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ECOLOGICAL AND GENETIC CONNECTIVITY OF SHREWS *(SOREX* SPP.*)* ACROSS INTERSTATE-90 IN THE WASHINGTON CASCADE RANGE

A Thesis

Presented to

The Graduate Faculty

Central Washington University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

Biology

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by

Jordan Dakota Marie Ryckman

November 2020

Central Washington University

Graduate Studies

We hereby approve the thesis of

Jordan Dakota Marie Ryckman

Candidate for the degree of Master of Science

APPROVED FOR THE GRADUATE FACULTY

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Dean of Graduate Studies

ABSTRACT

ECOLOGICAL AND GENETIC CONNECTIVITY OF SHREWS (*SOREX* SPP.) ACROSS INTERSTATE-90 IN THE WASHINGTON CASCADE RANGE

by

Jordan Dakota Marie Ryckman

November 2020

Roads negatively affect wildlife by degrading and fragmenting habitat. The I-90 Snoqualmie Pass East project was established to improve traffic safety for both humans and wildlife. Here, the Washington State Department of Transportation is constructing wildlife crossing structures to increase ecosystem connectivity as part of this project. The goal of my study was to investigate shrews – very small mammals with presumably low mobility and dispersal capacity – at sites on the eastern slopes of the Cascade Range where wildlife crossing structures will be built in the future. My main objectives were to verify shrew species composition, assess population genetic structure relative to the highway, and determine whether shrew abundance varied among macro- and micro-habitats. Using live-trapping techniques, I captured 136 individual shrews at three different paired sites north and south of the highway in the summer of 2019. Initial field identifications documented six sympatric species: Trowbridge's shrew (*Sorex trowbridgii*), montane shrew (*S. monticolus*), masked shrew (*S. cinereus*), vagrant shrew (*S. vagrans*), marsh shrew (*S. bendirii*), and the American water shrew (*S. palustris*). However, molecular analysis of the four terrestrial species determined that the study area was home to the Olympic shrew (*S. rohweri*) rather than the masked shrew (*S. cinereus*). This discovery represents a range extension for the Olympic shrew east of the Cascade Range and the surprising absence of the masked shrew. Analysis of population genetic structure for the three most abundant species indicated that montane shrew and Olympic shrew populations separated

by the highway still act as one population. Trowbridge's shrew populations had higher genetic variation, providing some evidence for population genetic structuring across the highway based on a limited sample size. Capture rates of shrews did not differ among habitat types or with most microhabitat characteristics. This study provides baseline information on an assemblage of shrew species near a major highway in central Washington and can be replicated in the future to assess potential effects of wildlife crossing structures on connectivity for these very small mammals.

ACKNOWLEDGEMENTS:

I was incredibly lucky to have an abundance of support from many people and organizations throughout this project. First, I want to acknowledge Kristina Ernest for her years of guidance and inspiration. Many times we had to adapt to the struggles of this research on this small mammal, but her continued encouragement and positivity kept me on track. Thank you to my other committee members Alison Scoville and Daniel Beck. Thank you to my incredibly hard-working field technicians Molly Robertson and Thomas McIntyre. Thank you to the other CWU students who took breaks from their own research to help with field work: Anneliese Myers, Alex McCarrel, Tyler Larson, and Steven Urakawa. Thank you to Laurel Baum and Conservation Northwest for spreading the word about us needing help installing pitfall arrays and to all the volunteers who came out to do so: Jamie Schultz, Robert Stevens, Cameron Sokoloski, Mallory Clarke, James Kern, Jen Ramsey, and Charlie Carmichael. Thank you to those who took time out of your week to help during live-trapping: Jenna Chapman, Gavin Scoville, Spencer Canada, and Zoe Rushton. Thank you, Jim Johnson, Jason Irwin, Adam Leache, and Joe Cook and his graduate student Schuyler Liphardt for the help and advice with the genetic analysis. Thank you, Lisa Shipley, for your advice and encouragement during the habitat analysis. A huge thank you to my graduate cohort for the support and laughs, even through the rough times. Thank you, Alexis Tiffany for being a supportive roommate and friend. Thank you to the individuals who have inspired me and supported this process by providing advice and equipment: Patty Garvey-Darda (USFS Cle Elum), Dana Stringer (WSDOT), Josh Zylstra (WSDOT), Mark Norman (WSDOT), Emil Babik, Cindy White, Mark Young, and Jonathan Betz. This project was made possible by the funding of the Washington State Department of Transportation and the Washington State Distinguished Fellowship Award.

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CHAPTER I

GENERAL INTRODUCTION

The continuous growth in human population size increases the need for housing, transportation corridors, and the use of other resources. This increase in human development fragments and degrades habitat at an exponential rate. Major interstate highways amplify these impacts on the environment and fragment wildlife populations, leading to reduced population sizes, lower genetic variability, and changing population structures (Jackson and Fahrig 2011). Roads create a physical and psychological barrier to the movement of wildlife. Physical barriers include wide expanses of pavement, guard rails, fencing, and vehicle collisions (Fahrig and Rytwinski 2009). Psychological barriers include the perceived risk of predation in open areas and disturbances such as noise and lights that may prevent successful crossing (Fahrig and Rytwinski 2009). Genetic studies have shown that highways genetically fragment many mammal populations , including the Florida black bear (*Ursus americanus floridanus,* Dixon et al. 2007), wolverines (*Gulo gulo,* Sawaya et al. 2019), and the bank vole (*Myodes glareolus*, Gerlach and Musolf 2000), but the need for continued research is crucial for mitigation and conservation efforts. Roads are of increasing concern worldwide for wildlife populations that are already struggling to find suitable habitat for resources and mates.

Washington State has about 11,265 km of roads (WSDOT 2019), with a minimum of 5,200 collisions with deer and elk each year (WSDOT 2020). This conservative estimate does not include roadkill of other animals including small mammals, amphibians, and reptiles, which is very difficult to determine due to removal by scavengers and rapid degradation (Hels and Buchwald 2001). Interstate-90 (I-90) bisects Washington State in a crucial part of central

Washington near Snoqualmie Pass where wildlife moves through the Cascade Mountains. This creates a pinch point where wildlife of all sizes is constrained from moving through their natural habitat. The Snoqualmie Pass area of I-90 gets used by 30-60,000 vehicles every day, making it a physical and psychological barrier for wildlife populations. WSDOT and many other organizations have worked together to transform a large-scale construction project, now called the I-90 Snoqualmie Pass East Project (I-90 SPEP), to include connectivity goals that help mitigate the impacts this highway has on wildlife and humans. The major goals of this project include expanding the roadway for increased traffic volumes, improving human and wildlife safety by reducing wildlife collisions, and improving the connection of wildlife populations and their habitat (WSDOT 2020).

Wildlife crossing structures are used to reconnect ecosystems that have been fragmented by roadways (Forman 2010). These structures range in size and design depending on the landscape, road size, and targeted species. For the I-90 SPEP, WSDOT has designed multiple structures of varied types that are suitable for a variety of species like elk, deer, bears, mountain lions, coyotes, small mammals, reptiles, amphibians, and fish. Bridges, overpasses, and bottomless culverts are just a few examples of the structures being constructed within the project area. After construction, different habitat features (e.g., rockpiles, woody debris, snags) are placed in and around the crossing structures in an effort to restore the connection of habitats along I-90 (WSDOT and U.S. DOT Federal Highway Administration 2006). The success of these structures can be assessed only by pre- and post-construction monitoring of wildlife (Clevenger 2005). This has been done effectively in Banff National Park, where data have been collected year-round since 1996 (Clevenger and Waltho 2003). Monitoring efforts often include

using wildlife cameras, sighting reports, roadkill data, and tracks for larger species; but small animals require more intensive sampling through live-trapping and other means.

Small mammals (typically defined as those weighing < 5 kg, Merritt 2010) are often overlooked when discussing species that are affected by roads. However, they suffer from the same negative effects of habitat degradation and fragmentation that larger mammals, reptiles, and amphibians do. With their relatively short lifespans and low dispersal distances, most small mammals are considered low-mobility species: they are unable to move large distances in their lifetime or even through generations. Small mammals are ecologically important for their ability to disperse seeds and fungal spores, as well as serving as prey to many raptors, reptiles, and other carnivores (Maser et al. 1978, Vander Wall 2001).

One group of small mammals, *Sorex* shrews, is abundant worldwide (Churchfield 1990). Shrews are the smallest mammals in North America. They are ecologically important as a food source for predators such as owls, kestrels and other raptors, weasels, foxes, and snakes, and as consumers of copious amounts of invertebrates in their lifetime (Korpimaki and Norrdahl 1989, Churchfield 1990). Six species of shrews have documented ranges within the I-90 SPEP area: Trowbridge's shrew (S. *trowbridgii*), masked shrew *(S. cinereus)*, montane shrew (*S. monticolus*), vagrant shrew (*S. vagrans*), marsh shrew (*S. bendirii*), and the American water shrew (*S. palustris*). Four of these species (*S. trowbridgii*, *S. cinereus*, *S. monticolus*, and *S. vagrans*) are terrestrial shrews that are similar in size and pelage color, and often can be identified only through skull, dental, and hindfoot characteristics or genetics (George 1989, Smith and Belk 1996, Gillihan and Foresman 2004, Rausch et al. 2007). The American water shrew (*S. palustris*) and the marsh shrew (*S. bendirii*) are semiaquatic species that forage for food in small streams and wetlands (Pattie 1973, Beneski and Stinson 1987). They can smell

food underwater and dive to eat aquatic invertebrates or small fish (Catania et al. 2007). These aquatic shrews are easily identifiable based on their pelage color and the fringe hairs on their hind feet that aid in swimming.

This study used shrew-specific methods to determine which species were present, their population genetic structure, and spatial distribution in both macro- and microhabitats. We also provide pre-construction baseline information for future evaluation of whether restoring the landscape with crossing structures and habitat has been successful. These sympatric shrews are difficult to identify, so my first goal was to verify or correct field identifications through phylogenetic inferences based on sequence data. Next, I aimed to determine the population genetic structure of the most abundant species to determine if the highway has fragmented shrew populations. Lastly, I evaluated both macro- and microhabitat associations of the shrew species in the project area. I-90 could have been a barrier to wildlife since the 1950s when it was built. Because this represents a potential impact on shrew populations for upwards of 70 generations, I predicted that populations on opposite sides of the highway would show some genetic differentiation. I also expected to see species-specific preferences for habitat, based on the coexistence of multiple shrew species within the area. Information derived from this study can serve as a baseline for comparisons with future studies after the crossing structures are built.

CHAPTER II

LITERATURE REVIEW

Wildlife requires room to disperse, breed, and live. Humans often affect their ability to do this by destroying, degrading, and fragmenting habitat through resource extraction or development. A large part of human development is the construction of transportation corridors. Roads fragment habitat by dividing large sections of land into smaller sections (Wilcox and Murphy 1985). Roads have impacts on the surrounding abiotic environment through changes in hydrology and contamination of air and water, and affect the biotic environment by causing changes in population size, composition, structure, and genetics (National Research Council 2005). Road mortality and avoidance of roads by wildlife populations cause decreased genetic diversity and species abundance (Jackson and Fahrig 2011). This has been shown in a variety of species from around the world: Florida black bear (*Ursus americanus floridanus*, Dixon et al. 2007), timber rattlesnakes (*Crotalus horridus*, Clark et al. 2010), and even ground beetles (*Carabus violaceus L.*, Keller and Largiader 2003). Research on the impacts of roads on wildlife has led to the exploration of ways to mitigate these impacts.

Wildlife on roadways is dangerous for animals and humans. The US has over 6.4 million km of public roads (USDOT and FHWA 2011). An estimated 1-2 million wildlife vs. vehicle collisions take place each year in the US (USDOT and FHWA 2008). The damages caused by these collisions are extensive, because most notable collisions are with large ungulates or carnivores (e.g., deer, elk, moose, bear, cougar), and have cost more than US$ 8 billion dollars annually (USDOT and FHWA 2008). Rerouting wildlife under or over the road through wildlife crossing structures reduces the number of collisions, saving lives (of humans and wildlife) and

costs. This has led state transportation departments and other agencies to work together to increase wildlife connectivity and decrease collisions by constructing wildlife crossing structures. The purpose of these crossing structures is to increase the connectivity of terrestrial and aquatic habitats and the species that inhabit them (Forman 2010). They can serve to connect a multitude of species including fish, amphibians, reptiles, and mammals.

Small mammals occupy most terrestrial ecosystems and play important roles as seed and spore dispersers, ground excavators, and prey to larger mammals, birds, reptiles, and some amphibians (Vander Wall 2001, Merritt 2010). A small mammal is frequently considered as one weighing less than 5 kg (Merritt 2010). Small mammals (especially rodents and insectivores in the order Eulipotyphla) often have short life spans, only living through one or two reproductive seasons (Merritt 2010). Populations that have short lifespans and high turnover rates can change genetically in a short amount of time (Merritt 2010), and these changes can be exacerbated by habitat fragmentation (Jackson and Fahrig 2011). Shrews, with their very small body sizes and short generation times, may be especially vulnerable to the barrier effects of highways.

Shrews are small "mouse-like" creatures with long snouts that are abundant worldwide (Churchfield 1990). The shrews in the genus *Sorex* are referred to as "long-tailed shrews" or "red-toothed shrews". *Sorex* shrews are the smallest mammal in North America, weighing as little as 3 grams as adults (George 1989, Smith and Belk 1996, Gillihan and Foresman 2004, Rausch et al. 2007). Shrews have distinct skull characteristics that distinguish them from other mammal species. Their skulls have small braincases with well-developed olfactory lobes suggesting that they rely heavily on smell (Churchfield 1990). Their very small eyes and lack of reaction to visual cues (unless accompanied by feeling, smell, or hearing) suggest that they do not rely on sight (Branis 1981). Shrew teeth are small but mighty and can crush invertebrate

exoskeletons. Iron deposits on the tips of shrew teeth cause red pigmentation and increase their dental strength (Merritt 2010). Each species has identifiable dentition; small differences in unicuspid size or incisor form can distinguish seemingly identical shrews as different species (Nagorsen 1996); however, skull characteristics and external features often need to be used in combination with dental characteristics to appropriately identify individuals (Rausch et al. 2007, Woodman and Fischer 2016). Among the shrews in the I-90 SPEP study area, terrestrial species are of similar size and pelage color, and semi-aquatic shrews are larger than terrestrial species of shrews. They have thicker and darker hair with fringe hairs on their hind feet that help them navigate the water (Nagorsen 1996). They dive and hunt for prey in small streams or wetlands. Interestingly, they can use scent to find prey by blowing bubbles underwater and inhaling those bubbles rapidly (Catania et al. 2007). High surface-area-to-volume ratios cause shrews to have tremendously high metabolic rates (Churchfield 1990). Consequently, these insectivorous creatures become opportunistic feeders when faced with a lack of food (Pearson 1954). They can eat worms, slugs, fish, or other small mammals. Unlike many other northern temperate mammals, shrews are active year-round. To do this, they go through what has been termed "Dehnel's phenomenon" whereby they can shrink or grow their body mass and braincase seasonally depending on food availability (Merritt 1995). *Sorex* shrews live 12-18 months depending on species and are completely independent 25 days after birth (Churchfield 1990). Mating season is primarily in early spring but can last to late summer. Shrews are solitary and asocial; only interacting during the breeding season (Churchfield 1990). Reproductive males have flank glands that produce a strong odor to deter predation and attract females (Stockley et al. 1995). Predators of shrews include owls, kestrels and other raptors, weasels, foxes, and snakes (Korpimaki and Norrdahl 1989).

Determining home ranges of small mammals like shrews can be difficult because they spend the majority of their time in abandoned runways or under leaf litter, making it difficult to estimate how far they move or defend territories (Churchfield 1990). Remarkably, multiple shrew species can occupy the same area even though they likely are competing for the same resources. This sympatry may be possible due to small changes in habitat use for foraging (Churchfield 2002), but this is one hypothesis and the true mechanism is largely unstudied. In captive studies, shrews of different species will either avoid each other or fight when encountered (Churchfield 1990).

Sorex shrews are found all over the world, but here in central Washington, we have six documented sympatric species. Four are terrestrial species: Trowbridge's shrew (S. *trowbridgii*), masked shrew (*S. cinereus*), montane shrew (*S. monticolus*), and vagrant shrew (*S. vagrans*); and two are semiaquatic: the marsh shrew (*S. bendirii)* and the American water shrew (*S. palustris*). Other species in Washington (but not known to inhabit central Washington) include the Olympic shrew (*S. rohweri*), pygmy shrew (*S. hoyi*), Merriam's shrew (*S. merriami*), and Preble's shrew (*S. preblei*).

Shrews are rarely the focal species of small-mammal monitoring projects, and this leads to insufficient data on many shrew species. During live-trapping studies, the bait used is not sufficient to sustain the shrews' metabolic needs overnight, causing mortality rates as high as 90%, mostly due to starvation or freezing (Shonfield et al. 2013). High mortality rates make it difficult to gain behavioral information and other important data that comes from recapturing individuals.

CHAPTER III

JOURNAL NOTE

RANGE EXTENSION OF THE OLYMPIC SHREW (*SOREX ROHWERI)* EAST OF THE CASCADE CREST IN WASHINGTON STATE

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***Abstract*—** The known geographic range of the Olympic shrew (*Sorex rohweri*) is western Washington, Oregon, and British Columbia. However, during a study on shrews in central Washington, we genetically verified the presence of this species on the eastern slopes of the Cascade Range. Field identifications of live shrews were deemed unreliable after genetic work was completed to confirm the identification of 127 terrestrial shrews captured in the summer of 2019. Of the 127 shrews captured, mtDNA analysis of cytochrome *b* gene sequences identified 41 of them as *S. rohweri*. Most of these individuals were misidentified in the field as *S. cinereus* (17) but others were field-identified as *S. trowbridgii* (13), *S. monticolus* (7), or *S. vagrans* (3). This discovery extends the documented geographic range of *S. rohweri* to the eastern slopes of the Cascade Range in Washington. We also suggest genetic verification of species identifications of museum specimens and of individuals captured in future live-trapping studies on species that are hard to identify.

***Note*—** Shrews in the genus *Sorex* are among the smallest mammals of North America, weighing as little as three grams (George 1989, Smith and Belk 1996, Gillihan and Foresman 2004). Multiple *Sorex* species often live sympatrically, occupying the same geographic range

(Churchfield 1990). Shrews are notoriously difficult to identify in the field during live-trapping studies. Proper identification often requires skull and dental measurements (Rausch et al. 2007, Nagorsen and Panter 2009, Woodman and Fischer 2016). This may be suitable for research in mammal collections but becomes more difficult during live-trapping studies that aim to keep mortality low. One of the most ethical and accurate ways to identify shrews is through genetic analysis, because the only other known way is to have access to the skull which is impossible on live animals (Rausch et al. 2007). As part of a larger study to determine the habitat preferences and population genetic structure of six sympatric shrew species in central Washington, we used DNA sequencing from the mitochondrial cytochrome *b* gene to verify species identifications (Dubey et al. 2007, O'Neill et al. 2005). Here, we report the genetic identification results from this set of sympatric shrews, and the discovery of the Olympic shrew (*S. rohweri*) on the east slopes of the Cascade Range in Washington State.

Our study area lies within the Okanogan-Wenatchee National Forest in central Washington on the east slopes of the Cascade Range between the southern end of Keechelus Lake and just east of Easton, WA. This area of mixed-coniferous forests contains many important habitat types: wetlands, talus slopes, and old-growth forest all host a wide variety of wildlife (WSDOT and USDOT FHWA 2006). Elevation along the highway varies from 700 to 850 m. We selected three sites straddling Interstate-90 (Fig. 1.1). Each site encompassed a stream that crossed the highway, and the surrounding forest.

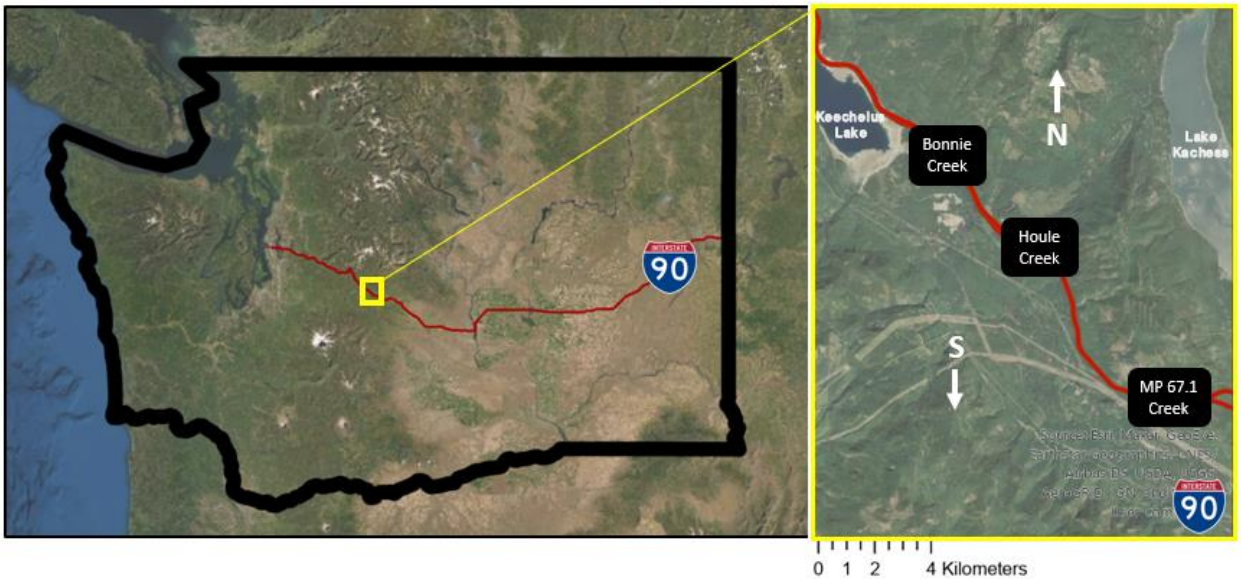

Fig. 1.1. Washington State outline showing Interstate-90 and study area (left). Study sites near I-90 in the central Cascades (right).

Trapping transects were placed in streamside habitat, "lowland" forest, and "upland" forest both north and south of the highway at all three sites for a total of 18 trapping transects. Streamside habitats were adjacent to a seasonal stream, lowland habitats were relatively flat forested areas at least 50 meters from the stream channel, and upland habitats occurred along or above an apparent slope. Each transect consisted of 20 Sherman traps, each spaced 5 meters apart. A pitfall array (four 5-gallon [19-liter] buckets dug level with the ground and connected by metal drift fencing) was placed 10-15 meters away from each transect. Up to three funnel traps were placed in each streamside habitat. We provided insulation and food to help sustain shrews overnight. All traps were opened for 2 consecutive nights from dusk until dawn (8-12 hours). Each site was trapped twice during the field season (1,772 total trap-nights).

Captured individuals were identified using a dichotomous key derived from multiple sources (Nagorsen 1996, Nagorsen 2002, Verts and Carraway 1998) and adapted for species expected in this region. We recorded standard body measurements (body length, tail length, and

hindfoot length), sex, age, and reproductive status. Body size, pelage color, dentition (observed with a hand lens), and hind feet (fringes and toepads) were evaluated to identify species. The distal 1-2 mm of the tail was clipped to provide a genetic sample of each individual. The end of the tail was dipped in coagulant powder (as recommended by a local veterinarian) to limit bleeding. Each genetic sample was placed directly into a 0.5-ml microcentrifuge tube filled with non-denatured ethanol and immediately placed on ice. Recaptured individuals (identified by nail polish applied to toes or a clipped tail) were quickly identified to species and released. Any shrews that died were collected as whole specimens. All species other than shrews were identified and released immediately. Samples and specimens were taken back to the lab and placed in a -20º C freezer. Mortalities were prepared as museum specimens, skulls were cleaned, and liver samples were retained for DNA extraction.

Genetic samples (tail clips or liver tissue) from all individuals except aquatic shrews (*S. bendirii* and *S. palustris*, which are clearly identifiable from the other species) were extracted, amplified, and sequenced by CD Genomics (New York, NY). Proteinase K and zirconia beads were added to each sample and vortexed with Qiagen Tissue Lyser II. The tissue was then incubated at 55ºC for no less than 3 hours. Genomic DNA was extracted from the tissue lysate using the magnetic beads extraction method. The mitochondrial cytochrome *b* gene was amplified using the primers L14723 and H15915 (Nicolas et al. 2012). The htt exon1 CAG repeat region was amplified using the 6-Fam labeled forward primer and reverse primer with Thermo HotStart PCR mix from ThermoFisher Scientific. Cycling conditions were 96ºC for 10 min, followed by 35 cycles of 95ºC for 30 seconds, 50ºC for 30 seconds, 72ºC for 45 seconds, then by 72ºC for 10 min and 4ºC forever. PCR products were purified with the PCR purification kit. The Sanger Sequencing method was used to sequence the mitochondrial cytochrome *b* gene

(1140 bp). Two Sanger sequences were performed with both PCR primers and Bigdye 3.1 and run on an ABI 3730XI sequencer. The forward and reverse sequences from the same sample were assembled using the CodonCode Aligner. Consensus sequences were then reported. Of the 128 samples, 127 were successfully sequenced using this method. We conducted phylogenetic and molecular evolutionary analyses using MEGA version X (Kumar et al. 2018). Sequences were aligned using ClustalW in MEGA with a northern short-tailed shrew sequence (*Blarina brevicauda*, Sample: AB175134.1 from GenBank) as the outgroup. Of the 1140 base pairs in the mitochondrial cytochrome *b* gene, 1,010 base pairs were preserved in the alignment.

We compared all samples to known samples in GenBank using BLAST (Basic Local Alignment Search Tool) with the criterion of being >99% identical to sequences of specific species. During this process, no samples were identified as masked shrew (*S. cinereus*), and 41 samples were most closely related (> 99%) to the Olympic shrew (*S. rohweri*). To verify these unexpected results, a maximum likelihood phylogenetic analysis was performed with 100 bootstraps in MEGA to create the phylogenetic tree that included all individuals trapped during this study plus two GenBank samples each of the Trowbridge's shrew (*S. trowbridgii*, GenBank samples: FJ667520.1 and AY014956.1), montane shrew (*S. monticolus*, GenBank samples: AB100273.1 and AB100272.1), Olympic shrew (*S. rohweri*, GenBank samples: EU088302 and EU088303.1), vagrant shrew (*S. vagrans*, GenBank samples: MK691376.1 and MK691381.1), and masked shrew (*S. cinereus*, GenBank samples: AY014951.1 and AY014952.1). The tree was rooted by the northern short-tailed shrew (*Blarina brevicauda*, GenBank sample: AB175134.1) sequence. This tree was condensed by 50% and color-coordinated by species (Fig. 1.2).

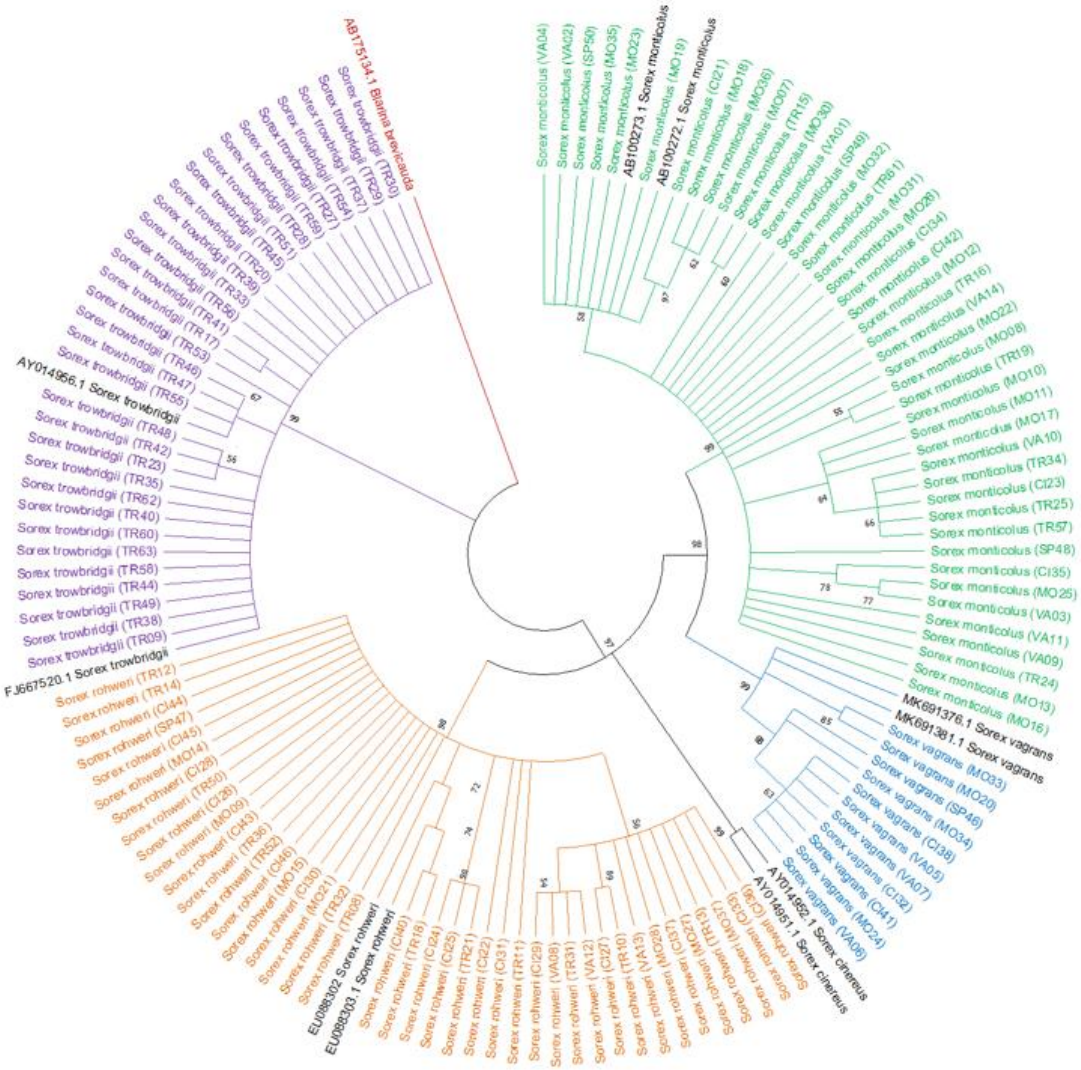

Fig. 1.2. Maximum-likelihood phylogenetic tree from 127 shrew samples collected east of Snoqualmie Pass, WA in 2019. A short-tailed shrew (*Blarina brevicauda*) sample from GenBank was included to root the tree, and two samples each of Trowbridge's shrew (*Sorex trowbridgii*), montane shrew (*Sorex monticolus*), Olympic shrew (*Sorex rohweri*), vagrant shrew (*Sorex vagrans*), and masked shrew (*Sorex cinereus*) from GenBank were included to verify species identifications.

Genetic verification showed that 43 montane shrews (*S. monticolus*), 41 Olympic shrews (*S. rohweri*), 32 Trowbridge's shrews (*S. trowbridgii*), and 11 vagrant shrews (*S. vagrans*) were captured in this study. The Olympic shrews were originally misidentified in the field as *S. cinereus* (17), *S. trowbridgii* (13), *S. monticolus* (7), *S. vagrans* (3), and *Sorex* sp. (1). Olympic shrews were captured only at Bonnie Creek (23) and Houle Creek (18) (Fig. 1.3); none of the 14

shrews captured at MP 67.1 Creek, just 4.5 km east of Houle Creek, was genetically identified as *S. rohweri*. The farthest east capture site was near Houle Creek (47.298561, -121.293831) at an elevation of about 730 m.

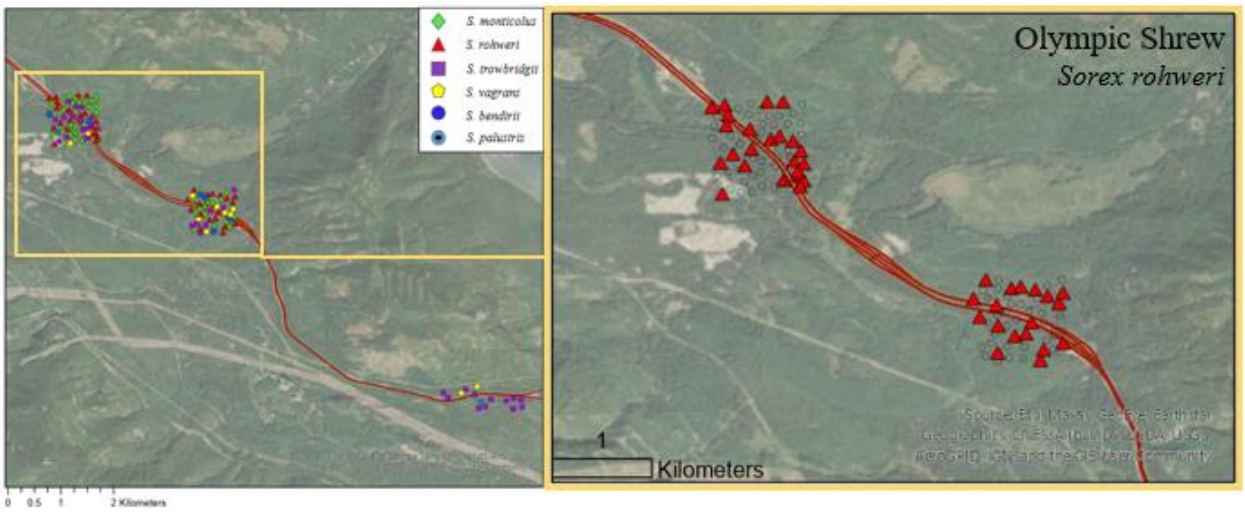

Fig. 1.3. Approximate locations of all shrews captured in 2019 (left). Map of approximate locations of the Olympic shrew (*Sorex rohweri*) (right).

The Olympic shrew (*S. rohweri*) was first discovered by Rausch et al. in 2007 through museum specimens. These museum specimens were originally identified as *S. cinereus* or *S. vagrans* and were from western Washington and British Columbia. The species range was later extended northward in British Columbia (Nagorsen and Panter 2009) and southward into western Oregon (Woodman and Fischer 2016). The shrews we report here are the first discovery of the Olympic shrew on the east slopes of the Cascade Range in Washington State and are the farthest east record of this species.

Only 43% of our field identifications were correct. Most of these identification errors were due to the discovery of the Olympic shrew (*S. rohweri*) in the area, as it was not included in our dichotomous key. However, the other errors were most likely caused by incorrect keying due

to the difficulty of seeing small characteristics (e.g., teeth and toepads) on live shrews. Genetic analysis proved to be crucial for the validity of this project and may be vital for future live-trapping studies on shrews that live sympatrically with other similar species.

Future work could include more extensive sampling in other locations along the eastern slopes of the Cascade Range and the review and genetic analysis of museum specimens from the area. Genetic expansion statistics from all samples of the Olympic shrew in Washington, Oregon, and British Columbia may help infer the source of the extension (Peter and Slatkin 2013). This might help determine the full extent of this range expansion as well as when and where it took place.

CHAPTER IV

JOURNAL ARTICLE

ECOLOGICAL AND GENETIC CONNECTIVITY OF SHREWS *(SOREX* SPP.*)* ACROSS INTERSTATE-90 IN THE CASCADE RANGE OF WASHINGTON STATE

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Abstract.— Roads negatively affect wildlife by degrading and fragmenting habitat. The I-90 Snoqualmie Pass East project was established to improve traffic safety for both humans and wildlife. Here, the Washington State Department of Transportation is constructing wildlife crossing structures to increase ecosystem connectivity as part of this project. The goal of our study was to investigate shrews – very small mammals with presumably low mobility and dispersal capacity – at sites on the eastern slopes of the Cascade Range where wildlife crossing structures will be built in the future. The main objectives were to verify shrew species composition, assess population genetic structure relative to the highway, and determine whether shrew abundance varied among macro- and micro-habitats. Using live-trapping techniques, we captured 136 individual shrews at three different paired sites north and south of the highway in the summer of 2019. Initial field identifications documented six sympatric species: Trowbridge's shrew (*Sorex trowbridgii*), montane shrew (*S. monticolus*), masked shrew (*S. cinereus*), vagrant shrew (*S. vagrans*), marsh shrew (*S. bendirii*), and the American water shrew (*S. palustris*). However, molecular analysis of the four terrestrial species determined that the study area was home to the Olympic shrew (*S. rohweri*) rather than the masked shrew (*S. cinereus*). This

discovery represents a range extension for the Olympic shrew on the eastern slopes of the Cascade Range, and the surprising absence of the masked shrew. Analysis of population genetic structure for the three most abundant species indicated that montane shrew and Olympic shrew populations separated by the highway still act as one population. Trowbridge's shrew populations had higher genetic variation, providing some evidence for population genetic structuring across the highway based on a limited sample size. Capture rates of shrews did not differ among habitat types or with most microhabitat characteristics. This study provides baseline information on an assemblage of shrew species near a major highway in central Washington and can be replicated in the future to assess potential effects of wildlife crossing structures on connectivity for these very small mammals.

Key words.— small mammals; populations; habitat fragmentation; live-trapping; road ecology; microhabitat; population genetic structure; highway.

INTRODUCTION

Major interstate highways affect the surrounding environment and fragment wildlife populations, leading to lower species abundance, less genetic variability, and changing population structures (Jackson and Fahrig 2011). Negative effects have been shown on a range of species from around the world from the Florida black bear (*Ursus americanus floridanus*, Dixon et al. 2007) to the timber rattlesnake (*Crotalus horridus*, Clark et al. 2010) and even ground beetles (*Carabus violaceus*, Keller and Largiader 2003). As human populations continue to increase, so do the need for transportation corridors, resulting in growing concern for the impacts roads have on wildlife populations that are already struggling to find suitable habitat for resources and mates.

Interstate-90 (I-90) runs through a crucial part of central Washington near Snoqualmie Pass where wildlife moves through the Cascade Range. This creates a pinch point where wildlife

of all sizes is constrained from moving either physically or psychologically. WSDOT and many other organizations have worked together to create a large-scale construction and connectivity project now called the I-90 Snoqualmie Pass East Project (I-90 SPEP) to improve traffic safety for wildlife and humans. The major goals of this project include expanding the roadway, improving human and wildlife safety by reducing wildlife collisions, and connecting wildlife populations by adding wildlife crossing structures (WSDOT 2020). Structures such as bridges, overpasses, and bottomless culverts were designed to be suitable for a variety of species. Habitat features like rock piles and snags are installed, and native vegetation is planted once crossing structures are complete in efforts to restore and connect the fragmented habitat. The project is split into four phases with two of them completed as of 2019, and the other two are planned for completion by 2029.

The success of these structures can be defined only by pre- and post-construction monitoring of wildlife (Clevenger 2005). Monitoring all species (large and small) within and around crossing structures is key to the planning of future restoration projects. Large mammals (mostly carnivores and ungulates) are typically the focal species for evaluating crossing structure success (Clevenger and Waltho 2005, Grilo et al. 2008, Simpson et al. 2016). However, species such as small amphibians, reptiles, and mammals are crucial for ecosystem health and function (Carey and Johnson 1995, Valencia-Aguilar et al. 2013). Small mammals (typically defined as those weighing less than 5 kg, Merritt 2010) are often overlooked when discussing species that are affected by roads (Porto Peter et al. 2013). Shrews have short lifespans and short dispersal distances (Churchfield 1990). They are ecologically important by being a food source for predators such as owls, kestrels and other raptors, weasels, foxes, snakes, and house cats, as well

as eating copious amounts of invertebrates in their lifetime (Korpimaki and Norrdahl 1989, Churchfield 1990).

Six species of shrews are documented in the central Washington Cascades: Trowbridge's shrew (S. *trowbridgii*), masked shrew *(S. cinereus)*, montane shrew (*S. monticolus*), vagrant shrew (*S. vagrans*), marsh shrew (*S. benderii*), and the American water shrew (*S. palustris*). Four of these species (*S. trowbridgii*, *S. cinereus*, *S. monticolus*, and *S. vagrans*) are terrestrial shrews that are similar in size and pelage color, and can often only be identified through the skull, dental, and hindfoot characteristics or genetics (Smith and Belk 1996, George 1989, Gillihan and Foresman 2004, Rausch et al. 2007). The American water shrew (*S. palustris*) and the marsh shrew (*S. bendirii*) are semiaquatic shrews that forage for food in small streams and wetlands (Pattie 1973, Beneski and Stinson 1987). The two species are easily identifiable based on their pelage color and the fringe hairs on their hind feet that aid in swimming.

We first genetically identified shrews captured in this study (methods and results found in chapter III). Then we evaluated genetic variation between populations on the same side of the highway compared to populations on opposite sides of the highway to determine if the highway has affected shrew genetic structure by acting as a barrier to the movement of shrews. This will provide baseline information for future evaluation of whether restoring the landscape with crossing structures and habitat has been successful. We also evaluated both macro- and microhabitat use of the shrew species in the project area. We hypothesized that these sympatric species would show differences in habitat use. The results of the habitat analysis can also be used to inform WSDOT of shrew habitat requirements for sites being restored within and adjacent to the crossing structures, and to provide microhabitat information for future restorations and the design of crossing structure features.

METHODS

Study Area

We studied shrews in forest adjacent to I-90 between the southern end of Keechelus Lake and just east of Easton, WA (Fig. 2.1). Our study area lies within the Okanogan-Wenatchee National Forest in central Washington State on the east slopes of the Cascade mountain range. This area is a mixed-coniferous forest that contains many important habitat types: wetlands, talus slopes, and old-growth forest stands, all of which host a wide variety of wildlife (WSDOT and USDOT FHWA 2006). Elevation along the highway ranges from 730 to 850 m. Interstate-90 (I-90) cuts through our study area, fragmenting the Cascade Range and blocking wildlife access to natural habitat. I-90 was constructed during the 1950s to connect the east and west sides of Washington state. This major interstate highway is used for commuting, visitor travel, and commercial transportation. Traffic levels vary in this area from 30,000 to 60,000 vehicles every day (WSDOT 2016). To allow for an increase in traffic and fewer road closures, the Washington State Department of Transportation (WSDOT) began construction in 2008 to expand the roadway to 6 lanes while also constructing a variety of crossing structures to increase wildlife connectivity. Construction is projected for completion by 2029.

We selected three study sites where wildlife crossing structures will be built within the next decade to connect the surrounding habitats on either side of the highway. At each site a stream intersects I-90; these are Bonnie Creek, Houle Creek, and an unnamed creek near milepost 67.1 (hereafter called MP 67.1 Creek) (Fig. 2.1).

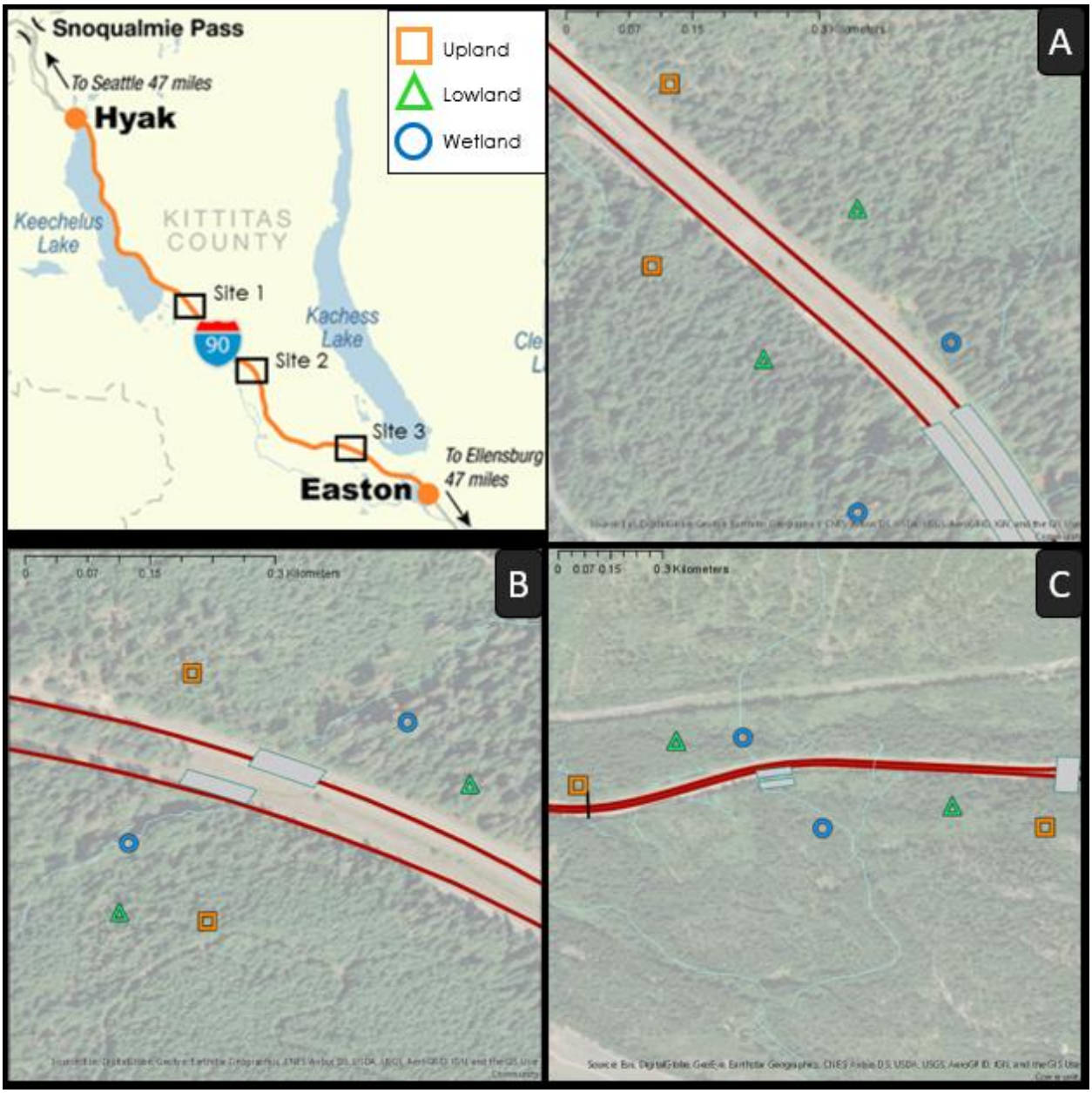

Fig. 2.1. Map showing I-90 Snoqualmie Pass East project area (top right) and locations of pitfall arrays in streamside (blue circle), lowland (green triangle), and upland (orange square) habitats in the three study sites: Bonnie Creek (A). Houle Creek (B). and MP 67.1 Creek (C).

Live-trapping

At each site, we identified streamside, lowland, and upland habitat types both north and south of the highway (Fig. 2.2). Streamside habitats were located within 10 m of the stream edge and represented either riparian forest or stream. Lowland habitats were flat, forested areas at least

50 meters away from streamside and upland habitats. Upland habitats were at least 50 meters away from lowland and streamside habitats, occurred along or above an apparent slope, and had drier, more well-drained soils. All trapping effort was done 40-100 meters from the highway edge (outside of planned construction zones).

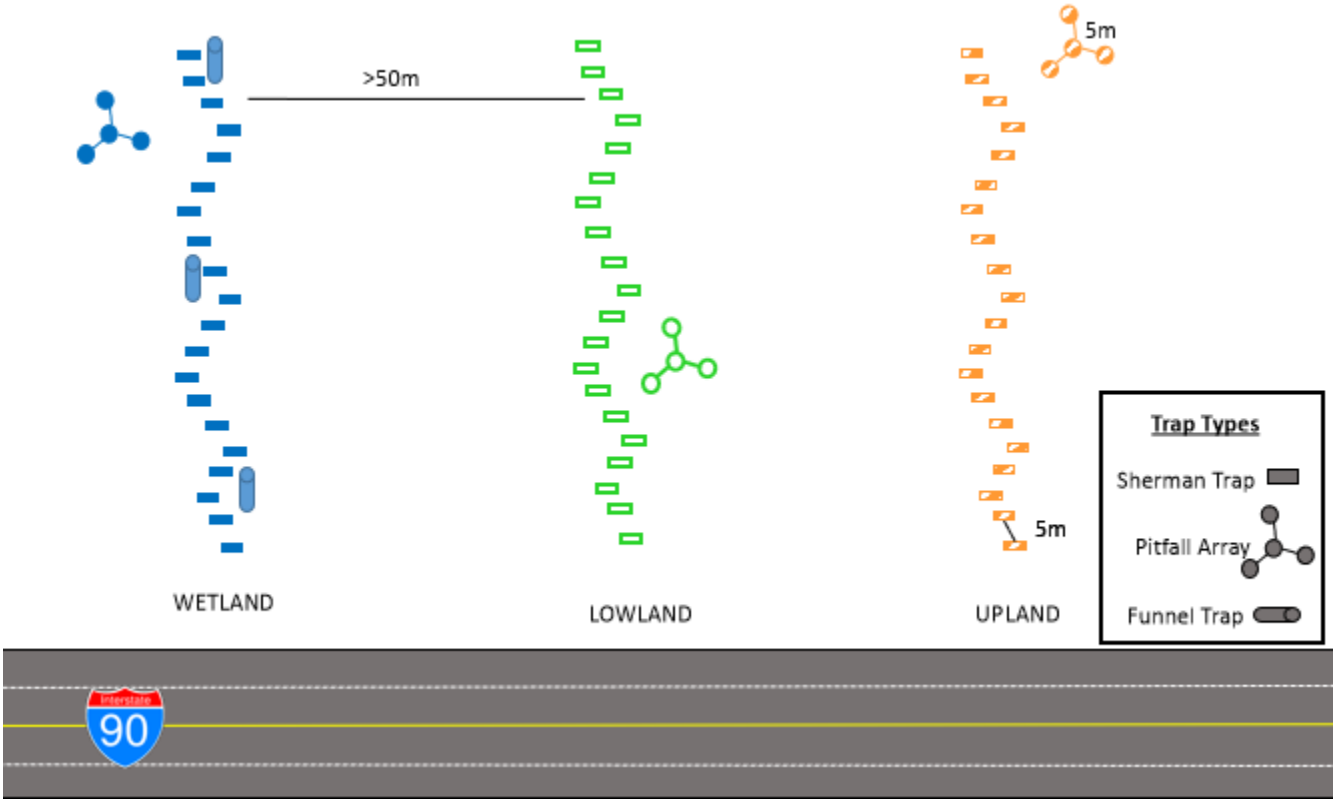

Fig. 2.2. Schematic example of live-trapping setup. Streamside (solid blue), lowland (hollow green), upland (striped orange). Placement of transect lines and pitfall arrays depended on habitat availability and landscape (see Fig. 2.1. for actual locations).

Three different types of live traps were used to increase our probability of capturing shrews in this study: Sherman traps, pitfall arrays, and funnel traps (Fig. 2.3). One transect line in each habitat was arranged with 20 Sherman traps spaced 5 m apart. Since the streamside transect lines followed the meandering stream course, we intentionally designed the lowland and upland transects to meander randomly through the forest. In each Sherman trap, we placed half a toilet paper tube with polyfill for insulation, and 20 mealworms as food. When rain was

expected, Sherman traps were covered for protection (Fig. 2.3A). One pitfall array was installed 10-15 meters from each transect line. Pitfall arrays consisted of four 5-gallon (19-liter) buckets buried so the rims were level with the ground. One bucket in the center was connected via metal fencing to 3 peripheral buckets 5 m away (Fig. 2.3B). Every bucket in the pitfall array contained 2 toilet paper tubes insulated with padding, a bottle cap with water, and 30 mealworms. When opened for trapping, we propped the bucket lid up with small sticks to provide cover from predators and weather but still allow shrews access. We recorded the locations of pitfall arrays and transect line endpoints with a handheld GPS. Up to three funnel traps were placed in each stream. We used funnel traps in this study to increase our probability of capturing aquatic shrews (*S. bendirii* and *S. palustris),* based on shrew captures in previous studies that used funnel traps for salamanders and other amphibians (Ernest, pers. comm.). Funnel traps were modified by adding a cork platform with a toilet paper tube insulated with padding wedged between the mesh and the cork (Fig. 2.3C). All traps were open for 2 consecutive nights from dusk until dawn (8-12 hours) during each trapping session. Each site was trapped twice during the field season for 588-592 trap-nights per site and a total of 1,772 trap-nights for this study (Table 2.1).

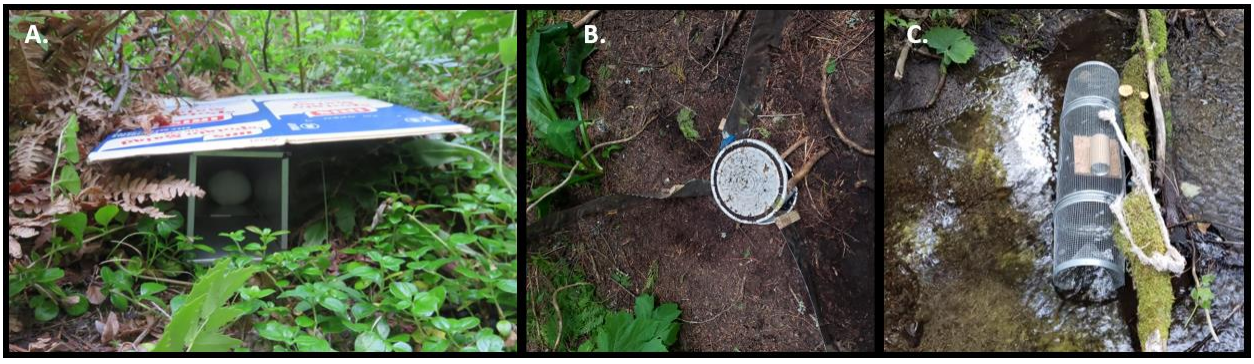

Fig. 2.3. Sherman trap with waxed carton covering to protect from rain (A). Middle bucket from pitfall array showing fencing leading to other buckets (B). Modified funnel trap (C).

Table 2.1. Sites were trapped (X) alternately throughout the summer weeks. Trap-nights are shown for all trap types (Sherman, pitfall, and funnel), as well as totals for the study.

Creek	July 10-11	July 17-18	July 31-Aug 1	Aug 7-8	Aug 22-23	Aug 28-29	Sherman Traps	Pitfall Traps	Funnel Traps	Total
Bonnie		X			X		480	96	12	588
Houle	X			X			480	96	16	592
MP 67.1			X			X	480	96	16	592
						All Sites	1,440	288	44	1,772

Animal Processing:

Captured individuals were identified using a dichotomous key derived from multiple sources (Nagorsen 1996, Nagorsen 2002, Verts and Carraway 1998) and adapted for species expected in this region. Standard body measurements (body length, tail length, and hindfoot length), sex, age, and reproductive status were recorded. Body size, pelage color, dentition, and hind feet (fringes and toepads) were evaluated to identify species. We took pictures of each individual's back, side, and tail to help establish a collection of photographs of each species. Genetic samples were taken from all individuals in the form of 1-2 mm tail clips. Each genetic sample was placed directly into a 0.5-ml microcentrifuge tube filled with non-denatured ethanol and immediately placed on ice. If the end of the tail began to bleed it was dipped in a veterinary approved coagulant. Nail polish applied to toes served as a temporary mark to avoid repeated sampling of the same individual. Recaptured individuals (identified by nail polish or a clipped tail) were quickly identified to species and released. Sluggish or cold shrews were fed sugar water until recovery and released without further stress of processing. All bycatch (species

other than shrews) was identified and released immediately to reduce time and stress of shrews waiting in traps to be processed. Animals that died in traps were collected as whole specimens. Genetic samples and specimens were taken back to the lab and placed in a -20º C freezer at the end of the trap week. Once back in the lab, mortalities were prepared as museum specimens, skulls were cleaned, and liver samples were retained for DNA extraction.

Population Genetics

DNA extraction, sequencing, alignment, and phylogenetic analysis was completed prior to evaluating population genetic structure (described in chapter III). Pairwise genetic distances (% divergence) between species were calculated using the Maximum Composite Likelihood model with 50 bootstraps (Tamura et al. 2004) in MEGA X. Samples of the three most abundant species were analyzed to determine each species' population genetic structure. The montane shrew (n = 43), Olympic shrew (n = 41), and Trowbridge's shrew (n = 32) were organized by capture site: Bonnie Creek (North), Bonnie Creek (South), Houle Creek (North), Houle Creek (South), MP 67.1 Creek (North), and MP 67.1 Creek (South) in Arlequin Version 3.5 (Excoffier and Lischer 2010). Fixation indices (F_{ST}) and their *P*-values were calculated in Arlequin 3.5 for all pairs of capture sites based on a 1,010-bp partial sequence of the mitochondrial cytochrome *b* gene. An Analysis of Molecular Variance (AMOVA) was performed with 1000 permutations to determine variance among and within populations.

Habitat Use

To evaluate microhabitats used by shrews, we measured microhabitat features at each pitfall bucket and 5 randomly-selected stations (using a random-number generator) along each transect line. A 1-m^2 square frame of PVC pipe was placed 1 m to the NE of each pitfall bucket or station (Fig. 2.4A) to avoid areas disturbed by setting and checking traps. Within this square, we estimated percent ground cover by soil, water, rock, woody debris, leaf litter, shrub, forbs,

graminoids, and moss; we also measured leaf litter depth (cm) and used a soil penetrometer to measure soil compaction (kg/cm^2). Using a spherical densiometer, we estimated canopy cover while facing north, south, east, and west. These four measurements were then averaged to determine canopy cover (%). Tree densities were calculated using the Point-centered Quarter Sampling Method (Brewer and McCann 1982), but we recorded the densities of all species by size class (medium 2.5 > 13 cm DBH, or large > 13 cm DBH) rather than by individual species (Fig. 2.4B). All plant species present within each habitat type were identified and recorded.

Fig. 2.4. Percent ground cover survey set-up (A). Measuring tree densities using the Point-centered Quarter Sampling method (B).

To test whether number of shrew captures (presumed to reflect relative abundance) was associated with certain habitat types (streamside, lowland, or upland) or microhabitat characteristics (soil compaction, canopy cover, tree densities, and ground cover: soil, rock, woody debris, leaf litter, shrub, forbs, and moss), we performed a generalized linear mixed model with a negative binomial distribution in R (version 1.3.959) using the "glmm" package (Knudson et al. 2020). The number of captures (over the trapping season) for the three most

abundant species (*S. monticolus*, *S. rohweri*, *S. trowbridgii*, using genetic identifications) and total shrew captures were used in this model with site, side of highway (north or south), and habitat type treated as fixed effects, and ground cover (soil, leaf litter, woody debris, rock, forbs, shrubs, and moss), soil compaction, canopy cover, and tree densities treated as random effects. These models were verified using the "DHARMA" package to test for outliers, dispersion, and zero-inflation (Hartig and Lohse 2020).

RESULTS

Captures

Of the 397 total mammal captures recorded during this study, 156 were shrews of six different species. The majority of shrew captures were unique individuals (136), with only 20 recaptures. In the field, we identified 54 individuals as Trowbridge's shrew (*S. trowbridgii*), 31 as montane shrews (*S. monticolus)*, 24 as masked shrews (*S. cinereus*), 14 as vagrant shrews (*S. vagrans*), 6 as marsh shrews (*S. bendirii*), and 2 as American water shrews (*S. palustris*). Molecular analysis (Chapter III, Fig. 1.2) verified that we actually caught 32 Trowbridge's shrews, 43 Montane shrews, 11 vagrant shrews, and 41 Olympic shrews (Table 2.2). No masked shrews were caught in this study area. Semi-aquatic shrews (*S. bendirii* and *S. palustris*) were not included in the genetic analysis because these species are easily distinguishable based on visible characteristics. Bonnie Creek and Houle Creek had higher species richness (catching all six species and five of the six species, respectively) than MP 67.1 Creek, which had lower capture numbers and only three species present. Trowbridge's shrews and vagrant shrews were the only terrestrial species captured at all three sites, but they were also the least abundant. Approximate capture locations of every individual can be seen in Fig. 2.5, and the representation of sympatric species captured in our study area can be found in Fig. 2.6. Bycatch included 228 deer mice

(*Peromyscus keeni* and *P. maniculatus*), 4 red-backed voles (*Myodes gapperi*), 3 unidentified voles (*Microtus* spp.), 3 shrew-moles (*Neurotrichus gibbsii*), 2 short-tailed weasels (*Mustela erminia*), and 1 American pika (*Ochotona princeps*).

Table 2.2. Shrew captures from genetic identification by site. Field identifications were included to show unreliability (Red). One genetic sample failed to sequence, resulting in one unidentified shrew (*Sorex* sp.).

Species	Bonnie Creek (North)	Bonnie Creek (South)	Houle Creek (North)	Houle Creek (South)	MP 67.1 Creek (North)	MP 67.1 Creek (South)	TOTAL	Field Identified
Sorex monticolus montane shrew	21	6	8	8	0	0	43	31
Sorex rohweri Olympic shrew	5	18	7	11	0	0	41	0
Sorex trowbridgii Trowbridge's shrew	2	11	3	4	4	8	32	54
Sorex vagrans vagrant shrew	1	1	1	6	2	0	11	14
Sorex bendirii marsh shrew	1	1	4	0	0	0	6	6
Sorex palustris American water shrew	1	0	0	0	0	1	2	2
Sorex cinereus masked shrew	0	0	0	0	0	0	0	24
Sorex sp. shrew	0	1	0	0	0	0	1	5
TOTAL	31	38	23	29	6	9	136	

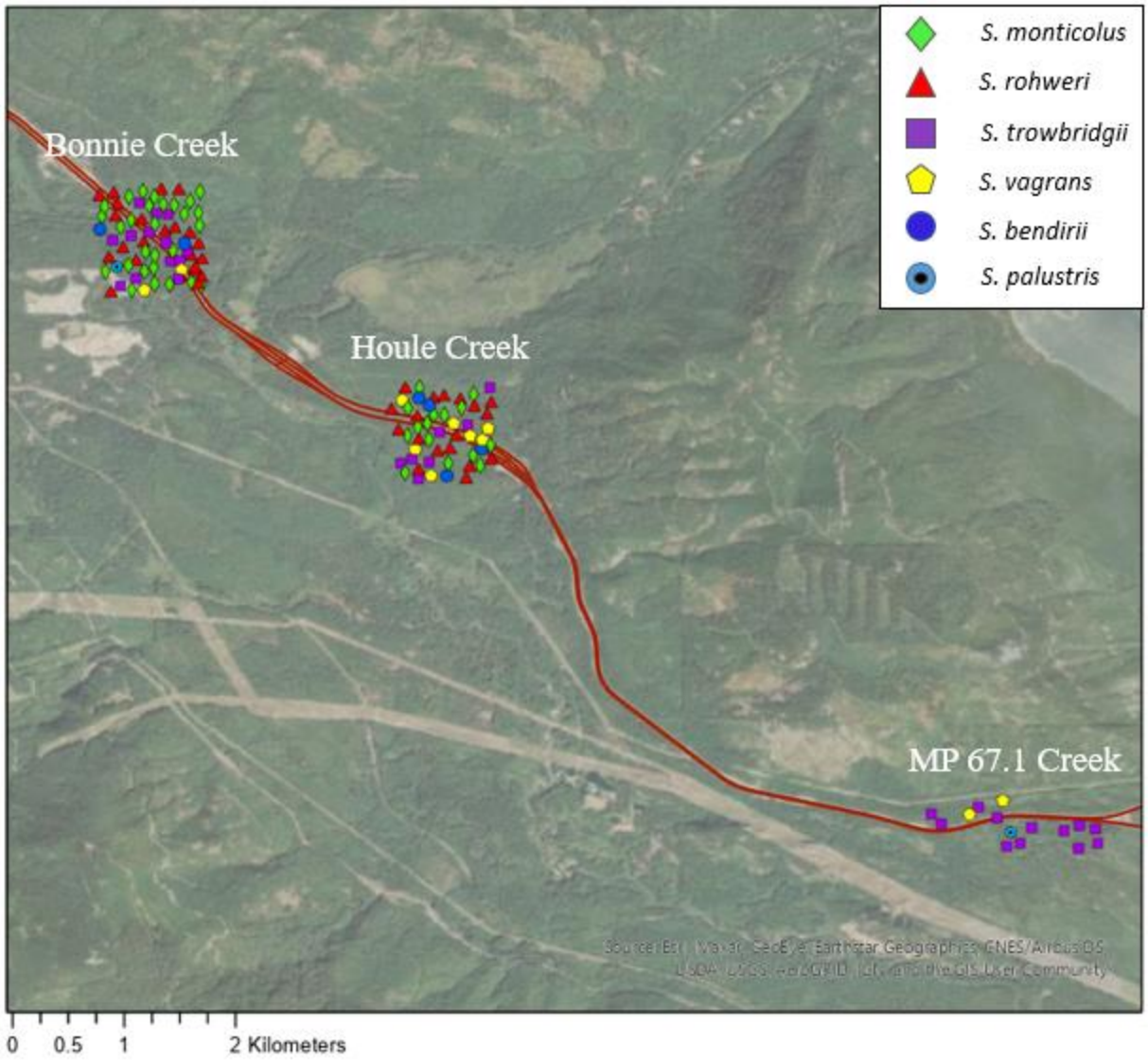

Fig. 2.5. Map of approximate locations of shrews trapped in 2019 based on their genetic ID. True locations were manipulated to avoid stacking of individuals that were captured in the same trap. 32 *Sorex trowbridgii*, 43 *Sorex monticolus*, 11 *Sorex vagrans*, 41 *Sorex rohweri*, 6 *Sorex bendirii*, and 2 *Sorex palustris* were captured during this study.

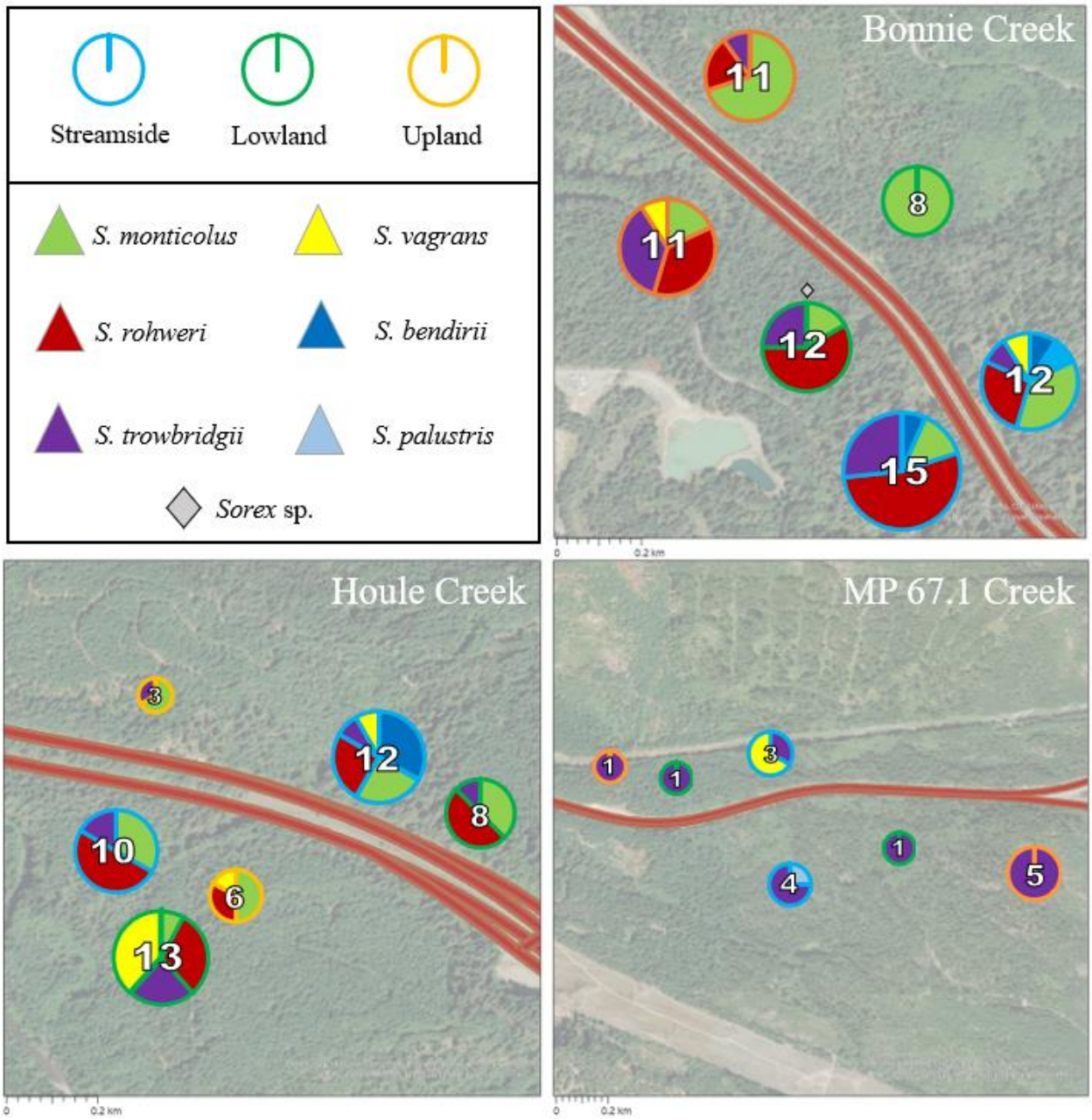

Fig. 2.6. Shrews of six different species captured at sites along I-90 in three separate macrohabitat types: streamside (blue outline), lowland (green outline), and upland (orange outline). Pie charts show species captured in pitfall arrays, Sherman traps and funnel traps, with the size of the circle and the number reflecting the total number of individuals captured and the slices representing the percentage of captures of each species. One unidentified individual is shown (gray diamond) above the pie chart.

Trap types

Although Sherman traps represented the highest trap effort (1,440 trap nights), they were the least successful in capturing shrews (success = 1%) and had the highest mortality rate (53%). The modified funnel traps were only 9% successful but doubled the number of captures of the two less common semi-aquatic shrews (*S. bendirii* and *S. palustris*). The pitfall arrays had the highest success rate (48%) with only 288 trap nights. The pitfall arrays also had the lowest mortality rate (25%). Our overall mortality rate was 28%.

Population Genetics

Analysis of the mitochondrial cytochrome *b* gene revealed 217 variable base pairs among the four terrestrial species (*S. monticolus*, *S. rohweri*, *S. trowbridgii*, and *S. vagrans*). The level of genetic divergence between species ranged from 8 to 17% (Table 2.3). The least divergence was seen between S. *monticolus* and *S. vagrans* (8%), and the greatest was seen between *S. trowbridgii* and *S. vagrans* (17%).

Table 2.3. Percent divergence in mitochondrial DNA cytochrome *b* gene sequences between species (*S. monticolus, S. rohweri, S. trowbridgii,* and *S. vagrans*) using the Maximum Composite Likelihood model with 50 bootstraps in MEGA X.

	S. monticolus (%)	*S. rohweri (%)*	*S. trowbridgii (%)*
Sorex monticolus (n = 43)			
Sorex rohweri (n = 41)	11		
Sorex trowbridgii (n = 32)	16	15	
Sorex vagrans (n = 11)	8	10	17

Montane shrews (*S. monticolus* n = 43) were captured north and south of the highway at both Bonnie Creek and Houle Creek (Fig. 2.7). All pairs of populations had low and non-significant fixation indices (F_{ST} values = 0, $P > 0.05$), and all variation was within rather than among populations (AMOVA, Table 2.4). This did not change when individuals were grouped by capture site (Bonnie Creek and Houle Creek) or by which side of the highway (north vs. south).

Table 2.4. Population genetic structure for different spatial groupings of the montane shrew (*Sorex monticolus*), Olympic shrew (*S. rohweri*), and Trowbridge's shrew (*S. trowbridgii*). Results from AMOVA (analysis of molecular variance).

	Genetic Variance	
Grouping	Among Populations (%)	Within Populations (%)
	Sorex monticolus	
All Together	0	100
By Site	0	100
By North vs. South of Highway	0	100
	Sorex rohweri	
All Together	3	97
By Site	3	97
By North vs. South of Highway	0	100
	Sorex trowbridgii	
All Together	21	79
By Site	39	61
By North vs. South of Highway	14	86

Olympic shrews (*S. rohweri* n = 41) were also captured north and south of the highway at both Bonnie Creek and Houle Creek (Fig. 2.7). All pairs of populations had low and non-significant ($P > 0.05$) fixation indices; F_{ST} values above 0 are shown in Figure 2.6. Most (97%) of the variation was within populations and 3% was among populations (AMOVA, Table 2.4). Similar results were obtained when individuals were grouped by capture site or side of the highway.

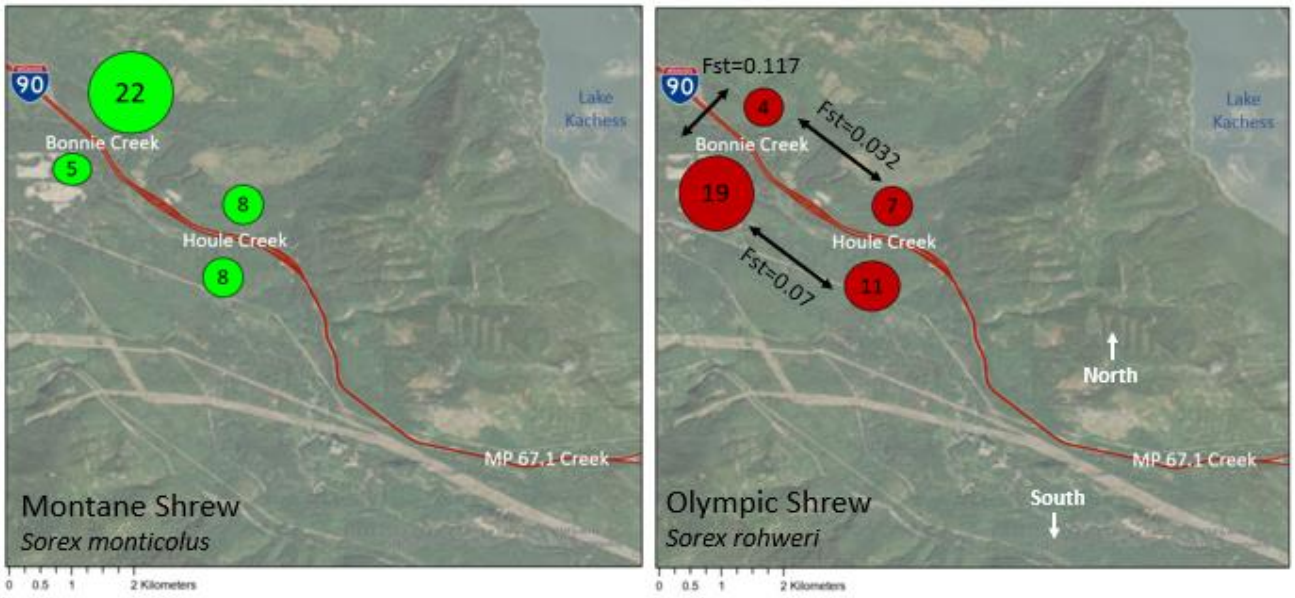

Fig. 2.7. Map of study area with number of captures for montane shrews (*Sorex monticolus*) (left) and Olympic shrews (*Sorex rohweri*) (right). Circle size represents number of captures, and arrows show F_{ST} values above zero between populations.

Trowbridge's shrews (*S. trowbridgii*, n=32) were captured on both north and south sides at all three sites (Fig. 2.7). These shrew populations had much higher fixation indices than the previous two species ($P < 0.05$). Bonnie Creek (South) and Houle Creek (North), Houle Creek (North) and MP 67.1 Creek (South), and MP 67.1 Creek (North) and MP 67.1 Creek (South) showed significant genetic differentiation (Fig. 2.8 and Table 2.5). About 79% of the variance was within populations and 21% of the variation was among populations (Table 2.4). The variance between populations was higher when groupings were organized by site rather than by what side of highway they were on.

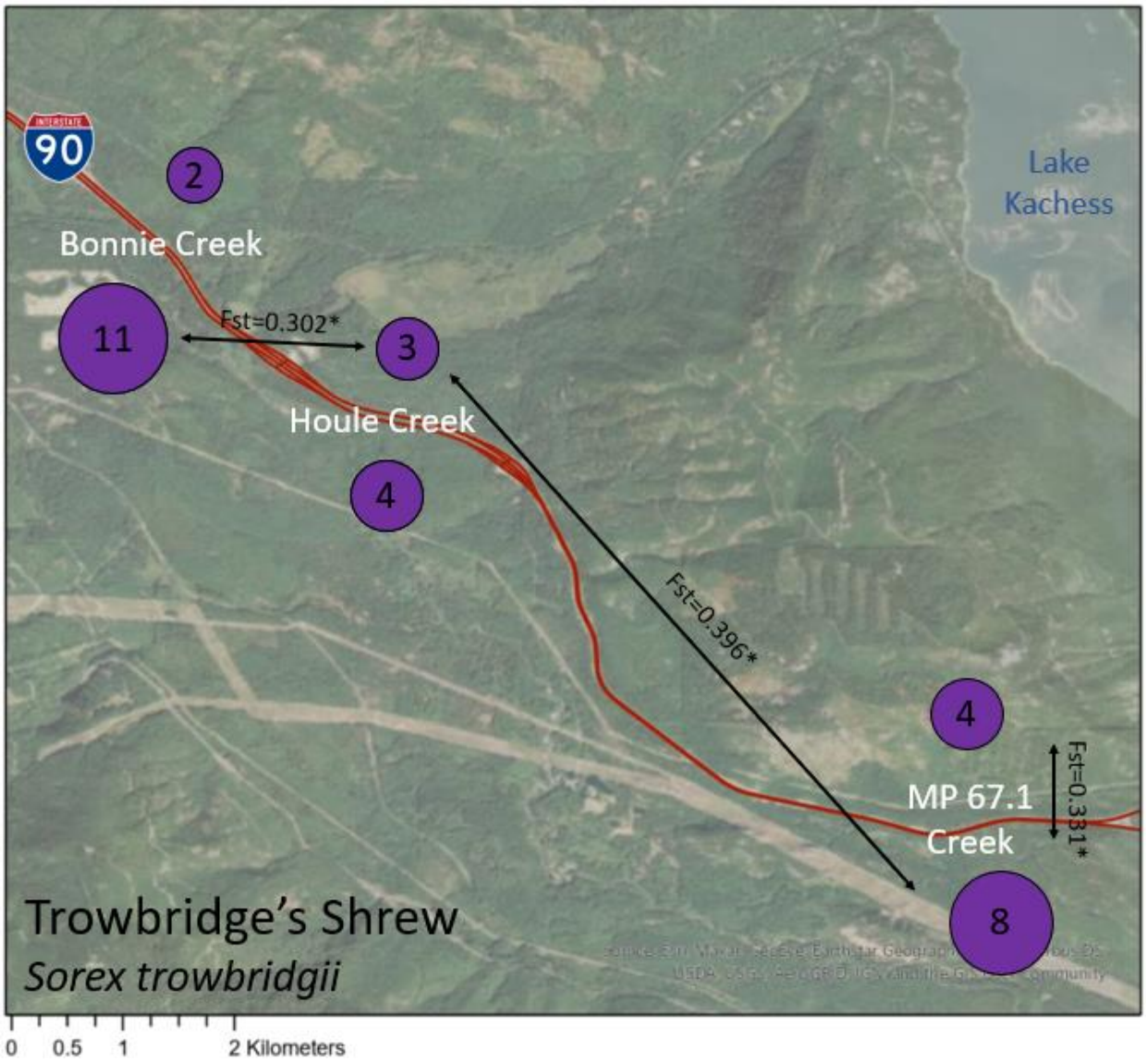

Fig. 2.8. Map of study area with population sizes for Trowbridge's shrews (*S. trowbridgii*). Circle size represents number of captures, and arrows show F_{ST} values significantly above zero between populations.

Table 2.5. Comparisons of pairs of population samples (F_{ST} values) for *Sorex trowbridgii*. North and south refer to direction from highway.

	Bonnie Creek (North) n = 2	Bonnie Creek (South) n = 11	Houle Creek (North) n = 3	Houle Creek (South) n = 4	MP 67.1 Creek (North) n = 4	MP 67.1 Creek (South) n = 8
Bonnie Creek (North)	0.000	___	___	___	___	___
Bonnie Creek (South)	0.382	0.000	___	___	___	___
Houle Creek (North)	0.221	0.302 *P* = 0.009	0.000	___	___	___
Houle Creek (South)	0.211	0.000	0.235	0.000	___	___
MP 67.1 Creek (North)	0.245	0.112	0.124	0.133	0.000	___
MP 67.1 Creek (South)	0.411	0.138	0.396 *P* = 0.018	0.000	0.331 *P* = 0.009	0.000

Habitat Use

Habitat surveys determined that the study area was dominated by western hemlock (*Tsuga heterophylla*), western red cedar (*Thuja plicata*), and Douglas-fir (*Pseudotsuga menziesii*). The forest floor was complex, with abundant leaf litter, woody debris, and nurse logs. Understory vegetation was dominated by vine maple (*Acer circinatum*), Oregon grape (*Mahonia aquifolium*), and vanilla leaf (*Achlys triphylla*). Skunk cabbage (*Symplocarpus foetidus*) and devil's club (*Oplopanax horridus*) were commonly found within or near the stream.

Semi-aquatic shrews (*S. bendirii* and *S. palustris*) were captured only in streamside habitats, but all other species were captured in all three macrohabitat types: streamside, lowland, and upland. Shrews (total captures) did not show significantly higher use of any certain habitat

type, nor did *S. monticolus, S. rohweri,* or *S. trowbridgii* individually ($P > 0.05$). The three individual species also did not differ significantly in their use of any microhabitat characteristics (ground cover, soil compaction, leaf litter depth, canopy cover, or tree densities) (Table 2.6). Shrews (total captures) showed no significant higher use of particular microhabitat characteristics ($P > 0.05$) besides using areas with lower tree densities ($P = 0.049$) (Fig. 2.9). Microhabitat characteristic data recorded during this study are shown in Table 2.7. Due to low capture numbers, we were unable to analyze habitat preferences by *S. vagrans, S. bendirii*, and *S. palustris*.

Table 2.6. A generalized linear mixed model with a negative binomial distribution was performed in R (version 1.3.959) using the "glmm" package. The number of captures (over the trapping season) for the three most abundant species (*S. monticolus, S. rohweri, S. trowbridgii*, using genetic identifications), and total shrew captures were used in this model. Shown below are the resulting *P*-values.

	Total Captures	**Montane shrew** (*S. monticolus*)	**Olympic shrew** (*S. rohweri*)	**Trowbridge's shrew** (*S. trowbridgii*)
Ground Cover (%)				
Bare Soil	0.20	0.99	0.06	0.29
Leaf Litter	0.63	0.41	0.16	0.25
Woody Debris	0.28	0.77	0.47	0.67
Rock	0.97	0.41	0.91	0.36
Forbs	0.28	0.53	0.36	0.34
Shrubs	0.82	0.66	0.25	0.39
Moss	0.65	0.90	0.56	0.67
Other Measurements				
Canopy Cover (%)	0.54	0.71	0.48	0.13
Soil Compaction (kg/cm2)	0.26	0.69	0.77	0.43
Leaf Litter Depth (cm)	0.45	0.51	0.79	0.74
Density of Medium Trees (trees/m^2)	0.12	0.19	0.06	0.43
Density of Large Trees (trees/m^2)	0.049*	0.42	0.61	0.06

Table 2.7. Microhabitat characteristic data (mean and standard deviations) around trap locations in different macrohabitat types: streamside, lowland, and upland. These data were collected near I-90 in the central Cascades as part of a shrew monitoring project in the summer of 2019.

	Streamside	Lowland	Upland
Ground Cover (%)			
Bare Soil	8.9 ± 17	0.05 ± 0.4	1.5 ± 4
Leaf Litter	73.5 ± 31	94.8 ± 17	92.5 ± 12
Woody Debris	21.4 ± 22	20.4 ± 17	21.9 ± 19
Rock	2.4 ± 11	0.04 ± 0.3	2.2 ± 4
Forbs	37.6 ± 34	13.4 ± 15	15.3 ± 17
Shrubs	10.3 ± 18	11.2 ± 14	18.9 ± 22
Moss	1.5 ± 10	9.3 ± 23	0.7 ± 5
Other Measurements			
Canopy Cover (%)	93.6 ± 5	93.5 ± 5	87.9 ± 10
Soil Compaction (Kg/cm2)	0.3 ± 0.3	0.6 ± 0.3	0.5 ± 0.4
Leaf Litter Depth (cm)	3.5 ± 3	2.2 ± 1.5	2.7 ± 2
Density of Medium Trees (trees/m^2)	0.06 ± 0.04	0.07 ± 0.09	0.09 ± 0.13
Density of Large Trees (trees/m^2)	0.04 ± 0.03	0.07 ± 0.04	0.07 ± 0.06

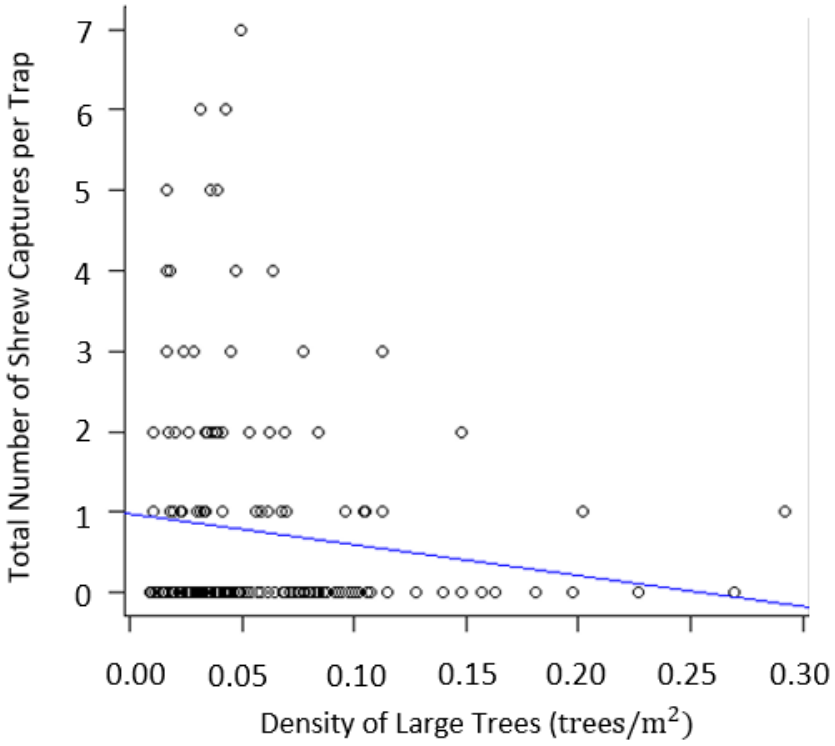

Fig. 2.9. Scatterplot showing total shrew captures per trap and the densities of large trees (DBH > 13cm) around that trap (trees/ m^2).

DISCUSSION

The first goal of this study was to verify or correct the species identifications of shrews trapped in the I-90 SPEP area. During this process, we discovered that only 43% of field identifications were correct. Most of the identification errors were due to the unexpected discovery of the Olympic shrew (*S. rohweri*) in the area, as it was not included in our dichotomous key. The other errors were likely due to the difficulty of seeing shrew characteristics (e.g., dentition and number of toepads) on live animals in the field. We captured three to six sympatric species in each site (Fig. 2.6), which is consistent with other studies (Brown 1967, Shchipanov et al. 2005). Including genetic analysis proved crucial for accurate species identification and may be vital for future live-trapping studies on shrews.

Our next objective was to evaluate population genetic structure for the three most abundant species (*S. monticolus*, *S. rohweri*, and *S. trowbridgii*). Montane shrew (*S. monticolus*) populations acted as one large population, showing no genetic differentiation between sites or on opposite sides of the highway. Similarly, Olympic shrew (*S. rohweri*) populations showed only 3% variation between populations, essentially acting as one large population. This suggests that I-90 is not a barrier to the movement of these species, or that not enough time has passed since I-90 was built to separate these populations significantly. Trowbridge's shrew (*S. trowbridgii*) populations showed greater genetic differentiation among populations than did the other two species analyzed. This variation was greater between sites rather than between opposite sides of the highway. However, fixation indices (F_{ST} values) between populations were significant only between populations that were on opposite sides of the highway (Fig. 2.8). This is a glimpse of the impact the highway may have on this species, but this species also had lower sample sizes than the other two species. The true impact the highway has on shrews is still unknown, but the

replication of this study post-construction could help determine the effectiveness of crossing structures for shrews. Future work should include more extensive sampling in other locations on the eastern slopes of the Cascade Range and the revision of museum specimens from the area. Genetic expansion statistics (Peter and Slatkin 2013) from all samples of the Olympic shrew in Washington, Oregon, and British Columbia may help infer the origin of the expansion. This could help determine the full extent of this range extension, when and where it took place.

Pitfall arrays were undoubtedly the most effective in capturing shrews. They required great effort to install but were easily maintained throughout the season. Sherman Traps, conversely, required 5 times more trap effort (in terms of trap-nights) than pitfall arrays, but secured less than 10% of the 156 shrew captures. Bycatch was greatest in Sherman traps (227 captures or 94% of bycatch captures), potentially increasing stress and the risk of death to individuals of those species (deer mice, voles, shrew-moles, weasels, and pika) and to the shrews waiting to be processed. Modified funnel traps had low trap effort and increased captures of the elusive semi-aquatic shrews (*S. bendirii* and *S. palustris*).

Many small-mammal monitoring projects are not species-specific, but by focusing on shrews we were able to increase shrew captures substantially from previous trap years in the I-90 SPEP area. We were also able to reduce the mortality rate by providing the necessary provisions (shelter, warmth, and food) for shrews in all traps. Throughout the study, we modified our methods to continually decrease mortality. The first trap session had a mortality rate of 48%, but once we increased bedding and bait inside the traps, we were able to decrease mortality to 7-27% in future trap sessions. By placing polyfill, cover (toilet paper tubes), and ample food, we were able to keep shrews alive throughout the trap night. Most mortality occurred when multiple shrews were captured in one trap, because they would attack and feed on each other. Total

mortality throughout the study was 28%, which is less than most studies that have their traps open for 8-12 hours (Shonfield et al. 2013). One way some studies lower mortality is by decreasing the amount of time traps are open between checks, but this increases cost, effort, and can be dangerous for humans walking in these forest sites at night (Shchipanov et al. 2005).

Lastly, we evaluated macro- and microhabitat use by shrews. We predicted that shrew species would partition their use of different habitat types or microhabitat characteristics to help explain how so many species can occupy the same area. We evaluated the three most abundant shrew species (*S. monticolus*, *S. rohweri*, and *S. trowbridgii*) to see if capture numbers varied among streamside, lowland, and upland habitat types. However, they were all caught in all three habitat types and showed no significant differences in use of one over the others. Microhabitat characteristics followed the same trend. Number of shrew captures was not associated with ground cover (soil, leaf litter, woody debris, shrubs, graminoids, forbs, rocks, and moss), soil compaction, leaf litter depth, canopy cover, or tree densities. When looking at total captures of all six species of shrews, the density of large trees was the only variable that was significantly associated with number of shrew captures. When large tree density was lower, captures were higher. Shrews use abandoned runways, loose litter, and other suitable understory protection from predators as they forage (Churchfield 1990). If tree densities are higher, the forest floor would be crowded with smaller areas for shrews to find food and this may be why shrew abundance was higher in areas with lower densities of large trees. Alternatively, lower tree densities could also allow for better understory growth, allowing more light and space for shrubs and forbs to grow (Harrington et al. 2003). This could be crucial to the everyday movements of shrews by providing shelter from predators. However, we would have expected that shrew abundances would be significantly higher in areas with lower canopy cover, and this was not the

case. Semi-aquatic species (*S. bendirii* and S. *palustris*) were captured only in streamside habitat types, which is consistent with other studies (Brown 1967, Galindo-Leal and Zuleta 1997). We collected and analyzed macro- and microhabitat data in hopes of shedding light on the way these six species partition resources, but we were unable to find any major differences for the most abundant species. This was surprising, and the mechanism of coexistence among multiple shrew species deserves more study.

This study provides baseline information on the shrews near I-90 in the central Cascades, but many other questions are still left unanswered and require further investigation. The surprising presence of the Olympic shrew (*S. rohweri*) and absence of the masked shrew (*S. cinereus*) could be a result of misidentifications in museum specimens, so verifying these identifications coupled with further sampling in the Cascade Range and surrounding areas could help clear up these distributions. Finally, our study sites were placed in areas that will be connected by wildlife crossing structures in the next decade. This study could be replicated in the future (post-construction) to see if genetic variability or species presence changes after crossing structures have been completed.

CHAPTER V

CONCLUSION

I used live-trapping, genetic analysis, and habitat data to acquire knowledge about the shrews that inhabit forest along a major interstate highway in central Washington. Pitfall arrays, modified funnel traps, and ample food and shelter in each trap proved most efficient in capturing shrews and keeping them alive. Sherman traps, despite being more numerous than other trap types, were far less effective during our study, capturing more bycatch than our focal species, shrews.

We verified six species living sympatrically within the study area. In the field, we misidentified some shrews as being to masked shrews (*Sorex cinereus*), but genetic analysis revealed that most of those individuals belonged to an unexpected species, the Olympic shrew (*Sorex rohweri*). This is the first discovery of the Olympic shrew along the eastern slopes of the Cascade Range, marking a distinct range extension in Washington State. This is also a hint that the distribution of the Olympic shrew may be substantially broader than currently recognized and masked shrew distribution may be different than previously published. We also captured the montane shrew (*Sorex monticolus*), Trowbridge's shrew (*Sorex trowbridgii*), vagrant shrew (*Sorex vagrans*), marsh shrew (*Sorex bendirii*), and the American water shrew (*Sorex palustris*). Future research should include the evaluation of museum specimens from Washington State (and surrounding areas) to verify shrew identifications and to correct the distributions of the masked shrew (*S. cinereus*) and the Olympic shrew (*S. rohweri*). Genetic expansion statistics could also be used to determine the origin and extent of the range expansion by the Olympic shrew.

Analyzing the population genetic structure of three shrew species caught in this study did not paint a clear picture. Populations of the two most abundant species (*S. monticolus* and *S. rohweri*) each acted as a single population, showing low and insignificant F_{ST} values and little genetic variation. Trowbridge's shrew (*S. trowbridgii*) populations had higher variation with significant and higher F_{ST} values, suggesting I-90 may have fragmented these populations. These trends must be considered with caution due to limited sample size.

Captures of terrestrial shrews seemed to be independent of macrohabitat and most microhabitat characteristics that we measured, but the presence of diverse understory characteristics is most likely crucial for shrew presence. All of our sites were in well-developed forest with some human disturbance due to the nearby highway. The two semi-aquatic shrews were captured only in and adjacent to streams, as expected. The best way to connect wildlife (specifically shrews) through crossing structures is to create a seamless habitat connection within the structure. This includes connecting the stream by establishing a flow channel and including a variety of features that create a diverse ground floor with woody debris, leaflitter, forbs, and shrubs. This study can be replicated in the future after crossing structures have been completed to evaluate the colonization of these crossing structures and the connection of shrew populations. Based on relative abundance, we expect *S. monticolus, S. rohweri*, and *S. trowbridgii* to be the first shrew species to use the crossing structures. We also expect *S. bendirii* and *S. palustris* to use the crossing structures once the stream has been successfully connected.

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APPENDIX A
Dichotomous Identification Key for Shrews in the I-90 Snoqualmie Pass East Project

1a. Total length > 130 mm; hind foot > 18 mm long, (usually) with a fringe of stiff hairs.…….2

1b. Total length < 130 mm; hind foot < 18 mm and lacking a fringe of stiff hairs……..….3

2a. Dorsal fur black; feet and belly lighter than dorsum, grey or silver-grey; tail sharply bicolored with a paler underside……...….*palustris*

2b. Dorsal fur dark brown; feet and belly as dark as dorsum; tail uniformly dark brown (not bicolored)……..……*bendirii*

3a. With head viewed laterally, 3rd uniscuspid as large or larger than 4th unicuspid (Fig. 31B); dorsal fur does not contrast sharply from the sides, body weight usually < 5.0 g …….*cinereus*

3b. With head viewed laterally, 3rd uniscuspid smaller than 4th (Fig. 32); dorsal fur variable…..4

4a. Tail distinctly bicolored, dark on dorsal surface and white underneath; medial edge of 1st upper incisor curved in front view….....……..*trowbridgii*

4b. Tail not distinctly bicolored, with underside pale brown or grey; medial edge of 1st upper Incisor straight in front view…….....5

5a. Five of more pairs of toe pads on toes 2 to 5 of hind feet (Fig. 34A); medial tine on 1st upper incisor is below the pigmented area (pigmentation not extending above tine)...*monticolus*

5b. Four or fewer pairs of toe pads on toes 2 to 5 (Fig. 34B); medial tine on 1st upper incisor positioned at or above pigmented area (pigmentation extending well above tine)……*vagrans*

Keys compiled by K. Ernest, Jan. 2017. Modified by J. Ryckman, July 2019.

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