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Elk Abundance Estimation and Road Ecology in Whatcom and Skagit Counties, Washington

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Elk Abundance Estimation and Road Ecology

in Whatcom and Skagit Counties, Washington

By

Nathan C. Rice

Accepted in Partial Completion
Of the Requirements for the Degree
Master of Science

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Nathan Caldwell Rice
November 17, 2017

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A Thesis
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The Faculty of
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Abstract

Chapter 1 – Elk abundance estimation using genetic mark-recapture in the South Fork Nooksack Valley, Whatcom County Washington

Non-invasive genetic mark-recapture is an increasingly useful method for estimating the abundance of elusive wildlife. This method was used to estimate the size of an elk population (*Cervus canadensis*) in the South Fork Nooksack River valley in northwestern Washington where dense forest cover can hamper aerial surveys. We genotyped 250 elk fecal DNA samples that were collected in a single sampling session. Only 103 samples amplified sufficiently after one PCR for genotype matching, which resulted in 49 unique genotypes. Program Capwire estimated a population size of 91 elk (95% CI = 83 - 130), possibly an underestimate of actual abundance. Unfortunately, funding limitations precluded necessary lab work to determine consensus genotypes so genotyping errors could not be corrected. For this reason, these results must be considered with caution. While genetic mark-recapture has many advantages over traditional mark-recapture methods, the potential for genotyping error can inflate laboratory expenses and should be carefully considered.

Chapter 2 – Elk road ecology on state Highway 20 in Skagit Valley, Skagit County, Washington

Wildlife-vehicle collisions pose a significant hazard to humans and wildlife. In Skagit Valley, Washington,158 elk (*Cervus canadensis)* roadkills were documented between 2002 and 2014 on 34.8 kilometers of state highway 20 between the towns of Sedro-Woolley and Concrete. In the current study, I documented road crossing activity between July and December 2013 between the towns of Sedro-Woolley and Concrete using string traps and remote cameras on game trails (*n* = 722 trail detections). Roadkill data were compiled from agency reports over

comparable time periods for spatial analysis (July to December 2013 ($n = 22$)) and modeling (January 2012 to January 2014 ($n = 103$)). Roadkill locations were weakly correlated with road crossing locations across the study area (Kendall's tau $= 0.23$, $P < 0.001$). Statistically significant hotspots were found for roadkills ($n = 4$) and road crossing activity ($n = 5$) ($P < 0.05$). One roadkill hotspot coincided with one road crossing hotspot. Presence / absence of road crossing activity and roadkills in 216 0.16-km road segments were each modeled against 10 habitat variables and 4 road variables using logistic regression. The best road crossing model indicated that road crossing activity was negatively associated with distance to forest, distance to streams, distance to crops, percent developed area, and guardrail length. Road crossing predictors with the highest relative importance values in the best model were *Distance to forest* ($RI = 1.00$), *Distance to crops* ($RI = 1.00$), and *Distance to streams* ($RI = 1.00$); however, *Distance to streams* had 95% confidence intervals containing zero. The best roadkill model indicated that roadkills were negatively associated with distance to pasture/hay, percent developed area, and roadside slope, and positively associated with percent forest cover. Roadkill predictors with the highest relative importance values were *Distance to pasture/hay* ($RI = 01.00$) and *Percent forest cover* ($RI = 1.00$). Understanding the spatial distribution of road crossing activity and roadkills, combined with the habitat and road factors associated with them, can inform management of wildlife and vehicles in rural areas.

Acknowledgements

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Chapter 1

Elk abundance estimation in the South Fork Nooksack Valley
using genetic mark-recapture

Introduction

The North Cascades elk herd (*Cervus canadensis[1]*), also known as the Nooksack herd, is the northernmost elk herd in western Washington and the smallest of 10 major herds in the state. Managed by the Washington Department of Fish and Wildlife (WDFW) and the nine Point Elliot treaty tribes, the herd is important to hunters, wildlife enthusiasts, local private landowners, government agencies and Native American tribes who use elk as a cultural and subsistence resource (Danilson 2012). Maintaining an elk population of adequate size is necessary to realize the herd's many values, including its role in the North Cascades ecosystem.

Elk have lived in western Washington for at least 6,000 years (Harpole and Lyman 1999). In Whatcom and Skagit counties, archeological elk remains between 100 and 3,000 years old have been found (Harpole and Lyman 1999) and Native American tribes in the area have hunted elk for millennia (McCabe 1981). With European settlement, hunting increased and elk abundance declined dramatically across the state; the historical North Cascades elk herd was apparently extirpated by overhunting around the turn on of the 20th century (Ware et al. 2014). Today's herd was re-established on historical range in the South Fork Nooksack River Valley and the Skagit River Valley following a series of reintroduction and augmentation efforts that began in 1912 (Figure 1-1). Most recently, the state and tribes relocated 98 animals from the Mount St. Helens area between 2003 and 2005. The genetic composition of today's North

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[1] Many authors continue to use *Cervus elaphus* to describe North American elk; however, phylogenetic research has shown them to be a distinct species (*Cervus canadensis*). *Cervus elaphus* now describes red deer species in Europe (Groves and Grubb 2011).

Cascades elk herd consists primarily of the introduced Rocky Mountain elk subspecies

(Cervus canadensis nelsoni) from as far away as Yellowstone, as well as reintroduced native

Roosevelt elk (*Cervus canadensis roosevelti*) from the Olympic Peninsula (WDFW 2017).
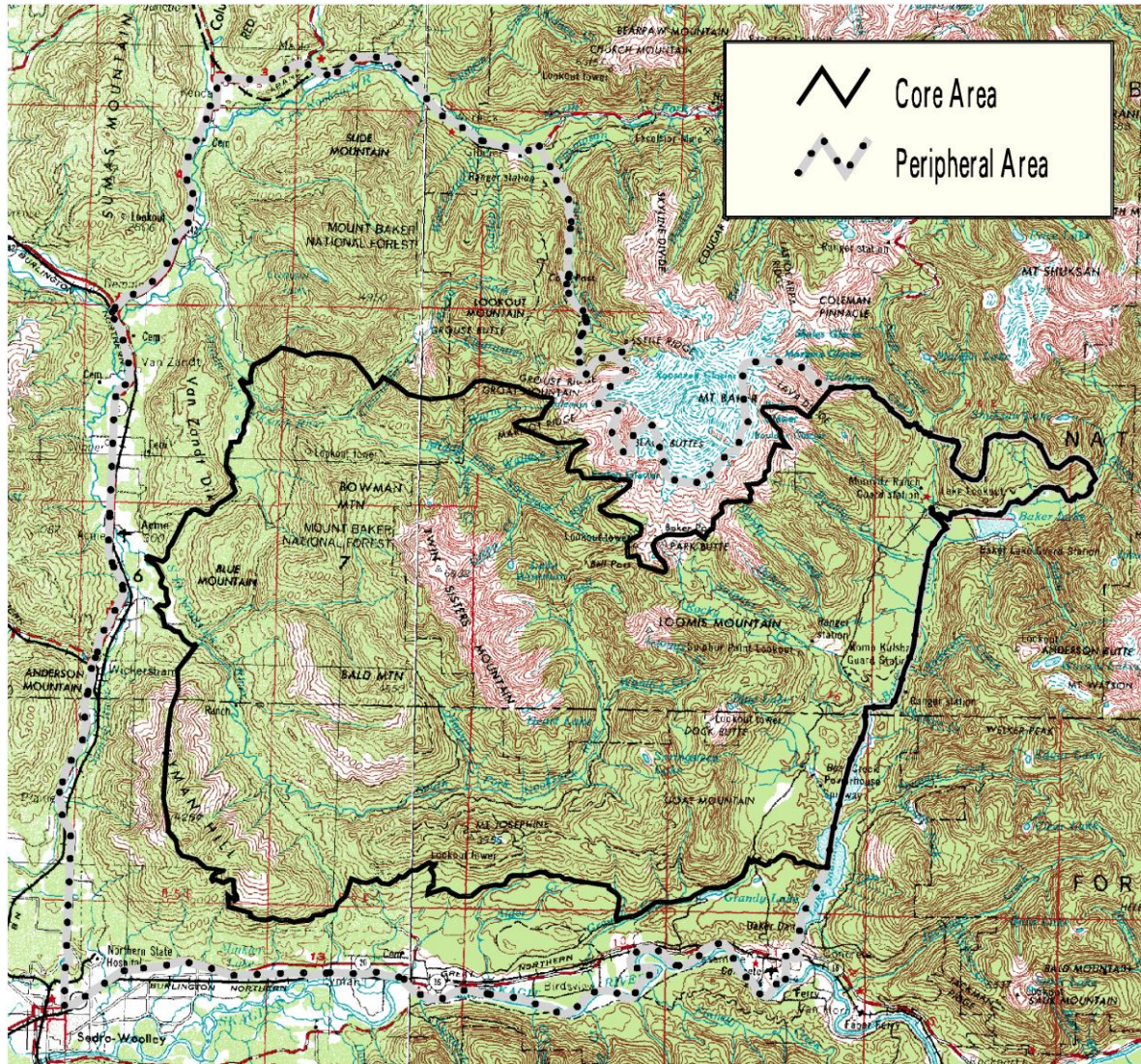
Figure 1-1. Core and peripheral areas of the North Cascades elk herd (Davison 2002).

The current abundance of the North Cascades elk herd is estimated to be between 1,569

and 1,769 animals (WDFW 2017). Annual mark-resight population surveys by WDFW suggest

that the population within the state Game Management Unit (GMU) 418 (Nooksack) and that
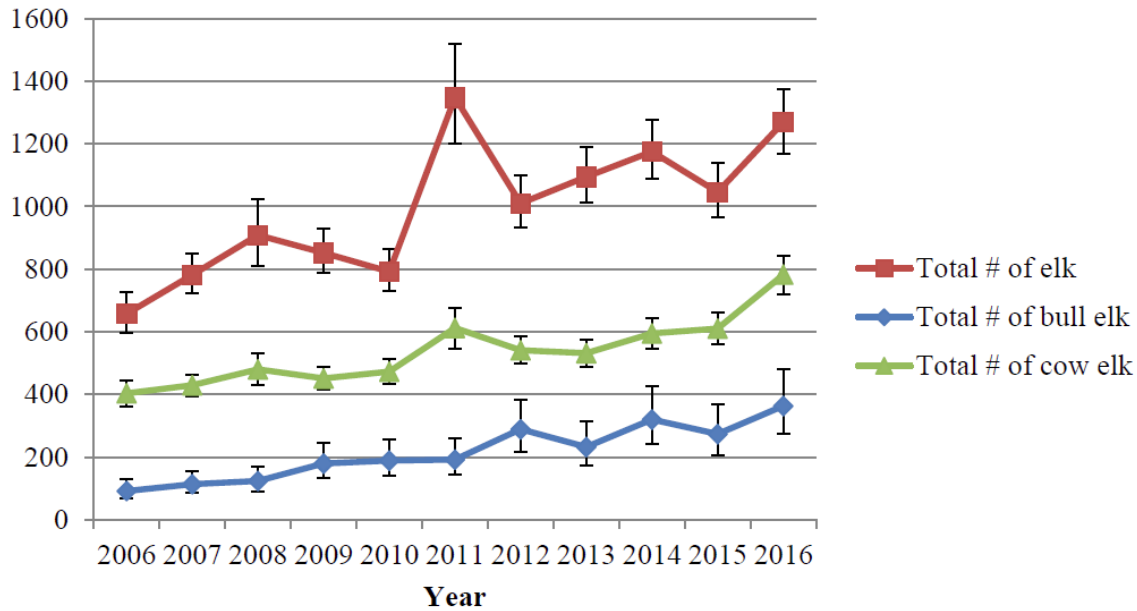
Figure 1-2. Mark-resight estimates of total elk, cow elk, and bull elk population size in GMU 418 (Nooksack), 2006–2016. Estimates also include elk within the northern portion of GMU 437 north of the Skagit River between Lyman and Concrete. (WDFW 2017)

portion of GMU 437 (Sauk) north of the Skagit River between Lyman and Concrete is 1,269 (95% CI = 1,170-1,379) elk. From 2006 to 2016, estimates of population size within the survey area indicate that the North Cascades elk herd has increased at a rate of 5-7% annually (Figure 1-2). In addition, 200-400 elk occur elsewhere in GMU 437, primarily south of the Skagit River between Sedro Woolley and Marblemount, and at least 100 more within the Sauk River valley south of Rockport, according to biologists' observations and other anecdotal information (WDFW 2017) (Figure 1-3).

Historically, the herd reached a peak of between 1,400 and 2,000 elk the mid-1980s before a major decline in the early-1990s drove the population down to a few hundred elk (WDFW 2017). Causes of the decline included intensive timber management practices (including herbicide use that reduced early seral habitat), associated traffic and disturbance, overhunting on an expanded road network, elk-vehicle collisions, loss of habitat to development and agriculture, and lethal removal on conflicted agricultural lands (Danilson 2012).
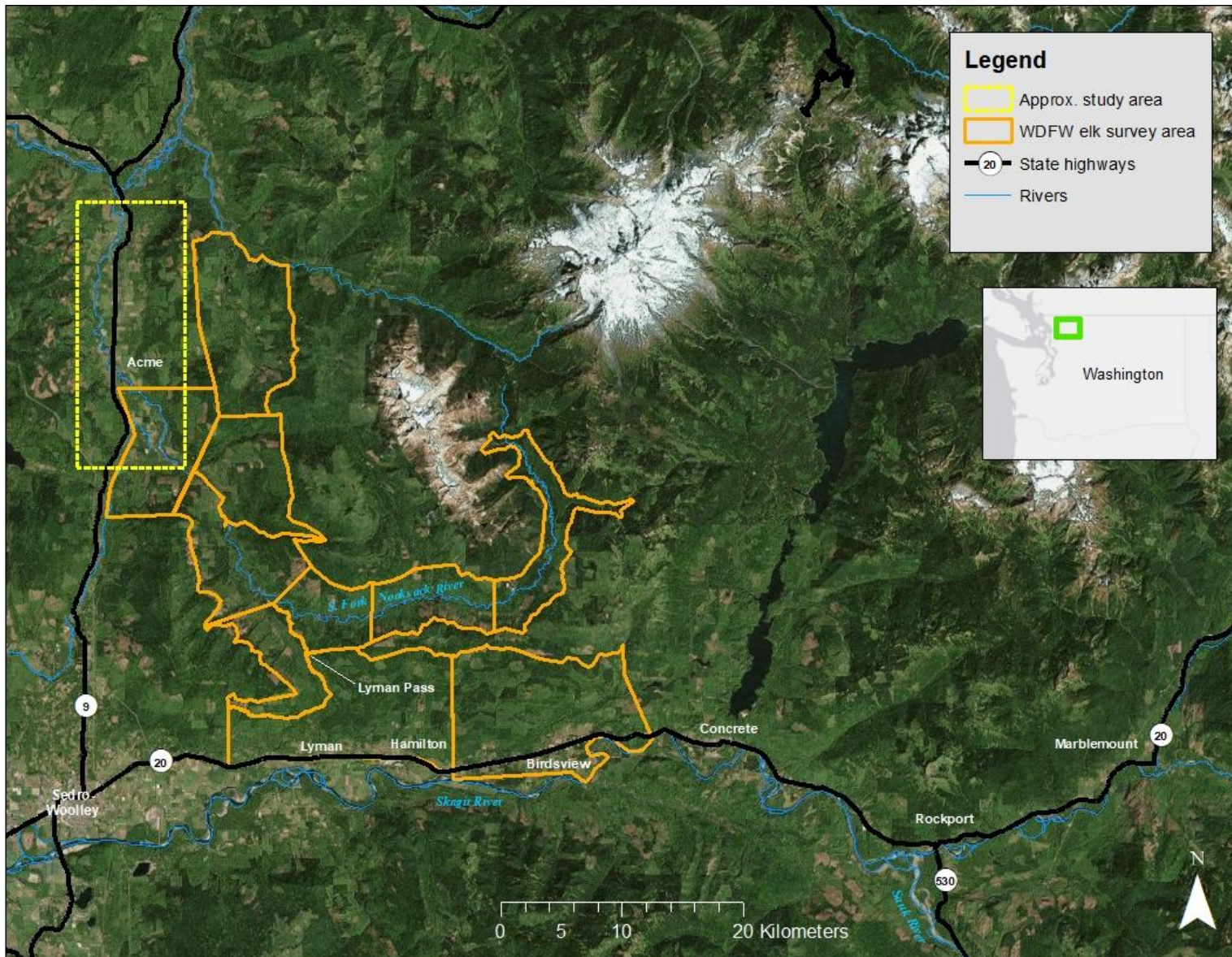
Figure 1-3. Overview map of study area. WDFW elk survey area is denoted in orange.

Disturbance from recreational use of logging roads by off-road vehicles has also increased in recent years (Danilson 2012, Davison 2002). The herd has since rebounded with the support of augmentation, a hunting moratorium from 1997 to 2007, habitat enhancement projects, restricted vehicle access, and changes in silvicultural practices that have created a more complex mosaic of habitats (WDFW 2017).

Managing agencies seek to increase the size of the herd to 1,950 elk (±10%, WDFW 2017). WDFW reports that the North Cascades elk herd is not limited by available habitat despite a reduction in overall elk carrying capacity in Washington. This is due to a marked reduction in timber harvest that created early seral habitat (including on federal land where herbicide is not used), as well as an increase in human population and associated development (WDFW 2014). Elk currently inhabit agricultural lands in the Skagit Valley and South Fork Nooksack Valley, leading to frequent landowner conflicts. A WDFW aerial survey in March 2014 counted 129 elk in the South Fork Nooksack Valley (Paul DeBruyn, pers. comm.).

Since 2006, the WDFW has conducted annual aerial mark-resight and sightability surveys to estimate and monitor abundance of the entire North Cascades elk herd (McCorquodale et. al 2012, WDFW 2017). The state surveys most of the herd's core range including areas around Mosquito Lake, the town of Acme, the south fork of the Nooksack River near the Whatcom-Skagit county line and the middle fork of the Skagit River north of Highway 20 between Sedro Woolley and Concrete. These areas fall within the WDFW Nooksack Game Management Unit (#418) and a small portion of the Sauk GMU (#437). Aerial detection is difficult in the North Cascades elk range due to low elk density and dense forest cover. McCorquodale et. al (2012) found "substantial limitations" in their sightability model, which likely underestimated abundance in 2011, while their mark-resight model may have

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overestimated population size, leading to "biologically implausible" results (Figure 1-2). The 2017 North Cascades Elk Herd Plan includes the objective to "implement a monitoring strategy that will provide a sound basis for herd size estimation using acceptable, cost-effective methodologies" by 2018 (WDFW 2017).

Genetic mark-recapture is a recent method used to estimate abundance that could be advantageous in areas of dense forest cover that hinder aerial survey methods**.** In this approach, DNA from blood, tissue, hair, or feces is used to identify (or "mark") individuals who may be detected again ("recaptured") in one or more sampling sessions. A mark-recapture model can then be used to estimate population size. Other advantages of this non-invasive method over helicopter-based surveys include fewer hazards, reduced animal disturbance, no need to deploy and maintain radio collars, and potentially lower cost and fewer detection biases. Single-session genetic mark recapture models (Miller et al. 2005) are particularly useful for cutting field costs and effort, and improving the chances of satisfying model assumptions, particularly that of population closure: no births, deaths, immigration or emigration during the sampling period.

Challenges associated with genetic mark-recapture include low DNA quality (particularly with fecal DNA) and resulting genotyping errors that can greatly reduce the accuracy of population estimates (Waits and Leburg 2000, Lampa et al. 2013)**.** Software programs such as Dropout (McKelvey and Schwartz 2005) and Microchecker (Van Oosterhout et al. 2004) can be used to detect genotyping errors in a dataset such as allelic dropout and null alleles (alleles that fail to amplify), stuttering (caused by errors in the PCR process), and false individuals (individuals created by genotyping errors). A number of laboratory protocols have been developed to reduce genotyping error by re-amplifying samples in order to create consensus

genotypes; however, these procedures can greatly increase laboratory costs. Due to funding limitations, we were not able to follow these protocols, which include:

1. Multiple tubes approach (Taberlet 1996) - Minimum 3 amplifications of all samples; accept heterozygous alleles detected twice; accept homozygous loci after up to 8 reamplifications (very conservative and costly).

2. Comparative multiple tubes approach (cMTA) (Frantz et al. 2003) - Accept heterozygous alleles after 2 detections. Follow step-wise protocol for up to 7 amplifications for ambiguous samples. Reamplify samples with 1-2 mismatched alleles 4 more times.

3. Mismatch (per Paetkau 2003) - Reamplify only those samples that mismatch by 1 to 3 loci 2 – 4 times. Accept heterozygous alleles detected twice. Reamplify homozygous loci 3 more times.

4. Singles (Hettinga et al. 2012, Woods et al., 1999, Poole et al., 2001) - Reamplify only singleton genotypes 2 – 3 times. This approach assumes that matches confirm each other.

As co-managers of the North Cascades elk herd, the Stillaguamish and Tulalip tribes are interested in monitoring the size of the elk population in the South Fork Nooksack River Valley near the towns of Acme and Saxon in Whatcom County, Washington. This area is of particular interest due to ongoing agricultural damage caused by elk in the valley bottom. Between 2002 to 2014, 17 elk damage claims were filed to WDFW in GMUs 407, 418, 437, 448, and 450, and the state paid a total of $78,555 to landowners for damage claims (WDFW 2017). Monitoring abundance on the South Fork Nooksack River valley floor could help determine the efficacy of efforts to reduce elk density such as hunting, hazing, killing problem elk, clearing and planting upland forage enhancement plots, and fencing agricultural fields.

Methods

Study area

The South Fork Nooksack River Valley is located on the west side of the Cascade Mountains in the Western Hemlock (*Tsuga heterophylla*) zone of northwest Washington State at about 100 meters elevation (Franklin and Dyrness 1973). The valley bottom is approximately 7,200 hectares and supports agriculture centered around the town of Acme (population 246). Elk habitat types in the valley bottom include agricultural fields of corn and hay, deciduous forests of primarily red alder (*Alnus rubra*) and big leaf maple (*Acer macrophyllum*), and second-growth evergreen forests dominated by Douglas fir (*Psuedotsuga menziesii*) and Western Red Cedar (*Thuja plicata*).

Elk scat sampling was restricted to the South Fork Nooksack River valley bottom. Given the relatively large area, the heterogeneous habitat distribution in the valley, and short sampling period required to assume a closed population, sampling effort was focused in areas where elk were reported by residents or detected by radio telemetry during the two weeks prior to collection. Elk fecal DNA has been shown to resist degradation for about 14 days in dry weather (Sager 2012 unpublished data).

Sample collection

Staff, students, and volunteers from Western Washington University, the Stillaguamish Tribe, and the Tulalip Tribe collected 357 fecal genetic samples from elk scat in the South Fork Nooksack Valley on September 24-26, 2013. Samples were collected using a toothpick method developed by Dr. David Paetkau of Wildlife Genetics International and tested by Kim Sager of the Lower Elwha S'Klallam Tribe (Paetkau pers. comm.; Sager, unpublished data). A coarse

toothpick was gently dragged over the translucent mucus on the surface of the elk pellet to collect epithelial cells sloughed from the intestinal tract. Collection of fecal material on the toothpicks was avoided because fecal enzymes can degrade DNA and interfere with the DNA amplification process (Paetkau pers. comm.). We completed a pilot study of this method in January 2013 and found improved genotyping success using toothpicks compared to samples collected using cotton swabs (See Appendix A). The sampling procedure was performed twice for each sampled scat pile -- once with a pellet from the top of a pile and a second time with a pellet from the middle of the pile in case the first DNA sample was degraded by weather. The two toothpicks were then placed in a coin envelope, sealed, and labeled with unique sample identification numbers and quality score from 1 to 3 based on freshness of scat. The sampled scat pile was then covered with vegetation (grass or sticks) to prevent resampling, and geographic coordinates were recorded using Garmin GPSMap 60 C and 60 CSx units. We made no attempt to exclude calves from our samples so this population estimate includes all age classes. We avoided sampling the same individual in the same area by not sampling scat piles within 4.5 meters (15 feet) of other scat piles.

Samplers working in groups of 2 to 4 were instructed to survey fields starting along the forest edge about 5 to 30 feet apart depending on vegetation cover and visibility of scat piles. When an elk trail into the forest was encountered, two or more samplers would follow the trail with the lead sampler searching the trail itself and the second sampler searching 1 m on either side of the trail. Once a given trail system was surveyed, samplers returned to the field to resume the field survey. Once edges and trails were surveyed around a given field, samplers were instructed to survey the remainder of the field area (Figure 1-4, Figure 1-5).
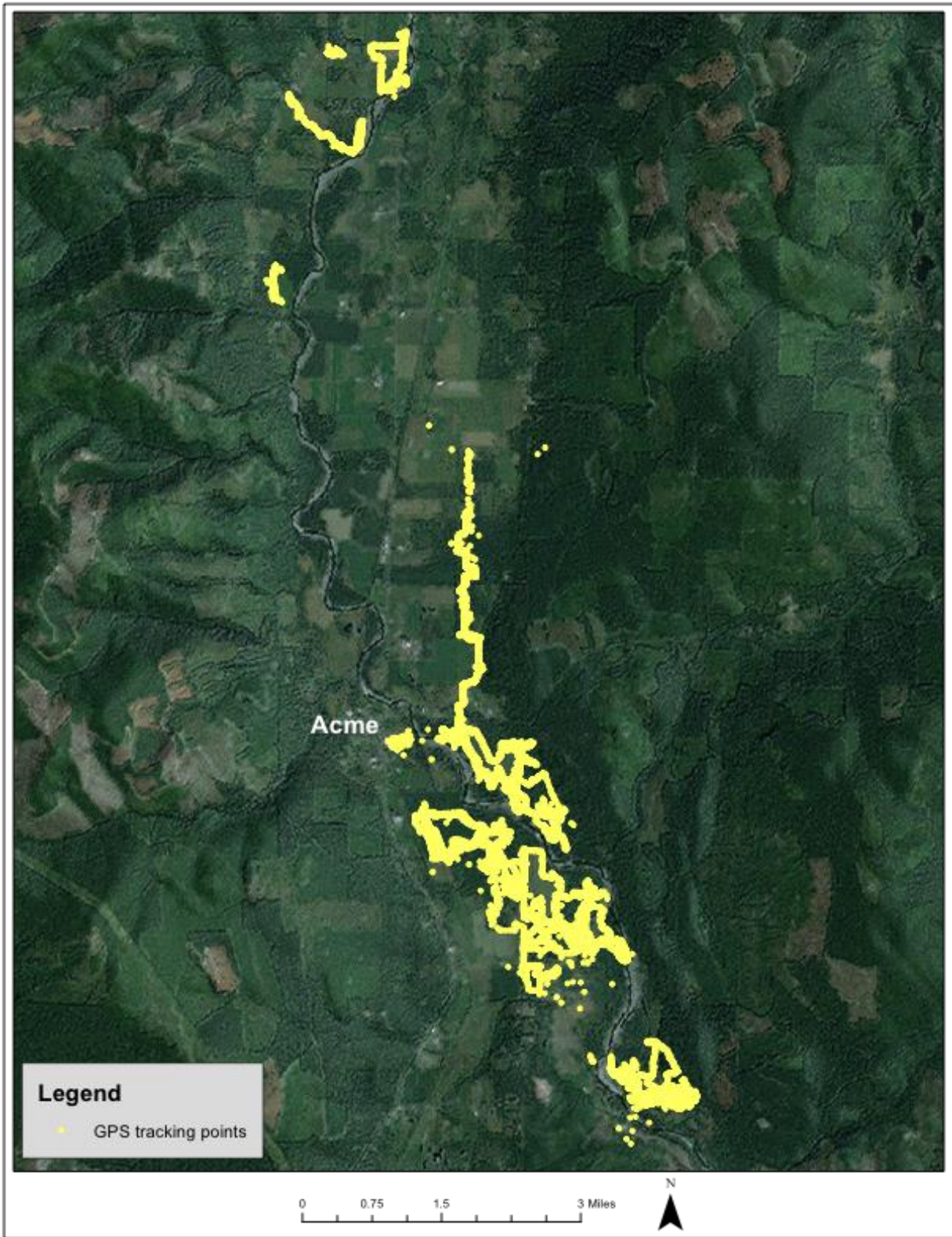
Figure 1-4. Approximate area sampled based on track points from GPS units carried by samplers.
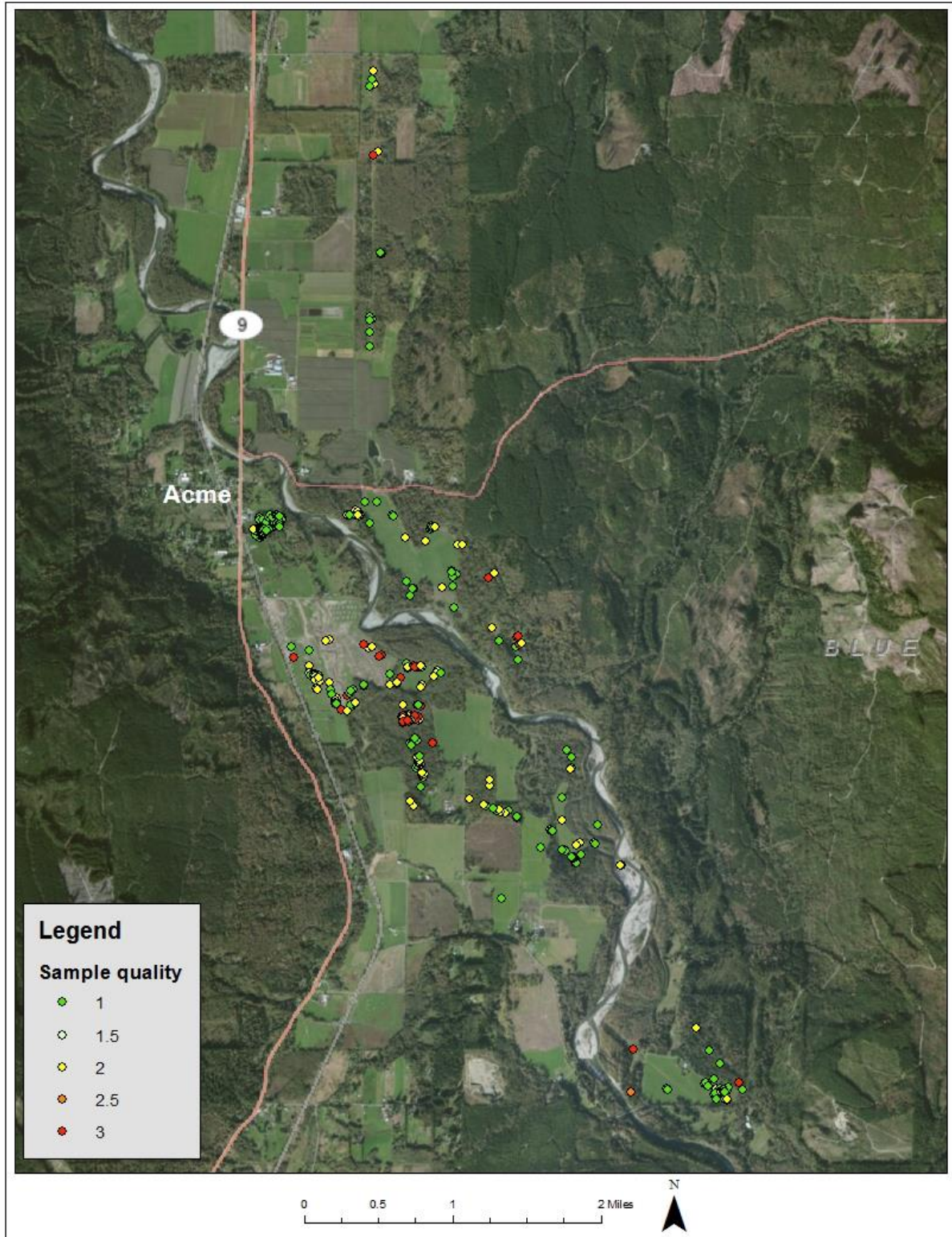
Figure 1-5. Geographic distribution of 357 elk fecal DNA samples in the South Fork Nooksack River Valley, Whatcom County, Washington. All 202 Quality 1 (green) and a random sampling of 48 Quality 2 (yellow) samples were genotyped for population estimation. No samples were found in northwestern-most sampling areas in Figure 1-4.

Genotyping

Due to limited funding for laboratory analysis, we selected 250 of 357 samples for

genotyping based on sample quality in order to maximize genotyping success and better ensure

population closure – a key assumption of the mark-recapture model (Miller et al. 2005). All 202

of the high-quality samples (quality score = 1) and a random sample of 48 medium quality

samples (quality score = 1.5 or 2) were sent to the Washington Department of Fish and Wildlife

genetics lab in Olympia, Washington for genotyping.

Genomic DNA was extracted from epithelial cells on sampled toothpicks following

standard recommendations for DNeasy commercial single tube silica-membrane blood and tissue

DNA isolation kit (Qiagen). Polymerase chain reaction (PCR) was used to amplify 13 previously

characterized microsatellite loci and two sex markers in 5 multiplex reactions (Multiplex Cca-B:

BM1225, BM4208, BM4513; Multiplex Cca-C: BM5004, ETH152, BMC1009; Multiplex Cca-

D: Texan4, BM888, BM4107, RT7; Multiplex Cca-E: OarCP26, BM203, RT27; Multiplex

Gender: ZFX/Y, SRY41/121rd). PCR results were scored independently by two WDFW lab

technicians. Only consensus allele base pair length scores were used for individual identification;

mismatching scores were nulled.

Genotyping error

Fecal DNA is generally of much lower quality than that from blood or tissue due to lower

DNA quantity and potential degradation from fecal enzymes and moisture. This can cause errors

in the genotyping process that can create false individuals and missing data, both of which cause

significant inaccuracies (overestimation, typically) in mark-recapture population estimates

(Waits and LeBurg 2000, Lampa et al. 2013). A number of protocols have been developed to

systematically reamplify problematic samples in order to reduce genotyping error (see above;

Taberlet 1996, Paetkau 2003TK, Lampa et al. 2013). However, additional lab work in this study was precluded by lack of funding. This is a significant limitation and our results should be considered with caution.

Matching genotypes

The R package Allelematch was used to determine unique genotypes and match similar genotypes in order to develop a capture history (Galpern et al. 2013). Allelematch performs a pairwise comparison of all genotypes using the allele base pair length values and calculates a similarity score between all genotypes. This similarity score is then used to cluster genotypes into similar groups. This program was advantageous in this study because it can "match" genotypes while allowing for a minimal amount of error and missing data. This is accomplished in two ways: 1) by matching genotypes using allele base pair values rather than the more commonly used Probability of Identity for Siblings (PIDsib) (Woods et al. 1999) and 2) by allowing matches to differ by a determined number of alleles (this is called the allele mismatch parameter). Allowing no allele mismatches would likely create false individuals since we know that errors and missing data exist in the genotypes – it would be too fine of a filter. On the other hand, allowing too many mismatched alleles would prevent Allelematch from differentiating between individuals – it would be too coarse of a filter. The optimum allele mismatch value is determined by Allelematch by calculating the second minimum number of samples that match multiple unique genotypes as the number of allowed allele mismatches increases (Figure 1-6) (Galpern et al. 2013).

Population modeling

The mark-recapture modeling R package Capwire estimates population size from a single sampling session rather than the multiple sampling sessions (a "mark" session followed by "recapture" sessions) required by traditional mark-recapture models (Miller et al. 2005, Pennell et al. 2013). While standard methods pool multiple observations of an individual within a sampling session into just one observation, Capwire uses these multiple observations when estimating population size from a single session -- hence the name Capwire: <u>cap</u>ture <u>wi</u>th <u>re</u>placement (Miller et al. 2005). This saves a great deal of cost and effort, and can aid in satisfying model assumptions. Miller et al. 2005 found that Capwire consistently performs as well as or better than comparable models such as Mh-jackknife (Burnham & Overton 1979), Mh-Chao (Chao 1988), the exponential rarefaction method of Eggert et al. (2003), and the hyperbolic rarefaction curve of Kohn et al. (1999).

The assumptions of the single-session mark-recapture model in Capwire must be considered during sample collection and analysis:

1) *Closed population:* There are no births, deaths, immigration, or emigration in the sampling area during the sampling period. This assumption was met by using a short sampling period of 3 days and genotyping only the freshest (most recent) scat samples. We estimate that all of our genotyped samples were no more than 3 days old when collected.

2) *Independent samples:* Each genetic sample should represent a separate "occurrence" of the individual animal. This can be problematic when sampling a herd whose

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individuals may defecate multiple times in one area. We avoided sampling the same individual in the same area by not sampling scat piles within 4.5 meters (15 feet) of another scat pile.

3) *Identical distribution:* Individuals are distributed identically across the sampling area. This assumption can be problematic in the real world; however, Miller et al. (2005) found that Capwire is robust to unequal distribution in both real world datasets and grid simulations.

4) *Equal capture probability:* All individuals have the same probability of being captured each time. This is less of an issue for scat sampling of herding animals than it would be for trapping carnivores, for example, where a trap effect could influence capture probability (Miller et al. 2005). Capture heterogeneity among individuals may still exist due to behavioral differences inherent to sex, age, or reproductive status. Missed or under-represented genotypes, possibly exacerbated by unequal sampling effort across an area, may introduce another source of capture heterogeneity. In this study, Capwire's Likelihood Ratio Test rejected the Equal Capture Model and so used the Two Innate Rates Model (TIRM) that accounts for distinct capture probabilities (i.e. easy and hard-to-capture individuals) when estimating abundance.

Results

Out of 3,750 potential base pair scores (250 individuals * [13 microsatellite loci + 2 sex ID loci]), 1,963 (52%) consensus scores were produced. In other words, 48% of the genotype data could not be amplified or scored. This low rate of amplification success indicates low-

quality, degraded DNA samples, likely due to exposure to moisture in the gastrointestinal tract

and the environment. This is consistent with the results of our genotyping pilot study (see

Discussion and Appendix A).

To reduce error and ambiguity in the genotypes dataset, I culled samples with consensus

scores at 8 or fewer loci ($n = 147$), retaining only those samples with consensus scores at 9 or

more loci ($n = 103$) for further analysis, following WDFW lab protocol (Ken Warheit, pers.

comm.). Next, I used program Dropout (McKelvey and Schwartz 2005) to estimate the error load

in the dataset by creating a distribution of genetic differences based on a pairwise comparison of

genotypes (Figure 1-6). A high number of genotypes that differ by 1 to 3 loci indicates a high

number of errors in the dataset since true individuals should differ by 4 or more loci while false

individuals – those created by isolated genotyping errors – will typically differ by 1 to 3 loci

(McKelvey and Schwartz 2005). Figure 1-3 shows all but 9 individuals differing by 1 to 3 loci

(not including the 25 matched genotypes differing by 0 loci), indicating many persistent errors.
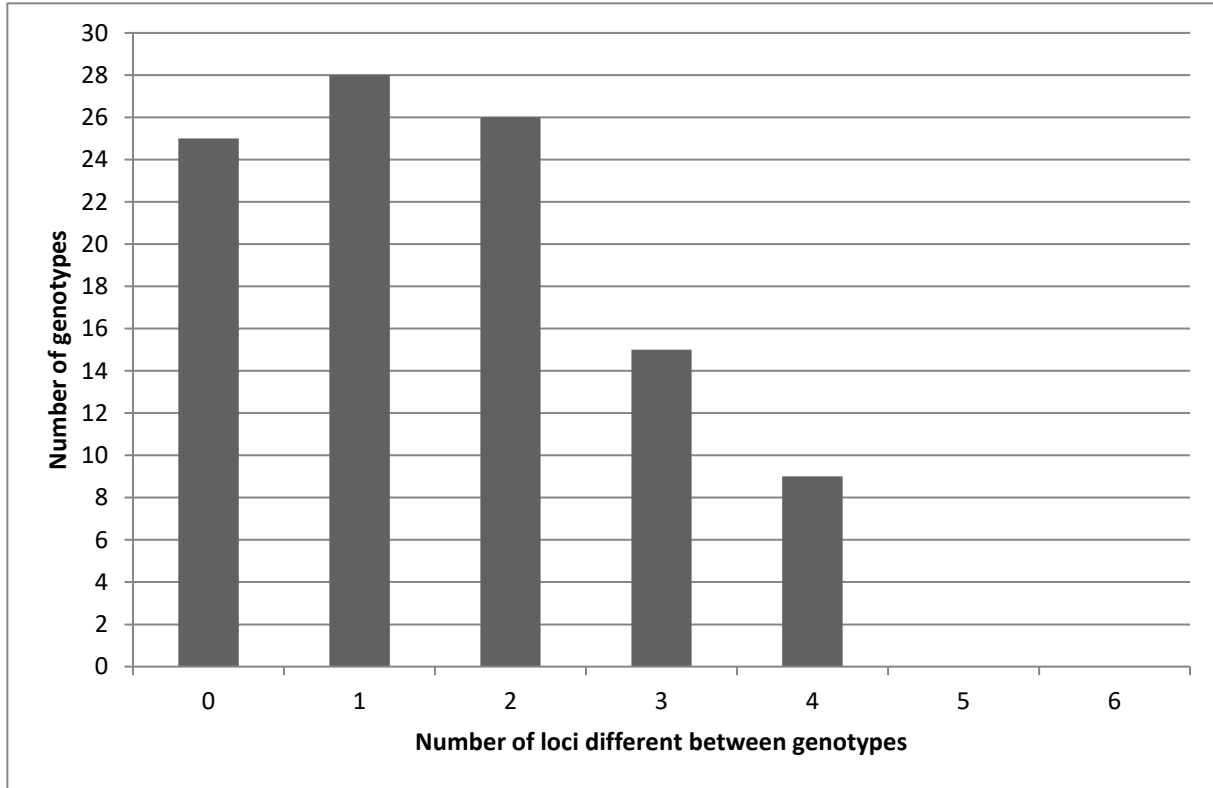
Figure 1-6. Results of the Dropout bimodality test on the 13-loci, 103-sample dataset, which found 78 genotypes that differ at 1 to 4 loci and 25 genotypes that match at all loci with non-missing data ("matches").

Additional software tests were used to detect errors so that problematic loci and samples could be removed from further analysis (Lampa et al. 2013, Micheline Manseau pers. comm.). The goal was to minimize loci that could introduce errors while retaining enough loci to differentiate between individuals (Waits and Leburg 2000, Lampa et al. 2013). Preliminary analysis using programs Genalex, MicroChecker, and Dropout found missing data, low observed heterozygosity (H_o), potential false individuals, potential null alleles, and potential stuttering at various loci (Table 1-1). To further remove errors, I culled four problematic loci from further analysis: BM203, RT27, RT7, and Texan4 (Table 1-2).

Table 1-1. Microsatellite loci and associated potential errors in 103 elk genotypes at 13 loci per Genalex, MicroChecker, and Dropout programs.

BM1225	34% missing data, no errors detected
BM203	False individuals, null alleles
BM4107	Stuttering
BM4208	Null alleles
BM4513	No errors detected
BM5004	Very low H_o (0.14), no errors
BM888	Stuttering
BMC1009	Null alleles
ETH152	26% missing data, stuttering
OarCP26	No errors detected
RT27	Very low H_o (0.011), null alleles
RT7	44% missing data, stuttering
Texan4	34% missing data, very low H_o (0.254), stuttering

Table 1-2. Summary statistics of elk genotypes before and after culls. (H_e = Expected heterozygosity. H_o= Observed heterozygosity. PIDsib=Probability of Identity for Siblings. Diversity = Allelic diversity (*n* total alleles / *n* loci). Missing data = *n* not amplified or nulled scores / *n* total scores)

	No cull (13 loci, *n* = 250)	Sample cull (13 loci, *n* = 103)	Loci cull (9 loci, *n* = 103)
Mean H_e	0.532	0.512	0.550
Mean H_o	0.368	0.411	0.452
PIDsib	0.00035	0.00051	0.0036
Diversity	3.7	3.7	3.6
Missing data	48.4%	16.8%	14.1%

Matching genotypes

The remaining 9 loci were sufficiently variable to differentiate between individuals in the

population (PIDsib for 9 loci = 0.0036^2, mean H_o = 0.452). In this dataset, allowing 5

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[2] Probability of Identity for siblings. This is the probability that two individuals in the dataset will be siblings due to chance (it's more stringent than PID). The accepted cut-off for PIDsib between two matched samples is 0.05, which is different from this overall value from all loci.

mismatched alleles between matching genotypes resulted in the fewest non-zero multiple-matched samples ($n = 17$, unclassified samples=6) (Figure 1-7). This is indicative of a "marginal" to "low-quality" dataset (Galpern et al. 2012).
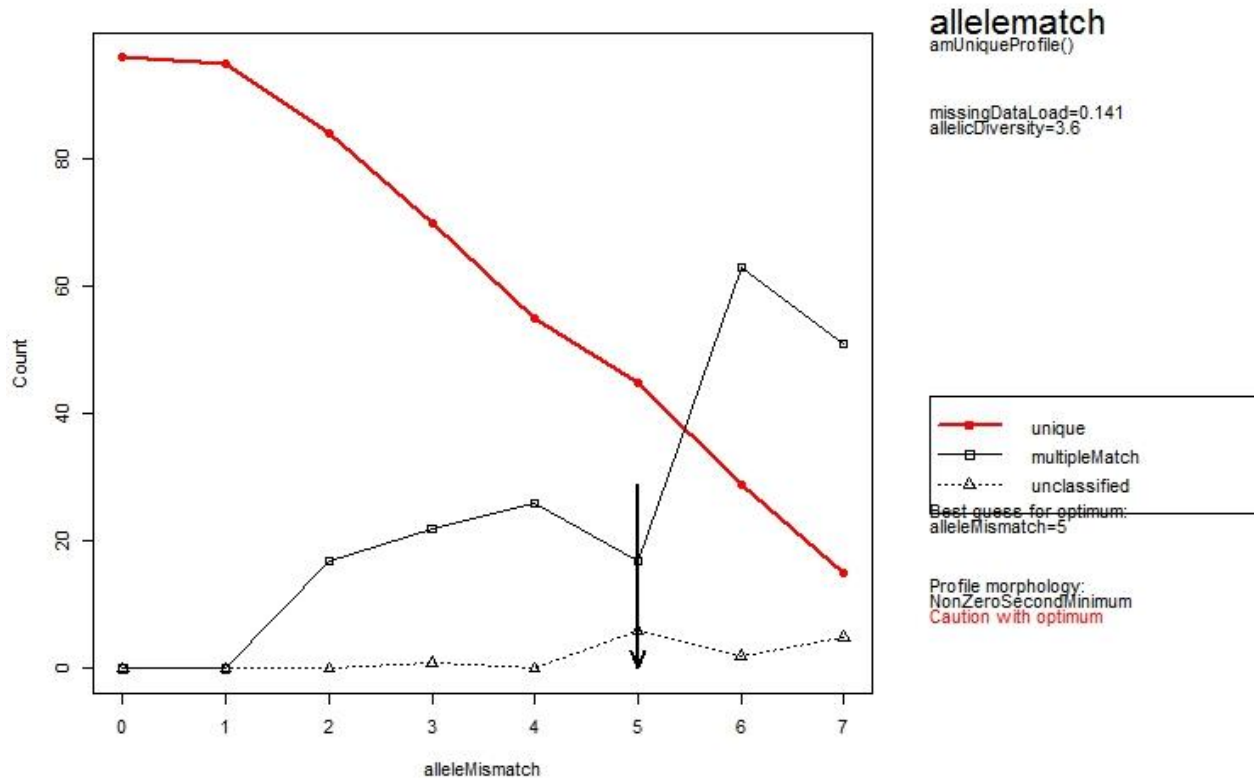
Figure 1-7. Allelematch unique profile output showing an optimum alleleMismatch value of 5. Note the emphasis to have "caution with optimum" since there are still multiple-matched samples. This is indicative of a "marginal" to "low-quality" dataset (Galpern et al. 2012)).

I resolved the 17 multiple-matched samples and the 6 unclassified samples by manually reviewing the genotypes and judging the best matches based on fewest mismatched alleles and least missing data (Galpern et al. 2012). Four of the unclassified samples appeared to be unique genotypes, bringing the total estimated "unique genotypes" to 49. These genotypes and their matches were counted to determine a history of total captures and recaptures.

From 103 elk genotypes, I used Allelematch to identify 49 unique genotypes and 54

matching genotypes. From these results, I developed a capture history. (Table 1-3).

Table 1-3. Capture history for 9-loci, 103-sample dataset with 49 unique genotypes per Allelematch (alleleMismatch parameter = 5).

Number of captures	Number of Individuals
1	31
2	5
3	6
4	2
5	1
6	2
9	1
10	1

I used this capture history with Capwire's Two Innate Rates Model (TIRM) to calculate a

maximum likelihood population estimate of 91 elk (95% CI = 83, 130) (Figure 1-5). Note that

the confidence interval does not take into account the genotyping error in the dataset. This may

be an underestimate of actual abundance since the allele mismatch parameter of 5 allowed

genotypes to match with up to 5 mismatched alleles, potentially resulting in false matches, fewer

unique individuals, and a lower population estimate in Capwire. As stated above, without further

laboratory testing of genotyping errors, the accuracy of this estimate is unknown. However, it is

the best estimate possible given the limitations of the error-ridden genotypes and Allelematch's

tolerance for some degree of error (See Discussion).

I repeated the analysis using an allele mismatch parameter of 2 in an attempt to estimate

the higher extreme of potential population estimates if in fact there were fewer genotyping errors

than the above tests suggest. Two mismatches were chosen because one mismatch was

unrealistic given the high potential for error in this dataset, and three mismatches would still

assume a high level of errors. This resulted in 84 unique individuals with 17 multiple matched

samples. Resolving the multiple matches resulted in 77 unique individuals and a capture history

(Table 1-3). Under TIRM, Capwire estimated a maximum likelihood population size of 236

individuals (95% CI = 192, 400) (Figure 1-5). This is very likely a significant overestimate of

actual elk abundance since the artificially low mismatch parameter of 2 results in fewer genotype

matches and more unique genotypes (likely false individuals) than the recommended allowance

of 5 mismatched alleles that would better account for genotyping error (Figure 1-8).

Table 1-4. Capture history for 9-loci, 103-sample dataset with 77 unique genotypes per Allelematch (alleleMismatch = 2).

Number of captures	Number of Individuals
1	61
2	10
3	2
4	4
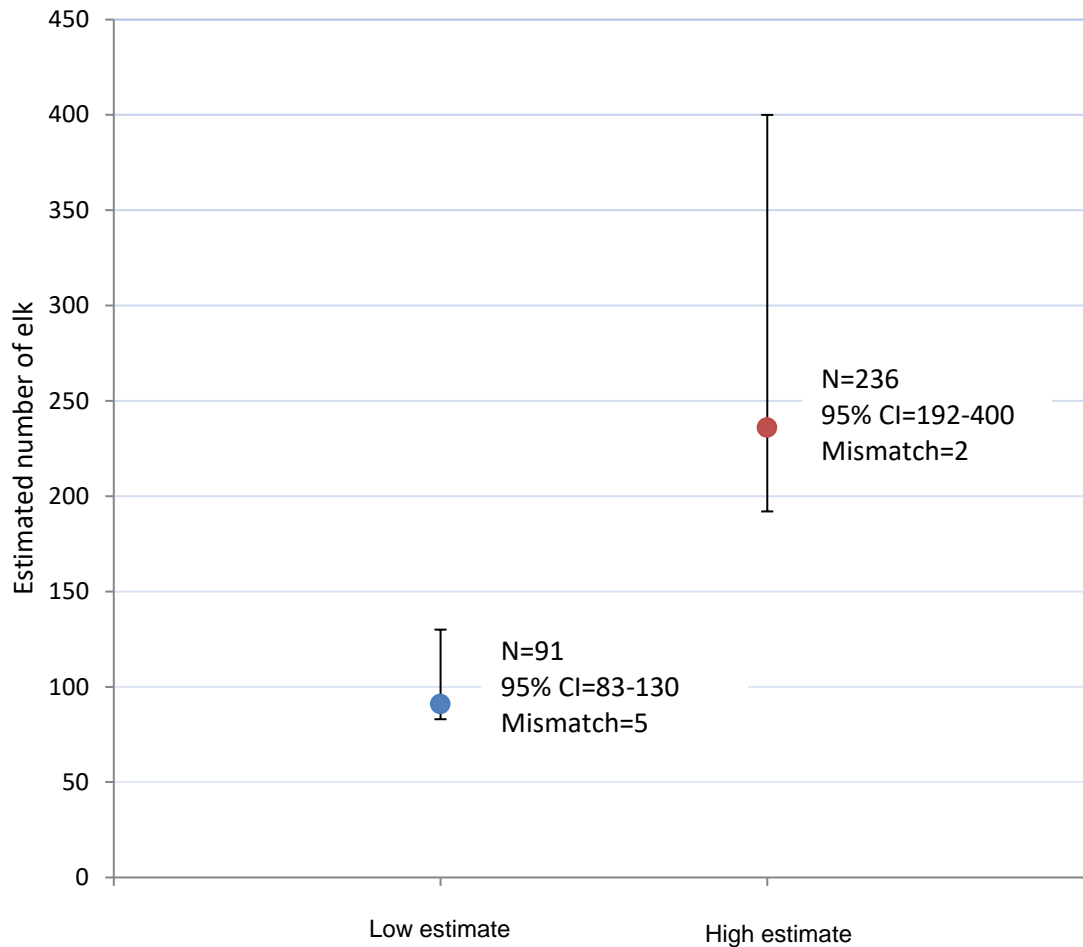
Figure 1-8. Two population estimates of elk in the South Fork Nooksack River Valley using Capwire with two capture histories based on different allele mismatch values in program Allelematch.

Discussion

Given the high potential for errors in the elk genotypes and the lack of funding to verify them in the laboratory, it is impossible to determine the accuracy of these population estimates. While Capwire assumes that genotype data is error-free when estimating population size, various error-checking programs suggest a high rate of genotyping error in our dataset. This hinders the reliability of our results.

A number of studies have found severely biased population estimates as a result of genotyping error. Using simulated data, Waits and Leburg (2000) found population estimates were overestimated by >200% when the probability of genotyping error was 0.05 per locus when 7–10 loci were used. In a study estimating the size of a Eurasian badger population, Lampa et al. (2013) found 70% of samples had erroneous genotypes at $1 - 5$ microsatellites after the first PCR when compared to consensus genotypes. These errors resulted in significant overestimation (~25%) of the population compared to error-controlled datasets using Capwire and other models.

While Allelematch appears to be robust to small amounts of genotyping error and missing data when matching genotypes, this dataset may be outside these bounds. Galpern et al. (2012) found that, in general, Allelematch identified unique genotypes reliably and accurately when allelic diversity $>=$ 7.8 alleles/locus, missing data $<=$ 5% and allelic dropout rate $<=$ 0.04. Elk genotypes were not diverse enough (3.6 alleles/locus) and lacked data (missing data $=$ 14%) to meet this general standard (Table 1-2); the allelic dropout rate is not known since samples were not reamplified. Allelematch can still perform reliably and accurately at lower levels of diversity and with more missing data depending on dataset characteristics (Galpern et al. 2012). However, without a more robust estimate of allelic dropout (and other genotyping errors) in our dataset, further analysis of the reliability of these genotypes and Allelematch's ability to match them is precluded.

Sources of genotyping error likely stem from the low quality of fecal DNA. Fecal enzymes degrade DNA from epithelial cells (David Paetkau pers. comm.) as does moisture from both the gastrointestinal tract and the environment. Due to scheduling limitations, the three-day sampling session began about 12 hours after heavy rains so most scat piles were likely rained on. Despite protocols to sample and analyze the highest quality DNA possible (i.e. sampling from

middle of scat pile and subsampling the freshest samples), samples nonetheless amplified poorly. The genotyping success rates for the three days of sampling were 55%, 47%, and 56%, respectively. The average quality scores for each day as (subjectively) assigned by field samplers were 1.16, 1.22, and 1.20, respectively (lower score is fresher). The higher genotyping success rate on the third day -- despite a similar average quality score on day two -- may be due to the longer period without rain and potentially drier scat piles; however, the subjectivity of the quality score precludes a clear conclusion. Scat DNA samples collected for our genotyping pilot study amplified better than samples collected during the population study, likely due to colder and drier weather (see Appendix A). In the pilot study, toothpick samples collected during cold and clear weather ($n = 13$) showed a genotyping success rate of 61% at 9 loci, meaning that 39% of loci did not produce consensus scores. Pilot samples collected during cold and drizzly weather ($n = 10$) had a 59% success rate. The genotyping success rate for the population study was 52% at 13 loci.

Model assumptions

Meeting all four assumptions of Capwire's single-session mark-recapture model (closed population, independent samples, identical distribution, and equal capture probability) proved difficult in the real world. In some cases, sampling protocols meant to ensure a closed population, for example, made it more difficult to meet other assumptions. Still, we believe that we satisfied Capwire's assumptions given its robustness and flexibility, except perhaps the assumption of sample independence. A discussion of model assumptions here is useful.

Population closure can be assumed given the short (3-day) sampling period and focus on fresh scat samples. It should be noted, however, that sampling took place during bow hunting

season as well as the fall rut when bulls actively move across the landscape searching for cows, which herd together during this time.

Rutting behavior also may have affected the distribution of elk across the valley, which was clearly not identical given the spatially clumped distribution of herds and heterogeneity of elk habitat. As stated above, Capwire is relatively robust to violations of the assumption of identical distribution (Miller 2005).

The assumption of equal capture probability was violated since Capwire's Likelihood Ratio Test rejected the Equal Capture Model. For this reason, Capwire used the Two Innate Rates Model to account for different rates of capture probability when estimating population size. A more systematic sampling approach may have resulted in equal capture probability; however, this assumption is exceedingly difficult to achieve in the field (Miller et al. 2005). A short and targeted sampling scheme was deemed necessary in order to assume a closed population and collect enough samples with limited time and resources. Sub-sampling fresh samples also likely influenced capture probability but was likewise necessary to maximize DNA quality and minimize the sampling period to assume population closure. Had we genotyped low quality (but randomly selected) scat samples, increased genotyping error and missing data would have also affected capture probabilities. Using low quality (older) samples would also expand the duration of the sampling window and would potentially violate the closed population assumption. Regardless, Miller et al. (2005) found that Capwire actually performs better with capture heterogeneity (i.e. unequal capture probability) across individuals in the dataset, reducing bias, narrowing confidence intervals, and lowering mean relative error compared to population estimates under the Equal Capture Model.

One risk of using Capwire is the potential for using samples from the same individual that may not be independent, possibly violating the model assumption of independent samples. While standard mark-recapture models using multiple sampling sessions pool repeated detections of an individual within the same sampling session into one observation, Capwire uses these repeated detections within a single sampling session (Miller et al. 2005). In general, we considered a sample to be independent if the animal defecated in a different place at a different time (spatiotemporal independence) but exact limits on these parameters are difficult to determine, particularly when animals are spatially clumped as with herding ungulates. We sampled 78 fresh scat piles in close proximity on a 2.5-hectare (6.2-acre) field behind the Acme firehouse where a herd of elk had been reported a few hours prior. We avoided sampling scat piles within 4.5 meters of each other to maintain sample independence; however, this may not have been sufficient given the high number of recaptures found in this field.

Of the 17 recaptured elk, 7 were captured more than once in the firehouse field. The elk captured a total of 10 times (W12631) was captured 5 times in this field within 50 meters and as close as 11.5 meters to the next nearest sample. Similarly, the elk captured a total of 9 times (W12597) was captured 6 times in the same field with the nearest samples just 13 meters apart. Another elk (W12735) was detected three times only in that field and nowhere else, as were elk W12647 and W12806, which were each captured twice. In all, 30% of the 69 total recaptures were in the firehouse field. In this situation, it is difficult to determine if these samples are truly biologically independent. Harris et al. (2010) rejected a Capwire population estimate of Argali sheep because 63% of single-session recaptures were found within 3 meters at the same site (which they identified with one GPS location). Since the samples lacked distinct geographic information, they concluded that this violated the assumption of sample independence, while also

producing falsely high precision. Our samples were more dispersed but the more focused sampling effort in the firehouse field – and other areas of high scat density -- may have violated sample independence, resulting in an excessive number of recaptures and hence a potential underestimate of the population size. In addition, individuals in this subherd may be more likely to be closely related compared to other subherds in the valley, possibly further increasing the probability of false matches. Despite these limitations, we decided to genotype these samples anyway to better ensure a closed population (by using fresher, more recent scat), and to maximize sample quality since the wet weather had likely degraded the fecal DNA, as seen in our pilot study (Appendix A). The low amplification success rate of even our higher quality samples suggests that our concerns were justified.

Miller et al. (2005) suggest that the Capwire model could be improved by not requiring samples to be independent since this assumption is difficult to satisfy in the real world – samples will never be evenly distributed and samples in close proximity are always more likely to come from the same individual than random samples. This spatial autocorrelation is not accounted for in the Capwire model.

Advantages and limitations of single-session sampling

Capwire has given accurate estimates of abundance compared to multiple-session mark-recapture models (Miller et al. 2005). In this study, the single sampling session made possible by Capwire is perhaps necessary in order to assume a closed population during the fall rut and the hunting season. However, given the potential for unequal capture probability, multiple sampling session models could better account for this and other capture processes that may bias the population estimate (Lampa et al. 2013, Harris et al. 2010, Luikart et al. 2010).

Comparison to ongoing mark-resight population surveys

Despite the potential for genotyping errors, our results appear to be comparable to aerial mark-resight population estimates in the same area. WDFW has been monitoring the core North Cascades elk herd range since 2006 using aerial surveys and a mark-resight model to estimate annual population size. In March 2014, WDFW counted 129 elk in roughly the same study area of the South Fork Nooksack River valley that we sampled in September 2013 ($n = 91$). Local elk migration patterns could explain this apparent seasonal fluctuation in herd size. Our survey was performed during the fall rut when elk were likely moving in and out of the valley bottom; bow hunting season may have also caused elk to remain at higher elevations. During the winter months, elk descend to lower elevations, likely increasing the herd size in the valley bottom, as the aerial survey results suggest.

Cost

Noninvasive genetic mark-recapture has many potential advantages over other methods but costs can increase dramatically depending on the amount of genotyping error in the dataset, as was the case in this study. Additional laboratory expenses required to determine consensus genotypes in an error-ridden dataset should be carefully considered and anticipated in the budgeting process. Table 1-5 shows cost estimates for the first round of three genotyping error reduction protocols for this study. The least expensive option would be to follow the singles protocol for the 103-sample dataset, which would have a minimum cost of $2,450 (Table 1-5). Additional lab work would be likely and would depend on results from round 1. These costs would be in addition to the first amplification of 250 samples already completed, which cost approximately $12,000. The estimated cost of the WDFW aerial surveys for mark-resight estimates over the entire survey area in Figure 1-3 is $47,000 per year not including staff time

(McCorquodale et al. 2012). If the cost of genotyping analysis continues to decline, this approach will become more practical, particularly if the cost of aerial surveys by helicopter increases.

Table 1-5. Cost estimates for the first step of three genotyping error reduction protocols at $49 per sample (not including first amplification of all samples). Multiple tubes approach mentioned above is omitted due to excessive cost.

	cMTA	Mismatch	Singles
1st cull ($n = 129$)	($n = 129$) $6,321	($n = 90$) $4,410	($n = 65$) $3,185
2nd cull ($n = 103$)	($n = 103$) $5,097	($n = 70$) $3,430	($n = 50$) $2,450

Management implications

Genotyping errors preclude our results from being used directly for management purposes. However, our population estimates provide a reasonable, if broad, range of elk abundance in the South Fork Nooksack River Valley. Additional laboratory analysis and perhaps a more objective sampling protocol are encouraged in future non-invasive genetic mark-recapture studies.

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Appendix A

Elk fecal genetic sampling protocol pilot study

Methods

Sampling

On January 10 and 16, 2013, tribal biologists and WWU researchers collected 46 elk fecal genetic samples in order to test the effect of sampling method, weather, and scat condition on genotyping success. Samples were collected at Hurn Field in Skagit Valley, Washington, a known elk grazing area. Samples were collected using a toothpick method developed by Dr. David Paetkau of Wildlife Genetics International and tested by Kim Sager of the Lower Elwha S'Klallam Tribe (Paetkau pers. comm., Sager, unpublished data). A coarse toothpick was gently dragged over the translucent mucus on the surface of the elk pellet to collect epithelial cells sloughed from the intestinal tract. Collection of fecal material on the toothpicks was avoided because fecal enzymes can degrade DNA and interfere with the DNA amplification process (Paetkau pers. comm.). The sampling procedure was performed twice for each sampled scat pile -- once with a pellet from the top of a pile and a second time with a pellet from the middle of the pile in case the first DNA sample was degraded by weather. Intestinal mucus was also sampled in this process where found. The two toothpicks were then placed in a coin envelope, sealed, and labeled with unique sample identification numbers and a quality score from 1 to 3 based on freshness of scat. This same protocol was followed using cotton swabs. The sampled scat pile

was then covered with vegetation (grass or sticks) to prevent resampling, and geographic coordinates were recorded using Garmin GPSMap 60 C and 60 CSx units.

Genotyping

Samples were sent to the WDFW genetics lab for genotyping. Genomic DNA was extracted from epithelial cells on sampled toothpicks and cotton swabs following standard recommendations for DNeasy commercial single tube silica-membrane blood and tissue DNA isolation kit (Qiagen). Polymerase chain reaction (PCR) was used to amplify 9 previously characterized microsatellite loci (BM1225, BM4107, BM4208, BM4513, BM5004, BM888, BMC1009, ETH152, RT7). Lab technicians amplified and scored each sample twice, and counted number of amplified alleles, number of mismatched alleles between PCR attempts (a measure of PCR pair concordance), and number of mismatched alleles between collection method subsamples (a measure of concordance).

Laboratory staff conducted a Principal Components Analysis on three variables: PCR pair concordance, collection method subsample concordance, and average number of amplified alleles. The first PC accounted for 71% of the variance, with all three variables highly correlated with the axis and with positive weights. This means that high PC 1 scores indicates successful extraction; low PC 1 scores means poor extraction. PC 1 scores were compared with field collection data (quality score, weather, and collection method).

Results

Samples with a quality score of 1 extracted successfully while samples with a quality score of 3 performed poorly (Figure A-1). Samples collected when weather was clear and cold (January 10) outperformed the samples collected during cold and wet weather (January 16)

(Figure A-2). Toothpick samples collected during cold and clear weather ($n = 13$) showed a genotyping success rate of 61% at 9 loci, meaning that 39% of loci did not produce consensus scores. Pilot samples collected during cold and drizzly weather ($n = 10$) had a 59% success rate. This is consistent with other studies showing improved genotyping success in winter (Harris et al. 2010, Hettinga et al. 2012). There was no difference in extraction quality between the cotton swab and toothpick extractions (Figure A-3). However, laboratory staff indicated that toothpick samples were much easier to process than the cotton swab samples.
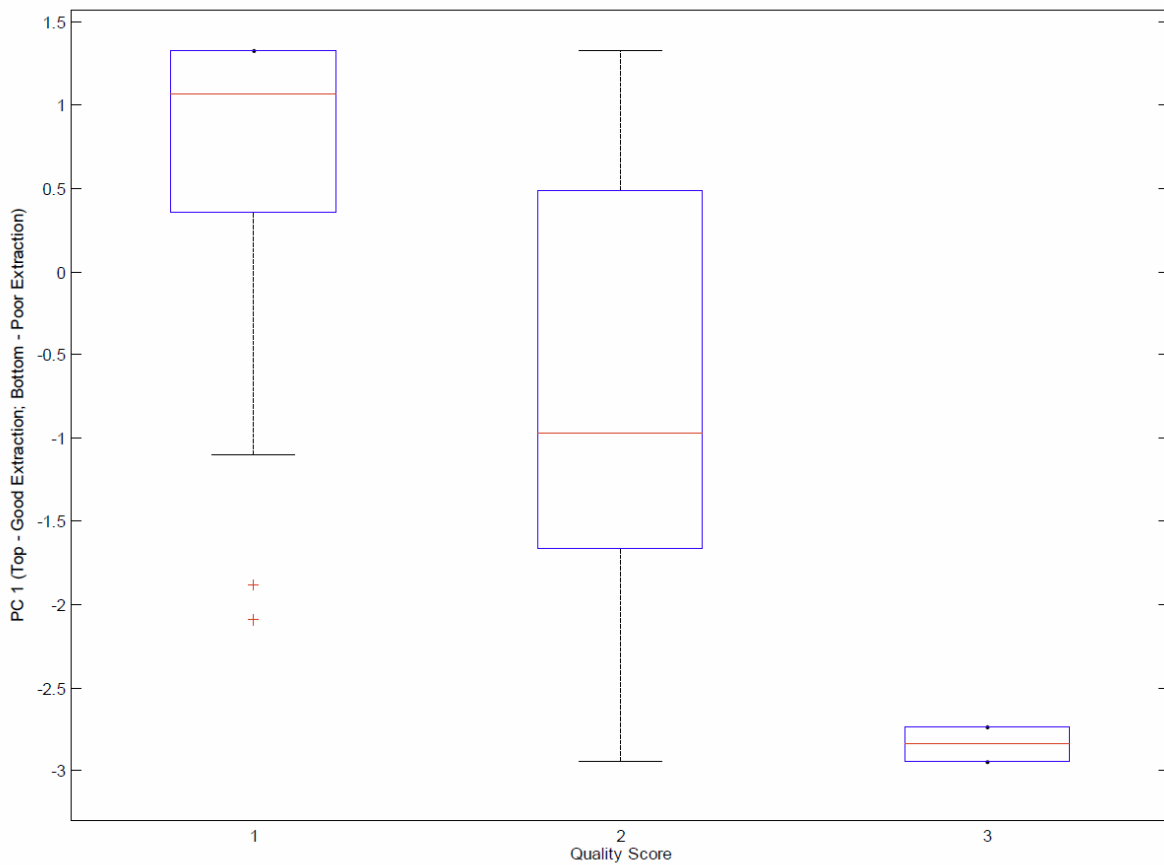
Figure A-1. Elk fecal genetic sample extraction success by field-determined quality score of scat sample freshness. The y-axis is Principal Component 1 in a Principal Component Analysis of PCR pair concordance, collection method subsample concordance, and average number of amplified alleles (see Methods). Figure courtesy Kenneth Warheit, WDFW Genetics Lab.
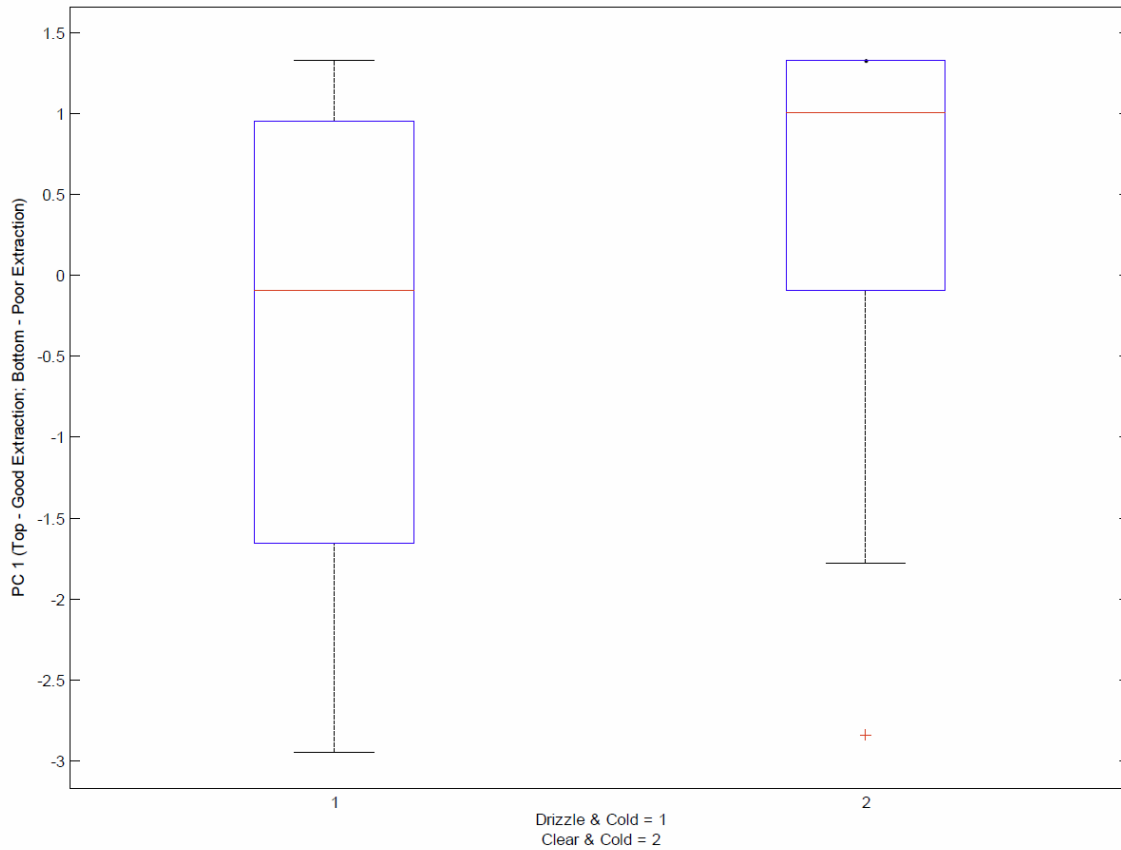
Figure A-2. Elk fecal genetic sample extraction success by weather conditions during sample collection. The y-axis is Principal Component 1 in a Principal Component Analysis of PCR pair concordance, collection method subsample concordance, and average number of amplified alleles (see Methods). Figure courtesy Kenneth Warheit, WDFW Genetics Lab.
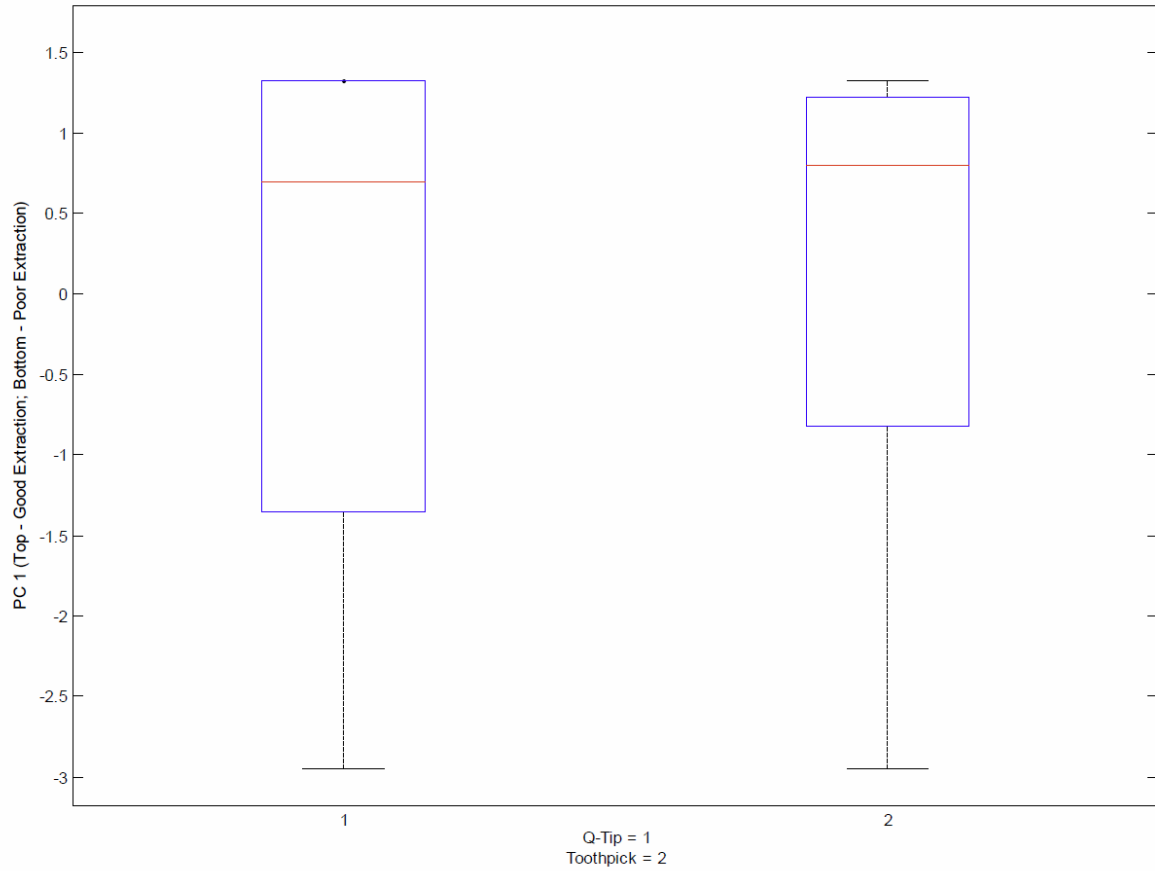
Figure A-3. Elk fecal genetic sample extraction success by sample collection method. The y-axis is Principal Component 1 in a Principal Component Analysis of PCR pair concordance, collection method subsample concordance, and average number of amplified alleles (see Methods). Figure courtesy Kenneth Warheit, WDFW Genetics Lab.

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Chapter 2

Elk road ecology on Highway 20 in Skagit Valley, Washington

Introduction

Roads can dramatically affect wildlife populations by degrading habitat, restricting migration corridors and gene flow, and increasing mortality from vehicular collisions (Forman et al. 2003, Dodd 2007, Epps et al. 2005). In the U.S., estimated deer-vehicle collisions exceed 1 million every year resulting in over 200 human fatalities, 26,000 human injuries and over $8 billion in damages and medical costs (Conover et al. 1995, Huijser et al. 2007). Each year in Washington State, approximately 3,000 collisions with deer (*Odocoileus* spp.) and elk (*Cervus canadensis[3]*) take place on state highways alone (Wagner and Carey 2006). Between 2000 and 2004, vehicle collisions on state and federal highways in the state killed at least 14,969 deer and 415 elk (Myers et al. 2008).

In Skagit Valley, Washington, elk-vehicle collisions are a common and dangerous occurrence on state Highway 20. Between 2002 and 2014, 158 elk-vehicle collisions were documented on 34.8 kilometers of Highway 20 between the towns of Sedro-Woolley and Concrete by Washington Department of Transportation (WDOT), Washington Department of Fish and Wildlife (WDFW), Washington State Patrol, and the Upper Skagit Indian Tribe (Figure 2-1). This is probably an underestimate of total elk-vehicle collisions since many collisions likely go unreported. Elk-vehicle collisions more than doubled between the periods 2001-2006 and 2007-2011 (WDFW 2017). In 2012, 62 elk collisions were documented on the 34.8 km between

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[2] Many authors continue to use *Cervus elaphus* to describe North American elk; however, phylogenetic research has shown them to be a distinct species (*Cervus canadensis*). *Cervus elaphus* now describes red deer species in Europe (Groves and Grubb 2011).

the towns of Sedro-Woolley and Concrete, which equals 53% of total hunting mortality that year (n =117). This marked a major increase in documented roadkills compared to previous years. However, this apparent increase may be due to improved reporting and coordination between agencies, rather than an actual increase in elk-vehicle collisions. The current annual number of elk-vehicle collisions is likely in the range of 20-30 (WDFW 2017). This apparent decrease in roadkills may be associated with the installation of nine flashing elk crossing signs that were installed on Highway 20 near areas of high elk activity in 2014. Temporary warning signs were shown to reduce deer-vehicle collisions by 50 percent and reduce vehicle speeds in Utah, Nevada, and Idaho; however, the effectiveness of the signs in reducing speeds declined a year after installation, suggesting that drivers may ignore warning signs over time (Sullivan et al. 2004). In addition to elk-vehicle collisions, WDFW reports that landowners have expressed concern about potential vehicle collisions with livestock when they escape from elk-damaged fencing, resulting in loss of property and creating an additional substantial threat to motorists (WDFW 2017).

Roadkill data on Highway 20 show wide spatial variation between Sedro Woolley and Concrete, with some road segments having dozens more collisions than others (Figure 2-1). This may be due to spatial variability in the frequency of highway crossings or because elk are more vulnerable to collisions in some areas due to landscape or road factors. Distinguishing between these two possibilities has significant management implications. Roadkill hotspots that are closely associated with road crossing hotspots could potentially be mitigated with additional warning signs, reduced speed limits, or crossing structures. In this case, the use of fencing would probably only result in shifting the location of both the roadkill and road crossing hotspots. Roadkill hotspots less associated with road crossing hotspots may occur because of land cover or

road factors that create a condition more conducive to a collision. This could be better mitigated with a different strategy such as fencing, which could restrict crossing activity to less dangerous locations.
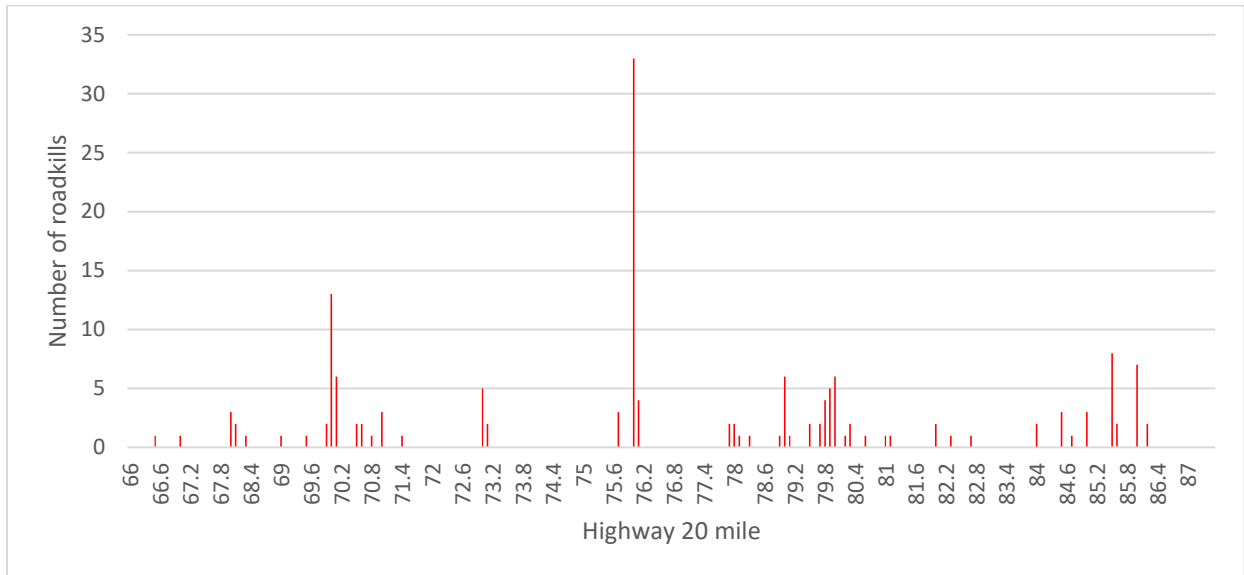
Figure 2-1. Elk roadkills by reported mileage on Highway 20 between Sedro-Woolley to Concrete, 2002-2014 (*n* =158).

The population size of the North Cascades elk herd likely influences the number of elk-vehicle collisions. Current North Cascades elk herd abundance is estimated to be between 1,569 and 1,769 animals (WDFW 2017). Annual mark-resight population surveys by WDFW suggest that the population within the state Game Management Unit (GMU) 418 and that portion of GMU 437 north of the Skagit River between Lyman and Concrete is 1,269 elk (95% CI = 1,170-1,379) (Figure 2-2). From 2006 to 2016, estimates of population size within the aerial survey area indicate that the North Cascades elk herd has increased at a rate of 5-7% annually (Figure 2-3). In addition, 200-400 elk occur elsewhere in GMU 437, primarily south of the Skagit River between Sedro Woolley and Marblemount, and at least 100 more within the Sauk River valley south of Rockport, according to biologists' observations and other anecdotal information

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(WDFW 2017). Elk regularly traverse between the Skagit and Nooksack watersheds via Lyman

Pass and other locations north of Hamilton and Birdsview (WDFW 2017). These movement

patterns likely play a role in determining elk crossing and roadkill locations.
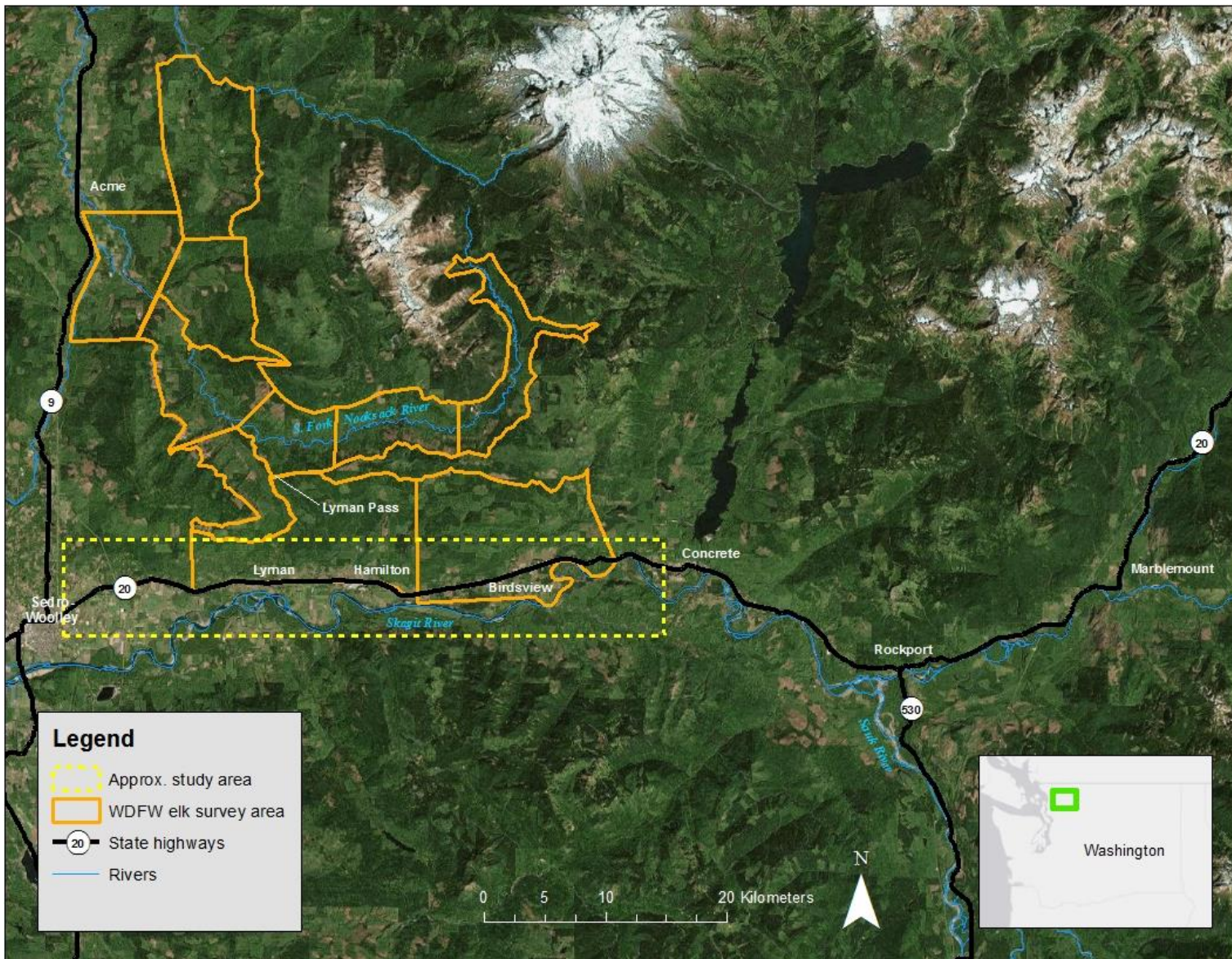
Figure 2-2. Overview map of study area. WDFW elk survey area is denoted in orange
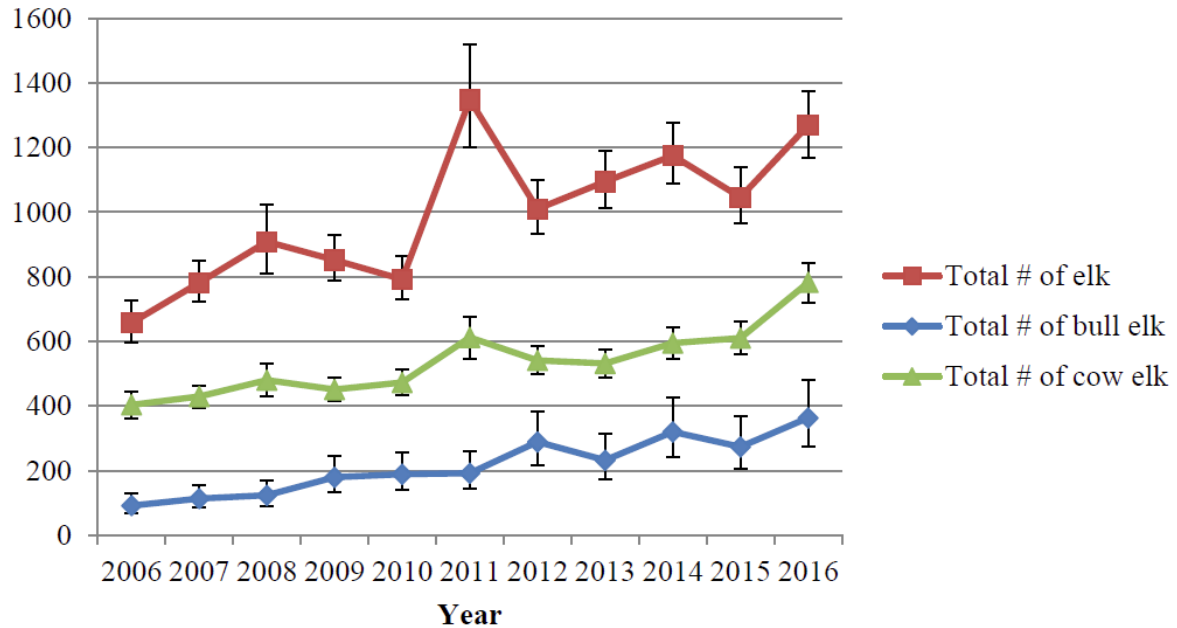
Figure 2-3. Mark-resight estimates of total elk, cow elk, and bull elk population size in GMU 418 (Nooksack) 2006–2016. Estimates also include elk within the northern portion of GMU 437 north of the Skagit River 372 between Lyman and Concrete. (WDFW 2017)

The herd reached a peak abundance of between 1,400 and 2,000 elk in the mid-1980s before a major decline in the early-1990s drove the population down to a few hundred elk (WDFW 2017). Causes of the decline included intensive timber management practices (including herbicide use that reduced early seral habitat), associated traffic and disturbance, overhunting on an expanded road network, vehicle collisions, loss of habitat to development and agriculture, and lethal removal on conflicted agricultural lands (Danilson 2012). Disturbance from recreational use of logging roads by off-road vehicles has also increased in recent years (Danilson 2012, Davison 2002). The herd has since rebounded with the support of augmentation, a hunting moratorium from 1997 to 2007, habitat enhancement projects, restricted vehicle access, and changes in silvicultural practices that have created a more complex mosaic of habitats (WDFW 2017).

Managing agencies seek to increase the size of the herd to 1,950 elk (±10%) (WDFW 2017). A larger population would likely increase the risk of vehicle collision. WDFW reports that the North Cascades elk herd is not limited by available habitat despite a reduction in overall elk carrying capacity in Washington. This is due to a marked reduction in timber harvest that created early seral habitat (including on federal land where herbicide is not used), as well as an increase in human population and associated development (WDFW 2014). Elk currently inhabit agricultural lands in the Skagit Valley and South Fork Nooksack Valley, leading to frequent conflicts with landowners. Human population growth in Whatcom and Skagit counties and the resulting increase in traffic volume may lead to more elk-vehicle collisions in the future (WDFW 2017, Gagnon et al. 2006).

Research elsewhere in Washington State has identified a number of factors associated with ungulate-vehicle collisions and road crossings (Table 2-1). Long et al. (2012) found that elk-vehicle collisions were associated with increased distance to forest cover, and negatively associated with the presence of concrete Jersey barriers on Interstate 90. Statewide, deer abundance strongly affected the number of collisions in a given area, as did roadside cover, forage, modest slopes, water sources, and southern exposure (Myers et al. 2008). In Arizona, Gagnon et al. (2006) identified traffic volume, proximity to riparian habitat, and season as factors contributing to the frequency with which elk cross roads, and Dodd et al. (2006) found that elk crossing and collision frequency was associated with proximity to riparian meadows. In Spain, Malo et al. (2004) found that non-riparian forest and diversity of forest and open habitat were positively associated with red deer (*Cervus elaphus*, a species closely related to elk*)* collisions while agricultural areas, urban areas, distance to forest, and guardrails were negatively associated with red deer collisions. In a review of spatial modeling of wildlife-vehicle collisions,

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Gunson et al. (2011) found that both forest and open habitat surrounding roads increased

ungulate collisions, as did landscape diversity, while agriculture and urban areas decreased them

(Hubbard et al. 2000, Seiler 2005, Bashore et al.1985, Finder et al., 1999, Gunson et al. 2009,

Malo et al. 2004, Nielsen et al. 2003, Puglisi et al.1974). Across the United States, deer-vehicle

collisions were most probable on two-lane highways with moderate traffic volume (similar to

Highway 20) rather than high volume interstate highways (Huijser et al. 2008). Time of day,

visibility, movement patterns, elk density, and other factors may also influence wildlife-vehicle

collisions.

Table 2-1. Significant variables in previous elk-vehicle collision and road crossing modeling studies

Predictor	Response	+/-	Species	Scale	Location	Reference
Habitat variables						
Distance to forest	Collisions	+	Elk	NA	WA USA	Long et al. 2012
Distance to forest	Collisions	-	Red deer*	NA	Spain	Malo et al. 2004
Proportion non-riparian forest cover	Collisions	+	Red deer*	1000 m	Spain	Malo et al. 2004
Percent forest cover	Crossings	+	Red deer*	100 m	Norway	Meisingset et al. 2013
Roadside cover	Collisions	+	Deer spp. [+]	30m,60m,0.8km	W WA USA	Myers et al. 2008
Distance to riparian meadows	Crossings	-	Elk	0.16 km	AZ USA	Gagnon et al. 2007, Dodd et al. 2007
Diversity of forest and open habitat**	Collisions	+	Red deer*	1000 m	Spain	Malo et al. 2004
Distance to pasture	Crossings	+	Red deer*	NA	Norway	Meisingset et al. 2013
Amount of herbaceous cover	Collisions	-	Deer spp. [+]	30m,60m,0.8km	W WA USA	Myers et al. 2008
Size of grass patches	Collisions	+	White-tailed deer	800 m	Iowa USA	Hubbard et al. 2000
Forage	Collisions	+	Deer spp. [+]	30m,60m,0.8km	W WA USA	Myers et al. 2008
Proportion open area	Collisions	+	Moose	500 m	Sweden	Seiler 2005
Proportion of agricultural area	Collisions	-	Red deer*	1000 m	Spain	Malo et al. 2004
Proportion of urban area	Collisions	-	Red deer*	1000 m	Spain	Malo et al. 2004
Proportion of crop fields	Collisions	-	White-tailed deer	800 m	Iowa USA	Hubbard et al. 2000
Proportion agriculture	Collisions	-	Moose	500 m	Sweden	Seiler 2005
Road variables						
Roadside slope	Collisions	-	Deer spp. [+]	30m,60m,0.8km	W WA USA	Myers et al. 2008
Speed limit	Collisions		Deer spp. [+]	30m,60m,0.8km	W WA USA	Myers et al. 2008
Traffic volume (AADT)	Collisions	-	Deer spp. [+]	30m,60m,0.8km	W WA USA	Myers et al. 2008
Traffic volume	Crossings	-	Elk	0.16 km	AZ USA	Dodd et al. 2007
Traffic volume	Crossings	-	Elk		AZ USA	Gagnon et al. 2007
Presence of guardrails	Collisions	-	Red deer*	100 m	Spain	Malo et al. 2004

*This study included red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and wild boar (*Sus scrofa*)

**As measured by Shannon Diversity Index.

[+] Northwest white-tailed deer (*Odocoileus virginianus ochrourus*), Rocky Mountain mule deer (*O. heminonus heminonus*)

In Skagit Valley, elk cross Highway 20 more frequently in the winter, possibly due to limited food availability and heavy snowpack at higher elevations. These elk tend to have small home ranges closely associated with riparian areas throughout the year and are known to frequent residential and agricultural areas, damaging lawns and gardens, tree farms, conifer

plantations, hay, pasture, alfalfa fields, green chop, feed corn, silage, blueberries, orchards, vineyards, potatoes, and other agricultural crops (WDFW 2017).

More information on local roadkill and crossing locations and the road and landscape factors associated with them is needed to better understand local elk road ecology and reduce road collisions (Gunson et al. 2011) This research will contribute to WDFW's management objective to "minimize public safety risk by reducing the average annual number of elk-vehicle collisions along the State Route 20 corridor between Sedro Woolley and Rockport by 50% over the next five years." (WDFW 2017)

In this study, I compared roadkill and road crossing locations and modeled road and land cover variables where elk cross and are killed on Highway 20 between Sedro-Woolley and Concrete. My objectives were to identify where elk roadkills and elk crossings occur and identify factors associated with each of them. This information can be used to help reduce elk-vehicle collisions with potential mitigation strategies such as signage, speed limits, fences, and crossing structures, with the goal of reducing mortality and improving traffic safety in Skagit Valley and beyond.

Methods

Study area

Skagit Valley is located west of the crest of the Cascade Mountains in the Western Hemlock (*Tsuga heterophylla*) zone of northwest Washington state (Franklin and Dyrness 1973). The town of Sedro-Woolley (population 10,645, elevation 17 m) is located up-valley from the broad alluvial plain that stretches to Puget Sound. Agricultural fields fill the valley as Highway 20 continues east to Hamilton (population 299), Lyman (population 437), and Concrete

(population 712) at 84 m elevation. Elk habitat types in Skagit Valley include agricultural fields, deciduous forests of primarily red alder (*Alnus rubra*) and big leaf maple (*Acer macrophyllum*), and second-growth evergreen forests dominated by Douglas fir (*Pseudotsuga menziesii*) and Western Red Cedar (*Thuja plicata*).

This study focuses on the 34.8 kilometers of Highway 20 between Sedro-Woolley and Concrete where the vast majority of roadkills, agricultural land, and conflicts with landowners are concentrated (Figure 2-4).

Data collection

Roadkills

Reported roadkills are routinely responded to by Washington Department of Transportation (WDOT), Washington Department of Fish and Wildlife (WDFW), Washington State Patrol, and the Upper Skagit Indian Tribe. These agencies collect carcasses and record locations, which are estimates based on the nearest milepost and are often recorded to the nearest 0.16 km (0.10 mile). For this reason, 0.16 km road segments will serve as the unit of observation for this analysis. GPS coordinates of roadkill locations were not consistently recorded, nor were sex or age data.

Road crossing activity

Road crossing activity data was gathered from two sources: elk trail monitoring and crowd-sourced observations from Skagit Valley residents and motorists.

1) Elk trail monitoring

From July to December 2013, I monitored elk trails adjacent to Highway 20 between Sedro-Woolley and Concrete. I surveyed the north and south sides of Highway 20 for elk trails by riding a bicycle on the road shoulders between Sedro-Woolley and Concrete. I repeated this bike survey monthly to locate new trails while monitoring existing trails weekly by car. To determine frequency of trail use, I used a "string trap" consisting of a length of thin, brown sewing string tied to vegetation on either side of the trail at a height of about 1.5 meters (Larry Baumann, retired WDFW elk biologist, pers. comm.). Trail substrate varied from packed gravel to unconsolidated soil to herbaceous vegetation, so tracks were only recorded where substrate allowed. If possible, I cleared herbaceous vegetation so that tracks could be seen better; tracks were then erased each week. I used a combination of broken strings and track observations to determine trail use. Since sampling periods varied depending on when individual trails were found, I divided the total number of detections on each trail by the total number of days elapsed from the trail's discovery to the end of the sampling effort to create an index of crossing activity (trail detections per day) for each trail. Trail detections per day were then totaled for all trails within each 0.16-km segment. This data has obvious limitations including the potential for other animals to break strings as noted in more detail in the Discussion.

Remote cameras were also used to monitor some trails. Cameras provide richer and more accurate data since they record date, time of day, and number of elk on a trail. However, substantial limitations precluded widespread use of cameras for trail monitoring, including limited range of motion sensor detection, the need to point cameras away from the road to avoid vehicle-triggered photos, and substantially increased costs. I deployed up to nine cameras (Reconyx PC800, Bushnell Natureview) provided by the Tulalip tribe and WDFW at high use

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crossings and incorporated these observations into the index of crossing activity. Successive photos documenting elk on both sides of the road counted as a trail detection for each trail, and only one such event per week was included in the crossing activity database in order to maintain consistency with string trap data across the study area.

With both the string traps and the cameras, trail use on both sides of the highway was used to infer probable elk road crossings since actual road crossings could not be documented using these methods.
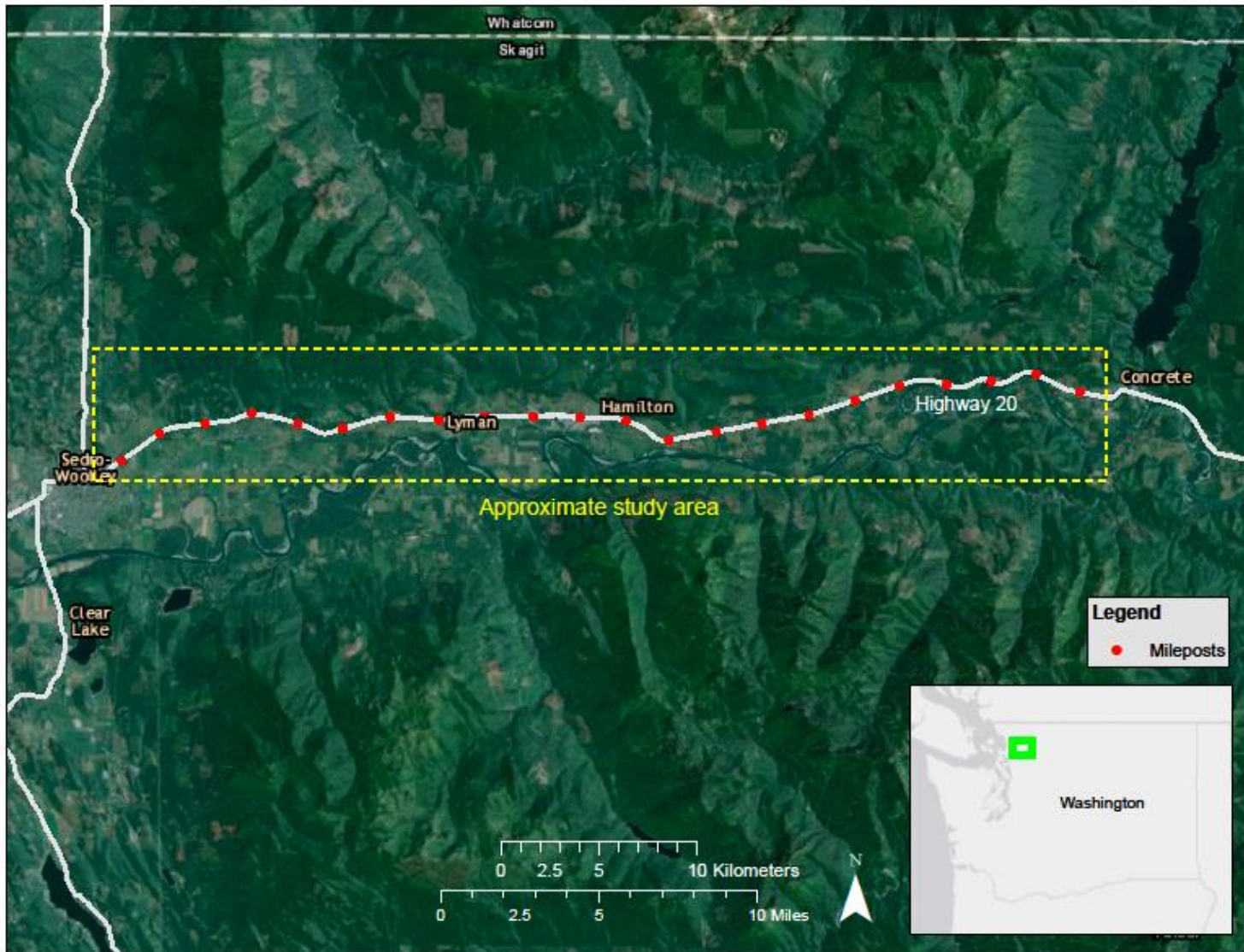
Figure 2-4. Study area in Skagit Valley, Washington on State Highway 20.

3) Crowd-sourced observations

Some motorists and residents in Skagit Valley reported elk roadkill and crossing observations by phone, email, or using a smartphone mapping application developed by WDFW, which could also be accessed by web browser[4]. This application allowed observers to report GPS locations of real-time elk observations (alive or dead) as well as number of elk, direction of crossing, sex, and other data. I contacted a selected group of residents and workers in Skagit Valley to report observations using the smartphone app, as there were some concerns from state employees regarding the safety of more widespread smartphone use while driving. For this reason, this application is no longer supported by WDFW. Furthermore, location accuracy was severely limited because users marked locations at the full extent of the map without zooming in, resulting in unusable location data. For this reason, these observations were omitted from analysis. I also posted signs requesting elk crossing observations by phone and email on gas station bulletin boards. However, participation was limited and reported locations were not sufficiently precise to use for further analysis.

Data preparation

In ArcMap, I located 0.16-km markers from WDOT mile marker spatial data and segmented a shapefile of Highway 20 into 0.16-km segments around these points to correspond to the estimated accuracy of roadkill data. Percent land cover variables were calculated using circular 1-km buffers from each 0.16-km point (the mid-point of each road segment). Proximity variables were calculated from the center of each segment. Roadkills and road crossing activity

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[4] Link to WDFW elk reporting options (http://wdfw.wa.gov/viewing/observations/elk_hwy20/)

data (response variables) for each 0.16-km segment was then converted to binary presence (1) / absence (0) data for logistic regression analysis.

I considered 10 habitat variables and 4 road variables for modeling of elk roadkill and crossing locations (Table 2-2). Variables were selected based on previous ungulate-vehicle collision research and those most relevant to elk ecology in the Skagit Valley (Table 2-1). The United States Geological Survey uses Landsat satellite imagery to classify terrestrial landscapes into 20 land cover types at a 30-meter resolution to create the National Land Cover Database (NLCD) (Jin et al. 2013). I reclassified some land cover types prior to modeling (Table 2-2). The Washington Department of Agriculture provides ground-truthed data of crop locations in the Skagit Valley that were used to calculate distances to crops and pasture/hay (WSDA 2013).

Percent cover of four land cover types within a 1-km buffer around the road segment midpoint was calculated using the *isectpolyrst* (Intersect Polygons with Raster) tool in Geospatial Modeling Environment (Beyer 2014). For proximity variables, I calculated distance to land covers or features from each road segment midpoint using the ArcMap Near tool.

I calculated road sinuosity within a 0.5-km buffer around each 0.16-km point (0.25 km on either side) using the ArcMap Calculate Sinuosity tool. Mean roadside slope angle was calculated within a 30-meter buffer on either side of the highway from a LiDAR-derived digital elevation model using the ArcMap Slope tool. I collected GPS locations of guardrails and summed the total guardrail length for both sides of the road within each 0.16-km segment.

Table 2-2. Model variables for elk roadkill and crossings analysis. Descriptions of land covers are abridged from National Land Cover Dataset metadata (Jin et al. 2013).

Variables	Description	Units
Habitat variables		
Developed (Medium/High)	NLCD Developed Medium, High combined	Percent area
All forest	NLCD Deciduous, Coniferous, and Mixed forest combined	Percent area
Hay/pasture/herbs	NLCD Herbaceous and Hay/Pasture classes combined	Percent area
Cultivated crops	Crops >20% of vegetation. Includes all land being actively tilled.	Percent area
Forest edge	Perimeter length of NLCD forest (deciduous, coniferous, mixed) in 1km buffer	Length (m)
Distance to developed land	Euclidean distance to NLCD dev. land cover (med. and high) from road segment midpoint	Distance (m)
Distance to nearest stream	Euclidean distance to nearest stream from road segment midpoint	Distance (m)
Distance to forest cover	Euclidean distance to any NLCD forest class from road segment midpoint	Distance (m)
Distance to pasture/hay	Euclidean distance to WSDA crop layer from road segment midpoint	Distance (m)
Distance to all crops	Euclidean distance to WSDA crop layer (food and tree crops) from road segment midpoint	Distance (m)
Road variables		
Mean slope	Mean slope in 30m and 60m buffers based on 6x6-ft resolution Lidar DEM	Angle
Guardrail	Total length of guardrail on both sides of road within each road segment	Length (m)
Traffic volume	2013 average daily traffic count (WDOT) per road segment	# of vehicles
Sinuosity	Deviation of highway route from shortest 0.5 km trail around segment midpoint	Total length/ shortest trail

Data analysis

I used the ESRI ArcGIS Hot Spot Analysis (Getis-Ord Gi*) tool with a fixed distance band of 0.16 km to determine statistically significant spatial hotspots of road crossing activity and roadkills in 0.16-km segments for the sampling period from July 2013 to December 2013. To determine a hotspot, the local sum of roadkills or crossings of a 0.16-km road segment and its two nearest neighbors was compared proportionally to the sum of all segments using *z* scores. Local sums that were significantly different from the expected local sum are hotspots (ESRI 2017). I used Kendall's Tau correlation to test for a correlation between all trail detections per

day and all roadkills per day across the study area during this period. Quasipoisson regression was used to model roadkills per day against trail detections per day due to the under-dispersed nature of the data (Quinn and Keough 2002).

I used Kendall's Tau correlations to test for collinearity between model variables and logistic regression to analyze candidate models. Though continuous data was available for both elk roadkills and road crossings, binary presence (1) / absence (0) data for each road segment provided several advantages: reduced error within each dataset, improved comparisons between roadkill and road crossing data and model results, and fewer confounding or undocumented variables likely contributing to differences in local elk abundance across the study area. Local abundance likely influences the amount and locations of roadkills and crossings (Dodd 2007, Gunson 2011); lacking an empirical estimate of local abundance, a binary response variable also better reflected the ecological scope of this study.

Model development

For the road crossings model, I used road crossing observations collected between July and December 2013 as the binary response variable in each 0.16-km road segment (1 = presence of a trail detection, 0 = no trail detection). For the roadkills model, I used elk roadkill data collected by state and tribal agencies between January 2012 and January 2014. This time period includes the peak of roadkills observed on this section of Highway 20 in 2012 as well as the sampling period for road crossings, providing comparable datasets.

Candidate models were determined *a priori* based on variables used in previous ungulate-vehicle collision research and knowledge of elk ecology in the Skagit Valley. Models were selected following an information theoretic approach using Akaike Information Criterion for

small sample sizes (AICc), pseudo-R^2, and Akaike weight (w_i) (Akaike 1973, Burnham and Anderson 2002, Symonds and Moussalli 2011). Variables were placed in groups pertaining to natural plant communities, agriculture, developed land, and road characteristics. Candidate models with the lowest AICc score from each category were then combined *post hoc* using an exploratory all-subsets approach to determine the best-fit models for elk roadkill and road crossing locations. The best models with cumulative Akaike weights up to 0.95 were included in a confidence set for multi-model inference to estimate parameter and error values derived from weighted averages across multiple models (Symonds and Moussalli 2011). The global model from each confidence set was tested for goodness-of-fit using the Hosmer and Lemeshow goodness-of-fit test for binary models (Quinn and Keough 2002). To evaluate the relative influence of variables in the averaged models, I considered confidence intervals and relative importance values derived from Akaike weights (*RI*) (Barton 2015, Meisingset et al. 2014)). I did not consider covariate interactions due to the small sample size of this study. I used the R program and R packages *stats* (R Core Team 2017), *MuMIn* (Multimodel Inference) (Barton 2015), *AICmodavg* (Model Selection and Multimodel Inference Based on (Q)AIC(c)) (Mazerolle 2017), and *generalhoslem* (Goodness of Fit Tests for Logistic Regression Models) (Jay 2017) for all statistical analyses.

Results

A total of 722 trail detections were documented in 31 of 216 highway segments between July and December 2013 (Table 2-3, Figure 2-5). The index of crossing activity, which normalized trail detections by the sampling effort for each trail, totaled 7.679 trail detections per day across the study area (Figure 2-6). Five statistically significant road crossing hotspots were found: Hotspot 1) km 110.1 (mile 68.4); Hotspot 2) km 122.1 - 122.6 (mile 75.9 - 76.2), Hotspot 3) km 125.2 - 125.4 (mile 77.8 - 77.9); Hotspot 4) km 137.7 – 137.9 (mile 85.6 - 85.7); and Hotspot 5) km 138.7 -138.9 (mile 86.2 - 86.3) (Table 2-4).

Table 2-3. Number of trail detections (Dets.) (total = 722), number of trails (total = 187), and detections per day (Det./day) (total = 7.679) are shown for each highway segment with at least one observed trail. Segments with no trails are omitted. All road segments were surveyed by bike six times between July and December 2013 to locate new trails. String traps were checked weekly (19 times). The number of trail detections in each trail was divided by the number of days elapsed between the discovery of the trail and the end of the sampling effort to determine trail detections per day. Trail detections per day were then totaled for all trails within each 0.16-km segment.

Km	Mile	Dets.	Trails	Det./day	Km	Mile	Dets.	Trails	Det./day	Km	Mile	Dets.	Trails	Det./day
109.6	68.1	19	5	0.185	122.1	75.9	17	7	0.179	129.4	80.4	11	2	0.145
110.1	68.4	84	12	0.945	122.3	76	86	18	0.896	136.0	84.5	7	6	0.055
110.2	68.5	8	3	0.117	122.4	76.1	144	21	1.455	136.1	84.6	45	9	0.300
110.7	68.8	10	7	0.137	122.8	76.3	1	3	0.009	136.4	84.8	0	2	0
110.9	68.9	2	1	0.026	122.9	76.4	0	1	0	137.1	85.2	0	1	0
112.8	70.1	25	5	0.205	125.2	77.8	6	3	0.114	137.4	85.4	0	1	0
113.0	70.2	1	1	0.011	125.3	77.9	74	9	0.563	137.6	85.5	5	3	0.037
113.9	70.8	0	1	0	125.5	78	2	3	0.022	137.7	85.6	68	15	0.579
114.1	70.9	18	7	0.143	125.7	78.1	0	1	0	137.9	85.7	12	5	0.088
115.8	72	0	1	0	127.0	78.9	0	1	0	138.7	86.2	3	3	0.101
116.0	72.1	1	1	0.008	128.4	79.8	0	1	0	138.9	86.3	12	9	0.519
116.5	72.4	3	2	0.023	128.6	79.9	23	1	0.371	139.8	86.9	0	1	0
117.6	73.1	10	1	0.081	128.7	80	3	1	0.039	140.3	87.2	0	1	0
117.9	73.3	11	2	0.089	129.0	80.2	0	1	0	140.5	87.3	4	6	0.032
121.2	75.3	0	1	0	129.2	80.3	7	2	0.206					

Table 2-4. Statistically significant road crossing hotspots by road segment on Highway 20 between Sedro-Woolley and Concrete with detections per day, z value, and p value. To determine a hotspot, the local sum of a road segment and its neighbors within 0.16-km is compared proportionally to the sum of all segments. Local sums that are significantly different from the expected local sum are hotspots.

Hotspot	Mile	Local sum of detections/day	z value	p value
Hotspot 1				
km 110.1	68.4	1.062	6.01	<0.001
Hotspot 2				
km 122.1	75.9	1.075	3.71	<0.001
km 122.3	76	2.531	9.29	<0.001
km 122.4	76.1	2.352	8.61	<0.001
km 122.6	76.2	1.455	5.20	<0.001
Hotspot 3				
km 125.2	77.8	0.677	2.19	0.029
km 125.3	77.9	0.677	2.84	0.005
Hotspot 4				
km 137.7	85.6	0.703	2.79	0.005
km 137.9	85.7	0.667	2.15	0.032
Hotspot 5				
km 138.7	86.2	0.620	1.97	0.049
km 138.9	86.3	0.620	2.57	0.010
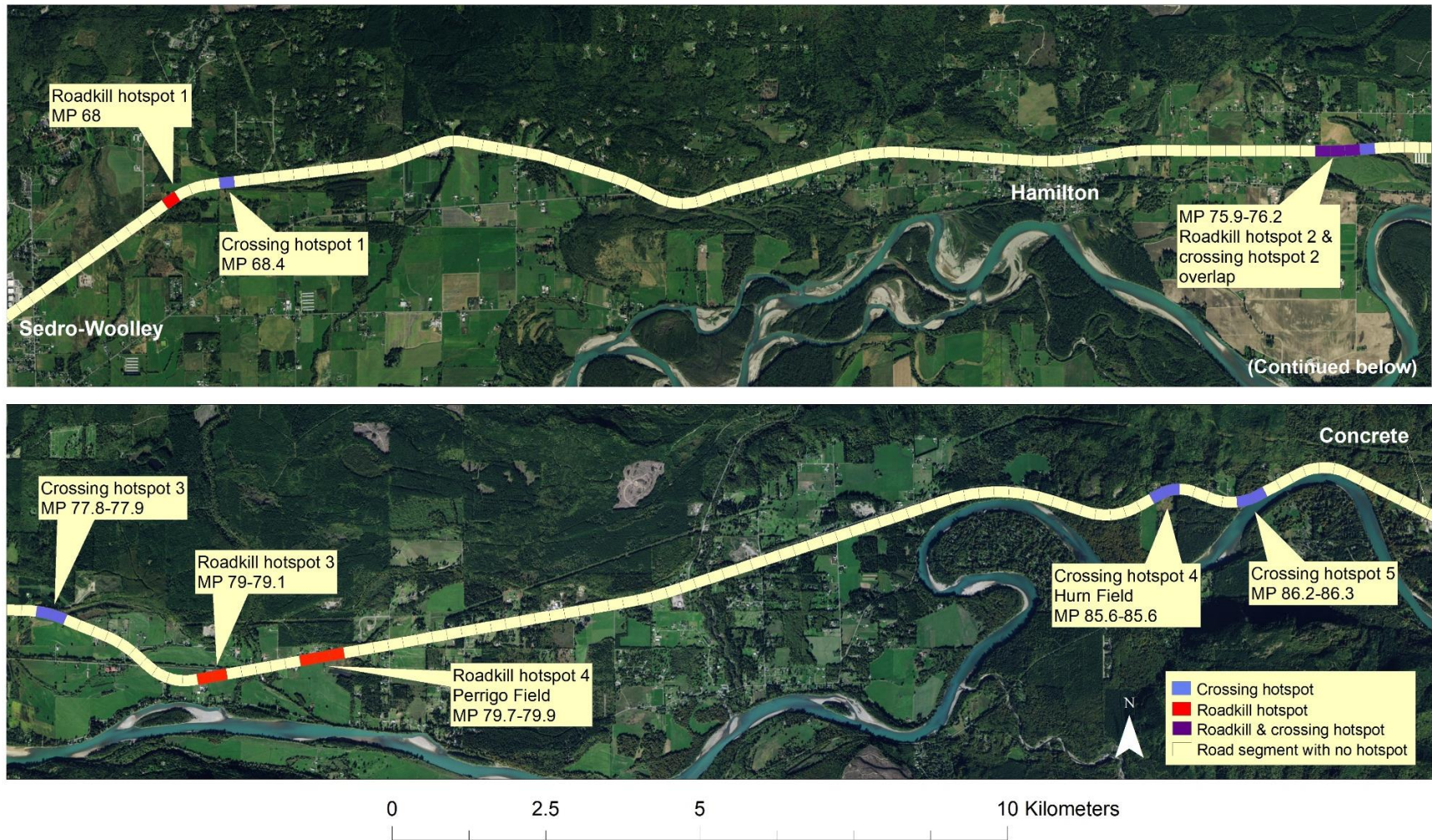
Figure 2-5. Roadkill and road crossing hotspots (p < 0.05) on Highway 20 between Sedro-Woolley and Concrete, Washington.
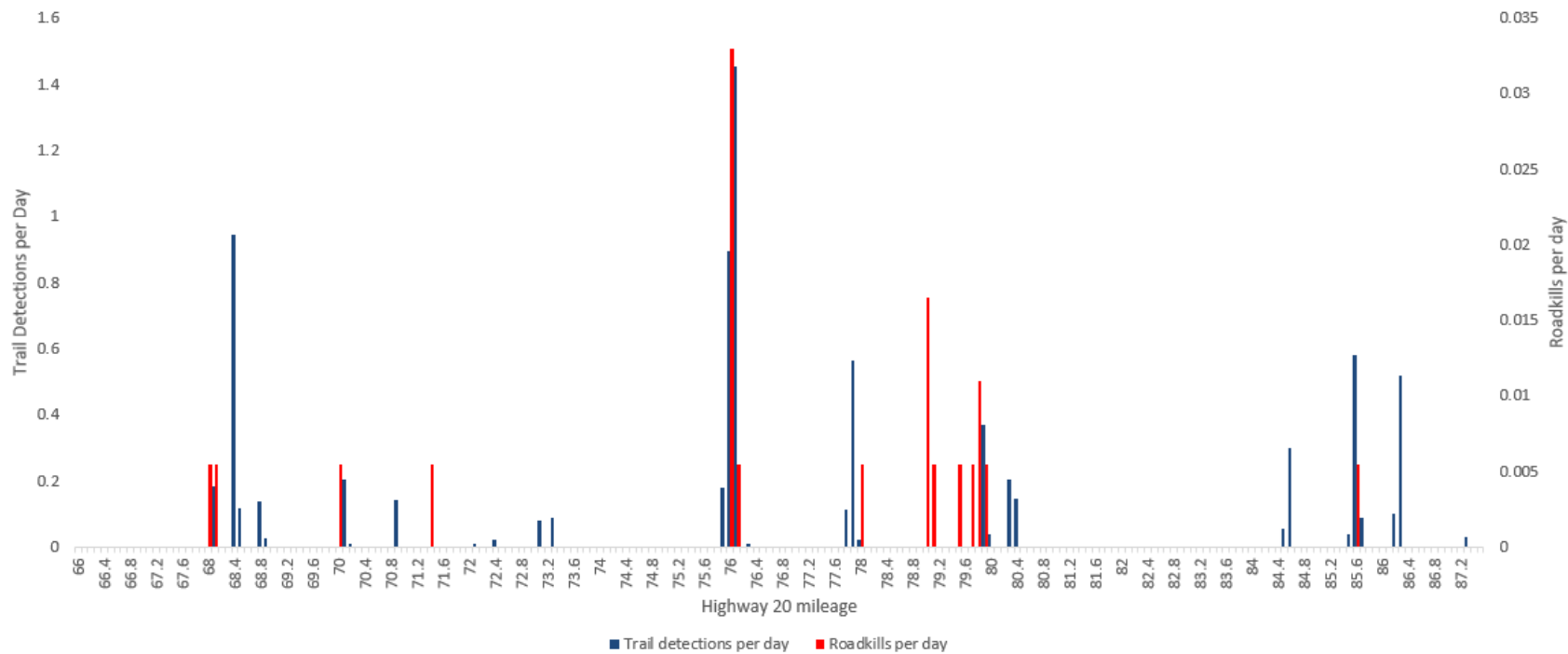
Figure 2-6. Trail detections per day and roadkills per day by mileage on Highway 20 between Sedro-Woolley to Concrete (7/2013 to 12/2013).

During the same time period (July - December 2013) elk roadkills were documented in 14 of 216 highway segments for a total of 22 roadkills (0.12 roadkills per day) (Figure 2-6). Four statistically significant roadkill hotspots were found: Hotspot 1) km 109.4 (mile 68); Hotspot 2) km 122.1 - 122.4 (mile 75.9 - 76.1); Hotspot 3) km 127.1 - 127.3 (mile 79 - 79.1); Hotspot 4) km 128.3 - 128.6 (mile 79.7 - 79.9) (Table 2-5). Roadkills per day and trail detections per day across the study area during this sampling period were correlated (Kendall's tau = 0.23, p < 0.001) (Figure 2-7).

Table 2-5. Statistically significant roadkill hotspots by road segment on Highway 20 between Sedro-Woolley and Concrete with roadkills per day, z value, and p value. To determine a hotspot, the local sum of a road segment and its neighbors within 0.16-km is compared proportionally to the sum of all segments. Local sums that are significantly different from the expected local sum are hotspots.

Hotspot	Mile	Local sum of roadkills/day	z value	p value
Hotspot 1				
km 109.4	68	0.011	2.47	0.014
Hotspot 2				
km 122.1	75.9	0.033	6.40	<0.001
km 122.3	76	0.038	7.53	<0.001
km 122.4	76.1	0.038	7.53	<0.001
Hotspot 3				
km 127.1	79	0.022	5.22	<0.001
km 127.3	79.1	0.022	4.15	<0.001
Hotspot 4				
km 128.3	79.7	0.016	3.03	0.002
km 128.4	79.8	0.022	4.15	<0.001
km 128.6	79.9	0.016	3.03	0.002
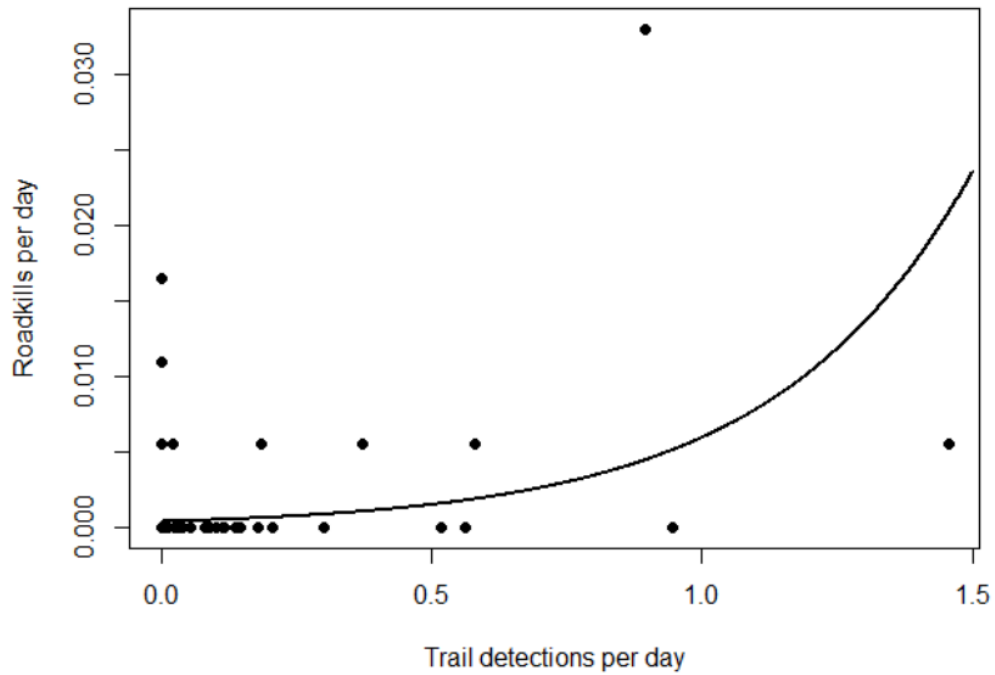
Figure 2-7. Quasipoisson regression of roadkills per day and trail detections per day between July 2013 and December 2013 (*P* < 0.001, Kendall's tau = 0.23)

Roadkill modeling

Ten habitat variables and four road variables were used to model elk roadkills between

January 2012 and January 2014 (Table 2-6). Elk roadkills were documented in 38 of 216

highway segments for a total of 103 roadkills (0.14 roadkills per day) (Figure 2-8).

Table 2-6. Summary statistics for model variables

Variables	Mean	Median	Range
Habitat variables			
% Developed (Medium/High)	10.8%	7.5%	3.5% - 82.7%
% Forest	33.4%	34.8%	0.1% - 64%
% Hay/pasture	24.2%	25.3%	1.2% - 45.8%
% Cultivated crops	1.7%	1.2%	0% - 10.1%
Forest edge length (m)	30,917	30,660	1,080 - 52,860
Distance to developed land (m)	517	404	0 - 2,137
Distance to nearest stream (m)	191	105	0 - 935
Distance to forest cover (m)	129	75	0 - 950
Distance to pasture/hay (m)	515	185	3 - 2,930
Distance to all crops (m)	437	155	3 - 2,477
Road variables			
Mean slope (degrees)	7.2	6	1.8 - 23.3
Guardrail length (m)	39.5	0	0 - 321.9
Traffic volume (AADT)	6,412	6,100	4,800 - 13,500
Sinuosity	0.991	0.998	0.93 - 1
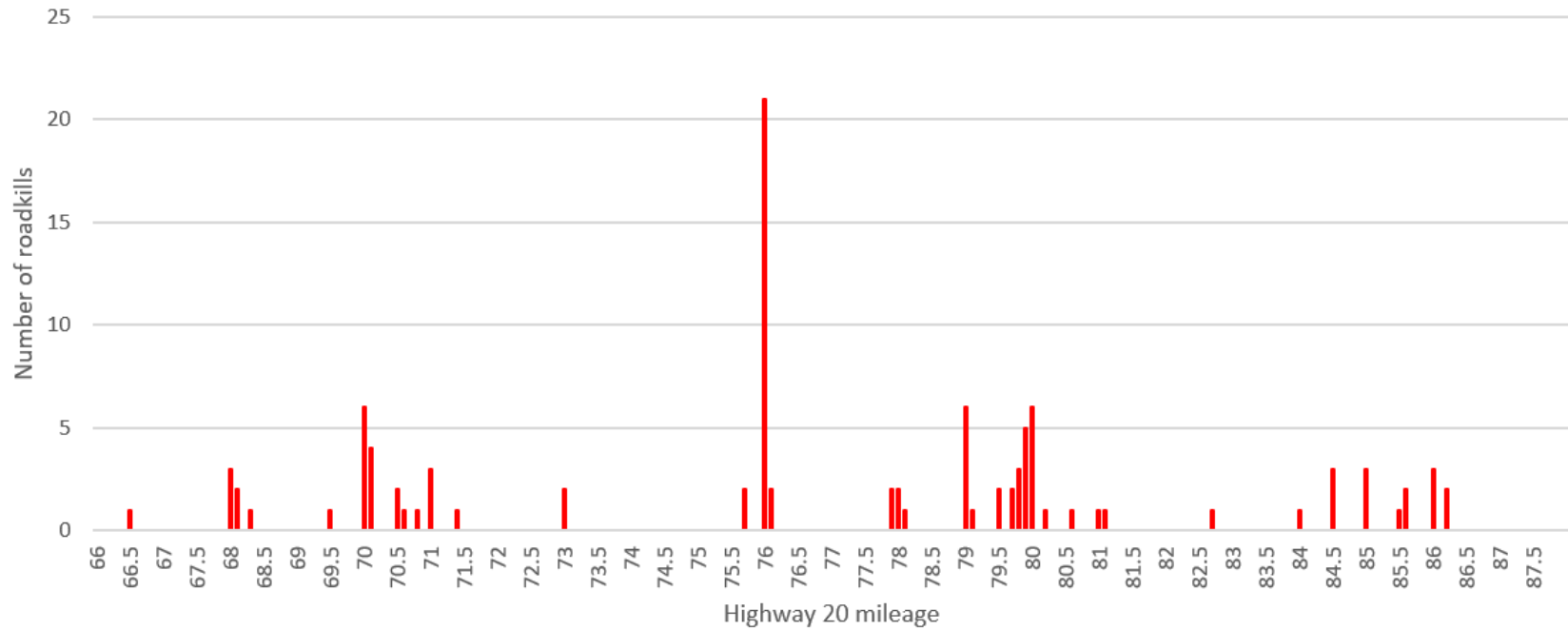
Figure 2-8. Elk roadkills reported by mileage on Highway 20 between Sedro-Woolley to Concrete (January 2012 to January 2014 (the time period used for modeling the presence /absence of elk roadkills at each road segment)) (n = 103).

Out of 17 candidate models, the best models (lowest AICc) from each category were

Distance to pasture/hay, Percent forest, Percent Developed area, and *Slope* (Table 2-7). All

subsets of the best models were tested *post hoc* to determine a confidence set of three models

(bolded in Table 2-7). Model-averaged parameter estimates indicate that the probability of an elk

roadkill increases with decreased distance to pasture/hay, increased percent forest, decreased

percent developed area, and increased guardrail length (Table 2-8). The Hosmer and Lemeshow

goodness-of-fit test for binary models showed that the global model in the confidence set is

correctly specified with no evidence of a poor fit, indicating the same for all confidence set

models ($X^2 = 4.304$, $df = 8$, $P = 0.829$) (Symonds and Moussalli 2011). Predictors with the

highest relative importance values were *Distance to pasture/hay* ($RI = 1.00$) and *Percent forest*

($RI = 1.00$) (Table 2-8). Figures 2-9 and 2-10 show predicted probabilities of the most important

predictors.

Table 2-7. Candidate models for North Cascades elk roadkills (Jan. 2012 – Jan. 2014) considered *a priori* with AICc, ΔAICc (difference from lowest AICc score in each category), pseudo-R², and Akaike weight (*wᵢ*). Best model combinations were determined *post hoc* from candidate models with the lowest AICc score in each category (italics). A confidence set (bold) included models with cumulative Akaike weights < 0.95.

Models by category	AICc	ΔAICc	Pseudo-R^2	w_i
Natural plant communities				
% Forest (1 km)	*202.9*	0	0.011	0.242
Distance to forest	203.03	0.13	0.010	0.227
Distance to streams	203.66	0.76	0.007	0.166
Distance to forest + Distance to streams	204.38	1.48	0.013	0.116
Distance to forest + Forest edge (1 km)	204.59	1.69	0.012	0.104
Forest edge (1 km)	204.96	2.06	0.000	0.086
Distance to streams + Forest edge (1 km)	205.71	2.81	0.007	0.059
Agriculture				
Distance to hay/pasture	*201.78*	0	0.016	0.530
Distance to crops	202.64	0.86	0.012	0.346
% Cultivated area (1 km)	204.69	2.91	0.002	0.124
Developed land				
% Developed area (1 km)	*202.23*	0	0.014	0.763
Distance to developed land	204.58	2.35	0.002	0.237
Highway characteristics				
Roadside slope	204.25	0	0.004	0.286
Sinuosity	204.58	0.33	0.002	0.243
Traffic volume	204.71	0.46	0.002	0.228
Guardrails	*204.81*	0.56	0.001	0.217
Roadside slope + Traffic + Guardrails + Sinuosity	209.07	4.82	0.011	0.026
Best models combined (post hoc)				
% Forest + Distance to hay/pasture + Slope	**193.45**	0	0.078	0.465
% Forest + Distance to hay/pasture	**194.86**	1.41	0.061	0.230
% Forest + Dist. to hay/pasture + % Dev. area + Slope	**195.38**	1.93	0.079	0.177
% Forest + Distance to hay/pasture + % Dev area	196.86	3.41	0.061	0.085
Distance to hay/pasture + % Developed area	200.44	6.99	0.033	0.014
Distance to hay/pasture + % Developed area + Slope	201.27	7.82	0.039	0.009
% Developed area + Slope	202.3	8.85	0.024	0.006
% Forest + Slope	202.67	9.22	0.022	0.005
% Forest + % Developed area + Slope	203.13	9.68	0.030	0.004
Distance to hay/pasture + Slope	203.56	10.11	0.017	0.003
% Forest + % Developed area	203.78	10.33	0.016	0.003

*Pseudo-R^2 is only comparative to other models

Table 2-8. Model-averaged parameter estimates from three elk roadkill models in the 0.95 confidence set with unconditional standard error (SE), 95% confidence interval (CI), p values, and relative importance (RI) (sum of Akaike weights over all models in which the variable appears).

Variable	Estimate	SE	95% CI	RI
(Intercept)	-2.559	0.683	-3.905 to -1.212	NA
Distance to pasture/hay	-0.001	0.000	1.832 - 9.847	*1.00*
% Forest (1 km)	5.840	2.034	-0.002 to -0.0003	*1.00*
Slope	-0.065	0.058	-0.187 - 0.011	0.74
% Developed area (1 km)	-0.268	1.627	-8.044 - 5.403	0.20
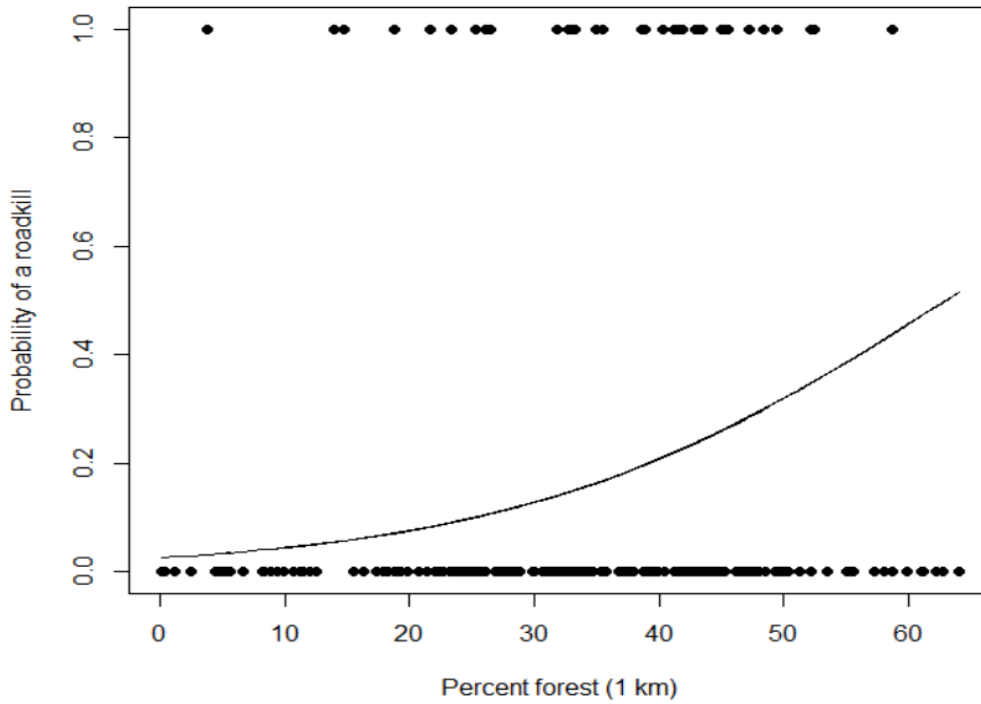
Figure 2-9. Predicted probability of a roadkill as a function of *Percent forest* in 1 km radius estimated by model-averaged parameters from the confidence set.
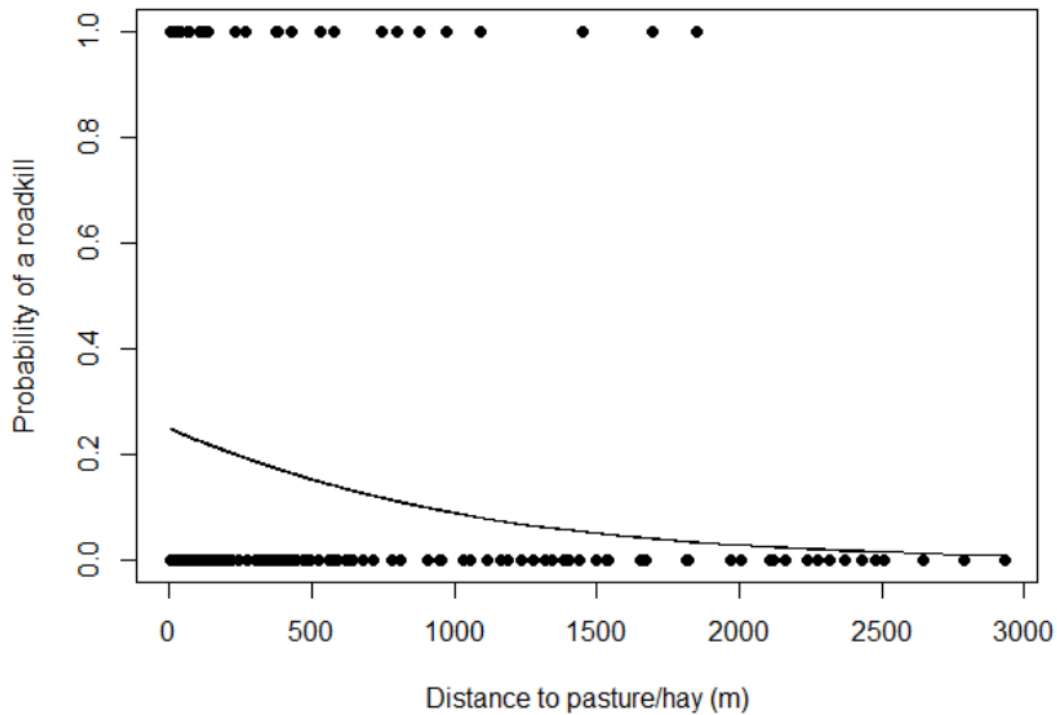
Figure 2-10. Predicted probability of a roadkill as a function of *Distance to pasture/hay* estimated by model-averaged parameters from the confidence set.

Road crossings modeling

From 17 candidate models, the best models (lowest AICc) from each category were

Distance to forest + Distance to streams, Distance to crops, % Developed area, and *Guardrails*

(Table 2-9). All subsets of the best models were tested *post hoc* to determine a confidence set of

four models (bolded in Table 2-9). Model-averaged parameter estimates indicated that the

probability of a road crossing decreases with increased distance to forest, increased distance to

streams, increased distance to crops, increased percent developed area, and increased guardrail

length (Table 2-10). The Hosmer and Lemeshow goodness-of-fit test for binary models showed

that the global model in the confidence set is correctly specified with no evidence of a poor fit,

indicating the same for all confidence set models ($X^2 = 5.366$, $df = 8$, $P = 0.718$) (Symonds and

Moussalli 2011). Road crossing predictors with the highest relative importance values in the

model-averaged model were *Distance to forest* (*RI* = 1.00), *Distance to crops* (*RI* = 1.00),

Distance to streams (*RI* = 1.00); however, confidence intervals for *Distance to streams* contained

zero, suggesting a lack of statistical influence (Table 2-10). Figures 2-11 and 2-12 show

predicted probabilities of the most important predictors.

Table 2-9. Candidate models for North Cascades elk road crossings considered a priori with AICc, ΔAICc (difference from lowest AICc score in each category), Pseudo-R², and Akaike weight (w_i). Best model combinations were determined post hoc from candidate models with the lowest AICc score in each category (italics). A confidence set of best models (bold) included models with cumulative Akaike weights below 0.95.

Models by category	AICc	ΔAICc	Pseudo-R²	w_i
Natural plant communities				
Distance to forest + Distance to streams	*165.9*	0.00	0.206	0.894
Distance to forest	171.64	5.74	0.168	0.051
Distance to streams	173.37	7.47	0.159	0.021
Distance to forest + Forest edge (1 km)	173.67	7.77	0.168	0.018
Distance to streams + Forest edge (1 km)	174.37	8.47	0.164	0.013
% Forest (1 km)	179.03	13.13	0.131	0.001
Forest edge (1 km)	179.56	13.66	0.128	0.001
Agriculture				
Distance to crops	*178.23*	0.00	0.135	0.647
Distance to hay/pasture	180.23	2.00	0.125	0.238
% Cultivated land (1 km)	181.69	3.46	0.118	0.115
Developed land				
% developed area (1 km)	*178.11*	0.00	0.136	0.764
Distance to developed land	180.46	2.35	0.124	0.236
Highway characteristics				
Guardrails	*179.4*	0.00	0.129	0.437
Sinuosity	180.82	1.42	0.122	0.214
Traffic volume	181.58	2.18	0.118	0.147
Roadside slope	181.73	2.33	0.118	0.136
Roadside slope + Traffic + Guardrails + Sinuosity	183.17	3.77	0.141	0.066
Best models combined (post hoc)				
Dist. to forest + Dist. to streams + Dist. to crops + % Dev.+Guardrails	**160.18**	0.00	0.266	0.384
Dist. to forest + Dist. to streams + Dist. to crops + Guardrails	**161.28**	1.10	0.250	0.221
Dist. to forest + Dist. to streams + Dist. to crops	**161.55**	1.37	0.238	0.193
Dist. to forest + Dist. to streams + Dist. to crops + % Developed area	**162.04**	1.86	0.246	0.151
Dist. to forest + Dist. to streams + % Developed area + Guardrails	164.97	4.79	0.232	0.035
Dist. to forest + Dist. to streams + % Developed area	166.84	6.66	0.212	0.014
Dist. to forest + Dist. to streams + Guardrails	170.33	10.15	0.184	0.002
Distance to crops + % Developed area + Guardrails	174.31	14.13	0.175	0
Distance to crops + % Developed area	175.89	15.71	0.157	0
Distance to crops + Guardrails	178.06	17.88	0.146	0
Guardrails + % Developed area	178.11	17.93	0.136	0

*pseudo-R2 is only comparative to other models

Table 2-10. Model-averaged parameter estimates from four road crossing models in the 0.95 confidence set with unconditional standard error (SE), 95% confidence interval (CI), p values, and relative importance (RI) (sum of Akaike weights over all models in which the variable appears).

Variable	Estimate	SE	95% CI	RI
(Intercept)	0.720	0.786	-0.827 - 2.266	NA
Distance to forest	-0.012	0.004	-0.020 to -0.004	*1.00*
Distance to streams	-0.003	0.002	-0.0066 - 0.0002	*1.00*
Distance to crops	-0.001	0.001	-0.0023 to -0.0001	*1.00*
% Developed area	-5.496	6.912	-22.713 - 3.210	0.64
Guardrails	-0.004	0.004	-0.014 - 0.002	0.56
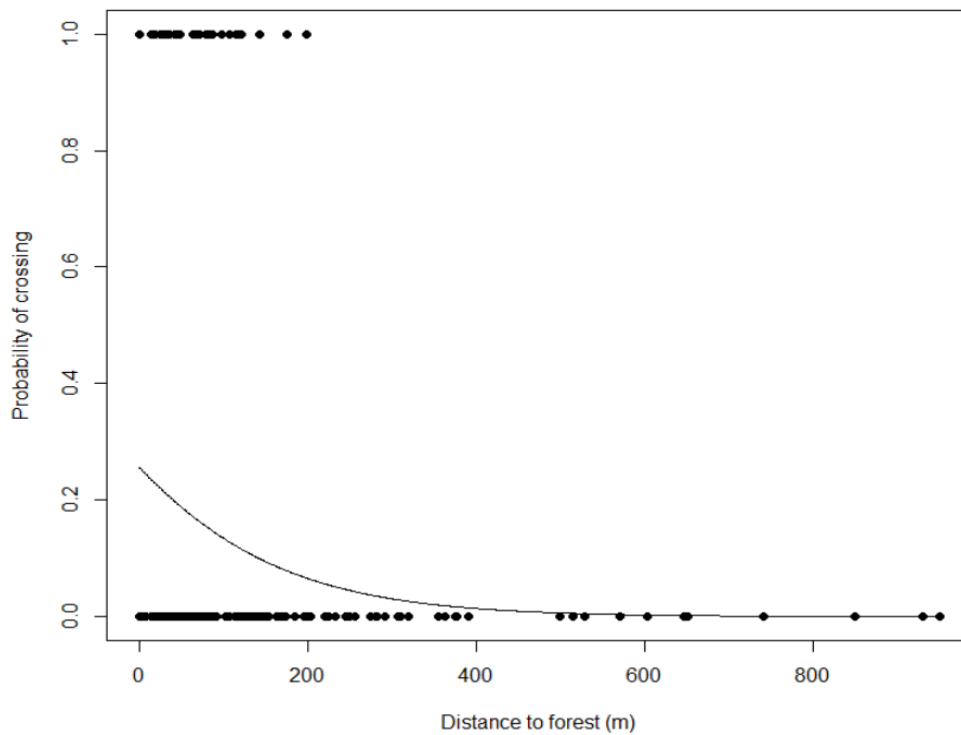
Figure 2-11. Predicted probability of a road crossing as a function of *Distance to forest* estimated by model-averaged parameters from the confidence set.
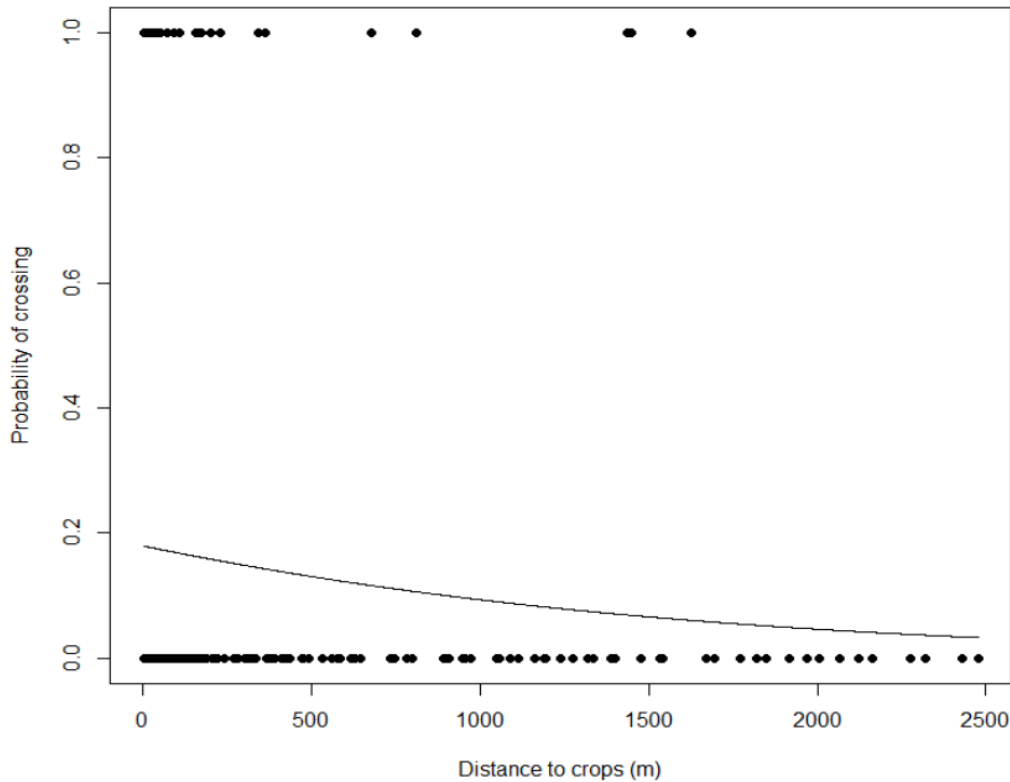
Figure 2-12. Predicted probability of a road crossing as a function of *Distance to crops* estimated by model-averaged parameters from the confidence set

Discussion

Elk roadkills and road crossings on Highway 20 in Skagit Valley showed wide spatial

variation across the 34.8-km study area between Sedro-Woolley and Concrete. Roadkill and

crossing locations were weakly correlated (Kendall's tau = 0.23, $P < 0.001$) and this was

reflected in the differential spatial distribution between road crossing and roadkill hotspots

(Figures 2-5 and 2-6). Roadkill hotspot 1 (km 109.4 (mile 68)) was 0.7 km from the nearest

crossing hotspot. Similarly, roadkill hotspot 3 (km 127.1 - 127.3 (mile 79 - 79.1)) was 1.1 km

from the nearest crossing hotspot, and roadkill hotspot 4 (km 128.3 - 128.6 (mile 79.7 - 79.9))

was 1.8 km from the nearest crossing hotspot. This spatial separation between roadkill and road

crossing hotspots suggests that the roadkill hotspots could be more dangerous crossing locations

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than the nearby crossing hotspots. Roadkill hotspot 2 (km 122.1- 122.4 (mile 75.9 - 76.1))

completely overlaps crossing hotspot 2 (km 122.1 - 122.6 (mile 75.9 - 76.2)). These spatial

patterns may indicate the need for very different mitigation strategies (see Management

Implications below).

Modeling landscape and road factors showed that roadkill locations have a negative

association with *Distance to pasture/hay* (*RI* = 1.00)) and a positive association with *Percent*

forest (*RI* = 1.00). Road crossing locations have a negative association with *Distance to forest*

(*RI* = 1.00) and *Distance to crops* (*RI* = 1.00), and have a weaker negative association with

decreased *Distance to streams* (*RI* = 1.00).

Roadkill model results

In the averaged roadkill model, *Distance to pasture/hay* and *Percent forest* were

important predictors (Table 2-8). These modeling results indicate that elk were more likely to be

killed by a vehicle as the distance to pasture/hay decreases and as percent forest in a 1-km radius

increases. In other words, roadkill risk may increase when pasture/hay is closer to the road and

percent forest increases.

Pasture and hay fields are sources of forage that attract elk and may motivate them to

cross roads to reach them (Gagnon et al. 2007, Meisingset et al. 2014). In this way, the

relationship between roadkills and pasture/hay may simply reflect a disproportionate use of this

habitat compared to other land covers. This is somewhat consistent with the crossing model,

which showed a significant negative association with *Distance to all crops* and *Distance to forest*

(see below for further discussion). The *Distance to pasture/hay and Distance to crops* variables

were highly correlated (Kendall's Tau = 0.91), which may confound a clear comparison between

these models (these correlated variables were never used in the same model). This degree of comparison between the roadkill and crossing models may also be confounded by the difference in accuracy and methodology of the response variables (documented roadkills v. trail detections per day).

The roadkill – pasture/hay relationship is somewhat unexpected since visibility should improve in open land covers. Sight distance likely increases when pasture/hay abuts the road as compared to forest cover, which could obscure the sight distance of both driver and elk. Increased visibility in open areas has been shown to decrease collisions with other ungulate species in other areas (Bashore et al. 1985, Nielsen et al. 2003, Seiler 2005). Since most roadkills on Highway 20 occur in low-light hours (dawn, dusk, night) (Fenner Yarborough, WDFW biologist, pers. comm.), however, the visibility advantage in open areas is greatly reduced. At the same time, elk may see headlights even from within forest cover, which would increase the sight distance and further reduce the visibility advantage of an open habitat. In addition, elk tend to use forest cover more intensely in areas near roads (Hurley and Sargeant 1991, Prokopenko et al. 2017). The lack of protective forest cover in pasture/hay habitat near the road may prevent elk from waiting near the road for cars to pass for a safer crossing, as has been observed in forested habitats on remote cameras on Highway 20 during this study. This could increase the probability of a collision in these open areas.

Previous studies show a similar relationship between open areas and ungulate-vehicle collisions. In Norway, red deer collision risk also increased with proximity to pasture as well as percent forest cover near the road (Meisingset et al. 2014). Hubbard et al. (2000) found that deer-vehicle collisions in Iowa increased with the size of grass patches in an 800-meter radius from the road. Tappe and Enderle (2007) found that deer collision locations were more likely with a

higher density of pasture and crop patches within 1200 m of a highway. In Sweden, Seiler (2005) found that moose-vehicle collisions increased with the proportion of open area in a 500-meter radius from the road. Though these are measures of area and not proximity, the mean distance from the mid-point of road segments on Highway 20 to pasture/hay was 515 meters, suggesting that the relationship in this study may be present at a scale comparable to previous studies (Table 2-6).

Percent forest within a 1-km radius was an important positive predictor of roadkill locations (*RI* = 1.00). This result is consistent with red deer collision studies in Spain and Norway (Malo et al. 2004, Meisingset et al. 2014), as well as deer collision research in Western Washington (Myers et al. 2008). A concurrent effect of pasture and forest cover was also seen with red deer collisions in Norway (Meisingset et al. 2014) and deer collisions in Arkansas (Tappe and Enderle 2007). This suggests that effects on roadkill locations from both open and forested habitat are not mutually exclusive, and their concurrence may be an indicator of the importance of land cover diversity and/or forest edge habitat. Forest edge habitat is particularly important for elk due to the close proximity of food and protective cover. Behavioral research has also shown that elk select areas near roads if vegetation and topography obscure visibility to the road (Lyon 1979, Edge and Marcum 1991, Rowland et al. 2005, Montgomery et al. 2012). This could explain the concurrent effects of pasture and forest on roadkills.

A positive relationship between land cover diversity and ungulate collisions is seen throughout the literature (Seiler 2005, Bashore et al.1985, Finder et al., 1999, Gunson et al. 2009, Malo et al. 2004, Nielsen et al. 2003, Puglisi et al.1974, Tappe and Enderle 2007). However, the methods used to measure land cover diversity are not consistent and include forest edge length, Shannon diversity index, density of forest patches, forest patch shape index, and ratio of open

and forested areas. In Skagit Valley, the *Forest edge* variable (length of forest edge within a 1-km radius) was used to quantify the distribution of open and forested habitats; however, *Forest edge* did not appear in the best roadkill or crossing models. The 1-km scale of this variable may have been too coarse to detect an effect closer to the road. Additional metrics of land cover diversity may have shown a different result. A better understanding of which measure of land cover diversity is most relevant to elk ecology and road ecology could be an area of future research.

The relationship between open and forested areas and roadkills appears to be inconsistent across other collision studies. Myers et al. (2008) found that herbaceous cover in Western Washington was negatively associated with deer-vehicle collisions while roadside cover was positively associated with them. This contrasts with the results in Skagit Valley and may be due to variation in the ungulate response to roads by species. Long et al. (2012) found a positive relationship between distance to forest and elk collisions, while Malo et al. (2004) found the opposite. These inconsistent findings across the literature suggest that the relationship between roadkill locations and open and forested habitats may be specific to a given study area. This echoes Gunson et al.'s (2011) recommendation for road-by-road and species-specific modeling to determine local roadkill factors and inform local management strategies when results over a broader geographic area are inconsistent. In Skagit Valley, elk movement patterns at a larger spatial scale (>1 km) may also influence roadkill and crossing patterns (WDFW 2017). This may be true in other areas where near-road factors appear to be inconsistent with other areas.

Road crossing model results

In the averaged road crossing model, *Distance to forest*, *Distance to crops,* and *Distance to streams* were negative predictors (Table 2-8). Relative importance values were equally high for *Distance to forest (RI =* 1.00*), Distance to crops* (*RI =* 1.00), and *Distance to streams* (*RI =* 1.00) because all models in the confidence set included these variables; however, the 95% confidence intervals for *Distance to streams* contained zero, discounting this variable (Table 2-10). These results suggest that elk may be more likely to cross the highway as the distance to forest and crops decreases.

Elk use forest cover for protection and may prefer to cross a road when they can approach it in the safety of forest cover. Elk are known to use forest cover more intensely in areas near roads (Hurley and Sargeant 1991, Propenko et al. 2017). This is consistent with observations from remote cameras on Highway 20 during this study in which elk were observed waiting near the road in forest cover as cars passed. Relatively few road ecology studies focus on road crossings rather than roadkills, likely due to the relative difficulty of documenting crossings. In Norway, red deer road crossings increased with percent forest cover near the road, as well as with proximity to pasture – another example of concurrent pasture-forest effect seen in collision studies and discussed above (Meisingset et al. 2013). A similar relationship between road crossings and forest was seen with moose, which crossed roads more frequently with proximity to forest and increased forest area (Becker 2011, Barnam et al. 2007).

Elk are also attracted to agricultural crops in Skagit Valley, which may result in more road crossings near these habitats (WDFW 2017). This relationship is less clear when comparing similar studies. Tappe and Enderle (2007) found that deer collision locations were more likely

with a higher density of pasture and crop patches within 1200 meters of a highway. Conversely, Malo et al. (2004) found a decrease in elk collisions associated with higher proportion of agricultural area at a similar scale. Seiler et al. (2005) saw similar results with moose in Sweden.

The effect of *Distance to crops* on road crossings may also reflect a more general association with open areas that provide forage since most crops in Skagit Valley are low to the ground for most of the year. This relationship is also seen in the literature. In Arizona, elk were more likely to cross the road near riparian meadows (Dodd et al. 2007, Gagnon et al. 2007). This habitat is similar to agricultural fields in Skagit Valley in that it provides forage in an open area. Barnam et al. (2007) also found that deer crossings increase with the presence of open cover types.

The high relative importance value of *Distance to streams* (*RI* = 1.00) in Skagit Valley is also consistent with the findings in Arizona. This result is expected since elk are known to travel along drainages (Kie et al. 2005). This effect is documented in collision studies as well (Tappe and Enderle 2007).

Comparison of roadkill and road crossing models

The difference in predictors between the model-averaged roadkill and road crossing models suggests that different landscape factors may influence roadkill probability compared to road crossing probability. Both roadkill and road crossing model-averaged models had the predictor *% Developed area* in common, though it wasn't influential. *Distance to crops* (*RI* = 1.00) , *Distance to forest* (*RI* = 1.00), *Distance to streams* (*RI* = 1.00), and *Guardrails* (*RI* = 0.56) were unique to the model-averaged road crossings model. *Distance to pasture/hay* (*RI* = 1.00), *Percent forest* (*RI* = 1.00), and *Slope* (*RI* = 0.74) were the unique to the model-averaged

roadkill model. These differences are most likely explained by the disparity in location accuracy and methodology between response variables (roadkills v. trail detections per day). A general association between open land covers that provide forage and both roadkills and road crossings appears to exist. Further research with more accurate roadkill locations and road crossing data that is more comparable to roadkill data may help clarify this relationship.

Relative abundance

An important limitation of this study is the lack of relative elk abundance data across the study area. WDFW produces annual population estimates of the North Cascades elk herd across its core range from a mark-resight model but these estimates cannot be parsed into discrete areas (WDFW 2017). WDFW biologists were not able to provide estimates of variation in abundance in different parts of the valley (Fenner Yarborough, WDFW biologist, pers. comm.) The distribution of elk across the study area likely influences the number and location of road crossings and collisions. Myers et al. (2009) found that deer concentration levels had a significant positive influence on collision counts in a rural setting. The absence of this data in the modeling process may contribute to the lack of a single, dominant, explanatory model in both the road crossing and roadkill confidence sets.

Spatial autocorrelation

Spatial autocorrelation occurs when nearby observations are more similar than distant observations. This can potentially violate independence assumptions of regression models. There is an ongoing discussion in the ecological literature about how much spatial autocorrelation actually biases results with some authors claiming that it does (Beale 2007, Kuhn and Dormann 2012) and others claiming that it needn't cause concern (Hawkins et al. 2007, Hawkins 2012).

Unpublished data simulations testing the effect of spatial autocorrelation on linear models show that the slope of a linear model can become inefficient with relatively high spatial autocorrelation but bias is not reported (Andy Bunn, WWU professor, pers. comm.). This discussion focuses on ordinary least squares regression and has only recently moved to spatial autocorrelation in generalized linear models, and what researchers can do about it (Saas et al. 2014). Unfortunately, suggested approaches within the scope of this study were not effective with binary response data. For these reasons, spatial autocorrelation was not explicitly incorporated in the modeling process.

Data limitations

String traps only showed presence/absence of an unknown species on monitored trails in a given week unless tracks could be found near the trail to confirm use by elk. Additional information such as time of day, date, number of elk crossing, number of crossing events, and number of animals in each crossing event would make it easier to compare road crossing data with roadkill data. String trap data also has some inherent bias toward existing trails. Dispersed crossing areas were more difficult to accurately sample unless there was a good substrate for documenting tracks. Spreading sand on road shoulders in open areas and near trails could improve track observations. Bike surveys can also be potentially dangerous due to high vehicle speeds, heavy traffic, and narrow road shoulders.

Remote camera detection of road crossings provides richer data but is significantly more expensive and has its own limitations. Detecting more dispersed crossings and crossings in open fields lacking trails remains a challenge given a lack of hidden camera locations, large area, and traffic-triggered cameras.

Starting in 2013, the Tulalip and Stillaguamish tribes collared up to nine elk in the Skagit

Valley with GPS collars. Adequate GPS collar data were not available for this study but could be

used in the future to better understand road crossing behavior. Using GPS collar data to

document road crossings has its own challenges, however, including the relatively low frequency

of recorded coordinates that can preclude accurately locating a road crossing. Increasing the

frequency of recorded coordinates would help but this results in reduced battery life and greater

expense. One potential solution is to install transmitters near the road that can trigger GPS collars

to increase the frequency of recorded locations when an elk is in range (Kelly McAllister,

WDFW biologist, pers. comm.). Another limitation is the inability to document the number of

elk that crossed with the collared individual.

Roadkill data had limited accuracy due to the subjective nature of location descriptions.

Including GPS coordinates with agency roadkill reporting would greatly improve roadkill

location accuracy and potentially clarify modeling results. In late 2013, the Washington

Department of Transportation installed 0.1-mile (0.16-km) mile markers near milepost 76 (km

122.3) in an effort to improve roadkill location reporting accuracy. A program to improve the

accuracy of roadkill documentation on Interstate 90 using GPS data collectors that automatically

upload data to a central electronic data repository helped researchers compile baseline data and

aid mitigation and monitoring efforts across multiple government agencies (Ament et al. 2011).

In the same project, a citizen outreach program took advantage of motorist observations of

wildlife (Long et al 2012); widespread handheld mobile technology may provide more

opportunities to crowd-source wildlife observations. However, distracted driving could create

additional hazards. Apps could be designed to only record observations when stationary. Users

should be instructed to zoom in on a location map to a scale that allows for accurate recording of

locations. Expanding coordination between stakeholders through such programs will improve research outcomes and inform road and wildlife management.

Management implications

This study and numerous others cited herein have shown that elk-vehicle crossings and collisions are not random, are spatially heterogenous, and are associated with particular land cover and road characteristics. For this reason, wildlife and transportation managers should consider these factors when planning road construction and mitigating wildlife-vehicle collisions. Skagit Valley is distinct in its concentration of private property around the highway, which is an important consideration when evaluating management strategies. The results of this study should be considered preliminary and should be interpreted with caution in terms of management applications given the low accuracy of roadkill locations and the limitations inherent in comparing hotspots from two datasets with varying accuracy and distinct data collection methodologies. Despite these limitations, this study presents an approach that could be used to guide the selection of alternative mitigation strategies.

Elk are clearly attracted to open areas that provide forage (pasture/hay and crop land covers) in Skagit Valley, which exist in the valley bottom near roads and population centers. In an effort to keep elk above the valley bottom, biologists at the Tulalip and Stillaguamish tribes have created habitat enhancement plots by removing stumps from old clearcuts and planting nutritious, non-native forage plants such as clover. Elk are regularly documented in these fields but whether they are diverted from the valley bottom is unclear. Recent use of GPS collars may help clarify this issue. Rowland et al. (2005) also suggest that forest road closures may allow elk to remain on public land and away from private land (and potentially busier highways).

In 2014, transportation managers installed nine flashing elk crossing signs in the Highway 20 study area that were followed by a decrease in annual reported elk roadkills. According to WDFW, annual reported elk roadkills have remained below about 30 since then, which is about half of the maximum annual roadkills recorded in 2012 ($n = 62$) (WDFW 2017). A causal relationship can't be confirmed, but flashing signs likely raise driver awareness, especially at night, dawn, and dusk when most roadkills occur. Despite the widespread use of wildlife crossing signs, there is little empirical evidence that they actually work to prevent wildlife-vehicle collisions; their use is likely influenced by financial considerations since they are considerably less expensive than fencing or crossing structures (Glista et al. 2009). Temporary warning signs were shown to reduce deer-vehicle collisions by 50 percent and reduce vehicle speeds in Utah, Nevada, and Idaho; however, the effectiveness of the signs in reducing speeds declined in the second year of the study, suggesting that drivers may ignore warning signs over time (Sullivan et al. 2004). Flashing signs triggered by a heat sensor that detects animal presence have reduced collisions in Europe (Bank 2002). Animal-triggered electronic signs were found to be most likely to cause drivers to reduce speeds according to driver surveys in Australia (Bond 2013). Electronic warning signs activated by the signal from radio-collared elk have been used near Sequim, Washington with mixed results. Uncollared elk can still cross the road without triggering the sign, which could be problematic if drivers are expecting a warning whenever an elk is present. In addition, collared elk can bed down near the highway and continually trigger the sign. Additional signage may help reduce roadkills on Highway 20 near roadkill and crossing hotspots, especially near km 122.3 (mile 76) where roadkill hotspot 2 and crossing hotspot 2 coincide.

Lower speed limits are another common and cost-effective mitigation strategy. High

speed limits have been shown to correspond with more animal collisions in Washington State

and elsewhere (Wang et al. 2010, Bashore et al. 1985, Seiler 2005, Meisingset et al. 2014).

Enforcement of lower speed limits in problem areas may help prevent elk-vehicle collisions.

Lower speed limits may help reduce roadkills on Highway 20 near roadkill and crossing

hotspots, especially near km 122.3 (mile 76) where roadkill hotspot 2 and crossing hotspot 2

coincide (Figures 2-5 and 2-6).

Another strategy discussed in the literature is the removal of vegetative cover near

roadways to improve visibility. Meisingset et al. (2014) tested the effect of vegetation removal

on red deer -vehicle collisions in Norway and found a 53% decrease, but only in winter. This

strategy has also prevented collisions in Europe in conjunction with wildlife crossing structures

(Banks 2002). Behavioral researchers studying deer response to roads have recommended this

strategy as well (Blackwell et al. 2014). Removal of edible vegetation also reduces habitat near

roads and decreases the number of potential crossings (Olsson 2007). These strategies should be

considered cautiously in Skagit Valley given the association with open areas like pasture/hay and

crop land covers with roadkills and crossings. Vegetation removal could also be an issue for

private landowners. Montgomery et al. (2012) recommend retaining vegetation that screen elk

from road networks in areas to improve elk habitat.

Fencing has also been used to mitigate wildlife road crossings. Clevenger et al. (2001)

found that ungulate-vehicle collisions decreased by 80 percent after the installation of highway

mitigation fencing. Ascensao et al. (2013) report that strategically placed fencing alone, even

without nearby crossing structures, may be the most effective and cost-effective option for

mitigating road impacts on wildlife. The spatial separation of Highway 20 crossing hotspots from

roadkill hotspot 1 (km 109.4 (mile 68)), roadkill hotspot 3 (km 127.1-127.3 (mile 79-79.1)), and

roadkill hotspot 4 (km 128.3-128.6 (mile 79.7-79.9)) suggests that these locations could

potentially be mitigated by fencing that may divert elk to safer crossing locations nearby. This

strategy would require further research into elk movement patterns at each of these sites, how

those patterns might be affected by fencing barriers, and how those changes might affect

collision risk. The concentration of private property along Highway 20 could complicate a

fencing strategy if it diverted elk to private property.

Fencing is often used in conjunction with wildlife crossing structures such as overpasses

or underpasses to funnel wildlife toward crossing structures (Glista et al. 2009). Crossing

structures have been successful in substantially reducing roadkills (Clevenger et al. 2001,

Langbein et al. 2011). A lack of research comparing crossing rates before and after construction

of structures hinders the robust evaluation of their efficacy but structures appear to be more

effective at reducing collisions and reconnecting habitat and populations than cheaper and more

widely used approaches such as signage (Glista et al. 2009). A crossing structure over or under

Highway 20 may help reduce elk roadkills; however, its location would be limited to public land

and/or willing private landowners. Based on the hotspot analysis, a crossing structure may be

most effective near km 122.3 (mile 76) where roadkill hotspot 2 and crossing hotspot 2 coincide.

This location abuts private land and construction of a crossing structure would require substantial

landowner approval and participation, or a change of land ownership. Alternatively, a nearby

bridge on Highway 20 over Red Cabin Creek could conceivably be raised to accommodate elk

passage in conjunction with fencing, though elk show a strong preference for overpasses

compared to underpasses (Clevenger 2009). Both of these options would require substantial

capital investment as well as further research into elk movement patterns at each of these sites,

how those patterns might be affected by fencing barriers, and how those changes might affect

collision risk.

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