# Mitigating Road Mortality of Wildlife in Rouge National Urban Park

by

Courtney G. Leermakers

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Department of Ecology and Evolutionary Biology

University of Toronto

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## Abstract

Rouge National Urban Park is one of the largest urban parks in North America, encompassing natural and developed areas, resulting in high numbers of wildlife road mortalities. Ecopassages are a common method to mitigate wildlife road mortality to provide safe crossing. To determine the placement of an ecopassage in the park, six kilometers of road was surveyed for five field seasons to record wildlife road-kill. Landscape features, traffic volume, distance to railway, seasonal movement, and weather were shown to be related to wildlife road mortality. After the installation of an ecopassage, a common tool used to assess its efficacy is PIT-tagging freshwater turtles, and strategically placing antennae at the entrances of an ecopassage. By completing arena experiments, this study investigated how PIT-tag placement, antenna type, environmental conditions, turtle orientation, and tag orientation influenced detection success. Overall, pass-over antenna, external placements, and vertical tag orientation had significantly higher detection.

# Acknowledgements

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# Chapter One: General Introduction

As urban development continues to expand globally, so do road networks and, consequently, the movement of wildlife across the natural landscape is increasingly disrupted. The study of wildlife movement and, more recently, road or movement ecology investigates the threat that road networks have on wildlife population stability and connectivity. Threats caused by fragmented landscape and road mortality include wildlife population distribution and abundance declines (Fahrig & Rytwinski, 2009), lower genetic diversity (Laporte et al., 2013), and populations with biased sex-ratios (Aresco, 2005).

Five main taxonomic groups have been studied in the field of road ecology: amphibians, birds, mammals, snakes, and turtles. Specifically, amphibians are at risk of road mortality because of their slow avoidance of cars and seasonal natal dispersal. For example, in July, the Northern Leopard Frog (*Lithobates pipiens*) disperses to neighboring waterbodies after they have undergone metamorphosis (Garrah et al., 2015; Langen et al., 2009). Both mammals and birds can have large home ranges and move more frequently compared to herpetofauna; however, few bird and mammal species have been documented to sustain population declines as a result of road mortality (Fahrig & Rytwinski, 2009; Kociolek et al., 2011). Population threats are less severe because certain mammal species behaviorally avoid roads because of noise and risk of mortality. Similarly, birds avoid nesting near roads as it disrupts their communication, and predatory birds have benefitted from scavenging road kill (Fahrig & Rytwinski, 2009). Additionally, both mammal and bird species reach maturity quickly compared to reptiles; therefore, herpetofauna are at a greater risk (Fahrig & Rytwinski, 2009). Snakes are at risk as they use roads as a heat source to thermoregulate and have a low car-avoidance capacity (Andrews & Gibbons, 2005; Fahrig & Rytwinski, 2009). Andrews and Gibbons (2005) observed that snakes would stop once a car approached, which increased the risk of mortality. Freshwater turtles are most at risk of road mortality because both males and females travel up to 1 km distances to mate or nest, often across roads (Aresco, 2005; Steen et al., 2006), and frequently nest along the shoulder of roads (Fahrig & Rytwinski, 2009). In addition, because turtles have a low hatchling survival rate and late maturity, it is crucial that, once turtles become mature, they survive to reproduce (Enneson & Litzgus, 2008).

One of the main goals of movement ecology is to determine successful mitigation techniques to reconnect fragmented landscapes, including building ecopassages or culverts underneath roads (Markle et al., 2017). Fencing is also important to funnel and encourage wildlife to use these ecopassages (Baxter-Gilbert et al., 2015). The success of mitigation measures has been evaluated by using monitoring tools, such as camera traps, radio tracking and PIT tagging, to confirm the successful passage of wildlife (Colley et al., 2017; Markle et al., 2017). Also, road-survey data are used to analyze road-mortality counts before and after mitigation (Colley et al., 2017; Markle et al., 2017; Rytwinski et al., 2016). A meta-analysis of road-mitigation success by Rytwinski et al. (2016) found that, among 50 different studies, overall mitigation reduced road mortality by 40% compared to control sites. In addition, mitigation measures with only fences and no crossing structure reduced road kill by 54% (Rytwinski et al., 2016). Baxter-Gilbert et al. (2015) found that when mitigation techniques were unsuccessful, it was mainly attributed to fencing not being well maintained (Baxter-Gilbert et al., 2015).

To evaluate the success of mitigation measures, PIT tagging is commonly used to assess the use of ecopassages by wildlife. This is achieved by attaching a PIT tag, which has a unique identification number, to individuals (Buhlmann & Tuberville, 1998). Use of an ecopassage by tagged individuals is recorded by antennae strategically placed in the passage (Markle et al., 2017). PIT tags are sometimes not read by antennae because of PIT-tag placement location, environmental conditions, or system errors such as faulty construction, noise, or weak receiver battery. For example, a study by Boarman et al. (1998) identified tortoise tracks entering a culvert being monitored with a receiver, however there was no tag recorded and, therefore, it was speculated that the PIT tag size or orientation was incorrect.

My research focuses on two aspects of movement ecology. In Chapter 2, I examine variables that predict wildlife road-mortality hotspots for a road being fitted with an ecopassage. These data were collected during summer road surveys recording dead-on-road wildlife. I hypothesize that road-mortality hotspots can be identified by analyzing variables including neighbouring landscape features, traffic volume, distance to nearest railways, seasonal movement, and weather. In Chapter 3, I investigate PIT-tag placement on freshwater turtles to determine the best conditions for the highest detection success. Experimental trials were completed to determine the most effective PIT-tag placement on freshwater turtles, and antenna type under wet and dry environmental conditions to yield the highest detection success. I hypothesize that PIT-tag placement, antenna type, environmental conditions, turtle orientation, and tag orientation will influence tag detection rate, distance read, and number of detections.

# Chapter Two: Predicting Road Mortality of Wildlife in Rouge National Urban Park

## Introduction

Road mortality has been identified as a major threat for many species of wildlife as roads are a barrier to movement. These movement barriers can affect individual fitness by increasing the risk of mortality and preventing movement between required resources (Fahrig & Rytwinski, 2009). With the realization that roads are a threat to wildlife, research investigating different mitigation techniques has become crucial to prevent further population decline and restore movement patterns. There are different options for mitigation, including ecopassage or fencing structures. However, before placement of mitigation, it is important to identify wildlife road-mortality hotspots for optimal placement. The location of hotspots may vary temporally and spatially as animals depend on resource availability and have peak movement times (Fahrig & Rytwinski, 2009).

Wildlife population sizes are often lower near habitats intersecting roads (Fahrig & Rytwinski, 2009; Laporte et al., 2013). For example, an empirical review analyzing the effects of roads on animal abundance for 79 studies, including 131 species and 30 taxonomic groups, found that the number of documented negative effects of roads on animal abundance outnumbered positive effects by a factor of 5 (Fahrig & Rytwinski, 2009). Decline in genetic diversity is another threat to wildlife as roads create barriers between subpopulations. This has been documented in a study that found Painted Turtles, *Chrysemys picta*, living within 500 m of a road, had fewer but larger families and lower mitochondrial diversity (Laporte et al., 2013). In addition, there is documented female-biased populations near roads as female turtles are more active during nesting season, making them more prevalent to cross roads and increasing the threat of road mortality (Steen et al., 2006).

Wildlife are threatened by roads because of peak movement times and resource availability. Amphibians are more active during July, after they undergo metamorphosis and must migrate to neighboring wetlands (Crawford et al., 2014; Garrah et al., 2015; Langen et al., 2009). Bird road mortality has been documented to increase during mating and fledgling season (Kociolek et al., 2011). Mammal movement generally increases during the warmer months, with no specific monthly patterns. (Červinka et al., 2015). Snakes are more active during the fall when they migrate back to hibernation locations (Crawford et al., 2014; Stinnissen, 2015). Turtles are documented to be more active during nesting and mating season (Steen et al., 2006). Movement is also influenced by resource availability and habitat type as animals move between different terrestrial and aquatic landscapes. It has also been documented that roads intersecting habitats with a wetland on both sides will have increased road mortality (Langen et al., 2009).

In Rouge National Urban Park, one of the largest urban parks in North America, there is currently an area of high road mortality of amphibians, birds, mammals, snakes, and turtles. To mitigate wildlife mortality occurring in the park, an ecopassage and eco-fencing installation is planned. To determine the placement of a future ecopassage in Rouge National Urban Park, three road segments, totaling 6 km, were surveyed during May-October 2010, 2011, 2017, 2018, and 2019, and the spatial and temporal aspects of road-kill documented. Using these data, I will identify the variables that explain wildlife road-mortality hotspots for a road with a planned ecopassage in Rouge National Urban Park.

With the primary objective to determine predictors of road mortality, I hypothesize that the threat of road mortality is spatially and temporally distributed, which can be related to factors such as neighbouring landscape features, traffic volume, distance to nearest railway tracks, seasonal movement, and weather. One of the main predictors of road-kill hotspots is distance to landscape features as wildlife migrate among terrestrial and aquatic landscapes. Distance to nearest wetland and shallow aquatic habitat is primarily associated with most life stages of reptiles and amphibians. Turtles stay in aquatic habitats, except during nesting and mating season. Snakes have been documented to be associated with wetland and forest habitat, and amphibians mate, lay there eggs, and metamorphize in aquatic ecosystems (Ashley & Robinson, 1996; Garrah et al., 2015). I predict that bird and mammal road mortality will be associated with forest and field habitats as these provide direct cover immediately adjacent to roads (Barthelmess, 2014; Kociolek et al., 2011), and herpetofauna will have a higher association to wetlands as they spend a majority of time in these habitats and similar studies have found the strong association between hotspots and wetland location (Garrah et al., 2015; Rowe & Dalgarn, 2010). Vehicle-traffic volume can influence hotspots as the likelihood of wildlife interacting with cars increases with increased vehicle volume, and herpetofauna have slow car avoidance (Crawford et al., 2014; Stinnissen, 2015; Teixeira et al., 2017). Additionally, increased traffic volume in the summer coincides with wildlife movement events (Langen et al., 2007). Therefore, I predict that slow moving wildlife will be associated with higher traffic volume. It has been reported that railway tracks are a barrier for wildlife, specifically small animals as they can get trapped between rails and die from collision or dehydration (Santos et al., 2017). Railway tracks intersect all three road segments surveyed; therefore, I predict train tracks are an obstacle for wildlife movement forcing them to cross at road crossings, where they can escape from being trapped by the two rails, but putting them at risk of vehicle collision. Reptiles and amphibians have temporal movement patterns during mating, nesting, hibernation, and natal dispersal (Aresco, 2005; Garrah et al., 2015). Therefore, I predict that turtle mortality will increase during June nesting and mating season, and snake mortality in October when returning to hibernation sites. Amphibian mortalities will increase during July when frogs metamorphosize and disperse to neighboring wetlands. Bird mortalities will be more frequent during mating and fledgling season in June (Kociolek et al., 2011) and mammal mortality will show no temporal variation, as studies have documented increased movement during the entire summer versus fall (Červinka et al., 2015). Daily weather, including precipitation, cloud cover and air temperature, has been documented to influence wildlife movement as reptiles and amphibians are ectothermic and, therefore, highly influenced by weather, moving more in warmer weather (Rowe & Dalgarn, 2010; Stinnissen, 2015). For example, Midland Painted Turtles (*Chrysemys picta marginata*) were found to move less on cloudy days because of decreased thermal energy availability for muscle activity (Rowe & Dalgarn, 2010). In addition, amphibians are more active on days with high precipitation and snakes on hot days with low precipitation (Stinnissen, 2015). Precipitation will have an affect on bird movement as it disrupts visibility and orientation (Kociolek et al., 2011). Additionally, mammals will have no association between weather and road mortality, as previous studies have not documented an association. Therefore, I predict that amphibians will be found on days with precipitation, reptiles will be found on hot days with little precipitation, birds on days with little precipitation and mammals will have no association.

# Methods

## Study Site

Rouge National Urban Park was established in 2017, and it encompasses areas of the Greater Toronto Area that extend from Lake Ontario to Uxbridge (Figure 1). The park is currently 79.1

km$^2$, making it the largest urban park in North America. It is home to 1,700 species, including 27 species at risk. Within the park, three road segments were surveyed, each 2 km in length, during 2010, 2011, 2017, 2018, and 2019. In 2010 and 2011 road segments were chosen based on there location next to recent restoration projects, including Reesor Wetland built in 2008, and tree planting by Friends of the Rouge, with the goal to determine if restoration projects near roads has an effect on wildlife road mortality. Road surveys were continued in 2017-2019 to continue analyzing road mortality near restoration projects and to inform the location of a future ecopassage and eco-fencing near the corner of Finch Avenue and Scarborough/Pickering Townline. To determine the location of a future ecopassage, this study will identify road-mortality hotspots based on spatial and temporal variables thought to influence species movement.

## Data Collection

The study was conducted on three road segments within Rouge National Urban Park: Reesor, Plug Hat, and Townline (Figure 1). The Reesor segment includes Reesor Road and Old Finch Avenue, Plug Hat includes Old Finch Avenue and Plug Hat Road, and Townline includes Scarborough/Pickering Townline and Finch Avenue East. Each road segment is 2 km long; totaling 6 km of road walked each day that surveys were conducted. Road surveys were completed in 2010, 2011, 2017, 2018, and 2019. In 2010, surveys were completed between May 31 and October 15 on Monday, Wednesday and Friday starting at 0900. In 2011, surveys were completed from May 10-October 29 on Tuesday, Thursday and Saturday starting at 0900. In 2017, surveys were completed June 20-29 and August 1-September 21 on Tuesday and Thursday starting at 1300. In 2018, surveys were completed May 7-September 28, Monday-Friday starting at 1300. Most recently, 2019 surveys were completed May 13-October 31, Monday to Friday starting at 1300. Refer to Table 1 for further supplementary data including search effort and site descriptions.

Data were collected by walking each side of the road towards traffic scanning for dead-on-road wildlife from the middle yellow line towards the shoulder. Each road was walked by two individuals, one recorded and the other scanned for road kill. For each road kill, the following data were recorded: species found; mortality status as dead or alive; proximity/position of road mortality on the road based on distance to shoulder and yellow line; GPS co-ordinates using a

Garmin eTrex 10 GPS; directional side of the road; and, environmental conditions. Once data were recorded for a dead-on-road sighting, the remains were discarded so that they were not re-counted. Real-time traffic data were collected October 6 to 12, 2011 for Reesor and Townline, and August 16 to 22, 2019 for Reesor, Townline, and Plug Hat sites by City of Toronto Transportation Services.

## Data Analysis

To analyze road-mortality locations, coordinates for each road-mortality record were plotted on a Toronto Region Conservation Authority (TRCA) Ecological Land Classification and railway layer by DMTI Spatial Inc. Using the near tool in ArcGIS, the minimum distance of each road-mortality coordinate to the nearest railway, wetland (marsh, swamp, fen), field (meadow and savannah), forest, agriculture, and shallow aquatic (river and pond) habitats was measured. To identify predictors of road mortality, data were analyzed using a binomial Generalized Linear Mixed Model (GLMM) to model the occurrence of wildlife road mortality. GLMMs incorporate both linear mixed models that include random effects, and generalized linear models, which controls for non-normal data (Bolker et al., 2009). Numeric data were scaled so that all values had similar units and then the severity of multicollinearity was tested using the variance inflation factor (vif), once complete values above four were removed. Finally, a dredge function was used, which ran all possible GLMM combinations and ordered them based on best AIC value. All statistical analyses were completed using MuMIn and car R packages (R Core Team, 2013). Response variables included presence and absence by taxonomic group. The entire dataset of all recorded road mortalities was used in the GLMMs, which were calculated separately for each taxonomic group. Records for the taxonomic group being analysed were coded as 1 and records for all other taxonomic groups coded as 0. Fixed effects include all temporal and spatial variables hypothesized to be related to road mortality. Time of survey, either morning or afternoon, was also used as a fixed effect because, when assessing multicollinearity using the variance inflation factor, this variable was independent of the other predictor variables. Random effects included year and road segment as data were collected at these different levels, which could influence the results (Table 2). Models were also run without road segment as a random effect, because removing variation between locations when analysing spatial features could remove the significance of the different habitat features present for each road. To analyse spatial relationships, an Optimized Hotspot Analysis was completed in ArcGIS, which plots statistically significant clusters of road mortality as hotspots in red and coldspots in blue using the Getis-Ord Gi* statistic (Getis & Ord, 1992). The Getis-Ord Gi* statistic assigns hot and cold spots by adding the number of road kills on a given segment with a neighboring segment and compares that value with an overall expected distribution. Once the Getis-Ord Gi* statistic is calculated, the resulting z-scores indicate where features with either high or low values cluster spatially. Positive z-scores indicate clustering of hotspots, whereas negative scores indicate clustering of coldspots, and the size of score indicates strength of clustering (Garrah et al., 2015; Getis & Ord, 1992).

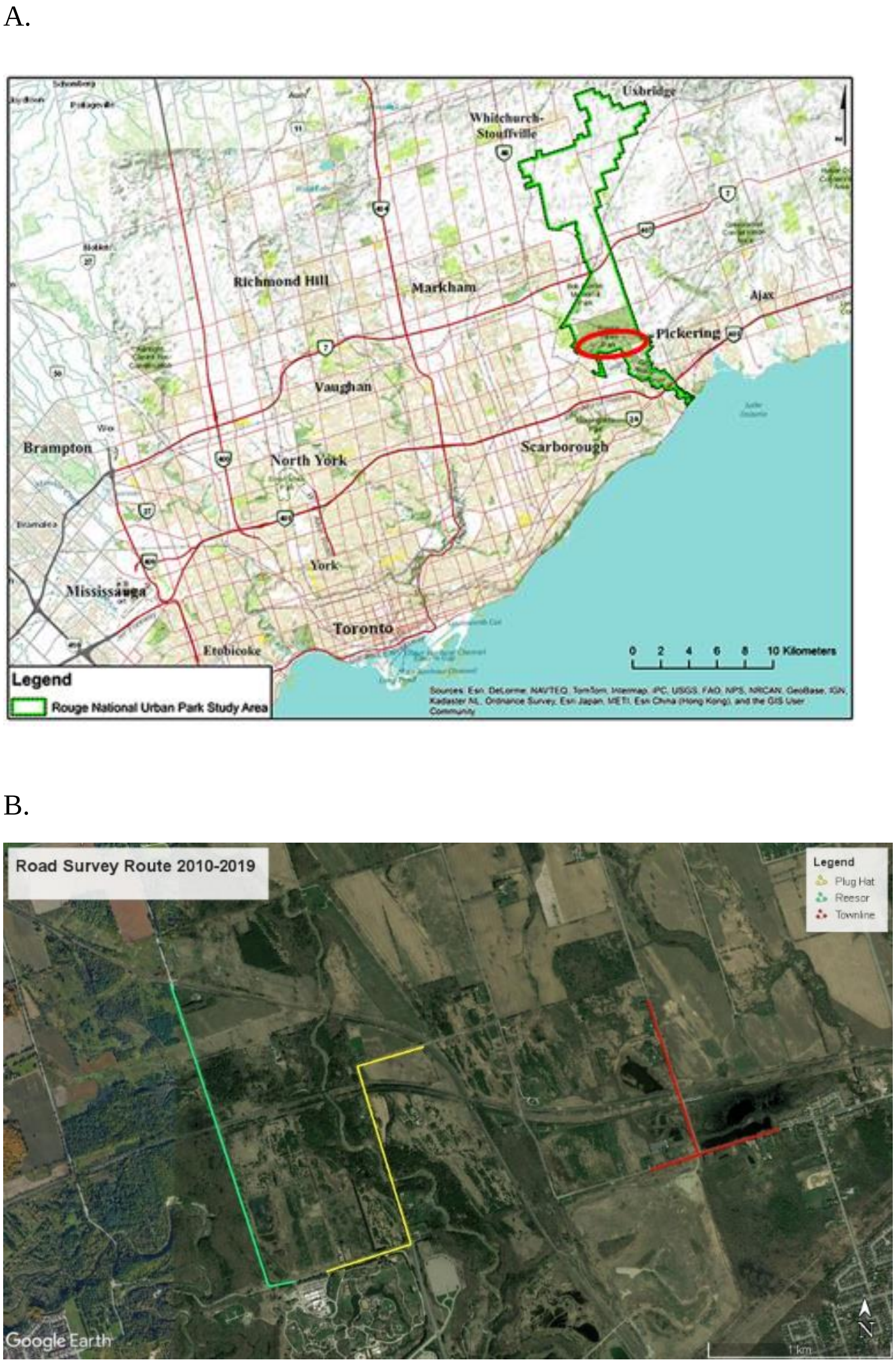


**Figure 1:** A. The study site is located within Rouge National Urban Park. B. Location of each road-survey route designed to count dead-on-road wildlife.

**Table 1:** Landscape characteristics and search effort by road segment for wildlife road mortalities in Rouge National Urban Park, 2010-2019. The survey route length on each road was 2 km.

| **Road** | **Roadside Habitat** | **Speed Limit (km/h)** | **Search Effort (Days)** | | | | | **Urban Features** |
|---|---|---|---|---|---|---|---|---|
| | | | **2010** | **2011** | **2017** | **2018** | **2019** | |
| Reesor | Field, Forest (ephemeral ponds), Wetland (built 2008), Roadside Ditches, Little Rouge Creek | 60 | 60 | 73 | 19 | 79 | 89 | Railway Crossing, <10 houses |
| Townline | Field, Forest, Wetland (Amos Pond, Townline Swamp, Marsh), Roadside Ditches, Petticoat Creek | 60 | 0 | 73 | 16 | 84 | 77 | Railway Crossing, <10 houses |
| Plug Hat | Field, Forest, Little Rouge Creek | 50 | 0 | 0 | 17 | 58 | 55 | Railway Crossing, Hillside Outdoor Education School, <10 houses |

**Table 2:** Variables used as predictors of wildlife road-mortality hotspots in Rouge National Urban Park in a Generalized Linear Mixed Model (GLMM) analysis.

| Variable | Definition | Variable Type | Code | Categories/ Range |
|---|---|---|---|---|
| Number Dead on Road | Binomial response variable for each taxonomic group. | Response | Number_ DOR | 0-1 |
| Air Temperature | Air temperature recorded based on Weather Channel. | Fixed | Temp | 0°C-40°C |
| Cloud Cover | Cloud cover present on day of study based on a 0-100% scale. | Fixed | Cloud | 0% |
| | | | | 25% |
| | | | | 50% |
| | | | | 75% |
| | | | | 100% |
| Distance to Agriculture | Distance measured from road mortality centroid to nearest agricultural field. | Fixed | Wetland | 0-1261 metres |
| Distance to Field | Distance measured from road mortality centroid to nearest field. | Fixed | Field | 0-339 metres |
| Distance to Forest | Distance measured from road mortality centroid to nearest forest. | Fixed | Forest | 0-280 metres |
| Distance to Railway | Distance measured from road mortality centroid to nearest railway. | Fixed | Rail | 0-935 metres |
| Distance to Shallow Aquatic Habitat | Distance measured from road mortality centroid to nearest shallow aquatic waterbody. | Fixed | Shallow_ Aquatic | 0-591 metres |
| Distance to Wetland | Distance measured from road mortality centroid to nearest wetland. | Fixed | Wetland | 0-358 metres |
| Month | Month that each roadkill was found. | Fixed | Month | May |
| | | | | June |
| | | | | July |
| | | | | August |
| | | | | September |
| | | | | October |
| Time of Day | Time of day that road survey was conducted. | Fixed | Time | Morning |
| | | | | Afternoon |
| Total Precipitation | Total precipitation fallen on time of survey, from Toronto City weather station data. | Fixed | Precip | 0-60 mm |

| | | | | |
|---|---|---|---|---|
| Traffic Volume | Daily mean vehicle count. | Fixed | Traffic | 500-7500 |
| Road Segment | Three road segments surveyed in Rouge National Urban Park. | Random | Location | Reesor |
| | | | | Townline |
| | | | | Plug Hat |
| Year | Year of road surveys. | Random | Year | 2010 |
| | | | | 2011 |
| | | | | 2017 |
| | | | | 2018 |
| | | | | 2019 |

# Results

From all 5 years surveyed, a total of 6021 dead-on-road wildlife observations were recorded. A total of 73% were amphibians, 13% snakes, 9% mammals, 4% birds and 1% turtles (Appendix Table A1). There were 64 identified vertebrate species: 31 bird species, 17 mammal species, 9 amphibian species, 4 snake species, and 3 turtle species (Appendix Table A1). Five species considered at-risk in Canada were recorded: Common Snapping Turtle, *Chelydra serpentina* (Special Concern); Midland Painted Turtle, *Chrysemys picta marginata* (Special Concern); Blanding's Turtle, *Emydoidea blandingii* (Threatened); Eastern Milksnake, *Lampropeltis traingulum* (Special Concern); and, Eastern Wood-Pewee, *Contopus virens* (Special Concern).

Across all years surveyed, temporal peaks in wildlife road mortality are evident. Specifically, amphibian mortality was highest in July (Figure 2), birds highest in August (Figure 3), mammals highest in summer months (Figure 4), snake mortality highest during September and October (Figure 5), and turtle mortality was highest in May and June (Figure 6). Overall, the most turtles found dead on the road were on Townline. Reesor and Plug Hat had the most snakes found dead on the road. Townline had the most amphibian's dead on the road in July, however for the other months surveyed Reesor had the most amphibian's dead on the road. Finally, there were more mammal and bird mortalities on Reesor and Plug Hat (Table 3).

Road-mortality locations were spatially clustered, as evident from the Optimized HotSpot Analysis (Getis & Ord, 1992). In 2010, only Reesor Road was surveyed, and there was a hotspot located near Reesor Wetland (Figure 7). In 2011, Reesor and Townline were surveyed and both roads had hotspots, including the same hotspot on Reesor identified in 2010 (Figure 8). In 2017, Reesor and Townline had the same hotspots as identified in previous years (Figure 9). In 2018, Reesor did not have a statistically significant hotspot, but the Townline hotspot remained in the same location near Amos Pond (Figure 10). A coldspot was identified for Plug Hat in 2018 (Figure 10), which remained the same in 2019 (Figure 11). In 2019, the hotpots remained in the same location for Reesor and Townline (Figure 11). Overall, the hotspot analysis showed that the identified hotspot locations did not change across the years except for Reesor in 2018.

There were also year-to-year fluctuations of road mortality between road segments and taxonomic groups. Based on the number of road kills per day based on search effort, amphibians in 2010 and 2011 on Reesor had the highest road mortality compared to more recent years. Between 2017 and 2018, amphibian mortality increased along Plug Hat and decreased for Townline and Reesor. In 2019, road mortality was highest for amphibians for Plug Hat and Townline, and Reesor had lower mortality compared to 2010-2011 (Figure 12). For bird mortality between 2010 and 2011, Reesor and Townline numbers decreased. Plug Hat bird mortality increased between 2017 and 2019, compared to Reesor and Townline which decreased (Figure 13). Mammal road mortality decreased on Reesor between 2010 and 2011. Between 2011 and 2017 mammal mortality increased on Reesor and Townline, however it decreased on these roads between 2017 and 2019. On Plug Hat, mammal road mortality numbers remained consistent from 2017-2019 (Figure 14). Snake road mortality decreased on Reesor between 2010 and 2011 and increased between 2011 and 2017, however, between 2018 and 2019 snake mortality on Reesor was lower compared to previous years. Townline had low snake mortality, and Plug Hat had similar fluctuations as Reesor, with highest snake mortality in 2017. All snake mortality decreased between 2017 and 2018, but increased again in 2019 (Figure 15). Lastly, there was no turtle mortalities recorded in 2010, and between 2011 and 2017 mortality increased along Reesor and Townline. Between 2017 and 2018 turtle mortality decreased, but increased in 2019 on Townline. Townline generally had higher turtle road mortality, and Plug Hat had few turtles counted (Figure 16).

**Table 3:** Number of dead-on-road wildlife observations for each taxonomic group and road segment surveyed from 2010, 2011, 2017, 2018, and 2019 in Rouge National Urban Park. Plug Hat was not surveyed from 2010-2011 and Townline in 2010 as indicated by NA.

| Year | Taxonomic Group | Reesor | Plug Hat | Townline |
|---|---|---|---|---|
| 2010 | Amphibian | 583 | NA | NA |
| | Bird | 35 | NA | NA |
| | Mammal | 67 | NA | NA |
| | Snake | 152 | NA | NA |
| | Turtle | 0 | NA | NA |
| 2011 | Amphibian | 664 | NA | 559 |
| | Bird | 15 | NA | 11 |
| | Mammal | 41 | NA | 31 |
| | Snake | 124 | NA | 28 |
| | Turtle | 5 | NA | 10 |
| 2017 | Amphibian | 74 | 12 | 96 |
| | Bird | 19 | 6 | 6 |
| | Mammal | 22 | 29 | 10 |
| | Snake | 53 | 72 | 9 |
| | Turtle | 6 | 0 | 5 |
| 2018 | Amphibian | 81 | 243 | 267 |
| | Bird | 19 | 29 | 6 |
| | Mammal | 42 | 110 | 22 |
| | Snake | 39 | 61 | 17 |
| | Turtle | 7 | 2 | 21 |
| 2019 | Amphibian | 379 | 473 | 967 |
| | Bird | 13 | 72 | 6 |
| | Mammal | 44 | 97 | 18 |
| | Snake | 118 | 82 | 39 |
| | Turtle | 10 | 1 | 15 |

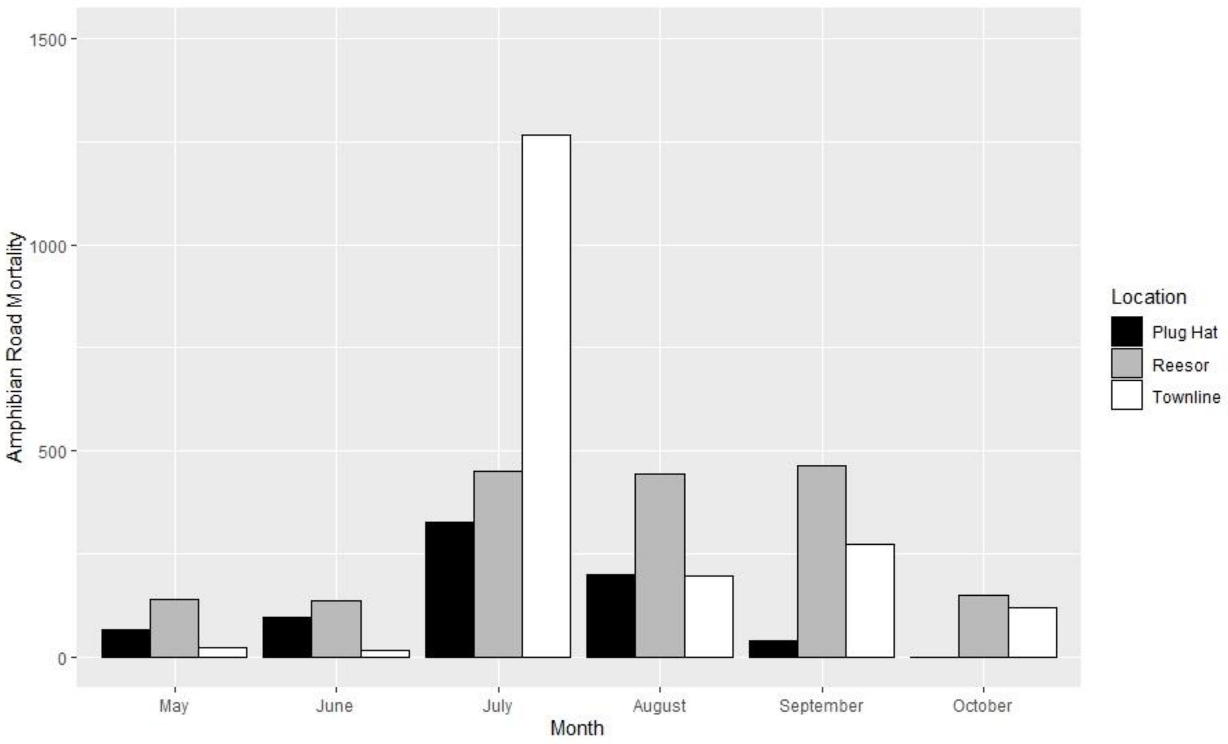


**Figure 2:** Total number of amphibian road mortality records per month across all three road segments surveyed in the vicinity of Rouge National Urban Park, totaling 6 km of road, May-October 2010, 2011, 2017, 2018, and 2019.

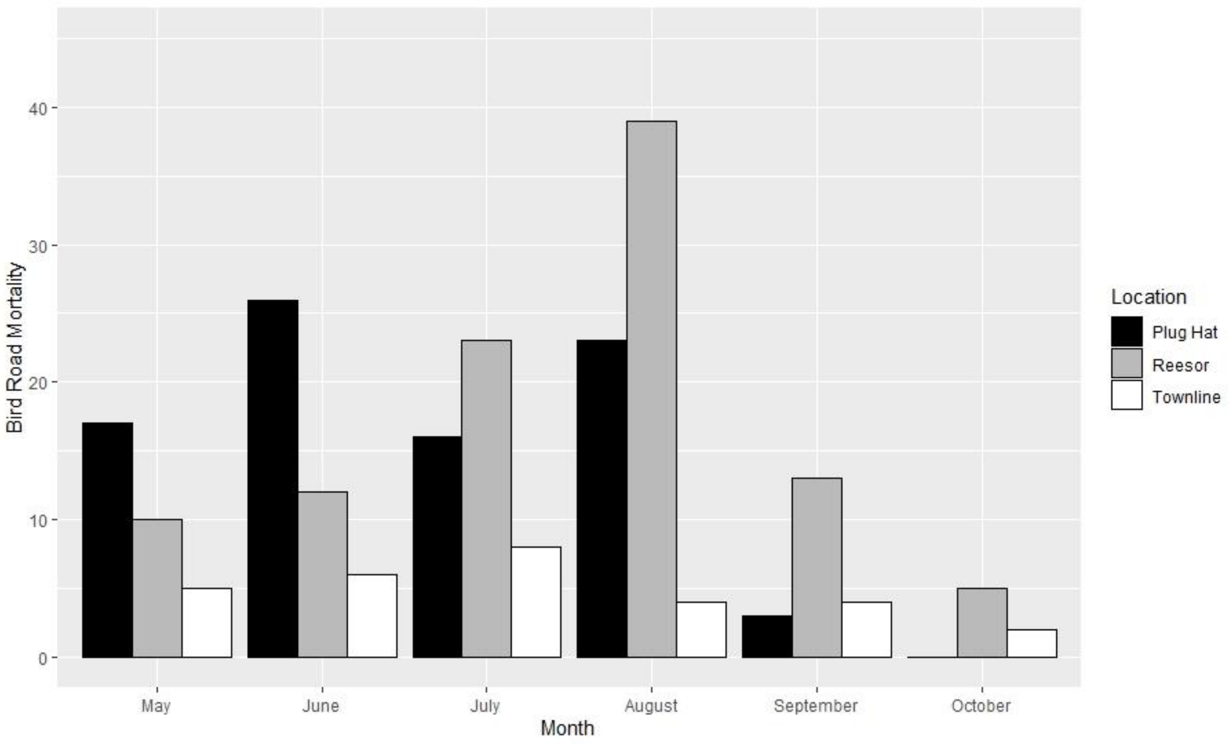


**Figure 3:** Total number of bird road mortality records per month across all three road segments surveyed in the vicinity of Rouge National Urban Park, totaling 6 km of road, May-October 2010, 2011, 2017, 2018, and 2019.

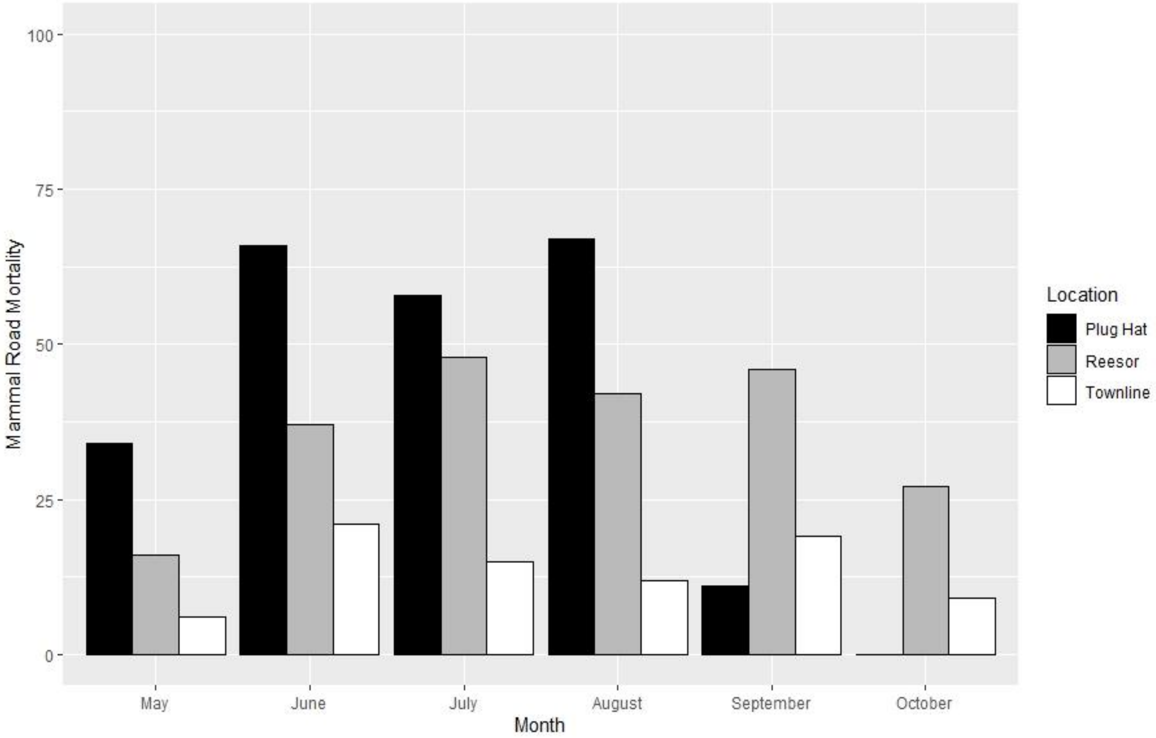


**Figure 4:** Total number of mammal road mortality records per month across all three road segments surveyed in the vicinity of Rouge National Urban Park, totaling 6 km of road, May-October 2010, 2011, 2017, 2018, and 2019.

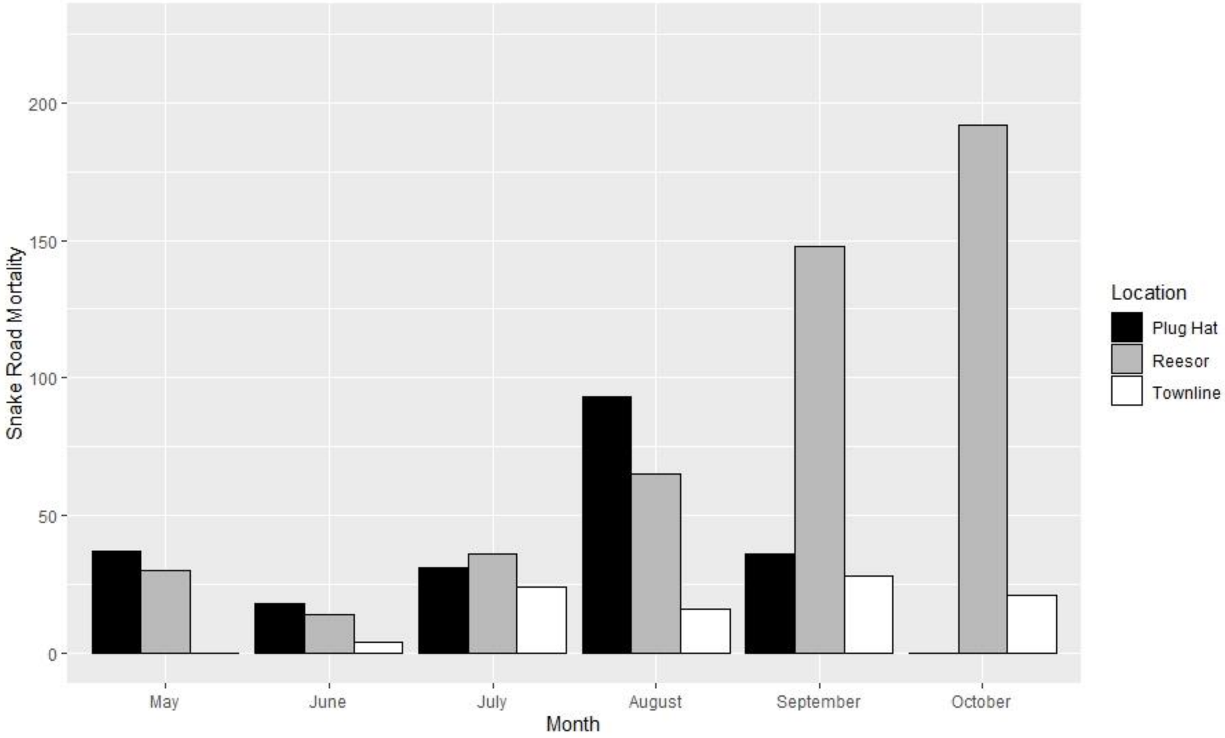


**Figure 5:** Total number of snake road mortality records per month across all three road segments surveyed in the vicinity of Rouge National Urban Park, totaling 6 km of road, May-October 2010, 2011, 2017, 2018, and 2019.

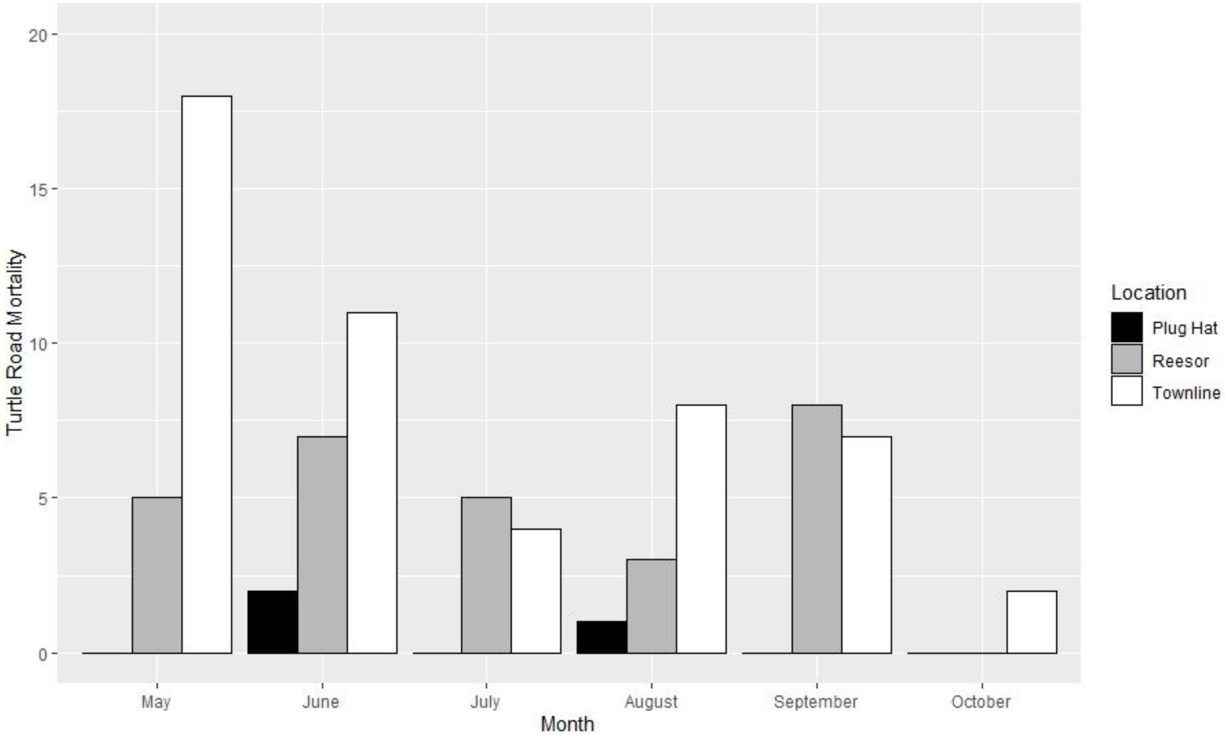
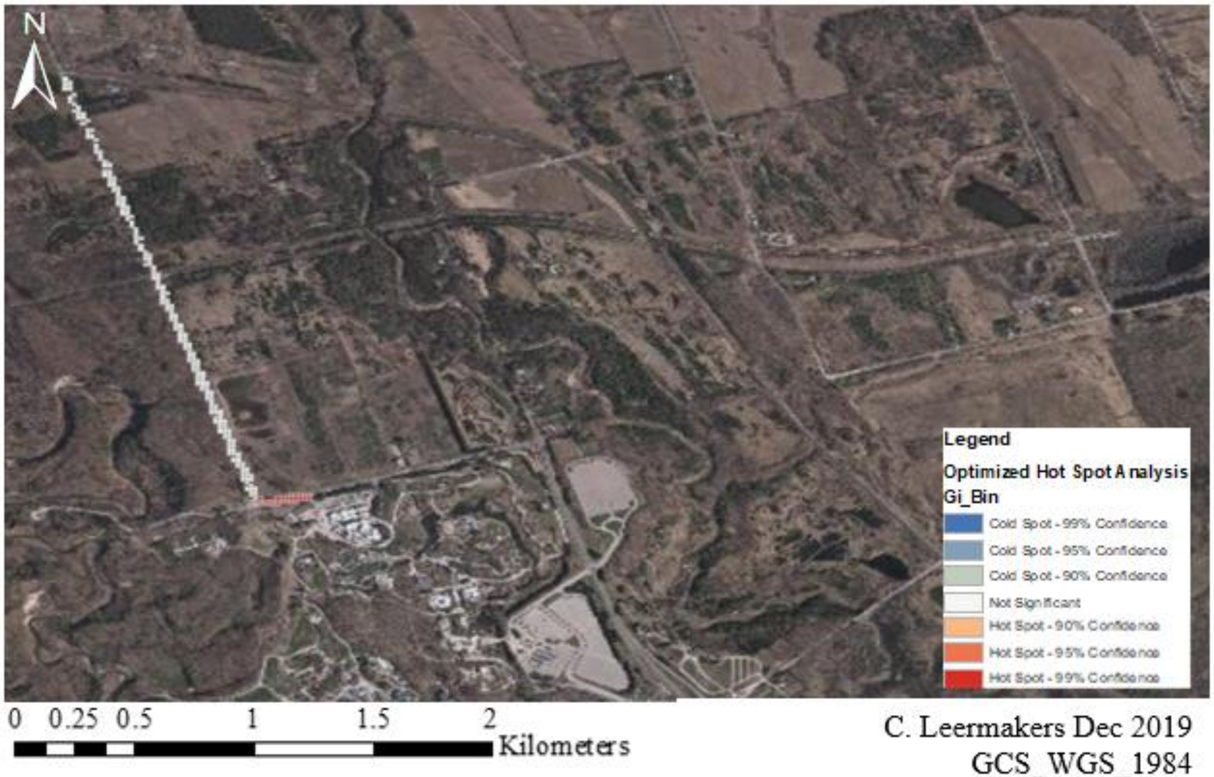
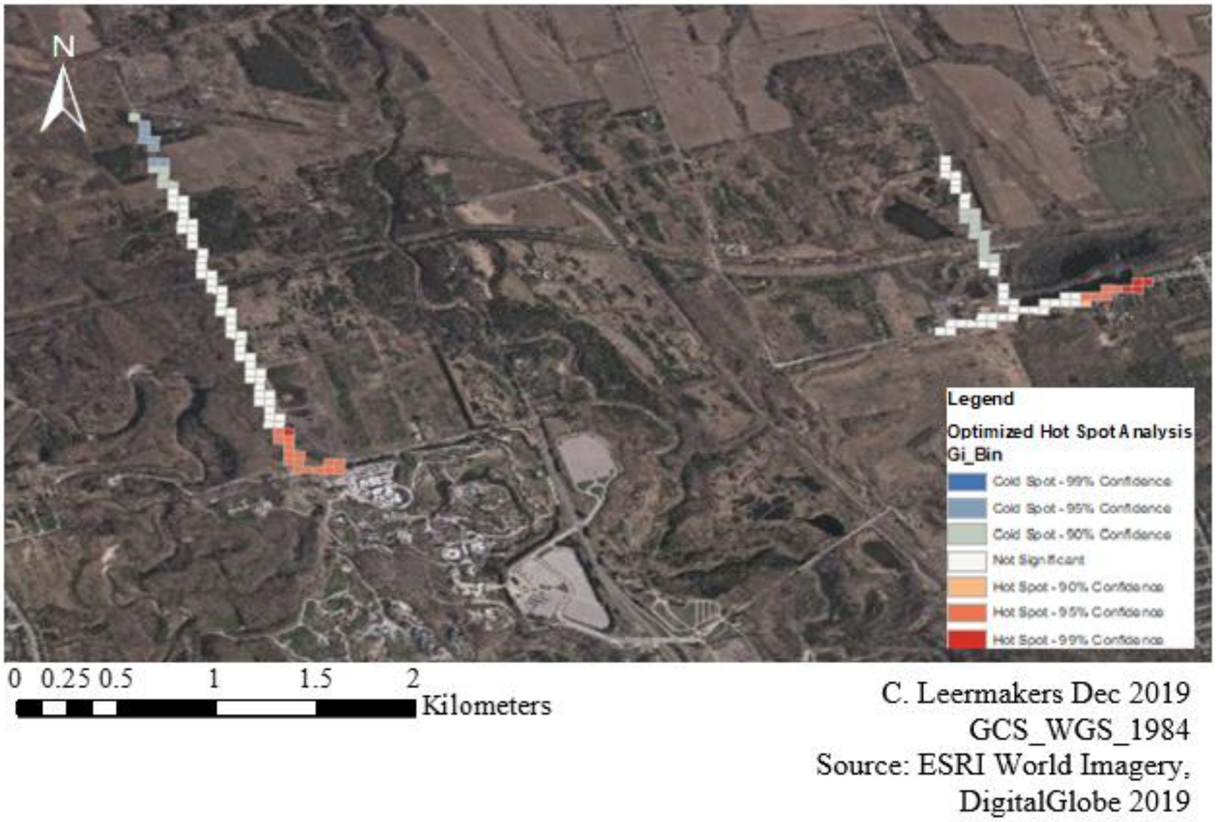


**Figure 6:** Total number of turtle road mortality records per month across all three road segments surveyed in the vicinity of Rouge National Urban Park, totaling 6 km of road, May-October 2010, 2011, 2017, 2018, and 2019.



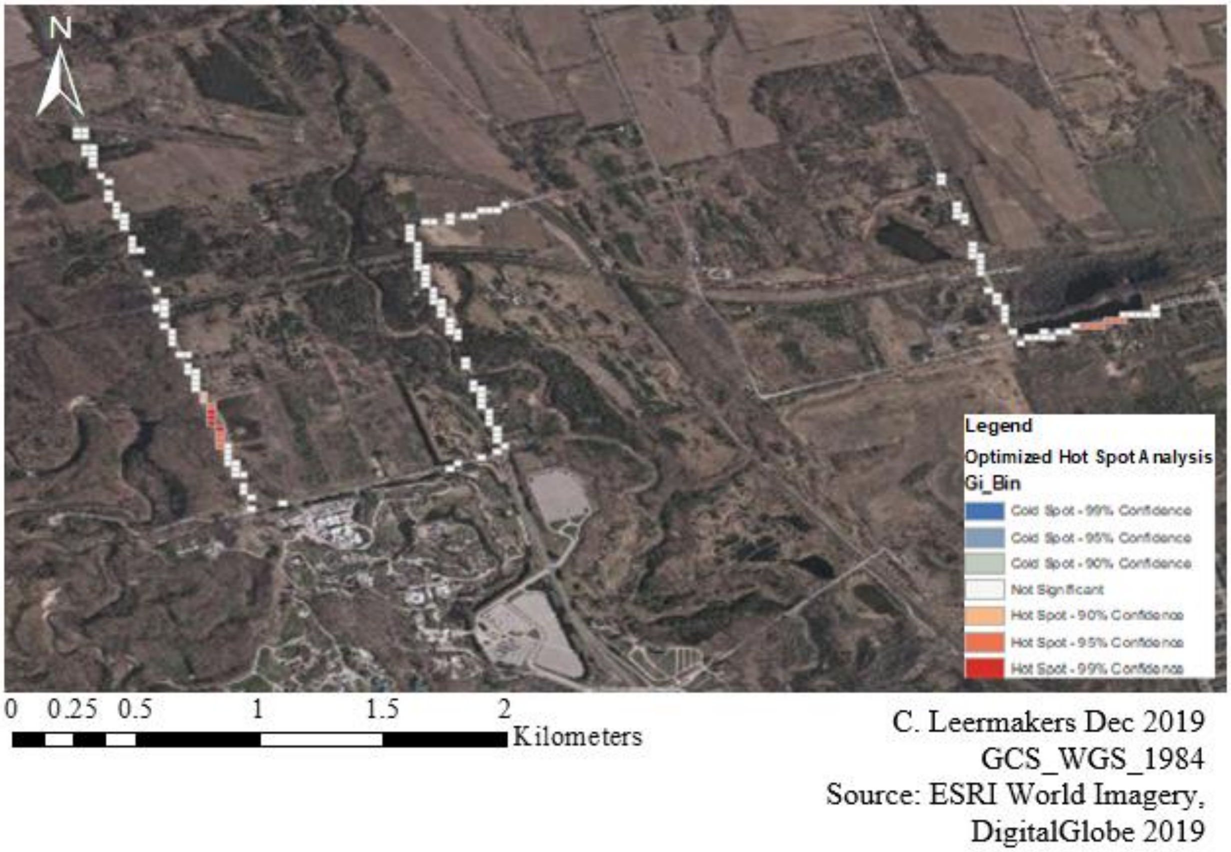
C. Leermakers Dec 2019
GCS_WGS_1984
Source: ESRI World Imagery,
DigitalGlobe 2019

**Figure 7:** Optimized Hotspot Analysis of road-mortality locations in 2010 in the vicinity of Rouge National Urban Park. Hotspot analysis identifies statistically significant clusters of high values (hotspots) and low values (coldspots), calculated using Getis-Ord Gi* statistic.



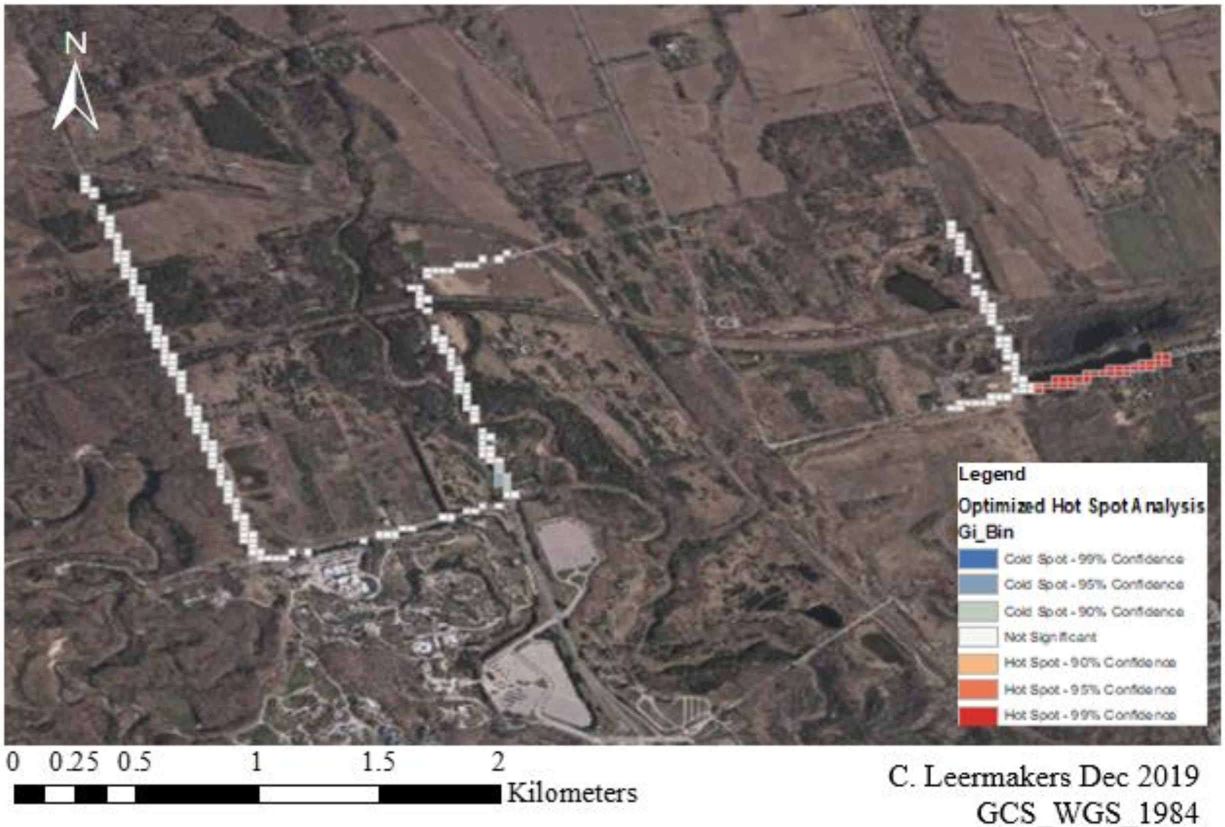
C. Leermakers Dec 2019
GCS_WGS_1984
Source: ESRI World Imagery,
DigitalGlobe 2019

**Figure 8:** Optimized Hotspot Analysis of road-mortality locations in 2011 in the vicinity of Rouge National Urban Park. Hotspot analysis identifies statistically significant clusters of high values (hotspots) and low values (coldspots), calculated using Getis-Ord Gi* statistic.



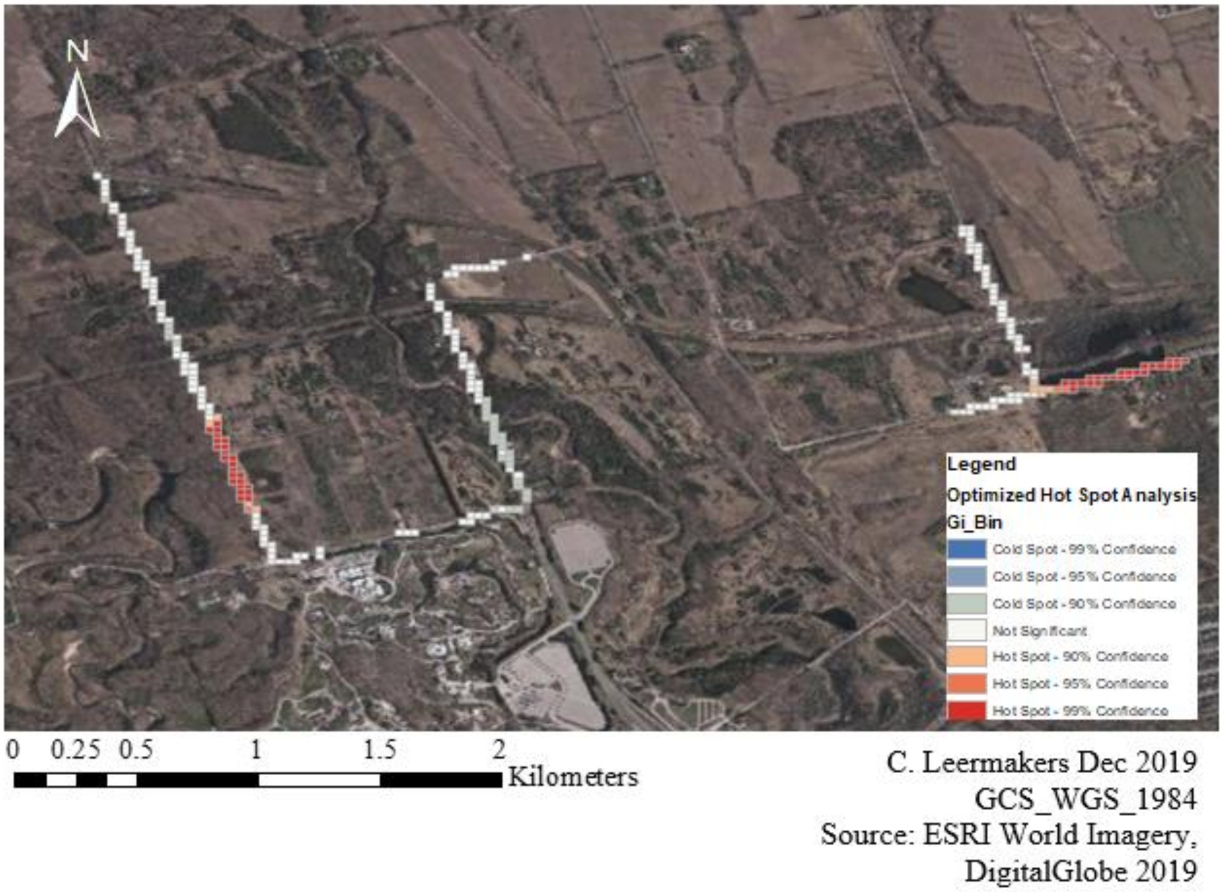
C. Leermakers Dec 2019
GCS_WGS_1984
Source: ESRI World Imagery,
DigitalGlobe 2019

**Figure 9:** Optimized Hotspot Analysis of road-mortality locations in 2017 in the vicinity of Rouge National Urban Park. Hotspot analysis identifies statistically significant clusters of high values (hotspots) and low values (coldspots), calculated using Getis-Ord Gi* statistic.



C. Leermakers Dec 2019
GCS_WGS_1984
Source: ESRI World Imagery,
DigitalGlobe 2019

**Figure 10:** Optimized Hotspot Analysis of road-mortality locations in 2018 in the vicinity of Rouge National Urban Park. Hotspot analysis identifies statistically significant clusters of high values (hotspots) and low values (coldspots), calculated using Getis-Ord Gi* statistic.



C. Leermakers Dec 2019
GCS_WGS_1984
Source: ESRI World Imagery,
DigitalGlobe 2019

**Figure 11:** Optimized Hotspot Analysis of road-mortality locations in 2019 in the vicinity of Rouge National Urban Park. Hotspot analysis identifies statistically significant clusters of high values (hotspots) and low values (coldspots), calculated using Getis-Ord Gi* statistic.

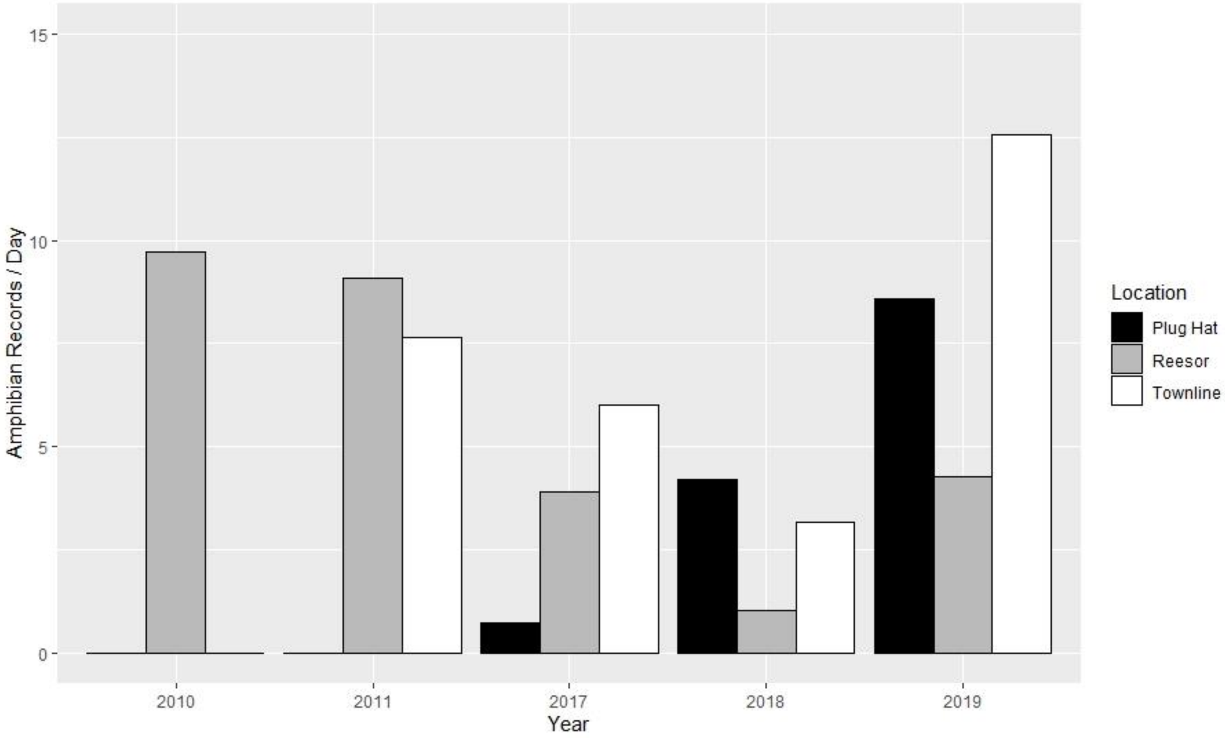


**Figure 12:** The number of dead-on-road amphibians found per day based on search effort for each year and road segment.

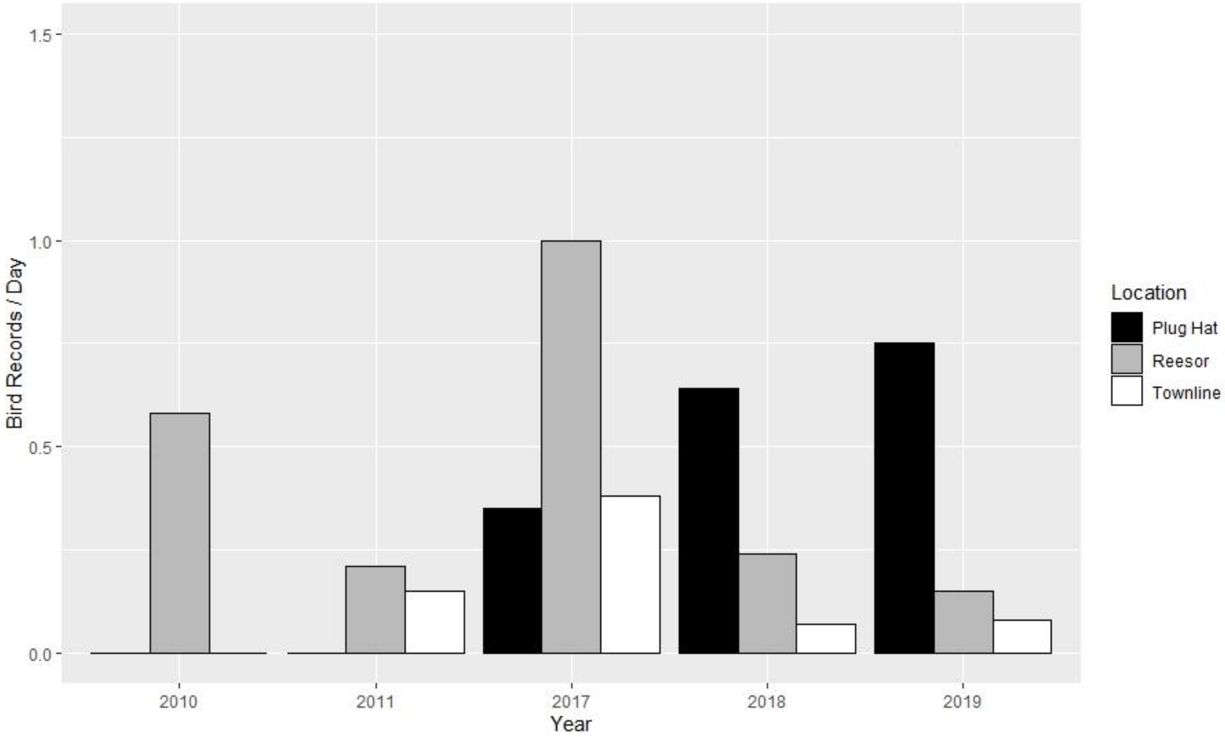


**Figure 13:** The number of dead-on-road birds found per day based on search effort for each year and road segment.

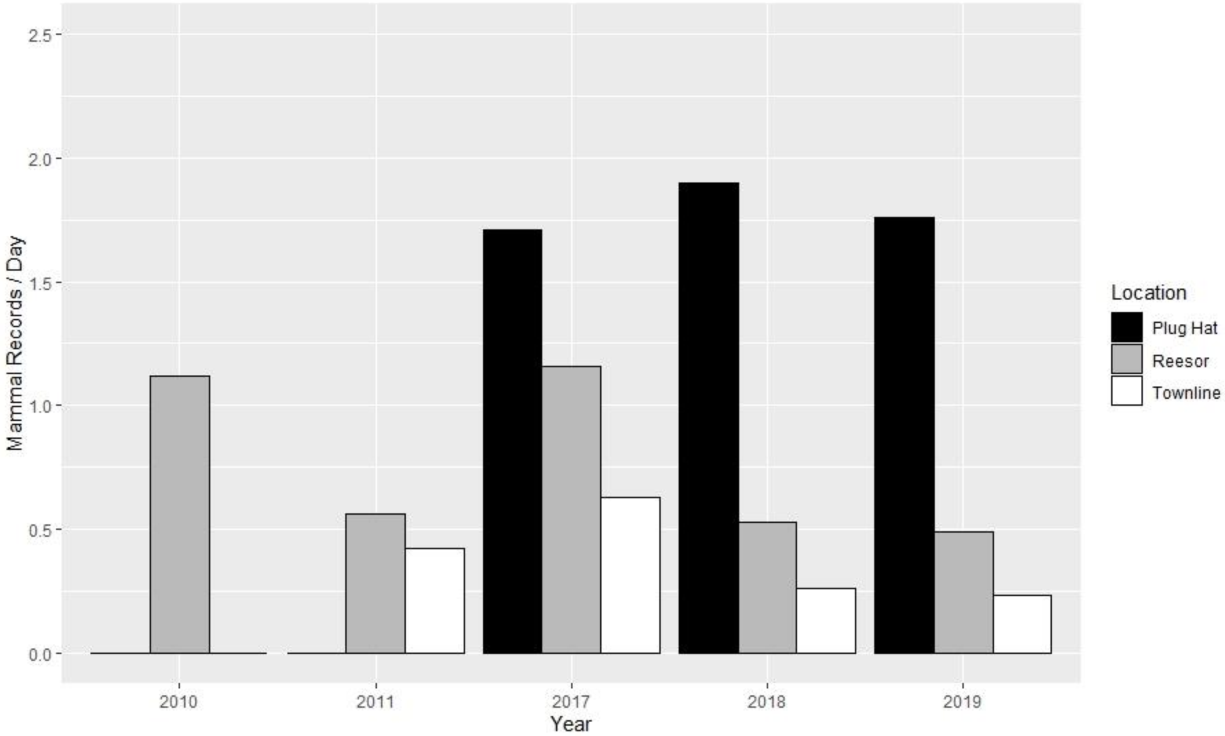


**Figure 14:** The number of dead-on-road mammals found per day based on search effort for each year and road segment.

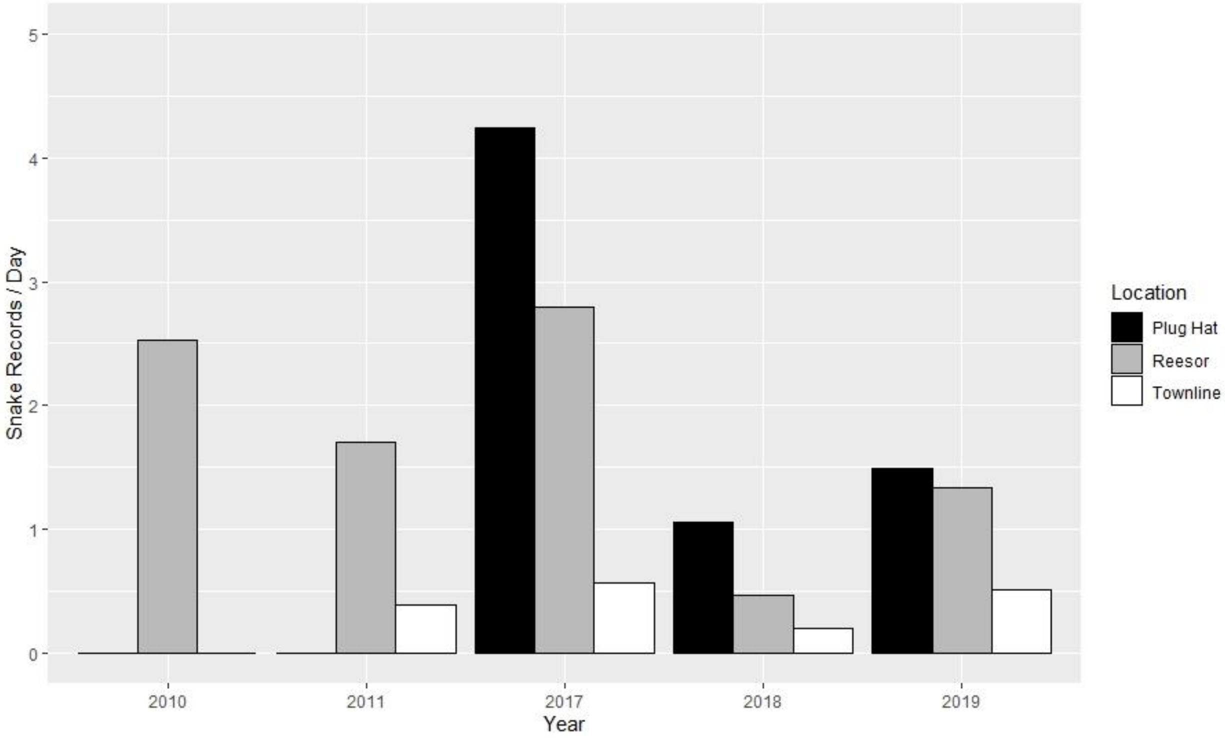


**Figure 15:** The number of dead-on-road snakes found per day based on search effort for each year and road segment.

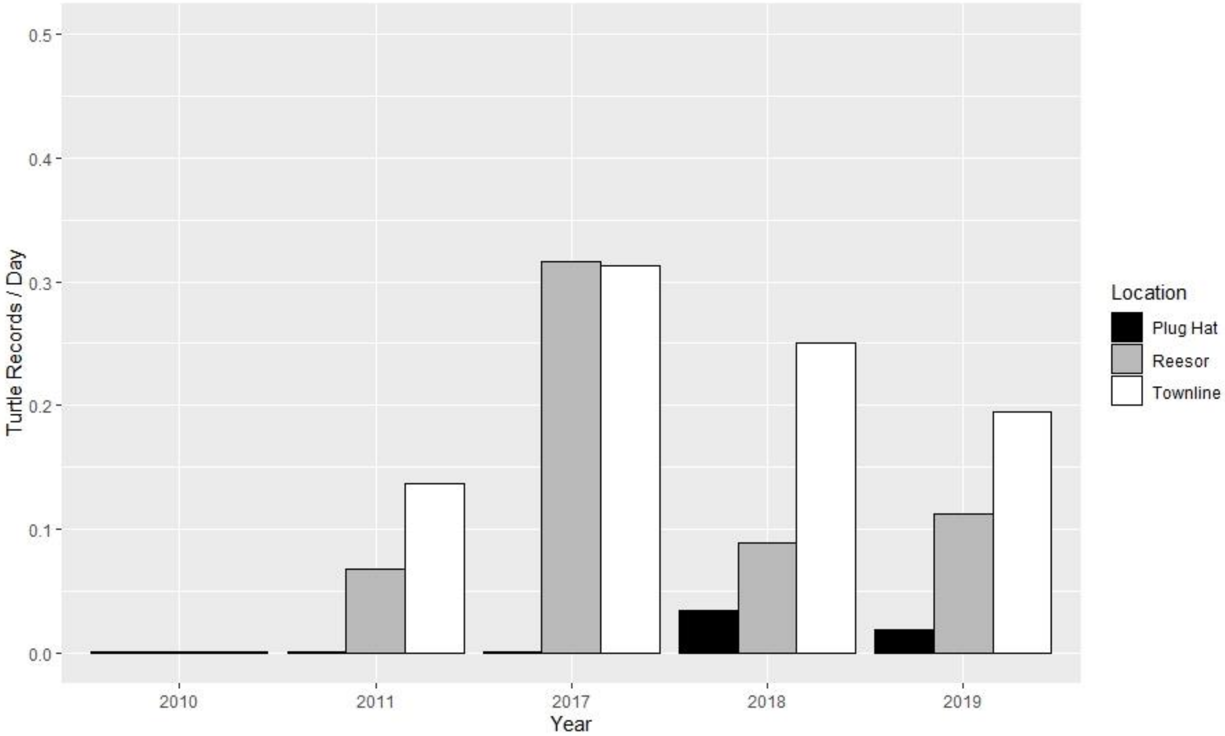


**Figure 16:** The number of dead-on-road turtles found per day based on search effort for each year and road segment.

Reesor was the busiest road, with total vehicle counts per day ranging 2140-2396 during October 6-12, 2011, and 3818-5422 during August 16-22, 2019. Plug Hat had similar data, with traffic volume ranging 2016-4791 during August 16-22, 2019. Townline had the least amount of traffic, with vehicle counts ranging 638-1103 during October 6-12, 2011, and 659-1297 during August 16-22, 2019. Across all roads and years surveyed, the highest daily counts occurred during weekdays versus weekends, indicating that these are highly used commuter roads. Additionally, summer vehicle traffic counts were higher compared to autumn (Table 4).

Across all years surveyed amphibian mortalities were located on average near forest, wetland, and field habitat. Maximum number of mortalities were found when cloud cover was 75% and in the month of July. Average precipitation was 3.16 mm and average air temperature was 22.5°C. Most amphibian mortalities were found in the afternoon, on Townline Road, and during 2019 surveys (Table 5). Bird mortalities were located on average near forest, field, and wetland habitat. Maximum number of mortalities were found when cloud cover was 0 %, and in the month of August. Average precipitation was 1.62 mm and average air temperature was

21.48°C. Most bird mortalities were found in the afternoon, on Reesor Road, and during 2018 surveys (Table 6). Mammal mortalities were located on average near forest, field, and wetland habitat. Maximum number of mortalities were found when cloud cover was 0% and in the month of June. Average precipitation was 2.51 mm and average air temperature was 21.78°C. Most mammal mortalities were found in the afternoon, on Plug Hat Road, and during 2018 surveys (Table 7). Snake mortalities were located on average near forest, field, and wetland habitat. Maximum number of mortalities were found when cloud cover was 0 % and in the month of October. Average precipitation was 2.24 mm and average air temperature was 19.4°C. Most snake mortalities were found in the morning, on Reesor Road, and during 2019 surveys (Table 8). Turtle mortalities were located on average near forest, wetland, and shallow aquatic habitats. Maximum number of mortalities were found when cloud cover was 100 % and in the month of May. Average precipitation was 1.96 mm and average air temperature was 21.23°C. Most turtle mortalities were found in the afternoon, on Townline Road, and during 2018 surveys (Table 9).

**Table 4:** Vehicle count data for October 6-12, 2011 and August 16-22, 2019 collected by Toronto Transportation for each road segment surveyed in Rouge National Urban Park.

| | **2011** | | **2019** | | |
|---|---|---|---|---|---|
| | **Reesor** | **Townline** | **Reesor** | **Plug Hat** | **Townline** |
| Monday | 2641 | 699 | 5005 | 3986 | 1199 |
| Tuesday | 2397 | 970 | 5214 | 4400 | 1282 |
| Wednesday | 2140 | 991 | 5173 | 4204 | 1297 |
| Thursday | 2396 | 1045 | 5504 | 4791 | 1266 |
| Friday | 2692 | 1103 | 5422 | 4558 | 1203 |
| Saturday | 2336 | 744 | 3818 | 2016 | 659 |
| Sunday | 2417 | 638 | 3942 | 2171 | 704 |

**Table 5:** Descriptive statistics (mean, variance inflation factor (vif), minimum and maximum values) for each predictor of road mortality for amphibian's dead-on-road in Rouge National Urban Park for all years and road segments surveyed. Variance inflation factor shows predictor variables are independent of each other, values between 1 and 5 are moderately correlated. Means that could not be calculated because they are factors are indicated by NA. Additionally, location and year were random effects and were not included in the vif analysis, indicated by NA.

| **Predictor of Road Mortality** | **Mean** | **VIF** | **Min** | **Max** |
|---|---|---|---|---|
| Distance to Agriculture (m) | 492.21 | 2.85 | 3.02 | 1261.81 |
| Distance to Field (m) | 98.86 | 1.46 | 0.00 | 339.20 |
| Distance to Forest (m) | 22.07 | 1.50 | 0.00 | 280.80 |
| Distance to Rail (m) | 398.60 | 3.16 | 0.18 | 934.55 |
| Distance to Shallow (m) | 145.34 | 2.03 | 7.04 | 591.98 |
| Distance to Wetland (m) | 49.49 | 1.40 | 0.00 | 355.09 |
| Location | NA | NA | Plug Hat | Townline |
| Cloud Cover (%) | NA | 1.38 | 100 | 75 |
| Month | NA | 1.82 | May | July |
| Precipitation (mm) | 3.16 | 1.09 | 0.00 | 58.20 |
| Temperature (°C) | 22.50 | 1.44 | 0.00 | 36.00 |
| Time | NA | 1.03 | Morning | Afternoon |
| Year | NA | NA | 2017 | 2019 |

Based on the best models produced by GLMM and GLM results, there were statistically significant predictors of road mortality (Table 10). Amphibian mortality had a positive association with morning survey times, July, and 75% cloud cover. Amphibian mortality had a negative association with distance to wetland and shallow aquatic habitat and with traffic volume for both 2011 and 2019 (Table 11). Bird mortality had a positive association with months May and June and a negative association with precipitation, wetland, field, and agriculture (Table 11). Additionally, bird presence was negatively associated with 2011 traffic volume data and positively associated with 2019 traffic volumes (Table 11). Mammal mortality was positively associated with months May and June and negatively associated with field, agriculture, and 2011 traffic volume (Table 11). Snake mortality was positively associated with months September and October, and 75% cloud cover and negatively associated with distance to railway and forest.

Snake mortality was positively associated with traffic volume in 2011 and 2019 (Table 11). Lastly, turtle mortality was positively associated with May and June, 50% and 100% cloud cover, and negatively associated with precipitation and shallow aquatic habitat. For 2011 data, turtle mortality had a positive association with traffic volume (Table 11).

**Table 6:** Descriptive statistics (mean, variance inflation factor (vif), minimum and maximum values) for each predictor of road mortality for bird's dead-on-road in Rouge National Urban Park for all years and road segments surveyed. Variance inflation factor shows predictor variables are independent of each other, values between 1 and 5 are moderately correlated. Means that could not be calculated because they are factors are indicated by NA. Additionally, location and year were random effects and were not included in the vif analysis, indicated by NA.

| Predictor of Road Mortality | Mean | VIF | Min | Max |
|---|---|---|---|---|
| Distance to Agriculture (m) | 357.40 | 3.02 | 3.50 | 1253.24 |
| Distance to Field (m) | 62.05 | 1.24 | 1.51 | 329.85 |
| Distance to Forest (m) | 25.97 | 1.69 | 0.00 | 167.46 |
| Distance to Rail (m) | 332.70 | 2.96 | 0.91 | 931.21 |
| Distance to Shallow (m) | 220.50 | 1.34 | 7.84 | 525.29 |
| Distance to Wetland (m) | 64.39 | 1.51 | 0.00 | 348.17 |
| Location | NA | NA | Townline | Reesor |
| Cloud Cover (%) | NA | 1.36 | 50 | 0 |
| Month | NA | 1.74 | October | August |
| Precipitation (mm) | 1.62 | 1.12 | 0.00 | 31.60 |
| Temperature (°C) | 21.48 | 1.45 | 0.00 | 34.00 |
| Time | NA | 1.07 | Morning | Afternoon |
| Year | NA | NA | 2011 | 2018 |

**Table 7:** Descriptive statistics (mean, variance inflation factor (vif), minimum and maximum values) for each predictor of road mortality for mammal's dead-on-road in Rouge National Urban Park for all years and road segments surveyed. Variance inflation factor shows predictor variables are independent of each other, values between 1 and 5 are moderately correlated. Means that could not be calculated because they are factors are indicated by NA. Additionally, location and year were random effects and were not included in the vif analysis, indicated by NA.

| Predictor of Road Mortality | Mean | VIF | Min | Max |
|---|---|---|---|---|
| Distance to Agriculture (m) | 489.09 | 3.46 | 1.56 | 1257.10 |
| Distance to Field (m) | 64.44 | 1.39 | 0.00 | 331.42 |
| Distance to Forest (m) | 26.25 | 1.67 | 0.00 | 196.41 |
| Distance to Rail (m) | 383.04 | 3.41 | 2.41 | 934.75 |
| Distance to Shallow (m) | 200.84 | 1.58 | 6.00 | 528.82 |
| Distance to Wetland (m) | 75.67 | 1.51 | 0.00 | 344.14 |
| Location | NA | NA | Townline | Plug Hat |
| Cloud Cover (%) | NA | 1.38 | 50 and 75 | 0 |
| Month | | 1.81 | October | June |
| Precipitation (mm) | 2.51 | 1.10 | 0.00 | 58.20 |
| Temperature (°C) | 21.78 | 1.43 | 0.00 | 36.00 |
| Time | NA | 1.05 | Morning | Afternoon |
| Year | NA | NA | 2017 | 2018 |

**Table 8:** Descriptive statistics (mean, variance inflation factor (vif), minimum and maximum values) for each predictor of road mortality for snake's dead-on-road in Rouge National Urban Park for all years and road segments surveyed. Variance inflation factor shows predictor variables are independent of each other, values between 1 and 5 are moderately correlated. Means that could not be calculated because they are factors are indicated by NA. Additionally, location and year were random effects and were not included in the vif analysis, indicated by NA.

| **Predictor of Road Mortality** | **Mean** | **VIF** | **Min** | **Max** |
|---|---|---|---|---|
| Distance to Agriculture (m) | 524.49 | 2.91 | 3.30 | 1259.12 |
| Distance to Field (m) | 58.48 | 1.30 | 0.00 | 333.03 |
| Distance to Forest (m) | 22.16 | 1.45 | 0.00 | 181.89 |
| Distance to Rail (m) | 362.67 | 2.97 | 0.03 | 935.22 |
| Distance to Shallow (m) | 199.47 | 1.57 | 8.09 | 518.31 |
| Distance to Wetland (m) | 62.54 | 1.44 | 0.00 | 358.75 |
| Location | NA | NA | Townline | Reesor |
| Cloud Cover (%) | NA | 1.43 | 75 | 0 |
| Month | NA | 1.83 | June | October |
| Precipitation (mm) | 2.24 | 1.14 | 0.00 | 58.20 |
| Temperature (°C) | 19.40 | 1.43 | 0.00 | 34.50 |
| Time | NA | 1.05 | Afternoon | Morning |
| Year | NA | NA | 2018 | 2019 |

**Table 9:** Descriptive statistics (mean, variance inflation factor (vif), minimum and maximum values) for each predictor of road mortality for turtle's dead-on-road in Rouge National Urban Park for all years and road segments surveyed. Variance inflation factor shows predictor variables are independent of each other, values between 1 and 5 are moderately correlated. Means that could not be calculated because they are factors are indicated by NA. Additionally, location and year were random effects and were not included in the vif analysis, indicated by NA.

| **Predictor of Road Mortality** | **Mean** | **VIF** | **Min** | **Max** |
|---|---|---|---|---|
| Distance to Agriculture (m) | 350.58 | 2.43 | 17.51 | 1254.04 |
| Distance to Field (m) | 124.06 | 2.30 | 6.51 | 327.41 |
| Distance to Forest (m) | 10.75 | 1.16 | 0.00 | 128.77 |
| Distance to Rail (m) | 299.44 | 3.49 | 3.05 | 910.58 |
| Distance to Shallow (m) | 102.19 | 1.83 | 11.39 | 300.89 |
| Distance to Wetland (m) | 42.28 | 1.33 | 0.85 | 201.41 |
| Location | NA | NA | Plug Hat | Townline |
| Cloud Cover (%) | NA | 1.97 | 75 | 100 |
| Month | NA | 2.17 | October | May |
| Precipitation (mm) | 1.96 | 1.25 | 0.00 | 18.50 |
| Temperature (°C) | 21.23 | 1.45 | 0.00 | 36.00 |
| Time | NA | 1.17 | Morning | Afternoon |
| Year | NA | NA | 2017 | 2018 |

**Table 10:** Models using wildlife road-mortality data from Rouge National Urban Park, surveyed 2010, 2011, 2017, 2018, and 2019 by taxonomic group. Models were chosen based on the best AIC value for GLMM (glmer) and GLM (glm) for each taxonomic group. Additional separate models for only 2011 and 2019 data were completed as traffic data were only surveyed during those years.

| **Package Type** | **Taxa** | **Best Model** | **AIC** |
|---|---|---|---|
| glmer | Amphibian | Number_DOR ~ Time + Month + Cloud + Precip + Rail + Wetland + Field + Agri+Shallow_Aquatic + Forest + (1\|Year) + (1\|Location) | 5557.222 |
| glm | Amphibian 2011 | Number_DOR ~ Time+Traffic +Temp +Cloud + Precip + Rail + Wetland + Field +Forest | 1211.069 |
| glmer | Amphibian 2019 | Number_DOR ~ Time+Traffic+Temp + Cloud + Precip +Wetland + Agri+Shallow_Aquatic+Forest + (1\|Location) | 1852.392 |
| glmer | Bird | Number_DOR ~ Month + Precip + Rail +Wetland + Field + Agri + Shallow_Aquatic + (1\|Year) + (1\|Location) | 1605.857 |
| glm | Bird 2011 | Number_DOR ~Traffic+Wetland+ Shallow_Aquatic | 242.187 |
| glm | Bird 2019 | Number_DOR ~ Traffic+Month+Precip+Agri+Shallow_Aquatic | 457.586 |
| glmer | Mammal | Number_DOR ~Month + Cloud + Wetland + Field + Agri+Shallow_Aquatic + Forest + (1\|Year) + (1\|Location) | 3190.341 |
| glmer | Mammal 2011 | Number_DOR ~ Traffic + Temp + Rail +Shallow_Aquatic + (1\|Location) | 560.592 |
| glmer | Mammal 2019 | Number_DOR ~ Traffic + Temp +Cloud+ Rail +Wetland+Field+Forest+ (1\|Location) | 998.271 |
| glmer | Snake | Number_DOR ~ Month + Cloud + Precip + Rail + Wetland + Shallow_Aquatic + Forest + (1\|Year) + (1\|Location) | 3854.070 |
| glm | Snake 2011 | Number_DOR ~ Time+Traffic + Temp + Cloud + Precip + Field + Agri | 800.190 |
| glm | Snake 2019 | Number_DOR ~ Time+Traffic +Temp + Cloud + Precip + Rail +Wetland + Field + Shallow_Aquatic | 1302.405 |
| glmer | Turtle | Number_DOR ~Temp+Month + Cloud + Precip+Shallow_Aquatic +(1\|Year) +(1\|Location) | 680.970 |
| glm | Turtle 2011 | Number_DOR ~ Traffic+Temp+Wetland | 161.294 |
| glmer | Turtle 2019 | Number_DOR ~ Traffic+Temp+Cloud+Wetland+Forest + (1\|Location) | 254.370 |

**Table 11:** Results of GLMM by taxonomic group using road-mortality data from Rouge National Urban Park, surveyed 2010, 2011, 2017, 2018, and 2019. Showing predictor variables included in the model, their significance to the taxonomic group response variable, and the affect as positive or negative on the response variable. Blank cells represent variables not included in the model based on best model AIC values. Results that are significant include p-value or labeled as not significant if p>0.05.

| Predictor Variables | Amphibian | Bird | Mammal | Snake | Turtle |
|---|---|---|---|---|---|
| Time | Morning (+) | NA | NA | NA | NA |
| Temperature (°C) | NA | NA | NA | NA | Not significant |
| Month | May (-), June (-), July (+), Oct (-) | May (+), June (+), Sept (-), Oct (-) | June (+) and May (+) | June (-), July (-), Sept (+), Oct (+) | May (+) and June (+) |
| Cloud (%) | Cloud75 (+) | NA | Not significant | Cloud100 (-) | Cloud 50 (+) and 100 (+) |
| Precipitation (mm) | Not significant | 6.34E-02 (-) | NA | Not significant | 4.72E-02 (-) |
| Rail (m) | 5.07E-04 (+) | 8.55E-03 (+) | NA | 1.58E-10 (-) | NA |
| Wetland (m) | 9.77E-12 (-) | 3.71E-03 (-) | 2.19E-04 (+) | 7.98E-08 (+) | NA |
| Field (m) | Not significant | 2.99E-02 (-) | 3.51E-03 (-) | NA | NA |
| Agriculture (m) | 1.43E-09 (+) | 1.34E-15 (-) | 4.87E-04 (-) | NA | NA |
| Shallow Aquatic (m) | 2.00E-16 (-) | 2.47E-12 (+) | 7.50E-07 (+) | 3.98E-04 (+) | 2.08E-03 (-) |
| Forest (m) | 1.29E-05 (+) | NA | 8.98E-03 (-) | 9.76E-03 (-) | NA |
| Traffic Volume 2011 | 2.48E-02 (-) | 2.16E-03 (-) | 1.69E-02 (-) | 8.20E-05 (+) | 1.44E-02 (+) |
| Traffic Volume 2019 | 1.26E-03 (-) | 5.11E-03 (+) | Not significant | 1.46E-02 (+) | Not significant |

# Discussion

Rouge National Urban Park has a high number of wildlife road mortalities as commuter roads intersect natural areas, creating barriers to movement for amphibians, birds, mammals, snakes, and turtles. This high number of road mortalities, especially amphibians with the highest mortality, can be compared to similar studies conducting road mortality surveys in protected parks (Stinnissen, 2015). The goal of this chapter was to determine variables that predict wildlife mortality hotspots to inform the location of an ecopassage. Results confirmed that road mortality is influenced by neighboring landscape features, traffic volume, distance to nearest railway, seasonal movement, and weather.

Amphibians have been frequently documented to face threats of mass road mortality because of there low car avoidance ability and large natal dispersal events. This study has similarly documented large numbers of amphibian mortalities. I found that amphibian mortality was positively associated with morning road surveys. It has been documented that frogs and salamanders do not persist on the roads long because they get picked up by scavengers, repeatedly get run over by cars, or get washed away or dried up depending on weather conditions (Santos et al., 2011). Amphibian road mortality was negatively associated with distance to wetland and shallow aquatic habitats. This is consistent with numerous studies that found that roads intersecting wetland habitats have higher numbers of amphibian road mortality (Langen et al., 2007; Stinnissen, 2015). Amphibians rely on wetlands for the developmental stages of their life cycle, including mating, oviposition, and larval growth. After breeding season, adults migrate to upland habitat and, following metamorphosis, juveniles emigrate to terrestrial habitat, putting themselves at risk of road mortality (Semlitsch et al., 1996). In my study, amphibian road mortality was significantly associated with July. In other studies, Northern Leopard Frogs (*Lithobates pipiens)* had a significant increase in road mortality during July, when they complete metamorphosis and travel to upland habitat (Garrah et al., 2015; Langen et al., 2009). Langen et al. (2007) found that frog mortality was low during May-June, peaked in July, and remained high until the animals became inactive in late October (Langen et al., 2007). Precipitation was not a significant factor, contrary to other studies finding that juvenile amphibian movement would increase following precipitation (Gravel et al., 2012). However, 75% cloud cover was positively associated with road mortality, which was likely related to rain events.

Birds are regularly hit by vehicles because they are highly mobile moving between available resources and annual migrations. Bird mortality were negatively associated with distance to wetlands, fields, and agriculture. Mortality increases in close proximity to wetlands, as many species nest and migrate to wetland habitat (Kociolek et al., 2011). Similar studies have found an association between bird mortality and fields because collisions can increase as they fly low towards low cover on the adjacent side of the road (Barthelmess, 2014; Kociolek et al., 2011). Traffic volume was negatively associated with birds in 2011 and positively associated in 2019. This could be the result of seasonal difference in traffic volume data collection, because October 2011 traffic was lower compared to August 2019. Similar studies found that there was no association between bird mortality and traffic volume, as birds have good car avoidance (Lepczyk et al., 2019). The positive association between bird mortality in May and June coincides with nesting and fledgling periods. Fledglings may have lower crossing success and experience compared to adults, increasing there risk of road mortality (Garrah et al., 2015). Precipitation was negatively associated with mortality, showing that birds were frequently hit by cars when there was little to no precipitation. This is similar to studies that have documented that birds are less active when it rains as it influences visibility and orientation (Erni et al., 2002).

Mammals are a commonly studied group for road ecology because they are commonly seen crossing roads and larger mammals are a danger to humans if hit. Mammals were negatively associated with distance to field, agriculture, and forest habitats. Distance to field and agriculture had a significant negatively associated with mammal mortality as small mammals, including mice and voles, rely on grassland habitats (Dickman & Doncaster, 1987). Similarly, studies have found a strong association of mammal road mortality and forests as they are common habitat types for large mammals, such as raccoons, and provide immediate cover adjacent to roads (Barthelmess, 2014; Carvalho & Mira, 2011). Traffic volume was negatively associated with mammal mortality, likely because mammals have a high car avoidance, because of their fast crossing speeds (Fahrig & Rytwinski, 2009). This study found that mammal road mortality was positively associated with the months of May and June, because of increased movement during breeding and juvenile dispersal. In addition, females are more active gathering resources for there young (Červinka et al., 2015).

Snakes are threatened by roads because they have a low car-avoidance ability and increased movement when returning to hibernation sites. Snake road mortality was negatively associated with distance to rail and forest features. Railways have been documented to be barriers for snakes, forcing them to cross over roads, putting them at risk of vehicle collision (Santos et al., 2017). Relationships are evident between forest habitat and snake road mortality because they are a primary habitat for many species. For example, a study investigating microhabitat of Dekay's Brownsnake, *Storeria dekayi*, found that snakes were located near forest edges and under log debris (Hecnar & Hecnar, 2011). Traffic volume was positively associated with snake road mortality for both 2011 and 2019, because they have slow crossing time and have been documented to stop when cars approach (Andrews & Gibbons, 2005; Stinnissen, 2015). Snake road mortality was positively associated with the months September and October. Snakes are often strongly associated with autumn peaks in mortality as they return to hibernaculum sites (Garrah et al., 2015). There was no association with spring spikes when snakes leave hibernaculum sites. This could be the result of snakes emerging over an extended period of time, as it is documented that snakes will begin to emerge once temperatures increase in April and they are slow and sluggish as they need time to thermoregulate and begin mating (Brown et al., 1974). Snake road mortality was negatively associated with 100% cloud cover, as snakes are more active on warmer, sunny days because they are ectothermic (Andrews & Gibbons, 2005; Stinnissen, 2015).

Turtles are documented to have increased road mortality during nesting and mating season and have low car avoidance. Turtle road mortality had a negative association with distance to shallow aquatic habitat, as studies have documented that after mating and nesting season, turtles spend majority of their time in aquatic systems (Rowe & Dalgarn, 2010). There was a positive association between traffic volume and turtle mortality because they have slow crossing speeds and have been documented to stop when cars approach (Gooley & Pauley, 2010). Turtle road mortality was positively associated with months May and June, during nesting and mating season, when they are most active. Turtles will travel up to 1 km during nesting and mating season and will nest in gravel shoulders along roads, making nesting adults and hatchlings vulnerable to road mortality (Langen et al., 2009; Rowe & Dalgarn, 2010; Steen et al., 2006). Both 50% and 100% cloud cover were positively associated with turtle mortality, contrary to other studies documenting limited movement during cloudy days because of decreased heat for muscle activity (Rowe & Dalgarn, 2010). In contrast, precipitation had a negative association with turtle mortality, showing that turtles were more active during low or no precipitation (Rowe & Dalgarn, 2010; Stinnissen, 2015). This contrast was similarly found in a study examining spatial and temporal influences of road mortality using road survey data that documented, after June, turtles were found following a day of 3 mm of rainfall (Garrah et al., 2015).

The Optimized Hotspot Analysis, which shows statistically significant clusters of road mortalities, identified two main hotspots, one on Reesor and Townline. Reesor and Townline hotspot locations varied slightly in size and location during all years surveyed. Additionally, the Reesor road hotspot was not identified in 2018. Identified hotspot locations were surrounded by large marsh and swamp ecosystems and, with GLMM results, it can be concluded that landscape characteristics influence road mortality. These results are important in illustrating the consistency of hotspot locations, as they remained generally the same throughout an eight-year period. Coldspots were also identified along Plug Hat from 2018-2019, near residential housing and a large bridge crossing over Little Rouge Creek. This bridge could be acting as an ecopassage, resulting in sparse clustering of wildlife road mortality. For example, a study found no significant association between bridges and mammal road mortality with the nearest bridges 3.4-4.4 km from mortality locations (Barthelmess, 2014). Another study investigated the installation of eco-fencing along pre-existing bridges, and documented turtles crossing under bridges using camera traps (Lake Simcoe Region Conservation Authority, 2018). Similar studies have completed Optimized Hotspot Analysis using the Getis-Ord Gi* statistic and found significance of hot and cold spots. For example, Garrah et al (2015) completed road surveys from 2008 to 2011 identifying amphibians, bird, mammals, snake, and turtle road mortality along a 37 km road that runs adjacent to the St. Lawrence River (Garrah et al., 2015). Using the Getis-Ord Gi* statistic, they found that the general location of significant hotspots did not vary between years surveyed. However, similar to my study, significant hotspots were not identified in some years (Garrah et al., 2015). This highlights the importance of collecting long-term data to identify the location of hotspots to inform placement of mitigation structures.

There were yearly fluctuations of road mortality between road segments and taxonomic groups that may be influenced by population abundances, which can vary annually due to varying environmental factors. One common trend was the lowest number of snake mortalities in 2018 on all road segments surveyed. This could be related to overall variation in snake abundance, which is influenced by a variety of factors including food supply that controls clutch and litter size. For example, in Ring-necked Snake, *Diadophis punctatus,* it has been documented that clutch size and rainfall were correlated, because their diet mainly consists of earthworms, which depend on soil surface moisture. Similarly, Dekay's Brownsnake, *Storeria dekayi*, which had low mortality numbers in 2018, eat an invertebrate diet, including insects, slugs, and earthworms. Typically, snake populations consist of newly mature individuals, and, therefore, poor clutch size in the previous year could have influenced 2018 abundance (Seigel & Fitch, 1985). Amphibian mortality was also lowest in 2018 on Reesor and Townline. Amphibian population abundance depends on food availability for larvae, temperature tolerance, predator avoidance, and length of the larval period (Semlitsch et al., 1996). Overall, there are many factors to consider that could have affected amphibian and snake numbers in 2018 and these low mortality numbers is likely the reason for Reesor losing its hotspot during this survey year. Turtle mortality was lowest for Reesor and Townline in 2011, which indicates turtle mortality increased from 2011 to 2019, which could be the result of the newly built Reesor Wetland, in 2008. The newly installed Reesor Wetland provides ideal habitat for freshwater turtles, attracting turtles to travel between neighboring wetlands and putting them at risk of mortality.

Overall, there was significant evidence for predictable spatial and temporal patterns of wildlife road mortality. These results were consistent with the literature showing different taxonomic groups can be influenced by different landscape features, traffic volume, distance to railways, seasonal movement, and weather. Road mortality for turtles and amphibians was influenced by aquatic habitat features, wetland and field habitats for birds, field and forest habitats for mammals, and railway tracks and forest habitat for snakes. These landscape features are important wildlife resources for reproduction and overall fitness. Traffic volume had a significant affect on snakes and turtles because they move slowly across roads and have been documented to stop when cars approach. Seasonal movement was a significant factor as wildlife have peak movement times. Amphibians were vulnerable during July after natal dispersal, birds in June during fledging season, mammals in summer when they are most active, snakes in autumn as they return to hibernation sites, and turtles in May and June during mating and nesting season. Air temperature did not have a significant affect on any taxonomic group surveyed, however, precipitation and cloud cover were significant. Birds and turtles were found when there was little precipitation, amphibians and snakes had a positive association with 75% cloud cover, and turtles had a positive association with 50% and 100% cloud cover. The hotspot analysis showed the consistency of hotspot location and size, with some variability between years, indicating the importance of long-term data to inform the placement of road-mortality mitigations. This research illustrates the usefulness of road-mortality data to predict wildlife road-mortality hotspots and will help better inform the placement of a future ecopassage in Rouge National Urban Park.

# Chapter Three: Experimental Evaluation of PIT-tag Detection Rates in Freshwater Turtles based on Tagging Location, Antenna Type, and Environment

## Abstract

Road mortality has been documented as one of the major threats facing freshwater turtles. One method to mitigate road mortality is building an ecopassage and fencing to provide safe passage. After the installation of an ecopassage, a common tool used to assess its efficacy is PIT-tagging wildlife, such as freshwater turtles, and strategically placing antennae at the entrances to the ecopassage to determine use by tagged wildlife. However, there is a lack of research that examines the detection success of tags, thus hampering the development of best-practice recommendations. Therefore, by completing arena experiments, this study investigated how PIT-tag placement, antenna type, environmental conditions, turtle orientation, and tag orientation influenced detection success. Overall, pass-over antenna, external placements (top plastron and bridge), and vertical tag orientation had significantly higher detection then pass-through, internal placement and horizontal orientation.

## Introduction

Freshwater turtles are often threatened by roads because of their increased movement during nesting and mating season, late maturity, and frequent nesting along the shoulder of roads (Aresco, 2005; Fahrig & Rytwinski, 2009; Steen et al., 2006). Common mitigation strategies that allow safe movement for turtles include ecopassages and fencing (Markle et al., 2017). Once an ecopassage is installed, it is important to test its successful use by turtles. The currently accepted technique involves injecting a PIT tag into the left hind-leg pit, giving the turtle a unique identification number. Once injected, an antenna is strategically placed within an ecopassage to detect tagged turtles passing through it (Markle et al., 2017). The insertion of a PIT-tag in the left hind-leg pit has become the accepted norm since Buhlmann and Tuberville (1998) tested the injection of tags into young Red-eared Sliders, *Trachemys scripta elegans* (Buhlmann & Tuberville, 1998). Internal tagging has many benefits as it confirms an individual's unique identity, is permanent, and can be read by automated systems. However, the accepted technique is invasive and, if not injected properly, could result in tag loss or mortality (Gibbons &

Andrews, 2004). To date, no studies have investigated alternative external tag placements or options for constructing an automated tag reader that will yield the highest detection success. However, recent studies have used both external placement with a pass-through antenna (Markle et al., 2017) and internal placement with a pass-over antenna (Baxter-Gilbert et al., 2015) for freshwater turtle detection in an ecopassage.

Turtles are threatened by roads because they intersect primary habitat, and their vulnerability increases during peak movement times (Steen et al., 2006). In a study tracking the movement of 52 Blanding's Turtles (*Emydoidea blandingii),* each turtle crossed roads, on average, twice in a season, with one turtle making 12 crossings (Refsnider & Linck, 2012). This puts turtles at risk as they travel to mate, nest, or between available resources. Turtle populations are also more vulnerable to losing an adult in the population because they have a low hatchling survival rate and late maturity (Enneson & Litzgus, 2008). With road mortality a significant threat for freshwater turtles, mitigation is an important tool to allow safe passage. For studies investigating the successful use of ecopassages by turtles, constructing antenna readers within or outside of the passage has become a common method for identifying individuals using the structure. With these data, researchers can make important conclusions about ecopassage use and future mitigation requirements.

PIT tags have become a common tool used to investigate freshwater turtle movement and study the efficacy of ecopassage placement (Markle et al., 2017). The widely used method of these tags is internal injection, however few studies have investigated the effectiveness of different tag placements. External placement is a non-invasive method, which allows easy identification of individuals in a study. Additionally, external placement would prevent a turtle from accidently being internally tagged twice, if the tag reader did not correctly pick up the already inserted tag. Internal tags can migrate away from the tagging location on the body, which could cause issues with the researcher not able to detect the tag (Camper & Dixon, 1988). If a turtle was injected with a tag twice, this could interfere with body function, result in death, or the tags may not be read by an antenna because of tag collision (Gibbons & Andrews, 2004; Oregon RFID Inc., 2018). External tags would also be more appropriate for short-term studies, so that turtles are not living with an internal tag for their lifetime. When constructing an automated tag reader, there are two common construction designs: pass-over, which is laid flat to allow an animal to walk or swim over the antenna; or, pass-through, which sits upright and is placed on its long edge to allow animals to travel through. Currently, no studies have evaluated the effectiveness of antenna type for freshwater turtle detection, however both antenna types have been used in recent studies (Baxter-Gilbert et al., 2015; Markle et al., 2017). Other variables that could disrupt the reading of tags include environmental conditions, such as water depth, and the tag or turtle orientation as it has been documented that orientation of the tag to the antenna can influence detection rate (Connock et al., 2019; Oregon RFID Inc., 2018).

In this chapter, I investigate PIT-tag placement on freshwater turtles, antenna type, and environment to determine the optimal conditions for highest detection success. Three experimental setups were completed for live Midland Painted Turtle (*Chrysemys picta marginata*), live Blanding's Turtle (*Emydoidea blandingii*), Western Painted Turtle (*Chrysemys picta bellii)* cadavers, and Painted Turtle (*Chrysemys picta*) decoys. The live trials tested tag placement (external and internal), antenna type (pass-through and pass-over), and different environments (wet and dry). Cadaver trials tested these variables and additionally tested turtle orientation entering the antenna (60°, 90°, 120°) and tag orientation (vertical and horizontal) in a dry environment, and decoy trials tested these variables in a wet environment. Therefore, my study aims to determine how PIT-tag placement, antenna type, environmental conditions, turtle orientation and tag orientation influence detection rate, read distance to the antenna, and number of detections.

# Methods

## Study Area and Study Species

To determine PIT-tag placement, antenna type, environmental conditions, turtle orientation and tag orientation with the highest detection success, three response variables were measured to assess detection success including, detection rate, which provides the percentage of successful detections, distance to antenna identifying the read range for the tag, and number of detections showing the reliability of the antenna (Oregon RFID Inc., 2018). Three arena experiments were conducted at the Toronto Zoo, to determine PIT-tag placement, antenna type, environmental conditions, turtle orientation and tag orientation with the highest detection success. The goal of the first arena experiment was to test tag placement, antenna type, and environmental conditions with the highest detection success for two species of native, at-risk freshwater turtle species. The second arena experiment used Western Painted Turtle, *Chrysemys picta bellii*, cadavers to manipulate the orientation that the turtle entered the antenna to test how turtle orientation affects detection success. The third arena experiment tested Painted Turtle decoys, to simulate a turtle floating in deeper water (13 cm) with different turtle and tag orientations.

The first arena experiment used one of the Toronto Zoo's education turtles, a male Blanding's Turtle, which has a TROVAN, ID-100A 11m tag inserted in the left hind-leg pit, and four Midland Painted Turtles (three females and one male) from the Americas Pavilion, which had not been previously injected with an internal PIT tag. Out of the four Midland Painted Turtles, two were inserted with a 12mm HDX tag internally and two were tagged externally, and each turtle kept the same PIT tag for all their trials (Figure 17) (Table 12). Each turtle was placed in a large tub (1.6 m long by 0.6 m wide by 0.5 m deep) and left to naturally cross two types of antennae constructed using an Oregon RFID Multi-Antenna HDX Reader (Oregon RFID Inc., 2018). A pass-through antenna was a 0.5 m by 0.25 m rectangle, made of a 14AWG wire wrapped around five times in a corrugated plastic sheet to prevent noise and create structure and placed upright on its long edge. A pass-over loop was a 14AWG wire wrapped five times in a circular shape with a diameter of 0.5 m and laid flat (Figure 18). To evaluate read range for each antenna, the distance from the antenna at which a 12 mm HDX tag was first read, facing perpendicular towards the centre of the antenna, towards the long edge of the antenna, and 45 degrees towards the antenna, was tested as recommended by Oregon RFID (Oregon RFID Inc., 2018) (Table 13). A measuring tape was used to record the turtle's distance from the antenna when the tag was first read by the receiver.

The second arena experiment included four cadaver sub-adult Western Painted Turtles, one was inserted with a 12mm HDX tag and the remaining three cadavers were used to test external tag placements. The internal placement used the same PIT tag and external placement was tested with two different tags for each, bridge and top plastron placement (Table 12). Trials were conducted in the same tub, with the pass-through and pass-over antennae tested separately. However, for the cadavers to cross, they were placed on a 24 cm by 20 cm vapor barrier sheet 20 cm from the antennae and pulled towards the antenna using a string at a rate of 0.05 m/sec (Gooley & Pauley, 2010; Walker, 1971; Zani & Claussen, 2006). Additionally, cadaver trials tested external tag placements at both vertical and horizontal orientations on the turtle's shell. Vertical orientations were perpendicular, and horizontal parallel, to the antenna. Also, three different turtle orientations were tested for the turtle entering the antenna, 60°, 90°, and 120° (Figure 19). The cadaver position started 20 cm from the antenna and ended once the back of the shell crossed over the width of the pass-through antenna or over the first loop of the pass-over antenna. Number of detections was recorded for cadaver and decoy trials to document the number of times the tag was picked up by the receiver to test reliability.

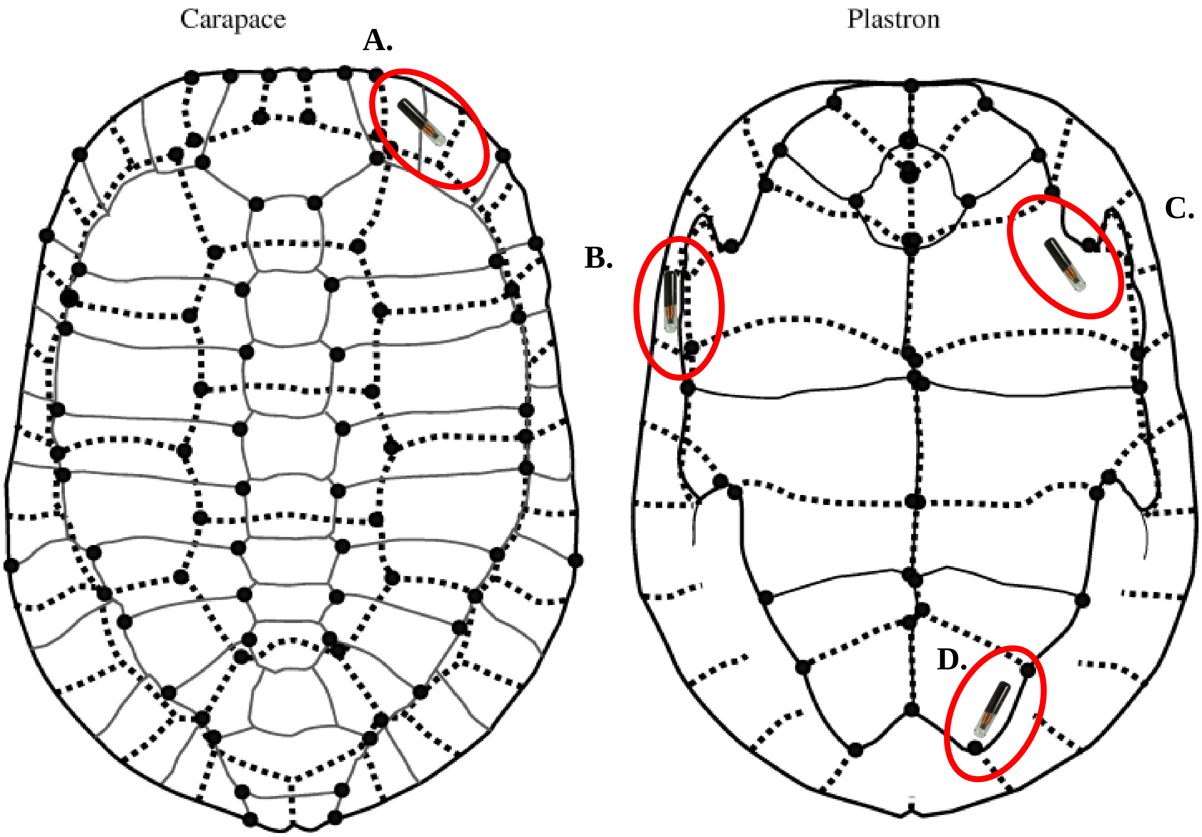


**Figure 17:** External PIT tag placement for experimental trials, **A.** top right corner of the carapace (notch 0-2) **B.** center of the right bridge (notch 0-6) **C.** top left corner of plastron (humeral scute) **D.** left back corner of plastron (anal scute) and internal injection left hind-leg pit. (from Claude et al., 2003)

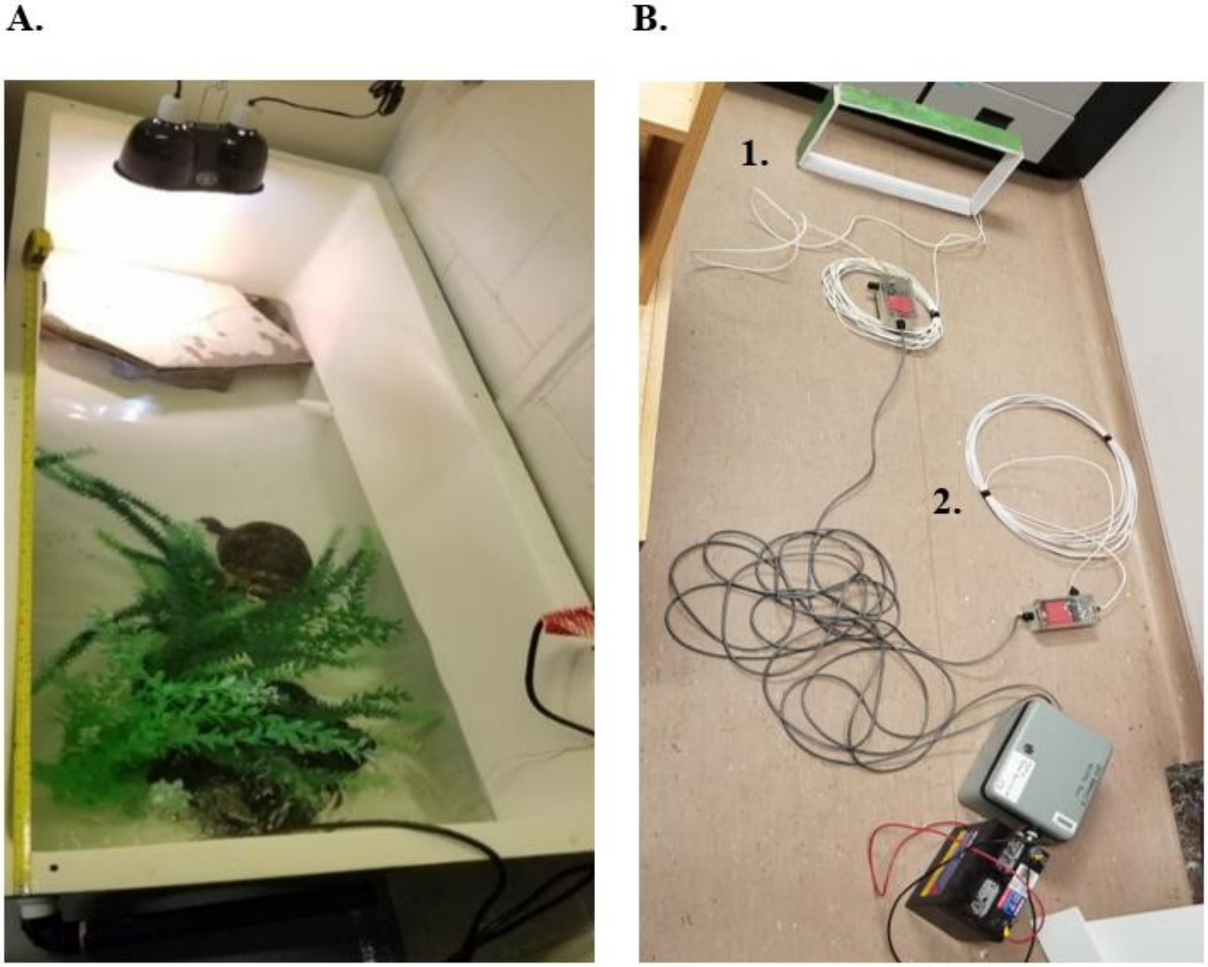


**Figure 18: A.** Tub used for the PIT-tag detection experiment and **B.** antennae setup for reading PIT tags. 1. Pass-through antenna. 2. Pass-over antenna. The receiver is powered by a 12 Volt battery attached to the receiver using jumper cables. A 20 AWG stranded wire twisted pair with a shield connects the receiver to the tuner and the antenna extends from the tuner using standard 14 AWG wire.

**Table 12:** PIT-tag number, the placement each turtle tested, gender, and morphological measurements for all experiment species.

| Turtle | PIT Tag Number | Placement Tested | Gender | Carapace Length (cm) | Height (cm) |
|---|---|---|---|---|---|
| PATU 1 | 900226000301864 | Internal | Female | 14.5 | 5.1 |
| PATU 2 | 900226000301872 | Internal | Female | 15.6 | 5.16 |
| PATU 3 | 900226000301831 | External | Male | 11.4 | 4.1 |
| PATU 4 | 900226000301794 | External | Male | 12.2 | 4.25 |
| BLTU | 900226000301826 and 900226000301847 | Internal and External | Male | 23 | 7 |
| Cadaver 1 | 900226000301873 | External Bridge | Male | 11.8 | 4.3 |
| Cadaver 2 | 900226000301712 and 900226000301805 | External Bridge and Top Plastron | Male | 12.4 | 3.8 |
| Cadaver 3 | 900226000301828 | Internal | Male | 13.1 | 4.6 |
| Cadaver 4 | 900226000301823 | Top Plastron | Male | 13.9 | 4.9 |
| Decoy | 900226000301896 and 900226000301851 | Internal and External | N/A | 12.8 | 3.8 |

**Table 13:** The distance a 12 mm tag was read entering both the pass-over and pass-through antenna at different angles and directions.

|  | 12mm Tag |  |  |  |
|---|---|---|---|---|
| **Antenna Type** | **Over (cm)** | **Z-axis (cm)** | **Outside (cm)** | **45d to Z-axis (cm)** |
| Pass-over | 20 | 35 | 15 | 20 |
| Pass-through | 1 | 10 | 2 | 2 |

In the third arena experiment, a decoy was made to test antenna type, tag placement, tag orientation and turtle orientation in water 13 cm deep. The Painted Turtle decoy was 12.8 cm L x 9.8 cm W x 3.8 cm H, based on the average size of the cadavers. Two tag placements were tested using the decoy: 1) 12mm HDX tag inserted in the bottom left corner of the decoy to simulate the left hind-leg pit; and, 2) 12mm HDX tag was externally attached on the centre right side of the decoy to simulate the bridge placement. Similar to cadaver trials, the external bridge placement tested both horizontal and vertical orientations placed on the shell. The decoy was placed on a vapor-barrier sheet and pulled from 20 cm away towards the antennae at a rate of 0.05 m/sec based on the cadaver preliminary testing, and entering the antenna at different angles, 60°, 90°, and 120°.

## Data Collection

### Live Trials PIT-Tag Detection

To decrease the number of trials and stress on the Midland Painted Turtles, two turtles were tested for external placement and two for internal placement, and each turtle was assigned their own PIT tag, which remained the same for the entire experiment. First, the detection success of external tags was tested for each antenna type (pass-through and pass-over) in a wet versus dry environment. PIT tags were externally placed using a reusable adhesive putty in the top right corner of the carapace (notch 0-2), center of the right bridge (notch 0-6), top left corner of plastron (humeral scute), and left back corner of plastron (anal scute). Ten trials were conducted for each tag location. The wet environment was created using a tub filled with water to 5 cm deep to test a water depth that partially submerged the turtle. Each turtle was tested in both wet and dry environments to determine detection rate, 10 times for each of two antenna types. For each trial, if the PIT tag was read, the distance to antenna was recorded from the front tip of the shell to the antenna using a one-centimeter tape grid. Once external placement was tested for two of the Midland Painted Turtles, the other two turtles were then injected with a 12 mm HDX PIT tag in their left hind-leg pit. The Blanding’s Turtle was previously inserted with a tag in the same location; however, it is a TROVAN PIT tag, which cannot be detected by the Oregon RFID reader and, therefore, the turtle was injected with an 12mm HDX tag. Each turtle with internal tags was then subjected to 10 trials for each of the two antenna types in both wet and dry conditions. Each trial was terminated at first detection of the PIT tag by the receiver and distance to antenna was recorded. If the PIT tag was not detected, the trial was terminated once the turtle crossed the antenna and passed the read range and recorded as no detection. Overall, 600 trials were completed with 20 different variable combinations and 30 trials per treatment (Table 14).

**Figure 19:** Second set of experimental trials using Western Painted Turtle, *Chrysemys picta bellii*, cadavers testing the affect of turtle orientation (60°, 90°, 120°) on detection success. The pass-through antenna is shown with a sub-adult, *Chrysemys picta bellii* cadaver on a sheet of vapor barrier, ready to begin trials with an internal tag facing the antenna at a 90° angle.

## Cadaver PIT-Tag Detection

The second experiment was designed to test PIT-tag detection by antenna type (pass-through and pass-over), external tag placement (center of the right bridge and top left corner of plastron) versus internal placement, turtle orientation (60°, 90°, 120°), and tag orientation (vertical and horizontal) on turtle cadavers. First, the detection rate of internal tags was tested for each antenna type and turtle orientation. The tag was injected in the left hind-leg pit, and trials were run for each antenna type and turtle orientation. For the next set of trials, the external bridge and left top plastron PIT-tag placement were secured using less than a gram of plumber's putty. Less than a gram was used, as when attaching radio transmitters, it is recommended that the size and weight should not exceed 5-10% of total body weight (Karch et al., 2013). Each external tag placement was tested for each antenna type, turtle orientation and tag orientation, 30 times for each variable combination. For each trial, if the PIT tag was read, the distance to antenna was recorded using the tape grid. If the PIT tag was not detected, the trial was terminated 4 seconds after the turtle crossed the antenna and recorded as no detection. Overall, 900 trials were completed with 30 different variable combinations and 30 trials per treatment (Table 14).

**Table 14:** Summary of each experiment completed testing the affect of tag placement, antenna type, environmental conditions, turtle orientation, and tag orientation on detection success. The number of variable combinations, number of trials per treatment, and total number of trials for each experiment is outlined below.

| **Experiment** | **Treatments** | **Number of Variable Combinations** | **Number of Trials per Treatment** | **Total Number of Trials** |
|---|---|---|---|---|
| Live | Tag Placement, Antenna Type, and Environmental Conditions | 20 | 30 | 600 |
| Cadaver | Tag Placement, Antenna Type, and Dry Environment, Turtle Orientation, and Tag Orientation | 30 | 30 | 900 |
| Decoy | Tag Placement, Antenna Type, and Wet Environment, Turtle Orientation, and Tag Orientation | 18 | 30 | 540 |

### Decoy PIT-Tag Detection

To test the detection rate for different PIT-tag and turtle orientations in a deeper wet environment (13 cm deep) in which turtles would swim, a Painted Turtle decoy was constructed. Before creating a decoy, a generalized linear model (GLM) and generalized linear mixed model (GLMM) were completed to determine if detection was influenced by turtle height and length in the cadaver experiment. A preliminary dry-environment experiment testing the decoy was completed to compare decoy and cadaver results for each treatment. Multiple ANOVAs were completed for each treatment comparing detection between the cadaver and decoy. From these results, only the bridge and internal placements were tested using the decoy, as decoy and cadaver mean detection rates were significantly different for the top plastron placement. Therefore, the decoy experiment tested external tag placement (center of right bridge) versus internal, antenna type (pass-through and pass-over), turtle orientation (60°, 90°, 120°), and tag orientation (vertical and horizontal) in a wet environment. The decoy was placed on a vapor-barrier sheet and pulled crossed the antenna at a rate of 0.05 cm/sec. A total of 540 trials were completed with 18 different variable combinations and 30 trials per treatment (Table 14).

## Data Analyses

Detection rate was calculated using the proportion of trials that the PIT tag was read, and the number of trials completed. A binomial ANOVA was completed for detection as all values were 1 (tag detected) and 0 (tag not detected) and used to test if treatment types resulted in statistically different means. If means were different, then one treatment was significantly more effective than another. In addition, a GLMM was calculated for live and cadaver data to test if random effects affected detection rate. The random effects used in the model included date of trial, ID of the turtle used, and PIT tag number. For the Midland Painted Turtle analysis, trial date and turtle ID were used, and the Blanding’s Turtle analysis used date and PIT tag number as only one individual was used. In the GLMM model, the Midland Painted Turtle pass-over and top plastron data and Blanding’s Turtles bridge data were excluded as detection rate was 100% detection. To compare treatments, Tukey pairwise comparisons were completed after each GLMM. To test the significance of read distance to the antenna between treatment types, an unpaired t-test was completed for each treatment because of unequal sample sizes due to lack of detections in some trials. One-factor ANOVA tests were completed to determine if the detection rate was significantly different between treatments. All statistical analyses were completed using lme4, multcomp and dplyr R packages (R Core Team, 2013).

# Results

## PIT-Tag Detection – Live Trials

Binomial ANOVA results showed that detection was significantly higher for Midland Painted Turtle (85.77%) than Blanding's Turtle (69.23%) (Tables 15, 16). Midland Painted Turtle top-plastron placement had 100% detection. Bridge placement (98.75%) was significantly higher than back-plastron (91.25%) and top-carapace position (66.25%) (Tables 15, 16). Results were significantly different between antenna types and the pass-over antenna had the highest detection (100%) (Tables 15, 16). Detection in the wet environment was significantly higher (91%) than the dry environment (90.5%) (Tables 15, 16). Blanding's Turtle, bridge placement had 100% detection, therefore its significance could not be calculated. Top-plastron (97.5%) placement had a significantly higher detection than back-plastron (55%), and top-carapace placement (52.5%) (Tables 15, 16). Pass-over antenna detection (91.82%) was significantly higher than for the pass-through antenna (60%) (Tables 15, 16). Detection in the dry environment (75%) was not significantly different from the wet environment (80%) (Tables 15, 16).

Detection rate was also analysed using a GLMM with different treatments as the fixed effects and turtle ID, date of test, and PIT tag number as random effects (Table 17). For Midland Painted Turtle, both the bridge and carapace placement significantly affected detection. Pairwise comparison results showed that there was a significant difference between external placements, with bridge having the highest detection rate (Table 17). For Blanding's Turtle, top-plastron placement, antenna type, and environment, significantly affected detection rate. Date of test had higher variance than PIT tag used. Pairwise comparison results showed that there were significant differences between top-plastron, back-plastron, and carapace placements (Table 17).

**Table 15:** Mean detection rate of PIT tag for each predictor variable and experimental trial.

| **Predictor Variables** | **Midland Painted Turtle (%)** | **Blanding's Turtle (%)** | **Western Painted Turtle Cadavers (%)** | **Painted Turtle Decoys (%)** |
|---|---:|---:|---:|---:|
| Species | 85.77 | 69.23 | | |
| Internal | 97.50 | 82.50 | 88.33 | 56.67 |
| Bridge | 98.75 | 100.00 | 85.56 | 52.50 |
| Back Plastron | 91.25 | 55.00 | | |
| Top Plastron | 100.00 | 97.50 | 89.17 | |
| Top Carapace | 66.25 | 52.50 | | |
| Pass-Over Antenna | 100.00 | 91.82 | 99.78 | 83.70 |
| Pass-Through Antenna | 81.50 | 60.00 | 75.33 | 24.70 |
| Dry Environment | 90.50 | 75.00 | 87.56 | 87.56 |
| Wet Environment | 91.00 | 80.00 | 53.89 | 53.89 |
| 60° Orientation | | | 84.67 | 50.56 |
| 90° Orientation | | | 80.67 | 62.78 |
| 120° Orientation | | | 97.33 | 48.33 |
| Vertical Tag Orientation | | | 85.56 | 50.00 |
| Horizontal Tag Orientation | | | 89.17 | 55.00 |

**Table 16:** Results of binomial ANOVA analysis for the three experiments testing the difference of means between detection rate and each predictor variable including species, antenna type, environment, tag placement, tag orientation and turtle orientation. Variable with Significantly Highest Detection Rate (Mean %) = if predictor variable significantly different, then the treatment with the highest detection rate is identified.

| | **Response Variable** | **Predictor Variable** | **Number of Trials** | **Pr(>F)** | **Variable with Highest Detection Rate (Mean %)** |
|---|---|---|---|---|---|
| **Live Trials** | Detection rate | Turtle Species | 600 | 9.17E-10 | Painted Turtle (86) |
| Midland Painted Turtle | Detection rate | Tag Placement | 400 | 8.04E-14 | Top plastron (100) |
| | Detection rate | Antenna Type | 400 | 1.16E-13 | Pass-over (100) |
| | Detection rate | Environment | 400 | 6.59E-08 | Wet (91) |
| Blanding's Turtle | Detection rate | Tag Placement | 200 | 1.64E-11 | Bridge (100) |
| | Detection rate | Antenna Type | 200 | 0.04361 | Pass-over (92) |
| | Detection rate | Environment | 200 | 0.2283 | No difference |
| **Cadaver Trials** | | | | | |
| Western Painted Turtle | Detection rate | Tag Placement | 900 | 0.3232 | No difference |
| | Detection rate | Antenna Type | 900 | 0.4514 | No difference |
| | Detection rate | Environment (Decoy Wet (13cm) Vs Cadaver Dry) | 1440 | <2.2E-16 | Dry (88) |
| | Detection rate | Turtle Orientation | 900 | 9.39E-12 | 120° (97) |
| | Detection rate | Tag Orientation | 900 | 0.0003211 | Horizontal (89) |
| **Decoy Trials** | | | | | |
| Painted Turtle | Detection rate | Tag Placement | 540 | 0.3594 | No difference |
| | Detection rate | Antenna Type | 540 | 3.92E-12 | Pass-over (84) |
| | Detection rate | Turtle Orientation | 540 | 0.01204 | 90° (63) |
| | Detection rate | Tag Orientation | 540 | 0.294 | No difference |

**Table 17:** Results from GLMM (glmer) and post-hoc (mytukey) tests for live and cadaver trials testing the difference of means between the response variable (detection rate) and predictor variables including antenna type, environment, tag placement, tag orientation, and turtle orientation. The random effects included turtle used (TurtleID) and date of test (DateofTest) for Midland Painted Turtle trials, and PIT tag number (PITTag) and date of test for the Blanding's Turtle trials. The Midland Painted Turtle pass-over and top plastron data and Blanding's Turtles bridge data were excluded as detection rate was 100%.

| **Live Trials** | **Package** | **Response Variable** | **Predictor Variable** | **Random Effects** | **Number of Trials (n)** | **Statistically Significant Variables** |
|---|---|---|---|---|---|---|
| Midland Painted Turtle | glmer | Detection rate | Tag Placement<br>+Antenna Type<br>+Environment | (1 \| TurtleID) + (1 \| DateofTest) | 400 | Bridge, and Carapace Placement |
|  | mytukey |  |  |  | 400 | Carapace-Back Plastron, and Carapace-Bridge |
| Blanding's Turtle | glmer | Detection rate | Tag Placement<br>+Antenna Type<br>+Environment | (1 \| PITTag) + (1 \| DateofTest) | 200 | Environment, Top Plastron, Antenna Type |
|  | mytukey |  |  |  | 200 | Top Plastron-Back Plastron, and Top Plastron-Carapace |
| **Cadaver Trials** |  |  |  |  |  |  |
| Western Painted Turtle | glmer | Detection rate | Tag Placement<br>+Antenna Type<br>+Environment<br>+Tag Orientation<br>+Turtle Orientation | (1 \| TurtleID) + (1 \| DateofTest) | 900 | Environment, Antenna Type, Tag Placement, Turtle Orientation, And Tag Orientation |
|  | mytukey |  |  |  | 900 | 60°-120°, 90°-120°, and Pass-through-Pass-over |

Midland Painted Turtle read distance to the antenna was not significantly different from Blanding's Turtle (Tables 18, 19). Midland Painted Turtle read distance was significantly greater for top plastron (0.606) and internal tag placement (0.012) (Tables 18, 19). For Midland Painted Turtle, pass-over (0.408) antenna detection had a significantly greater read distance than the pass-through antenna (Tables 18, 19). Read distance was significantly greater in the wet environment (0.377) than dry (Tables 18, 19). Blanding's Turtle similarly found that top plastron placements (0.244) were read significantly farther from the antenna, and internal, bridge, and back plastron placements were only read once they reached the antenna (Tables 18, 19). Pass-over antenna (0.435) detection had a significantly greater read distance than the pass-through antenna (0) (Tables 18, 19). Read distance was significantly greater in the dry environment (0.52) than wet (0.06) (Tables 18, 19).

## PIT-Tag Detection – Cadaver Trials

Using Western Painted Turtle cadavers, binomial ANOVA results showed that detection rates were significantly different between turtle orientations, with 120° having the highest detection rate (97.33%) (Tables 15, 16). Horizontal tag placement (89.17%) has a significantly higher detection rate than vertical placement (85.56%) (Tables 15, 16).

The results of the GLMM found that differences in detection rate based on tag placement, antenna type, turtle orientation and tag orientation were statistically significant, except when comparing the cadaver dry trials with decoy wet trials (Table 17). Similar to the live trial results, the pass-over antenna had a significantly higher detection rate (99.78%) than pass-through antenna (75.33%), and top plastron tag placement had the highest detection (89.17%) (Table 17). Similar to the binomial ANOVA results, detection was significantly higher when the cadaver was entering the antenna at a 120° angle (Table 17).

Read distance was significantly greater for bridge (2.013 cm) and internal (0.883 cm) placements (Tables 18, 19). Detection by the pass-over antenna (2.37 cm) had a significantly greater read distance than the pass-through antenna (0 cm) (Tables 18, 19). The dry environment (1.35 cm) had a significantly farther read distance than the wet environment (0 cm) (Tables 18, 19). The 60° turtle orientation (1.69 cm) and vertical tag orientation (2.35 cm) had significantly greater read distances than 90° (1.17 cm) and horizontal orientations (0.13 cm) (Tables 18, 19).

**Table 18:** Mean distance from the antenna (cm) that PIT tag was read for each predictor variable and experimental trial.

| **Predictor Variables** | **Mean Distance (cm)** | | | |
|---|---|---|---|---|
| | **Midland Painted Turtle** | **Blanding's Turtle** | **Western Painted Turtle Cadavers** | **Painted Turtle Decoys** |
| Species | 0.22 | 0.28 | | |
| Internal | 0.01 | 0.00 | 0.88 | 0.00 |
| Bridge | 0.01 | 0.00 | 2.01 | 0.00 |
| Back Plastron | 0.00 | 0.00 | | |
| Top Plastron | 0.61 | 0.24 | 0.46 | |
| Top Carapace | 0.59 | 0.00 | | |
| Pass-Over Antenna | 0.41 | 0.44 | 2.37 | 0.00 |
| Pass-Through Antenna | 0.00 | 0.00 | 0.00 | 0.00 |
| Dry Environment | 0.07 | 0.52 | 1.35 | 1.35 |
| Wet Environment | 0.38 | 0.06 | 0.00 | 0.00 |
| 60° Orientation | | | 1.69 | 0.00 |
| 90° Orientation | | | 1.17 | 0.00 |
| 120° Orientation | | | 1.21 | 0.00 |
| Vertical Tag Orientation | | | 2.35 | 0.00 |
| Horizontal Tag Orientation | | | 0.13 | 0.00 |

**Table 19:** Results of unpaired t-tests for all three experiments testing the difference of means for read distance to antenna between response and predictor variables including species, antenna type, environment, tag placement, tag orientation and turtle orientation. Variables with Significantly Highest Read Distance (Mean cm) = if values were predictor variable significantly different, then the treatment with the highest read distance is given.

| | **Response Variable** | **Predictor Variable** | **Number of Trials (n)** | **Pr(>F)** | **Variables with Significantly Highest Read Distance (Mean cm)** |
|---|---|---|---|---|---|
| **Live Trials** | Distance read | Turtle species | 518 | 0.5176 | No difference |
| Midland Painted Turtle | Internal | Carapace | 131 | 6.12E-06 | Carapace (0.59) |
| | Internal | Top plastron | 158 | 3.77E-04 | Top plastron (0.60625) |
| | Internal | Bridge | 157 | 0.7164 | Internal (0.01282) |
| | Internal | Back plastron | 151 | 0.3204 | Internal (0.0128) |
| | Pass-through | Pass-over | 363 | 7.89E-08 | Pass-over (0.408) |
| | Dry | Wet (5cm) | 363 | 2.43E-04 | Wet (0.3769) |
| Blanding's Turtle | Internal | Carapace | 54 | 2.16E-03 | Carapace (1.6428) |
| | Internal | Top plastron | 72 | 0.03757 | Top plastron (0.2435) |
| | Internal | Bridge | 73 | NA | Internal (0) Bridge (0) |
| | Internal | Back plastron | 55 | NA | Internal (0)<br>Back plastron (0) |
| | Pass-through | Pass-over | 155 | 0.0005489 | Pass-over (0.435) |
| | Dry | Wet (5cm) | 155 | 0.006482 | Dry (0.52) |
| **Cadaver Trials** | | | | | |
| Western Painted Turtle | Internal | Top plastron | 501 | 1.20E-10 | In (0.8833) |
| | Internal | Bridge | 488 | 4.00E-10 | Bridge (2.0129) |
| | Top plastron | Bridge | 629 | 5.40E-16 | Bridge (2.0129) |
| | Pass-through | Pass-over | 291 | <2.2e-16 | Pass-over (2.373) |
| | Dry | Wet | 1079 | <2.2e-16 | Dry (1.352) |
| | 60° | 90° | 496 | 1.07E-02 | 60° (1.68897) |
| | 90° | 120° | 534 | 8.26E-01 | 120° (1.2106) |
| | 120° | 60° | 546 | 2.13E-02 | 60° (1.68897) |
| | Vertical | Horizontal | 189 | <2.2e-16 | Vertical (2.353) |
| **Decoy Trials** | | | | | |
| Painted Turtle | Internal | Bridge | 291 | NA | 0 |
| | Pass-through | Pass-over | 291 | NA | 0 |
| | Dry | Wet | 1079 | <2.2e-16 | Dry (1.352) |

|  |  |  |  |  |  |
|---|---|---|---|---|---|
|  | 60° | 90° | 204 | NA | 0 |
|  | 90° | 120° | 200 | NA | 0 |
|  | 120° | 60° | 178 | NA | 0 |
|  | Vertical | Horizontal | 189 | NA | 0 |

**Table 20:** Mean number of times PIT tag was read by the antenna, for each predictor variable for experimental trials based on Western Painted Turtle cadavers and Painted Turtle decoys.

| Predictor Variables | Western Painted Turtle Cadavers | Painted Turtle Decoys |
|---|---:|---:|
| Internal | 8.82 | 4.22 |
| Bridge | 7.66 | 2.53 |
| Top Plastron | 5.33 | |
| Pass-Over Antenna | 11.44 | 5.604 |
| Pass-Through Antenna | 2.47 | 0.5778 |
| Dry Environment | 6.96 | 6.957 |
| Wet Environment | 3.09 | 3.091 |
| 60° Orientation | 7.34 | 2.667 |
| 90° Orientation | 5.75 | 3.917 |
| 120° Orientation | 7.78 | 2.689 |
| Vertical Tag Orientation | 6.68 | 3.244 |
| Horizontal Tag Orientation | 6.31 | 1.81 |

**Table 21:** Results of ANOVA tests for cadaver and decoy experiments testing the difference of means between number of detections and predictor variables including antenna type, environment, tag placement, tag orientation and turtle orientation. Variables with Significantly Highest Number of Detections (Mean) = if values were predictor variable significantly different, then the treatment with the highest read distance is given.

| Western Painted Turtle Cadavers | **Cadaver Trials** | | | | |
|---|---|---|---|---|---|
| | **Response Variable** | **Predictor Variable** | **Number of Trials** | **Pr(>F)** | **Variables with Significantly Highest Number of Detections (Mean)** |
| | Number of Detections | Tag placement | 900 | 2.34E-12 | Internal (8.817) |
| | Number of Detections | Antenna type | 900 | 0.00000025 | Pass-over (11.44) |
| | Number of Detections | Environment (Decoy wet (13cm) vs Cadaver dry) | 900 | 0.000957 | Dry (6.957) |
| | Number of Detections | Turtle orientation | 900 | 3.50E-05 | 120° (7.78) |
| | Number of Detections | Tag orientation | 900 | <2E-16 | Vertical (6.678) |
| | **Decoy Trials** | | | | |
| Painted Turtle Decoys | Number of Detections | Tag placement | 540 | <2e-16 | Internal (4.222) |
| | Number of Detections | Antenna type | 540 | <2e-16 | Pass-over (5.604) |
| | Number of Detections | Turtle orientation | 540 | 0.000728 | 90° (3.917) |
| | Number of Detections | Tag orientation | 540 | 0.00011 | Vertical (3.244) |

Internal tag placement (8.82) had significantly greater number of detections than external placements and the pass-over antenna (11.44) had significantly greater detections than the pass-through antenna (2.47) (Tables 20, 21). There were significantly more detections in the dry environment (6.96) than the wet environment (3.09) (Tables 20, 21). Additionally, 120° turtle orientation (7.78) and vertical tag orientation (6.68) had significantly greater number of detections than the other orientation angles and horizontal tag orientation (6.31) (Tables 20, 21).

## PIT-Tag Detection – Decoy Trials

Using Painted Turtle decoys, binomial ANOVA results showed that antenna type, environment, and turtle orientation had significantly different results between treatments (Table 16). Detection rate was significantly higher for the pass-over antenna (83.7%), dry environment (87.56%), and 90° turtle orientation (62.78%) detection (Tables 15, 16). The tag placement and tag orientation results were not significantly different.

Read distance was significantly higher during the cadaver dry (1.35 cm), experiment then wet (0 cm) decoy experiment (Tables 18, 19). The only unpaired t-test analysis completed was for the environment as all other detection results were zero (Tables 18, 19).

The number of detections by the antenna were all significant for each variable combination (Table 21). Therefore, internal placement (4.22), pass-over antenna (5.604), dry environment (6.957), 90° (3.917) and vertical orientation (3.244) yielded significantly higher number of detections by the antenna than other treatments (Tables 20, 21).

# Discussion

Internal injection of PIT tags in freshwater turtles and the use of automated tag readers in a point of passage to detect movement is a universal technique. However, there is a lack of studies investigating the detection rate of these widely accepted techniques or alternative placements, antenna types, environments conditions, and orientations. This study completed arena experiments testing tag placement, antenna type and environmental conditions on live Midland Painted Turtles and Blanding's Turtles. Additionally, cadaver and decoy experiments were completed to manipulate the angle the turtle entered the antenna and create less stress on live turtles.  This experiment found that PIT-tag placement, antenna type, environmental conditions, turtle orientation, and tag orientation influence detection rate, read distance, and number of detections for all experiments. Detection rate was highest across all experiments when a pass-over antenna was used, however the best tag placement and orientations varied among experiments. Read distance to the antenna was highest across all experiments when a pass-over antenna was used, except for decoy trials, and there were different successful placements and orientations dependent on the experiment type (i.e. live, cadaver, decoy). Lastly, number of detections was highest when a pass-over antenna was used, internal injection, vertical tag placement and turtle orientation varied between cadaver and decoy results. Overall, the pass-over antenna consistently provided the highest detection rate, read distance to the antenna, and number of detections across all experiment and treatment types.

Detection rate was significantly higher across all treatments for Midland Painted Turtle than Blanding's Turtle. This could be the result of the 12mm tag being too small for the adult Blanding's Turtle, highlighting the importance of using the appropriate tag size for different-sized turtles. Appropriate tag sizes are important as smaller tags have a lower read range, resulting in not being detected in larger animals. This was similarly observed when an external tag placed on a Desert Tortoise was not detected by a strategically placed antenna, and it was speculated that this was due to tag size relative to the turtle's body size (Boarman et al., 1998). However, read distance to the antenna was not significantly different between the two species in my study, showing that tag size may not influence read distance, only detection.

Turtles with external tags had a significantly higher detection rate and read distance compared to the traditional internal placement. Specifically, top plastron and bridge placement for the live trials had consistently higher detection and read distance. Both external placements were near the front of the turtle, which would be closest to the antenna. Similar to live trials, cadaver top plastron placement had a significantly higher detection rate. However, decoy detection rate for tag placement were not significantly different; this could be the result of only two different tag placements being tested, internal and bridge. For cadaver experiments, bridge placement had significantly farther read distance compared to other placements, and tags in the decoys were only read once they crossed the antenna. For both cadaver and decoy trials, internal placement had highest number of detections. Both external and internal placements have been used in the literature, however no studies have previously tested the reliability of these methods. Based on these results, it appears that external placement is more reliable for higher detection rate and greater read distance.

The pass-over antenna had significantly higher detection rate, read distance, and number of detections for all experiments (Baxter-Gilbert et al., 2015; Markle et al., 2017). This is contrary to what PIT-tag companies recommend. For example, Oregon RFID suggests a pass-through antenna as the best type because the tag coil orientation is the same as the loop (Oregon RFID Inc., 2018). However, my results show that the pass-over antenna had the highest detection rate. Pass-through antenna detection could have been significantly less than pass-over antenna detection because Oregon RFID suggests larger tags for pass-through antennas as they have a low read range in the centre (Oregon RFID Inc., 2018). In addition, the recommendations are general for all wildlife species injected with PIT tags, however different taxon may need different antenna type requirements, as indicated by the results of this study. Similar studies testing PIT-tag antenna type to detect fishes have tested both pass-through and pass-over antenna systems and showed that pass-over antennas had a slightly lower read range then pass-through (Beeman et al., 2012; Connolly et al., 2008). These differ from my results; however, this could be because studies tested different taxa and in deep, fast-flowing streams. Pass-over antennas have been thought to be a superior design as they are less likely to be caught by floating debris in an aquatic system (Zydlewski et al., 2006). While testing antenna designs, both studies had antenna failures because of floating debris hitting pass-through antennas (Beeman et al., 2012; Connolly et al., 2008). Overall, there are currently no studies testing the detection rate of antenna types for freshwater turtles. However, based on my results, pass-over antennas have a high detection rate for freshwater turtles.

The local environmental conditions had important effects on detection. The Midland Painted Turtle live-trial results showed that detection rates and read distance were significantly higher in the wet environment, whereas, there were no significant differences when using a Blanding's Turtle. Midland Painted Turtle results showed that the wet environment had the highest detection success because the water was only 5 cm deep, which only partially submerged the turtle and, therefore, did not affect the height that the turtle entered the antenna. Additionally, the size of the turtle may have been a factor, as the Blanding's Turtle was larger then the Midland Painted Turtle and, according to previous studies, the turtle and tag size can affect tag detection (Boarman et al., 1998). In the cadaver and decoy trials, detection rates and read distance were significantly higher in the dry environment.  This is most likely because the wet environment was in a water depth of 13 cm, which allowed the decoy to float in the centre of the pass-through antenna. Additionally, live-turtle trials and decoy trials yielded different results for environmental conditions because live trials were completed in a water depth of (5 cm), which only partially submerged the turtle not allowing it to float, compared to the decoy trials, which were undertaken in water 13 cm deep, depths at which turtles would have to swim. This is similar to studies testing antenna types in deep and fast-flowing aquatic systems, which found that water did not disrupt the detection, but rather increased range from the antenna (Beeman et al., 2012; Connolly et al., 2008).

The orientation angle at which the turtle entered the antenna range had a significant effect on detection rate, however the optimal orientation angle varied among experiments. Tagged cadavers had highest detection rates at 120°and highest read distances at 60°. In comparison, the decoy experiment had highest detection rates and read distances at 90°. Although these results are unclear, there is documented affects of tag orientation disrupting detection, as tags should be the same orientation as the antenna (Oregon RFID Inc., 2018). No other studies have examined the angle that turtles enter an antenna and how this might disrupt readings.

Tag orientation is another important factor when considering antenna type and tag placement. Tags have the highest read range when the coil is the same direction as the antenna (Oregon RFID Inc., 2018). Horizontal placement resulted in significantly higher detection rate for cadaver, but not decoy, trials. The cadaver results support vertical placement as the highest number of detections and read distance and decoy trials had significantly higher number of detections for vertical placement. Similarly, in a study testing the detection rate of PIT tags in Eastern Hellbender, *Cryptobranchus alleganiensis*, vertical oriented tags were 18% more likely to be detected then horizontally placed tags (Connock et al., 2019). Boarman et al. (1998) found that, when testing the external horizontal and vertical placement of 14-mm tags on Desert Tortoises, vertical placement was most successful. In one instance when a tag was not detected by the antenna, it was hypothesized that this was due to tag orientation being horizontal instead of appropriate vertical placement (Boarman et al., 1998).

Overall, this study found that different tag placements, antenna types, environments, turtle orientation and tag orientations can affect the detection rate, distance read, and number of detections. Specifically, external placement and pass-over antenna yielded the highest detection rate and read distance. Environmental conditions only affected tag detection when it disrupted read range, turtle orientation was different across all experiments, and vertical tag orientation generally had higher detection success. These findings highlight the importance of considering the most appropriate tag placement and antenna construction parameters to yield the highest detection success. For studies investigating the movement or use of ecopassages by freshwater turtles, pass-over antennas should be used on either side of the passage as they yield high detection success. In addition, for short-term studies, external placement of tags on freshwater turtles should be considered as my results show that they yield 100% detection and are a non-invasive method. Depending on the location of the antenna, environmental conditions could impact detection success depending on how depth disrupts read range. Tag orientation should also be considered as my study showed that vertical placement generally have higher detection success, and similar studies have found that vertical placement yields higher detection. Turtle orientation was different across the experiments and needs further investigation. Further experiments testing turtle orientation should examine entering at sharper angles, such as 20°, and testing live freshwater turtles instead of cadavers by controlling the angle they enter the antenna in wet and dry conditions.

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# Appendices

**Table A1:** Total number of dead-on-road sightings for each taxonomic group, species, and year surveyed.

| **Common Name** | **Scientific Name** | **2010** | **2011** | **2017** | **2018** | **2019** | **Total** |
|---|---|---|---|---|---|---|---|
| American Bullfrog | *Lithobates catesbeianus* | 0 | 0 | 0 | 2 | 4 | 6 |
| American Toad | *Anaxyrus americanus* | 129 | 103 | 1 | 2 | 9 | 244 |
| Eastern Newt | *Notophthalmus viridescens* | 1 | 0 | 6 | 35 | 2 | 44 |
| Eastern Red-backed Salamander | *Plethodon cinereus* | 1 | 1 | 0 | 0 | 0 | 2 |
| Gray Treefrog | *Hyla versicolor* | 103 | 90 | 3 | 16 | 242 | 454 |
| Green Frog | *Lithobates clamitans* | 169 | 150 | 5 | 18 | 49 | 391 |
| Northern Leopard Frog | *Lithobates pipiens* | 102 | 542 | 124 | 36 | 153 | 957 |
| Spotted Salamander | *Ambystoma maculatum* | 2 | 0 | 0 | 0 | 0 | 2 |
| Wood Frog | *Lithobates sylvaticus* | 6 | 5 | 1 | 0 | 1 | 13 |
| Unidentifiable Amphibian | | 70 | 332 | 42 | 482 | 1359 | 2285 |
| **Total Amphibians** | | **583** | **1223** | **182** | **591** | **1819** | **4398** |
| American Crow | *Corvus brachyrhynchos* | 0 | 0 | 1 | 0 | 0 | 1 |
| American Goldfinch | *Spinus tristis* | 8 | 5 | 10 | 11 | 4 | 38 |
| American Robin | *Turdus migratorius* | 1 | 6 | 4 | 4 | 8 | 23 |
| American Yellow Warbler | *Setophaga petechia* | 0 | 0 | 0 | 3 | 3 | 6 |
| Baltimore Oriole | *Icterus galbula* | 1 | 0 | 0 | 0 | 2 | 3 |
| Barn Swallow | *Hirundo rustica* | 0 | 0 | 0 | 0 | 2 | 2 |
| Black-capped Chickadee | *Poecile atricapillus* | 0 | 1 | 0 | 0 | 0 | 1 |
| Blue Jay | *Cyanocitta cristata* | 2 | 1 | 1 | 3 | 0 | 7 |
| Cedar Waxwing | *Bombycilla cedrorum* | 1 | 0 | 0 | 8 | 2 | 11 |
| Common Grackle | *Quiscalus quiscula* | 0 | 0 | 0 | 0 | 1 | 1 |
| Common Starling | *Sturnus vulgaris* | 0 | 1 | 0 | 0 | 0 | 1 |
| Common Yellowthroat | *Geothlypis trichas* | 0 | 0 | 0 | 0 | 2 | 2 |
| Eastern Kingbird | *Tyrannus tyrannus* | 0 | 0 | 0 | 0 | 1 | 1 |
| Eastern Wood-Pewee | *Contopus virens* | 1 | 0 | 0 | 0 | 0 | 1 |
| Gray Catbird | *Dumetella carolinensis* | 0 | 0 | 2 | 2 | 3 | 7 |
| Hairy Woodpecker | *Leuconotopicus villosus* | 0 | 0 | 1 | 0 | 0 | 1 |
| Hermit Thrush | *Catharus guttatus* | 0 | 1 | 0 | 0 | 0 | 1 |
| House Sparrow | *Passer domesticus* | 0 | 0 | 0 | 1 | 0 | 1 |
| House Wren | *Troglodytes aedon* | 1 | 0 | 0 | 0 | 0 | 1 |
| Ruby-throated Hummingbird | *Archilochus colubris* | 0 | 0 | 0 | 0 | 1 | 1 |
| Indigo Bunting | *Passerina cyanea* | 0 | 0 | 0 | 1 | 0 | 1 |
| Mallard | *Anas platyrhynchos* | 0 | 1 | 0 | 0 | 0 | 1 |
| Mourning Dove | *Zenaida macroura* | 1 | 0 | 0 | 3 | 1 | 5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Northern Cardinal | *Cardinalis cardinalis* | 0 | 0 | 0 | 3 | 0 | 3 |
| Northern Flicker | *Colaptes auratus* | 0 | 0 | 1 | 0 | 0 | 1 |
| Pileated Woodpecker | *Dryocopus pileatus* | 0 | 0 | 0 | 1 | 0 | 1 |
| Red-eyed Vireo | *Vireo olivaceus* | 1 | 0 | 0 | 0 | 1 | 2 |
| Red-winged Blackbird | *Agelaius phoeniceus* | 0 | 0 | 0 | 0 | 3 | 3 |
| Rose-breasted Grosbeak | *Pheucticus ludovicianus* | 0 | 0 | 0 | 1 | 1 | 2 |
| Song Sparrow | *Melospiza melodia* | 0 | 0 | 1 | 0 | 0 | 1 |
| Sora | *Porzana carolina* | 0 | 0 | 0 | 1 | 0 | 1 |
| Unidentifiable Bird | | 18 | 10 | 10 | 20 | 25 | 83 |
| **Total Birds** | | **35** | **26** | **31** | **62** | **60** | **214** |
| American Beaver | *Castor canadensis* | 1 | 0 | 0 | 0 | 0 | 1 |
| American Mink | *Neovison vison* | 0 | 0 | 0 | 2 | 2 | 4 |
| American Red Squirrel | *Tamiasciurus hudsonicus* | 7 | 6 | 2 | 14 | 14 | 43 |
| Brown Bat spp. | *Myotis* | 0 | 1 | 0 | 1 | 2 | 4 |
| Eastern Chipmunk | *Tamias striatus* | 15 | 17 | 20 | 56 | 29 | 137 |
| Eastern Cottontail | *Sylvilagus floridanus* | 1 | 0 | 3 | 11 | 5 | 20 |
| Eastern Gray Squirrel | *Sciurus carolinensis* | 4 | 4 | 3 | 13 | 6 | 30 |
| Groundhog | *Marmota monax* | 0 | 0 | 0 | 1 | 1 | 2 |
| Hairy-tailed Mole | *Parascalops breweri* | 0 | 6 | 0 | 0 | 0 | 6 |
| Meadow Vole | *Microtus pennsylvanicus* | 1 | 2 | 7 | 6 | 3 | 19 |
| Mouse spp. | *Mus* | 17 | 7 | 15 | 38 | 38 | 115 |
| Northern Short-tailed Shrew | *Blarina brevicauda* | 3 | 4 | 0 | 1 | 1 | 9 |
| Star-nosed Mole | *Condylura cristata* | 1 | 0 | 0 | 2 | 2 | 5 |
| Stoat | *Mustela erminea* | 0 | 0 | 0 | 4 | 2 | 6 |
| Striped Skunk | *Mephitis mephitis* | 0 | 1 | 0 | 3 | 6 | 10 |
| Raccoon | *Procyon lotor* | 4 | 6 | 2 | 5 | 10 | 27 |
| Virginia Opossum | *Didelphis virginiana* | 0 | 1 | 2 | 0 | 2 | 5 |
| Unidentifiable Mammal | | 13 | 17 | 7 | 17 | 36 | 90 |
| **Total Mammals** | | **67** | **72** | **61** | **174** | **159** | **533** |
| Dekay's Brownsnake | *Storeria dekayi* | 70 | 75 | 48 | 51 | 114 | 358 |
| Eastern Gartersnake | *Thamnophis sirtalis sirtalis* | 10 | 27 | 58 | 35 | 55 | 185 |
| Eastern Milksnake | *Lampropeltis triangulum* | 4 | 3 | 1 | 3 | 5 | 16 |
| Red-bellied Snake | *Storeria occipitomaculata* | 60 | 42 | 26 | 11 | 56 | 195 |
| Unidentifiable Snake | | 8 | 5 | 1 | 17 | 9 | 40 |
| **Total Snakes** | | **152** | **152** | **134** | **117** | **239** | **794** |
| Blanding's Turtle | *Emydoidea blandingii* | 0 | 0 | 1 | 0 | 0 | 1 |
| Common Snapping Turtle | *Chelydra serpentina* | 0 | 5 | 0 | 11 | 5 | 21 |
| Midland Painted Turtle | *Chrysemys picta marginata* | 0 | 10 | 10 | 19 | 20 | 59 |
| Unidentifiable Turtle | | 0 | 0 | 0 | 0 | 1 | 1 |
| **Total Turtles** | | **0** | **15** | **11** | **30** | **26** | **82** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Total Road Mortality** | **837** | **1488** | **419** | **974** | **2303** | **6021** |