Do Ecopassages Affect Daily Movements and Home Ranges of Blanding's Turtles

(*Emydoidea blandingii*)?

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Thesis submitted to the

Faculty of Graduate and Postdoctoral Studies

University of Ottawa

In partial fulfillment of requirements for the

Master of Science degree in the

Ottawa-Carleton Institute of Biology

Department of Biology

Faculty of Science

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ABSTRACT:

Global biodiversity has been decreasing at an alarming rate due to factors such as over-exploitation of species, habitat loss, and fragmentation. A major contributor to habitat loss and fragmentation is road development. Massive road networks span our landscapes and contribute to loss of biodiversity in many ways, such as road mortality. Mitigation techniques, such as ecopassages and exclusion fencing, are implemented across the world to reduce road mortality, though few studies have explored their effectiveness at maintaining movement. Within the Canadian Nuclear Laboratories (CNL) in Chalk River, Ontario, Canada, there is a small population of Blanding's turtles (*Emydoidea blandingii*), a federally protected species. In 2014/15, a radiotelemetry study was conducted to assess habitat use and movement patterns of this species at CNL. Seven ecopassages and exclusion fencing were installed along the main access roads following this study. I wished to determine whether the installation of ecopassages at CNL has improved habitat connectivity and spatial distribution of Blanding's turtles. Thus, I repeated the radiotelemetry study after the installation of the ecopassages. Movement patterns and home ranges were not significantly impacted by ecopassage presence, however, population spatial distribution decreased after ecopassage installation. Considering the widespread use of ecopassages by animals, these findings highlight the need for further investigation into the effectiveness of ecopassages for reptiles prior to continued application of this road mitigation strategy.

RÉSUMÉ:

La biodiversité mondiale diminue à un rythme effréné en raison de facteurs tels que la surexploitation des espèces, la perte d'habitat et la fragmentation. Le développement des routes est un contributeur majeur à la perte et à la fragmentation de l'habitat. Des réseaux routiers massifs s'étendent sur nos paysages et contribuent à la perte de biodiversité de nombreuses manières, notamment en termes de mortalité routière. Des techniques d'atténuation, telles que les écopassages et les clôtures d'exclusion, sont mises en œuvre dans le monde entier pour réduire la mortalité routière, même si peu d'études ont exploré leur succès à maintenir les mouvements. Sur le site des Laboratoires Nucléaires Canadiens (CNL), à Chalk River, Ontario, Canada, il existe une petite population de tortues mouchetées (*Emydoidea blandingii*), une espèce protégée par le gouvernement fédéral. En 2015, une étude radiotélémétrique a été réalisée pour évaluer l'utilisation de l'habitat et les habitudes de déplacement de cette espèce aux CNL. Sept écopassages ont été aménagés le long des routes d'accès principales suite à cette étude. Nous souhaitons déterminer si l'installation d'écopassages aux CNL a amélioré la connectivité de l'habitat et de la répartition spatiale des tortues mouchetées. Ainsi, j'ai réitéré l'étude radiotélémétrique après l'installation des écopassages. Les schémas de déplacement et les domaines vitaux n'ont pas été significativement affectés par la présence de l'écopassage. Cependant, la répartition spatiale de la population a diminué après l'installation de l'écopassage. Compte tenu de l'utilisation généralisée des écopassages par les animaux, cela souligne la nécessité d'étudier plus en profondeur l'efficacité des écopassages pour les reptiles avant de poursuivre l'application de cette solution d'atténuation.

ACKNOWLEDGEMENTS:

Thank you to my supervisor Dr. Gabriel Blouin-Demers for his continuous support and expertise throughout this project. From developing industry relationships to providing invaluable feedback on my writing, your support was always unfaltering. I would also like to thank my thesis committee members Dr. Jessica Forrest and Dr. Lenore Fahrig for their assistance with the development of this project. Thank you to the members of the Blouin-Demers lab for their continuous advice and guidance, especially Audrey Turcotte and Francesco Janzen.

I would also like to thank Annie Morin and Emily Hawkins from CNL for their support and encouragement throughout this project. Also, to the many other CNL staff without whom this project would not have been possible including Jamie Carr, Shelly Ball, and Meghan Murrant. Thank you for your time, effort, and support during my time at CNL. I would also like to acknowledge the following CNL summer students for their contribution to the field work: Spencer Kielar, Eve Schroeder, Aidan Chaput, Emily Wong, Emily Woodcox, and many others. I greatly appreciate your efforts in supporting my field work. An additional thanks to Spencer Kielar for his photography skills which ensured that my presentations were always filled with great nature shots.

Data collection would not have been possible without the hard work of Tanya Li and Céline Lafrance. Thank you both for your positivity and commitment to the project. I would also like to thank my close friends and family, especially my partner Brendan and my parents for your encouragement and support.

Finally, this research would not have been possible without the financial support of the Canadian Nuclear Laboratories, Dr. Gabriel Blouin-Demers, and the University of Ottawa.

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INTRODUCTION:

Habitat and Biodiversity Loss:

Global biodiversity has been decreasing at an alarming rate due to factors such as pollution, climate change, land use, and over-exploitation of species (Pereira et al., 2012; Rawat & Agarwal, 2015; Sih et al., 2011). The greatest cause for reduced global biodiversity appears to be habitat loss and fragmentation (Hanski, 2011; also see Sala et al 2000). A major contributor to habitat loss and fragmentation is road development. Roads alter typical behaviour and movement patterns of animals (Bélanger-Smith, 2014), cause population isolation (Clark et al., 2010), and increase animal mortality (Fahrig et al., 1995).

Road Impacts:

Massive road networks span our landscapes with continuous development; there were over 43,000 km of roads built in Canada in 2019-2020 (Statistics Canada, 2022). The presence of this anthropogenic disturbance contributes to loss of biodiversity in many ways including habitat fragmentation, habitat loss (Rawat & Agarwal, 2015), declines in population density through wildlife-vehicle collisions (Fahrig et al., 1995), movement restriction, and isolation which all decrease population persistence (Clark et al., 2010).

The degree to which roads are detrimental to animals is determined by their particular behaviours and life history traits (Forman et al., 2003). Roads alter typical behaviour and movement patterns potentially resulting in increased energy expenditure to complete normal activities (Bélanger-Smith, 2014). Some animals display road-avoidance, increasing energetic costs of accessing resources as individuals must travel greater distances (Lusseau, 2004). This isolation fragments habitats and can create subdivisions in populations (Mader, 1984), thereby disrupting typical metapopulation dynamics and obstructing gene flow (Clark et al., 2010).

Interference with metapopulation dynamics decreases typical subpopulation interactions (i.e. immigration and emigration) and can have severe consequences on population persistence (Brown & Kodric-Brown, 1977). Individual dispersal is especially important for the maintenance of smaller populations and recolonization of essential habitats as it ensures gene flow in a population (Epps et al., 2005). Loss of gene flow combined with habitat fragmentation raises the risk of extinction in isolated populations (Lande, 1988). Additionally, population isolation restricts the ability of individuals to migrate from neighbouring populations to alleviate the threat of extinction by providing genetic input (Lande, 1988). Animal populations in close proximity to roads experience greater stress, meaning they may have increased resting heart and metabolic rates as well as decreased reproductive success (Anthony and Isaacs, 1989). These negative physiological effects occur in animals living more than 1,000 m from the roadside (Forman, 1995). There are numerous ways that roads influence wildlife, but potentially the greatest threat is road mortality.

Mitigation Strategies Against Road Effects:

Mitigation techniques can be implemented to reduce the negative effects of roads on wildlife. Some techniques include community outreach, improved road signage, exclusion fencing, and wildlife crossing structures such as ecopassages (Figure 1) (Glista et al., 2009; Martinig & Bélanger-Smith, 2016). Ecopassages are installed in high-traffic areas to enable safe animal movement between habitats without crossing on roads (Glista et al., 2009; Parks Canada, 2021). Exclusion fencing acts as a physical barrier, installed along roadsides to prevent animals from accessing roads and, often, funnels wildlife towards crossing structures such as ecopassages (Bélanger-Smith, 2014; van der Grift et al., 2013).

Ecopassage Effectiveness:

The efficacy of ecopassages has yet to be determined as most studies assessing effectiveness of these crossing structures only include data after installation. Knowledge of crossing frequency before intervention is essential to understand the resulting changes in crossing frequency. The ideal study design to gather reliable evidence on ecopassage effectiveness is a BACI (before-after control-impact) experiment (Soanes et al., 2024). Currently, we know that ecopassages are used by a variety of animals, however, with so many studies lacking data collection periods prior to installation, it is difficult to determine whether movement has changed as a result of the installation of ecopassages.

Ecopassage efficacy is influenced by multiple factors including location, size, sound disturbances, length, and openness (Glista et al., 2009; Lesbarrères & Fahrig, 2012). Ecopassags are typically located close to known road-kill hotspots (Glista et al., 2009; Lesbarrères & Fahrig, 2012) and areas where frequent large animal wildlife-vehicle collisions occur (i.e. moose; Healy et al., 2016). The size of the ecopassage should reflect the target species. For example, the impact of ecopassage length depends on the size of the animals which they serve. For example, smaller reptiles and amphibians are more likely to make use of shorter passages, with a maximum length of 50-60 m recommended (Sisson, 2017). Noise disturbance is a difficult factor to control, however, it has been suggested that animals are less likely to use ecopassages if there is frequent human or vehicle activity nearby (Glista et al., 2009). Finally, openness (i.e., light and visibility) is essential when designing an ecopassage (Glista et al., 2009; Martinig & Bélanger-Smith, 2016). Ecopassages that offer direct lines of sight and allow sunlight to enter are more likely to be used by animals (Colley et al., 2017; Glista et al., 2009; Martinig & Bélanger-Smith, 2016). With these characteristics in mind, researchers and policy makers need to know whether ecopassages have assisted with animal mobility and reconnected habitats fragmented by road development. Assessing the efficacy of ecopassages will allow for design refinement and road development projects that limit the impact on surrounding animal populations.

The Effectiveness of Partial Exclusion Fencing at Reducing Road Mortality:

Partial exclusion fencing is characterized by gaps between sections of fencing that create access points for wildlife to enter roadways. In a long-term study conducted by Markle and colleagues (2017), they discovered that turtle abundance on roads increased after partial fencing was installed compared to pre-mitigation assessments. Meaning that partial fencing can cause increased turtle presence at the end of the fencing and, therefore, no reduction in (Markle et al., 2017) or increased mortality (Baxter-Gilbert et al., 2015). This has been referred to as a 'corralling effect' in the literature (Baxter-Gilbert et al., 2015). The most effective method to decrease road mortality is the installation of complete exclusion fencing (Markle et al., 2017; Rytwinski et al., 2016). In cases where complete fencing cannot be installed, i.e. driveways management strategies need to be improved to reduce the corralling effect (Markle et al., 2017).

The Effects of Road Mortality on Reptiles:

Reptiles are at significant risk to road mortality because they are often slow-moving (Ashley & Robinson, 1996) and engage in seasonal migrations (Bodie, 2001). Reptiles frequently end up on roads as they move in search of food, mates, and nesting sites (Haxton, 2000). Snakes may even pause on roads to use the warm asphalt for thermoregulation (Ashley & Robinson, 1996). These behavioural characteristics paired with reptiles' general inability to avoid approaching vehicles, augments the threat of road mortality they face (Glista et al., 2009).

Turtle populations are especially at risk from the impacts of road mortality due to their delayed sexual maturity and naturally low annual juvenile recruitment (Haxton, 2000). Though lifespan and reproductive lifetimes vary by species, turtles are long-lived and continue to reproduce until late in life (Brooks et al., 1991; Congdon et al., 2001). These characteristics render turtle populations particularly vulnerable to the effects of surplus adult mortality (Congdon et al., 1993). Should ecopassages prove effective at reducing adult mortality and reconnecting fragmented critical habitats, they will help play a role in stabilizing or recovering turtle populations. Diminishing road mortality is extremely important as Findlay & Bourdages (2000) found that the effects roads have on surrounding reptile populations can be detected within 8 years of construction, however, it may take decades before the full effect is evident in individual populations. This is of great concern as the impact of road development on these species may not yet be fully clear where long-term population monitoring data are not available.

Blanding's Turtle:

The Blanding's Turtle (*Emydoidea blandingii)* is a federally protected species under the Species at Risk Act. There are two distinct populations of Blanding's Turtles in Canada, the Nova Scotia and Great Lakes-St. Lawrence populations (Davy et al., 2014).

The Blanding's Turtle is well recognized by its distinctive yellow chin and neck (Baker and Gillingham, 1983). Blanding's Turtles are a medium sized turtle with an approximate maximum carapace length of 20 cm. An adult Blanding's Turtle typically weighs 800-1,600 g (Congdon & van Loben Sels, 1993); however, this value will fluctuate when females are gravid. Their carapace is smooth, highly domed and dark (Panella & Rothe-Groleau, 2021) and is also typically streaked with yellow markings (Rowe et al., 2017).

Known for their longevity, sexual maturity is achieved around the age 16 for females (Congdon et al., 1983) and 13 for males (Graham & Doyle, 1977). Females produce a clutch size of 3 to 19 eggs, every 1 to 3 years (Congdon & van Loben Sels, 1993; Congdon et al., 1993).

Blanding's Turtles are semi-aquatic because they spend most of their time in wetlands (Beaudry et al., 2008), such as marshes and bogs, and complete overland migrations to use overwintering hibernacula (Edge et al., 2009), find mates, or access nesting sites. As a result of the migrations required to complete necessary biological functions, they have large home ranges (Innes et al., 2008). These inter-wetland movements increase an individual's risk of road mortality due to unavoidable road crossings in fragmented areas (Beaudry et al., 2008).

<u>Population Spatial Distribution and Individual Home Ranges:</u>

Understanding how population spatial distribution and individual home range areas differ after installation of ecopassages in the area is essential for determining effective conservation strategies. By assessing the movement and area use of a species, we can identify their critical habitats, as well as focus preservation and management efforts in these areas. Long-term studies allow researchers to identify migration corridors and connectivity between habitats to allow protection of these areas and reduce the impact of development.

For Blanding's Turtles, critical areas include nesting sites, overwintering sites, and habitats used during general activity. Corridors may be used to connect nesting or overwintering sites to their summer wetlands. Understanding the location of these habitats and corridors may allow for mitigation against anthropogenic threats. By identifying these areas of high concern, such as a wetland expected to be fragmented by a new road build, land managers can plan ahead and install ecopassages when land use changes.

An animal's home range, the smallest area where it is 95% likely to be found (Millar & Blouin-Demers, 2011) and which fulfills all biological requirements. Population spatial distribution is described as the area used by a population, this area may change over time. One method often used to assess home range and population spatial distribution is Minimum Convex Polygons (MCPs). MCPs are a method whereby all recorded locations of an individual or a population are contained within the smallest convex polygon possible (Row & Blouin-Demers, 2006). Some limitations of MCPs are the sensitivity of the results to outliers, sometimes leading to overestimated home range sizes and the assumption that all habitats within the home range are used equally.

Objectives:

The purpose of my study is to determine whether the installation of ecopassages and partial exclusion fencing at Chalk River Laboratories in Ontario, Canada, has reduced road mortality and improved the habitat connectivity for the local Blanding's Turtle population. The population was studied in 2014-2015 to investigate habitat use and movement patterns (Hawkins, 2016). The results of this study led to the construction of seven ecopassages (Figure 1) and 200 m of exclusion fencing on either side of the passages along the main access roads.

I aim to determine the efficacy of the ecopassages and partial exclusion fencing, through documenting use to assess if and when movements take place. Comparison with activity observed in 2014-2015 to that in 2022-23 will enable me to investigate whether movement patterns have been altered or new habitats have been adopted since the ecopassage and fencing installations. Should population spatial distribution and individual home ranges of individuals followed in both studies have increased, this may be an indication that ecopassages contribute to habitat re-connectivity. This comparison will provide general insight into the effectiveness of ecopassages and partial exclusion fencing in protecting reptiles, particularly turtles. Furthermore, the results will inform future conservation projects on whether these costly structures should continue to be used to fight species loss.

METHODS:

Study Area:

My study was conducted at the CNL site in Chalk River, Ontario, Canada (Figure 2), from May 2022 to August 2023. The Canadian Nuclear Laboratories is located on the Ottawa River. At the CNL site, the majority (3820 ha) of land is undeveloped and includes wetlands, forested areas, and monitoring stations (Hawkins, 2016). One main road runs through site for employees to access the facilities, this is a two-lane road with a speed limit of 60 km/h. Gravel roads and dirt paths exist to connect the main road to the undeveloped areas to facilitate operations.

Wetland Sampling:

Sampling protocol was consistent between the two study periods though some sampling locations varied. However, this variation did not result in the discovery of novel critical habitats. Nineteen wetlands were sampled in 2022 and 2023 for the presence of Blanding's Turtles (Cranberry Marsh, Dew Drop Lake, Duke Swamp, DWL, Gusts Creek, Lake 233, Lower Bass Lake, Lower Bass Swamp, M12, Maskinage Lake, No Name Lake, Odd Swamp, Road. 7I Wetland, Skinny Wetland, Sturgeon Lake, Toussaint Lake, Twin Lakes, Upper Bass Lake, and West Swamp (Figure 3)). While fourteen wetlands were sampled in 2014 and 2015. Between the months of May and September, large hoop nets (Figure 4) baited with sardines were set in these wetlands for periods ranging from five to twelve days. Every 24-hours, the hoop nets were checked for captures (every 12 hours in June). In addition to hoop nets, turtles were caught opportunistically by hand.

Lab Procedures:

When Blanding's Turtles were caught, they were transported back to a lab onsite for processing following the Animal Care and Handling protocol (ECCC - SARA-OR-2022-0663; University of Ottawa - #BLf-3846). Various measurements were taken to determine the sex and approximate age (stage class) of captured individuals including mass and carapace length. Sex was determined based on the plastron curvature and location of the cloaca (Forrester, 2022; Graham & Doyle, 1977; Lefebvre et al., 2011). In males, the plastron is concave (Graham & Doyle, 1977) and the pre-cloacae tail length is longer (Forrester, 2022; Lefebvre et al., 2011). Using the Ontario Ministry of Natural Resources (OMNR) notching system, individuals were marked with a unique number for future identification (Hawkins, 2016).

Radiotelemetry:

A transmitter (Holohil Systems Ltd. SI-2FT, 13g) was attached to individuals heavier than 300 g on the posterior marginal scutes of the carapace (Figure 5). A Dremel 100 series rotary tool was used to create two holes for small stainless-steel bolts. Bolts were inserted from the bottom of the carapace to prevent injury and to avoid obstructing typical hindlimb movement. Two stainless steel nuts were then affixed to each bolt and all hardware coated in marine-grade silicone. Turtles were kept in the lab overnight to allow the silicone to cure before being released at the capture site.

Individual locations were determined on average every 6.8 days in 2014-15 and every 7.0 days in 2022-23. Turtles were relocated on foot using hand-held receivers (Wildlife Materials Inc. TRX 1000S) and three-element Yagi antennas (Advanced Telemetry Systems), with GPS locations being taken when the turtle was found. Unless visual inspection of hardware or medical intervention was necessary, individuals were not recaptured during radiotelemetry tracking. At the end of August 2023, the fourteen individuals with working transmitters had their hardware removed.

Number of Road Crossings:

All radiotelemetry data was mapped using ArcGIS online. A shape file which outlined the positioning of roads was superimposed on the map. I counted each time an individual was assumed to have crossed a roadway based on radiotelemetry evidence. Producing an inferred minimum number of crossings for each study period.

Daily Movement:

Nineteen individuals (13 females, 6 males) were tracked during the 2022 and 2023 active season (May to August – 514 locations) and infrequently throughout the autumn-spring for a total of 549 locations (Supplemental Material). To measure the distance moved between relocations, the 'Measurement' tool in ArcGIS was used. This tool determines the minimum linear distance between relocation points (i.e. Euclidean distance).

A linear mixed-effect model was used to assess whether the presence of ecopassages resulted in longer distances moved per day. The model compared distance moved by each individual between the two study periods. Fixed variables included reproductive status (gravid female, non-gravid female, and adult male), sex, and study period, while turtle ID was a random variable.

Individual Home Ranges:

A linear mixed-effect model was used to assess whether the presence of ecopassages allowed for larger individual home ranges. The model compared the home range of individuals between the 2014-15 study and the 2022-23 study, accounting for ID and reproductive status.

Prior to analysis, if necessary, data were transformed using the square root function to meet the assumption of normality and homogeneity of variances.

Population Spatial Distribution:

To examine the spatial distribution of the local Blanding's Turtle population, I used R. Following Paterson (2019), MCPs were calculated using the *'adehabitatHR'* (Calenge, 2006), '*ggplot2*', *'ggmaps'*, '*scales*', '*nlme*' (Pinheiro et al., 2021) and '*sp*' packages on RStudio. I created MCPs for each study and maps to display the overall difference between the two.

Statistical Analyses:

All analyses were conducted using R Studio (version 3.6.3) (R Development Core Team, Vienna, Austria, 2008) and ArcGIS Online.

RESULTS:

Demography:

In total, I captured 22 Blanding's Turtles (15 females, 7 males) between May 2022 and August 2023. The frequency of new captures decreased over the course of the study, where I captured 17 new individuals in 2022 while only five were captured in 2023. In 2014-15, 21 Blanding's Turtles (15 females and 8 males) were captured. Of the nineteen Blanding's Turtles tagged with transmitters, 89% were caught using large hoop nets. The majority of turtles captured were adults (18 individuals; 81.2%). Four adult females were determined to be gravid in both 2022 and 2023. Ten of these individuals were captured during both study periods.

Radiotelemetry Data:

On average, I tracked an individual turtle 13.5 times per year (range 1-25) in 2022-23 (Supp. Mat. Table 2.1), the same frequency as those tracked in 2014-15 (range 1-20) (Supp. Mat. Table 2.2). Tracking duration varied as some transmitters failed prematurely, most likely due to dead batteries. Additionally, some antennas broke from their transmitter, attenuating the signal.

Number of Road Crossings:

There was no difference in the minimum number of road crossings observed between the two study periods. Eight road crossings were observed both before and after the ecopassages and exclusion fencing were installed. Despite some evidence based on radiotelemetry data, no ecopassage crossing events were confirmed with photos in 2022-23, though one of the crossings may have been through an ecopassage based on exclusion fence positioning.

Daily Movement:

Turtles moved further in 2022-23 (range 2-57 m/day) than in 2014-15 (range 14-60 m/day) (Figure 6). I compared the average distance moved between relocations between the two study periods using a linear mixed model. Turtles in 2022-23 moved farther on average in between relocations (6 ± 5 m). However, this difference was not significant ($t = -1.23$, $df = 8$, $p = 0.25$).

When comparing individuals followed during both study periods, turtles moved farther between relocations in 2022-23 (range 17-57 m/day) than in 2014-15 (range 14-44 m/day) (Figure 7). I ran a linear mixed model to compare average distance moved between relocations. In 2022-23, turtles moved greater distances on average (6.1 ± 4.9 m). However, this difference was not significant ($t = -1.23$, $df = 8$, $p = 0.25$), meaning that average movement did not vary for individuals followed in both studies with the construction of ecopassages and partial exclusion fencing in the area.

When I ran this model excluding an outlier, I found that individuals moved farther between relocations in 2022-23 than in 2014-15. The individuals in 2022-23 moved 9.4 ± 4 m more on average. However, this difference was again not significant ($t = -2.34$, $df = 7$, $p = 0.052$), meaning that average movement did not vary significantly for individuals followed in both periods with the construction of ecopassages and partial exclusion fencing in the area.

Finally, the model was run again excluding known nesting migrations. The difference in daily movement was still small and non-significant (0.0023 ± 0.054 m/day; p-value = 0.97), with individuals moving slightly more before the installation of ecopassages and exclusion fencing.

<u>Individual Home Ranges:</u>

When comparing all individuals found in both study periods, turtles had larger home ranges in 2014-15 than in 2022-23 based on the transformed data. On average the turtles in the 2014-15 study had larger home ranges by 0.18 ± 0.36 ha (Figure 8a). However, the difference in home range was not significant ($t = 0.52$, $df = 8$, $p = 0.62$). When the model was run again to include only individuals followed during both study periods, with one outlier removed, individuals in the 2014-15 study occupied a smaller area (-0.27 ± 0.56 ha; $t = -0.48$, $df = 8$, $p = 0.64$; Figure 8b). Therefore, home ranges tended to be larger, but not significantly different after the construction of ecopassages and partial exclusion fencing. Additionally, the fixed and random effects did not have a significant effect on home ranges either.

Population Spatial Distribution:

Turtles tracked in 2014-15 occupied a larger area than those tracked in 2022-23 (Figure 9). The population occupied 142 ha in 2022-23, while it occupied 342 ha in 2014-15. Contrarily, when comparing individuals followed in both study periods, the population occupied a 3% greater area in 2022-23 (Figure 10). The population occupied 98 ha in 2022-23, while it occupied 95 ha in 2014-15. Though greater overall population distribution was documented in the 2014-15 study, turtles tracked in both study periods expanded their range to occupy a greater portion of the property by 2022-23.

DISCUSSION:

Summary:

The installation of ecopassages and partial exclusion fencing in the area did not result in increased habitat connectivity or movement for Blanding's Turtles. The number of road crossings were the same before and after installation. There was no difference in daily movement before and after the installation of the mitigation solutions. Home ranges tended to be larger before ecopassage installation when comparing all individuals and larger after installation when only comparing individuals followed in both study periods, but those differences were not significant. The population spatial distribution of all individuals decreased after installation, whereas it increased after installation when comparing individuals followed in both study periods.

Number of Road Crossings:

The number of road crossings did not vary before and after the installation of ecopassages and exclusion fencing. Since the connectivity on site did not change with the installation, it is not surprising that no major differences in movement were observed. Though the ecopassages and exclusion fencing may have improved safety, as no Blanding's Turtle road mortality events were observed after installation. Prior to installation, 4 mortality events were observed between 2011-2018, the connectivity (i.e. crossings) and daily movements may not have improved as a result.

Daily Movement:

The daily movements of the Blanding's Turtles in both study periods were similar to those found in previous studies (Table 1), except when compared to studies by Millar and Blouin-Demers (2011) and Edge (2010), where the documented movements were about six times greater than in my study. At CNL, daily movement did not vary significantly before and after the installation of ecopassages and partial exclusion fencing. As daily movements were similar both before and after installation as well as in comparison to other studies in natural settings, I presume that roads do not constrain Blanding's Turtles daily movements at CNL. Therefore, the installation of ecopassages and exclusion fencing would not impact daily movement.

Though daily movement did not vary significantly between study periods based on the linear mixed effect model, individuals followed in both periods moved longer distances in 2022-23 by approximately 9 metres per day (31% increase). Daily movement patterns may be sex dependent due to nesting and mating requirements (Edge et al., 2010). This increased movement could be due to nesting migrations in 2022-23, as several gravid females made long migrations. Travel to these areas was not documented in 2014-15. When models were rerun without the nesting migrations, the difference in daily movement between study periods disappeared. As Blanding's Turtles appear to display nest site fidelity (Congdon et al., 1983; Standing et al., 1999), it is likely these females were not gravid in 2014-15. As it is common for Blanding's Turtle females to nest less than once annually (Congdon et al., 1983; Standing et al., 1999), this is consistent with their nesting ecology. The presence of ecopassages and exclusion fencing does not appear to be a factor impacting the daily movement of Blanding's Turtles.

Individual Home Ranges:

Individual home ranges tended to be larger before ecopassage and partial exclusion fencing installation comparing all individuals, whereas they tended to be larger after installation when only comparing repeated individuals, but these differences were not significant. Compared to other studies on Blanding's turtles, the CNL population displayed small to intermediate home-range areas (Table 1). The variation in home-range areas between these studies may be due to factors such as sample size differences, population age structure, procedural differences, and habitat variation (Cagle 1944; Bury 1979; Millar & Blouin-Demers, 2011). As the home ranges in my study are comparable to those of previous studies, it is possible that the roads at the Canadian Nuclear Laboratories are not constraining the movement of Blanding's turtles. If this is the case, then it is unsurprising that the installation of ecopassages did not affect space use on site. Blanding's Turtles live up to 83 years (Erickson, 2016); therefore, they may not yet have had sufficient time to find and acclimatize to the ecopassages. As a result, I recommend follow-up studies in the coming years (Martinig & Bélanger-Smith, 2016) to confirm whether turtles adjust to the ecopassages and habitat re-connectivity occurs.

Additionally, Blanding's Turtles are generally known to be a species with large home ranges (Innes et al., 2008), however, it is possible that individual variation could affect this. The results displayed that on average, individual turtles at CNL smaller to intermediate home ranges than those found in the literature (Edge et al., 2010 and other references in Table 1). If individual variation is at play, the average home range observed could be because more mobile individuals have been eliminated over the years via road mortality. It is known that individuals who are more dispersive and live near roads are more likely to be impacted by their effects (Gibbs, 1998). Meaning, dispersive individuals are likely more negatively affected by the habitat fragmentation and dangers that roads pose. This is because with greater movement they are more likely to end up on roads and to cross roads at a higher frequency than their sedentary counterparts (Beaudry et al., 2008; Gibbs, 1998). As a result, this once beneficial trait which allowed individuals to seek out better habitat or resources, became a detriment in the changed landscape (Gibbs, 1998). Over the course of operations at CNL, the more mobile individuals could have been wiped out, leaving only the more sedentary individuals and thus resulting in smaller home ranges.

Population Spatial Distribution:

The population spatial distribution of Blanding's Turtles differed after the installation of ecopassages and partial exclusion fencing depending on the group of individuals. With individuals followed in both study periods, the population spatial distribution was larger after installation. I observed the opposite when comparing all individuals in the study, with a larger distribution before installation.

Climatic conditions influencing habitats, for instance affecting water levels, can transform hospitable areas into hostile ones, affecting space use. In 2014-15, Hawkins (2016) discovered a Blanding's Turtle in a wetland in the southwest corner of her study area, however, none were caught there in 2022-23 despite extensive trapping efforts. This absence could be due to a dam breakage in 2016 which caused a drastic drop in water levels in the area. As a result, the residing Blanding's Turtles may have needed to relocate. To account for this possibility, I sampled nearby water bodies for Blanding's Turtles in 2022-23, however, again none were found. Thus, it appears that the reduction in the spatial distribution of the population after the installation of ecopassages and partial exclusion fencing could be due to a reduction in available habitat that is unrelated to roads.

Hibernacula locations were confirmed for 11 Blanding's Turtles in winter 2023. Nine Blanding's Turtles were using a pond neighboring Lake 233, with two Blanding's Turtles remaining in Lower Bass Swamp. In 2014-15, two additional overwintering sites were identified by Hawkins (2016), adding to the difference in population spatial distribution. One of the female turtles tracked in 2015 switched to the Lake 233 overwintering site in 2022-23. This may have happened for a few reasons, such as the habitat becoming unsuitable for overwintering or limited mate availability, restricting mating opportunities to the early active season.

Limitations:

Certain limitations and variations between study periods may have affected the results of my study. These varied from methodological to technological constraints and should be considered when interpreting the findings. The main limitations of my study are that I did not complete a true BACI (Before-After-Control-Impact) study and the limited number of individuals I tracked. I was unable to compare changes in home ranges and movements to a control site where ecopassages were not installed, which limits the strength and applicability of my results. Ideally, I would have before and after movement data for a set of sites where ecopassages and partial exclusion fencing were installed, and simultaneous before and after movement data for a set of sites where they were not installed, although this would represent a formidable task. In future, BACIs should be the gold-standard for ecopassage studies (Soanes et al., 2024).

Though continuous monitoring of ecopassage use was done via wildlife cameras taking pictures every 15-30 sec, these data could not be included because a systematic analysis of all the pictures (~10 million) has not been completed at this time. Without powerful AI technology, this analysis would require an estimated 1000 person-hours. Should researchers wish to use this type of data, it would be beneficial to collaborate with someone experienced in the use of artificial intelligence to analyze and sort photos. This was outside of my knowledge and the currently available pre-trained artificial intelligence technology, to my knowledge, at the time of my project. Certain roadblocks we experienced in trying to train an AI program to recognize turtles included the background constantly changing as plants grow and the sun changing position. Additionally, there were fourteen backgrounds that we would need to program because there was a camera set up at each end of the seven ecopassages. AI programs are commonly trained to identify mammals or birds (Albardi et al., 2021; Bodesheim et al., 2022; Carl et al., 2020), but have not yet been trained to identify turtles.

Management Implications:

To assess the effectiveness of ecopassages, understanding road use before and after construction is essential. A systematic review by Soanes and colleagues (2024) found that most studies reported that ecopassages did not restore typical movement. It is generally accepted that some decline in movement across roads is unavoidable post-construction and aiming for a 'limited net loss' (van Der Grift et al., 2013) may be more achievable. Though ecopassages are often a requirement for new road developments, it is important to compare use in a specific area (Soanes et al., 2024). This will allow a clearer understanding of ecopassage effectiveness and avoid experimental bias, as this rigorous assessment creates a baseline for comparison and a method to confirm success which is not possible with after-impact data alone. As highlighted in this review, the use of ecopassages does not necessarily equate to successful mitigation.

Ecopassages are not a one size fits all solution to road mortality for all taxa. The ideal structure varies based on the targeted species in terms of openness, passage length, and light exposure. Smaller animals may prefer shorter ecopassages with greater visibility and openness, to allow predator surveying (Martinig & Bélanger-Smith, 2016). The ecopassages at CNL provide limited light, except for one grated-top ecopassage and vary in openness as they range in size from approximately 50-120 cm tall and 36-180 cm wide. The length and spacing between ecopassages should also be related to the size and average daily distance moved by the target species. Though site specific, acquiring data on dispersal and migration pathways for species is important for determining appropriate ecopassage placement. Currently, this information is more widely available for mammals than for other groups. Generally, species that travel shorter distances require more ecopassages to allow for a greater chance of encounters (Bissonette & Adair, 2008).

Only one Blanding's Turtle followed with radiotelemetry appeared to use the ecopassages. Based on a Lincoln Petersen estimate (Bailey, 1952) of population size, however, I only tracked one third of the population. Thus, it is possible that ecopassages were used by turtles that were not equipped with radio-transmitters.

The majority of the turtles were located in an area that appears to allow fulfillment of their biological requirements (nesting sites, mate availability, hibernacula, etc.). Additionally, for turtles to access some other areas of the site, they did not necessarily need to use ecopassages. Turtles could access about half of the site by moving through surrounding forests, something Blanding's Turtles are particularly adept at doing (Beaudry et al., 2010).

Despite the limited use of ecopassages by Blanding's Turtles, the overall impact of ecopassages at CNL may be positive. Other turtle species (Painted and Snapping) and various mammals were documented using the ecopassages based on the haphazard perusal of approximately 1 million photos out of the 10 million available. Though a management practice may work for Snapping and Painted Turtles, it does not necessarily mean it will work for other turtle species. However, at the time of this study the exclusion fencing was largely incomplete so a decisive conclusion on the effectiveness cannot be drawn. The primary goal of CNL management at this time should be to complete the length of exclusion fencing on site. Any gaps in the fencing along roads maintains the potential for animals to gain access to roadways and become involved in vehicle-wildlife incidents. Though ecopassages are essential in maintaining habitat connectivity, the installation of fencing is the most critical component in reducing animal road mortality (Rytwinski et al., 2016). To keep smaller animals off roads such as turtle hatchlings or frogs, it would be effective to modify existing fencing by adding an 80 cm tall metal fence addition to the base of existing fencing (Woltz et al., 2008). This fencing should also be buried a minimum of 20 cm to prevent future lifting at the base (Dodd et al., 2004) with smaller holes in the material to ensure smaller amphibians and reptiles cannot evade the mitigation and access roads.

In addition to the construction of ecopassages and exclusion fencing, the speed limit along the main access road, Plant Rd., was reduced from 90 km/h to 60 km/h, thus presumably reducing the risk of animal road mortality. As the exclusion fencing does not line the entirety of Plant Rd., it is possible that animals can still cross on the road far from ecopassages. During my study, three Blanding's Turtles were found alive on roads. It is difficult to say if these animals would have been killed if the speed limit had not been reduced, but it is a possibility. Before the fencing installation, four Blanding's Turtles were killed on roads at CNL between 2012-2018 (one – 2011, one – 2014, and two – 2018). Based on this anecdotal evidence, speed limitation paired with public education could be an effective short-term solution and should be considered when the cost of conservation tools exceeds budget allowances.

<u>Conclusion:</u>

While ecopassages are not known to have been used by Blanding's Turtles thus far, they seem to benefit other species and do not seem to have affected connectivity. There may have been insufficient time for the Blanding's Turtles to acclimate and learn to use these structures. They may not yet be aware of, or comfortable with the crossing of ecopassages and could start to use them in the future, especially once exclusion fencing has been completed. It could also be that the wetlands they reside in meet all their needs and negate the need to use ecopassages to access other habitats. Finally, since approximately one third of the population was radio tracked in each study period, turtles that were not equipped with radio-transmitters may be occupying other areas on site or making occasional use of the ecopassages without detection.

My study suggests that ecopassages may be ineffective at reconnecting habitat and expanding home range for Blanding's Turtles without complete connectivity of exclusion fencing. Few studies have properly investigated movement changes after ecopassage installation, particularly for turtles, and the results of my study accentuate the need for further research. I recommend the use of a BACI study design to assess animal response to ecopassages before continuing the widespread installation of such structures (Soanes et al., 2024). BACI studies will better inform land management on the effectiveness of ecopassages at maintaining habitat connectivity and movement, while reducing when selecting ecopassages for animal road mortality. Should further studies prove ecopassages are indeed ineffective at maintaining habitat connectivity more well-suited mitigation solutions will need to be designed and tested.

FIGURES:

Figure 1. A Blanding's Turtle (*Emydoidea blandingii*) in the Dew Drop Lake ecopassage, image captured using a wildlife camera.

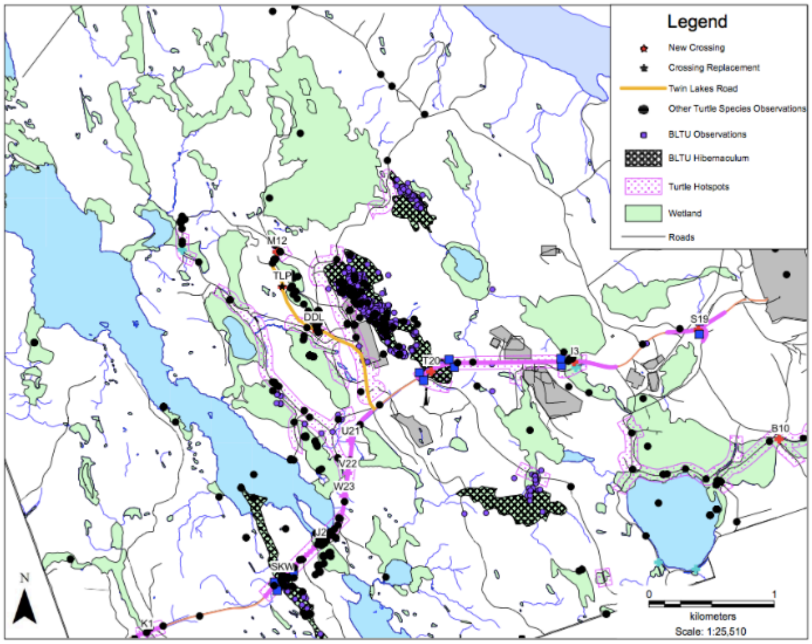

Figure 2. Map of study area. Pink line depicts the main access road (Plant Road) and the yellow line represents Twin Lakes Road. Each label along these roads indicates the presence or a future ecopassage (SKW, T20, I3, S19, M12, TLP, DDL - established ecopassages, while the remaining are to be constructed).

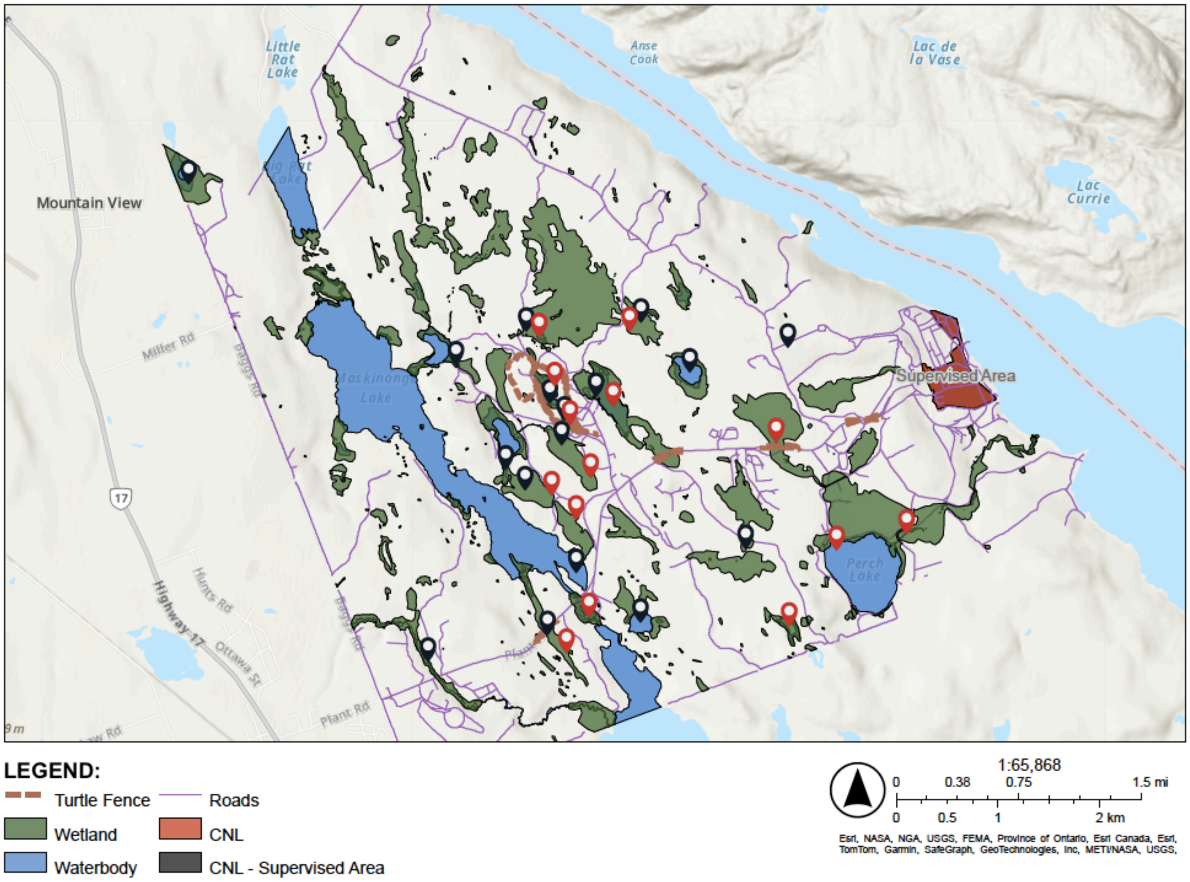

Figure 3. Nineteen wetlands sampled total in both study periods (2014-15 and 2022-23). Where the red points represent the nets in the 2014-15 period and black represent the nets in 2022-23. Wetlands include Cranberry Marsh, Dew Drop Lake, Duke Swamp, DWL, Gusts Creek, Lake 233, Lower Bass Lake, Lower Bass Swamp, M12, Maskinage Lake, No Name Lake, Odd Swamp, Rd. 7I Wetland, Skinny Wetland, Sturgeon Lake, Toussaint Lake, Twin Lakes, Upper Bass Lake, and West Swamp.

Figure 2. Large hoop net set up in Lower Bass Swamp, Canadian Nuclear Laboratories, Chalk River, Ontario.

Figure 3. A transmitter fitted on the posterior marginal scutes of a Blanding's Turtle (*Emydoidea blandingii*) carapace.

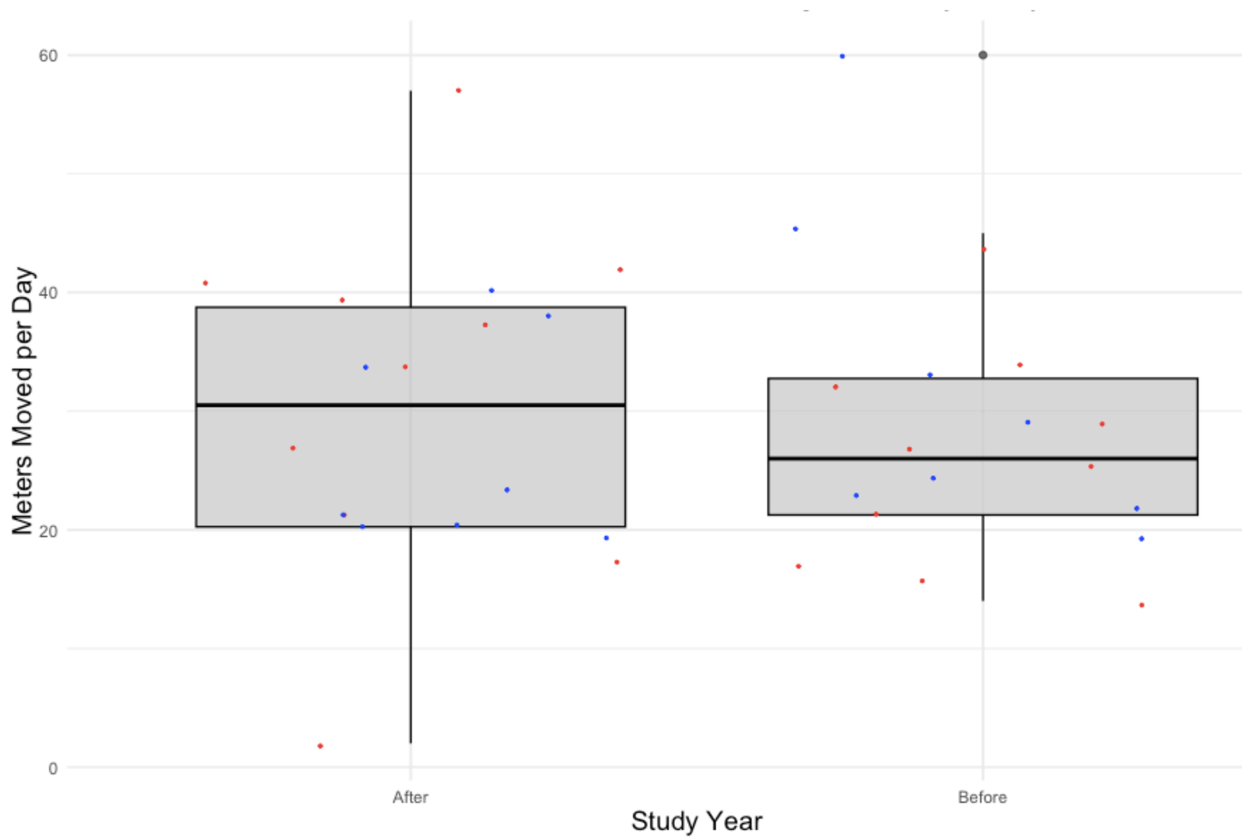

Figure 4. Distance moved per day (m) by study for Blanding's Turtles (*Emydoidea blandingii*) (n = 28) in Chalk River, Ontario. Blue points represent distances moved by individuals unique to that study, whereas the red points represent individuals tracked in both studies.

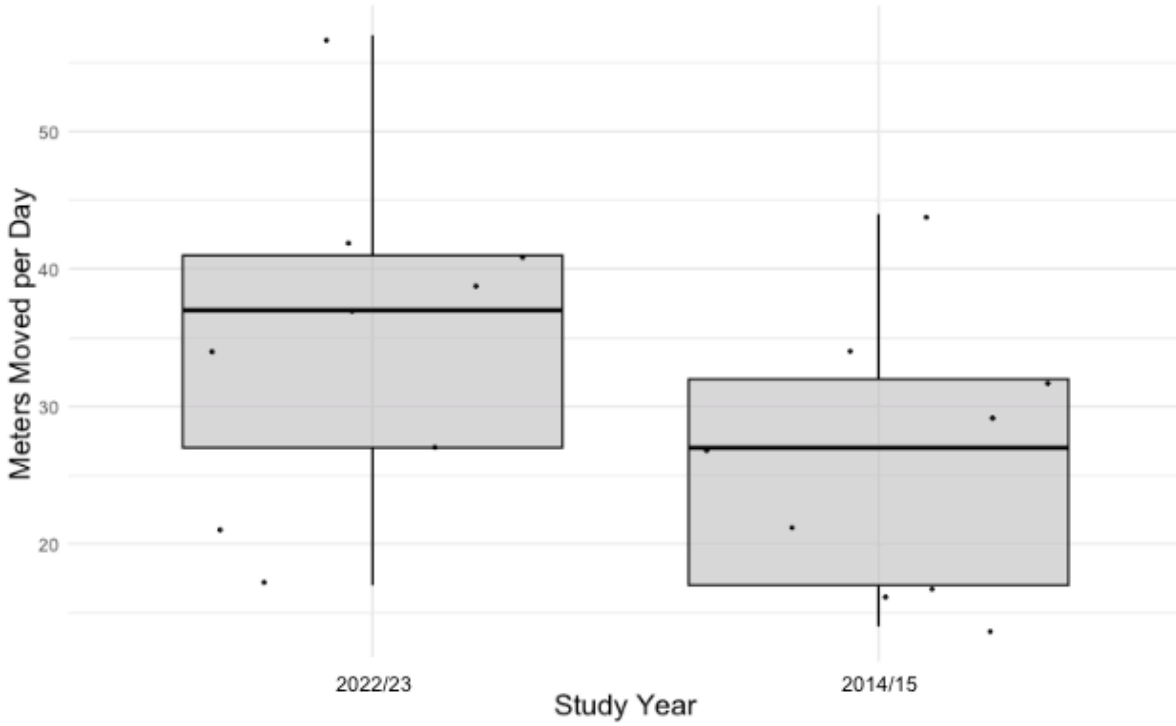

Figure 5. Distance moved per day (m) by period for Blanding's Turtle (*Emydoidea blandingii*) individuals followed in both studies (n = 9) in Chalk River, Ontario. Black points represent individual movement distances in each respective study.

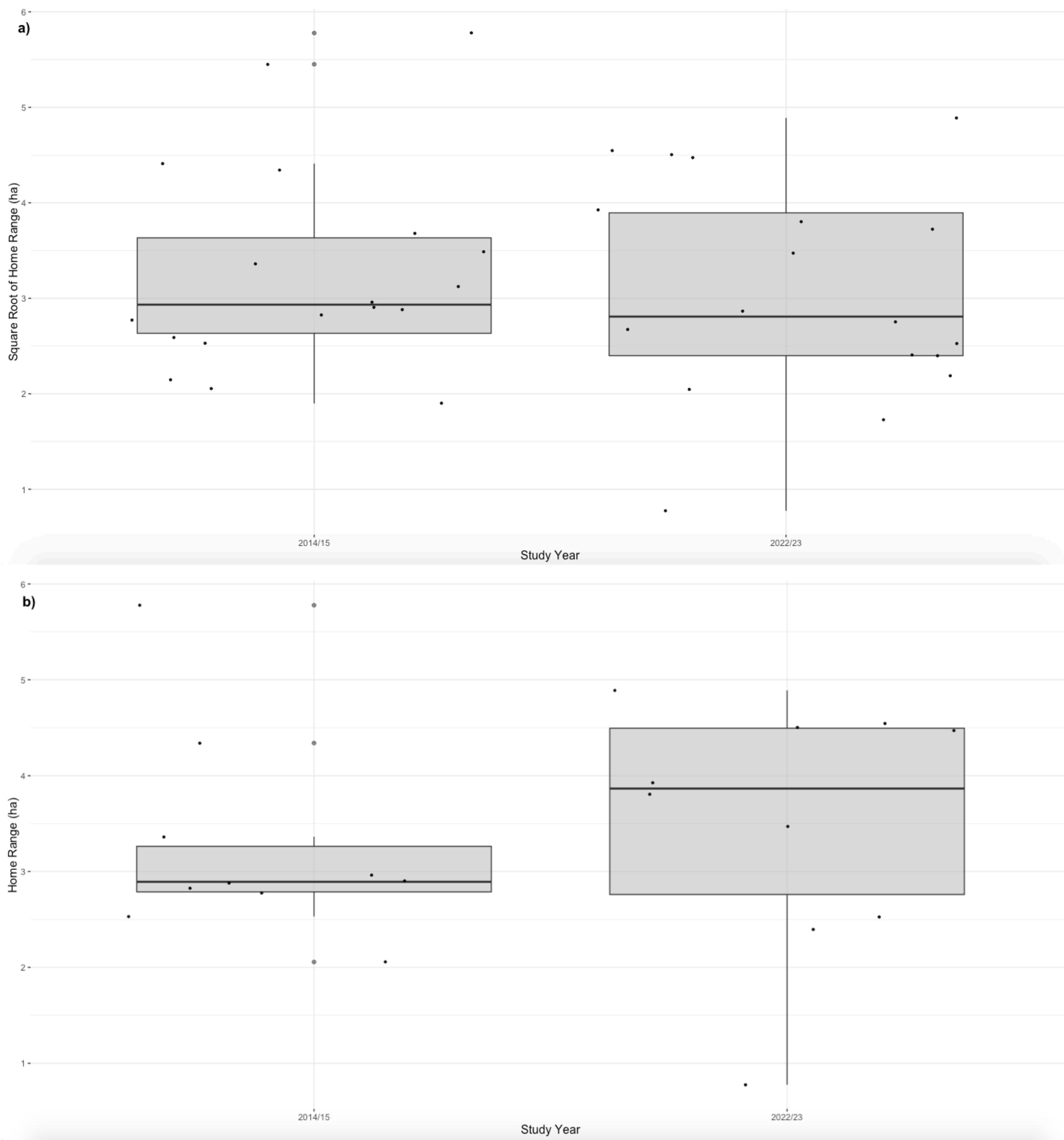

Figure 6. Solid black bars represent the mean area (ha). Black points indicate individual home ranges (ha). (a) Home range (ha) area for Blanding's Turtles (*Emydoidea blandingii*) (n = 29) in Chalk River, Ontario in 2014-15 and 2022-23. (b) Home range area (ha) for individuals followed in both study periods, 2014-15 and 2022-23.

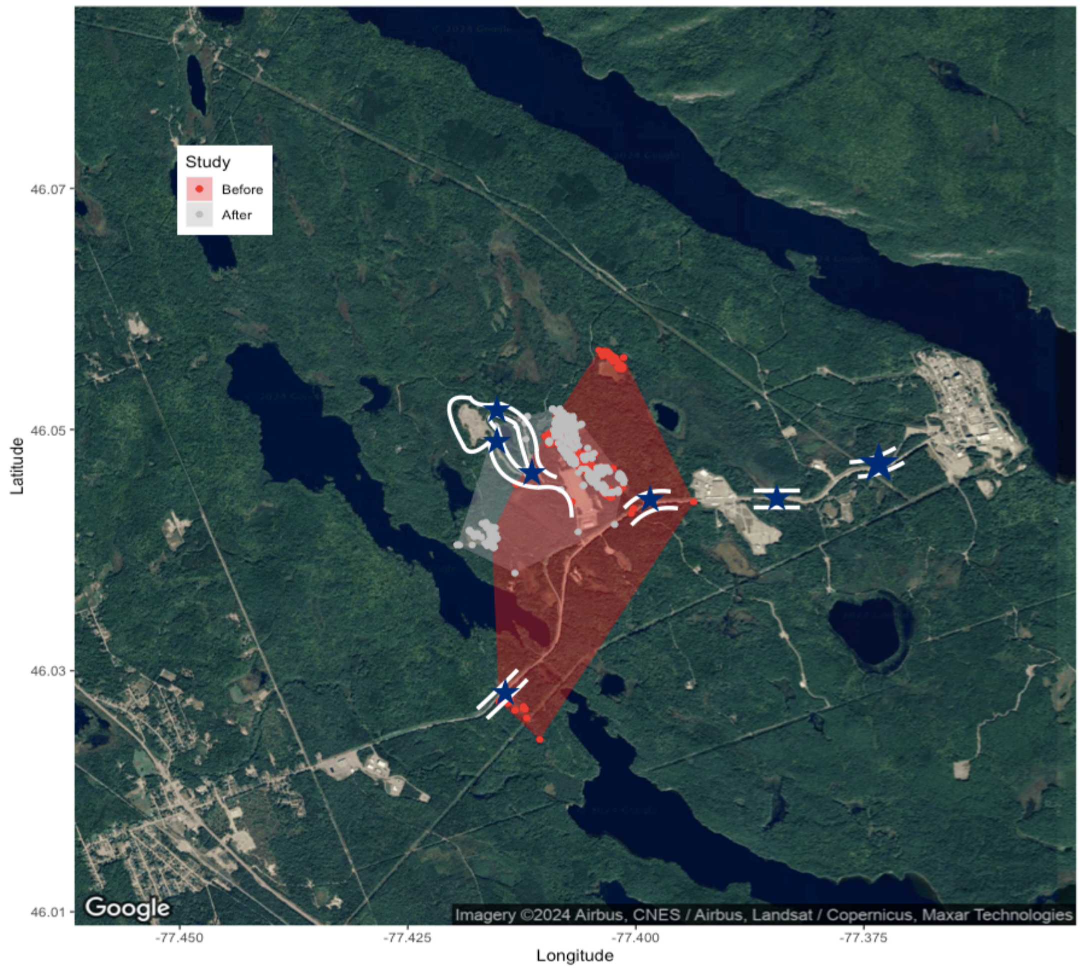

Figure 7. Population spatial distribution comparison for Blanding's Turtles (*Emydoidea blandingii*) from 2014-15 (red) and 2022-23 (gray) in Chalk River, Ontario. Ecopassages are represented by blue stars and wildlife exclusion fencing is represented by white lines. Map of all individuals (n = 29).

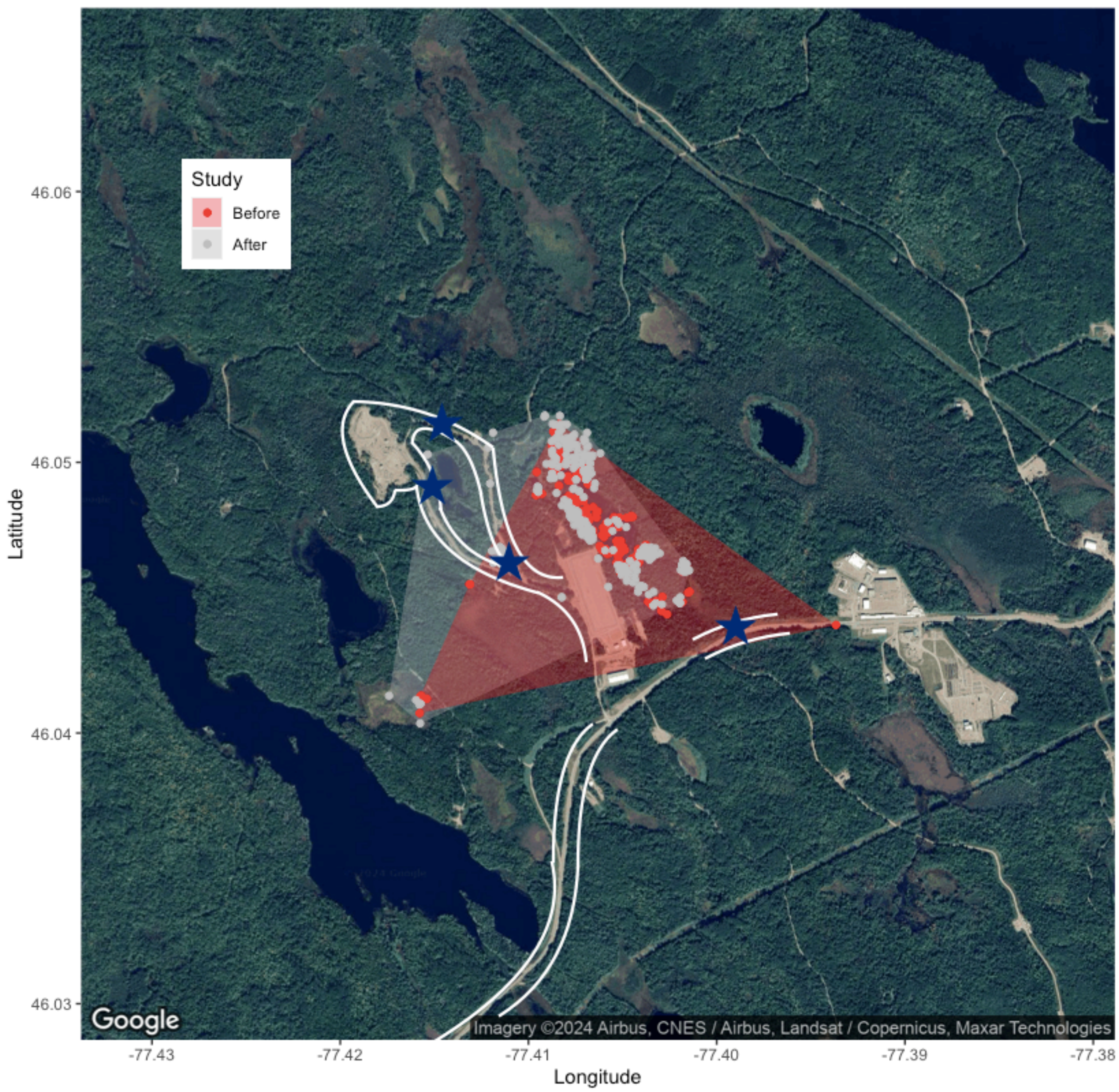

Figure 8. Population spatial distribution comparison for Blanding's Turtles (*Emydoidea blandingii*) from 2014-15 (red) and 2022-23 (gray) in Chalk River, Ontario. Map of individuals repeated in both studies (n = 10).

TABLES:

Table 1 - Literature review of daily movement (m) and home range (ha) by male (M) and female (F) Blanding's Turtles (*Emydoidea blandingii*):

Reference	**Study Location**	**Mean Movement (m/day)**	**Mean Home Range Area (ha)**
O'Halloran and Blouin-Demers, 2024	Ontario	29.56 ± 3 M - 23.4 ± 9.1 F - 31.92 ± 2.41	M - 8.23 ± 2.49 F - 12.35 ± 1.96
Hamernick et al., 2020	Minnesota		M - 94.92 F - 60.75
Paterson et al., 2019**	Ontario	45 ± 2 (w/o roads) 31 ± 2 (w/ roads)	
Markle and Chow-Fraser, 2018	Ontario		M - 20 ± 3 F - 46 ± 11.9
Starking-Szymanski et al., 2018**	Michigan	26.38	2.8 ± 0.95[3]
Hawkins, 2016	Ontario	28.56 ± 2.75 M - 29.67 ± 3.24 F - 28 ± 3.89	M - 14.23 ± 3.97 F - 10.79 ± 2.7
Hasler et al., 2015	Ontario		19.06 ± 6.37[3]
Walston et al., 2015	New Hampshire		M - 10.7 ± 0.1 F - 19.6 ± 3.5
Anthonysamy, Dreslik, & Phillips, 2013	Illinois	M - 19.6 ± 9.9 (2005) 33.5 ± 8.3 (2006) F - 18.6 ± 6 (2005) 27.0 ± 3.4 (2006)	
Fortin, Blouin-Demers, and Dubois, 2012	Quebec		29.7 ± 32.3[3]
Millar and Blouin-Demers, 2011	Ontario	M - 199.42 NGF - 195.32 GF - 249.50*	M - 8.2 NGF - 7.3 GF - 20.3[2]
Edge et al., 2010[1]	Ontario	M - 83.5 ± 39.9	M - 57.1 ± 15.3

		F - 91 ± 37.4	F - 61.2 ± 30.4
Innes et al., 2008	New Hampshire	M - 27.55 ± 6.89 F - 30.08 ± 14.15	M - 3.7 F - 6.6/1.5
Grgurovic and Sievert, 2005[1]	Massachusetts		M - 27.5 ± 0.1 F - 19.9 ± 0.07
Hamerick, 2000	Minnesota		M - 38.4 F - 35.4
Piepgrass and Lang, 2000	Minnesota		M - 94.9 ± 58.4 F - 60.7 ± 12.6
Sajwaj et al., 1998	Minnesota	M - 22.75 ± 8.34 F - 41.75 ± 8.83	
Rowe and Moll, 1991	Illinois	M - 48.9 ± 20.85 F - 32.4 ± 22.66	M - 1.4 F - 1.2
Ross and Anderson, 1990	Wisconsin	M - 48.4 ± 8.99 F - 95.1 ± 10.56	M - 0.76 ± 0.19 F - 0.56 ± 0.15

**NGF = non-gravid female; GF = gravid female*
***Mean not available by sex as individuals were juveniles*
1 - All home ranges determined using MCPs except Grgurovic and Sievert, 2005 and Edge et al., 2010
2 - NGF = non-gravid female; GF = gravid female
3 - Mean not available by sex

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SUPPLEMENTARY MATERIAL:

Table 2.1. Summarized telemetry data for individual Blanding's Turtles (*Emydoidea blandingii*) (n = 19) in Chalk River, Ontario. Date of transmitter attachment and total number of tracking events per individual throughout the 2022-23 study period.

Turtle ID	Transmitter Attachment Date (Y-M-D)	Number of Relocations (2022-23)
702	2022-05-27	17/24
703	2022-05-28	17/21
707	2022-05-28	21/21
712	2022-06-30	18/11
715	2022-05-27	25/13
716	2022-05-27	22/17
719	2022-07-19	17/22
720	2022-05-29	18/7
722	2022-08-16	5/20
723	2022-09-22	2/3
724	2022-09-22	2/23
731	2023-06-20	0/16
734A	2022-05-30	19/18
734B	2023-06-28	0/1
738	2023-06-16	2/5
739	2022-06-14	14/15
740	2022-06-14	13/20
742	2023-05-30	0/18
743	2023-07-28	0/17

Table 2.2. Summarized telemetry data for individual Blanding's Turtles (*Emydoidea blandingii*) (n = 22) in Chalk River, Ontario. Date of transmitter attachment and total number of tracking events per individual throughout the 2014-15 study period.

Turtle ID	Transmitter Attachment Date (Y-M-D)	Number of Relocations (2014-15)
702	2014-06-13	2/12
703	2015-05-05	0/19
706	2015-07-07	0/12
707	2015-05-09	0/19
709	2014-05-14	5/0
710	2014-05-30	3/17
711	2014-06-15	2/16
712	2014-06-13	2/14
713	2014-06-24	1/1
714	2014-06-30	1/1
715	2014-07-16	4/19
716	2014-07-16	3/20
717	2015-05-07	0/15
719	2015-05-09	0/16
720	2015-05-09	0/1
721	2015-05-09	0/20
722	2015-05-11	0/16
723	2015-05-21	0/14
724	2015-05-27	0/19
725	2015-05-29	0/20
726	2015-05-30	0/3
727	2015-06-20	0/17

732	2015-05-08	0/16