A Rocky Solution: Evaluating the use of Common Construction Materials as Road-Effect Mitigation for Turtle Communities in a Rock Barren Landscape

by

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Abstract

Roads are pervasive linear features that bisect landscapes, altering how female turtles use and move between critical habitats during nesting migrations. While turtle population viability depends on the survivorship of reproductive females, few cost-effective mitigation strategies directly address their vulnerability to roads. The objective of my study was to evaluate a new cost-effective mitigation strategy that used common construction materials to reduce road threats for turtle communities in eastern Georgian Bay, Ontario. The mitigation design aimed to deter females from nesting in roadside habitats by replacing ~ 300 m of exposed gravel at 5 wetland crossings with rock rip-rap and paved road shoulders (using tar-and-chip), while the rest of the road shoulders remained unchanged. The success of the mitigation strategy was assessed by whether it successfully prevented females from using the road as nesting habitat. First, I used a Before-During-After comparison of nesting observations and nest hot spots on the road. I found a 15% decrease in the number of females nesting at Mitigated sites in the After period; however, females continued to nest in the nearest available Unmitigated roadside habitat, including in semi-compact tar-and-chip road shoulders at Mitigated sites. In addition, nesting hot spots remained at Mitigated sites in the After period. Second, I investigated the availability and suitability of natural nesting habitats in the surrounding rock barren landscape relative to nesting habitats used by female turtles on road shoulders. I conducted systematic habitat surveys (in 10800 1m x 1m plots) to quantify the availability of suitable nesting habitats on open rock barrens based on soil depth and canopy openness requirements for Species at Risk (SAR) turtles. I found road shoulders met nesting habitat requirements for three local turtle species, whereas only 1% of rock barrens in the 231-ha study area were suitable for turtle nesting. Overall, I found the availability of suitable nesting habitats was limited across the natural landscape, which may contribute to females' selection of roadside nesting habitats. My findings suggest that the mitigation strategy was unsuccessful at deterring female turtles from nesting on roads and should not be applied without further research, especially in areas where natural nesting habitat may be limited. I identified additional recovery actions, such as mortality mitigation (i.e., fence-underpass mitigation) and nesting habitat restoration, that may be required to reduce road effects for the turtle community. Overall, my project contributes to studies evaluating road-effect mitigation and highlights several important findings that can be incorporated into Best Management Practices for turtles during road development.

Keywords: road ecology; road-effect mitigation; freshwater turtles; species at risk; nesting; rock barrens

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General Introduction

Importance of Turtles

Persisting in the environment for over 200 million years, turtles play crucial roles in the structure and function of healthy ecosystems (Lovich et al. 2018). Turtles can reach high population densities making them vital for nutrient cycling, energy flow, seed dispersal, the food web, and keeping waterways clean (Lovich et al. 2018, Santori et al. 2020). However, in the 21st century, turtles and the ecosystem services they provide are threatened by humans rapidly changing the environment (Gibbons et al. 2000, Stanford et al. 2020, Cox et al. 2022). The threats to turtle populations are multifaceted and include habitat loss and degradation, road mortality, illegal collection for the pet trade, and invasive species (Gibbons et al. 2000, Stanford et al. 2020). With global declines in turtle populations, there is an urgency to evaluate turtle communities and their conservation needs (Gibbons et al. 2000, Lovich et al. 2018, Stanford et al. 2020, Cox et al. 2022). In Ontario, seven of eight turtle species are listed as Species at Risk (SAR) (COSSARO 2015, 2017), with local populations facing high risk of extirpation (Enneson and Litzgus 2009, Howell and Seigel 2019). If conservation actions are not taken, vulnerable turtle populations in Ontario may become extirpated (Enneson and Litzgus 2009, Howell and Seigel 2019).

Road Effects

The expansion and use of road networks are a significant threat to turtle communities (Gibbs and Shriver 2002, Howell and Seigel 2019, Piczak et al. 2019). Turtles require connectivity between aquatic and terrestrial habitats to carry out biological functions of overwintering, foraging, mating, and nesting (Beaudry, et al., 2008; Joyal et al., 2001;

Rasmussen & Litzgus, 2010). However, roads and their associated traffic reduce the availability of high-quality habitat and result in mortality from vehicle-turtle collisions (Aresco 2005, DeCatanzaro and Chow-Fraser 2010). Road-effects on turtle populations can vary spatially and temporally (Beaudry et al. 2010b, Cureton and Deaton 2012, Patrick et al. 2012). In road ecology, hot spots are defined as road sections with spatially clustered species occurrences of either alive or dead individuals (Cureton & Deaton, 2012). Common indicators of road mortality hot spots for turtles are traffic volume, surrounding landscape features, and species-specific dispersal corridors (Langen et al. 2009, Gunson et al. 2012, Patrick et al. 2012). Specifically, studies have found that because turtles make inter-wetland movements, road mortality hot spots are often associated with road sections that bisect or are near wetland habitats (Beaudry et al. 2008, Cureton and Deaton 2012).

The risk of road mortality can also vary temporally, with seasonal peaks during terrestrial migrations (Cureton and Deaton 2012). Studies have found that road mortality can disproportionally impact mature female turtles as they undertake nesting forays and nest on road shoulders (Steen and Gibbs 2004, Steen et al. 2006); the consequences are population declines from female mortality, reduced nest success rates, and fewer females contributing to the genetic diversity of a population (Marchand and Litvaitis 2004, Aresco 2005, Laporte et al. 2013). Studies have found that high rates of female road-mortality can result in male-biased populations in wetlands adjacent to roads (Steen and Gibbs 2004, Steen et al. 2006, Patrick and Gibbs 2010). Contrastingly, some studies have found that there is no bias in the sex-ratio of turtle populations adjacent to roads or in road mortality rates, indicating that road mortality can be a threat to both adult male and female turtles as they make upland movements (Buchanan 2017, Bowne et al. 2018, Carstairs et al. 2018). In addition, the mortality of juvenile turtles during dispersal movements can also contribute to population declines, especially in populations that may already be experiencing adult mortality (Keevil et al. 2022). Thus, the construction, maintenance, and daily use of roads threaten turtles' ability to safely navigate the landscape (Steen and Gibbs 2004, Beaudry et al. 2008, Piczak et al. 2019).

The viability of turtle populations experiencing road mortality is of specific concern due to their life history (Congdon et al. 1993, 1994, Heppell 1998, Enneson and Litzgus 2008). Turtles have long-life spans, late maturity and low juvenile recruitment, which are characteristics that prevent populations from absorbing the loss of mature adults (Congdon et al. 1993, 1994, Heppell 1998, Enneson and Litzgus 2008). Annually, adult mortality as low as 2–3% can impact population persistence (Congdon et al. 1993, Gibbs and Shriver 2002). Some turtle populations without any additive anthropogenic mortality are predicted to have high risks of extirpation (Enneson and Litzgus 2009); thus, populations experiencing road effects require extra conservation attention to prevent population declines.

Road-Effect Mitigation

Mitigation designs will vary depending on the specific life-history traits, behaviour, and movement patterns of a target species (Woltz et al. 2008, van der Grift et al. 2013, Macpherson et al. 2021). Traditionally, road-effect mitigation for turtles targets hot spot locations with the installation of roadside fencing coupled with a crossing structure (e.g., Baxter-Gilbert et al., 2015; Gunson et al., 2016). This combination allows for habitat connectivity with safe passage under the road during seasonal migrations (Boyle et al. 2021). The materials used can vary depending on the availability of resources (e.g., funding, effort, materials) and local landscape features. Crossing structures can connect habitats over the road with wildlife bridges or under the road with culverts, drainage pipes, or tunnels (Woltz et al. 2008, Lesbarrères and Fahrig 2012, van der Grift et al. 2013). Road-side fencing can include material such as geotextile fabric (Baxter-Gilbert et al. 2015, Markle et al. 2017), concrete walls (Aresco, 2005), or HDPE (plastic) half-pipe (Heaven et al. 2019). The application of road-effect mitigation strategies has become a common practice to reduce road mortality for turtle populations (Lesbarrères and Fahrig 2012, van der Grift et al. 2013). However, retrofitting fence-underpass mitigation strategies on roads is not feasible for all conservation projects because of the high installation costs, demanding long-term maintenance, and the difficulty of applying in some landscapes (Lesbarrères and Fahrig 2012). Therefore, it is crucial that studies continue to explore and evaluate alternative mitigation strategies that are cost-effective, low-maintenance, and easily incorporated into road development projects.

Post-Installation Monitoring

The success and long-term outcomes of road-effect mitigation strategies are greatly unknown because few studies evaluate mitigation strategies post-installation (van der Grift et al., 2013). The success of mitigation is often determined based on a direct assessment of road mortality rates before and after mitigation. Studies following before-after (BA) designs are useful in evaluating if a mitigation strategy shows a reduction in turtle abundance on roads; however, they are limited in their ability to distinguish if the observed effect is the result of mitigation or other temporal factors (e.g., weather, seasonality, climate change) (van der Grift et al. 2013). Alternatively, a before-after-control-impact (BACI) is a more rigorous study design that can help distinguish the effects of mitigation from other temporal factors by simultaneously evaluating changes at a control and impact site over time (van der Grift et al. 2013). Although, in the absence of a detailed population study, a BA or BACI study design fails to evaluate the effectiveness of mitigation on a population level (Boyle et al. 2021). Thus, there is a need for rigorous study designs (i.e., BACI design) that include population-level studies to quantify the effects of roads and mitigation strategies to help inform conservation initiatives for turtle communities (Lesbarrères and Fahrig 2012, van der Grift et al. 2013).

Despite well intended mitigation strategies, introducing new structures into an environment can adversely affect an ecological community. Studies evaluating exclusion fencing found that a fence's structural integrity and connectivity are crucial for successfully reducing turtle road mortality (Baxter-Gilbert et al., 2018; Markle et al., 2017). Turtles can become trapped on the road if the integrity of a fence is compromised with an opening, thereby increasing their risk of road mortality (Baxter-Gilbert et al., 2015). Fencing can also create an ecological trap for non-target species seeking thermoregulation opportunities (Boyle et al., 2019). Herpetofauna were unable to escape heat exposure due to improperly installed fencing, resulting in their desiccation (Boyle et al., 2019). These documented secondary consequences of mitigation strategies demonstrate the need for rigorous post-installation studies to evaluate the efficacy of mitigation strategies. In some cases, the secondary consequences caused by mitigation may be inconsequential compared to their success in reducing road effects for a priority species. However, efforts must be made during the design, construction, monitoring, and management of mitigation strategies to adaptively respond to the outcomes for both target and non-target species.

Objectives

It is important to consider the nesting behaviour of female turtles when designing and implementing road-effect mitigation strategies. Despite models showing that turtle population persistence depends on the survivorship of reproductive females (Congdon et al. 1994, Heppell 1998, Enneson and Litzgus 2008), few cost-effective mitigation strategies directly address female vulnerability to roads. However, using common construction materials may provide a cost-effective strategy in areas where fence-underpass mitigation designs are not feasible. In eastern Georgian Bay, a new road-effect mitigation strategy made use of scheduled road works to prevent female turtles from nesting in roadside habitats. The mitigation strategy reduced roadside nesting habitat at wetland crossings by replacing gravel with rock rip-rap and paved road shoulders (paved using tar-and-chip). The mitigation strategy's success depended on the design's ability to deter females from nesting in road shoulders at Mitigated sites, without causing females to use other Unmitigated roadside habitat for nesting. If females were successful deterred from the road and chose to nest in the natural landscape, the design could reduce females' exposure to road mortality and risks associated with roadside nesting (i.e., low success rates from predation and compaction).

The overarching goal of my thesis was to evaluate if the rip rap and paved road shoulder mitigation strategy effectively reduced road threats for the local turtle community. The chapters of my thesis evaluated two critical components to determine the success of this mitigation strategy. In my first chapter, I evaluated the effectiveness of this mitigation strategy using a Before-During-After comparison of nesting observations and hot spots on the road. In addition, I estimated the relative risk of road effects for the local turtle community using a mark-recapture study. In my second chapter, I investigated the availability and suitability of nesting habitats in the area surrounding nesting hot spots on the road to determine if an alternative to the road was available to females. Suitable nesting habitat was quantified based on soil depth and canopy openness requirements for three focal species of Blanding's Turtles (*Emydoidea blandingii*), Painted Turtles (*Chrysemys picta*), and Snapping Turtles (*Chelydra serpentina*). Overall, the mitigation strategy was considered successful if it deterred females from nesting on the road, without causing their use of other risky roadside habitat for oviposition.

Study Area

The study area is in the Parry Sound Ecodistrict along the eastern shores of Georgian Bay, situated within the Robinson-Huron Treaty of 1850 and Williams Treaty of 1923 and located on Anishinabek territory. The landscape is characterized by a habitat mosaic of mixed forest with open gneissic rock barrens and large wetland complexes. The diverse landscape features of the area provide a significant wildlife refuge for several SAR turtle species approaching their northern range limit. The habitat in the eastern Georgian Bay area is unique, and compared to turtles more southern ranges, few studies have described the critical habitats turtles use within this region (Markle and Chow-Fraser 2014). Specifically, this study area provides nesting habitat for turtles unlike that in other regions (Burke et al. 2018, Markle et al. 2021), consisting of moss and lichen-covered shallow soil deposits on open rock barrens (Litzgus and Brooks 1998, Markle and Chow-Fraser 2014, Markle et al. 2021). With increasing development in the area, conservation efforts are required to protect these critical habitats and vulnerable turtle communities.

Study Species

My study focuses on three freshwater turtle species known to nest in roadside habitats and occupy the wetland complexes within the study area. All three species are federally listed as SAR and are at risk from anthropogenic threats, including habitat fragmentation and degradation; road mortality; and increasing predator populations (COSEWIC 2008, 2016, 2018). Populations of all three species are susceptible to additive mortality because of their life history characteristics (i.e., long life-span, delayed maturity, and low recruitment; Congdon et al. 1993, 1994, Heppell 1998, Enneson and Litzgus 2008); thus, making them important species to target with conservation efforts to prevent further population declines.

Painted Turtle

Midland Painted Turtles (*Chrysemys picta marginata*, hereafter "Painted Turtle") are a medium-sized freshwater turtle with unique red and yellow markings on their upper head and limbs. Painted Turtles are widely distributed and relatively abundant across their range (COSEWIC 2016). Provincially, Painted Turtles SAR status is not assessed, and federally they are listed as Special Concern (COSEWIC 2018). Painted Turtles will occupy several wetlands during the active season (April – October) with abundant basking sites and vegetation for foraging (Rowe 2003, Moldowan et al. 2015). Females can have slightly larger home ranges (3.8 ha) compared to males (2.7 ha) because of females nesting migrations (Ernst and Lovich 2009, Brown 2016). Typically, females' nest near their resident wetlands in open areas with sandy-loam or gravel substrate (Rowe et al. 2005). However, in areas where nesting habitat is limited, females will travel farther on land to locate nesting habitat, which often includes road shoulders, lawns, or agricultural fields (Baldwin et al. 2004). In eastern Georgian Bay, Painted Turtles will nest in the shallow soil deposits on open rock barrens (Markle et al. 2021).

Snapping Turtle

Snapping Turtles (*Chelydra serpentina*) are the largest freshwater turtle in Canada and are recognizable by their long tails with triangular crests, olive-coloured carapaces with serrated marginal scutes, and their cross-shaped plastron (COSEWIC, 2008). In Ontario, Snapping

Turtles are relatively abundant and reach some of their most northern ranges (COSEWIC, 2008). However, their populations are sensitive to additive mortality from anthropogenic and natural threats (Keevil et al. 2018), and as a result, they are designated as Special Concern both provincially and federally (COSEWIC, 2008). Snapping Turtles are habitat generalists and can be found in ponds, streams, wetlands, bogs, rivers, or lake edges (Paterson et al. 2012); however, their preferred habitat consists of slow-moving waters, soft mud bottoms, and dense aquatic vegetation (Ernst and Lovich, 2009). Snapping turtles are mostly aquatic but will undertake terrestrial movements to migrate between habitats (Obbard and Brooks 1980). Females will travel great distances during the nesting season, with distances documented of up to 16 km to find suitable nesting sites (Obbard and Brooks 1980). Snapping Turtles typically nest in open areas with well-drained soils, including sandy shorelines, abandoned beaver lodges, rocky outcrops, and road shoulders (Obbard and Brooks 1980, Francis et al. 2019). Females can exhibit strong nest site fidelity, returning to nest in the same location each year (Obbard and Brooks 1980, Congdon et al. 1987).

Blanding's Turtle

Blanding's Turtles are semi-aquatic turtles that are highly recognizable by their distinct bright yellow-orange chin (COSEWIC 2016). Blanding's Turtles are at risk across most of their range; provincially they are listed as Threatened (COSSARO 2017) and federally as Endangered (COSEWIC 2016). They inhabit a variety of wetlands, creeks, rivers, marshes, lakes, or bays and will make extensive upland movements using forests and vernal pools as travel corridors (Edge et al. 2010, Markle and Chow-Fraser 2014). Their home range can vary from 12–60 ha (Ernst and Lovich 2009, Edge et al. 2010), with gravid females having greater home ranges because of their nesting migrations (Edge et al. 2010). Females in their more southern ranges nest in open areas such as sandy shores, gravel roads, forest clearings, or meadows (Beaudry et al. 2010a, Edge et al. 2010, Mui et al. 2016); however, in their more northern ranges females will nest in moss and lichen-covered soil on rock barrens (Markle and Chow-Fraser 2014, Markle et al. 2021).

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Chapter One: Modifying Roadside Habitat with Common Construction Materials does not Mitigate Road Threats to Nesting Turtles

Abstract

Roads may create an ecological trap for female turtles during nesting forays because exposed road shoulders are attractive as nesting habitats despite the risk to female fitness. Road-effect mitigation for turtle communities has become widely recognized as necessary for protecting at-risk turtle populations. The objective of my study was to evaluate a cost-effective and low-maintenance mitigation strategy to reduce road threats to turtle communities by deterring females from nesting on road shoulders at wetland crossings in eastern Georgian Bay, Ontario. The goal of the mitigation design was to prevent females from nesting on the road by covering gravel on road embankments near wetlands with rock rip-rap (large angular rocks; in ~ 100 m-long sections) coupled with paved road shoulders (paved using tar-and-chip; in ~ 300 m-long sections). I predicted that females would not nest along road sections with rip-rap and paved shoulders but may continue to find nesting habitats along the road beyond these mitigated sites. I evaluated the mitigation strategy using a Before-During-After comparison of daily nest counts on the road between Mitigated and Unmitigated sites. I found a significant reduction in the number of females nesting at Mitigated sites in the After period, and no females of any species nested in the rip-rap on road embankments. Although hardening embankments at wetland crossings with rip-rap prevented turtles from nesting in the rip-rap itself, turtles continued to find nesting habitat on the road, including at Mitigated sites in areas with non-compact tar-and-chip pavement. In addition, I evaluated spatial changes in the nesting locations between periods using a hot spot analysis and found that nesting hot spots remained at Mitigated sites in the After period. Furthermore, I used a mark-recapture study to estimate the risk of road-effects for the turtle community based on the proportion of females found on the road. I found a high proportion of the local Species-at-Risk populations of Blanding's Turtles (*Emydoidea blandingii*) and Snapping Turtles (*Chelydra serpentina*) nesting on the road, indicating a risk of road-effects to the turtle community. My findings suggest that the mitigation strategy was unsuccessful at deterring female turtles from nesting on roads and indicate that the application of rip-rap and paved road shoulders as a road-effect mitigation strategy should not be applied without further research. Females will likely continue to nest on the road if a barrier (i.e., exclusion fencing) is not deployed. My study elucidates the importance of incorporating species-specific behaviour and movements into all aspects of road planning and highlights that any new road-effect mitigation strategies should be continuously explored and empirically tested.

Introduction

Roads can disproportionally affect female turtles because of their migrations during nesting season and tendency to nest in road shoulders (Gibbs and Shriver 2002, Aresco 2005a, Steen et al. 2006). During nesting forays, the availability of nesting habitat can influence female nesting behaviour, and in areas where nesting habitat is limited, females may travel greater distances and spend more time on land searching for suitable nest locations (Baldwin et al. 2004, Rowe et al. 2005). Anthropogenically-created habitats, such as road shoulders, can provide females with suitable microhabitats for oviposition (Baldwin et al. 2004, Beaudry et al. 2010a). Because females can exhibit site fidelity, they may return to the same nesting site each season (Freedberg et al. 2005, Rowe et al. 2005), including road shoulders (Beaudry et al. 2010a), and this can impact maternal survival. Threats to maternal survival and recruitment from roads include mortality of hatchlings and mature females (Aresco 2005b, Steen et al. 2006), nest success from soil compaction and pollution, and nest predation (Marchand and Litvaitis 2004). Ultimately, roads can have population-level consequences because of the risks imposed on female turtles' fitness (Beaudry et al. 2008, Enneson and Litzgus 2008).

Road mortality mitigation strategies need to consider both the spatial and temporal factors influencing turtle communities, with special attention given to road-effects on female turtles (Cureton and Deaton 2012). To ensure the long-term viability of turtle populations, reproductively active females may be an important demographic on which to focus conservation efforts (Gibbs and Shriver 2002, Enneson and Litzgus 2008). Commonly, road mortality mitigation strategies designed to reduce the abundance of turtles on roads consist of exclusion fencing paired with an underpass (Boyle et al. 2021). While this method can reduce road crossings by females, it may not prevent females from nesting in exposed gravel on road shoulders (Langen et al. 2012). In addition, if the integrity of fencing is compromised, turtles will be directed to cross the road at fence gaps that lead to the road surface, which can increase the risk of road mortality (Baxter-Gilbert et al. 2015, Markle et al. 2017).

Although road mortality mitigation strategies have been explored, mitigation strategies directly targeting female nesting behaviour on road shoulders are less common in the literature. In eastern Georgian Bay, a new road-effect mitigation strategy was applied to deter females from nesting in roadside habitats. The new strategy uses a combination of rip-rap and paved road shoulders at wetland crossings along a road. Rip-rap is a common construction material that can be used on road embankments to reduce erosion of sediment and consists of layered angular rocks, cobble, or boulders (Province of British Columbia 2000). The intention of the new strategy was to reduce the risk of road mortality for females and hatchlings by eliminating suitable nesting habitats in areas where females typically congregate on road shoulders; however, the specific application of rip-rap and paved road shoulders as a road-effect mitigation strategy for turtles has not yet been investigated.

Female turtles often nest near wetland edges (Steen et al. 2012), and when along roads, in the loose gravel area immediately adjacent to the paved or packed surface (Marchand and Litvaitis 2004). Therefore, hardening (e.g., rip-rap, paving) the surface material on road shoulders and embankments where roads bisect critical wetland habitats could eliminate roadside nesting habitats, and thus reduce interaction with roads and vehicles. Studies have evaluated the effects of rip-rap on geomorphic processes and on the ecology of aquatic organisms including fish and macroinvertebrate communities (Reid and Church 2015, Chhor et al. 2020); however, little is known about the effects of rip-rap on other ecological communities, such as freshwater turtles. Therefore, it is important to evaluate how turtles interact with materials commonly used on roads, such as rip-rap and paved road shoulders, to help inform best management practices for turtle communities during road development.

Objectives

The application of rip-rap and paved road shoulders during road construction could provide a cost-effective and low-maintenance road-effect mitigation strategy that specifically targets the most vulnerable and valuable demographic group in turtle populations (i.e., mature females; Congdon et al. 1994). The objective of the work described in this chapter was to evaluate the effectiveness of this mitigation strategy at deterring female turtles from nesting in roadside habitats, using a mark-recapture study and a Before-During-After comparison of nest observations and hot spots on the road. First, I evaluated the changes in number of nests on the road in Mitigated and Unmitigated road sections between the Before, During and After periods of mitigation. I predicted that females would not nest in the rip-rap and paved road shoulders in Mitigated road sections, but that females would continue to gain road access and nest in Unmitigated road sections. Second, I conducted a hot spot analysis to identify if nests were spatially aggregated on the road and compared the spatial distribution of nests between the Before, During and After periods of mitigation. In addition, I evaluated changes in the distances of nests to the nearest waterbody between periods. I expected that the distances of nests from the edge of wetlands would increase from the Before to After period if the mitigation deterred females from nesting at wetland crossings, but also that females would continue to find nesting habitat along the road. If the mitigation did not prevent females from nesting near wetland crossings, I expected distances would not change between periods because turtles would continue to nest in road shoulders at wetland crossings. Third, I used data from the mark-recapture study to investigate the local abundance and structure of the turtle community that was likely to interact with the road and mitigation. I estimated the risk of road-effects for the turtle communities and observed the nesting behaviour of individual females that nested in consecutive years on the road (During and After periods). Overall, the mitigation strategy was considered successful if it prevented females from nesting on the road without causing their use of other risky roadside habitat for oviposition.

Methods

Road Construction and Mitigation Design

My study occurred within the Township of the Archipelago (TOA) along eastern Georgian Bay, situated within the Robinson-Huron Treaty of 1850 and Williams Treaty of 1923 and located on Anishinabek territory. The study site includes an 11.7 km section of a road and its surrounding wetland habitat (Figure 1.1). In 2020 and 2021, the road was scheduled for routine road maintenance and culvert replacements. The timing of construction coincided with turtle nesting season (i.e., June); therefore, the road was identified as a priority area for conservation of Species at Risk (SAR) turtles. As part of developing Best Management Practices (BMP) during road maintenance and construction, the Georgian Bay Mnidoo Gamii Biosphere (GBB) and Shawanaga First Nation (SFN) collaborated with the TOA, engineers, and road construction companies to implement road-effect mitigation. The design was developed to be a cost-effective and low-maintenance road-effect mitigation design for turtle communities in areas where fence-and-underpass mitigation strategies are not feasible. The goal of the design was to deter females from nesting on road shoulders, thereby reducing road-effects including mortality of females and hatchlings, nest predation, and nest substrate compaction.

Mitigation was applied during construction at 5 locations where the road directly bisects critical wetland habitat (Figure 1.1). In 2020, culverts were upgraded to act as aquatic crossing structures and the gravel on either side of the culverts was replaced with angular rip-rap (300 mm in diameter) that was layered 450 mm deep (Figure 1.2). Nesting data were collected from June 9th – June 26th in 2020 which was considered the Before period of the study because construction occurred post-nesting season. In 2021, the roadbed was raised by 2.5 cm and resurfaced using the tar-and-chip method. This process consisted of preparing the road by pulverizing the existing road base, raising the roadbed with granular A gravel, grading (making the surface smooth) and leveling the new surface. A liquid asphalt emulsion (tar) was then sprayed on the roadbed followed by the application of aggregates (chip) to bind to the asphalt, and a steam roller was used to embed the chip into the tar. In addition to a steam roller, the tar-and-chip method of paving typically requires further compaction by vehicular traffic to create a smooth paved surface over time. To further eliminate exposed gravel, the road shoulders were paved with tar-and-chip to meet the rip-rap and in ~100 m sections on either side of the rip-rap (Figure 1.2). The culverts, length of rip-rap, and paved road shoulders varied slightly at each culvert location depending on the landscape (Appendix 1.1). The timing of road resurfacing coincided with nesting season, making nesting data collected from June 1st – June 21st, 2021, the During period of the study. In 2022, the wetland crossings were fully mitigated with rip-rap and paved road shoulders, making nesting data collected from May 30th – July 5th, 2022, the After period of the study. Despite paving the road, in the After period, loose gravel remained in most sections of the road shoulder. In addition, because the road was raised and paved following the application of rip-rap, there was a gap of exposed gravel that remained between the rip-rap and paved road shoulders following road resurfacing (Appendix 1.1).

Turtle Community Ecology

In 2021 and 2022, I conducted a mark-recapture study to describe the turtle community adjacent to the road. The field season was divided into three capture seasons: pre-nesting (April to late May), nesting (late May to late June), and post-nesting (mid July to August), with different capture methods used in each. Wetland surveys conducted in the pre-nesting and post-nesting seasons used baited hoop-nets as the primary method of capture. However, I attempted to reduce biases towards male and adult captures by also opportunistically capturing turtles by hand and dip-net while moving through the wetlands (Mali et al. 2014, Tesche and Hodges 2015). During nesting season, I focused survey efforts on the road where females were captured post-oviposition, in addition to any other opportunistic captures of male or juvenile turtles crossing the road. The survey types differed in their overall methods, survey effort, and capture location. Wetland surveys occurred in 6 wetlands adjacent to the road (Figure 1.3) with 760 trap nights in 2021 and 772 trap nights in 2022. Nesting surveys occurred on the road during the month of June and included a total of 26 survey days in 2021 and 27 survey days in 2022.

Wetland Trapping

Six wetlands in two wetland complexes were selected for live-trapping as part of the mark-recapture study (Figure 1.3). I selected wetlands if they were bisected by the road and were suitable for trap deployment (i.e., deep enough, free of vegetation and debris). Four of the wetland trapping sites were associated with mitigation in 2021 and 2022, with upgraded culverts, rip-rap, and paved road shoulders. The other two wetland trapping sites were not associated with mitigation (Figure 1.3). I did not consider the wetland trapping locations to be independent from each other because they were part of two larger wetland complexes that surround the road, and females are known to make large inter-wetland movements (Edge et al. 2010). Because of the overall size of wetlands, I focused trapping efforts to be within ~500 m of the road at wetland crossings to capture turtles most likely to interact with the road (Figure 1.3).

At the beginning of each survey, the wetland was observed from shore for basking turtles. While moving between hoop nets within a wetland, two surveyors captured turtles that were basking, floating, or swimming with dip nets or by hand. Trapping occurred on a rotational schedule 6 days per week during the pre-nesting and post-nesting seasons. Traps consisted of a 3-ring hoop net with 2.54 cm-gauge mesh (Miller Net Company, Inc., USA). Traps were deployed with a 30 cm headspace that was not submerged, and floats were placed in the middle ring of the hoop-net to ensure there was breathing space for turtles or bycatch if water levels changed unexpectedly (Robertson et al. 2013). Surveyors set traps around the edge of wetlands and baited traps with canned sardines; bait was refreshed every 3 days. Five hoop-nets were deployed at 3 wetland trapping locations (15 nets in total) for a given trapping event, which lasted 3 days. Surveyors checked traps twice daily, once in the morning and again in the evening, with no more than 12 hours between checks. On the third day of a given trapping event, all 15 traps were removed from the wetlands and prepared to be set in the next rotation of wetland locations.

Surveyors processed all turtles in the field immediately following their capture (Robertson et al. 2013). New turtles were individually marked with 1-4 notches in the anterior marginal scutes of the carapace using a triangular file (Cagel 1939). Morphometric and demographic data were collected once for each turtle upon their initial capture of the season. Surveyors recorded turtle species, and each turtle was sexed by visual inspection of secondary sex characteristics (Ernst and Lovich 2009). Midline plastron and carapace lengths were measured with tree calipers (40 cm ± 1 cm, Haglof Inc., Langsele, Sweden) or Vernier calipers (200 mm ± 0.05 mm, Wondersunm). Body mass was measured using spring scales (Pesola, Präzisionswaagen, AG, Switzerland); the scales varied in maximum weighing capacity from 100 g (± 1 g) to 30 kg (± 1 kg), and the scale used depended on the size of the turtles. Following processing, turtles were released back into the wetland within 250 m of their capture location.

Nesting Surveys on the Road

Nesting surveys along the road were conducted by car every morning and evening during the nesting season. To determine the start date of nesting season, females captured during wetland surveys in mid-late May were palpated in the inguinal leg pockets to identify the stage of egg development (Robertson et al. 2013). Nesting surveys began once females were confirmed to be gravid and started to exhibit nesting behaviours of staging, searching, or ground nuzzling (Morjan and Valenzuela 2001). Typically, morning surveys began between 5:30 and 6:00 and ended at 12:00 to 13:00, and evening surveys began at 17:00 and ended at 24:00. At the start and end of each nesting survey, surveyors recorded the personnel, date, time, air and road temperatures (± 0.1ºC, Accu Temp, New Haven, USA), and weather conditions (rain and % cloud cover). During the survey, two surveyors slowly drove the road (20 – 30 km/hr) to scan for nesting turtles or signs of nesting activity. At least once per survey, surveyors walked road sections to look for nesting activity or turtles staging in the water at wetland crossings. In addition, during morning surveys, the road was biked by two observers to search road shoulders for recent nesting activity and depredated nests. Bike surveys occurred 7 days of the week starting at 7:30 and commenced once all road observations (e.g., active, or inactive nesting activity) on both sides of the road were recorded. When a potential nest location was detected based on surface soil disturbance, the presence of eggs was confirmed by carefully removing the top layer of soil. When confirmed nests were identified, surveyors flagged all nests to ensure they were not recorded twice. The location of any depredated nests was recorded and, if possible, identified to turtle species based on the characteristics of the nest chamber and the remnants of depredated eggs. When females were found nesting on the road, they were periodically monitored until oviposition was complete. For each nesting observation, the species, notch code ID (if a recapture), nest status (predated, partially predated, or intact), and location (accuracy ± 5 – 20 m) were recorded. I recorded capture location using ArcGIS survey 123 app (ESRI, Redlands, California, USA) on a Samsung tablet (TAB Active2, Android operating system). If an unmarked turtle was found nesting, the turtle was notched and processed post-oviposition.

Statistical Analyses

Before – During – After Mitigation Comparison

Temporal Analyses: To analyze the data collected from nesting surveys, I divided the road into 17 sections (labelled A – Q; Table 1.1) in ArcGIS Pro 3.0.3 (ESRI, Redlands, California, USA). I categorized road sections based on the habitat surrounding the road as either 'wetland' if there was a wetland crossing within the road section or 'terrestrial' if there was no wetland crossing and the adjacent habitat was forest or rock barren. In addition, road sections were further distinguished as either 'Mitigated' or 'Unmitigated' sites based on the presence of mitigation in the After period. Overall, the resulting three categories consist of 'Mitigated wetland', 'Unmitigated wetland' or Unmitigated terrestrial'. The road sections varied in length (600 m – 750 m) to ensure that areas with mitigation were fully encompassed within a road section, which did not allow for the road to be divided into equal length sections.

I evaluated temporal trends in the number of turtles nesting on the road among study periods using Generalized Additive Models (GAMs). I used GAMs because they can control for variation in seasonality using smoothing functions and allow the inclusion of repeated measures as random effects. I created GAMs with a Poisson error distribution using the package "mgcv" (Wood 2017) in program R version 4.2.1 (R Core Team 2022). I used the daily count of nests for each road section as the response variable to compare the number of nests on the road among study periods and sites (Appendix 1.2). A set of candidate models were constructed with the main fixed effects of period (Before, During, and After), site (Mitigated or Unmitigated), habitat (wetland or terrestrial), and the period × site interaction (Appendix 1.3). Models varied in their inclusion of smoothing functions applied to Julian date and the random effects of date and road section (Appendix 1.3). I assessed candidate models using Akaike's information criterion (AIC; Akaike 1974) and the main effects of the highest ranked model were examined with a Wald χ^2 test using the "anova.gam" function in the "mgcv" package (Wood 2017). Specifically, I evaluated the period × site interaction for After × Mitigated because this would show if there was a relative change in the daily count of nests on the road after mitigation. However, because my study had a During period, I evaluated both individual period × site interaction levels of During × Mitigated and After × Mitigated to distinguish the effects of partial mitigation (During period) from full mitigation (After period). All analyses were conducted in program R version 4.2.1 (R Core Team 2022).

Spatial Analyses: Hot spot analyses were conducted in ArcGIS Pro 3.0.3 (ESRI, Redlands, California, USA) to evaluate differences among study periods in the spatial distribution of nest locations on the road. Nesting hot spots in each period (Before, During, and After) were quantified using combined positional data (GPS coordinates) of nests of Blanding's

Turtles (*Emydoidea blandingii*), Painted Turtles (*Chrysemys picta*) and Snapping Turtles (*Chelydra serpentina*), that I observed nesting on the road. I used Getis-Ord Gi* Hot Spot Analysis to elucidate statistically significant clusters of nest locations based on the sum of nest occurrences in a road segment relative to nests in neighboring segments and an overall expected distribution (Getis and Ord 1992). The output from the analysis indicates whether nest occurrences fall within hot spots with significantly high clusters of nests (positive z-score) or cold spots with significantly low clusters of nests (negative z-score; Getis and Ord 1992). The False Discovery Rate (FDR) correction was applied to the hot spot analysis to help control for spatial dependency in the data. The road segments used for hot spot analyses consisted of 230 50-m sections, the finest scale possible within the accuracy of my GPS device.

Characteristics of Nesting Locations

I calculated inter-nest distances, which is the distance between nest locations of marked females that nested in two consecutive years in ArcGIS Pro 3.0.3 (ESRI, Redlands, California, USA). I evaluated inter-nest distances to determine if females exhibited site fidelity to nesting locations on the road between the During and After periods. In addition, I calculated the straight-line distance from nests to the nearest wetland edge in ArcGIS Pro 3.0.3 (ESRI, Redlands, California, USA) and evaluated if the distance changed between periods (Before, During, and After). Female turtles typically nest within 300 m of the edge of a wetland (Joyal et al. 2001, Baldwin et al. 2004, Steen et al. 2012) and in my study I observed females nesting on the road at wetland crossings in each study period. Therefore, I assessed nest distances to the nearest wetland between periods using Kruskal-Wallis rank sum tests ($\alpha = 0.05$). I used a Kruskal-Wallis rank sum test because the residuals were not normally distributed based on a Sharpiro Wilk test.

All reported means were calculated in R Studio (R Core Team, 2022) and are followed by ± 1 SE, range of data, and the number of observations.

Turtle Community Ecology

Minimum abundance of each species in the turtle community adjacent to the road was estimated based on the total number of individuals marked from two years of mark-recapture data collected during wetland trapping and nesting surveys. I did not estimate population size from the mark-recapture data because of insufficient years of data (i.e., only two-years) and known biases in capture methods (Mali et al. 2014, Tesche and Hodges 2015). Studies have found trapping to be biased towards adult and male captures (Mali et al. 2014, Tesche and Hodges 2015), and I assumed a female-bias in turtle captures during nesting surveys on the road because these surveys were focused on observing female nesting behaviour.

I used the estimated minimum abundance to describe the risk of road-effects for three local turtle species based on the proportion of individual females on the road. I assumed species with a higher proportion of females using the road during nesting season may have a higher risk of road mortality. I used the mark-recapture data to identify the number of individual females detected on the road over a two-year period (During and After periods). I then estimated the risk of road-effects based on the total number of individual females found on the road relative to the estimated minimum abundance for each Blanding’s Turtles, Painted Turtles, and Snapping Turtles.

Results

Before – During – After Mitigation Comparison

Temporal Analyses: Relative to the Before period, there was a 15% decrease in the number of nests at the Mitigated site in the After period, a 43% increase of nests at Unmitigated wetland sites, and a 38% increase at Unmitigated terrestrial sites (Table 1.1). To evaluate trends in the number of nests on the road, I assessed the fixed effects of the top-ranked GAM model of daily nest counts on the road. I found that there was a significant interaction between periods (Before, During, and After) and sites (Mitigated and Unmitigated) for the number of nests on the road (Wald $\chi^2 = 6.2$, df = 2, P = 0.044). I further evaluated the individual Period x Site interaction level to determine the impact of partial mitigation in the During period and full mitigation in the After period on the number of nests on the road. I did not find a significant difference in the number of nests on the road with partial mitigation (During period) as indicated by the During x Mitigated interaction level (Figure 1.4; Z = 0.27, P = 0.78). In the During period there was a decreasing trend in the number of nests at all sites on the road, indicating that the decrease in the number of nests at Mitigated sites was not specific to the effects of mitigation (Figure 1.4). However, in the After period, when wetland crossings were fully mitigated, I found Mitigated sites continued to experience a decrease in the number of nests compared to an increase in nests at Unmitigated sites. Thus, I found a significant decrease in the number of nests at Mitigated sites in the After periods as quantified by the After x Mitigated interaction level (Figure 1.4; Z = -2.01, P = 0.044).

Spatial Analyses: Nesting hot spots were evident on the road in the Before, During and After periods of my study. The spatial distribution and size of hot spots fluctuated between periods, with all nesting hot spots concentrated around wetland crossings (Figure 1.5 and Table 1.2). The Before period had the fewest number of hot spots on the road with 3% (300 m /1170 m) of the road having significant nest clusters (Figure 1.5 and Table 1.2). The two areas on the road that were identified as hot spots in the Before period were sites that were planned for mitigation (Figure 1.5 and Table 1.2). Despite an overall decrease in the number of nests on the road in the During period (Table 1.1), the number of hot spot clusters increased to 5% (550 m / 1170 m) of the road (Figure 1.5 and Table 1.2). In the After period, 4% (500 m / 1170 m) of the road consisted of two hot spots at Mitigated wetland crossings and one at an Unmitigated wetland crossing (Figure 1.5 and Table 1.2).

Characteristics of Nesting Locations

I found nests of three local turtle species on the road, with Snapping Turtles constituting the majority of nesting observations (n = 107 in 2020, n = 80 in 2021, n = 94 in 2022). In the During period, when road resurfacing coincided with nesting season (Figure 1.6a), I observed turtles nesting in the newly exposed gravel in traffic lanes (Figure 1.6b). I did not find any turtles nesting in the rip-rap on road embankments in the During or After periods. However, in the After period, adult Blanding's Turtles, Painted Turtles, and Snapping Turtles were able to traverse the rip-rap and were found nesting in the tar-and-chip sections of the road, including directly above the rip-rap in an exposed gap (Figure 1.6c-d). Across all the Mitigated sites in the After period, a total of 58 turtles were found nesting in the semi-compact-tar-and-chip pavement on road shoulders, including 11 turtles that nested above the rip-rap in the exposed gap.

I compared the average straight-line distances from nests to the nearest wetland among periods for each of the three species (Figure 1.7). Although not significant, on average

Blanding's Turtles (χ^2= 2.32, df = 2, p = 0.31; Figure 1.7) and Painted Turtles (χ^2= 0.62, df = 2, p = 0.73; Figure 1.7) nested further from the edge of a wetland in the During and After period relative to the Before period; whereas on average Snapping Turtles nested furthest from the edge of a wetland in the During period but the distance between the Before and After periods were similar (χ^2 = 4.65, df = 2, p = 0.09; Figure 1.7).

The mean straight-line inter-nest distance for individual females nesting in both the During and After periods varied among species. In the After period, a total of 38 females, marked in the During period, returned to nest on the road; this consisted of 6 Blanding's Turtles, 30 Snapping Turtles, and 2 Painted Turtles. Blanding's Turtles had the greatest mean inter-nest distance of 595 m (± 360 SE, 160 – 2392, n = 6; Figure 1.8). Painted Turtles had a small sample size of only two females, with the mean inter-nest distance of 303 m (± 202 SE, 100 – 505, n = 2; Figure 1.9). Snapping Turtles had the smallest mean inter-nest distance of 228 m (± 68 SE, 1 m – 1280 m, n = 30; Figure 1.8). In the After period, most Snapping Turtles nested within 300 m of their nest location from the During period; however, there were 5 outliers with inter-nest distances greater than 650 m (Figure 1.8). Similarly, one Blanding's Turtle had a notable inter-nest distance of 2392 m (Figure 1.8).

Turtle Community Ecology

The turtle community was described using wetland trapping and nesting surveys on the road to evaluate the minimum abundance and demographics of turtle species most likely to interact with the road. The combined efforts from wetland and nesting surveys (2021-2022) resulted in a total of 437 individual turtle captures of three species, with Painted Turtles being the most abundant (n = 191 individual turtles) followed by Snapping Turtles (n = 166), and

Blanding's Turtles ($n = 80$; Table 1.3). Minimum abundance and demographics differed between capture methods, with a total of 264 turtles marked from wetland trapping and 173 turtles marked during nesting surveys (Figure 1.9). The number of unique Blanding's Turtles captured did not differ between survey types (Figure 1.9; $\chi^2 = 0.05$, df =1, $p = 0.82$); however, more Painted Turtles were captured in wetlands than on the road during nesting surveys (Figure 1.9; $\chi^2 = 64.5$, df =1, $p < 0.05$) and although not significant, there were slightly more Snapping Turtles captured during nesting surveys than during wetland trapping (Figure 1.9; $\chi^2 = 1.48$, df =1, $p = 0.2$). Not surprisingly, because the road surveys were focused on nesting behaviour, the majority of turtle captures on the road were female (Figure 1.9). Of the total number of individual females found during the two-year study, only 11% (5/46) of Blanding's Turtles, 12% (8/65) of Painted Turtles, and 14% (16/115) of Snapping Turtles were identified in both survey locations, indicating that a large proportion of females were not captured during trapping in the wetlands.

I estimated the risk of road-effects to the local turtle community by comparing the number of individual females found on the road relative to the total number of turtles marked during the mark-recapture study (During and After periods). I calculated proportions separately for each species and found 50% (40/80) of Blanding's Turtles, 21% (40/191) of Painted Turtles, and 61% (102/166) of Snapping Turtles to be females found on the road during nesting season (Figure 1.10).

Notably, over the three years of this study, I found several adult female turtles injured or dead on the road while conducting nesting surveys. One female Blanding's Turtle was found injured and another was found dead on the road from a vehicle strike. In addition, one adult female Painted Turtle was found injured and two more were found dead on the road from vehicle strikes.

Discussion

The need for, and importance of, applying road-effect mitigation strategies has become widely recognized as necessary to reduce road threats to turtle communities (Lesbarrères and Fahrig 2012, van der Grift et al. 2013). The mitigation strategy commonly applied to reduce road mortality of turtles consists of exclusion fencing coupled with underpasses (Heaven et al. 2019, Boyle et al. 2021). Studies on the effectiveness of the fence-underpass design show mixed results, with success often depending on the structural integrity of materials used, long-term maintenance, and monitoring (Baxter-Gilbert et al. 2015, Markle et al. 2017). The objective of my study was to evaluate a cost-effective and low-maintenance alternative mitigation strategy to reduce road threats to female turtles on a low-traffic road. The strategy made use of scheduled road works to add mitigation aimed at deterring females from nesting in road shoulders by covering exposed gravel with rip-rap and paved road shoulders. As predicted, females of all three local turtle species did not nest in the rip-rap on road embankments; however, females continued to nest in the nearest available roadside habitat, including the tar-and-chip paved road shoulders. My observations of both temporal and spatial changes in nesting locations suggest that if turtles are not explicitly prevented from gaining road access, they will continue to nest on the road, thus putting themselves at risk of mortality. My findings are reinforced by other studies of road-effect mitigation strategies that have found the integrity of exclusion barriers to be the most important factor in reducing road threats for turtles (Baxter-Gilbert et al. 2015, Markle et al. 2017). My data show that the mitigation strategy was unsuccessful at preventing female turtles from nesting on roads and indicate that the application of rip-rap and paved road shoulders as a road-effect mitigation strategy for turtles should not be applied without further research.

Effectiveness of Mitigation at Deterring Roadside Nesting

Before-During-After Mitigation Comparison

I found a significant reduction in the number of turtles nesting in the After period at mitigated wetland crossings. As expected, the rip-rap covered suitable nesting substrate (i.e., gravel) on the embankments at mitigated wetland crossings which prevented turtles from nesting in these ~ 100 m sections of the road. Few studies have evaluated the effects of rip-rap for freshwater turtle communities; however, the preventative effect is not surprising. In fact, the potential for rip-rap to remove nesting habitat was mentioned in the proposed Species at Risk Management Plan for Snapping Turtles (Environment and Climate Change Canada 2016). In addition, Roosenburg et al (2014) documented that shoreline hardening, including the application of rip-rap, acted as a barrier to movement and removed suitable nesting habitat for Diamondback Terrapin (*Malaclemys terrapin*). Similarly, shorelines modified with rip-rap reduced available nesting habitat for sea turtles in coastal environments (Witherington et al. 2011). The few studies documenting the use of rip-rap as a nesting deterrent corroborate my findings that rip-rap can harden surfaces and prevent turtles from nesting.

Despite the decrease in nests at mitigated wetland crossings, I found that females continued to nest at Mitigated sites in the After period. Road shoulders paved using the tar-and-chip method did not prevent turtles from nesting in the semi-compact surfaces. In fact, 42% of the nests in the after period were laid in the tar-and-chip pavement at mitigated sites, including 11 nests that were laid in the exposed gap between the road surface and rip-rap. Other studies evaluating the effectiveness of mitigation strategies have identified that mitigation success is dependent on the integrity of the design. Studies evaluating fence-underpass mitigation designs found structural integrity (i.e., no gaps in fence design; Baxter-Gilbert et al. 2015) and continuity (i.e., no partial fencing; Markle et al. 2017) is crucial for successfully mitigating road threats to turtles. The After period of my study was conducted only one-year post road resurfacing, therefore the road shoulders may continue to harden over time and prevent nesting. However, at present, without preventing turtles from accessing the road, nests may continue to be laid in the tar-and-chip road shoulders. Notably, the persistent nesting behaviour of females was also of concern in the few earlier studies evaluating the use of rip-rap because the loss of suitable nesting habitat may result in concentrated nests within the remaining available habitat, and thereby decrease overall nest success (Witherington et al. 2011, Roosenburg et al. 2014).

Consistent with other studies that evaluated road-effect mitigation with years (Boyle et al. 2021) or road sections (Markle et al. 2017) of partial mitigation, my study found that partial mitigation (i.e., rip-rap without paved road shoulders) did not deter turtles from nesting on the road. Moreover, the During period may have presented a greater risk to females because road resurfacing coincided with nesting season and thus created suitable nesting areas in active traffic lanes. I observed females nesting in this fresh gravel in active traffic lanes (Figure 1.6b), just as females are well-known to nest in exposed gravel on road shoulders (Baldwin et al. 2004, Beaudry et al. 2010a). Females' selection of active traffic lanes with exposed gravel as nesting sites in my study emphasizes the overall threat of construction activities to turtles during nesting season and the importance of conducting road work activities outside of critical time periods for turtles.

Spatial Observations of Nests

Overall, the total number of turtles that nested on the road were similar between the Before and After periods; however, based on the hot spot analysis, I detected a shift in the distribution of nests between Mitigated and Unmitigated road sections. In the After period, two of the five Mitigated road sections remained as hot spots, and although not significantly clustered, nests were also found at the three other Mitigated sites. In addition, there was one Unmitigated wetland crossings near a Mitigated site that became a hot spot in the After period. The mitigation reduced the number of nests at some of the Mitigated sites; however, the hot spot analyses clearly indicated that the mitigation did not prevent turtles from nesting on the road shoulder. Instead, the hot spots show a shift in the location of nests to Unmitigated road sections, specifically to sections near wetland crossings. Hot spots of turtle observations on roads are known to be associated with species-specific behaviour and critical habitat, and consistent with other studies (Langen et al. 2009, Beaudry et al. 2010b, Cureton and Deaton 2012), wetland crossings on my study road appeared to be an important area for nesting female turtles.

Straight Line Nest-to-Water Distances

I expected the distance from nests to water to change between periods depending on females' response to the mitigation. However, there was no significant change detected in the distance from nest to water between periods for all three study species, indicating that females continued to find suitable nesting habitat on the road in close proximity to wetlands with Mitigated and Unmitigated road shoulders. Although not significant, the distance from nests to water consistently increased from the Before to After period for Blanding's Turtles and Painted Turtles. Any increase in nest-to-water distance associated with mitigation may compromise hatchling turtle dispersal from nest to water. Hatchling turtles may already be highly vulnerable to mortality post-nest emergence from predation and desiccation (Finkler 2001) and with increased time spent on land in unfavourable environmental conditions (e.g., low humidity) or habitats (e.g., exposed habitats and roads), hatchlings may have a greater risk of mortality.

Inter-Nest Distance

The availability of nesting habitat can influence female nesting behaviour and migration (Baldwin et al. 2004, Rowe et al. 2005); thus, I observed how individual females responded to the mitigation in the During and After periods. The inter-nest distances confirmed that some Snapping Turtles and Painted Turtles nested at Mitigated wetland crossings in both periods. Although females are known to exhibit site fidelity to nesting locations (Obbard and Brooks 1980), large distances between yearly nest locations are not uncommon (Congdon et al. 1983, Rowe et al. 2005). Interestingly, five Snapping Turtles had large inter-nest distances between the During and After periods, consisting of two females that moved from Mitigated to Unmitigated sites to nest, one that nested at two different Mitigated sites, and two that nested at different Unmitigated sites. All Blanding's Turtles observed nesting on the road in both periods were at Unmitigated road sections. My mark-recapture study only encompassed two years of nesting (the During and After periods), and the changes in nest locations I observed may not have been in response to mitigation. However, the movements by individual females indicate that turtles in the community make inter-wetland or terrestrial movements to nest at multiple sites on the road. Although most females nested at wetland crossings, the inter-nest distances demonstrate that despite removing nesting habitat at hot spots, females may continue to seek suitable nesting habitat elsewhere on the road. Langen et al (2009) found that in addition to wetland crossings, wetlands within 100 m of a road are also associated with road mortality hot spots. Thus, the large wetland complexes surrounding my study road likely complicate the potential success of mitigation because turtles may encounter Mitigated and Unmitigated road sections during nesting migrations. These findings are supported by studies identifying that modest buffer zones (i.e., protected areas based on short-distance movements) around wetlands are unlikely to protect female turtles from the development and use of roads (Congdon et al. 2011, Refsnider and Linck 2012) because of their large inter-wetland movements and nesting migrations (Edge et al. 2010, Steen et al. 2012, Paterson et al. 2019). Specifically, inter-wetland movements by female Blanding's Turtles can reach > 6000 m (Edge et al. 2010), therefore buffer zones that are based on Blanding's Turtle movements would likely sufficiently encompass other species (Congdon et al. 2011).

Caution with Rip-Rap and Paved Road Shoulders

Rip-Rap: Rip-rap was not a barrier to movement for adult turtles. I observed most turtles navigating the rip-rap with ease (Snapping Turtles in particular); however, a three-year-old Painted Turtle was found trapped in a gap of the rocks during the study (T. Burke and J. Kentel, pers. obs.). The Painted Turtle had a mid-carapace length of 4.9 cm and was found alive but showed signs of dehydration (T. Burke and J. Kentel, pers. obs.). Although adult turtles did not become trapped in the rip-rap in my study, a gravid female Snapping Turtle was found uninjured but wedged in rip-rap (~ 30 – 100 cm diameter rock) along with a broken egg on an embankment in Algonquin Provincial Park (M. Keevil pers. obs.). In addition, Langton and Clevenger (2017) reported the potential for Desert Tortoises (Gopherus agassizii) to become trapped in rip-rap. Currently, there is a gap in the literature about the effects of common construction materials on the ecology of herpetofauna communities and, more specifically, of turtle populations. Future research should continue to explore and evaluate the effects of different commonly used construction materials for vulnerable species to ensure there are no secondary consequences from their application. Specifically, research should be conducted on the potential entrapment of different age-classes and sizes of turtles relative to the sizes of rocks used in rip-rap.

Paved Road Shoulders: Despite paving the road, loose gravel remained in most sections of the road shoulder in the After period (2022 field season), likely because the tar-and-chip method of paving requires compaction by vehicular traffic to create a smooth paved surface over time (Pasindu et al. 2020). Unlike the main road surface where vehicles compacted the road, the road shoulders were not fully compacted because cars do not generally drive on the shoulder. In addition, because the road was raised and paved following the application of rip-rap, there was a gap (ranging 0 – 117 cm) of exposed non-compact tar-and-chip pavement that remained between the rip-rap and road surface following construction (Appendix 1.1). Therefore, applying tar-and-chip pavement without ensuring compaction may contaminate the roadside nesting habitat because females are likely to continue to nest in non-compact sections. Few studies have evaluated the effects of different road surfaces and substrates (i.e., tar-and-chip pavement, asphalt, gravel) on hatchling development. However, the use, construction and maintenance of roads may contaminate (e.g., fuel, calcium chloride, de-icing agents, etc.) turtles roadside nesting habitats. de Solla and Gugelyk (2018) documented a Snapping Turtle that nested in non-compact freshly laid asphalt mixture and found turtle embryos had low levels of polycyclic aromatic hydrocarbons (PAHs) after only eight days of exposure. In addition, studies have found that exposure to contaminants in the nesting environment can cause developmental abnormalities in hatchling turtles (Bishop et al. 1991, Bell et al. 2006). Because females are likely to continue nesting in road shoulders, further research should be conducted on the potential effects of chemicals used in building and maintaining roads on nest survival and long-term hatchling health.

Community Ecology

Few studies have evaluated mitigation strategies for turtles on a population level, even though such studies are important for determining if mitigation supports populations in a biologically meaningful way (Lesbarrères and Fahrig 2012, van der Grift et al. 2013). Boyle et al (2019) found that a high proportion of turtle and snake populations will use tunnels to cross roads; thus, a fence-underpass mitigation design not only reduced mortality but also successfully connected habitats on opposite sides of the road. Although I did not contextualize the mitigation strategy evaluated in my study with modeled population size estimates, the mark-recapture data from the road and adjacent wetlands provided some insight into the risk posed by the road to the local turtle community. Studies evaluating turtle communities in landscapes modified by roads have found mixed results with some studies showing that populations can become male-biased because of increased mortality of females during nesting season and to offspring during hatching (Aresco 2005a, Steen et al. 2006). In contrast, other studies have found no difference in turtle population sex ratios in wetlands adjacent to roads (Buchanan 2017, Bowne et al. 2018). Notably, over the course of my study, I found several adult female turtles injured or killed from vehicle strikes during nesting season, including gravid Blanding's Turtles and Painted Turtles. Although I did not evaluate sex ratios in the wetlands adjacent to the study road, the observed mortalities are significant because additive anthropogenic mortality can have serious implications for turtle population persistence (Enneson and Litzgus 2008, Crawford et al. 2014, Howell and Seigel 2019). Howell and Seigel (2019) found that 4 adult female road mortalities represented 13% of a small turtle population, emphasizing that even a few observed mortalities can have substantial implications for the long-term viability of turtle populations.

I estimated the risk of road-effects to the turtle community based on the number of individual females found on the road relative to the total number of individuals of each species. Although I observed a modest proportion (21%) of adult female Painted Turtles on the road relative to the total number of locally abundant individuals, 3 of the individuals were adult females found dead on the road. Therefore, it is possible that I did not observe an abundance of female Painted Turtles on the road because the population may already be depleted from road mortalities. Alternatively, the low proportion of female Painted Turtles found on the road may be related to their nesting behaviour. Baldwin et al. (2004) found that Painted Turtles may not nest in road shoulders if other suitable nesting habitat is abundant within 30 m of a wetland, therefore I may have observed fewer females because many were nesting in natural landscapes. Comparatively, a high proportion of the observed Snapping Turtles (61%) and Blanding's Turtles (50%) were females that encountered or used the road during nesting season. Snapping Turtles and Blanding's Turtles will travel great distances during nesting (Obbard and Brooks 1980, Millar and Blouin-Demers 2011) and may travel further to suitable nesting sites when their home range includes roads (Paterson et al. 2019). Thus, the observed abundance of Snapping Turtles and Blanding's Turtles on my study road may be the result of females' nesting migrations and behaviour. Overall, the road may present a risk to the turtle community living in wetlands adjacent to the road.

Based on the observed and potential risk of road mortality for all three species, efforts to reduce road-effects for this turtle community should be continued to ensure the viability of turtle populations. Turtles are long-lived, making it difficult to evaluate the health of a turtle population without long-term studies that consider population recruitment (Congdon et al. 1993, Heppell 1998, Enneson and Litzgus 2008, 2009). Turtle communities that are evaluated several years after road development may have already experienced declines from adult mortality or limited recruitment (Howell and Seigel 2019). My study described the turtle community likely to interact with the road, including at-risk Blanding's Turtles (Endangered; COSEWIC 2016), Snapping Turtles (Special Concern; COSEWIC 2008), and Painted Turtles (Special Concern; COSEWIC 2018). Hopefully, these mark-recapture data can serve as a baseline to allow for future monitoring to evaluate changes in the turtle community. In addition, future studies in the area should include radio-telemetry, specifically of females found nesting on the road, to determine their aquatic home range relative to the road.

Conclusion

I found the combined application of rip-rap and tar-and-chip pavement to be unsuccessful as a road-effect mitigation strategy for deterring female turtles from nesting in roadside habitat. Although hardening embankments at wetland crossings with rip-rap prevented turtles from nesting in the rip-rap itself turtles continued to find nesting habitat on the road, including areas paved with non-compact tar-and-chip pavement. It is important to understand successes and failures regarding road-effect mitigation so that we can make well-informed decisions on cost-effective best management practices for vulnerable species. Continuing to include species-specific behaviour and movements into all aspects of mitigation, road planning, and construction schedules will be critical for success. Although females primarily nested at wetland crossings, turtles were observed nesting along the entire length of the road. The persistent behaviour of turtles to bypass mitigation is a common challenge for implementing successful road-effect mitigation strategies (Baxter-Gilbert et al. 2015, Markle et al. 2017). At present, the most successful mitigation strategies for turtle communities have consisted of fence-underpass designs that use durable materials (Dodd et al. 2004, Heaven et al. 2019) and may be cost-effective by reducing cost associated with maintenance (Aresco 2005b) if incorporated into road construction schedules. However, scaling mitigation strategies to the landscape level will remain a challenge because females use habitat mosaics and make nesting migrations. In addition, although the fence-underpass design can redirect turtles' movement onto a road, it may not address risks associated with females roadside nesting. Alternatively, a multifaceted approach including several low-cost strategies to alter human and turtle behaviour on the road, could aid in reducing road threats for turtles (see *General Conclusion*).

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Figures and Tables

Figure 1.1: Aerial imagery of the study area includes the study road and surrounding wetland complexes along eastern Georgian Bay, Ontario. The 11.7 km study road is highlighted in grey, and the labelled white circles (1 – 5) identify Mitigated road sections. Each Mitigated road section consisted of ~ 300 m of paved road shoulders (dark grey sections) and ~ 100 m of rip-rap on road embankments (yellow sections) that are approximately centered within the 300 m paved section. Other wetland crossings that did not receive the mitigation strategy are indicated with red dots. The inset photo shows a Mitigated wetland crossing.

Figure 1.2: Example photo of the mitigation design at study site number 3 (refer to Figure 1.1), showing upgraded culverts, rip-rap on road embankments, and the paved road including road shoulders.

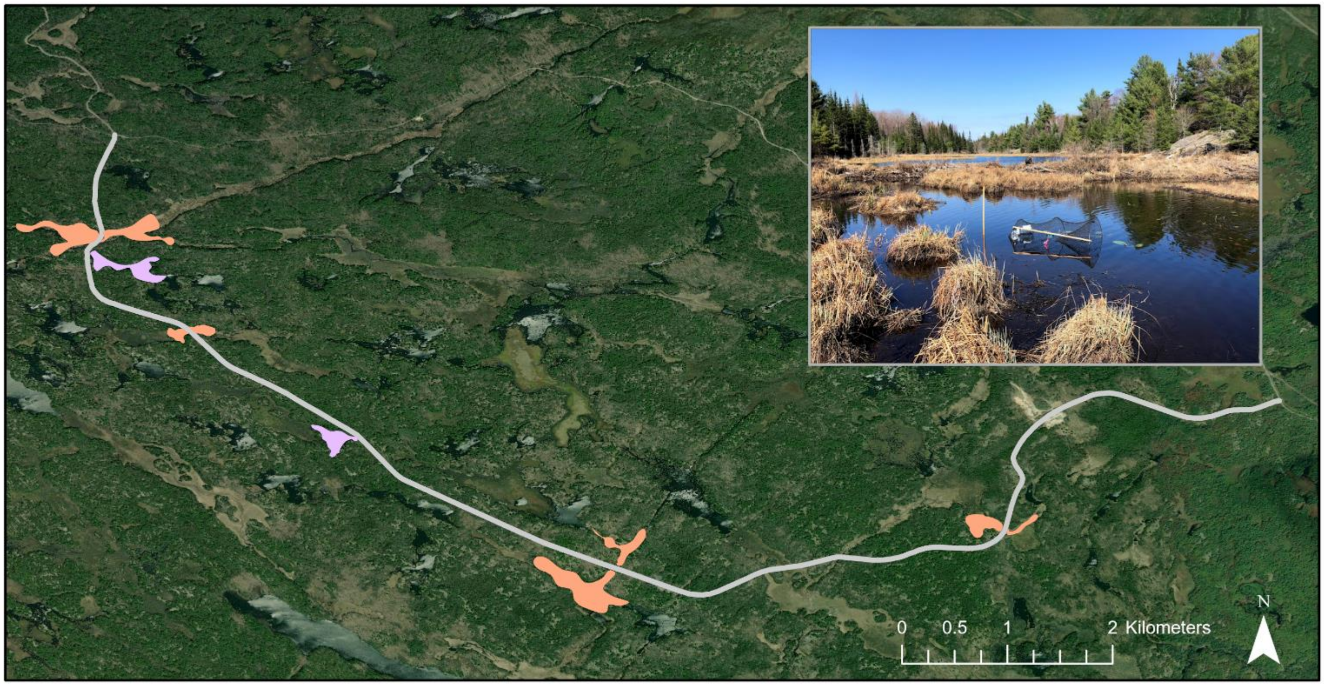

Figure 1.3: Location of wetlands where trapping surveys occurred adjacent to the road. Inset photo shows a hoop trap deployed in a wetland. Wetland surveys occurred within a 500 m buffer of the road in six locations. There were 4 Mitigated sites where trapping occurred on both sides of the road (coloured orange) and 2 Unmitigated sites (coloured purple) where trapping was only possible on one side of the road.

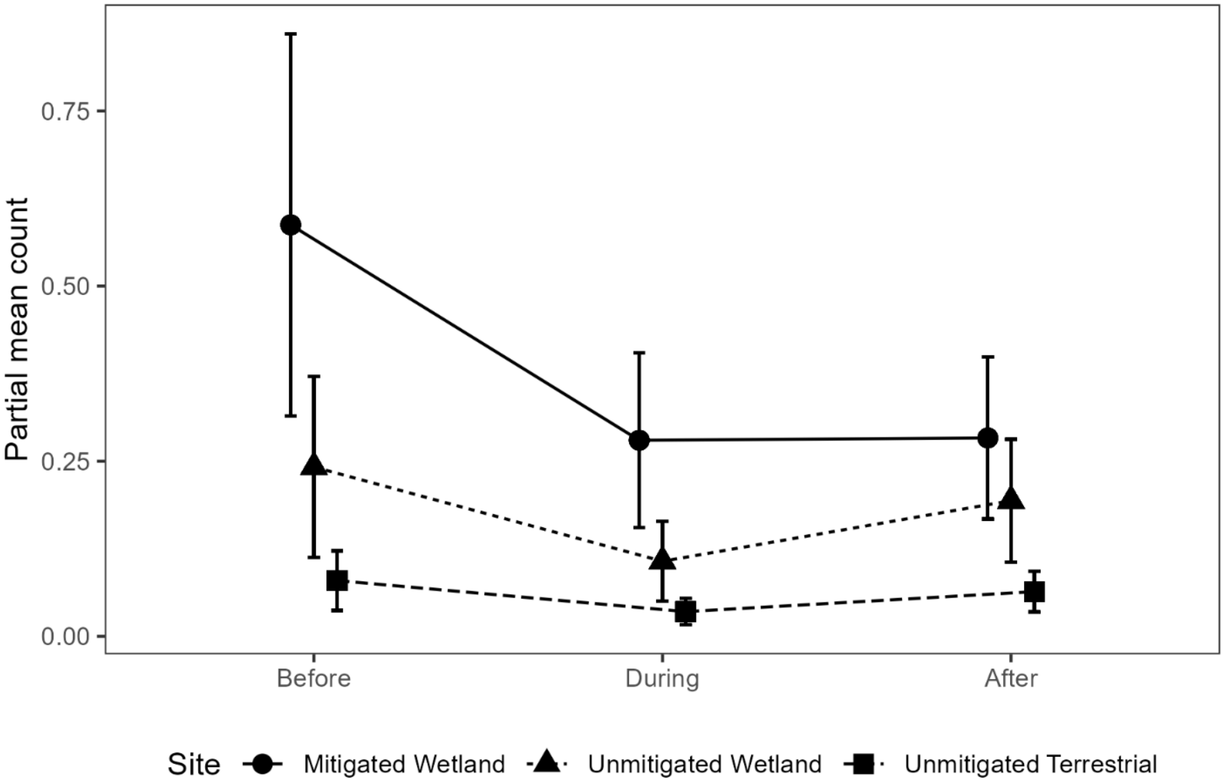

Figure 1.4: Partial effects plot on the response scale of mean daily nest count with 95% confidence intervals for the fixed effects (period, site, period × site interaction, and habitat) from the top-AIC ranked Poisson Generalized Additive Model (GAM; Appendix 1.3). Fixed effects were plotted while holding the smoothing term on Julian-day-of-year and random effects of date and road section constant. The Mitigated Wetland (solid line) were road sections that contained mitigation (rip-rap and paved road shoulders) at wetland crossings in the After period. The Unmitigated sites (dashed lines) did not contain mitigation and were distinguished by habitat as Unmitigated Wetland (dashed line-triangle) if there was a wetland crossing within the road sections or Unmitigated Terrestrial (dashed line-square) if there was no wetland crossing and the adjacent habitat was forest or rock barren.

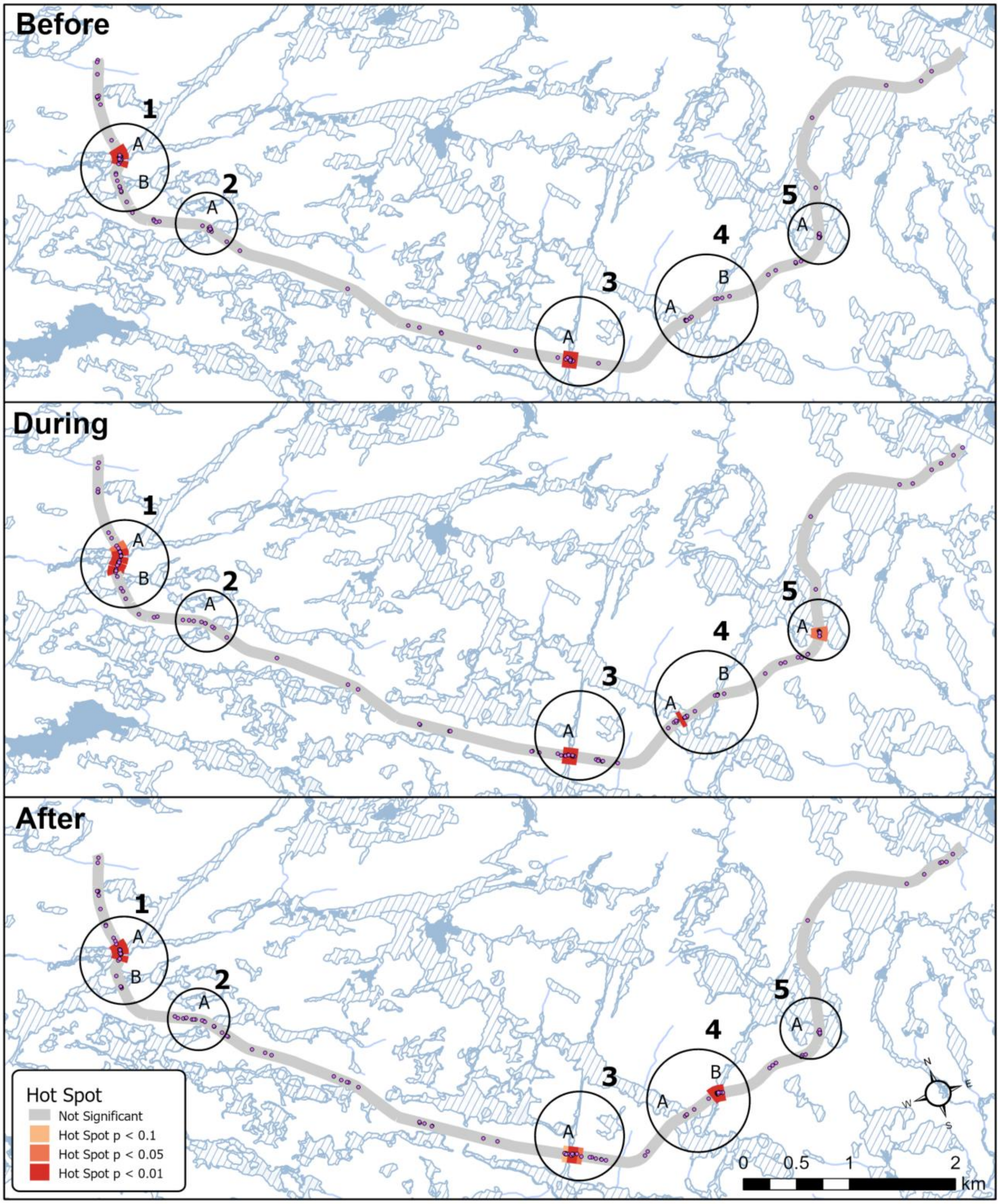

Figure 1.5: Hot Spot Analysis identifying significant clusters of nest locations on the study road in eastern Georgian Bay, Ontario. Hot spots were detected using the Getis-Ord Gi* statistic in ArcGIS Pro 3.0.3 based on number of nests in 50 m road segments in the Before (n=133 nests), During (n=118), and After (n=138) periods of mitigation. The map includes location data for each nest observation (purple points) and the surrounding wetland complexes (Land Information

Ontario 2019). Hot spots were grouped and given cluster ID’s (black circles numbered 1-5) based on their location and proximity to Mitigated sites. Clusters were further distinguished if there were two distinct hot spots within the cluster and labelled “A” if the hot spot was at a Mitigated road section and “B” if the hot spot was at an Unmitigated road section.

Figure 1.6: Example photos of Snapping Turtles (*Chelydra serpentina*) nesting observations at Mitigated wetland crossings in the During and After periods. The photos show: A) female nesting with heavy traffic in the During period; B) female nesting in a traffic lane in gravel exposed from road resurfacing in the During period; C) female nesting in an exposed gravel gap above rip-rap in the After period; D) a group of 3 females nesting simultaneously in non-compact tar-and-chip road shoulder at the edge of rip-rap in the After period.

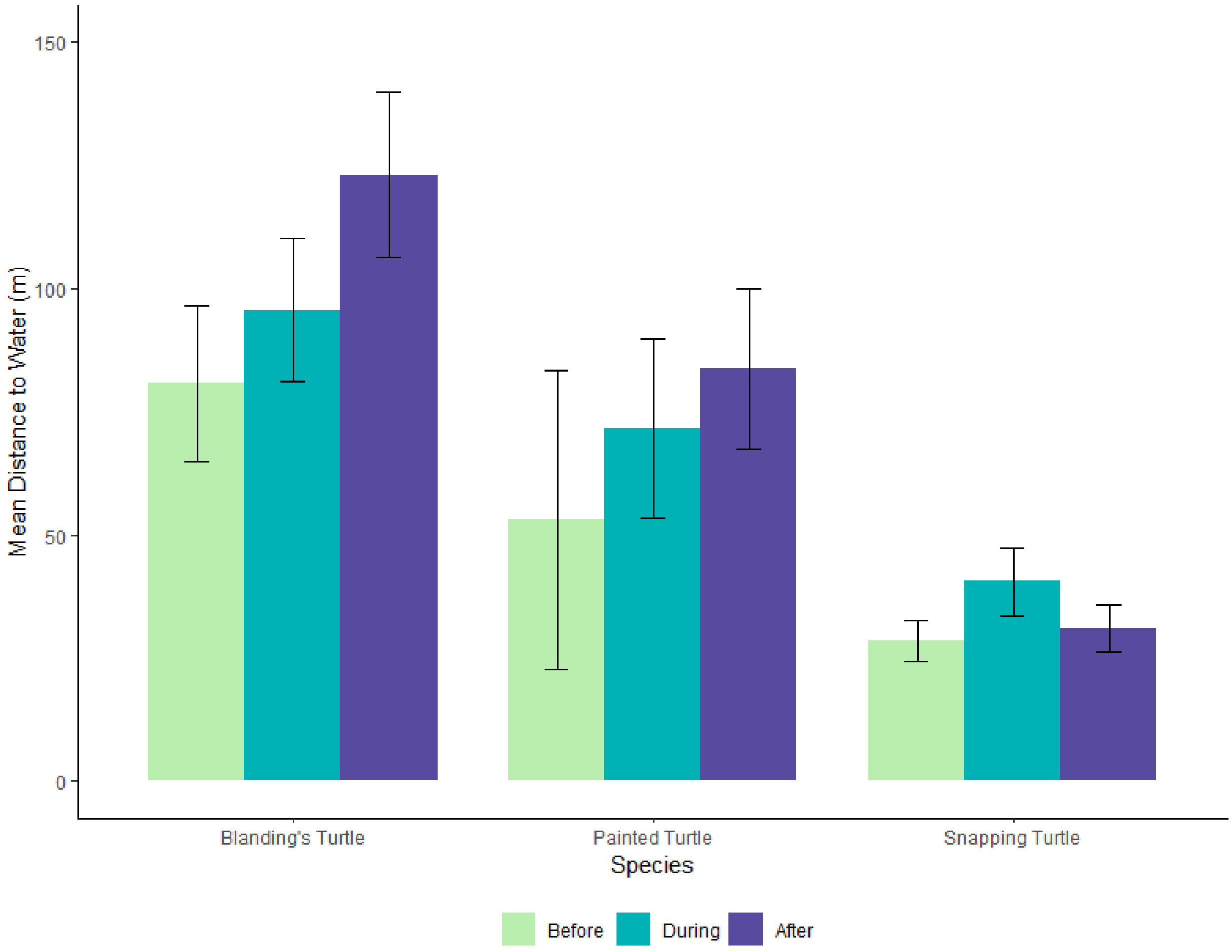

Figure 1.7: Comparison of the straight-line distance (mean ± 1 SE) from nests to the nearest wetland edge between periods (Before, During and After) for the Blanding's Turtles (*Emydoidea blandingii*), Painted Turtles (*Chrysemys picta*) and Snapping Turtles (*Chelydra serpentina*).

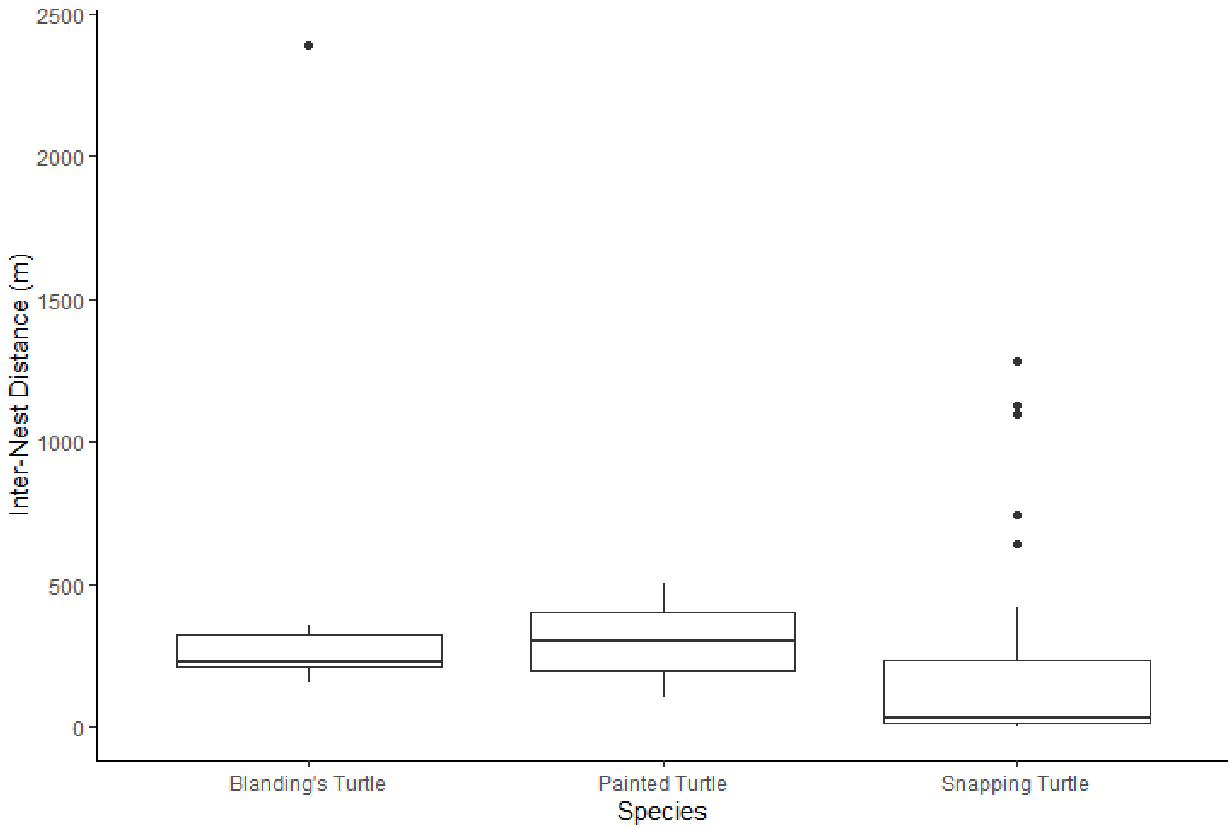

Figure 1.8: Box plot of inter-nest distances (m) of marked turtles that nested in 2021 (During construction period) and 2022 (After mitigation period) for Blanding's Turtles (*Emydoidea blandingii*; n=6), Painted Turtles (*Chrysemys picta*; n=2), and Snapping Turtles (*Chelydra serpentina*; n=30). The boxes are the interquartile range (IQR), the dark horizontal line is the median, the whiskers represent the range of data from minimum to maximum values, and the black dots show outliers.

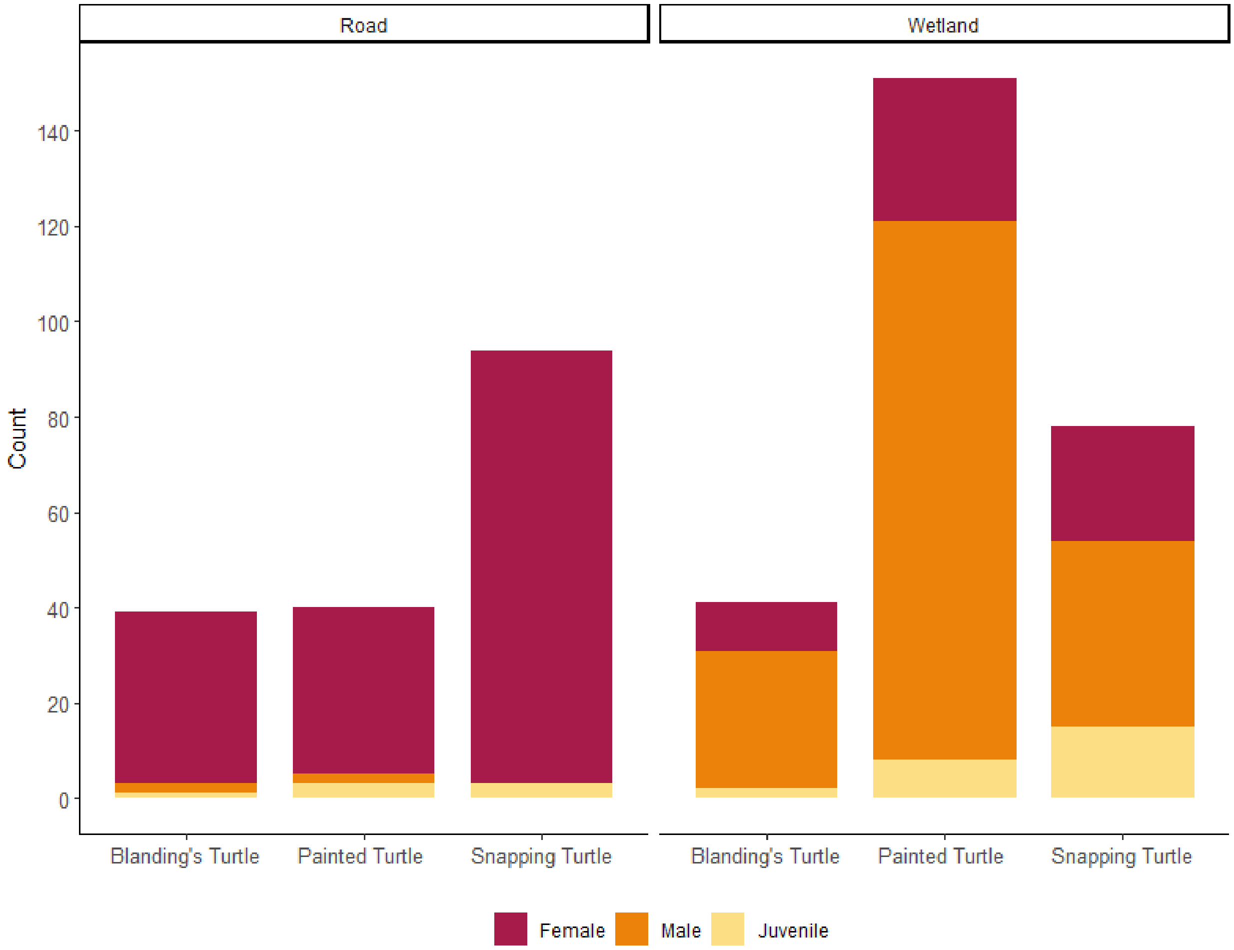

Figure 1.9: Total number of marked turtles of three species captured from road and wetland surveys in 2021 (During construction period) and 2022 (After mitigation period). The sex of turtles captured by survey type are depicted for each species, Blanding's Turtles (*Emydoidea blandingii*), Painted Turtles (*Chrysemys picta*) and Snapping Turtles (*Chelydra serpentina*). Road surveys were focussed on nesting females and occurred during the month of June. Wetland surveys included trapping and occurred in six wetlands adjacent to the road in the months of May, July, and August.

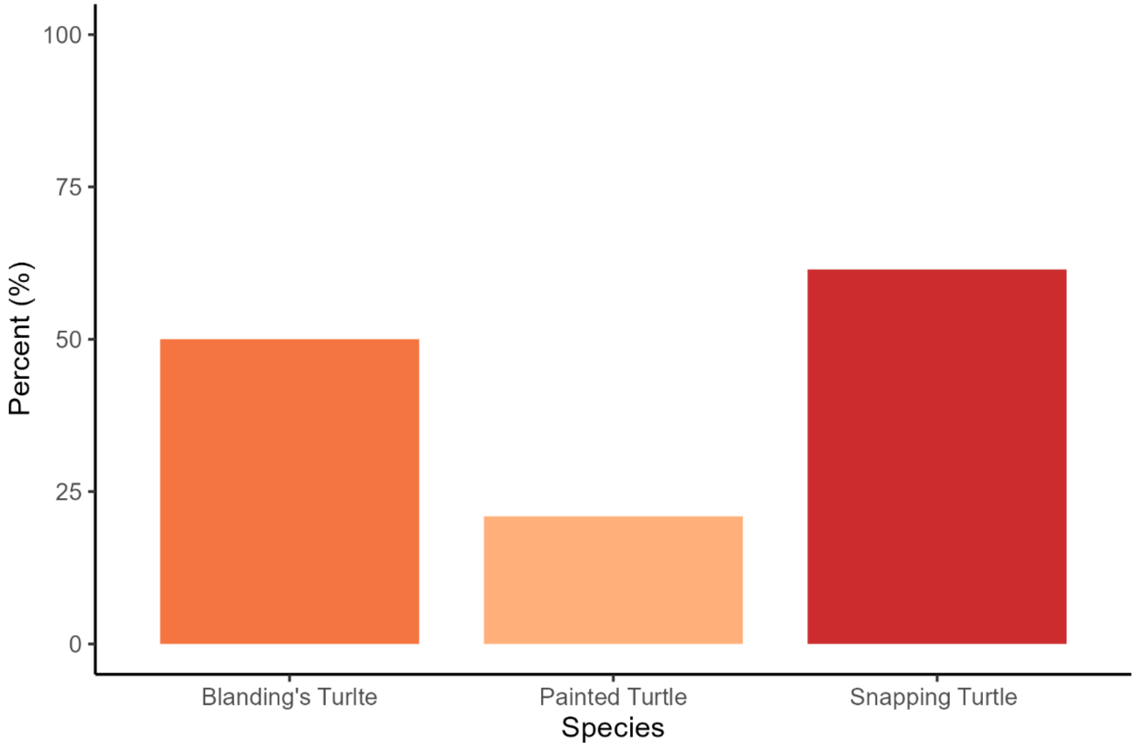

Figure 1.10: The proportion (%) of individual females that were found on the road relative to the estimated minimum local abundance of Blanding's Turtles (*Emydoidea blandingii*), Painted Turtles (*Chrysemys picta*) and Snapping Turtles (*Chelydra serpentina*). The relative proportions of females on the road roughly quantify the risk of road-effects to the turtle communities. Notably, there was a high risk of road-effects for Snapping Turtle and Blanding's Turtle communities.

Table 1.1: Total count of nests (depredated and intact) of three turtle species on the road at sites (Mitigated or Unmitigated) and habitats (Terrestrial or Wetland) between study periods (Before, During, and After mitigation), with survey effort.

Site / Habitat	**Road section**	**Period**		
		Before	**During**	**After**
Mitigated / Wetland Mitigation at wetland crossing	E	14	13	10
	G	6	12	7
	I	31	23	22
	N	11	9	17
	P	27	24	20
	Total	89	81	76
Unmitigated / Wetland Wetland crossing with no mitigation	F	6	8	20
	L	1	2	5
	O	16	6	8
	Total	23	16	33
Unmitigated / Terrestrial No mitigation or wetland crossing	A	3	5	6
	B	1	1	0
	C	1	1	1
	D	1	1	0
	H	0	3	5
	J	2	2	2
	K	4	2	5
	M	0	1	3
	Q	9	5	7
	Total	21	21	29
Total Nest Count		133	118	138
Total Survey Effort (days)		22	26	27

Table 1.2: Summary of nesting hot spot clusters on the 11.7 km study road in the Before, During and After periods of mitigation. Cluster Identification (ID) refers to road sections centered around Mitigated sites with distinct groupings of hot spots (Figure 1.5). The asterisk (*) in the Before period for the "Mitigated site" column indicates that a hot spot was an area planned for mitigation.

Period	Cluster ID	Count of hot spots			Total length of road (m)	Proportion of road	Count of nests in cluster	Mitigated site
		$P < 0.1$	$P < 0.05$	$P < 0.01$				
Before	1 A			3	150		23	No*
	3 A			3	150		29	No*
	Total			**6**	**300**	**3%**	**52**	
During	1 A		1	4	250		21	Partial
	3 A	2		2	200		15	Partial
	4 A			1	50		0	Partial
	5 A		2		100		8	Partial
	Total	**2**	**3**	**7**	**550**	**5%**	**44**	
After	1 A			3	150		14	Yes
	3 A			4	200		20	Yes
	4 B			3	150		16	No
	Total			**10**	**500**	**4%**	**50**	

Table 1.3: Total number of individual turtles of three species marked during wetland and road surveys from a two-year mark-recapture study (2021-2022). Wetland surveys were conducted using hoop traps and occurred in six wetlands adjacent to the road in May, July, and August. Road surveys occurred during the turtle nesting season in June.

Species	Sex	Wetland trapping	Nesting surveys on road	Total
Blanding's Turtle	Male	29	2	31
	Female	10	36	46
	Juvenile	2	1	3
	Total	41	39	80
Painted Turtle	Male	113	2	115
	Female	30	35	65
	Juvenile	8	3	11
	Total	151	40	191
Snapping Turtle	Male	39	0	39
	Female	24	91	115
	Juvenile	15	3	18
	Total	78	94	172
Survey Effort (2021/2022)		Trap Nights: 760 / 772	Days: 26 /27	

Appendix

Appendix 1.1: Descriptions of landscape characteristics and mitigation at Mitigated wetland crossings on the study road. Sites (1-5) described in this table correspond to those labelled in Figure 1.1. The mean exposed gap height describes the area between the rip-rap and paved road surface that provided a space with loose gravel substrate in which females could nest.

Site	Wetland description	Culvert		In bound lane			Out bound lane		
		Quantity	Specifications	Rip-rap length (m)	Paved shoulder length (m)	Mean exposed gap height (cm)	Rip-rap length (m)	Paved shoulder length (m)	Mean exposed gap height (cm)
1	Wide meandering watercourse through a shallow marsh with sandy banks	2 3	Main culverts 2000 mm x 16 m CSP Overflow culverts 1200 mm x 15 m CSP	92	400	33.5	96	400	49.5
2	A small channel that flows through a marsh with large areas of open water; controlled by beaver dam	2	1200mm x 17 m HDPE	62	200	8	67	200	11.1
3	Mainly open water with a large channel that flows through shallow marsh community	2	1200mm x 15m HDPE	44	370	46	36	370	84.6
4	Narrow meandering watercourse (approx. 1m wide) flows through a shallow marsh	2	1200mm x 15m HDPE	53	350	21.8	47	350	67.8

5	Meandering watercourse opens into an open marsh; controlled by beaver dam	1	1200mm x 15m HDPE	50	300	34.2	24	300	92

Appendix 1.2: Summary of variables used in the Generalized Additive Model (GAM) to analyze the temporal trends in the number of nests on the road between periods (Before, During, After) at sites (Mitigated and Unmitigated).

Variable	Description	Variable Type	Range / Category
Count of nests	Total daily number of nests found in each road section (A-Q).	Response	0 – 8
Period	Year of study categorized based on the presence of mitigation and construction: **Before** = 2020 before mitigation; **During** = 2021 with partial mitigation and construction; **After** = 2022 full mitigation.	Fixed variable	Before, During, After
Site	Road sections (A-Q) categorized as either 'Mitigated' or 'Unmitigated' based on the presence of Mitigation in the After period.	Fixed variable	Mitigated, Unmitigated
Period x Site	Interaction between period and sites.	Fixed variable	Mitigated x During, Mitigated x After
Habitat	Road sections (A-Q) categorized based on the presence of a wetland crossing within the section.	Fixed variable	Wetland, Terrestrial
Julian-day-of-year (jday)	A number between 1 and 365 that ignores year. Including jday as a smoothing function to control for time and seasonality between years.	Smoothing term	159-186
Date	Day of survey including year to account for within-season variability at road sections. Including date controls for observations that were not independent because they were observed on the same day.	Random effect	2020/06/09 – 2020/07/27; 2021/06/01 – 2021/06/21; 2022/05/31 – 2022/07/04
Road section	The 11.7 km road divided into 600 – 750 m sections. Sections with Mitigated wetlands contain the full length of mitigation.	Random effect	A-Q

Appendix 1.3: Summary of Generalized Additive Model (GAM) candidate models assessed for best fit using Akaike's Information Criterion (AIC) to analyze the temporal trends in number of nests on the road between periods (Before, During, After) at sites (Mitigated and Unmitigated). Note the best fit model (Model 1) included a smoothing function applied to Julian day and random effects of road section (sectionID) and date (see Table Appendix 1.2).

Model	Formula	AIC	ΔAIC
1	count ~ period + site + period:site + habitat + s(jday, bs = "tp", k=20, m=2) + s(date, bs = "re")	1450.9	0
2	count ~ period + site + period:site + habitat + s(jday, bs = "tp", k=20, m=2) + s(sectionID, bs = "re")	1507.9	57
3	count ~ period + site + period:site + habitat + s(jday, bs = "tp", k=20, m=2)	1581.7	130.8
4	count ~ period + site + period:site + habitat	1807.6	356.7

Chapter Two: Limited Suitable Nesting Habitat in a Rock Barren Landscape may Lead to Female Turtles Nesting on Road Shoulders

Abstract

Landscapes fragmented by roads can influence nesting migrations by female turtles, especially in areas where natural nesting habitat may be limited. Female nesting behaviour and nest site selection have important implications for turtle population persistence, and although roads can provide suitable nesting habitats, nesting on road shoulders may put females and hatchlings at risk of mortality. The objective of my study was to investigate the availability of natural nesting habitat for a turtle community inhabiting a rock barren landscape bisected by a road. To prevent females from using the road as nesting habitat, road shoulders and embankments at wetland crossings were resurfaced with rip-rap and paved road shoulders. The effectiveness of the mitigation strategy was gauged by whether it prevented females from nesting on the road, with the goal of redirecting females to instead nest in the natural rock barren landscape. However, natural nesting habitat may be limited; therefore, I conducted 36 300-m systematic habitat surveys to quantify the availability of suitable nesting habitat on open rock barrens. I measured soil depth and canopy openness at 1 m intervals in rock barren habitat as metrics to assess nesting suitability. I found that 16% (1736/10800 plots) of the 231-ha study area was rock barren habitat and only 2% (231/10800) of habitat surveyed consisted of roads. However, despite the availability of rock barrens, only 1% (144/108000) of plots on the rock barrens met the minimum soil depth (≥ 7.5 cm) and canopy openness (≥ 55%) required for turtle nesting. In addition, I found that the nesting habitat characteristics (cavity depth and canopy openness) on rock barrens differed from turtle-selected nest sites on the road shoulder, such that the road habitat met the nesting requirements for three local turtle species while the rock barrens did not. Overall, I found that suitable nesting habitat was limited by soil depth and canopy openness across the landscape, which may result in females continuing to nest in road shoulders, despite the rip-rap and paving mitigation. To encourage females use of the natural landscape, recovery actions that include preventing turtles access to roads and enhancing nesting habitat on rock barrens may be required. My findings emphasize the importance of understanding site-specific landscape characteristics and species-specific habitat use to help inform conservation efforts.

Introduction

All eight species of freshwater turtles in Ontario are federally classified as Species at Risk (SAR) and require conservation actions to prevent further population declines (COSEWIC 2018). Turtles have complex natural histories that require the protection of both terrestrial and aquatic habitats to support all biological functions (Burke and Gibbons 1995). Nesting is a critical life stage when reproductively active females make extensive upland movements in search of nesting habitat (Obbard and Brooks 1980, Edge et al. 2010). The nesting behaviour of females can have long-term consequences for turtle population persistence through impacts on maternal and hatchling fitness (Wilson 1998, Spencer and Thompson 2003, Hughes and Brooks 2006). Female turtles may travel great distances to nesting sites (Steen et al. 2012) and the availability of suitable microhabitats for nesting can influence females time spent and distance travelled on land (Baldwin et al. 2004, Rowe et al. 2005). Females may travel as far as 6 km from wetland habitat (Edge et al. 2010, Millar and Blouin-Demers 2011) and spend up to 17 days on land searching for nesting sites (Rowe and Moll 1991); these upland activities put females at risk of desiccation, depredation, and other hazards such as road mortality (Spencer 2002, Steen et al. 2006). Females prefer nesting habitats that exhibit suitable thermal qualities for egg incubation and reduce their predation risk (Wilson 1998, Spencer and Thompson 2003, Hughes and Brooks 2006).

Nesting habitat characteristics will vary depending on the turtle species and landscape but commonly include open canopy, no herbaceous vegetation cover, and well-drained soils (Hughes and Brooks 2006). Typically, these characteristics are found in habitats such as beaches, gravel or sandy waterway banks, fields, and beaver lodges (Rowe et al. 2005, Hughes et al. 2009, Francis et al. 2019). The nesting habitat for freshwater turtles occupying more southern locations has been well characterized, including females use of anthropogenic habitats such as road shoulders, lawns, or agricultural fields (Beaudry et al. 2010, Edge et al. 2010, Mui et al. 2016). However, along eastern Georgian Bay, where several turtle species reach their northern range limit, the landscape is largely dominated by rock barrens and wetland complexes that provide unique nesting habitat unlike that in other regions (Burke et al. 2018, Markle et al. 2021). The nesting habitat for Blanding's Turtles (*Emydoidea blandingii*), Spotted Turtles (*Clemmys guttata*), and Painted Turtles (*Chrysemys picta*) along eastern Georgian Bay includes moss and lichen-covered shallow soil deposits on rock barrens (Litzgus and Brooks 1998, Markle and Chow-Fraser 2014, Markle et al. 2021). However, naturally occurring nesting habitats on rock barrens may be limited by the availability of sites with suitable canopy openness and soil depth for oviposition (Markle et al. 2021), which are important habitat characteristics for providing a successful egg incubation environment. In addition, the thermal and hydric properties of nesting sites are influenced by the dominant cover types of moss and lichen (Moore et al. 2019, Markle et al. 2021), which may be highly sensitive to environmental changes (e.g., wildfires, Markle et al. 2020). Therefore, it is important to describe the nesting habitat for turtle populations occupying the Georgian Bay landscape, especially in areas fragmented by roads.

Roads can remove critical habitat and create anthropogenic nesting habitat that may put females and their offspring at risk of mortality (Baldwin et al. 2004, Marchand and Litvaitis 2004, Steen et al. 2006). Common road-effect mitigation strategies, such as barrier fences coupled with underpasses can prevent turtles, including migrating females, from crossing the road and thereby reduce potential road mortality (Boyle et al. 2021). In addition, artificial nesting mounds, typically constructed with sand and gravel mixtures, can provide alternative nesting habitats away from roads (Paterson et al. 2013). Although fence-underpass and nesting mound mitigation strategies can reduce turtle abundance on roads and provide alternative nesting habitat, they may not completely prevent turtles from nesting in road shoulders. Along Eastern Georgian Bay, a new mitigation design, that includes covering the gravel shoulders of roads with rock rip-rap and pavement, was used to prevent females from nesting on roads; thereby reducing road-effects if females choose to nest in the naturally occurring nesting habitat (*see Chapter 1*). Turtles may exhibit fidelity to nesting habitats (Freedberg et al. 2005, Rowe et al. 2005, Rasmussen and Litzgus 2010), sometimes displaying a preference for disturbed anthropogenic sites (Beaudry et al. 2010); therefore, in response to the removal of roadside nesting habitat (e.g., by covering with rip-rap and pavement), females may either continue to seek roadside habitat or return to the adjacent natural habitat to nest. However, if nesting habitat availability is limited in the rock barren landscape, then females may continue to nest on the road despite mitigation at wetland crossings.

Objectives

To investigate female nesting behaviour and nest site selection in a landscape modified by a road, it was necessary to quantify the nesting habitat in the surrounding rock barren landscape relative to the nesting habitat used by female turtles on road shoulders. My objective was to investigate the availability of suitable nesting habitats in the rock barren landscape surrounding wetland crossings to determine if an alternative to the road was available to females. I conducted 36 300-m systematic habitat surveys to quantify the availability of suitable nesting habitat on open rock barrens in terms of soil depth and canopy openness. To further investigate if the nesting conditions on rock barrens would provide similar habitat used by females on road shoulders, I compared the characteristics of potential nesting habitat on rock barrens to those of female-selected nests observed for three local Species at Risk (SAR), Blanding's Turtles

(Threatened, COSEWIC 2016), Painted Turtles (Special Concern, COSEWIC 2018) and Snapping Turtles (*Chelydra serpentina*; Special Concern, COSEWIC 2008), that nested on the study road in 2022. I expected that natural nesting habitat would be limited for large-bodied species requiring deeper soils for nest cavities (e.g., Snapping Turtles and Blanding's Turtles), but that suitable habitat may be more available for species with shallow nest cavity depths (e.g., Painted Turtles).

Methods

Study Area

The study area consists of an 11.7 km road and its surrounding habitat in the Parry Sound Ecodistrict along eastern Georgian Bay (Figure 2.1), situated within the Robinson-Huron Treaty of 1850 and Williams Treaty of 1923 and located on Anishinabek territory. The landscape surrounding the road is a habitat mosaic characterized by two large wetland complexes, mixed broad-leafed and conifer forests, and granite rock barrens. The rock barrens are distributed throughout the landscape and consist of a mixture of open areas; exposed bedrock; lichen or moss-covered depressions; and areas dominated with juniper (*Juniperus spp*.) or blueberry (*Vaccinium angustifolium*). In the study area, female turtles are known to nest in the exposed gravel on road shoulders and embankments at wetland crossings (see *Chapter 1*). During scheduled road maintenance, five wetland crossings on the road were mitigated with rip-rap and paved road shoulders to cover roadside nesting habitat (see *Chapter 1* for details).

Habitat Survey

I conducted habitat transect surveys that were adapted from methods described in the Markle et al. (2021) study characterizing turtle nesting habitat along eastern Georgian Bay. All transect surveys were conducted in 2022 during the months of August and September. I focused habitat surveys within a 350 m radius buffer around the center of six wetland crossings on the road (Figure 2.1). The six wetland crossings were selected to encompass the habitat surrounding known nesting hot spots on the road (Figure 2.1). The buffer area around wetland crossings totaled 231-ha and consisted of Mitigated wetland crossings (i.e., rip-rap and paved road shoulders), Unmitigated wetland crossings (i.e., gravel road shoulders), wetlands, rock barrens, and mixed forests (Figure 2.1). I conducted six 300-m habitat surveys within the buffer area at each wetland crossing, for a total of 36 transects in the study area. Prior to data collection, I randomly generated starting points at the edge of wetlands for all transects using ArcGIS Pro 3.0.3 (ESRI, Redlands, California, USA). All transects were surveyed perpendicular to the edge of a wetland using a tape measure and compass. I described the habitat every 1 m along the transect by recording the habitat type as either forest, wetland, road, or rock barren (Table 2.1).

In rock barren habitats only, I collected additional data to quantify suitable nesting habitats. At every 1 m along the transect within a rock barren, I determined soil depth, canopy openness, vegetation cover, slope, and aspect in a 1 m^2 plot. I measured soil depth by inserting a metal stake into the plot corners and center and recorded the maximum depth (to the nearest mm) represented in the plot using a ruler. At the point of soil depth measurement, I recorded the vegetation cover type, measured the cover height if the cover type was moss, lichen, or leaf litter and described the bedrock morphology (flat, crevice, ledge, or rubble; Table 2.1). For each plot, I determined canopy openness using a densiometer (Universal Field Supplies, Canada). I recorded the percent of vegetation cover by cover type and recorded the slope of the plot using a level with an adjustable vial (Milwaukee® 360° Pocket level, Brookfield, United States) positioned on the center of the plot. I recorded the aspect of a plot if the slope was greater than 5° using a compass. Additional data were not collected for other habitat types (i.e., wetlands, forest, roads) because my survey objective was to characterize natural nesting habitats typical for the local turtle species.

Nesting Surveys

The study road was monitored for nesting activity every morning and evening during the month of June in 2022. Surveyors slowly drove the road (20-30 km/hr) and walked road sections to locate signs of nests or nesting turtles. Surveyors recorded the species, GPS location (accuracy ± 5 – 20 m; TAB Active2, Android operating system), and the nest status (intact, partially predated or predated), of all identified nests. Additional information was collected for each nest found on road shoulders if the nest was found intact. Eggs were carefully excavated from the chamber and surveyors recorded the depth to the top and bottom of the nest cavity. Following nest measurements, eggs were placed back in the nest chamber in the order and position they were removed. Canopy openness, slope, and aspect were recorded for all nests (intact and predated) found on the road following the same methods used to describe the rock barren habitat plots.

Statistical Analyses

Habitat Availability and Suitability

I assessed the availability of potential nesting habitat based on the number of plots characterized as rock barren relative to the total number of plots surveyed (n = 10800 plots). I deemed plots suitable for nesting on rock barrens if they contained a depression (soil-filled bedrock, hereafter “depression plots”); suitable vegetation cover (e.g., moss, lichen, or leaf litter); and met the minimum soil depth and canopy openness requirements for Blanding's Turtles, Painted Turtles, and Snapping Turtles (Appendix 2.1). I determined the minimum soil depth (≥ 7.5 cm) and canopy openness (≥ 55%) requirements for all three species based on averages reported in the literature (Appendix 2.1) and minimums observed for nests found on the road in this study.

For rock barren sites to meet minimum nesting requirements of all three turtle species, plots were required to have relatively deep soils with low canopy cover. Therefore, I described the relationship between soil depth and canopy openness in depression plots using a Spearman's rank correlation test. Although other factors can contribute to the suitability of nesting sites, including soil properties (i.e., texture, organic content, and bulk density), Markle et al. (2021) did not find that soil properties were a limiting factor for nesting suitability across the rock barren landscape.

Rock Barren Site Characteristics

To describe the plot characteristics that may contribute to nesting site selection by females, I investigated if there was a relationship between soil depth and cover type or bedrock morphology. For depression plots that met soil depth and canopy openness requirements, I used Kruskal-Wallis's rank sum tests ($\alpha = 0.05$) to assess differences in soil depth and cover height among cover groups (lichen, moss, and litter) and soil depth among bedrock morphology groups (flat, crevice, ledge, rubble). I used a Kruskal-Wallis's rank sum test because the residuals did not meet assumptions of normality based on a Sharpiro Wilk test. If a significant difference was detected, I conducted a Dunn's post-hoc test with a Bonferroni adjustment for multiple comparisons. In addition, I summarized bedrock morphology, cover height, slope, and aspect in suitable nesting depression plots.

Road Nest Characteristics

I described the characteristics of nests observed on the road for all three local turtle species during the 2022 nesting season. I compared the characteristics of nests on the road to the habitat characteristics described on rock barrens to determine their suitability as nesting habitat. I evaluated differences in soil depth and canopy openness of nests observed on the road to those described on rock barrens using a Kruskal-Walli's rank sum test ($\alpha = 0.05$). I used a Kruskal-Wallis's rank sum test because the residuals of soil depth and canopy openness did not meet assumptions of normality based on a Sharpiro Wilk test. All summary statistics and analyses were conducted in R version 4.2.1 (R Core Team, 2022). Reported means are followed by ± 1 SE, range of data, and number of observations.

Results

Habitat Availability

I found forests to be the dominant habitat type on the landscape, comprising 62% (6699/10800 plots) of the plots surveyed along transects, followed by rock barrens (16%, 1736/10800), wetlands (20%, 2134/10800), and roads (2%, 231/10800). Rock barrens comprised almost 1/5 of the habitat surveyed, with 87% (1506/1736) of the rock barren plots containing depressions with potentially suitable nesting habitats, and the remaining 13% (230/1736) containing exposed bedrock or dense juniper and shrub patches. I found forest and rock barren habitats were uniformly distributed across most transects, roads were sparsely distributed across the transects, whereas wetland habitats were commonly found furthest from the starting point of the transect (Figure 2.2).

Habitat Suitability

Soil Depth and Canopy Openness

Average soil depth across the rock barrens was 4.2 cm (± 0.14 SE, 0 – 45 cm, n = 1506 plots), and mean canopy openness was 63% (± 0.9 SE, 0 – 100%, n = 1506 plots). I found that only 10% (144/1506) of depression plots met the minimum soil depth (≥ 7.5 cm) and canopy openness (≥ 55%) nesting requirements (Figure 2.3 and Appendix 2.1), which constituted only 1% (144/10800) of all transect plots. However, the proportion of plots suitable for nesting differed on a species-specific basis. I found that only 1% of the depression plots met the minimum requirements for Snapping Turtles, 3% for Blanding's Turtles, 15% for Painted Turtles, and 23% for Spotted Turtles, a species that also occurs in eastern Georgian Bay (Figure 2.4). Available nesting habitat for the community of turtles decreased as soil depth requirements increased (i.e., because larger-bodied species nest in deeper soils; Figures 2.3 and 2.4). I detected a negative correlation between soil depth and canopy openness ($r = -0.07$, $p < 0.05$) such that plots with deeper soils had greater canopy cover.

Suitable Nesting Site Characteristics

Soil depth differed among cover types and bedrock morphologies, with the deepest soils associated with moss and flat or a ledge bedrock. Moss was the dominant cover type in suitable depression plots with 23% (33/144) of plots having 80% or greater moss cover, 3% (5/144) of plots with lichen, and 3% (5/144) of plots with leaf litter. I detected significant differences in soil depths among cover groups ($\chi^2 = 6.2$, df = 2, p = 0.04) with a post hoc analysis identifying that only lichen and moss differed in soil depths (z = -2.3, p adjusted = 0.05; Figure 2.5). I also detected significant differences in cover heights among cover groups ($\chi^2 = 84.2$, df = 2, $p < 0.05$; Figure 2.5) with a post hoc analysis identifying that moss, lichen, and litter all differed in cover height (Appendix 2.2). Moss was associated with the deepest soil (12 cm ± 0.49 SE, 7.6 – 26, n = 86) and greatest cover height (5.9 cm ± 0.4 SE, 0.5 – 15, n = 86). Litter was associated with a mean soil depth of 10.6 cm (± 0.5 SE, 7.6 – 21, n = 37) and shortest cover height of 0.3 cm (± 0.06 SE, 0.1 – 1.6, n = 37). Lichen was associated with a mean soil depth of 10.1 cm (± 0.6 SE, 7.5 – 18, n = 21) and a cover height of 3.4 cm (± 0.4 SE, 1 – 10, n = 21). I detected significant differences in soil depths among bedrock morphologies ($\chi^2 = 15.1$, df = 3, p = 0.001; Figure 2.6). A post hoc analysis identified that areas with ledges had significantly deeper soil than crevice or rubble bedrock morphology (Appendix 2.2). Similarly, areas with flat bedrock had significantly deeper soil than crevices or rubble bedrock morphology (Appendix 2.2). Ledge bedrock morphology was associated with the deepest soil (12.2 cm ± 0.9 SE, 8.7 – 21, n = 17), followed by flat (11.8 cm ± 0.4 SE, 7.5 - 34, n = 103), crevices (9.2 cm ± 0.3 SE, 7.8 – 11.5, n = 18), and rubble (8.5 cm ± 0.5 SE, 7.6 – 11.2, n = 6). Of the plots that met the soil depth and canopy openness requirements, 72% were on flat bedrock, 33% had a south-facing aspect, and 63% had a slope that fell within the range of 0 – 10 degrees (Appendix 2.3).

Observed Nest Characteristics

I detected the nests of 138 females on the road in 2022, consisting of 26 Blanding’s Turtles, 18 Painted Turtles, and 94 Snapping Turtles. I collected nest characteristics of intact nests found on the road for 15 Blanding’s Turtles, 9 Painted Turtles, and 74 Snapping Turtles. Nest cavity depths differed among species (Figure 2.7) with Snapping Turtles having the greatest mean nest depth (17.4 cm ± 0.5 SE, 7.9 – 28.5, n = 74), followed by Blanding's Turtles (11.5 cm ± 1.4 SE, 5 – 25, n = 15), and Painted Turtles (5.9 cm ± 0.4 SE, 5.3 – 8, n = 9). The canopy openness at nesting sites was similar among species (Figure 2.7) with an average of 73% (± 7.8 SE, 31 – 100%, n = 74) for Blanding's Turtles, 80% (± 7.2 SE, 46 – 100%, n = 15) for Painted Turtles and 84% (± 2.4 SE, 0 – 100%, n = 9) for Snapping Turtles. Snapping Turtle nests had Northeast (23%), East (20%), or Southwest (16%) aspects, whereas Blanding's Turtles had mostly North (46%) and Painted Turtles had mostly Southwest (44%) aspects. Snapping Turtles laid nests in road shoulders with the steepest average slope of 18° (± 1.5 SE, 0 – 48, n = 73), whereas the average slope for Painted Turtle nests was 9° (± 3.4 SE,1 - 34, n = 9) and Blanding's Turtles was 6° (± 2.2 SE, 0 - 26, n = 15).

Road Nest and Rock Barren Comparison

I compared the nest depth (as an index of minimum required soil depth) and canopy openness measured at nests of all three turtle species that nested on the road to the soil depth and canopy openness documented in depression plots on rock barrens. I found Painted Turtle nest cavity depth was significantly greater on the road compared to data collected on the rock barrens (χ^2= 7, df = 1, p < 0.05); however, there was no significant difference in canopy openness (χ^2= 0.8, df = 1, p = 0.4). Similarly, I found that Blanding's Turtles nest cavity depth on the road was significantly greater than soil depth available on the rock barrens (χ^2= 22.2, df = 1, p = < 0.05); however, there was no difference in canopy openness between nests on the road and available habitat on the rock barrens (χ^2= 0.4, df = 1, p = 0.5). For Snapping Turtles, I found significant differences in nest cavity depth (χ^2= 176.8, df = 1, p = < 0.05) and canopy openness (χ^2= 17.8, df = 1, p = < 0.05) on the road compared to data collected on the rock barrens.

Discussion

Natural Nesting Habitat

Despite the abundance of rock barrens on the landscape, I found only a small proportion of the habitat was suitable for nesting based on the minimum soil depth and canopy openness requirements for the local turtle community. The rock barrens primarily consisted of shallow soil deposits that may support the nesting requirements for small-bodied species such as Spotted Turtles and Painted Turtles, but suitable sites were limited for large-bodied species such as Blanding's Turtles and Snapping Turtles. Typically, females will select oviposition sites based on environmental cues that can maximize hatchling success (Spencer and Thompson 2003, Hughes and Brooks 2006), which include areas that have both adequate soil depths and high canopy openness (Congdon et al. 2000, Litzgus and Brooks 2000). Canopy openness and soil depth can be important indicators of the thermal and moisture properties of a nest chamber, and consequently nest success and hatchling fitness (Brooks et al. 1991, Wilson 1998, Janzen and Morjan 2002, Morjan 2003). Generally, deeper nests are cooler, experience less temperature fluctuations, and may prevent desiccation in dry conditions (Wilson 1998, Booth and Astill 2001, Moore et al. 2019). However, overall I found the average soil depth on the rock barrens was less than that recorded in other studies (7.9 ± 6.9 cm; Markle et al 2021) and did not meet typical soil depth requirements for Painted Turtles (8.0 – 10.5 cm, Markle et al 2021; 6 – 13 cm, Morjan, 2003), Blanding's Turtles (10 – 13.5 cm, Markle et al 2021; 12 cm, Standing et al 1999) and Snapping Turtles (12 – 18 cm Congdon et al 1987). Although, the shallow soil depths likely provide suitable nesting habitats for other species, such as Spotted Turtles that have shallower nest cavities (7.5 cm, Markle et al 2021; 2 – 6 cm, Rasmussen and Litzgus 2010). In contrast, I found the average canopy openness across rock barrens to meet the minimum requirements described for some species (Painted Turtle 46 % - 100 %, Snapping Turtle 63% - 100 %, Riley et al. 2014; 55% - 94 %, Markle et al 2021). I found that areas with greater canopy openness were associated with shallower soils; therefore, limiting the availability of suitable nesting sites for species with deeper soil depth requirements. Similarly, other studies have found that suitable nesting sites on rock barrens were limited by canopy openness, such that the canopy was too closed in areas that met soil depth requirements (Litzgus and Brooks 2000, Markle et al. 2021). Overall, my findings were similar to the Markle et al. (2021) study characterizing nesting habitat in eastern Georgian Bay, which found that 25% of a 660-ha study area was rock barren habitat, however with shallow soils and low canopy openness only 3% was suitable for nesting.

Although shallow soils may not provide ideal thermal incubation environments in an open rock barren landscape (Wilson 1998, Booth and Astill 2001), some species may select nesting sites with shallow soils, instead prioritizing the desired canopy openness. Blanding's Turtles and Painted Turtles were documented nesting on rock barrens with nests cavities ≤ 10 cm deep, and in the absence of depredation had a nest success rate of 59% (Markle et al. 2021). Few studies have described Snapping Turtles nesting in rock barren habitats in the literature. Francis et al (2019) documented two Snapping Turtle nests in similar rocky outcrop areas in Algonquin Provincial Park with canopy cover consisting of 5% and 61% and intermediate nest depths of 14.5 cm and 16.5 cm, showing that in some locations Snapping Turtles can find suitable nesting sites on open rocky habitat. However, I found that nesting habitat on rock barrens may be especially limiting for Snapping Turtles because only 1% of the rock barrens provided deep enough soil with suitable canopy openness. Interestingly, I found evidence of Snapping Turtle nesting activity, consisting of test pits and plowing into moss and lichen-covered soil, on a rock barren directly adjacent to the road and wetland edge in 2022; however, no nests were found on the rock barrens during my study. Other studies have found Snapping Turtles nesting in muskrat (*Ondatra zibethicus*) and beaver (*Castor canadensis*) lodges (Obbard and Brooks 1980, Francis et al. 2019); therefore, in areas where natural upland nesting habitat is limited, females may find suitable habitat within the wetland. Further research should describe the nesting behaviour and site selection of Snapping Turtles in a rock barren landscape.

In addition to soil depth and canopy openness characteristics, rock barrens can create unique nesting microenvironments with differences in thermal and moisture properties between cover types and bedrock morphologies (Markle et al. 2021). Studies have found that females typically nest in areas with soil deposits at the base of a ledge or in a crevice covered with lichen (Litzgus and Brooks 1998, Markle and Chow-Fraser 2014, Zagorski et al. 2019). Soil deposits on flat granite bedrock may experience greater temperature fluctuations compared to crevices and ledges that can create warmer and more stable environments from contact with granite rock that holds heat (Markle et al. 2021). Crevasses and ledges in rock barren habitats can also provide areas for deeper soil accumulation (10 cm – 30 cm, Zagorski et al 2019; 0 - 31 cm this study), that may support nest cavity requirements for Snapping Turtles. However, I found the majority of plots described on the rock barrens to have a flat bed rock morphology compared to only a small proportion described as a ledge or crevice, therefore suitable sites may be further limited by bedrock morphology. The cover height and type of vegetation may also contribute to nest site suitability, as Blanding's Turtles and Painted Turtles were found nesting in areas with moss and lichen cover height less than 5.5 cm (Sandler 2020). In addition, moss and lichen cover can regulate soil moisture and prevent desiccation (Moore et al. 2019); therefore, in a landscape with shallow soils, cover types of moss and lichen likely play an important role in nest success. Overall, I found moss was the dominating cover type in depression plots and was associated with the deepest soil; however, moss also had the greatest cover height which may be a deterrent for species if they select areas with shorter cover height (i.e., lichen).

Roadside Nesting Habitat

The road consisted of only a small proportion of habitat described from the transect surveys relative to the surrounding natural habitat. Nonetheless, because roads are a pervasive linear feature on the landscape that bisects critical habitat, the presence of roads can alter how turtles use and navigate the landscape (Baldwin et al. 2004, Paterson et al. 2019). For example, roads can reduce natural nesting habitat and instead create anthropogenic nesting habitat on road shoulders to which females migrate (Baldwin et al. 2004). I found that the potential nesting habitat conditions on the rock barrens differed from the nest characteristics of three species using open gravel road shoulders for nesting. Interestingly, I found a high proportion of all species that nested on the road to be Snapping Turtles, suggesting that larger bodied turtles in the turtle community may be more likely to use nesting habitat on the road if natural habitat is limited by soil depth and canopy openness. However, further population demographic studies are required to determine if the high proportion of Snapping Turtles on the road was due to their nesting behaviour or their overall greater abundance relative to other species in the turtle community. Because most natural nesting habitat that I surveyed did not meet both soil depth and canopy openness requirements compared to the conditions available on the road shoulders for all three species, females in the local turtle community may continue to use road shoulders as nesting habitat. Females are known to travel great distances in search of nesting sites in areas with roads (Paterson et al. 2019) and may readily adopt freshly-disturbed habitat if encountered during nesting forays (Beaudry et al. 2010). Therefore, because roads are a permanent feature with road shoulders that consist of well drained gravel substrate in areas that are regularly maintained to be clear of vegetation, females may prefer road shoulders over potentially less predictable natural nesting habitat.

In addition, for turtle species at their northern range limit where the duration of nesting is thermally restricted, anthropogenic nesting habitat may provide ideal thermal conditions for oviposition (Francis et al. 2019). Francis et al. (2019) found that anthropogenic sites were consistent in creating high-quality thermal nest environments compared to natural sites, suggesting that females may prefer the thermal environment of road shoulders. Although the road may appear as favourable nesting conditions for female turtles, roadside habitat can act as an ecological trap by exposing females and their offspring to road mortality and reducing nest success from predation, compaction, or pollution (Baldwin et al. 2004, Marchand and Litvaitis 2004, Steen et al. 2006). Mui et al. (2016) suggests that despite the risk to female fitness from nesting in anthropogenic sites, females may not recognize roads as a threat because the environmental cues used in natural landscapes may not be reliable predictors of nest success in anthropogenic habitats.

The well documented road-effects for female turtles emphasize the need for mitigation strategies to reduce road threats to turtle communities. However, my findings suggest that the overall lack of suitable natural nesting habitat may complicate mitigation strategies that attempt to deter females from nesting on road shoulders. The success of the rip-rap and paved road shoulder mitigation strategy partially depended on females adopting natural nesting habitat available on rock barrens. However, removing roadside nesting habitats at wetland crossings did not effectively encourage females to seek suitable nesting habitat in the natural landscape; instead, females continued to use roadside nesting habitats available further down the road (*see Chapter 1*). The deterrent mitigation strategy is unlikely to be successful without additional mitigation to prevent road access (e.g., fencing; Boyle et al. 2021) or encouraging alternative nesting locations (e.g., natural nesting habitat restoration). Currently, the road provides nesting conditions that may be more favourable for female turtles, especially large-bodied species, relative to the surrounding rock barrens. Further research should be conducted to investigate if females select roadside nesting habitats because of preference or necessity. In addition, research should investigate the cost-benefit of females nesting in anthropogenic habitats compared to natural habitats, especially if natural nesting habitats may be limited. In some areas, road shoulders may provide suitable nesting conditions that could support recruitment if other road threats are properly mitigated, such as preventing road crossings and predator protection (e.g., nest cages or ex situ incubation and headstarting). However, if road threats are not effectively mitigated for turtles during multiple life stages (Keevil et al. 2022), road shoulders may continue to create an ecological trap for turtles.

Habitat Restoration

In road ecology projects, artificial nesting mounds (ANMs) are often used to supplement the loss of naturally occurring nesting habitat and may reduce the need for migrating females to cross the road if constructed in opportune locations (Beaudry et al. 2010, Buhlmann and Osborn 2011, Paterson et al. 2013). ANMs can be a beneficial nesting alternative for females, especially in landscapes degraded by human development or with low naturally occurring nesting habitat; however, their success is often dependent on size (i.e., enough surface area to support multiple turtles to nest), the use of protection from predators, and annual maintenance (Beaudry et al. 2010, Dowling et al. 2010, Buhlmann and Osborn 2011, Paterson et al. 2013). ANMs are typically constructed using a sand and gravel mixture to mimic nesting conditions on beaches, shorelines, or road shoulders; however, this substrate may not be suitable in a rock barren landscape (Markle et al. 2021). Therefore, as suggested by Markle et al. 2021, a more appropriate strategy to encourage nesting in a rock barren landscape may consist of supplementing suitable crevice and ledge sites on rock barrens with substrate characteristic of the landscape (i.e., sandy-loam soil and moss or lichen).

If suitable nesting habitat is available in a rock barren landscape, it may provide additional benefits for female turtle fitness compared to roadside or ANM habitat. Females and hatchlings are vulnerable to predators (Spencer 2002, Spencer and Thompson 2003) and environmental stressors while navigating terrestrial habitats (Paterson et al. 2014); therefore, females may show selective behaviour for oviposition sites that minimize predation risk during nesting forays and for hatchlings post-emergence (Spencer 2002). Typically, females' nest within 300 m of the edge of a water body (Steen et al. 2012), which may reduce the time females and hatchlings spend on land. Therefore, the scattered distribution of rock barrens within a complex landscape (e.g., multiple habitat types) may provide greater predator protection (Paterson et al. 2014) compared to other habitats where nests may be clustered in small areas or linearly concentrated, such as on beaches, pond edges, or road shoulders (Marchand et al. 2002, Crawford et al. 2014) or even in ANMs (Dowling et al. 2010). In addition, hatchlings can be vulnerable to desiccation during the nest-water migration (Kolbe and Janzen 2002); therefore, increased nesting habitat near water may be beneficial for hatchlings survival. The rock barrens are uniformly scattered among forest and wetland habitats within 300 m of a wetland at my study site. Therefore, if nesting habitat is restored on the rock barrens, it may provide an ideal distribution for female and hatchling survival during upland movements. However, a potential limitation with restoring rock barren nesting habitat in a landscape modified by a road is ensuring that females will encounter and select the restored habitat instead of the road shoulders.

Further studies should investigate the females encounter rates of restored habitat in rock barren landscapes adjacent to a road.

Conclusion

The large wetland complexes and rock barrens in eastern Georgian Bay provide habitats that support several SAR turtle species. With increasing development pressures in the area, it is important to understand the specific regional challenges turtle populations face in this unique environment. In a rock barren-dominated landscape where naturally-occurring nesting habitat is limited, road construction, maintenance, and use may create an ecological trap for turtles. I found that the success of the rip-rap and paved road shoulder mitigation strategy at deterring females from nesting on the road may be further limited by the availability of suitable natural nesting habitats. Despite removing nesting habitat on the road, females may continue to seek nesting habitat on road shoulders because of microhabitat preferences or out of necessity from limited natural habitat. Further conservation efforts, such as enhancing nesting habitat on rock barrens, may be required to encourage females to use the natural landscape. My findings emphasize the importance of understanding site-specific landscape characteristics and species-specific habitat use to inform conservation efforts. Mitigation strategies should consider the distribution of critical habitats surrounding the road, such as rock barrens for nesting, to help understand turtles' behaviour in a modified landscape.

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Figures and Tables

Figure 2.1: Study areas for habitat transect surveys occurring within 350 m radius buffers (light pink circles ○) at 6 locations identified as nesting areas for Blanding's Turtles, Painted Turtles, and Snapping Turtles on the road. The buffered area around wetland crossings totaled 231-ha and consisted of 5 Mitigated wetland crossings (white diamond ◊) with ~100 m of rip-rap and ~300 m of paved road shoulders to deter nesting and 3 Unmitigated wetland crossings (black diamond ♦). The starting location of habitat transect surveys (pink points ●) were determined in Arc GIS pro 3.0.3 (ESRI, Redlands, California, USA) using the random points generator.

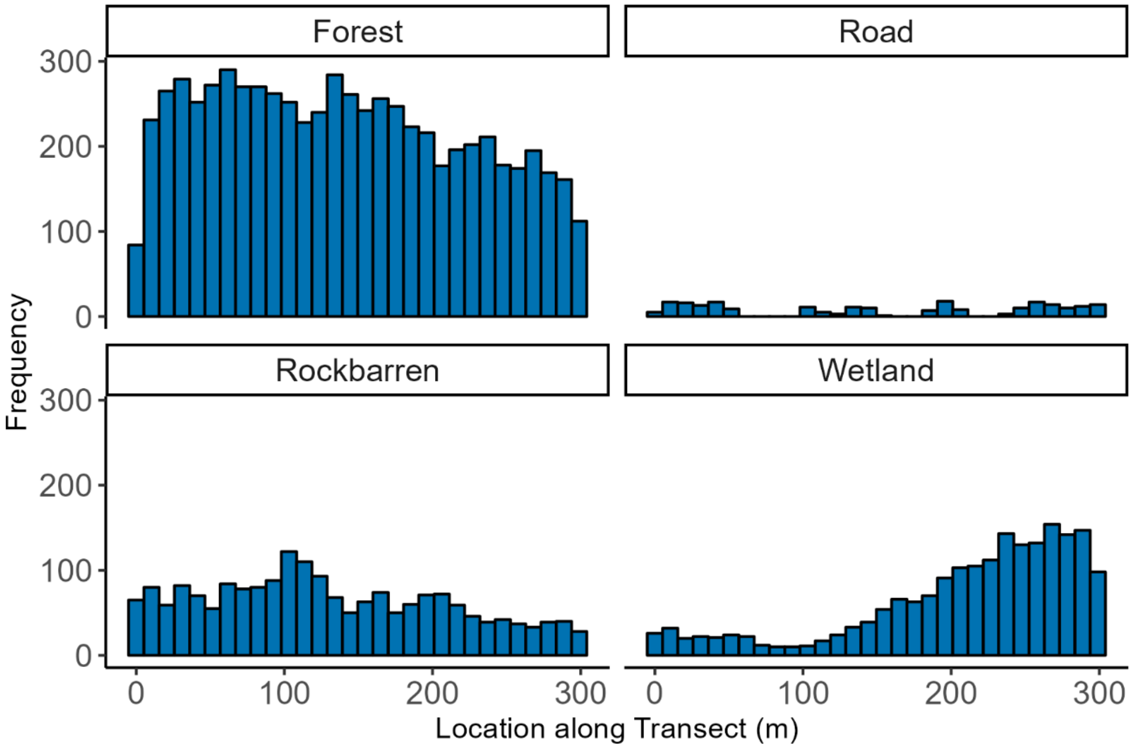

Figure 2.2: Frequency of the total number of survey plots (n = 10800) characterized as forest (6699/10800), road (231/10800), rock barren (1736/10800), or wetland (2134/10800) distributed along a 300-m transect running perpendicular from the edge of a wetland. All transect surveys were conducted in a 231-ha study area surrounding six wetland crossings on the study road (Figure 2.1) that were known nesting areas for female turtles.

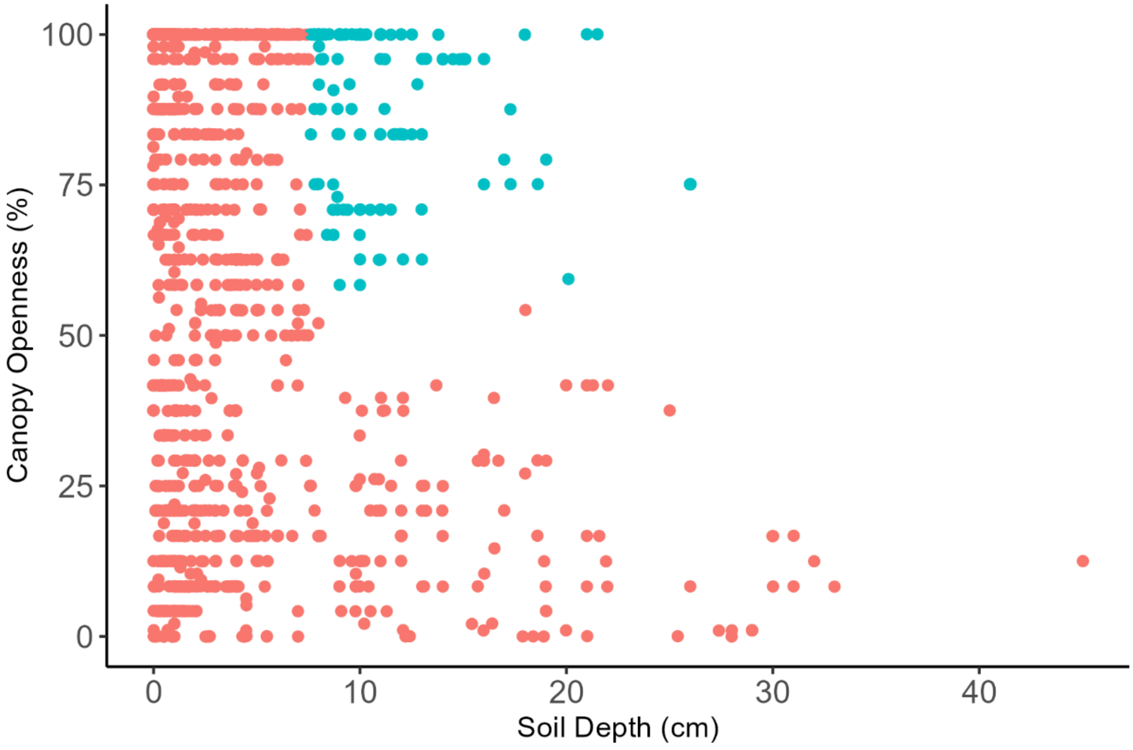

Figure 2.3: Canopy openness (%) and soil depth (cm) of plots described as depression areas (soil deposits) on rock barrens. Data were collected from habitat transect surveys in a 231-ha area surrounding the study road to characterize suitable nesting habitat for the local turtle community. All depression plots provided habitat potentially available for nesting (pink points); however, only 10% (blue points) were suitable for nesting. Suitability was determined based on the number of plots that met generalized nesting requirements for Blanding's Turtles (*Emydoidea blandingii*), Painted Turtles (*Chrysemys picta*) and Snapping Turtles (*Chelydra serpentina*) with a minimum soil depth of ≥ 7.5 cm and canopy openness of ≥ 55 % (Appendix 2.1).

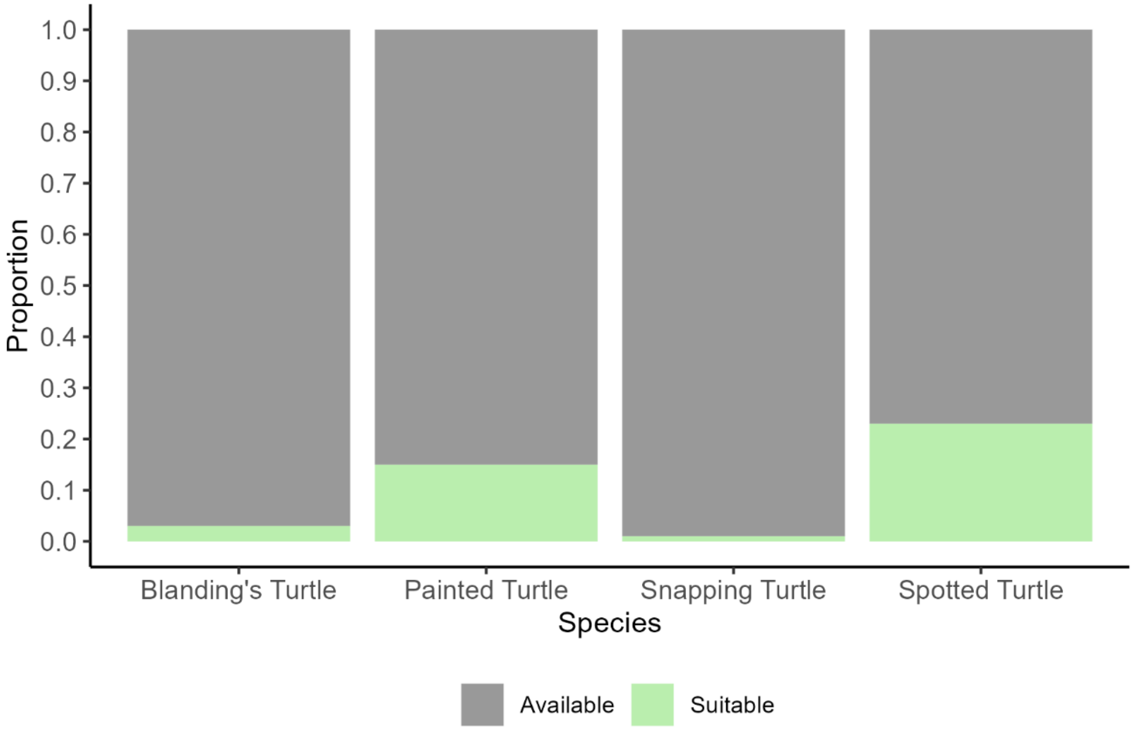

Figure 2.4: The proportion of suitable nesting habitat (green) compared to that which was available (grey) based on minimum soil depth and canopy openness requirements for four turtle species. The minimum required canopy openness for all species was ≥ 55 %, with varying minimum soil depth requirements of ≥ 4 cm for Spotted Turtles (*Clemmys guttata*), ≥ 6 cm for Painted Turtles (*Chrysemys picta*), ≥ 12 cm for Blanding's Turtles (*Emydoidea blandingii*) and, ≥ 16.5 cm for Snapping Turtles (*Chelydra serpentina*) (Appendix 2.1).

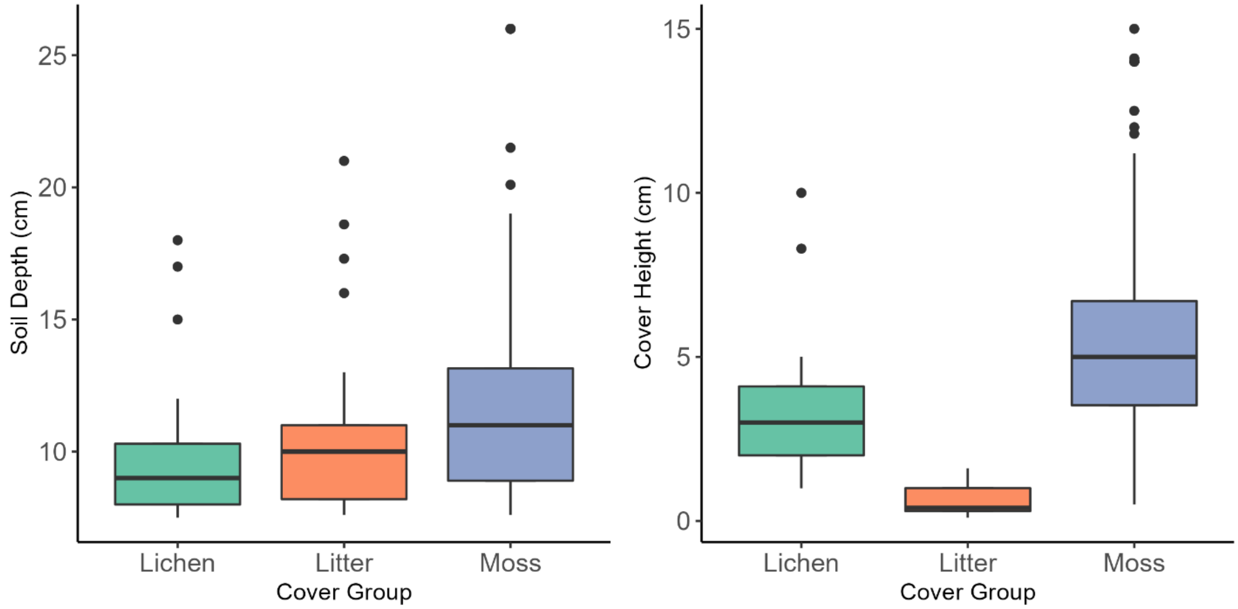

Figure 2.5: Differences in soil depth (cm) and cover height (cm) among vegetation cover groups (lichen, leaf litter, and moss) in suitable depression plots (n = 144 plots) on rock barrens quantified from habitat transect surveys. The boxes are the interquartile range (IQR), the dark horizontal line is the median, the whiskers represent the range of data from minimum to maximum values, and the black dots show outliers. A Kruskal-Wallis's rank sum tests ($\alpha = 0.05$) and post hoc analysis identified that moss had significantly deeper soil than lichen ($z = -2.3$, p adjusted = 0.05) and that moss, lichen, and litter all significantly differed in their cover height (Appendix 2.2).

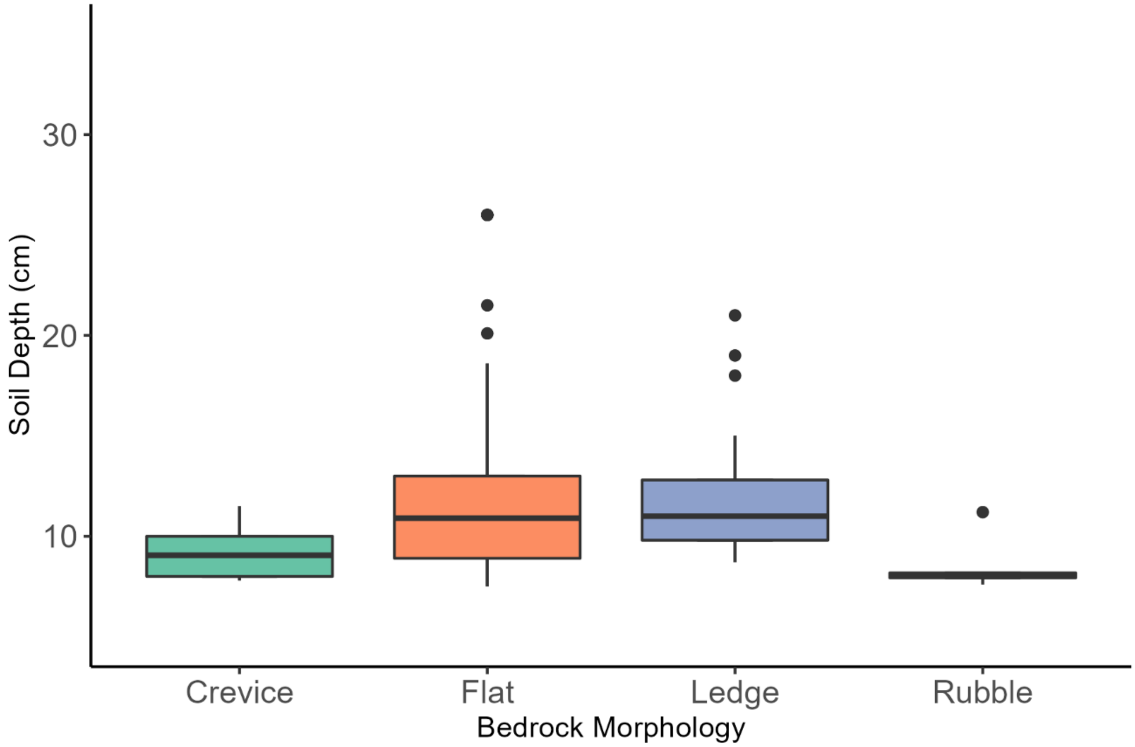

Figure 2.6: Differences in soil depth (cm) and among bedrock morphologies (crevice, flat, ledge, and rubble) in suitable depression plots (n = 144 plots) on rock barrens quantified from the habitat transect surveys. The boxes are the interquartile range (IQR), the dark horizontal line is the median, the whiskers represent the range of data from minimum to maximum values, and the black dots show outliers. A Kruskal-Wallis's rank sum tests ($\alpha = 0.05$) and post hoc analysis identified that soil was significantly deeper in flat areas compared to crevices ($z = -2.6$, $p_{\text{adjusted}} = 0.05$) and rubble ($z = 2.7$, $p_{\text{adjusted}} = < 0.04$) bedrock morphologies. Similarly, soil depth was significantly deeper in areas with ledges compared to crevices ($z = -2.6$, $p_{\text{adjusted}} = 0.05$) and rubble ($z = 2.9$, $p_{\text{adjusted}} = < 0.04$) bedrock morphologies.

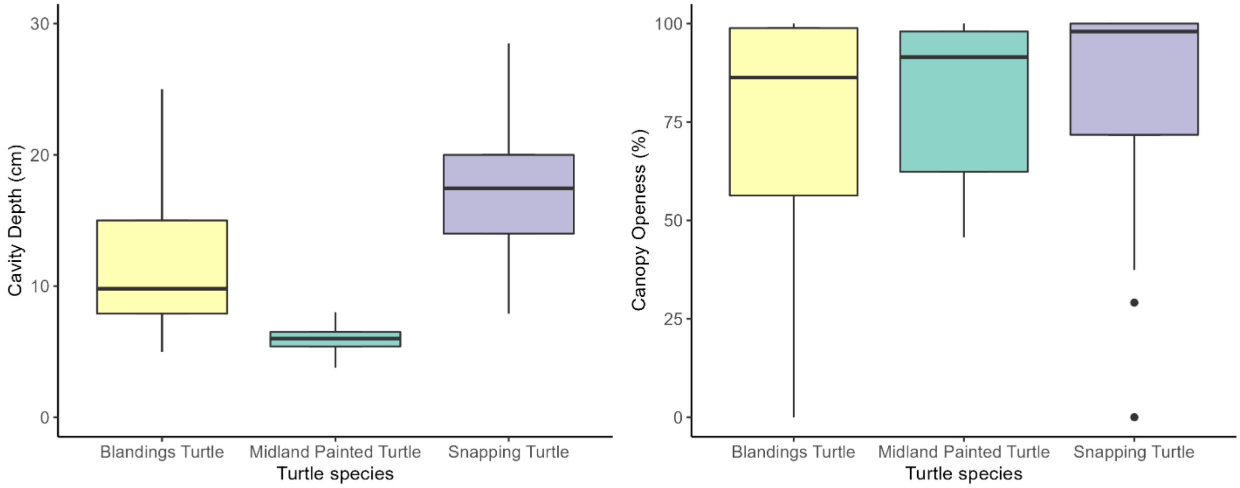

Figure 2.7: Cavity depth (cm) (left panel) and canopy openness (%) (right panel) collected at nests of Blanding's Turtles (*Emydoidea blandingii*), Painted Turtles (*Chrysemys picta*) and Snapping Turtles (*Chelydra serpentina*) laid on the study road in June of 2022. The boxes are the interquartile range (IQR), the dark horizontal line is the median, the whiskers represent the range of data from minimum to maximum values, and the black dots show outliers.

Table 2.1: Descriptions of habitat types characterized during transect surveys, including descriptions of additional habitat data collected on rock barrens. Habitat groups and features on rock barrens that contained depression plots that were considered potentially suitable for nesting are identified (*).

Habitat Classification	**Habitat Characteristics**		**Description**	**Depression plots**
	Group	**Feature**		
Rock Barren	Cover Type	Moss	Moss present on the surface of soil	*
		Lichen	lichen present on the surface of soil	*
		Litter	Area with pine or leaf litter covering soil	*
		Other	Herbaceous vegetation or exposed soil	
		Bedrock	Exposed bedrock with no vegetation, soil, or loose rock on the surface	
		Thicket	Area with dense vegetation, typically Juniper or blueberry	
	Bedrock Morphology	Flat	Smooth surface area	*
		Crevice	Opening in rock with rock walls on either side, typically with soil accumulation in opening	*
		Ledge	Area with one vertical rock wall, typically with soil accumulation at base	*
		Rubble	Area with rock fragments either covered with growth (moss or lichen), bare, and / or soil accumulation between rocks	*
Forest	Forest Type	Mixed Forest	Area dominated with hard and soft wood trees consisting of high canopy cover	
		Deciduous Forest	Area dominated with hard wood trees, typically consisting of sugar maple (*Acer saccharum*), red maple (*Acer rubrum*) and black ash (*Fraxinus nigra*)	
		Coniferous Forest	Area dominated with soft wood trees, typically consisting of jack pine (*Pinus banksiana*) and white pine (*Pinus strobus*)	
Wetland	Wetland Type	Marsh	Permanently flooded with herbaceous vegetation (shallow and open water marshes)	
		Meadow Marsh	Seasonal water fluctuations with herbaceous vegetation	

		Ephemeral Wetlands	Temporary wet patches in low lying areas near rock barrens typically dominated with *Sphagnum* moss
		Swamp	Permanently flooded coniferous forest
		Open Water	Area with water free of vegetation
Road	Road Section	Ditch	Transition area from forest or wetland habitat and road
		Road shoulder	Surface of road adjacent to traffic lanes with exposed gravel, clear of vegetation, typically with open canopy cover
		Road surface	Paved traffic lanes

Appendix 2.1: Summary of nest depths and canopy openness requirements documented in the literature for Blanding's Turtles (*Emydoidea blandingii*), Painted Turtles (*Chrysemys picta*) and Snapping Turtles (*Chelydra serpentina*) from natural and anthropogenic habitats. Minimum nesting requirements were generalized among species based on these literature values to a soil depth of ≥ 7.5 cm and canopy openness of ≥ 55% to assess suitability of nesting habitat on rock barrens along eastern Georgian Bay.

Species	**Nesting requirements**	**Location**	**Study**
Soil Depth			
Painted Turtle	10.1 ±SD 1.2 cm	Algonquin Provincial Park, Ontario	Schwarzkopf and Brooks 1987
	6 – 13 cm	Illinois and New Mexico	Morjan 2003
	8 – 10.5 cm	Parry Sound Eco District, eastern Georgian Bay	Markle et al. 2021
Blanding's Turtle	12 cm	Kejimkujik National Park, Nova Scotia	Standing et al. 1999
	10 - 13.5 cm	Parry Sound Eco District, eastern Georgian Bay	Markle et al. 2021
Snapping Turtle	12 -18 cm	Livingston County, Michigan	Congdon et al. 1987
	14 – 21.25 to middle of nest	Algonquin Provincial Park, Ontario	Francis et al. 2019
Spotted Turtle	2 – 6 cm	Lake Huron, Ontario	Rasmussen and Litzgus, 2010
	7.5 cm	Parry Sound Eco District, eastern Georgian Bay	Markle et al. 2021
Canopy Openness			
Painted Turtle	83 %	Algonquin Provincial Park, Ontario	Hughes and Brooks, 2006
	46 – 100 %	Algonquin Provincial Park, Ontario	Riley et al. 2014
	70 ± 2% (56% - 81 %)	Parry Sound Eco District, eastern Georgian Bay	Markle et al. 2021
Blanding's Turtle	70 ± 2% (56% - 81 %)	Parry Sound Eco District, eastern Georgian Bay	Markle et al. 2021
Snapping Turtle	63 – 100 %	Algonquin Provincial Park, Ontario	Riley et al. 2014
Spotted Turtle	70 ± 2% (56% - 81 %)	Parry Sound Eco District, eastern Georgian Bay	Markle et al. 2021

Appendix 2.2: Results of Dunn post-hoc tests using a Bonferroni p-value adjustment following Kruskal Wallis Rank sum tests comparing differences in soil depth among vegetation cover groups and bedrock morphologies and differences in cover height among cover groups. Asterisks (*) indicate the level of significance.

Difference among groups	**Comparison**	**Z**	**Adjusted P value**
Soil depth ~ cover group	Lichen – Litter	-1	0.87
	Lichen – Moss	-2.3	0.05 *
	Litter – Moss	-1.4	0.47
Cover height ~ cover group	Lichen – Litter	4.38	< 0.001 ***
	Lichen – Moss	-2.4	< 0.001 ***
	Litter – Moss	-9.2	< 0.001 ***
Soil depth ~ bedrock morphology	Crevice – Flat	-2.6	0.05 *
	Crevice – Ledge	-2.7	0.04*
	Flat – Ledge	-0.9	1.0
	Crevice – Rubble	0.9	1.0
	Flat – Rubble	2.7	0.04 *
	Ledge – Rubble	2.9	0.02*

Significance codes: 0 *** 0.001 ** 0.01 * 0.05

Appendix 2.3: Number of depression plots that met suitable soil depth and canopy openness requirements (Table Appendix 2.1; total = 144) categorized by habitat characteristics of directional aspect, bedrock morphology and slope.

Habitat Characteristic		Number of plots
Directional Aspect	E	10
	N	16
	NE	43
	NW	14
	S	3
	SE	31
	SW	9
	W	14
Bedrock Morphology	Flat	103
	Crevice	18
	Ledge	17
	Rubble	6
Slope	0-5°	7
	6-10°	84
	11-15°	14
	16-20°	18
	21-25°	10
	26-30°	2
	31-35°	6
	36-40°	1

General Conclusion

The importance of road effect mitigation has become widely recognized as necessary to protect turtle populations from further declines (Lesbarrères and Fahrig 2012, van der Grift et al. 2013); however, during the road development process, turtles, and the habitats on which they rely for survival are often overlooked. Although mortality mitigation strategies have been explored (Aresco 2005, Baxter-Gilbert et al. 2015, Markle et al. 2017, Boyle et al. 2021), challenges for conservation managers and turtles remain. My project provided a unique opportunity in the priority area of eastern Georgian Bay to evaluate a cost-effective and low-maintenance road-effect mitigation strategy developed through a partnership among multiple stakeholders during the road planning process. The overarching goal of my thesis was to evaluate if rip-rap and paved road shoulders (tar-and-chip pavement) could be effectively applied as a mitigation strategy to deter females from nesting in risky roadside habitats. The success of the mitigation strategy depended on (1) females being deterred from nesting in mitigated road sections and (2) being redirected to nest in the natural landscape. The chapters of my thesis evaluate these two critical components to determine the success of this mitigation strategy in a rock barren landscape.

First, I conducted a mark-recapture study in which I identified a high proportion of Species at Risk (SAR) turtles nesting on the road, highlighting the potential risk of road mortality to the vulnerable turtle community and the need for effective mitigation. However, I found that the rip-rap and paved road shoulder mitigation strategy did not effectively deter females from nesting on the road. Although females were prevented from nesting in the rip-rap applied on wetland embankments, turtles continued to find nesting habitat on the road, including areas paved with non-compact tar-and-chip pavement. In addition, I detected individual females making large inter-nest movements and nesting in multiple locations on the road between study periods (During mitigation construction and After mitigation was in place). Therefore, I suggested that females will likely continue to find nesting habitat on the road if suitable habitat remains in Mitigated or Unmitigated road sections because of their nesting behaviour and migrations. The persistent behaviour of turtles to bypass mitigation is a common challenge for implementing successful road-effect mitigation strategies (Baxter-Gilbert et al. 2015, Markle et al. 2017), especially if the integrity of mitigation is compromised (i.e., non-compact tar-and-chip pavement in my study). Overall, I concluded that rip-rap and paved road shoulders should not be applied as a road-effect mitigation strategy without further research. Similar to other mitigation designs in their infancy stage, my study highlights the importance of empirically testing mitigation strategies to adaptively manage them based on the outcomes.

In addition, in the During period of my study, I documented females nesting in freshly exposed gravel in active road lanes because road resurfacing activities coincided with nesting season. Thus, I emphasize the overall threat of construction activities to turtles during nesting season and the importance of conducting road work activities outside of critical life cycle time periods for turtles. If the goal is to conserve turtles, then it is imperative that local turtle communities are considered and prioritized during road planning, construction, and maintenance activities. Considering the effects of road activities early in the planning process may not only reduce the cost associated with retrofitting mitigation, but more importantly, can avoid harm to vulnerable turtle populations.

Second, I found that suitable nesting habitat was limited across the natural rock barren landscape, which may encourage females to continue to use road shoulders for nesting. Therefore, despite applying a nesting deterrent mitigation strategy, females may continue to seek roadside nesting habitats because of microhabitat preferences or out of necessity from limited natural habitats; however, further research should be conducted to identify why females select roadside habitats. Recovery actions, such as enhancing nesting habitat on rock barrens, may be required to encourage females use of the natural landscape. My findings emphasize the importance of understanding site-specific landscape characteristics and species-specific habitat use when developing road-effect mitigation strategies for turtle communities. Overall, I found that mitigation to deter females from nesting in roadside habitats may not successfully reduce road threats for turtle communities in a landscape with limited natural nesting habitat.

Turtles are struggling to survive in a world that humans are rapidly changing (Gibbons et al. 2000, Stanford et al. 2020, Cox et al. 2022). Therefore, it is crucial that we adapt our ways as we learn how to responsibly share the land with turtle species. To reduce road threats for vulnerable turtle populations, it is important that turtles' movements and behaviour are incorporated into the road planning process, and that we take a collaborative approach among multiple stakeholders to develop, apply, and empirically test site- and species-specific mitigation designs.

Considerations and Recommendations

My findings can help inform Best Management Practices for turtle communities during road development projects. My project highlights several important successes that can be incorporated into the road development process to reduce road threats for vulnerable turtle populations. In addition, I have identified several important areas requiring further research and consideration to develop our understanding of road effects and mitigation for turtle communities.

Collaboration: The work described in my thesis demonstrated the importance of collaboration among First Nations, non-for-profits, municipalities, and construction companies for the protection of SAR. Through collaborative efforts during all stages of project planning, execution, monitoring, and evaluation, SAR were at the forefront of management decisions. In addition to the application of mitigation, as part of the partnership with the Township and road construction companies, a qualified biologist from the Georgian Bay Mnidoo Gamii Biosphere (GBB) or Shawanaga First Nation (SFN) was on site while road construction occurred to ensure the safety of any SAR encountered. During nesting surveys in 2020 and 2021, GBB collected and incubated all turtle eggs laid on the road to prevent compaction or mortality from construction. Although the incubation efforts were not evaluated as part of the mitigation strategy or my thesis, studies have shown that increasing recruitment can help to offset losses to turtle populations from road mortality when combined with strategies targeting protection of juveniles and adults (Bougie et al. 2022, Keevil et al. 2022).

Further Evaluation: Rigorous evaluation of mitigation strategies should include several years of data collection prior to and following mitigation implementation (Lesbarrères and Fahrig 2012). A limitation with my study is that my evaluation of the rip-rap mitigation strategy is only based on three years of data, including a Before and After period, and was confounded with a During period. Therefore, I recommend that nesting activities continue to be monitored and recorded for several years to observe the number and location of females nesting on the road.

Turtle Community Ecology: Although the population demographic data I collected as part of a 2-year mark-recapture study provided insight into the local abundance and structure of the turtle community; I did not have sufficient data (minimum three-years) to estimate population sizes using open-population models. There are few studies that have evaluated mitigation strategies for turtles on a population level (e.g., Boyle et al., 2021), even though such studies are important for determining if mitigation supports populations in a biologically meaningful way (Lesbarrères and Fahrig 2012, van der Grift et al. 2013). Hopefully my mark-recapture data can serve as a baseline to allow for future monitoring to evaluate changes in the turtle community.

Rip-rap and Paved Road Shoulders: Overall, hardening the road shoulders using a method other than tar-and-chip may be more successful in prohibiting turtles from nesting on road shoulders. However, increasing paved surface area may also have consequences for other species, such as SAR snakes seeking thermoregulating habitat, increasing their exposure to predators and road mortality (Mccardle and Fontenot 2016). In addition, paved road shoulders may not reduce the threat of roads to female turtles on nesting migrations because paved roads are not barriers to movement (Refsnider and Linck 2012). Although rip-rap prevented turtles from nesting, it may not be a practical (or cost-effective) application on all sloped road edges. Moreover, there are other effects to aquatic (changes to geomorphic processes, fish and macroinvertebrates; Reid and Church 2015) and terrestrial (entrapment of hatchlings or smaller-bodied turtles) animal communities that should be considered prior to the widespread application of rip-rap. Backfilling the rip-rap with smaller aggregate to fill gaps and crevices could serve as a short-term solution (i.e., smaller aggregate may wash out over time). Future research should continue to evaluate effects of common construction practices and materials on SAR turtle communities. While recognizing that road development decisions need to consider economic, social and human safety factors, it is important to carefully consider the effects of introducing new materials into an environment on SAR and their habitats.

Nesting Habitat Restoration: Currently, because the mitigation strategy did not deter some females from nesting on the road, the mitigation strategy is unlikely to support recruitment into the turtle population. Although female turtles can be one of the more vulnerable demographics to road mortality, mitigation strategies need to consider protecting multiple life stages for population persistence (Bougie et al. 2022, Keevil et al. 2022). Replacing the nesting habitat removed from the road with safer alternative sites away from the road may aid in reducing road threats to females. In the absence of predation, nests in artificial nesting mounds (ANM) can have greater hatchling success compared to natural nesting sites (Paterson et al. 2013). However, the success of ANM is contingent upon females encountering them (Paterson et al. 2013). In addition, nesting habitat created adjacent to the road without mitigation to prevent road access may not resolve all road threats. An alternative approach is to enhance nesting conditions on existing rock barrens away from roads, to encourage turtles to nest in the natural landscape (Markle et al. 2021).

Low-cost Strategies: In general, the most effective mitigation strategies for turtles are multifaceted and include the protection of turtles during multiple life stages (Bougie et al. 2022, Keevil et al. 2022). For conservation projects on low-use roads that require low cost and maintenance strategies, efforts to encourage changes in human behaviour on the road may be beneficial in supporting turtle communities if they are coupled with mitigation strategies that target human behaviour. Strategies may include increasing public awareness, “brake for turtles” signs, reduced temporary speed limits during peak movement times, or community science initiatives to protect nests laid on the road. Ultimately, engaging residents and raising community awareness on the importance of sharing the land with turtles may instil a long-term responsibility to protect turtles from road mortality. However, it should be noted that the success of methods is dependent on human behaviour and may not significantly reduce road threats (Seburn and

McCurdy-Adams 2020). Although these actions will not eliminate the threat of roads, they could assist in protecting turtles that continue to interact with roads.

Timing of Road Work Activities: My study documented females using traffic lanes as nesting habitat when road resurfacing activities provided exposed gravel, which shows the importance of conducting road work outside of critical life cycle time periods for turtles. Observations made during my study also emphasize the importance partnerships between construction companies and qualified biologists to ensure SAR are protected at times of increased risk, if road construction activities must occur during critical time periods.

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