Appendix K

Oregon Deer and Elk Crossing Prioritization Project (ODECPP) Fecal DNA Analysis

Summary

Wildlife crossing structures (WCSs) are regularly monitored using camera traps to detect wildlife utilization and determine the rate of successful crossings, but few studies have attempted to account for detection errors, most notably the degree to which crossing rates reflect repeated use by habituated individuals rather than population-level use. Habituated use by relatively few individuals has implications for reported success rates as well as the biological and ecological relevance of WCS. Established techniques for marking and tracking individuals (e.g., GPS-collars, tagging, etc.) can be very effective, but these approaches are often invasive (as they require capture), expensive, and limiting in terms of sample size. In contrast, extraction of DNA from indirect sign (e.g., scat, hair, and feathers) is non-invasive, utilizes abundant material, and can be used to generate credible population estimates based on individual markers.

We implemented a capture/recapture fecal-DNA pilot study for ODOT to determine if mule deer (*Odocoileus hemionus*) scat within the vicinity of the North Gilchrist WCS located on U.S. South Highway 97 (US-97) in central Oregon could be used to:

1. estimate local population sizes and,
2. characterize individual animal use of the WCS as serial (repeat) use by resident animals versus distinct migratory use.

Per the first question, though our initial pellet sample was sufficient (n = 211), a relatively small portion of those samples (n = 82) were viable for DNA sequencing. This limited our ability to estimate credible population sizes for mule deer. The general methodology used in this study has been successful elsewhere, so we conclude that a larger sample and shorter periods between sample collections could increase sample viability and allow for credible local population estimates. Our results also demonstrate that repeat use of crossing structures by the same individuals is occurring at the North Gilchrist WCS, though the limited viable sample size constrains our ability to generalize our findings to camera trap capture histories at the structure.

Methods

This study was implemented at the Milepost 180.13 (MP 180) WCS on U.S. South Highway 97, just north of Gilchrist, Oregon. The MP 180 structure is a wildlife-specific underpass completed in 2020 and includes approximately five miles of exclusion fencing on both sides of the highway (three miles to the south and two miles to the north). Habitat in the immediate vicinity of the MP 180 WCS is characterized primarily by dense, early-seral lodgepole (*Pinus contorta*) stands with an antelope bitterbrush (*Purshia tridentata*) understory and mid to late-seral ponderosa pine (*Pinus ponderosa*) stands with a bunchgrass understory, primarily consisting of Idaho fescue (*Festuca idahoensis*). The study area on the east side of US-97 is managed by the Oregon Department of Forestry (ODF) and has been logged extensively. Landownership in the western portion of the study area (west of US-97) is split between an active livestock ranch and logged private forest.

To capture animal movement patterns within and between migration periods, fecal pellets were collected in June (end of spring migration), September (pre-fall migration), October (fall migration), and November 2025 (end of fall migration). Fecal pellets of mule deer were collected following methods described in Pfeiler et al. (2021). Rocky Mountain elk pellets were also collected for validation of species identification. Prior to field data collection, 15 transects were established on each side of the MP 180 crossing structure along 1.5km radii extending from a baseline perpendicular to wildlife fencing with a center point aligned with the center of the MP 180 crossing structure (see Figure 1). The 1.5 km buffer was selected to capture local animal movement, as prior literature has indicated the average daily movement for mule deer is approximately 1.5 km (Bissonette and Adair, 2008). Radii were established at 0º (along the exclusion fenceline), 45º, and 90º from the baseline center point at the crossing structure entrance on both sides. Wherever possible, radii were adjusted to game trails visible from a given radii and generally leading in a similar direction. Transects in the western portion of the study area were limited by the Little Deschutes River and property access. As a result, transects in the western portion of the study area did not extend the full 1.5 km except along the fencelines.

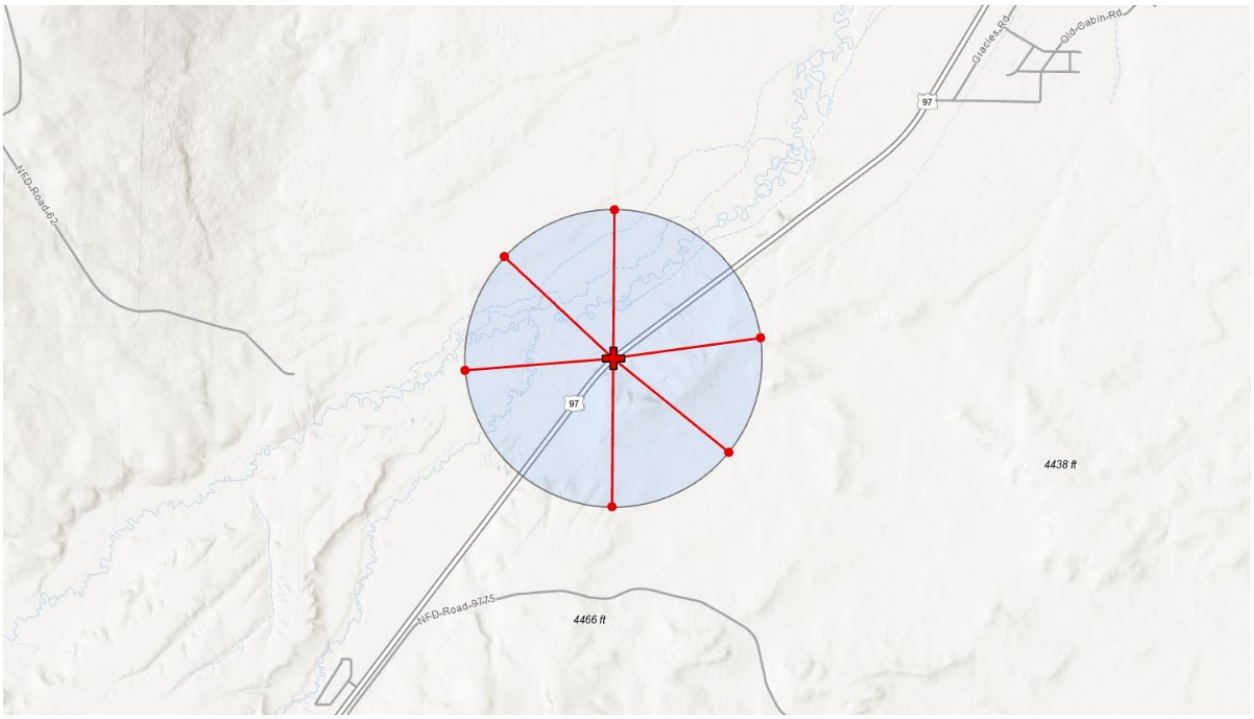


Figure 1. Study area for fecal pellet collection and DNA analysis. The blue shaded area indicates the 1.5km buffer surrounding the Milepost 180 wildlife crossing structure (red cross) located on U.S. South Highway 97 near Gilchrist, Oregon. Red lines indicate radii that served as starting points for identification of game trails for pellet collection.

The sampling unit for scat and subsequent DNA analysis was fecal pellet groups consisting of 15-20 pellets from all pellet piles encountered within visible distance of a surveyed transect. Two weeks prior to pellet sample collection, transects were walked and visually surveyed and all existing pellet piles were scattered to establish a “baseline” condition. During each collection session, field technicians were assigned 1-3 transects to walk while searching for deer pellet piles. The intention was to collect only “fresh” pellets during each sampling session, where a fresh pellet could be distinguished by dark brown appearance and minimal surface cracking. Pellet samples were collected using sticks found on-site adjacent to each pellet pile. Sticks used to collect materials were discarded after one use to protect against contamination of sample DNA. Pellet samples were placed in 6” x 9” manila envelopes left unclasped and open to allow moisture to escape and reduce potential molding. Pellet samples were marked with the transect number, geographic coordinates, date, and species. After samples were collected, remaining pellets were scattered immediately to avoid double-sampling of pellets in future collection sessions.

Following each sampling session, all samples were returned to a laboratory environment where they were stored in collection envelopes and allowed to air-dry. Once the five sample collection periods were concluded, samples were visually examined for signs of mold development. Any contaminated samples were discarded. Following inspection, all remaining samples were shipped via express shipping to the Epps Conservation and Genetics Laboratory at Oregon State University for DNA sequencing.

After sequencing, DNA data were tabularized and input into a geographic information system and mapped using the geographic coordinates logged for each detection event. Individuals detected on both sides of the highway, particularly on the same date, were assumed to have used the WCS as the exclusion fencing would have prevented crossing for several miles in either direction of the structure.

Results

A total of three hundred and thirty-one samples were collected across the five sampling sessions in 2025. Of those 331 original samples, 20 were discarded during the pre-shipping examination process due to signs of mold development. Upon receiving the samples, the Epps Lab discarded two additional samples due to mold contamination. Three hundred and nine samples (98 elk, 211 mule deer) were processed for DNA extraction and amplification against seven microsatellite loci and two additional markers to identify individuals and sex. None of the elk samples amplified at more than 3 of the 7 microsatellite markers, which confirmed correct species identification for samples identified as mule deer. Of the 211 mule deer samples, 82 (39%) generated data at five or more microsatellite loci and allowed for individual identification and recapture analysis. Results of the DNA sequencing indicated mule deer samples were derived from 31 distinct individuals, with 17 males, 10 females and 4 individuals for which sex could not be determined.

The majority of samples (87%) for which individual identification was possible were detected on only one side of the highway (Figure 2). Fifteen individuals were detected on the west side of US-97 and 12 were detected on the east side. Twenty individuals were detected only once, two individuals were detected twice, and nine individuals were detected three or more times.

Only four individuals were detected on both sides of the highway (Figure 3). Deer 079 was detected at three locations, with all sample collections occurring on the same date (June 5). The distance between sample locations and path of travel for this individual indicates migratory movement. Deer 136 was detected on two dates, September 17th and October 9, with a single October sample collected within 56m of the crossing structure and five

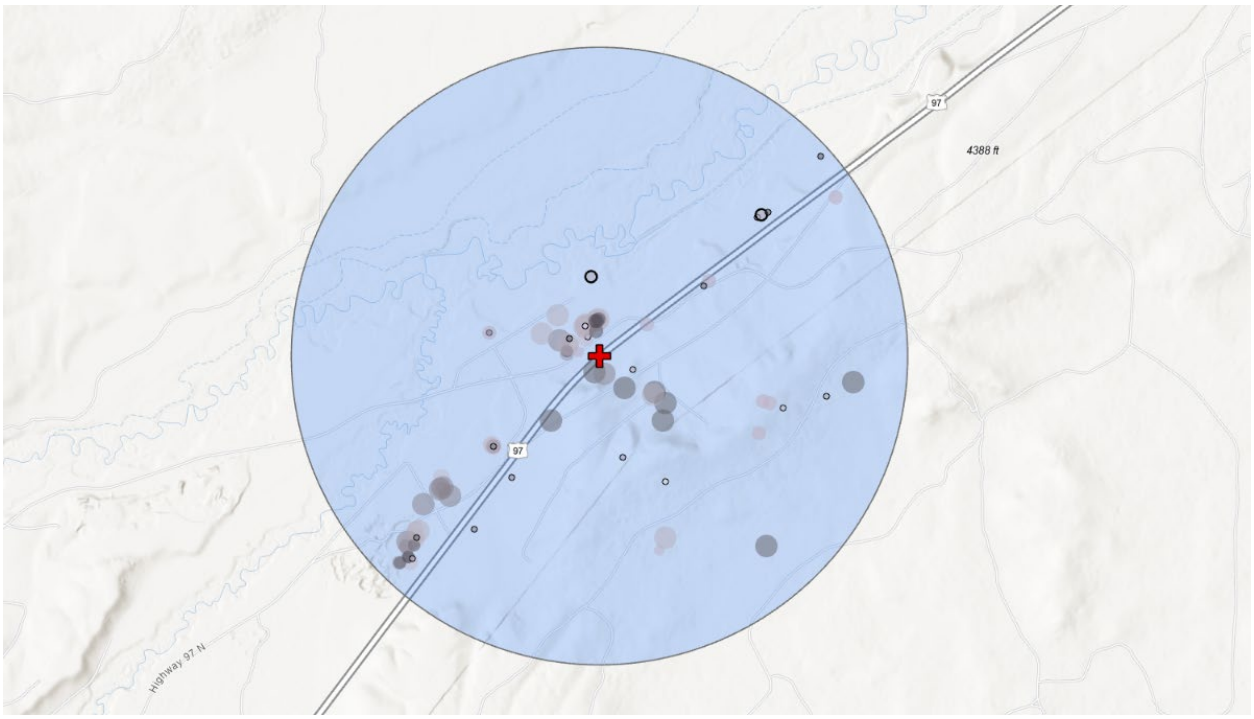


Figure 2. DNA detection history for fecal pellet collection within a 1.5 km buffer of the MP 180 wildlife crossing structure on U.S. South Highway 97, Gilchrist, Oregon. Larger spheres indicate individuals detected multiple times.

samples collected on the west side of the highway, four of which were concentrated at a single habitat patch adjacent to the fenceline south of the structure. Deer 137 was detected twice on the same day (October 9), with one sample collected within 90 m of the crossing structure and the second sample collected over 1000 m away on the east side of the highway, indicating possible migratory movement. Deer 150 was detected 10 times: twice on September 17, eight detections on October 9, and once on October 30. Two detections for this individual on the east side of the highway were from different dates (September 17 and October 9), indicating that this individual used the crossing structure multiple times during the sampling period.

Viable DNA samples were unevenly spread between collection dates. Fifteen viable samples were collected on June 5 (11 individuals), five samples were collected on September 17 (four individuals), 54 samples were collected on October 9 (17 individuals), five samples were collected on October 30 (five individuals), and three samples were collected on November 6 (three individuals). Rain during the October 30 sampling date may have contributed to lower sample viability. Additionally, many of the samples

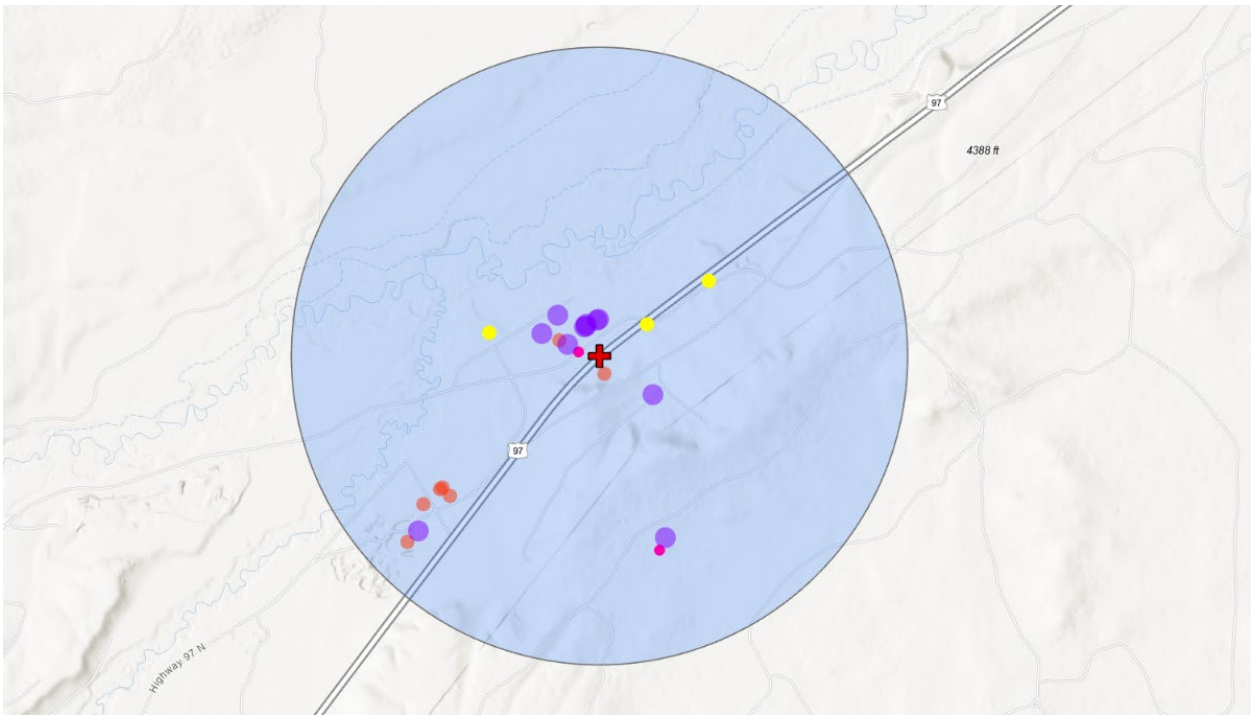


Figure 3. Detection histories for four mule deer detected on both sides of U.S. South Highway 97 within the exclusion fencing zone of the MP 180 wildlife crossing structure (red cross). Each color represents a distinct individual, where the size of the sphere correlates to a higher number of dates on which the animal was detected.

collected on November 6th exhibited high moisture content and were difficult to collect as distinct pellets.

Sampled individuals exhibited substantial clustering, predominately on the west side of the highway (Figure 4). Seven individuals (15 collected samples) were detected at a mixed habitat patch within a 100-meter radius of each other, adjacent to the fenceline south of the crossing structure. Eleven individuals (17 sample collections) were detected in a cluster within 150 meters of the western entrance to the crossing structure. While no pellet piles were detected in the crossing structure or on entrance abutments on any sampling date, six individuals were detected within 100 meters of the structure, the closest of which was collected within approximately 50 meters of the structure ingress.

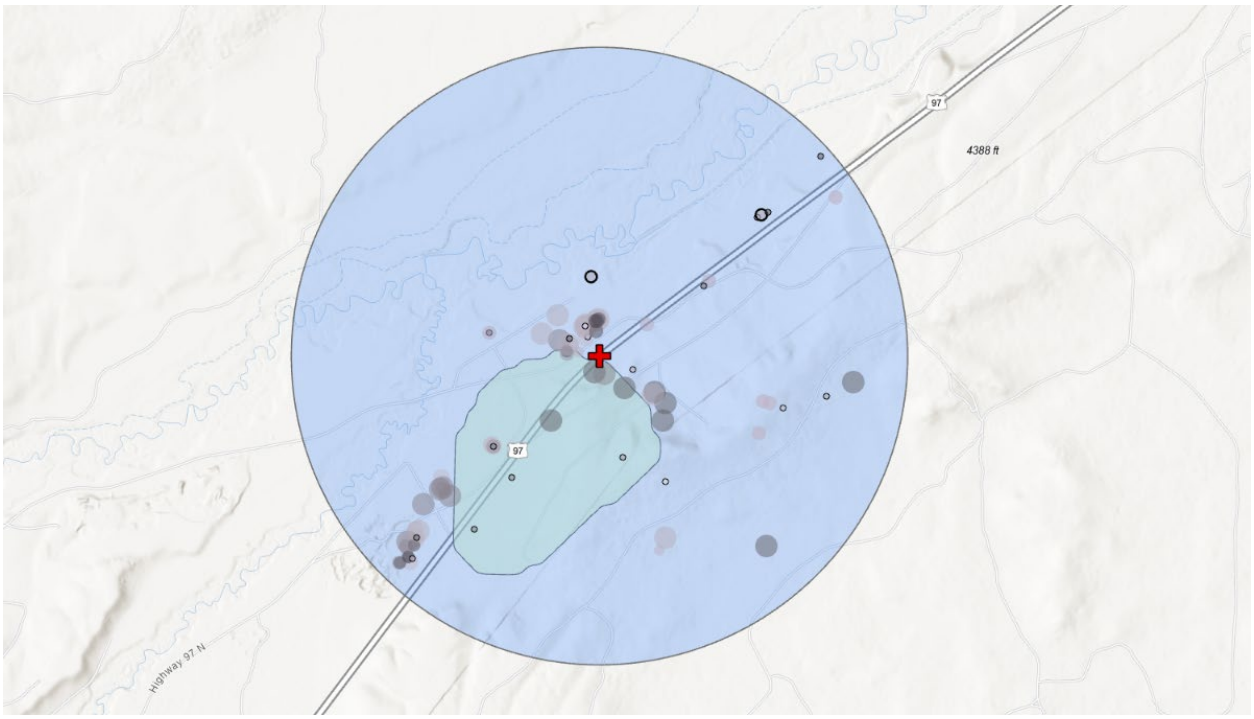


Figure 4. Individual mule deer DNA detections within a 1.5 km buffer of the MP 180.13 wildlife crossing structure on U.S. South Highway 97. The green shaded polygon within the buffer zone represents a mule deer stop-over location based on established movement patterns of radio-collared animals (Kaufmann et al., 2025).

Discussion

DNA sample collection was conducted to determine if fecal samples could be used to:

1. estimate local population sizes and
2. characterize individual animal use of the WCS as repeat use by resident animals versus distinct migratory use.

Although a sufficient number of samples were collected, the low proportion of viable samples (39%) and insufficient recapture history (i.e., 20 of 31 individuals were only detected once) were deemed too low for population estimation. Sample viability may have been compromised by several factors, including precipitation or freezing events immediately prior to or during the October 30 and November 6 sampling dates, as moisture can break down the mucous coating on fecal samples from which DNA is derived. Time between sampling sessions may also have contributed to degradation of pellet DNA. This study relied on volunteers for data collection, and challenges with schedule coordination resulted in less than optimal time spans between pellet scattering and sampling periods.

Finally, although field technicians were trained to select only fresh pellet piles, some older pellets (e.g., gray and scaly) were included in the samples sent for analysis.

One additional limitation of our sampling approach in this study involves the duration of the collection period. Funding constraints and timing limited our ability to sample across several full migratory periods. Capturing fecal samples during the full spring, summer, and fall periods would have improved our capture histories for all individuals, thus allowing for credible population estimation.

The second objective of this study was to evaluate the potential for using fecal DNA to characterize repeat (resident) versus migratory use of the structure. Camera trap capture histories are often used to estimate crossing rates to evaluate project success, but it can be difficult to determine how many crossing events detected by cameras are made by the same individuals moving back and forth through the structure between local habitats versus distinct individuals crossing during migratory periods. Despite the low sample size (four individuals) found on both sides of the highway, our results confirm both migratory and repeat use of the MP 180 WCS. However, the genetic data we obtained does not allow generalization of results to camera trap capture histories (i.e., discrete camera trap capture events should not be interpreted as crossings by distinct individuals).

Additionally, our results indicate resident use of habitat areas on both sides of the structure, with no evidence of crossing derived from fecal samples in the collection area. It is certainly possible that those individuals are crossing at other points along the highway, such as the exclusion fencing terminus points, or that we missed pellet piles deposited by those individuals, but the density of detection locations on multiple dates indicates that some individuals may be using habitat in close proximity to the highway without crossing during a given season. A longer sampling period encompassing multiple migratory seasons across years and replicated collection areas at exclusion fencing termini would likely allow for statistically robust characterization of resident versus migratory patterns.

Clustered detections within our study area, on both sides of the highway, fall within or just outside an established mule deer stopover location (Figure 4). Stopover sites are habitat areas used by migrating animals for rest and foraging, most often as they wait for snowmelt and spring green-up in higher elevation environments. Our detections occurred primarily during the fall migration period, and several animals were detected on two or more dates more than three weeks apart. While our data does not allow us to extrapolate if these animals use this habitat seasonally as part of a local ecological neighborhood, it is clear that MP 180 and the surrounding area is frequented by multiple individuals for weeks at a time.

We conclude that fecal DNA sampling has the potential to inform pre- and post-construction ungulate use of habitat in the vicinity of WCSs and the crossings themselves, and to generate credible local population estimates if strict pellet collection protocols are followed. A larger viable sample would allow for estimation of the ratio of residential versus migratory movement at a particular structure. Obtaining a larger viable sample could be achieved by increasing the overall sampling effort (several capture dates during each season) and by reducing the amount of time between seasonal sampling dates. More specifically, pellet capture sessions within a given season should be no more than seven days apart to ensure pellets are fresh. Pellet collection following precipitation events should be avoided, and collection teams should make efforts to scatter pellets following precipitation events prior to initiating a post-precipitation sample collection.

Selected References

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