

FIGURE 6 Frequency of occurrence (FOO) of taxonomic groups by season and disturbance in western spotted skunk (*Spilogale gracilis*) scats ($n = 128$) collected from 2017 to 2019 in the Willamette National Forest. Contents are determined by DNA metabarcoding and mechanical sorting. Left panel shows FOO by season (dry in red, wet in blue), and the right panel shows FOO by amount of disturbance in the location we collected the scat (previously logged in tan, no record of logging in green). The asterisk represents significant differences in FOO by taxonomic group ($\alpha = 0.05$).

Invertebrate remains mainly consisted of exoskeletons and head parts, indicating that skunks primarily consumed individuals in the adult life stage. We also found feathers ($n = 3$), snakeskin ($n = 4$), and plant material such as Douglas fir needles and bark (likely consumed incidentally along with other food items), indicating that primers and DNA quality in some scats did not allow for detection of all diet items.

Diet composition of western spotted skunks differed based on season (season, likelihood ratio test (LRT) $= 32.0$, $p = 0.001$), but not based on the collection site's logging history (logged, LRT $= 10.7$, $p = 0.35$). During the dry season, diet was composed primarily of insects (season, insect, LRT $= 25.4$, $p = 0.001$) (Figure 6), and wasps were detected in 75% ($n = 43$) of scats. Although plant material consumed was similar across seasons and the collection site's logging history (season, plant, LRT $= 0.36$, $p = 0.996$; logged, plant, LRT $= 0.007$, $p = 0.98$), there were more plants from genera that produce fruit during the dry season, including *Rubus* ($n = 6$), *Vaccinium* ($n = 1$), and *Gaultheria* ($n = 10$). During the wet season, western spotted skunks consumed *Gaultheria* ($n = 3$) and *Vaccinium* ($n = 3$), but no *Rubus*. Similarly, although mammals and amphibians consumed were similar across season and collection site's logging history (season, mammal, LRT $= 5.8$, $p = 0.12$; season, amphibian, LRT $= 0.05$, $p = 0.999$), wet season scats consisted of more small mammals (*N. townsendii*, *N. gibbsii*, *Myodes* spp., *Sorex* spp.) and amphibians, such as salamanders (*Rhyacotriton*, *Plethodon*, *Ambystoma*, and *Aneides*) (Figure 6).

When examining the effect of the amount of disturbance in the area surrounding each scat at multiple scales, the composition of scats only differed with the percentage of logged area within a 1-km buffer (p_logged 1 km, LRT $= 20.3$, $p = 0.03$). Insect presence in the scat decreased with an increasing percentage of area that was previously logged and was the only taxonomic class that showed a slight response to the percentage of area logged (p_logged 1 km, insect, LRT $= 6.43$, $p = 0.105$) (Figure 7).

DISCUSSION

This study provides the first data on the diet of western spotted skunks in the Pacific Northwest and represents the first use of DNA metabarcoding for high-resolution spotted skunk diet analysis. In the coniferous forests of the Oregon Cascades, the western spotted skunk diet was highly diverse and included mammals, birds, amphibians, reptiles, insects, gastropods, and plants. The combined methods of DNA metabarcoding and mechanical sorting revealed that invertebrates were the primary diet items and mammals were the secondary diet items for western spotted skunks, which is consistent among other food habit studies conducted on skunks (Baker & Baker, 1975;

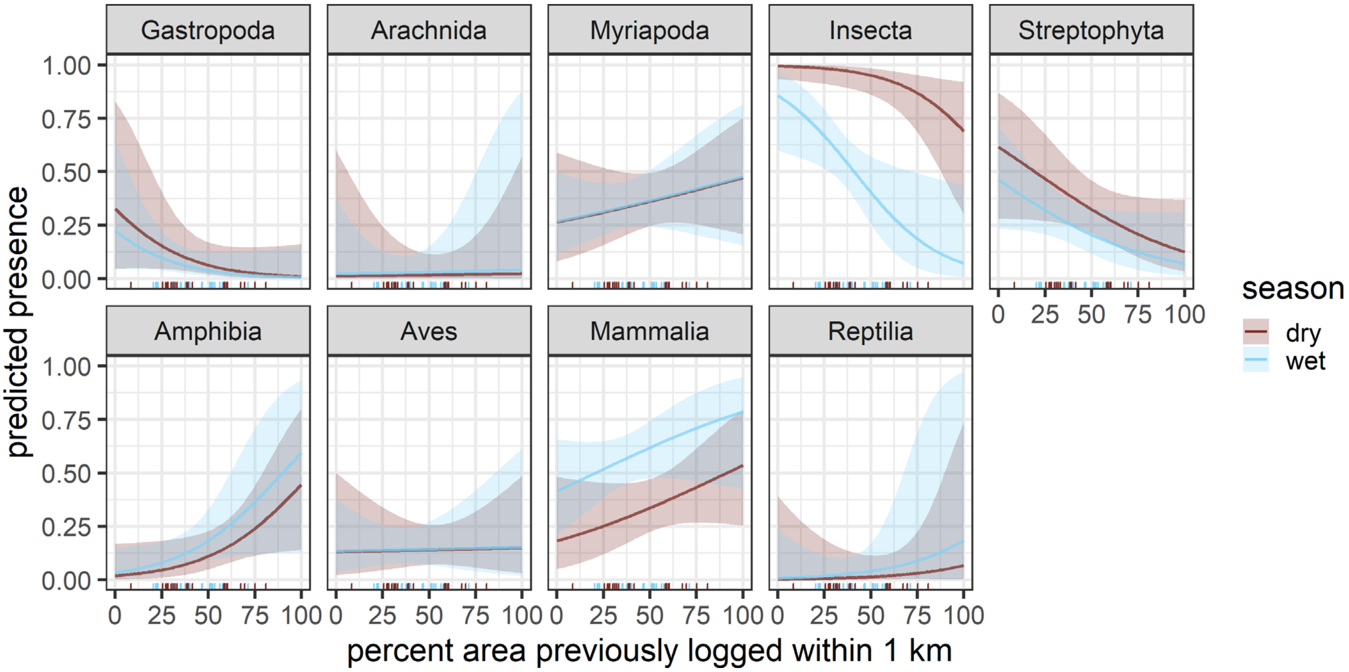

FIGURE 7 Predicted response curves representing the probability of presence of each taxonomic class in western spotted skunk scat to percent area that was previously logged within a 1-km buffer of the location where scat was collected. Response curves for dry season (June–September) are in red and wet season (October–May) in blue. Ribbons represent 95% confidence intervals.

Cantú-Salazar et al., 2005; Crabb, 1941; Selko, 1937). The importance of these diet items shifted by season, where skunks consumed more insects during the dry season.

We detected substantially higher frequency of Vespidae in this study than in diets reported for the island spotted skunk (Crooks & Van Vuren, 1995), the pygmy skunk (Cantú-Salazar et al., 2005), and the plains spotted skunk (Crabb, 1941; Howell, 1906; Selko, 1937). In these studies, grasshoppers and crickets (Orthoptera) and beetles (Coleoptera) were consumed more frequently (Baker & Baker, 1975; Cantú-Salazar et al., 2005; Crabb, 1941; Howell, 1906). The scats collected for this study were collected during a year when the Oregon Cascades were unusually dry during the spring and summer (Appendix S1: Figure S1). These conditions have been shown to be correlated with irruptions in wasp populations (Akre & Reed, 1981; Dejean et al., 2011), and wasps were observed to be more abundant on the landscape (W. Gerth, personal communication, 2020). Thus, higher consumption of wasps during these irruptions suggests that western spotted skunks can switch from one prey item to another and capitalize on abundant resources as generalist predators.

As expected, the vertebrate prey base of the western spotted skunk in Oregon was more diverse than that of the island spotted skunk, which only consumed one mammal species (*Peromyscus maniculatus*) (Crooks & Van Vuren, 1995), considering mammal diversity is greater in the Oregon Cascades. Unlike many of the other studies on skunks (except Howell, 1906; Sprayberry & Edelman, 2016), the western spotted skunk in the Oregon Cascades consumed a variety of amphibian species, which are tightly associated with aquatic systems. DNA metabarcoding also revealed that western spotted skunks consumed 11 avian species that would have been difficult to identify with mechanical sorting. During mechanical sorting, we were only able to identify avian species in three scats through the presence of feathers, suggesting that skunks may be consuming eggs rather than adult birds. Still, these avian and flying squirrel prey species connect western spotted skunks to the arboreal system. Together, western spotted skunks consumed a great diversity of prey, including arthropods, small and large mammals, amphibians, and birds, which is more diverse than all other mammalian carnivores in this region. Given their generalist diet, relatively high abundance, and terrestrial habits, the western spotted skunk may provide substantial linkages among terrestrial, aquatic, and arboreal systems in the Pacific Northwest by facilitating energy and nutrient transfer.

In addition to linking these disparate systems, western spotted skunks may perform important roles in ecosystem as seed dispersers, scavengers, and disease reservoirs. We identified plants in western spotted skunk scats with fruiting bodies, including berries (e.g., *Vaccinium*, *Rubus*, *Gaultheria*) and mast (e.g., *Acer*, *Quercus*, *Corylus*).

Western spotted skunks may provide key movements that allow for long-distance dispersal of seeds and may influence plant communities similar to martens and foxes (González-Varo et al., 2013; Jordano et al., 2007). We also identified black-tailed deer in three scats that we collected opportunistically. We observed radio-collared western spotted skunks scavenging on kills made by mountain lions (*Puma concolor*) on trail camera videos on multiple occasions and tracked western spotted skunks to rest sites adjacent to mountain lion kill sites. This behavior has been observed in other systems (e.g., California scrub oak forest), where the western spotted skunk has the ability to displace gray fox (*Urocyon cinereoargenteus*) from carcasses and mountain lions from their kill sites (Allen et al., 2013; Allen et al., 2016). Furthermore, this signifies the importance of these kill sites and carrion as food resources that are worth the risks associated with being near or directly encountering larger predators (Allen et al., 2016; Briffa & Sneddon, 2007; Ruprecht et al., 2021). Finally, we identified possible direct and indirect pathways for transmission of nematode parasites such as *Skrjabingylus* spp., which require spotted skunks as definitive hosts to complete their life cycle (Higdon & Gompper, 2020; Kirkland Jr. & Kirkland, 1983; LaRose et al., 2021; Lesmeister, Millspaugh, et al., 2008). Western spotted skunks have been shown to exhibit a high prevalence and high severity of *Skrjabingylus* spp. infection (Higdon & Gompper, 2020). Direct transmission of this nematode may occur through consumption of gastropods, which are the obligate intermediate host (Kirkland Jr. & Kirkland, 1983; Lankester & Anderson, 1971), and indirect transmission may occur through consumption of gastropod-consuming vertebrates that serve as paratenic hosts such as chipmunks (*N. townsendii*), shrew moles (*N. gibbsii*), shrews (*S. trowbridgii*), voles (*Myodes* spp.), and amphibians (Gamble & Riewe, 1982). Given that *Skrjabingylus* spp. can cause significant osteologic damage to the cranium, it is possible that this parasite could have significant impacts on individual fitness and population dynamics (Hughes et al., 2018; Lankester & Anderson, 1971).

Seasonal changes in diet for western spotted skunks were similar to other skunk species, where skunks switched from consuming more insects during the dry season to more vertebrate prey during the wet season (Crabb, 1941; Crooks & Van Vuren, 1995). Island spotted skunks increased their consumption of crickets during the dry season and mice during the wet season (Crooks & Van Vuren, 1995). Prairie spotted skunks primarily consumed rabbits and mice during the winter and spring and insects during the summer and fall (Crabb, 1941). These seasonal changes in diet likely reflect changes in availability and abundance of invertebrate resources and plasticity in spotted skunk diet (Cantú-Salazar et al., 2005). In addition to the changes in availability of invertebrate resources, western spotted skunks may switch their diet to one that includes more vertebrates because they need more caloric and protein-rich input to thermoregulate and survive the harsher, colder weather (Moors, 1977). Moreover, western spotted skunks breed during the fall during the wet season (Mead, 1968), and if, like eastern spotted skunks (Lesmeister et al., 2009), males have larger home ranges when questing for mates, this could increase their energetic requirements, and therefore require an increase in caloric input.

Although they are challenging to collect because they are difficult to find, western spotted skunk scats could serve as a rapid and efficient biodiversity sampler (Shao et al., 2021). As widely distributed and relatively abundant predators with a broad, generalist, and opportunistic diet, western spotted skunks could provide valuable information on species co-occurrence and interspecies relationships, ecosystem dynamics, and biodiversity through noninvasive collection of their scats (Boyer et al., 2015; Shao et al., 2021). Western spotted skunk diet analysis may detect cryptic species such as shrew moles, coast moles (*Scapanus orarius*), Pacific tree frogs, and other amphibians that may be difficult to detect using traditional methods.

This was the first study to examine western spotted skunk diet across scat collected from areas with different logging histories. Similarities in diet across logging histories across most scales, however, are not surprising. As a generalist species and as an efficient sampler of biodiversity, spotted skunk diet may indicate that many prey species, such as chipmunks, may be distributed equally across these forest types and that these areas may have similar biotic communities. Although some prey species are associated with old-growth forest, such as flying squirrels, these species still occur in logged forest at lower densities (Carey, 1989). A relatively large portion of our study area (41.5%) is still composed of old-growth forest (Davis et al., 2022), which may help support old-growth-associated species within logged areas. Results may differ in landscapes with few remaining old-growth stands, a different configuration of old-growth stands, or in landscapes with more intensive logging operations. Alternatively, spotted skunks are highly mobile (Lesmeister et al., 2009) and can easily move between logged and unlogged areas in this area. This landscape can be characterized as a mosaic of previously logged and unlogged areas without clear boundaries, and scats do not necessarily represent the prey consumed within the stand in which they were defecated. This is supported by the difference in scat composition at the 1-km buffer size (Figure 7), which corresponds to the size of a spotted skunk home range (Lesmeister et al., 2009), and fewer insects in the diet if more area in the buffer had been logged. Since many insects, such as wasps and beetles, depend on the presence of dead wood (e.g., nesting substrate and nutrition; Siitonen & Jonsson, 2012), change in diet composition likely reflects differences in insect abundance within these areas.

Although we detected a wide variety of diet items in western spotted skunk scats using DNA metabarcoding, we did not amplify and detect any DNA besides western spotted skunk in 51 scats (39.2%) and only amplified predator and bait DNA in 67 scats (51.5%). Upon manual inspection, discrepancies between DNA metabarcoding and mechanical sorting indicated that the ANML invertebrate primers that we used poorly amplified Vespidae, even though we detected Vespula in some of our samples. The potential for mismatch in the universal invertebrate primers and the biases introduced by the ANML primers we used is well known because of a lack of conserved regions across all invertebrates (Deagle et al., 2014). Still, we used these primers because of the extensive COI reference library and prior research suggesting that they outperformed other primers for invertebrate biodiversity surveys (Elbrecht et al., 2019; Jusino et al., 2019). This highlights the need for taxa-specific primers, better universal invertebrate primers, a panel of invertebrate primers, or shotgun sequencing that does not rely on PCR to amplify target sequences so that key prey items are not missed in the future (Alberdi et al., 2018).

We detected a wide variety of plants in the western spotted skunk diet through DNA metabarcoding, but many of the genera identified may not have been consumed by western spotted skunks as a food source. When mechanically sorting scats, we discovered many intact Douglas fir needles and bark imbedded in the scat that were likely consumed incidentally, environmentally contaminated following defecation, or contaminated during scat collection (Tercel et al., 2021). Although we tried to reduce the amount of contamination by extracting DNA from fecal material from the interior of the scat, care should be taken when interpreting some DNA metabarcoding results using plant primers.

Noticeably missing from our analysis are the fungal components of the western spotted skunk diet. Fungi are likely to contribute important nutrients (e.g., vitamins) to the western spotted skunk diet (Maser et al., 2008), and fungal dispersal by mammals is essential to plants, fungal diversity, and ecosystem function (Nuske et al., 2017). Eastern spotted skunks have been documented bringing fungal sporocarps to den sites (Sprayberry & Edelman, 2016), and we have also recorded western spotted skunks carrying fungal sporocarps on trail cameras during this study. The importance of fungal diet items remains unknown for western spotted skunks and is an important area of future research. In addition, we did not characterize differences in diets between individuals and sexes. Although there may be preferences for certain prey items by individual or sex, we were unable to link scats to individuals in this study.

Western spotted skunks occupy a key position in the Pacific Northwest food web. Through their broad diet and omnivory, they serve as a hub species that creates high connectivity across arboreal, terrestrial, and aquatic systems. Their generalism and habitat plasticity suggest that western spotted skunks may possess greater ability to withstand environmental change (Ducatez et al., 2020; Reed & Tosh, 2019). Due to their ability to switch prey to group-living invertebrates, they may even be a beneficiary of climate change if hotter summers increase the availability of wasps, which were by far the most consumed taxa during our study. Although these traits suggest that spotted skunks are resilient, the congeneric eastern spotted skunk has experienced a precipitous decline, and a lack of natural history information stymies recovery efforts. By studying the natural history of western spotted skunks while they are still abundant, we hope to provide key information necessary to achieve the conservation goal of keeping this common species common.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Tosa et al., 2022) are available from the Environmental Data Initiative Data Portal: https://doi.org/10.6073/pasta/c703a61ec22260d83ad855039a8f03af.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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