Variation of the Trowbridge's shrew (*Sorex trowbridgii*) diet across forest types in the Oregon Cascades

by
Margaret Yates

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
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degree of

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Abstract approved: _____

Taal Levi

Investigating the insectivorous diet of the Trowbridge's shrew (*Sorex trowbridgii)* across forest types can illustrate how forest management affects biotic communities. I used DNA metabarcoding methods with ANML primers to identify stomach and intestines contents from shrews caught in pitfall traps in the HJ Andrews Experimental Forest (HJA) and Willamette National Forest in the Oregon Cascades. I caught 54 Trowbridge's shrews between July and September of 2018 and identified diets from 37 shrews (12 intestines, 35 stomachs). Shrew diet consisted of Orthoptera, Lepidoptera, Diptera, Araneae, and Scolopendromorpha. Specifically, Tettigoniidae were consumed most consistently (FOO = 0.41) and constituted a large portion of the diet (mean RRA = 0.39). When comparing the mean relative read abundance of taxonomic orders against site variables, more Lepidoptera were consumed outside of HJA (t_{10}= -2.445, P = 0.03) and more Araneae were consumed at mid elevations than low elevations (t_5 = 2.229, P = 0.08). Although Trowbridge's shrew diets were similar across forest types, I found that they consumed more species in recently disturbed areas and areas lacking old-growth characteristics. This suggests shrews have a more diverse diet in younger stands. Further research should be done to assess the species' fitness in different forest types.

Key Words: *Sorex trowbridgii*, Oregon Cascades, metabarcoding, diet analysis, forest management

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APPROVED:

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Toni Doolen, Dean, Oregon State University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

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Margaret Yates, Author

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INTRODUCTION

Small mammals play an important role in the ecosystem. They influence plant production and composition, alter the microtopography of their habitat, and contribute to the chemical nature of the soil (Stoddart 1979). As prey to other animals they affect the reproductive success, survival, and general fitness of their predators, and as secondary consumers it is suggested that small mammals, especially insectivores, regulate their prey populations (Stoddart 1979). Small mammal populations are commonly limited by their habitat and available resources while predators suppress their growth rates (Stoddart 1979). Though small mammals can be found in many habitats, forests are a common habitat, many of which are affected by logging.

There are many methods, each with their own limitations, to determine small mammal diets. Microscopic analysis is the classic way to conduct diet analysis besides direct observation, which is difficult with small mammals (Symondson 2002). This can be done on gut contents or on scats. Microscopic analysis on gut contents is limited by investigator knowledge, magnification level needed to properly identify items, the variability of recognizable items between seasons (Hansson 1970), and having to kill the animal. Microscopic analysis specifically on feces is especially problematic when observing insectivorous diets because it underestimates the proportion of soft-bodied and small invertebrates since those are more easily digested and harder to identify (Dickman and Huang 1988). Fecal analysis can also cause rare prey items to be overlooked, especially if one species dominates the diet (Dickman and Huang 1988) and potentially introduces contamination from the surrounding environment (Esnaola et al. 2018). Overall microscopic analysis relies heavily on

proper identification by the investigator and the level of digestion; even partial digestion can cause remains to become unrecognizable (Symondson 2002).

Stable isotope analysis is a newer method to examine diet. In stable isotope analysis, researchers must compare isotopic ratios of specific elements, such as carbon and nitrogen, between tissues from the animal of interest and the food sources. This means researchers need to collect and analyze all potential food of the species of interest (Phillips 2001) which might not always be possible resulting in gaps in the analysis. The main drawback of this technique is that it cannot give taxonomic resolution in prey. Rather, it gives information on the animal's trophic level and summarizes recently eaten items to up to a few weeks (Baugh et al. 2004), which is useful when studying a species' ecology but not when attempting to identify diet composition. Isotopes occurring from non-diet items such as fertilizer can also be detected (Baugh et al. 2004).

DNA metabarcoding is considered the most effective and efficient way to identify even trace amounts of an animal's diet (Ji et al. 2013). This method identifies species via DNA sequences from a specific gene. To do this DNA is extracted from samples and then targeted sequences are replicated using universal polymerase chain reaction (PCR) primers that can be used on multiple taxa (Ji et al. 2013). These amplified sequences (an 'amplicon') are then sent to be read by a DNA sequencer machine which returns taxonomic information of a sequence based off a reference database (Ji et al. 2013). Metabarcoding does not require the same level of taxonomic expertise and allows for the identification of soft and hard-bodied invertebrates through DNA analysis. By metabarcoding gut contents, the samples are not exposed

to environmental conditions as is the case for scats (Esnaola et al. 2018). Metabarcoding can also be successful with minimal amounts of starting material and can still retrieve a majority of dietary components with a relatively high taxonomic resolution unlike microscopic analysis where indistinguishable parts can go unidentified (Iwanowicz et al. 2016).

Using metabarcoding to determine insectivorous diets of small mammals like shrews has become more popular in recent times (Iwanowicz et al. 2016). Shrews have fast metabolisms requiring frequent feedings, especially when breeding (Rust 1978). Previous studies indicated that they predominantly eat invertebrates such as centipedes, spiders, beetles, slugs, and snails (Jameson 1955, Whitaker and Maser 1976, McCay and Storm 1997, Churchfield and Rychlik 2006, Klenovšek et al. 2013). Shrews also consume plants, notably Douglas fir seeds (Moore 1942). Diet composition can also change due to competition and temporally.

When multiple species of shrews inhabit the same area, niche partitioning occurs, where the larger competitively dominant species get the larger prey (Churchfield and Rychlik 2006, Klenovšek et al. 2013). In harsher habitats this is not the case and trophic niches of differently sized shrews have a greater overlap (Klenovšek et al. 2013). In Britain, a study on *Sorex araneus* found that there was a greater variety in diet per sample in the winter (mean = 4.5) than the summer (mean = 3.5) but when the number of types of invertebrates were summed for each month and averaged there was no significant difference in diversity (Churchfield 1982). An isotopic diet analysis study in Utah determined that niche partitioning occurred between six species of shrews but researchers were not able to identify prey species

(Baugh et al. 2004). Knowing these factors can help form a better understanding of a shrew's diet choice.

Trowbridge's shrew (*Sorex trowbridgii*) of the family Soricidae is the most common shrew species in Oregon and the most adaptable species of shrew (Whitaker and Maser 1976, George 1989). Trowbridge's shrews are important prey to barred owls (*Strix varia;* Livezey 2007), Pacific giant salamanders (*Dicamptodon* spp.*;* Maser et al. 1981), and other small mammals (Harris 2000). They also compete with *Sorex vagrans* in marshy habitats as species abundances were observed to be inversely correlated (Dalquest 1941). This is potentially related to breeding habits since *S. vagrans* breed year round while *S. trowbridgii* only breed in late April to early May (Dalquest 1941). The diet of *S. trowbridgii* is also poorly known. Jameson (1955) only recorded the presence or absence of prey. Whitaker and Maser (1967) provided a more detailed look into their diet but had very loose category definitions to determine prey items. For example, some items are identified to the genus such as '*Endogone*' and other times they are grouped as simply as 'spider.' Gunthert and Horn (1983) mentioned various plant types like lichen or grasses but grouped all invertebrates together. Accurate identification of *S. trowbridgii*'s prey items and how their habitat may influence their selection would allow for a better understanding of this shrew's ecological role.

Few studies that observe small mammals across different forest types due to anthropogenic effects include diet changes. One study compared the diet of *Hylaeamys megacephalus,* the large-headed rice rat, between oil palm plantations and the Amazon rainforest (Pena and Mendes-Oliveira 2019). On plantations, populations

of *H. megacephalus* ate a greater variety of species, but individually there was less overlap indicating that on the plantations the rats were expanding their niche (Pena and Mendes-Oliveira 2019). In the Washington Cascades, Gunther and Horn (1983) investigated how small mammal abundances changed due to timber harvest but also included dietary information. Plant matter was eaten more often than invertebrate parts by most rodents in all stands but *Peromyscus maniculatus* ate more invertebrate parts in forests and burned areas than in clear cuts (Gunthert and Horn 1983). They also found that *S. monticolus* and *S. trowbridgii* had an overall preference for invertebrate parts across all stands (Gunthert and Horn 1983). This is the only study in the Pacific Northwest to look at diet composition across forest types but does not identify invertebrate species.

Limited research has been done on the insectivorous diet of small mammals (Stoddart 1979) in the Pacific Northwest, and no study has compared invertebrate species eaten across different forest types that occur from logging in the area. The impact of logging on ecological communities is important in creating effective conservation practices. Old-growth forests in Pacific Northwest forests support 1.5 times more small mammal individuals than managed forests, which is attributed to understory development and differences in plant species composition (Carey and Johnson 1995). When small mammal species were studied across different age stands, the trend in abundance was dependent on species (Morrison and Anthony 1989, Carey and Johnson 1995, Cole et al. 1998). Habitats of small mammals such as shrews are characterized by fallen trees and dense understory vegetation, which is not necessarily correlated with age stand (Carey and Johnson 1995) but is affected by

forest management. Shrews in particular were significantly more abundant in old growth (Carey and Johnson 1995) and the strongest indicator for increased abundance was dense forb and grass cover (Morrison and Anthony 1989).

In boreal forests where logging has occurred, it has been suggested that forest fragment size influences invertebrate species richness (Niemelä 1997). Smaller fragments appear to have more species, but this may be due to an influx from surrounding areas as well as a loss of species that were specialized for old-growth habitats (Niemelä 1997). In Finland forest type (*Vaccinium vitis-idaea* or *Vaccinium myrtillus*) and age class significantly affected the biomass of larvae and certain invertebrate orders; mature forests had the highest biomass (Lakka and Kouki 2009). Atlegrim and Sjöberg (1995) found that certain species of spiders are more abundant in clear cuts, but the general pattern appears to be lower abundance in clear cuts. Temporal patterns indicate that clear cuts have a decrease in invertebrate biomass while there was an increase in unharvested areas from spring to summer (Duguay et al. 2000).

As humans continue to impact the environment, it is important to understand if shrews require a certain type of forest to thrive or can adapt to these changes. Previous studies that specifically focused on or included shrews found that shrews vary in abundance across habitat types depending on the species. Many attribute this difference in abundance to the differences in undergrowth cover (Morrison and Anthony 1989, Carey and Johnson 1995, Nicolas et al. 2009). Also, previous research on *S. trowbridgii* diet has focused on how each species differ in diet rather than how one species' diet may change across habitats (Whitaker and Maser 1976). It is

possible that diet variation plays a role in how abundant shrews are within each habitat as invertebrates are also affected by age stands (Atlegrim and Sjöberg 1995, Duguay et al. 2000).

The purpose of this study is to examine the diet of *Sorex trowbridgii* via DNA metabarcoding and to determine if there are correlations between forest type and invertebrate consumption. I hypothesize that forest stands of varying age will support different invertebrate communities thus *S. trowbridgii*'s diet composition will change depending on the forest type.

METHODS

Study Site

The HJ Andrews Experimental Forest (HJA) is located in the central part of Oregon's Willamette National Forest (WNF) and was established in 1948 by the U.S. Forest Service as a place to conduct ecosystem research (Figure 1). HJA consists of 15,800-acres of the Lookout Creek Watershed (Figure 1). The elevation ranges from 410 m to 1,630 m. Forests above 1,050 m are predominantly Pacific silver fir (*Abies amabilis*) whereas forests are a mix of western hemlock (*Tsuga heterophylla*) and Douglas fir (*Pseudotsuga menziesii*) below 1,050 m (Bierlmaler and McKee 1989). Before harvesting began in the 1950's about 65% of the forest was characterized by old-growth and the remaining stands had regenerated from wildfires in the mid-1800's. Currently, about 40% of HJA remains covered in old-growth forests. Winters are wet and mild while summers are warm and dry. From 1972-1984 yearly average

rainfall was 230 cm and yearly average daily air temperature was 8.5°C (Bierlmaler and McKee 1989).

Shrew Collection

I collected shrews as bycatch during invertebrate trapping using pitfall traps as part of a larger research project focused on biodiversity across forest gradients. I collected samples from July-September of 2018. I checked pitfall traps 7 days after setting them with 400 ml of 50% propylene glycol mix. After discovering shrews in the pitfall traps, I stored them at room temperature in 50mL tubes in 100% ethanol until dissection.

Dissection

I dissected the shrews in Oregon State University's Department of Fisheries and Wildlife necropsy room. I removed both stomach and intestine contents, placed them into separate 2.0 ml microcentrifuge tubes, and stored them at -20°C. I split intestine contents into two samples. The remaining part of the sample was placed back in 100% ethanol in 50ml tubes. Although it is difficult to identify species based off physical characteristics, I identified shrews to species using characteristics from Maser et al. (1981). Shrews were not sexed since morphological characteristics are often misleading (Carraway 2009) and because they were stored in ethanol for 7-10 months before dissection. It has also been noted that sex does not influence shrew diets (Klenovšek et al. 2013).

DNA Extraction

I extracted DNA from the stomach contents and one of the intestine replicate samples, resulting in two extracts per shrew. I also extracted two blanks to use as negative controls. I extracted DNA using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). I followed the Purification of Total DNA from Animal Tissues protocol (Spin-Column Protocol) with modifications made by J. Allen for dried scats. Modifications consisted of adding 550 ul Buffer ATL and 50 ul of proteinase K to the sample, lysing at 61°C for 2-4 hours, adding 400 ul of Buffer AL and 400 ul of ethanol during precipitation, multiple rounds of pipetting the mixture into the spin column until it is gone, two washes of Buffer AW1, and adding 100ul of Buffer AE for elution. Extracts were then stored in a freezer at -20°C.

PCR Amplification and Sequencing

I amplified the COI region of the mitochondrial DNA using ANML primers (forward: 5′-GGTCAACAAATCATAAAGATATTGG-3′, reverse: 5′-GGWACTAATCAATTTCCAAATCC-3′), These primers have been shown to detect a greater number of arthropod taxa than other primer pairs (Jusino et al. 2019). Each 15 ul PCR consisted of 2.5 ul of deionized H_20, 7.5 ul of Amplitaq, 3 ul of ANML f/r primers, and 2 ul of DNA extract. Touchdown PCR was done for cycling. The initial denature occurred at 95°C for 10 minutes followed by 30 seconds at 95°C, 30 seconds at 62°C, (touchdown begins) 25 cycles of 30 seconds at 72°C, 30 seconds at 95°C, 30 seconds at 45°C (touchdown ends), 10 cycles of 30 seconds at 72°C, 7 minutes at 72°C, and then held at 10°C until removed. Each plate had three replicates of each

sample, a water sample, and two blank DNA extracts. The amplicons were double-tagged to differentiate each sample, normalized, and pooled.

Following PCR, we sent amplicons to the Center for Genome Research and Biocomputing at Oregon State University for 150-bp paired sequencing on the Illumina HiSeq 3000 platform (Illumina Inc., San Diego, CA). We demultiplexed and clustered sequences with a custom bioinformatics shell script, and taxonomically assigned sequences using the reference library from BLAST against all COI sequences in GenBank (www.ncbi.nlm.nih.gov/genbank/). I removed any species within a replicate with less than 20 reads due to low counts thus normalizing detection requirements across samples (Deagle et al. 2018). I then confirmed taxonomic assignment using BLAST and the BOLD System v4.

Site Variables

I looked at four site variables: location (HJA or WNF), the old-growth structural index (OGSI), elevation, and the number of years since disturbance. OGSI was extracted from gradient nearest neighbor maps fitted to forest structure data derived from remotely sensed Landsat images (https://lemma.forestry.oregonstate.edu/data/structure-maps), and elevation was from LiDAR data. OGSI was categorized into 'simple structure' (< 22), 'plantation' (22-45), and 'complex' (> 45) forests based on Davis et al. (2012), while elevation was split into 'low' (<610 m), 'mid' (610-914 m) and 'high' (>914 m) elevations. The number of years since disturbance was determined by taking the smaller value of either data derived from satellite imagery (Ray Davis, US Forest Service) or the

number of years since logging operations as of 2018. All areas without documented disturbance were given a value of 200 years. This was reclassified as ‘recently disturbed’ (< 40 years), ‘mature forest’ (40-80 years) and ‘old growth’ (> 80 years).

Analysis

Statistical analysis was done in R v3.6.3 (www.r-project.org, accessed 7 April 2020) using the package *car* (socialsciences.mcmaster.ca/jfox/Books/Companion/, accessed 28 April 2020) and function *lm*. Figures were produced using the package *ggplot2* (ggplot2.tidyverse.org, accessed 7 April 2020). I removed sequences identified as algae, freshwater sponge, and water mold before analysis because they only appeared in one sample and could be a result of sequencing error. These items also had low read counts and the species are not found in the United States. I summarized the data first into the number of unique species and if invertebrate DNA was detected in the stomach or intestines of the shrew.

I calculated the frequency of occurrence (FOO) for each phylum, class, order, family, and species. This allowed me to determine the proportion of shrews that ate the taxon of interest. Unique detections of the taxonomic level of interest per shrew were divided by the total number of shrews that returned results.

I computed the relative read abundance (RRA) for each species by summing counts of a unique species across all samples and dividing by the sum of all sample counts. I calculated the RRA for each taxonomic order per shrew which tells us how common one order is compared to the others within a shrew. I summed the reads for each order per replicate, averaged the unique order reads per replicate for each shrew

and then divided by the total order reads per shrew. Any shrew with a total count below 20, after averaging across replicates and adding counts from both stomach and intestines, was removed. I also found the mean RRA of each order across all shrews with the order of interest.

I ran linear regressions on the logit transformed RRA if the sample contained the taxonomic order of interest against categorical classifications of elevation, OGSI, number of years since disturbance, and if the sample was within the HJA. I logit transformed RRA since the response variable is a proportion between 0 and 1. This allowed me to run a linear regression to model the relationship between the different classifications. Tukey's HSD test was used to evaluate differences between each site variable classification. A p-value of 0.10 was used as the cut-off for statistical significance due to the small sample size.

RESULTS

I collected 54 shrews and conducted 108 DNA extractions. Of the 108 DNA extractions that I sequenced, 47 returned sequences; 12 of them were from intestines and 35 were from stomach contents (Figure 2). 10 shrews returned sequences from both stomach and intestines. There were 19 shrews collected within the HJA, 14 of which returned DNA sequences (Table 1, Figure 1). Outside of thee HJA 23 of the 35 collected shrews returned DNA sequences (Table 1, Figure 1). There were no detections from the water samples or blank extracts. Shrews collection and sequence returns were equal within most site variables except there were less than 10 shrews that returned data at high elevations (Table 1).

While not all sequences could be identified to the genus or species, assuming if each of the unique sequences represent a different species, I detected 57 different species. Most shrew gastrointestinal tracts contained between one to four species (mean = 1.5). I detected 21 species in one individual. Species with the highest FOO were *Tettigoniidae* sp. (FOO = 0.41), *Scolopocryptops capillipedatus* (FOO = 0.12), *Callobius canada* (FOO = 0.08) and *Blastobasis glandulella* (FOO = 0.08) (Table 2). The species with the highest RRA were *Tettigoniidae* sp. (RRA = 0.22), *Leptarctia californiae* (RRA = 0.21), *Callobius canada* (RRA = 0.20) and *Dolichovespula norvegicoides* (RRA = 0.13) (Table 2).

When detections occurred in both stomach and intestines the number of species in each differed by at most two (Appendix A). If the number of species detections was the same for each then the species were also the same. This was not the case for the five shrews with unequal detections. Shrew 29 had Rhagionidae sp. in both stomach and intestines but only had *Hypatopa simplicella* in the stomach. Shrew 39 had *Cladara* sp., *Drosophila affins,* and *Melanolophia imitate* in the intestines and *Negha longicornis* in the stomach. Shrew 43 had *Dolichovespula norvegicoides* in both but only *Vespula consobrina* in the stomach. Shrew 49 had *Leptarctia californiae* in both but only *Blastobasis glandulella* in the intestines. Shrew 56 had *Phyllodesma Americana* in both but only *Tettigoniidae* sp. in the stomach. Shrews 58 and 59 were the only samples with a detection solely in the intestines which were *Hydriomena edenata* and *Enypia venata* respectively. In general, we detected more species in stomachs than in intestines.

Shrews from low elevation sites consumed the most families of prey items (n = 23) (Figure 3). Diets of shrews at mid elevation consisted of 14 families with Tettigoniidae having the highest count (n=7) and at high elevations there were nine family detections. Shrews at simple structure and recently disturbed sites for OGSI and years since disturbance respectively had 23 families in their diet (Figure 4, Figure 5). There were 15 families in the diet of shrews in mature forests and nine families for old growth (Figure 4). OGSI had 12 families in plantation diets and 11 families in complex forest diets (Figure 5).

All sequences were identified down to family allowing me to calculate FOO for those taxonomic levels (Figure 6). I identified two phyla: Arthropoda, which was consumed by all shrews, and Mollusca. There were four classes which Insecta dominated (FOO = 0.84). 13 orders were detected of which Orthoptera was the most frequent (FOO = 0.41). Tettigoniidae was the most common (FOO = 0.41) in the 35 distinct families.

The mean RRA was dominated by Orthoptera (mean RRA = 0.3902) followed by Lepidoptera (mean RRA = 0.2020) and Araneae (mean RRA = 0.1277) (Figure 7). The regression analysis indicated whether the diet of shrews was different depending on the site characteristics. Shrew 59 was removed before analyzing RRA due to total average counts being below 20. This reduced the number of shrews with detections to 36. Regression analysis only yielded two significant results (Figure 8). Mean Lepidoptera RRA was higher in shrew diet outside of the HJA ($t_{10} = -2.445$, $P = 0.03$). Mean Araneae RRA was higher in shrew diet at mid elevations than low elevations ($t_5 = 2.229$, $P = 0.08$) but was not detected in high elevations. Since there was one shrew

where I detected 21 different species, I reran the analysis by removing this individual. Removing this shrew did not affect the Lepidoptera results since this order did not appear in the shrew that was removed. Removal did change the mean Araneae RRA difference to no longer be significant ($t_4 = 1.927$, $P = 0.13$) between mid and low elevations.

DISCUSSION

The purpose of this study was to quantify shrew diet and to determine if shrew diet varied across different forest types. My results indicate that Lepidoptera and potentially Araneae are eaten more by *S. trowbridgii* in certain site variables, shrew diet does not vary significantly by forest type. However, since Lepidoptera (moths/butterflies) is more common in the diet of shrews outside of the HJA, it may indicate that shrews are consuming more Lepidoptera in areas where the landscape is more disturbed. It is also possible that the reason Araneae (spiders) are consumed more at mid elevations than low is that they prefer more humid locations (Pearce and Venier 2006) and higher elevations in Oregon tend to be wetter. With the removal of shrew 12 which contained 21 species, only one of which was part of Araneae, this is no longer supported.

While no supported conclusions can be made about shrew diet across forest types, general trends indicate that their diet has a greater species richness when in younger, more recently disturbed forests at low elevations (Figures 3, 4, and 5). Shrew 12 has influenced these trends though since 21 species were detected and the sample came from a simple structured, more recently disturbed, low elevation site. Removing

this shrew from the summary statistics resulted in greater similarity across elevation, disturbance, and location variables (Appendix B).

Species detections also align with previous studies on *Sorex* spp. diet with the Class Insecta composing a majority of the diet (Jameson 1955, Whitaker and Maser 1976, Churchfield 1991, Churchfield et al. 1997, McCay and Storm 1997, Klenovšek et al. 2013). The similarity of diets across forest types also supports previous findings of studies that found that most (or in this study's case, all) invertebrates do not differ significantly across forest habitats (Atlegrim and Sjöberg 1995).

A majority of the taxonomic orders detected in this study were found in other studies that documented general shrew diet. Interestingly I did not find any Oligochaeta (earthworms) which were detected in other shrew diet studies albeit in mostly low proportions. (Whitaker and Maser 1976, Dickman and Huang 1988, McCay and Storm 1997). Whitaker and Maser (1976) noted that earthworms comprised 6.3% of *S. trowbridgii* stomach contents. Another interesting comparison to other studies is my detection of Orthoptera all of which was Tettigoniidae (katydids). They do not appear common in other studies and when they are it is in mid to low abundance (Dickman and Huang 1988, McCay and Storm 1997).

It is important to note that these shrews were a bycatch of another project focused on invertebrates. They were found in pitfall traps that contained other species. Therefore, invertebrate detections may not accurately reflect Trowbridge's shrew's true diet as they may have resorted to eating species that fell in the traps, especially since seven days passed between setting and returning to the traps. This amount of time may also have introduced digestion bias where DNA becomes

degraded, causing us to miss species that would have otherwise been detected (Thomas et al. 2014). It is also possible that since traps were not checked for seven days any shrews that fell in shortly after the traps were set would have empty gut contents by the time we collected them.

By removing any initial reads below 20, many other species were excluded from the summarization and final statistics. However, by using data from both stomach and intestine contents of each shrew we were able to detect a few species we may have missed, for example *Drosophila affins* and *Melanolophia imitate*. Due to the methodology, we are also unable to account for the life stage of the organism consumed; it has been indicated that larvae comprise a portion of diet (Whitaker and Maser 1976, Churchfield 1991).

Although metabarcoding is more efficient and effective at determining diet of shrews than traditional methods, there are still limitations with metabarcoding. One limitation of metabarcoding results from amplification error as primers must bind to the DNA. If primers do not perfectly match the target DNA, they will not effectively amplify it. Thus, I checked the effectiveness of the forward and reverse primer binding site in BLAST to taxonomic orders of *S. trowbridgii*'s prey items identified in this study and those listed in Whitaker and Maser (1976). There was no mismatch between the forward primer and identification to all the orders. There may, however, be bias against detections of Chilopoda, and Pseudoscorpiones as query cover is below 70% for most sequences producing significant alignments with the forward primer. The reverse primer also did not have any mismatches but about a third of the results for Raphidioptera had a query coverage below 70%. I also checked the

effectiveness of primer binding sites in BLAST by families of prey items found in this study. The forward and reverse primer appear to be less reliable at family identification as many families had less than 70% query coverage for a majority of their results. However percent identity (similarity between target and query sequence) remained at 100% for all families. Other limitations of metabarcoding stem from incomplete reference databases, which could prevent full identification of a species (Elbrecht et al. 2018). Moreover, while metabarcoding is better at detecting small amounts of a specimen than mechanical sorting, it is still possible that small or rare prey items can still be missed due to differences in biomass (Elbrecht et al. 2018).

For future research, examining diet temporally may result in more species identifications since larvae appear to be a common food item of shrews (Whitaker and Maser 1976) and since invertebrate abundance changes seasonally (Duguay et al. 2000). Another change to this study would be to compare detected species to what was available to the shrew. This would provide a better understanding of selection and niche partitioning especially if compared to other species of shrews. While no clear management implications can be concluded from this study, further research is needed to effectively assess *S. trowbridgii's* adaptability to forest management.

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Table 1: Number of *Sorex trowbridgii* that were caught in the HJ Andrews Experimental Forest (HJA) and the surrounding Willamette National Forest (WNF) from July-September 2018 using pitfall traps. Number of shrews with detections indicate number of shrews with gut samples from which invertebrate species were detected using DNA metabarcoding with ANML primers. Site locations where shrew were caught were classified based on four variables: old-growth structural index (OGSI), years since disturbance, elevation, and location.

	OGSI			**Years Since Disturbance**			**Elevation (m)**			**Location**	
	Simple Structure (<22)	Plantation (22-45)	Complex (>45)	Recently Disturbed (<40)	Mature Forest (40-80)	Old Growth (>80)	Low (<610)	Mid (610-914)	High (>914)	HJA	WNF
Total Shrews	17	22	15	13	15	26	16	23	15	19	35
Shrews with detections	12	15	10	11	12	14	13	16	8	14	23

Table 2: Frequency of occurrence (FOO) and relative read abundance (RRA) of invertebrate prey species in gut contents across all *Sorex trowbridgii* collected. Collection occurred in the Willamette National Forest and the HJ Andrews Experimental Forest from July-September 2018. Identifications were done via DNA metabarcoding with ANML primers.

Scientific Name	Frequency of occurrence (FOO)	Scientific Name	Relative read abundance (RRA)
Tettigoniidae sp.	0.40541	Tettigoniidae sp.	0.22213
Scolopocryptops capillipedatus	0.10811	*Leptarctia californiae*	0.21233
Blastobasis glandulella	0.08108	*Callobius canada*	0.1974
Callobius canada	0.08108	*Dolichovespula norvegicoides*	0.13185
Dolichovespula norvegicoides	0.05405	*Blastobasis glandulella*	0.05145
Keroplatidae sp.	0.05405	*Arcuphantes arcuatulus*	0.04058
Negha longicornis	0.05405	*Enypia packardata*	0.0389
Agulla unicolor	0.02703	*Hybomitra affinis*	0.02178
Agyneta perspicua	0.02703	*Caradrina meralis*	0.00657
Arcuphantes arcuatulus	0.02703	*Ozophora picturata*	0.00624
Bathyphantes orica	0.02703	*Ozyptila americana*	0.00558
Caradrina meralis	0.02703	*Zonitoides arboreus*	0.00553
Cecidomyiidae sp.	0.02703	*Negha longicornis*	0.005
Chalcosyrphus libo	0.02703	*Scolopocryptops capillipedatus*	0.00433
Chironominae sp.	0.02703	*Palmadusta asellus*	0.00413
Chironomus cf. decorus	0.02703	*Pleromelloida conserta*	0.00374
Chironomus sp.	0.02703	Rhagionidae sp.	0.00322
Chrysobothris trinervia	0.02703	*Vespula consobrina*	0.00301
Cladara sp.	0.02703	*Chironomus cf. decorus*	0.00295
Culiseta minnesotae	0.02703	*Chironomus* sp.	0.00288
Dichelotarsus sp.	0.02703	*Dicranomyia haeretica*	0.00269
Dicranomyia haeretica	0.02703	*Agyneta perspicua*	0.00246
Dolichovespula adulterina	0.02703	Psychodinae sp.	0.00242
Dolichovespula arenaria	0.02703	*Drosophila affinis*	0.00237
Drosophila affinis	0.02703	*Phyllodesma americana*	0.00213
Enypia packardata	0.02703	*Dolichovespula arenaria*	0.00173
Enypia venata	0.02703	*Chrysobothris trinervia*	0.00171
Glyptotendipes meridionalis	0.02703	*Rhagio* sp.	0.00169
Hybomitra affinis	0.02703	*Dolichovespula adulterina*	0.00132
Hybomitra sp.	0.02703	*Bathyphantes orica*	0.0013
Hydriomena edenata	0.02703	*Hypatopa simplicella*	0.00087
Hypatopa simplicella	0.02703	Keroplatidae sp.	0.00084
Leptarctia californiae	0.02703	*Rhipidia* sp.	0.00066
Limonia nubeculosa	0.02703	*Nowickia marklini*	0.00059
Machilidae sp.	0.02703	Cecidomyiidae sp.	0.00057
Melanolophia imitata	0.02703	*Glyptotendipes meridionalis*	0.00057

Nowickia marklini	0.02703	*Culiseta minnesotae*	0.00052
Nycteola cinereana	0.02703	*Nycteola cinereana*	0.00052
Ochlerotatus fitchii	0.02703	*Hydriomena edenata*	0.00044
Ozophora picturata	0.02703	Chironominae sp.	0.00042
Ozyptila americana	0.02703	*Schizura ipomoeae*	0.00041
Palmadusta asellus	0.02703	*Sympetrum madidum*	0.00041
Phyllodesma americana	0.02703	*Sympetrum* sp.	0.00039
Pleromelloida conserta	0.02703	*Enypia venata*	0.00037
Pseudolycoriella sp.	0.02703	*Agulla unicolor*	0.00035
Psychoda sp.	0.02703	*Cladara* sp.	0.00034
Psychodinae sp.	0.02703	*Hybomitra* sp.	0.00032
Rhagio sp.	0.02703	*Limonia nubeculosa*	0.00032
Rhagionidae sp.	0.02703	*Ochlerotatus fitchii*	0.00032
Rhipidia sp.	0.02703	Machilidae sp.	0.00019
Schizura ipomoeae	0.02703	*Melanolophia imitata*	0.00019
Steatoda bipunctata	0.02703	*Chalcosyrphus libo*	0.00017
Sympetrum madidum	0.02703	*Steatoda bipunctata*	0.00017
Sympetrum sp.	0.02703	*Dichelotarsus* sp.	0.00016
Thallophaga hyperborea	0.02703	*Psychoda* sp.	0.00016
Vespula consobrina	0.02703	*Thallophaga hyperborea*	0.00016
Zonitoides arboreus	0.02703	*Pseudolycoriella* sp.	0.00015

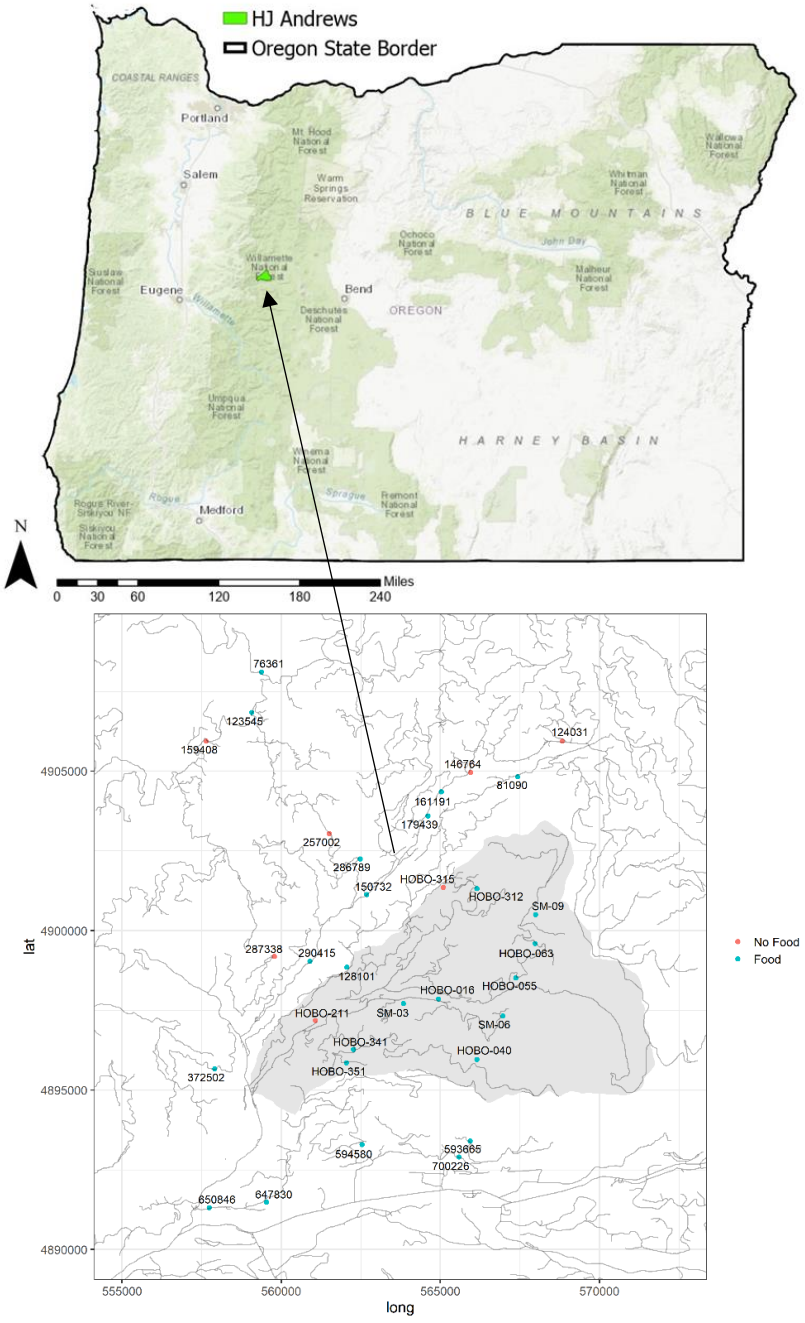

Figure 1: Map of study area showing site locations where shrews were collected and if there was invertebrate DNA detected in stomach contents via metabarcoding from that site (Food). Sites where shrews were caught but no invertebrate DNA was detected are marked as 'No Food.' The shaded area indicates the HJ Andrews and lines indicate roads. The base map of Oregon is a clipping of the World Topographic Map from these sources: Esri, DeLorme, HERE, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, and the GIS User Community.

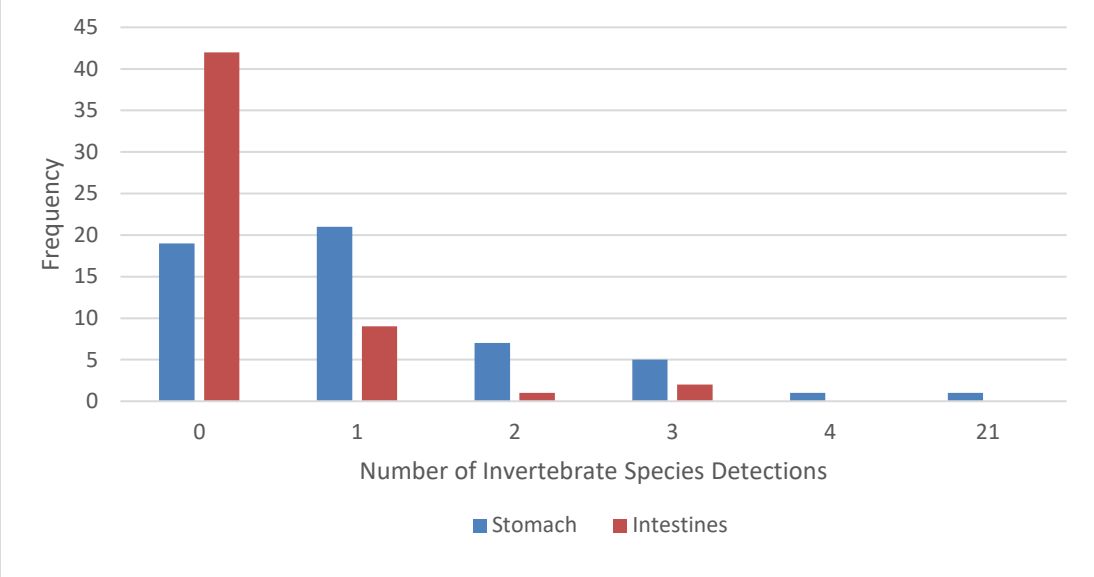

Figure 2. Frequency of total number of unique invertebrate species detected per gut part in *Sorex trowbridgii* via DNA metabarcoding. Shrews were collected from July-September 2018 in the Willamette National Forest and the HJ Andrews Experimental Forest**.** See Appendix A for shrew specific species frequency.

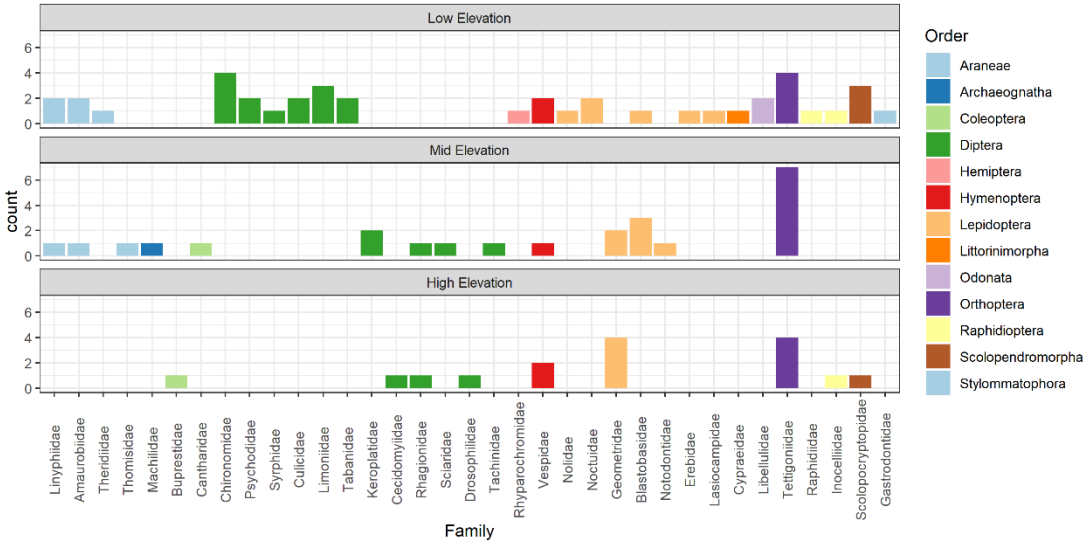

Figure 3: Unique species detections in gut contents of 54 *Sorex trowbridgii* summarized by family are organized by elevation classification (Low: < 619 m, Mid: 610-914 m, High: >914 m). 'Count' refers to how many times that family is detected. Shrews were caught in the Willamette National Forest and the HJ Andrews Experimental Forest from July-September 2018. Species identifications were determined by DNA metabarcoding. The mean number of families per classification is 11.67

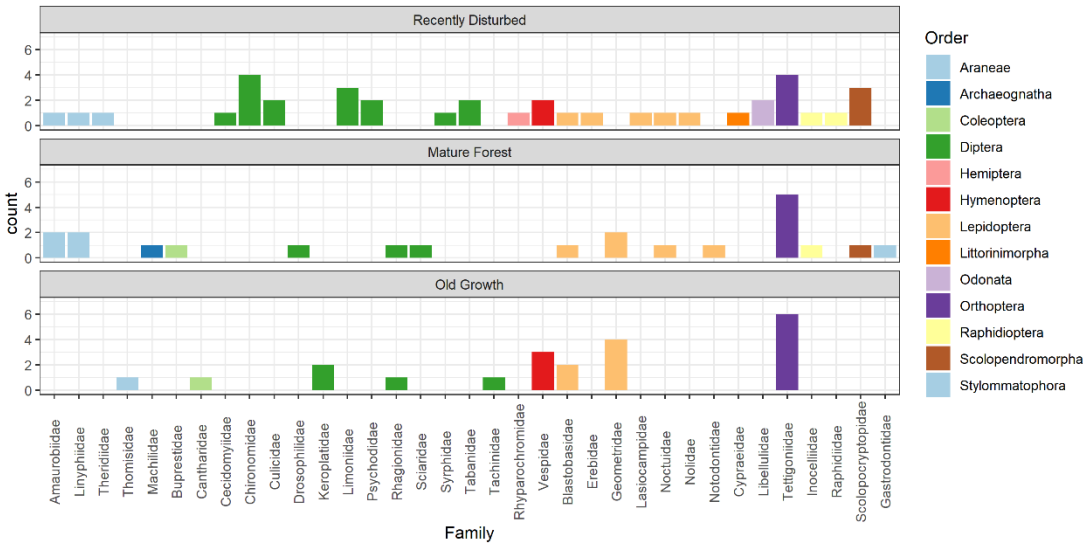

Figure 4: Unique species detection in gut contents of 54 *Sorex trowbridgii* summarized by family are organized by the number of years since disturbance (Recently Disturbed: <40 years, Mature Forest: 40-80 years, Old Growth: >80 years). 'Count' refers to how many times that family is detected. Shrews were caught in the Willamette National Forest and the HJ Andrews Experimental Forest from July-September 2018. Species identifications were determined by DNA metabarcoding. The mean number of families per classification is 11.67

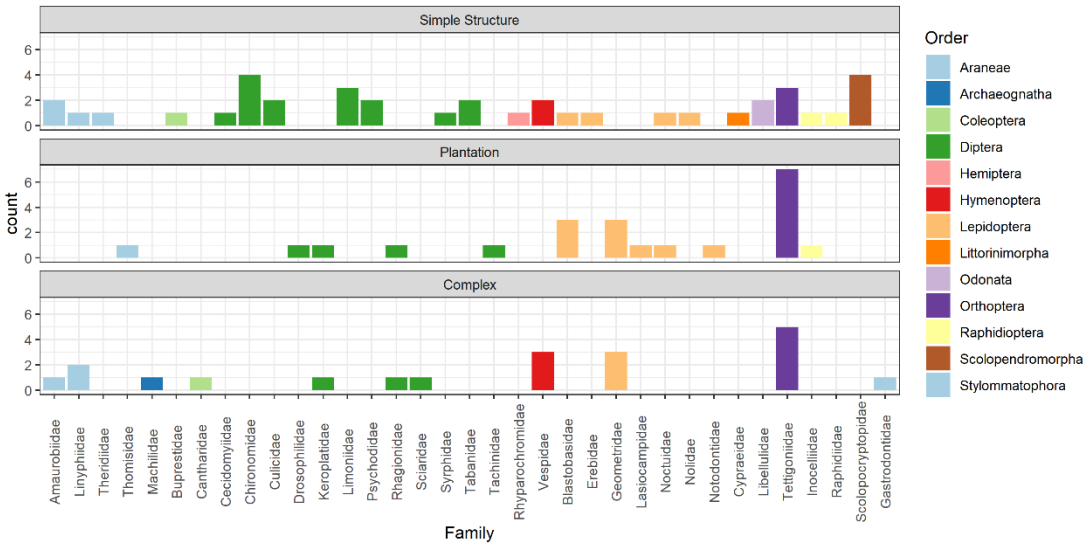

Figure 5: Unique species detections in gut contents of 54 *Sorex trowbridgii* summarized by family are organized by old growth structure index (OGSI) (Simple Structure: <22, Plantation: 22-45, Complex: >45). 'Count' refers to how many times that family is detected. Shrews were caught in the Willamette National Forest and the HJ Andrews Experimental Forest from July-September 2018. Species identifications were determined by DNA metabarcoding. The mean number of families per classification is 11.67

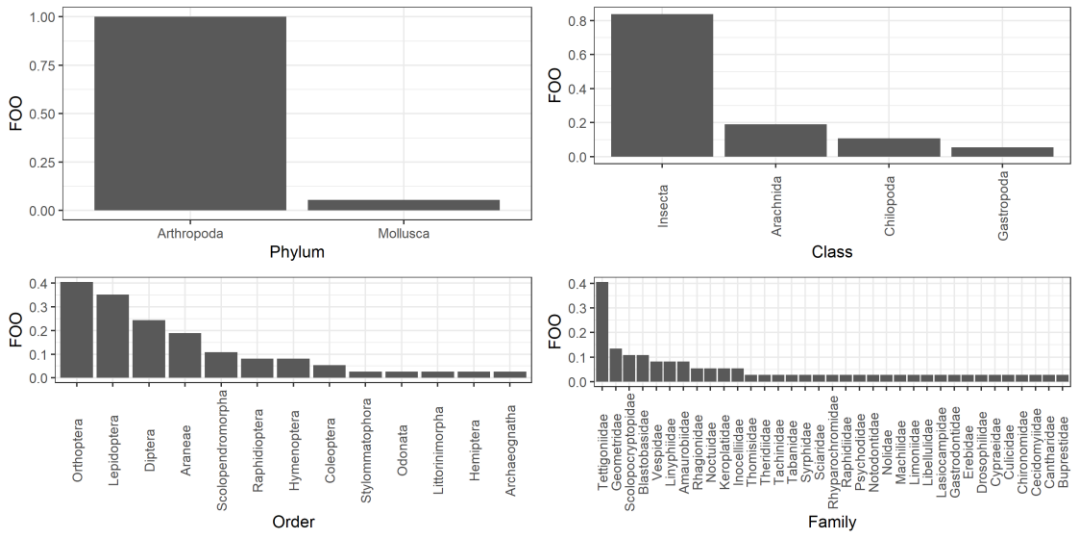

Figure 6: Percent frequency of occurrence (FOO) of phylum, class, order, and family across all *Sorex trowbridgii*. There were 2 phyla, 4 classes, 13 orders, and 35 families delectated in gut contents. Shrews were caught in the Willamette National Forest and the HJ Andrews Experimental Forest from July-September 2018. Species identifications were determined by DNA metabarcoding.

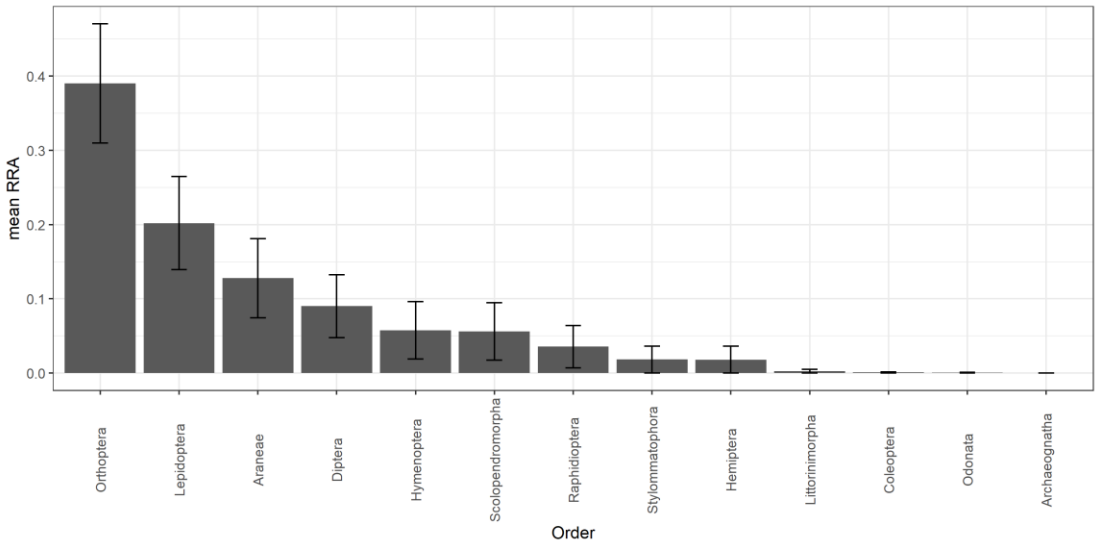

Figure 7: The mean relative read abundance (RRA) and standard error for orders detected in gut contents of *Sorex trowbridgii*. Shrews were caught in the Willamette National Forest and the HJ Andrews Experimental Forest from July-September 2018. Species identifications were determined by DNA metabarcoding.

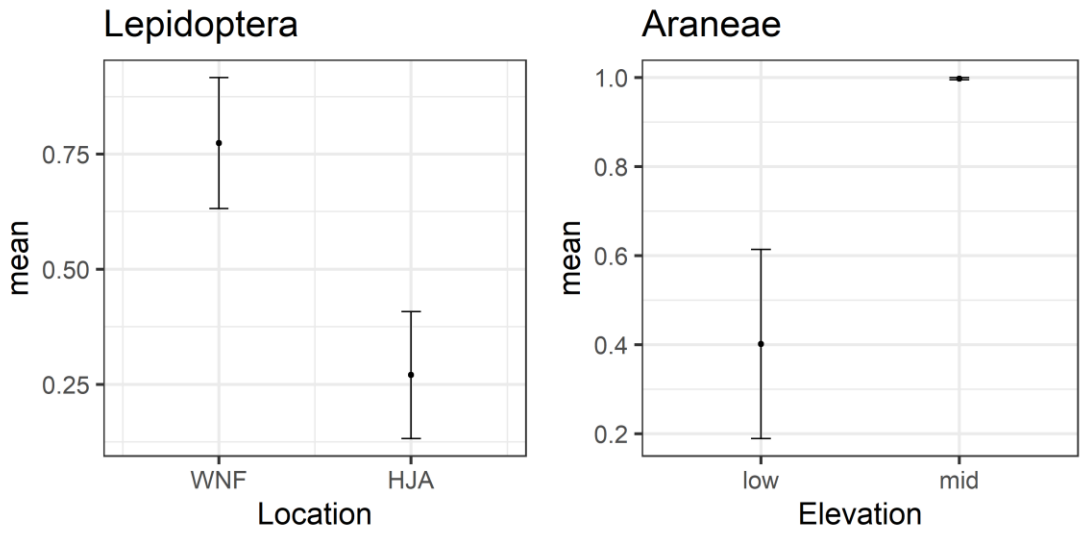

Figure 8: The mean relative read abundance (RRA) and standard error of significant site variables. Mean Lepidoptera RRA was higher in shrew diet in the Willamette National Forest (WNF) than within the HJ Andrews Experimental Forest (HJA) boundary (t_{10} = -2.445, *P* = 0.03). Mean Araneae RRA was higher in shrew diet at mid elevations than low elevations (t_5 = 2.229, *P* = 0.08) but was not detected in high elevations. Shrews were caught in the Willamette National Forest and the HJ Andrews Experimental Forest from July-September 2018. Species identifications were determined by DNA metabarcoding.

APPENDIX A. SHREW SPECIFIC SPECIES FREQUENCY

Table 1: Number of species detected per gut part of *Sorex trowbridgii* via metabarcoding. Shrews were collected from July-September 2018 in the Willamette National Forest and the HJ Andrews Experimental Forest**.** Species detections were determined by DNA metabarcoding.

Shrew	Intestines	Stomach
1	0	0
2	0	3
3	0	4
11	0	1
12	0	21
13	0	2
14	0	0
15	0	0
16	0	1
17	0	0
18	0	0
19	1	1
20	0	2
21	0	1
22	0	1
23	0	1
24	0	0
25	0	1
26	0	1
27	0	2
28	0	0
29	1	3
30	3	3
31	0	1
32	0	3
33	0	1
34	0	0
35	0	0
37	0	1
38	1	1
39	3	1
40	0	1
42	1	1
43	1	2
44	0	0
45	0	3
47	1	1

48	0	1
49	2	1
50	0	1
51	0	0
53	0	0
55	0	2
56	1	2
58	1	0
59	1	0
60	0	0
61	0	1
62	0	2
63	0	0
65	0	0
66	0	1
67	0	0
69	0	0
Median	0	1

APPENDIX B. SUMMARY STATISTICS WITHOUT SHREW 12

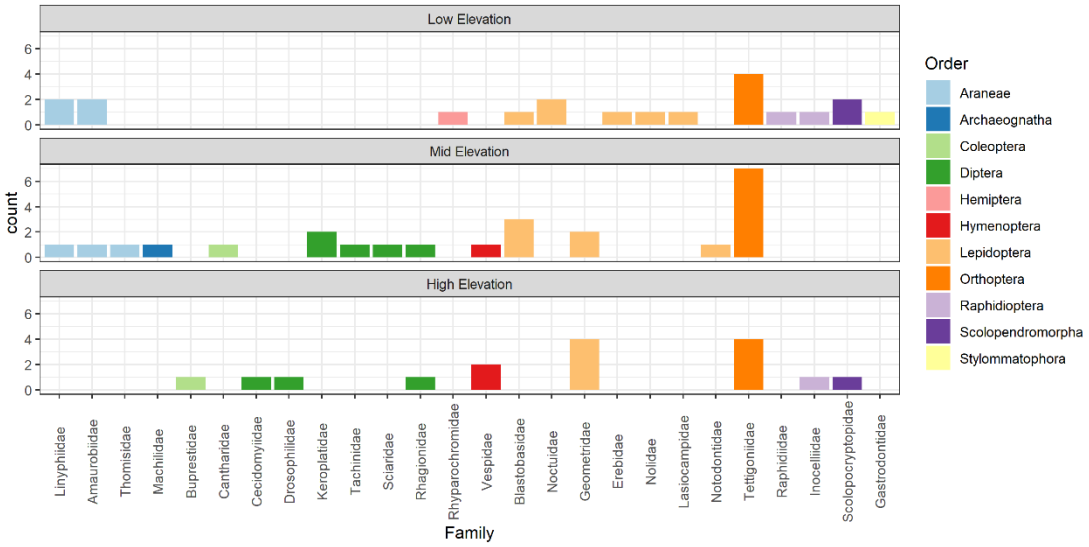

Figure 1. Unique species detections in gut contents of *Sorex trowbridgii* summarized by family are organized by elevation classification (low: < 619 m, mid: 610-914 m, high: >914 m). Shrews were caught in the Willamette National Forest and the HJ Andrews Experimental Forest from July-September 2018. Species identifications were determined by DNA metabarcoding. Shrew 12 was removed from this summary. The mean number of families per classification is 8.67.

Figure 2: Unique species detection in gut contents of *Sorex trowbridgii* summarized by family are organized by the number of years since disturbance (Recently Disturbed: <40 years, Mature Forest: 40-80 years, Old Growth: >80 years). Shrews were caught in the Willamette National Forest and the HJ Andrews Experimental Forest from July-September 2018. Species identifications were determined by DNA metabarcoding. Shrew 12 was removed from this summary. The mean number of families per classification is 8.67.

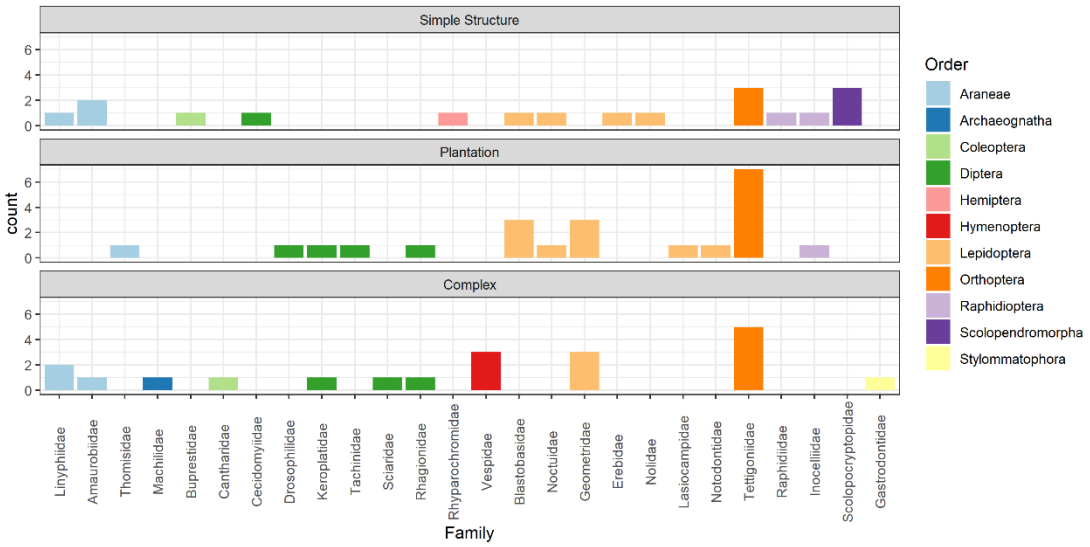

Figure 3: Unique species detections in gut contents of *Sorex trowbridgii* summarized by family are organized by old growth structure index (OGSI) (Simple Structure: <22, Plantation: 22-45, Complex: >45). Shrews were caught in the Willamette National Forest and the HJ Andrews Experimental Forest from July-September 2018. Species identifications were determined by DNA metabarcoding. The mean number of families per classification is 8.67.