A Road to Conservation: Understanding the Dynamics of Road-Effects and Road-Effect Mitigation

by

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Thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Boreal Ecology

Faculty of Graduate Studies

Laurentian University

Sudbury, Ontario, Canada

THESIS DEFENCE COMMITTEE/COMITÉ DE SOUTENANCE DE THÈSE
Laurentian Université/Université Laurentienne
Faculty of Graduate Studies/Faculté des études supérieures

Title of Thesis Titre de la thèse	A Road to Conservation: Understanding the Dynamics of Road-Effects and Road-Effect Mitigation
Name of Candidate Nom du candidat	Boyle, Sean
Degree Diplôme	Doctor of Philosophy
Department/Program Département/Programme	Boreal Ecology
Date of Defence Date de la soutenance	May 30, 2019

APPROVED/APPROUVÉ

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Abstract

Globally, roads are one of the most ubiquitous forms of human infrastructure, and have been identified as a serious conservation concern. Though roads impact wildlife in a variety of ways, their effects are often negative. Perhaps the most concerning threats are habitat fragmentation and mortality via wildlife-vehicle collisions. Attempts to manage the negative effects of roads have had mixed results, and major gaps in our understanding of how roads affect wildlife populations and the effectiveness of strategies to mitigate road-effects remain. Many factors contribute to these gaps, particularly the logistical constraints associated with road-effect monitoring and weak study designs that inhibit strong inferences crucial for effective management. To this end, I approached road-effect mitigation in a holistic way. First, I documented and analyzed the local-scale population and spatial ecologies of large mammals around a newly twinned highway. I found that highway twinning, a common strategy to accommodate increased traffic volume, had little effect on large mammals. Second, I focused on the optimization and evaluation of road-effect mitigation (i.e., exclusion fencing and road-crossing structures). I developed a procedure for identifying ideal locations for mitigation features by comparing road monitoring data to landscape resistance models for both large mammals and herpetofauna. Using this approach, I designed a mitigation plan for reptiles and amphibians, which I evaluated using a robust 6-year Before-After-Control-Impact design that included road surveys, trapping, and two methods of monitoring tunnel usage: trail cameras and PIT tag scanners. I found that exclusion fencing was effective for turtles and amphibians but had no impact on the number of snakes detected on the road. Crossing tunnels were well used by reptiles and amphibians and I demonstrated that for turtles, tunnels effectively facilitated connectivity at the population-level. Finally, I investigated the value of outreach as a long-term

conservation strategy in the context of road ecology. I demonstrated that outreach programs significantly increase the perceptions of youth concerning their own likelihood to participate in conservation. Further, using a mixed-methods approach, I identified the aspects of outreach that were most effective at eliciting this change, creating broadly applicable guidelines to optimize future outreach endeavors. By addressing knowledge gaps pertaining to each phase of road-effect mitigation, I have provided a structural framework from which the field of road ecology can continue to flourish. My findings have serious implications for wildlife management and conservation because they increase our understanding of road-effects and importantly, how to increase the success of road-effect mitigation.

Acknowledgements

I must first express my thanks to my two amazing supervisors, Dr. David Lesbarrères and Dr. Jacqueline Litzgus. When I first began at Laurentian, I had no idea what the experience would become. Shortly after beginning, you took a chance on me by recommending that I transfer to the PhD program, thus beginning the journey I have been on over the last six years. Your encouragement and mentorship has played an integral role in who I am today, and has shaped my approach to science, academia, and life in general. Thank you for the long hours you put in, many questions you answered and friendship and support you have given me. Thank you also to my fantastic committee members, Dr. Josef Hamr and Dr. Jeff Bowman for your insight and guidance throughout my PhD.

I would also like to thank the crew of people that have helped me collect the data for my dissertation. Vincent Billy, Kaitlyn Read, Dave McGeachy, Wes Kowbasniuk, Bill Carrigan and Kari Gunson – collecting snow-track data is both physically and logistically challenging. I owe you all a huge debt of gratitude for helping me collect as much data as I did, both before and during my stay at Laurentian. Thanks to Chad Cordes for helping me develop and run the *Circuitscape* models I use in my second chapter and thank you to Chantal Barriault and Michelle Reid for their assistance with the qualitative analyses in my fourth chapter. Cristen Watt, Natasha Noble, Mary Balsdon, Rachel Dillon, Cory Trowbridge and Brookelyn Jones – all of you deserve a huge round of applause for the time you spent slogging through the swamp, batting away mosquitoes, and biking in the pouring rain. All of you did a tremendous job. Thank you for keeping a smile on, having a positive attitude and working your butts off! In addition to everyone

I named above, thank you to everyone who came out for a day here and there volunteering your time – there were a lot of you!

To the lab mates and friends I have travelled along this journey with, you guys are great! I couldn't have asked for a more welcoming group of people, or a more excited and enthusiastic team to be a part of. I will long remember the hours learning with you all, bouncing ideas off of you and asking for help. I especially would like to thank James Baxter-Gilbert and Julia Riley, who played a huge role in my interest in road ecology, as well as Matt Keevil for the endless stats and GIS questions I bombarded him with. I won't soon forget the good times had at the Herp House/Ecological Estates.

Corina Brdar, Don Tyerman, David Bree and Rob Cunningham – you four were incredibly helpful and patient throughout the entire process. I am thrilled that we were able to work together and create something truly important in Presqu'ile that will last for years to come, protecting the animals that call Presqu'ile home. Thanks also to the Friends of Presqu'ile Provincial Park. You welcomed me in as one of your own, and supported my research both financially and logistically. Steve Sinclair deserves special mention. Without your efforts, the mitigation in Presqu'ile could not have happened! Thanks also to Brent Barnes for keeping our PIT-tag scanner batteries fresh when I had to go back to Sudbury.

To Mom, Dad and Jeannie, thank you for helping me become the person I am today. I am truly lucky to have had the relentless love, support, encouragement, and level-headedness you have provided me over the last 29 years! To all of my friends and family, thank you for your patience with me missing events (because who knows, it might snow in the next 48 hours, or ya know, I only have 3 hours off between surveys), talking endlessly about turtles, and living so far away. I have appreciated all of your love and support.

Finally, to the person who has pushed me further than I thought I could go, has been the most patient with my sometimes ridiculous schedule, and who has unwaveringly stood beside me through all of it. Jenn, I honestly can’t express the gratitude I have for everything you have done to help me through this process. From sorting over a million pictures, helping as a part-time field tech, and as my long-suffering editor, to being the best friend a guy could ever have. I couldn’t have done this without your support, guidance, insight and love. I can’t wait for the next chapter in our adventure together!

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General Introduction

The Road so Far

In 1925, Dayton Stoner documented, for the first time in published literature, the 'Toll of the Automobile' (Stoner 1925). Following Stoner's seminal research, the ecological implications of roads and automobiles remained largely ignored by the scientific community for decades. In 1981, Ellenberg et al., coining the term 'Road Ecology' (Sraßen-Ökologie, in the original German), highlighted and gave name to a field of growing interest within the scientific community concerning the impacts of roads on wildlife. Investigations to understand these impacts became substantially more common as the new millennium approached, and mounting evidence pointed to roadways as a major disturbance to wildlife requiring targeted efforts to mitigate their effects (Yanes et al. 1995; Forman & Alexander 1998; Trombulak & Frissell 2000). In 2000, Forman and Deblinger documented the 'road-effect zone', highlighting that the impact of roads on wildlife was far greater than simply a point source of mortality. They described how roads had the potential to modify behavioural and population ecology, and strikingly, that approximately 25% or more of the continental United States was already severely affected in this way.

Road-Effects and Road-Effect Mitigation

Since those critical papers were published, the interest in understanding and managing road-effects has increased substantially. Although road ecology is still in its infancy compared to other branches of ecology, the impacts of roads are broad in scope and magnitude (Trombulak & Frissell 2000; Seiler 2001; Coffin 2007) and roads are recognized as a major threat to

biodiversity globally (Laurance et al. 2014). Roads have been shown to affect mammals (Rytwinski & Fahrig 2011; Sawaya et al. 2014), birds (Kociolek et al. 2011; Brown & Bomberger Brown 2013; Morelli et al. 2014), reptiles (Steen et al. 2006; Andrews et al. 2015; Baxter-Gilbert et al. 2015a), amphibians (Gibbs & Shriver 2005; Mazerolle et al. 2005), pollinating (Suárez-Esteban et al. 2014; Baxter-Gilbert et al. 2015b) and non-pollinating insects (Blaho et al. 2014; Muñoz et al. 2014), plants (Tikka 2001; Brisson et al. 2010), and microorganisms (Pichette 2016). The broad range of taxa affected, combined with the expansiveness of the global road network, make the consideration of roads critical to conservation.

Although roads impact many species, the nature of responses to them can vary as a result of species-specific variation in life history traits (Rytwinski & Fahrig 2012). For example, plant species rapidly propagate along road verges (Brisson et al. 2010), canids use roads and other linear corridors as movement pathways (Whittington et al. 2005; Zimmermann et al. 2014), and scavengers feed on road-killed animals (Coleman & Fraser 1989; Beckmann & Shine 2014; Ratton et al. 2014). Despite several species benefitting from roads, these benefits can be one-sided as they often have indirect negative consequences for species with which the benefactors interact (i.e., competitors, predators/prey, etc.). Indeed, most impacts on wildlife are negative (Fahrig & Rytwinski 2009; Rytwinski & Fahrig 2012, 2013), including landscape modification and fragmentation (Vos & Chardon 1998; Poessel et al. 2014), constraint of genetic connectivity (Lesbarrères et al. 2006; Balkenhol & Waits 2009; Holderegger & Di Giulio 2010), mortality through wildlife-vehicle collisions (Aresco 2005; Huijser et al. 2008; Baxter-Gilbert et al. 2015a, 2015b), physiological (Karraker et al. 2008; Kight & Swaddle 2011), reproductive (Karraker & Gibbs 2011; Laporte et al. 2013) and behavioural modifications (Mazerolle et al. 2005;

Lengagne 2008; Prokopenko et al. 2016), increased stress (Hopkins et al. 2013; Owen et al. 2014; Dananay et al. 2015), and in some cases, these impacts are strong enough to elicit microevolution (Brown & Bomberger Brown 2013).

A wide variety of strategies to mitigate the negative effects of roads have been applied (Glista et al. 2009; Rytwinski & Fahrig 2015). Some of these approaches aim to modify human behaviour (e.g., outreach, road closures, reduced speed limits, signage, warning systems - Sullivan et al. 2004; Farmer & Brooks 2012; Crawford & Andrews 2016; Linton et al. 2018; Whittington et al. 2019), while others aim to modify animal behaviour (e.g., habitat modification, reflector systems, exclusion fencing and connectivity structures - Glista et al. 2009; Grosman et al. 2009; Brieger et al. 2017). Of these however, the most commonly-studied approach combines roadside fencing and road-crossing structures. These infrastructures work together to accomplish two main goals: (1) create a physical barrier to prevent animals from reaching the road's surface, and (2) reconnect portions of the landscape fragmented by the road.

Depending on the target species, landscape characteristics (e.g., drainage, soil type/depth), available funding, and road construction/improvement plans and schedules, mitigation designs vary greatly. Exclusion barrier/fencing material can consist of hardware cloth (Colley et al. 2017), drift fencing (Jackson & Tyning 1989), heavy geotextile fencing (Baxter-Gilbert et al. 2015a; Markle et al. 2017), HDPE piping (Heaven et al. 2019), concrete walls (Aresco 2005), chain-link fencing (Mccollister & van Manen 2010), as well as several prefabricated fences specifically targeting wildlife (i.e., ACO 2019; Animex 2019). Crossing structures can be built either under or over the road. Generally, underpasses consist of elliptical or box-culverts (Clevenger & Waltho 2000; D'Amico et al. 2015; Baxter-Gilbert et al. 2017; Markle et al. 2017), drainage culverts (Clevenger et al. 2001; Heaven et al. 2019), engineered

box culverts with steel grate tops (Colley et al. 2017), or prefabricated wildlife tunnels (i.e., ACO 2019), and these vary in size and the amount of light and moisture that can enter. Overpasses typically come in the form of span bridges (Clevenger & Waltho 2005; D'Amico et al. 2015) but other examples include rope bridges and glider poles (Soanes et al. 2013).

Although these mitigation structures have rapidly increased in number, few studies have rigorously evaluated their success in meaningful ways, with many studies using insufficient experimental designs or weak success criteria (as highlitghted by Lesbarrères and Fahrig 2012, van der Grift et al. 2013). Studies that have used appropriate designs have had mixed results, with some showing limited or even increased road mortality rates (Baxter-Gilbert et al. 2015a; Markle et al. 2017; but see: Gilhooly et al. 2019; Ottburg & van der Grift 2019). Similarly, the effects of roads on connectivity can vary (Holderegger & Di Giulio 2010), but functional population-level connectivity across mitigation structures has been evaluated for very few species (Sawaya et al. 2013, 2014; Ford et al. 2017; Heaven et al. 2019). With such a broad array of both road-effects and mitigation strategies, there is a clear need for rigorous evaluation of mitigation effectiveness, contextualized by population-level data.

The Road Less Traveled

Although our understanding of the ways in which roads impact wildlife and the approaches we use to mitigate said threats has increased greatly, many gaps in knowledge remain. For example, road ecology literature is taxonomically biased towards large mammals, leaving other taxa vulnerable to unknown consequences (Popp & Boyle 2017). This is especially concerning in situations involving species at risk, where the loss of only a few individuals can

have severe implications for population persistence. There is also limited research regarding the effects of new roads or roadway twinning (Taylor & Goldingay 2014; Ciocheti et al. 2017); popular strategies used to accommodate increasing traffic volumes as road networks expand. Understanding the implications of these changes, as well as the effects of new roads, will allow conservation biologists to properly manage these systems from their onset.

In addition to a thorough understanding of road-effects, proper planning is critical to maximize mitigation effectiveness (van der Grift et al. 2013). This includes having a strong understanding of the impact of roads on specific target populations, using robust strategies to plan the location of mitigation structures (Baxter-Gilbert et al. 2017; Boyle et al. 2017), beginning projects early enough to have appropriate baseline data to determine mitigation effectiveness (Lesbarrères & Fahrig 2012; van der Grift et al. 2013), determining mitigation success criteria (van der Grift et al. 2013), and having a plan for continued adaptive management following evaluation.

Finally, the success of conservation initiatives is intrinsically linked to societal support (Mascia et al. 2003; McNutt 2013), and the effective mitigation of road-effects relies on collaboration between multiple partners (i.e., NGOs, private organizations, government agencies, and research institutions). Communication and outreach not only foster these partnerships, but also garner public support for conservation. There is little doubt that education is vital to the success of conservation initiatives (Pagdee et al. 2006; McNutt 2013; Scyphers et al. 2015), but little is known about how to augment engagement or maximize outreach effectiveness (Bennett et al. 2017). This is especially true in the context of road ecology, despite the common use of outreach by road ecologists (Jensen & Buckley 2014).

Objectives

The specific objectives of my dissertation were determined by identifying key aspects of road-effect mitigation, from its planning phases, to its long-term success. These objectives and the corresponding questions I investigated will be organized in four chapters as follows:

1) *Identify and quantify the problem*. While many road effects have been previously quantified (Forman & Alexander 1998), several questions remain. One example of this is the changing effects of shifting traffic volumes, new roads, and road twinning. Given the diverse array of effects associated with roads, the creation of new roads is typically detrimental to wildlife. Highway widening/expansion is a common approach used to accommodate increased traffic volume; however, the ecological implications of expansion are mixed. While increased mortality rates have been observed (Ciocheti et al. 2017), some suggest that the expansion of existing roads is a more ecologically sustainable option compared to building entirely new roads because incorporating and resurfacing large portions of old roads reduces the overall footprint (Rhodes et al. 2014). South of Sudbury, Ontario, the main thoroughfare connecting northern and southern portions of the province, and by extension eastern and western portions of Canada, has undergone large-scale twinning over approximately 250 km of highway over the last 20 years. I questioned if the process of twinning had an effect on the population and spatial ecology of large mammals living in the vicinity of the highway. This question has serious implications both for understanding and managing populations near this highway, but also for developing a global road building strategy.

2) *Optimize solutions to maximize success*. Mitigation design and location are crucial considerations required for mitigation success. The spatial distribution and clumping of

road mortality (i.e., road mortality hotspots) can be readily used to estimate where road-effect mitigation should be placed (Gunson et al. 2012). However, these hotspots can vary spatially and temporally (Cureton & Deaton 2012; Crawford et al. 2013; Lima Santos et al. 2017). Further, relying solely on road mortality data may underestimate the importance of specific locations that were important for connectivity historically, but have experienced high mortality rates and thus appear to have fewer individuals than expected in the immediate area (Eberhardt et al. 2013; Teixeira et al. 2017). In an effort to clarify and optimize the process of identifying ideal locations for mitigation, I compared the size and location of road mortality hotspots to those generated by landscape resistance models.

3) *Evaluate solutions*. One of the biggest questions currently facing road ecologists is the effectiveness of efforts to mitigate road-related threats to wildlife. Mixed-results, logistical constraints, study designs with low inferential strength, and limited population-level context have confounded our understanding of the actual conservation value of such initiatives (Lesbarrères & Fahrig 2012; van der Grift et al. 2013). To address this concern, I designed a six-year herpetofauna monitoring project, spanning several years both before and after mitigation features were implemented, in Presqu'ile Provincial Park, Ontario. Combining this design with trapping efforts to contextualize road mortality trends and crossing structure usage rates with population size estimates, allowed me to robustly evaluate the success of both connectivity structures and exclusion fencing.

4) *Alternative approaches to solving the problem of road-effects*. While infrastructure designed to mitigate road-effects holds great promise, the economic cost of this approach causes hesitation among both conservation practitioners and the public, despite evidence

suggesting that reducing the impact of roads on wildlife would actually be beneficial to the economy in the long run (Huijser et al. 2008). As such, education is equally important to conservation initiatives (McNutt 2013). Effective education engages the public to participate in conservation and/or citizen science efforts and stimulates interest in, and support for, conservation initiatives. Public engagement is crucial for political action and consequent policy changes, thus the optimization of outreach programs is vital to conservation initiatives. Using a mixed-methods approach allowed me to determine how youth perceived their own likelihood to engage in conservation, and identify specific ways in which outreach affects participants in order to optimize future efforts.

Significance

The overall goal of this dissertation is to investigate road-effect mitigation in a holistic way. This can be broken down into four parts: (1) identifying threats and determining the impact they have on wildlife populations, (2) identifying and optimizing mitigation planning in order to increase mitigation success, (3) incorporating study designs and analyses which facilitate strong and reliable inferences concerning the effectiveness of road-effect mitigation and 4) analyzing the value of conservation engagement in the context of road ecology and optimizing its ability to promote public participation in conservation action. By addressing gaps in our understanding of each phase in the process of mitigating road-effects, I have provided a structural framework from which the field of road ecology can continue to flourish. The following works have serious implications for wildlife management and conservation in that they increase our understanding of the effects of roads on wildlife, and importantly, how to increase the success of road-effect mitigation initiatives.

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Chapter 1

Highway widening has limited effects on large mammal population and spatial ecologies

Abstract

Roads are arguably one of the most severe and far reaching threats to wildlife globally because of their pervasive and linear nature, and this threat is expected to increase in the future as road networks expand. Despite considerable attention afforded to road-effects on animals generally, little information is available concerning the specific impacts of road widening. To address this knowledge gap, I monitored the abundance and distribution of large mammals adjacent to an 18 km section highway in Ontario, Canada that underwent expansion from a two-lane to a four-lane, divided highway, over four years. Specifically, I expected to observe an initial decrease in species abundance and increased distance from the highway, followed by a period of habituation, where abundance and proximity to the highway would return to baseline levels. To quantify the effects of highway expansion, I monitored snow tracks of three ungulates, two felids, and three canids on transects extending perpendicular and parallel to the highway. My analyses revealed that proximity was species-specific and varied seasonally, but I found little evidence that the new four-lane highway alignment had a negative effect on the population and spatial ecologies of the local large mammal community. Limited increases in traffic volume could partially explain why I detected no change; however, traffic volume will continue to rise. My findings have important management implications as road widening is a common infrastructure modification for accommodating increased traffic volume, yet no studies have explicitly investigated its effects on animal spatial ecology.

Introduction

Globally, biodiversity is declining at an unprecedented rate because of rapid human-induced environmental changes and the destruction of important habitats (Ceballos et al. 2017). One of the largest anthropogenic drivers of habitat alteration is the ever-increasing sprawl of road networks (Laurance et al. 2014). To wildlife, roads are both a source of mortality via wildlife-vehicle collisions and a source of habitat fragmentation, either because they create a physical barrier, or because animals actively avoid them (Trombulak & Frissell 2000; Seiler 2001; Jaeger & Fahrig 2004; Coffin 2007). These interactions can inhibit gene flow (Epps et al. 2005), alter spatial ecology (Poessel et al. 2014), modify hunting behaviours (Dickie et al. 2017) and result in a stressful environment for wildlife (Ditmer et al. 2018). Unfortunately, the extensive linear nature of roads means that even small zones of avoidance can degrade large amounts of habitat (Forman & Alexander 1998), and as such, roads require consideration for management and conservation of wildlife.

On a global scale, road networks are expected to continue expanding, nearly doubling in length by 2050 (Dulac 2013). While the field of road ecology has rapidly developed alongside growing road networks, several knowledge gaps remain. One such gap concerns the impacts of road expansion on local wildlife communities where roads already exist. A common strategy used to accommodate increasing traffic volume includes highway realignment and twinning, the process by which a new set of lanes is built alongside an existing highway, often incorporating portions of the original alignment in the twinned highway (Laurance et al. 2014). Several studies have evaluated mitigation effectiveness (i.e., crossing structures/exclusion fencing) in highway widening scenarios (Clevenger et al. 2001; Baxter-Gilbert et al. 2015; Ciocheti et al. 2017); however, little research has quantified the impact on wildlife of the road widening itself (Rhodes

et al. 2014; Taylor & Goldingay 2014; Ciocheti et al. 2017). Understanding the relationship between highway widening and animal ecology is important because the scope and magnitude of a widened highway's effects on wildlife may not be equivalent to those of the original alignment. As such, understanding the effect of a widened highway (i.e., an increased footprint, disturbance from construction, and eventually increased traffic volume) is imperative for sustainable management (Ciocheti et al. 2017). Combined with the fact that road avoidance behaviour is also typically species- and context-specific (Rytwinski & Fahrig 2013), it is clear that more research specifically addressing the effects of road widening is needed. Ultimately, an increased understanding of the spatiotemporal dynamics of populations adjacent to highway widening projects will support effective management decisions and help optimize road-effect mitigation.

I examined the effects of highway widening on the population and spatial ecologies of a large mammal community at a local-landscape scale, which I defined as the area within approximately 2 km of a new highway alignment in Central Ontario, Canada. To this end, I monitored large mammal activity both parallel to the highway and in adjacent habitats. I hypothesized that if opening a new highway alignment (i.e., fully constructed with active traffic) negatively impacted large mammals, then fewer large mammals would be detected in the surrounding habitat, and large mammals that were detected would be found farther away from the highway as compared to the period before the highway was open. Second, however I expected large mammals to habituate to the new highway over time, following an initial period of increased avoidance after the highway opened. Further, I predicted that if habituation occurred, large mammal abundance and proximity to the highway would return to pre-open highway levels during my 4-year study. Conversely, if large mammals are not affected by a new highway alignment opening, I expected to observe no changes in the number of large mammals

detected, nor their proximity to the highway throughout my study. Given that relatively little information is available concerning the impact of road expansion on wildlife, my findings will be important for informing future management plans.

Methods

Study Site

My study was conducted along Highway 69, approximately 25 km south of Sudbury, ON, Canada (46.2799 N, -80.7987 W; Figure 1.1). Highway 69 is a major thoroughfare connecting southern and northern portions of the province that, since 2005, has been undergoing widening from a two-lane highway to a twinned, four-lane highway with a median approximately 30 m in width (Ontario Ministry of Transportation 2005). During the twinning process, the original alignment was either converted to low traffic secondary roads within ~700 m of the new alignment, or was resurfaced and incorporated into the twinned alignment of the highway. This scenario presented me with the opportunity to investigate the impact of an increase in road size (four divided lanes versus two lanes) prior to substantial increases to traffic volume (Ontario Ministry of Transportation 2005). My study site is located in the Ontario shield ecozone (Crins et al. 2009), which in general has low road density and large contiguous matrices of mixed and evergreen forest and natural wetlands (e.g., lakes, muskeg, marsh, and fen). Although not the focus of this paper, it is noteworthy that the section of highway twinned during my study also included wildlife exclusion fencing and three large mammal crossing structures (Gunson 2017).

I surveyed for snow-tracks to quantify large mammal abundance and highway-proximity over the course of four winter field seasons (2011/12 – 2014/15; hereafter 2011 - 2014). The large mammal assemblage in the area consists of three ungulates (moose - *Alces alces*, white-

tailed deer - *Odecoileus virginianus* and a reintroduced population of elk - *Cervus canadensis*), two felids (Canada lynx – *Lynx canadensis* and bobcat – *Lynx rufus*), and three canids native to Ontario (coyote – *Canis latrans*, eastern fox – *Vulpes vulpes* and grey wolf – *Canis lupus*). There is documented genetic mixing and size overlap among coyote, eastern wolf (*Canis lupus lycaon*), and grey wolf in my study area (Benson et al. 2012). Therefore, to ensure consistency, I defined canid species in my study by footprint size (Halfpenny 1987), where tracks of fox are ~4 cm, coyote are ~6 cm and grey wolf are ~8 cm or greater in width.

Survey methodology

Driving surveys were conducted along north and southbound lanes of both new and old alignments of the highway. Approximately half of the new highway alignment was opened prior to the onset of my study, while the remaining portion of the new alignment opened in July 2012, between my first two winter field seasons (Figure 1.1). In 2011, the unfinished portion of the new alignment was inaccessible because it was an active construction site. I also monitored six transects extending on average ~1500 m (range: ~700 – 1800 m) perpendicular to the highway (Figure 1.1). Thus, in 2011, approximately 11 km of new active highway (100 km/h), 7 km of old active highway (90 km/h), and 9 km of adjacent habitat transects were surveyed. From 2012 – 2014, with the new alignment completed, approximately 18 km of new active highway alignment (100 km/h), 7 km of old highway converted to secondary roads (80 km/h), and 9 km of adjacent habitat transects were surveyed. Surveying both along the highway, and in adjacent habitat allowed us to estimate large mammal ecology at two distances from the road (i.e., immediately at road edge and within approximately 1.5 km of the new alignment) Surveys in adjacent habitat were either along low use side roads (50 km/h) and were driven at ~10 km/h, or along highway construction access roads no longer accessible to vehicle traffic, and thus were

conducted by snowshoe. I assumed that on perpendicular surveys, detection rates were similar between methods (by car versus on snowshoe) because I drove slowly and encountered additional tracks only a few times over four years when I stopped to examine those I had detected from the car, similar to while snowshoeing.

All surveys were conducted by 1 – 2 observers. Surveys along old and new highway alignments (hereafter parallel surveys) and surveys through habitat adjacent to the highway (hereafter perpendicular surveys) were carried out approximately 48 hours (range: 12 - 128 hours) after each snowfall, unless the snowpack was too thin or patchy to allow reliable data collection, or at least an additional 2 cm of snow had fallen in the interim. Upon detecting signs of large mammals (i.e., footprints, beds, craters, feces, hair, blood and urine), researchers noted what species created the tracks and recorded geospatial coordinates (± 3 m; Garmin eTrex 10, USA). To control for the effect of spatial autocorrelation, especially for gregarious species (e.g., elk, deer, and wolves), groups of animals were treated as individual detections. If multiple tracks of the same species were found within ~100 m to one another, tracks were followed to determine if they were from the same animal, and unless definitively different (i.e., large size discrepancy), only the track closest to the highway was included in analyses.

Analyses

To identify changes in large mammal abundance, I compared species-specific total daily track abundances on both parallel and perpendicular surveys across years. This allowed me to determine if large mammal abundance varied over time at two distances from the road (i.e., directly at the roads' edge and within the area surrounding the highway). I built sampling distributions by permuting daily abundance counts (N_{2011} = 13 days, N_{2012} = 13 days, N_{2013} = 12 days, N_{2014} = 7 days) for each species (except felids, which were grouped because of infrequent

detection). To avoid violating assumptions of independence, I excluded fox and coyote from parallel survey analyses because they frequently travelled parallel to the highway, making it difficult to discern independent detections. To further delineate the effects of road size and traffic volume on the population ecology of large mammals, I analyzed yearly changes in daily abundance counts along each alignment separately, and compared daily abundances recorded on old and new alignments. I corrected for multiple comparisons using the Bonferroni method.

All geospatial data were handled using ArcGIS v.10.3.1 (ESRI 2015) and visually inspected for outliers. Using the 'Near' function from the ArcGIS Analysis toolbox, I measured the proximity of large mammal tracks detected during perpendicular surveys to the new alignment of the highway. Data were exported and analyzed using R v.3.4.1 (R Development Core Team 2018) and plots were made using ggplot2 (Wilkinson 2011).

Levene's test indicated that the variance associated with my proximity data was heteroscedastic across years and Shapiro-Wilk's test indicated that my proximity data was non-normal. Subsequently, I performed a Kruskal-Wallis analysis, with the Scheirer-Ray-Hare extension (Sokal and Rohlf 1995) in order to interpret multiple factors simultaneously and to identify interaction effects. Specifically, I tested if minimum proximity to the new highway alignment varied significantly with year, species, within-season date, and snow depth. Snow depth data were acquired from the Sudbury A weather station, ~40 km from my study site (Environment and Climate Change Canada 2019). Within-season dates were Julian dates which I transformed by subtracting 200 so that data collected within a single season would be continuous. To identify interspecific differences in proximity, I applied Dunn's test with a Bonferroni correction.

Results

Coyote and fox were the most common species recorded on perpendicular surveys, and elk was the most common species on parallel surveys. In contrast, felids were the least common on either survey (Tables 1.1, 1.2). Interestingly, elk, although detected on all perpendicular survey transects, appeared to favour two of the six transects, making up 54 of a total 79 detections. On average, it appeared that the abundance of moose and deer on perpendicular surveys declined over the 4-year study; however, these trends were not significant (moose: $H = 4.95$, $P > 0.05$; deer: $H = 5.38$, $P > 0.05$). In fact, the only significant shift in large mammal abundance on either parallel or perpendicular surveys was that in 2011, when there were significantly fewer foxes detected on perpendicular surveys compared to subsequent years ($H = 0.18$, $P = 0.01$). Moreover, when abundance counts of old and new highway alignments within the same latitude (Figure 1.1) were compared to elucidate the effect of traffic volume, I detected no significant differences ($P > 0.05$; note this section of the new alignment was not monitored in 2011).

As expected, I observed species-specific trends in proximity-to-highway measurements (Figure 1.2). Because when animals moved along perpendicular transects, I analyzed only the point at which they were closest to the highway, large mammal detections were recorded less frequently as distance from the road increased; however, moose and felids did not demonstrate this trend. In general, fox, coyote, and deer were the closest to the new highway alignment. Conversely, moose were the furthest away, and were detected substantially less often within 500 m of the new alignment (Table 1.3).

Scheirer-Ray-Hare analysis indicated significant effects of species (H = 7.98, P = 0.005; Figure 1.3), snow depth (H = 7.56, P = 0.006), and within-season date (H = 8.29, P = 0.004) on the proximity of large mammals to the new highway alignment, but no significant effect of year (H = 3.26, P = 0.07). I also detected three significant interaction effects between (1) year and within-season date (H = 12.13, P = 0.0005), (2) species, year and snow depth (H = 4.44, P = 0.04), and (3) year, within-season date and snow depth (H = 1.82, P = 0.03). After correcting for multiple comparisons, my post-hoc Dunn's test indicated that throughout my study, the only species-specific proximity differences were that moose were found further from the new highway than coyote, fox and deer.

Discussion

Contrary to my predictions regarding the habituation dynamics of large mammals around a newly twinned highway alignment, I detected little change in the abundance of animals or their proximity to the new highway alignment across time, suggesting that highway twinning has a limited effect on the population and spatial ecologies of large mammals. An alternative explanation for my non-significant results is that large mammals did not habituate to the new highway within my 4-year study period. This is particularly concerning because ungulate fitness is negatively correlated with longer habituation periods (Sawyer et al. 2017). However, this seems unlikely because although habituation responses to anthropogenic disturbance can vary (Sawyer et al. 2017), several studies indicate that habitation to roads and other human infrastructure is detectable in under four years (Haskell and Ballard 2008; Marino and Johnson 2012; Jacobson et al. 2016). Moreover, the fact that large mammals were frequently detected immediately adjacent to the active highway throughout my study suggests that this is not the case.

A more likely explanation for why I observed no effect of highway twinning is that the local large mammal community had been exposed to disturbances associated with the existing highway for several decades, and thus required little to no habituation period. Road avoidance by ungulates is positively related to traffic disturbance (Gagnon et al. 2007). However, in my study area, the increase in traffic volume over the course of my study was negligible, changing from an annual average daily traffic volume (AADT) of 6850 vehicles in 2010 to 7550 vehicles in 2016 (Ontario Ministry of Transportation 2010, 2016). Despite an increase of approximately 10%, this total AADT is relatively small, especially given that winter traffic (i.e., when tracking data were collected) would be lower than the annual average, which is inflated by summer recreation. This explanation is supported by the fact that I found no significant difference in the number of large mammals detected along either old or new highway alignments over time, and that large mammal abundance counts along both alignments did not vary significantly from one another.

Although I detected few significant changes, proximity measurements varied among species in several ecologically relevant ways. Perhaps the most interesting is the spatial distribution of elk. Elk were predominantly detected on two of my six perpendicular transects, both of which were associated with large, commonly used open areas corresponding to previously published telemetry data (Popp et al. 2013; McGeachy 2014). This is not surprising because elk in Ontario have historically selected deciduous forests that are more open than the conifer stands in my study area (Hamr et al. 2016). Unfortunately, the predictable nature of this elk herd may have artificially biased my data, suggesting that I may not have been successful in controlling for pseudoreplication. Thus, my results pertaining to elk should be considered carefully.

I also observed species-specific patterns of apparent road avoidance. While fox and coyote have been shown to adapt well to human presence (Bateman & Fleming 2012; Lombardi et al. 2017), wolves, although known to use linear corridors to improve hunting efficiency (Dickie et al. 2017), tend to avoid high-use roads (Whittington et al. 2004). Similarly, moose tended to be detected further from the road compared to deer or elk. While the selection of different microhabitats may account for this variation, ungulates have been shown to avoid preferred habitat close to roads (Leblond et al. 2013), and the road avoidance patterns of moose specifically are positively correlated with road size and traffic intensity (Wattles et al. 2018). The possible avoidance of optimal habitat by ungulates is of particular importance, especially given the recent decline of moose populations in several jurisdictions across Eastern North America (Timmermann & Rodgers 2017). Therefore further attention is required to fully discern the effects of highway twinning, and linear infrastructure in general, on moose spatial ecology (Popp & Boyle 2017).

Interspecific interactions within the local large mammal community likely played an additional role in the spatial distributions I observed. Ungulate proximity patterns overlapped with those of canids, specifically the distribution patterns of wolves with both elk and moose, and those of deer and coyote. Because of their size, wolves are more likely than coyote to prey upon larger ungulates (Benson & Patterson 2013; Benson et al. 2017) and in my study area wolves appear to preferentially prey upon elk (Popp et al. 2018). While predator-prey dynamics are a simple and likely explanation for the overlapping spatial distributions I observed, a more multi-faceted explanation is also possible. Because wolves have been shown to out-compete and attack coyote (Thurber et al. 1992), coyote may prefer road-adjacent habitat avoided by wolves. However, the selection of habitat nearer to roads may also act as an ecological trap by exposing

coyotes to increased road mortality (Benson et al. 2015), especially as the width of roads increase. Thus, while my study indicates that the effects of highway twinning may be negligible, it is important for managers to consider existing road-effects and interspecific interactions when coordinating future expansion projects.

Although I detected no variation in the population or spatial ecologies of large mammals between years, proximity to the highway varied seasonally. Seasonal variation in large mammal spatial ecology is well documented (Laurian et al. 2008; Popp et al. 2013), and winter severity and length can have long lasting negative effects on deer and moose populations (Mech et al. 1987). Snowpack conditions and depth have also been shown to affect large mammal habitat selection, foraging behaviour and predator-prey interactions (Thompson & Vukelich 1981; Whittington et al. 2004; Popp et al. 2013). My results suggest that this had a larger impact on large mammal spatial ecology than the widened highway. This effect was also significant when interacting with within-season date, indicating that the observed effects are likely the result of snow accumulation through the winter season. Climate change and fluctuating weather patterns make investigations specifically targeting seasonal variation of road-avoidance patterns particularly important and underscore the need for multi-year studies such as mine.

Road effects are complicated and diverse, but an understanding of such effects is critical to the effective management and conservation of road adjacent wildlife populations. A common strategy to support additional traffic volume is expanding existing roads, or creating wider, updated alignments. I specifically targeted a section of road undergoing twinning to tease apart the effects of increased road width and shifting traffic volume. Although I predicted that this shift would initially intensify road avoidance behaviour from the local large mammal community followed by a period of habituation where large mammal spatial ecology returned to pre-

expansion levels, I found that generally, the new alignment had little effect on their population and spatial ecologies. From an ecological viewpoint, limiting the total number of roads is preferable (Laurance et al. 2014). However, as the human population continues to grow and road networks expand, infrastructure will need to be strategically and purposefully planned in order to minimize environmental effects. My findings suggest that highway twinning has limited negative effects on large mammals and thus may be an ecologically favourable option to accommodate traffic increases. It is important to highlight however, that other taxa likely experience greater impacts from highway realignment, especially those with small home ranges, specific habitat requirements, or those which are already rare. Highway widening has also been associated with higher animal mortality rates (Baxter-Gilbert et al. 2015; Ciocheti et al. 2017), which is especially important for taxa sensitive to additive mortality. My study only investigated the impacts of a new highway on the population and spatial ecologies of large mammals at the local scale, but further research on migratory and reproductive ecologies as well as inter- and intraspecific interactions should be considered for future studies. Despite this, my findings are promising given that highway realignment is a widely used technique, and the neutral effect it appears to have on wildlife support its continued application.

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Figures

2011	2012-2014
Fully twinned 4-lane divided alignment of Highway 69, completed before study	Fully twinned 4-lane divided alignment of Highway 69, completed before study
Two-lane alignment of highway 69 active. New 4-lane alignment ~700 m east of old highway under construction	Old two-lane alignment converted to secondary roads. New 4-lane alignment of Highway 69 active

Figure 1.1. *Left:* Schematic of highway twinning alignments approximately 25 km south of Sudbury, ON, and their condition throughout my study. The twinning of the highway was completed in the northern portion of the study site prior to my study. The opening of the southern portion of the new highway alignment occurred between the 2011 and 2012 field seasons. Braces link highway condition to northern and southern portions of study site on map. *Right:* Aerial image of my study site (Google 2019) along Highway 69, which runs north-south. The new alignment is indicated by the thick yellow line, the old alignment is indicated by the thin white line. Red lines indicate location of perpendicular survey transects adjacent to the highway.

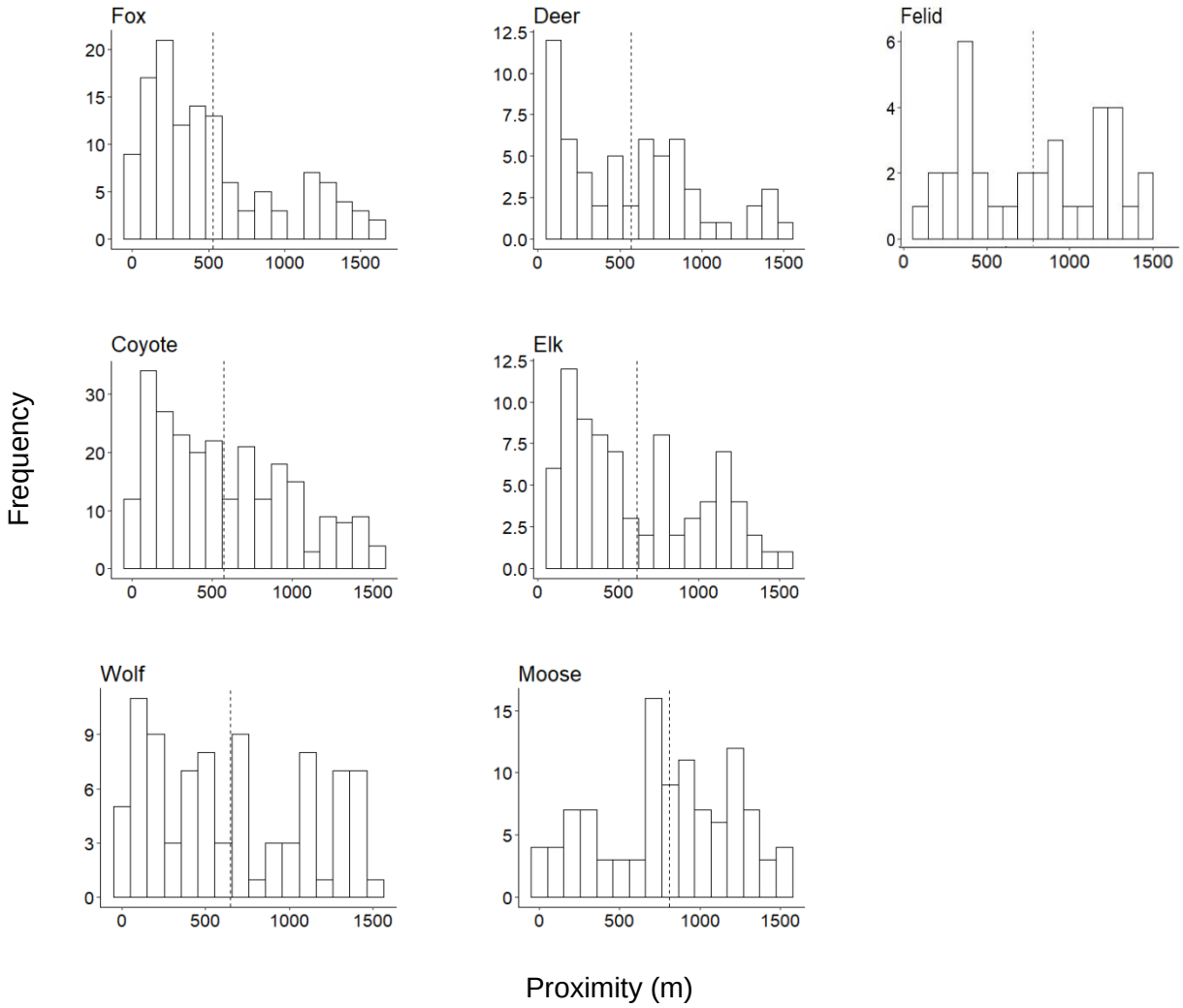

Figure 1.2. Frequency distributions of large mammal minimum proximity to the recently re-aligned Highway 69, approximately 25 km south of Sudbury, ON, Canada. Data collected during snow-tracking surveys over 4 winter seasons (2011/12 – 2014/15). Dashed line represents mean proximity.

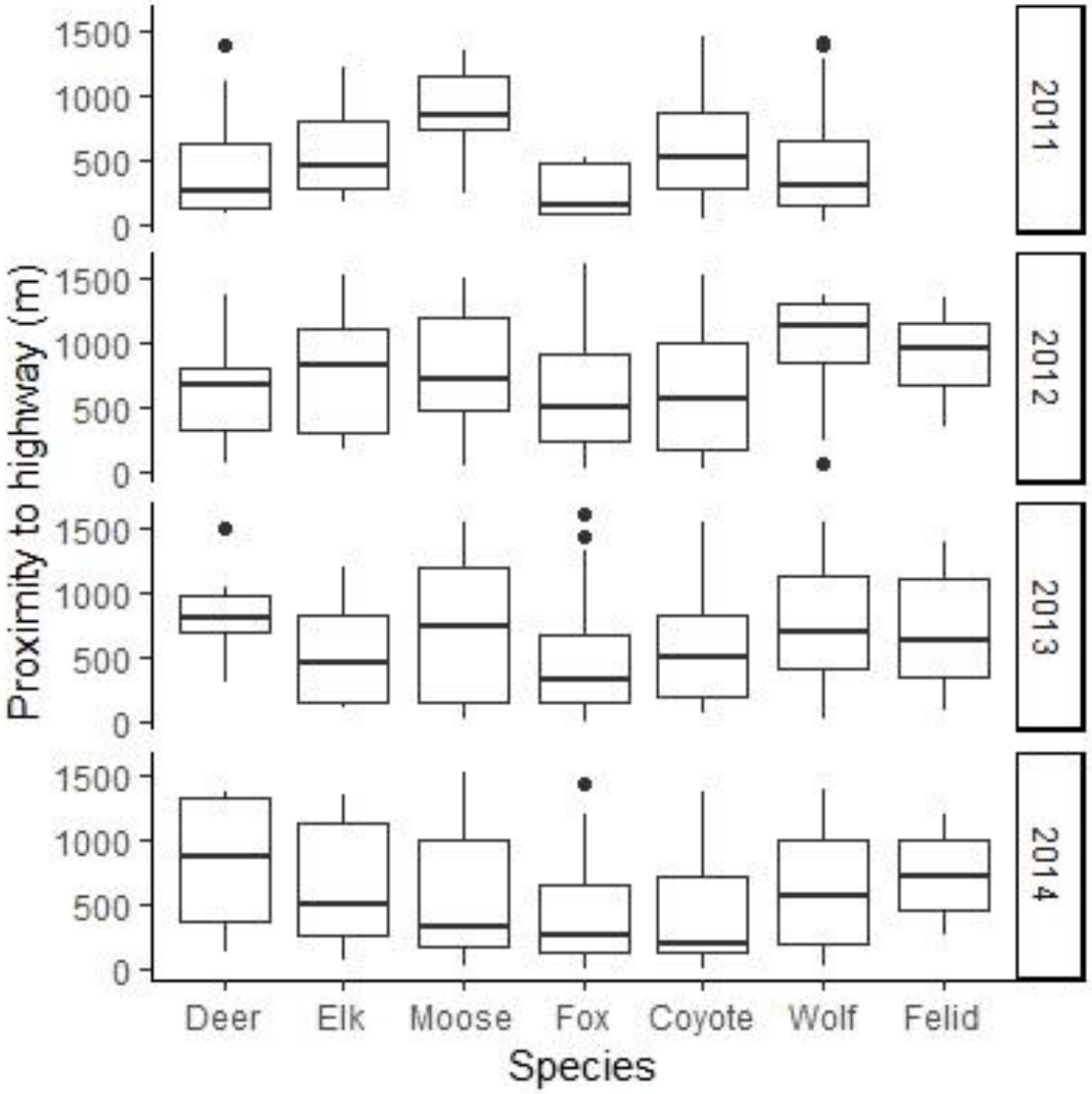

Figure 1.3. Minimum proximity (m) of large mammals to the newly twinned highway alignment near Sudbury, ON, Canada, collected over four winters (2011/12 – 2014/15). No significant differences were observed over time; however, moose were detected significantly further from the new highway alignment than coyote, fox or deer throughout my study. Lynx and bobcats were grouped together as felids because of the low number of detections.

Tables

Table 1.1. Abundance of large mammals detected on perpendicular snow-tracking surveys displayed as mean number of large mammal detections per survey day (± 1 SD). The only significant variation detected was the number of fox detected in 2011. Note the high variance (SD) in nearly all categories.

Year	Moose	Deer	Elk	Coyote*	Fox*	Wolf	Felid	All
2011	4.2 (5.1)	2.4 (3.2)	2.1 (2.6)	10.6 (9.0)	0.4 (1.2)	2.0 (3.0)	0.0 (0.0)	24.6 (13.8)
2012	3.1 (2.5)	1.7 (2.0)	2.0 (2.2)	4.0 (3.1)	5.2 (3.8)	1.4 (1.1)	0.6 (1.5)	18.0 (8.6)
2013	1.5 (1.4)	0.5 (1.0)	1.9 (2.7)	5.1 (4.2)	4.2 (4.9)	2.9 (1.9)	1.8(1.6)	18.8 (12.6)
2014	1.4 (1.1)	0.8 (1.3)	2.0 (1.0)	4.0 (1.0)	3.4 (0.9)	3.6 (1.5)	1.4 (1.6)	16.8 (3.7)

* Possibly due to differences in interpretation between those collecting data; 2011 had different technicians than 2012-2014.

Table 1.2. Abundance of large mammals detected on parallel snow-tracking surveys (new and old alignments grouped) displayed as mean number of tracks per survey day (± 1 SD). Note the high variance (SD).

Year	Moose	Deer	Elk	Wolf	Felid	All
2011	0.6 (1.6)	0.9 (2.0)	1.9 (4.0)	0.5 (1.5)	n/a	3.9 (5.4)
2012	0.7 (1.0)	0.4 (1.5)	0.6 (1.3)	0.1 (0.3)	0.1 (0.6)	1.9 (2.1)
2013	0.2 (0.4)	n/a	0.5 (0.9)	0.5 (0.8)	0.1 (0.3)	1.3 (1.8)
2014	0.1 (0.3)	0.1 (0.3)	0.9 (1.2)	0.4 (0.5)	0.1 (0.3)	1.4 (1.4)

Table 1.3. Descriptive statistics for large mammal track proximity to the new 4-lane highway alignment ~25 km south of Sudbury, Ontario, Canada. Tracks detected along standardized 1600 m transects running approximately perpendicular to the highway through adjacent habitat. Note the proportion of individuals found beyond 500 m of the new highway alignment is low for moose compared to other species.

	Distance to highway (m)			
Species	Minimum	Mean (± 1 SD)	Maximum	Proportion within 500 m of the new 4-lane highway
Moose *(Alces alces)*	14.1	812.2 (± 411.2)	1540.0	26/106 (24.5%)
Elk *(Cervus canadensis)*	80.4	615.3 (± 408.2)	1523.3	40/79 (50.6%)
White-tailed Deer *(Odecoileus virginianus)*	72.7	567.5 (± 409.5)	1488.6	27/59 (45.8%)
Wolf *(Canis lupus)**	19.4	650 (± 467.5)	1534.5	39/86 (45.3%)
Coyote *(Canis latrans)**	18.0	573.3 (± 414.6)	1544.4	123/249 (49.4%)
Red Fox *(Vulpes vulpes)**	4.6	529.2 (± 440.8)	1611.1	74/125 (59.2%)
Canada Lynx *(Lynx canadensis)*	85.3	731.7 (± 405.6)	1388.2	13/33 (39.4%)

*Note: all observations were recorded within *Canis* hybridization zone and species identification for wolves, coyote, and fox was determined based on track size.

Chapter 2

Comparison of road surveys and circuit theory to predict ideal locations for implementing road-effect mitigation

Published as:

Boyle SP, Litzgus JD, Lesbarrères D (2017). Comparison of road surveys and circuit theory to predict ideal locations for implementing road-effect mitigation. Biodiversity and Conservation. **26**(14): 3445-3463. https://doi.org/10.1007/s10531-017-1414-9

Abstract

The mitigation of road-effects on wildlife, especially road mortality and habitat fragmentation, has become increasingly common in the last 20 years. However, exclusion fencing and habitat connectivity structures can be very costly and several questions remain regarding how to best determine locations that will optimize mitigation success. Based on data collected across several years and across multiple landscapes and taxa, I present a comparative analysis of two methods: road surveys and circuit theory, and review their benefits and challenges to better inform decision making. Road surveys were completed in two locations over three years for large mammals and herpetofauna to identify road crossing hotspots. Circuit theory was also applied to these systems to identify crossing hotspots using habitat resistance models. The location, number and width of hotspots were compared between methods. Hotspot distributions were similar between methods for some herpetofauna, but different for mammals, and road surveys produced a significantly greater number of smaller hotspots compared to circuit theory, implying that road surveys provide better hotspot resolution. As circuit model complexity increased, the number and width of hotspots decreased, diffusing across the landscape. Road surveys were better at predicting optimal crossing structure location at a local scale; however, circuit theory is less costly, and can be useful at large scales. As both methods can offer valuable information, I argue that the combination of these two approaches provides a strong basis for managers and biologists to make informed decisions about costly mitigation measures, optimizing both conservation benefits and limited funding.

Key Words: connectivity, *Circuitscape*, *Sireima*, wildlife management, reptiles, amphibians, large mammals, road ecology

Introduction

Roads are recognized as a global threat to biodiversity (Laurance & Balmford 2013; Laurance et al. 2014), and the negative effects of roads on wildlife have become increasingly well studied as the trend for increased urbanization and continued road expansion (Dulac 2013) intersects with efforts to conserve biodiversity (Trombulak & Frissell 2000; Seiler 2001; Fahrig & Rytwinski 2009). The combined effects of habitat fragmentation, direct mortality, and other indirect effects (Fahrig & Rytwinski 2009; Jackson & Fahrig 2011; Votsi et al. 2016) demonstrate the crucial need for mitigation of road effects. Furthermore, the road-effect zone is far larger than the footprint of the road itself (Forman 2000; Marsh et al. 2017). Measures taken to mitigate road effects can be costly (Huijser et al. 2008), and as conservation dollars are often limited, efforts should be made to optimize mitigation planning and implementation.

Although mitigation of road effects can take several forms, including community involvement (Cosentino et al. 2014) and even closure or removal of roads (Switalski et al. 2004; Palis 2016), these approaches are not always pragmatic or effective on a large scale. Predominantly, road ecology literature refers to one or a combination of exclusion fencing and crossing structures. Fencing is designed to prevent access to the road surface thus reducing animal mortality, and crossing structures are designed to restore access to resources (e.g. seasonal habitat, food) and genetic connectivity (Glista et al. 2009). Both types of mitigation are critical for population persistence (Jaeger et al. 2005; Glista et al. 2009); however, for mitigation to be effective, careful planning is required well before implementation of costly mitigation features. Factors such as the configuration and design of exclusion and connectivity structures must be evaluated during the planning stages of every roadway mitigation project to ensure that the mitigation will be useful (Lesbarrères & Fahrig 2012) across taxa and locations.

Information about the locations of road crossings and road mortality of animals is highly valuable for road ecologists and managers. When these data are more spatially aggregated than expected (usually compared to random distributions), they are referred to as hotspots (Gunson et al. 2011). Hotspots have been identified at multiple spatial and temporal scales (Mountrakis & Gunson 2009) and can be used for prioritization and planning of roadway mitigation projects to optimize the effectiveness of mitigation features. Hotspots have been correlated with a variety of landscape characteristics such as the presence of an ecotone (Gomes et al. 2009; Cureton & Deaton 2012; Gunson et al. 2012; Barthelmess 2014) and similar habitat types on both sides of a road (Langen et al. 2012), but driver behaviour and road characteristics are also generally considered important (Gunson et al. 2011, Langen et al. 2012, Barthelmess 2014; but see Bissonette and Kassar 2008). Incorporating habitat variables and landscape patterns is therefore critical to effectively predict hotspots (Roger & Ramp 2009). Hotspot locations are often species-specific due to behavioural or morphological characteristics. For example, certain species are more at risk of road mortality due to their high vagility (Carr & Fahrig 2001; Jochimsen et al. 2014). Similarly, some species are drawn to roads, including freshwater turtles looking for nesting opportunities (Steen & Gibbs 2004; Steen et al. 2006; Langen et al. 2012). Community variables such as prey abundance are also associated with hotspots of predator mortality (Barrientos & Bolonio 2009). Animal movement patterns through a landscape have been compared to the movement of electricity through a circuit board with a preference for the path of least resistance but not an absolute aversion to paths with higher resistance (McRae et al. 2008; Koen et al. 2014).

Road surveys are commonly used for determining road crossing hotspots for several taxa including: birds (Eberhardt et al. 2013), insects (Baxter-Gilbert et al. 2015b), herpetofauna

(Langen et al. 2007; Eberhardt et al. 2013; Baxter-Gilbert et al. 2015a) and mammals (Eberhardt et al. 2013; Schuster et al. 2013). Many variables, both natural (e.g. landscape composition, inter- and intraspecific interactions) and anthropogenic (e.g. traffic volume, road type, driver behaviour), affect hotspot location (Gunson et al. 2011). Although road crossing data have been shown to be reliable in some situations (Langen et al. 2009), road surveys may not be an accurate reflection of road mortality due to factors including: scavenging, limited carcass persistence (Beckmann & Shine 2014) and the frequency of surveys (Santos et al. 2011). Additionally, hotspots can vary considerably through time, and can be either spatially diffuse or concentrated (Crawford et al. 2013). Road surveys do not typically include historical data, and wildlife-vehicle collision hotspots based on recently collected data alone may neglect important areas that experienced high mortality in the past (Eberhardt et al. 2013). Despite a large amount of literature regarding wildlife-vehicle collision hotspots, there seem to be few, if any, broadly applicable rules (Gunson et al. 2011), which has led to management and conservation difficulties.

Decisions about costly conservation initiatives (i.e., road-effect mitigation) need to optimize conservation benefits and minimize resources consumed (McDonald-Madden et al. 2008). Limited funding can undermine well informed decisions (Wilson et al. 2006), and managers are often faced with uncertainty regarding best practices due to the lack of evaluation of, publication of, or access to, previously completed projects (Pullin et al. 2004; Regan et al. 2005; McDonald-Madden et al. 2008; Lesbarrères & Fahrig 2012). It is thus imperative that robust and diversely applicable tools are readily available for use by those responsible for adaptive management.

I compared and contrasted two approaches for identifying optimal locations for road-effect mitigation: road surveys, and habitat resistance modelling using circuit theory and aerial

imagery. By using multiple taxa and study sites, I demonstrate the benefits and shortfalls of each method. I compared the number and width of hotspots produced by each method, as well as their spatial distributions, to determine if the methods corroborated one another. I expected that road surveys would produce a greater number of narrower hotspots, but that in general the hotspot distributions would not vary between methods. Further, I assessed how complexity of circuit theory models affects the results, predicting that overall hotspot width would be narrower as complexity increased. Additionally, I address issues associated with funding, logistical support, and time commitment required for each method. My comparison is broadly applicable for managers and road ecologists to increase the informative value of their hotspot predictions, leading to more effective mitigation.

Methods

Study Sites and Survey Methodology

I counted road crossings by animals at small and large spatial scales. At a small scale, road surveys for herpetofauna were completed in Presqu'ile Provincial Park, ON, Canada (PQPP), over three summers (2013-2015) from May to August. The road surveyed was 8 m wide, and experienced mid-day traffic of 161 – 212 cars per hour during June – August (Boyle et al. *unpublished data*). Daily surveys were used to account for all weather types. The survey route was 1.25 km in length and was surveyed daily by bicycle at 9:00, 18:00 and 22:00 and complemented by a survey on foot immediately following the 9:00 survey. Bright headlamps were used during surveys beginning at 22:00 allowing field staff to easily see both live and dead animals (including small amphibians) from several meters away. Live and dead herpetofauna

were identified and referenced geospatially with a handheld GPS unit (Garmin, eTrex10, Olathe, Kansas, USA; acc. ± 3 m). Over 524 survey days, I detected 116 turtles (4 species, but dominated by *Chelydra serpentina* and *Chrysemys picta*), 133 snakes (4 species, but dominated by *Thamnophis sirtalis*), and 5338 amphibians (9 species).

At a larger scale, snow tracking surveys for large mammals were completed over three winters (2012/2013 – 2014/2015), along Highway 69, ~50 km south of Sudbury, ON, Canada (Hwy 69). The surveyed highway was a twinned 2-lane highway with approximately 40 m of median between north and southbound lanes and an annual average daily traffic volume of approximately 10800 (Ontario Ministry of Transportation 2013). Surveys took place approximately 48 hours after each snowfall (>1 cm) and consisted of 1 – 2 observers driving an approximately 25 km stretch of twinned highway at 80 km/h. This tracking schedule was chosen as animal activity is high after a storm, and the weather is moderately consistent. Tracking conditions were typically ideal, and tracks were apparent along the side of the highway at this speed. When tracks were spotted, observers pulled the vehicle over onto the road shoulder to inspect the tracks more closely. Tracks of all felids and ungulates were identified, and referenced geospatially with a handheld GPS unit (acc. ± 3 m). Apparent groups of same-species tracks were counted as a single track to avoid spatial autocorrelation of potentially gregarious species. In total, 34 surveys were completed over this time period and the number of detections consisted of 87 large mammals tracks (3 species of ungulate and *Lynx canadensis*; canids were not recorded).

Road Survey Spatial Aggregation Analysis

Crossing locations elucidated from surveys at both sites (PQPP, Hwy 69) were analyzed using the 2-D Hotspot Identification Tool from the program *Siriema* (Coelho et al. 2008, 2014).

In all instances, three years of survey data were grouped by taxa (Turtles, Snakes, Amphibians, Mammals), and GPS coordinates of both live and dead individuals, and signs of individuals (for mammals only), encountered during surveys were combined, hereafter referred to as road-crossing points. Prior to hotspot identification, *Siriema*'s 'Fit Events to the Road' function was used to account for any minor GPS errors, and the program's 2-D Ripley's K function was used ensure hotspots were not resulting from a sample distribution which was in fact uniform (Coelho et al. 2014). Parameters selected for *Siriema* analyses were chosen to maximize graphical clarity of hotspots. For herpetofauna analyses, *Siriema* parameters used were: radius = 10 m, # simulations = 100, # road divisions = 500. Because the survey area was much larger for mammals, the mammal analysis incorporated the following parameters: radius = 100 m, # simulations = 100, # road divisions = 500. *Siriema* randomly simulates road crossing points along the survey route. The number of randomly simulated crossing points was subtracted from the number of actual survey crossing points in a road division to generate a hotspot value ($N_{events} - N_{simulated}$). At points where this function exceeds the 90% upper confidence limit associated with the data, the spatial aggregation was considered a significant hotspot (Coelho et al. 2014). Road survey hotspots were quantified graphically by measuring their length and proximity to other hotspots to determine the location and size of hotspots.

Circuit Theory Landscape Resistance Analysis

Land use classification rasters acquired from the Ontario Land Cover Data Base (OLCDB; mammals; OMNRF, 2004) and Southern Ontario Land Resources Information System (SOLRIS; herpetofauna; OMNRF, 2008) were prepared for analyses using ArcGIS (ESRI 2015) and loaded into *Circuitscape* (McRae & Shah 2011). Circuit theory has been shown to be a useful predictor of animal occupancy (Walpole et al. 2012), gene flow (McRae & Beier 2007) and movement

patterns (Koen et al. 2014), but has not be applied to small scale road mitigation initiatives. Native map resolutions were used for all analyses (OLCBD = 25 m^2, SOLRIS = 15 m^2). Land use classification values were reassigned with resistance values of 1, 10, 25, or 100, specific to either herpetofauna (animals that are mainly aquatic) or large mammals (mainly terrestrial), representing the ease with which animals would be able to travel through each square depending on the habitat type present in the square, with 1 as the easiest to move through and 100 the most difficult. This type of parameterization was chosen because it has been used previously to create multi-species functional connectivity maps for general forest- and wetland-dwelling species (Koen et al. 2014)

Five models were created for herpetofauna using established knowledge of their general ecology and habitat use (Appendix A2.1). By adjusting resistance values assigned to each habitat type and using buffers to create gradients of resistance around preferred habitat (acknowledging that habitat use may not be fully categorical), it became possible to determine how manipulation of model complexity affects the number and width of predicted hotspots. Only relative values of habitat types were modified between models, acknowledging basic habitat use was likely to be consistent within taxa, but that additional variation may be present (i.e., wetlands would be used by herpetofauna preferentially compared to upland, but certain types of wetland may be used more than others). Model 1 was the simplest, with any wetlands given a resistance of 1, uplands given a resistance of 10 or 25 depending on their tree cover, and impassable terrain (e.g. cliffs) and infrastructure given a resistance of 100. Model 2 differed by increasing open water areas to a resistance of 10. Because aerial imagery provides only a coarse depiction of habitat type, Model 3 included a 250 m buffer around wetlands with a resistance of 10, making the assumption that changes between habitat types are gradual rather than discrete, and to account for seasonal

fluctuations in wetland size, which vary substantially over the course of the active season for herpetofauna in PQPP due to snow melt and summer drying. Model 4 included both the 250 m buffer with resistance 10 from Model 3, as well as an additional 30 m buffer surrounding wetlands with a resistance of 1. Model 5 included a resistance of 100 in far shore open water (i.e., Lake Ontario), in an effort to force current through the road, and limit its diffusion into the surrounding area.

One circuit model was created to represent large mammal movement through the landscape on Hwy 69 to qualitatively compare the effectiveness of each method for detecting hotspots across taxa. Upland habitat was assigned the lowest resistance (i.e., 1), while medium resistance habitat included wetlands (i.e.10), and infrastructure and large bodies of water were assigned a high resistance (i.e., 100). A buffer representing 20% of the size of the map was placed around study areas to minimize edge effect bias (Koen et al. 2010) and pair-wise node placement followed Koen et al. (2014). Raster cell connections were made using average resistance, and 8 neighbours. Locations along the road were considered hotspots if the amount of current in each raster square exceeded the average amount of current produced by the model. Hotspots were quantified in ArcGIS by measuring their length and proximity to other hotspots.

Statistical Analyses

The number and width of hotspots were compared between approaches (circuit theory and road surveys). I chose narrowness and the number of hotspots as identifiers of methodological value because they allow those implementing mitigation to more readily prioritize optimal locations for installation. Hotspot metrics were compared both individually and as hotspot groups to determine the sensitivity to scale of each method and because individual hotspots alone may represent fragmented sections of larger hotspots. I defined ‘hotspot groups’

as hotspots within close proximity to each other, specifically, within 4% of the total survey length (herpetofauna – 50 m; mammals – 1000 m). I chose this number as it could be easily understood by managers implementing mitigation. All statistical analyses were conducted using R v. 3.2.3 (R Development Core Team 2018). Number and width of hotspot estimates were compared using Wilcoxon Rank-Sum tests as the data did not meet the assumptions of parametric tests. Specifically, the width of hotspots and width of hotspot groups produced by each method were compared on a taxa-specific basis but the number of hotspots and the number of hotspot groups produced by each method were compared across taxa due to small sample size. I believe grouping taxa for this comparison is unlikely to produce spurious results. Although it is possible that taxa-specific behaviours could affect the number of hotspots (i.e., via site fidelity), I argue that this did not affect my results as I compared the same animal assemblage with each method.

To compare the distribution of hotspots, the focal roads were divided into 25 equal length segments (herpetofauna – 50 m long; mammals – 1000 m long). For both methods, each segment was scored as a "1" if at least one whole or partial hotspot fell within its boundaries, and a "0" if no hotspots fell within them. Hotspot distributions were compared between methods using unweighted Cohen's Kappa coefficients; typically a test used to quantify agreement of data coded by two researchers, Cohen's Kappa is also applicable when comparing two methods and the goal of each is the same. Cohen's Kappa was chosen as a robust analysis of methodological agreement because it takes into account agreements which occur by chance. This analysis produces coefficients on a scale of [-1 to +1], where higher positive values represent a greater degree of agreement (Landis & Koch 1977). Amphibian, snake and turtle road survey hotspots were grouped and compared to each of the five circuit theory models because the circuit model

parameters were applicable to all herpetofauna. Cohen's Kappa coefficient was used to compare how often results from circuit theory models and road surveys agreed (i.e., a segment had the same score – hotspot or not – in both methods). Road survey hotspots for each herpetofaunal taxon were then compared separately to the circuit model that best approximated the road survey (Model 1). Mammalian road survey hotspots were compared to the single model developed to represent mammalian movement, also using Cohen's Kappa coefficients. Predictions of the width and number of hotspots were compared among models using Kruskal-Wallis tests.

Results

Across taxa, there was high variation in the number of individual hotspots produced via road surveys (# of individual hotspots: Turtles = 5, Snakes = 9, Amphibians = 11, Mammals = 18; Figure 2.1). Comparatively, the number of hotspot groups produced by road surveys displayed substantially less variation (# of groups: Turtles = 4, Snakes = 4, Amphibians = 2, Mammals = 7). Hotspot width also varied among taxa, and for amphibians nearly half of the survey route was considered a hotspot (% of survey route considered a hotspot: Turtles = 10.6%, Snakes = 14.2%, Amphibians = 40.3%, Mammals = 10.7%)

Herpetofauna current density maps (Figure 2.2 a-e) based on circuit resistances demonstrated animal movement corridors throughout the landscape with relatively few hotspots (n = 1 to 3), compared to road surveys (Table 2.1). Moreover, circuit theory predicted that 22-53% of the survey route was considered a hotspot for herpetofauna. For mammals, a large number of circuit theory hotspots were predicted (n = 9; several of which were small) and 20.7% of the survey route was considered a hotspot (Table 2.1). This resistance model displayed

relatively high current spread evenly across the landscape, with cold spots primarily at roads and large bodies of water, and hotspots at pinch points between these features.

Individual hotspot widths for herpetofauna ($W = 13$, $p = 0.0001$) and mammals ($W = 0$, $p < 0.0001$), and hotspot group widths for herpetofauna ($W = 11$, $p = 0.01$) were significantly narrower when estimated using road surveys versus circuit theory, but there were no significant differences between methods regarding mammal hotspot group width ($W = 4$, $p = 0.18$). Circuit theory produced a significantly greater number of individual hotspots across taxa compared to road surveys ($W = 23$, $p = 0.03$), and the number of hotspot groups was nearly significantly higher using the road survey method ($W = 21$, $p = 0.064$). No significant differences were detected among herpetofauna models of varying complexity with regards to number or width of hotspots ($X^2 = 4$, $df = 4$, $p = 0.41$ in both cases). However, as circuit theory model complexity increased, there was a general trend towards fewer hotspots and narrower overall hotspot width (Table 2.1) with models 3 and 5 displaying substantially smaller total hotspot widths than the two simplest models; however, this trend was not consistent.

My evaluations of Cohen's Kappa coefficients for comparing hotspot distributions between road surveys and circuit theory follow the methods and terminology used by Landis and Koch (1977). Herpetofauna hotspots developed from road surveys displayed a high level of agreement with circuit theory model 2 ($K_2 = 0.96$) and a moderate level of agreement with model 1 ($K_1 = 0.58$), while models 3 – 5 demonstrated a disagreement with road survey results ($K_{3, 4, 5} = -0.353, -0.353$ and -0.397, respectively). In all cases, $p < 0.05$ indicating that these measures of agreement were not by random chance. When compared at a taxa-specific level, the circuit theory model with the highest expected agreement with road survey results for herpetofauna in general (model 1 with 20/25 road segments in agreement), displayed only a fair to moderate level

of agreement for turtles and amphibians which was not due to random chance ($K_{turtle} = 0.348$, $K_{amphibian} = 0.426$; $p < 0.05$ in both cases). The locations of hotspots for snakes demonstrated a fair level of agreement, and in the case of mammals, a disagreement ($K_{snakes} = 0.24$, $K_{mammals} = -0.23$); however, these results may have been due to random chance ($p_{snakes} = 0.17$, $p_{mammals} = 0.20$).

Discussion

My data reinforce the fact that no single metric or method of road crossing hotspot identification can provide researchers and managers with prescient knowledge of how mitigation will work in a given system. I demonstrate that on a local scale for herpetofauna, road surveys provide a finer resolution estimate of hotspot location than circuit theory, but that for large mammals the relationship is not as strong, highlighting the importance of considering hotspots both individually and in groups, for specific taxa separately. Differences observed only when comparing the number of hotspots and width of mammalian hotspots individually imply that road surveys subdivide larger hotspots more than circuit theory models, or that map resolution is a limiting factor in circuit theory (i.e., hotspots cannot be smaller than the map resolution used). This is encouraging, especially because mitigation for large mammals can be very costly (Huijser et al. 2008). Given that individual hotspots differ in size between methods, and the fact that circuit theory hotspots were greater than the minimum size possible given map resolution (3-192 times wider than map pixels), it is possible that both subdivision, and to a lesser extent, map resolution errors, are occurring. While it is important to recognize grouped hotspots as they may indicate spatially autocorrelated movement, visualization of hotspot subdivision can help identify optimal locations for mitigation on a finer scale. However, the value I used as a cut off for

grouping hotspots (4% of total survey route) was chosen for simplicity for those implementing mitigation, and as such some hotspots that were not grouped could be functionally the same hotspot. Although it could be argued that more hotspots imply the need for more crossing structures, a large number of narrow hotspots should be preferred by mitigation planners because individual structure effectiveness can be increased through prioritization and strategic placement, thereby increasing both mitigation efficiency and cost-effectiveness.

Model complexity and hotspot distribution

Increased complexity in circuit theory models did not have a significant effect on overall hotspot width or the number of hotspots (likely due to small sample size). The trend, however, was for lower overall width and fewer hotspots as model complexity increased (in partial agreement with my predictions). As model complexity increased, hotspots of herpetofauna movement became more diffuse across the landscape. Compared to isolated hotspots of high intensity which are easily identified, these diffuse movements through the landscape make specific prioritization based on hotspot magnitude more difficult, and reduce the ability of researchers to locate optimal mitigation sites. Further, my distribution analysis became less predictive of hotspots when considering my most complex models (models 3 to 5). Although model 2 produced the most similar hotspot distribution, the only variation between model 1 and 2 was an increased resistance value for open water, a habitat rarely used by the herpetofauna in the area. Therefore, based on my results, simple models with few parameters are more accurate which should make circuit theory a feasible tool for managers when planning mitigation.

The distributions of crossing hotspots along roads varied significantly between analytical methods for several taxa. Circuit theory was able to replicate the distribution of herpetofauna crossings patterns generally (i.e., road survey hotspots), but failed to replicate distributions of

specific crossing patterns for snakes and mammals. Further, it was only moderately successful for turtles and amphibians suggesting that circuit theory may be a valuable tool for predicting localized crossing and mortality patterns for these taxa given my model parameters. My findings for snakes and mammals warrant further research however, as they may have been the result of random chance. Snakes are likely to use more upland habitat than turtles or amphibians, which could explain the discrepancy within herpetofauna. Koen et al. (2014) applied circuit theory across a large spatial extent and found that circuit theory does accurately predict road mortality patterns for herpetofauna, suggesting that resolution of available maps relative to study area size is an important consideration when determining what method to choose. Although maps with higher resolution may increase the value of the results, their creation would require more time and potentially on-the-ground classification. These findings indicate that selection of an approach should be guided by the mobility of the species for which mitigation is targeted, especially when map resolutions below 15 m^2 are unavailable (i.e., for my herpetofauna analyses). While circuit theory models have been shown to be effective at predicting wildlife occupancy and road crossing patterns of both mammals and herpetofauna across large landscapes (Walpole et al. 2012; Koen et al. 2014), their use across small landscapes (10 - 250 km^2) appears to be limited.

Trade-offs for each method

When choosing which method to use, there are several considerations that must be weighed (Table 2.2). Road surveys can provide excellent data about road crossing patterns (Langen et al. 2007), but their major downfall is the rigour required to make those surveys effective. In addition to potentially ignoring temporal variation in spatial ecology, an infrequent survey regime can bias results through limited carcass persistence (Beckmann & Shine 2014).

Further, using multiple years and study sites to control for stochastic variation is important if any analyses beyond hotspot identification are needed (Lesbarrères & Fahrig 2012; van der Grift et al. 2013; Rytwinski et al. 2015); however, such large studies require extensive coordination with partners and large amounts of funding and people-power (van der Ree et al. 2011; Lesbarrères & Fahrig 2012). Yet, even extensive road surveys may fail to identify historical road mortality hotspots (Eberhardt et al. 2013), especially on older roads (Teixeira et al. 2017), and this could partially explain differences between hotspot locations predicted by each method.

While broad assemblage-based models are becoming more common (Koen et al. 2014; Santini et al. 2016), the variation in my road survey hotspots among taxa highlights the importance of data collection specific to the species for which mitigation is intended (Lesbarrères et al. 2004). Species-specific data collection can be difficult when target species are inconspicuous or rare, but is critical if feasible. In contrast, although circuit theory hotspots are wider, the method is a relatively fast, inexpensive and flexible tool, and allows for immediate cartographic depiction. Although complexity of models did not increase their usefulness in my study, in agreement with Koen et al. (2012), this flexibility allows them to be easily adapted to many situations or species assemblages. The biggest benefit to using circuit theory is its efficiency. The circuit theory analyses I carried out each took approximately 2-3 days for parameterization of each site, and less than a week to run each model in *Circuitscape* (computer usage but not staff time). In comparison, approximately 45 days of road surveys were executed for mammals and 300 days of road surveys were executed for herpetofauna. The greatly reduced time and resources required for circuit theory analyses are its greatest benefits over road surveys. As circuit theory uses fewer resources than road surveys to execute (Table 2.3), it can be used in preconstruction projects, which are generally more economical than retrofitting existing roads

with mitigation (Glista et al. 2009). Such anticipated planning is especially valuable because road surveys are impossible in these situations (i.e., there are no roads to survey), and visual encounter surveys may experience limited success and fail to provide information on animal movement patterns.

Conclusions

I recommend road surveys as the first choice for identification of optimal locations for wildlife connectivity structures on a local scale; however, neither method is perfect in isolation. Although circuit theory is much less costly, its results are less defined than those from road surveys. So, if resources are available, I suggest that both methods be used in combination for increased effectiveness. Ultimately, the cost of using each method is minimal compared to the actual cost of mitigating road effects, and the additional cost of using circuit theory is small compared to the overall cost of road surveys. The main benefits of using road surveys and circuit theory in combination are: 1) multiple results allow managers to prioritize hotspots predicted by both approaches and 2) running circuit theory analyses first can guide monitoring and increase efficiency of surveys by identifying broad areas that are worthy of further observation. Incorporating multiple spatial scales and sources of data would further increase the efficacy of this dual circuit theory – road survey approach (Schuster et al. 2013). For example, it may be useful to compare road mortality rates to population sizes in adjacent habitat, and use per-capita values to select ideal mitigation locations (Teixeira et al 2017). Approximation of population metrics would also benefit subsequent evaluations of mitigation effectiveness (Clevenger & Waltho 2005). Road surveys are a more informative method compared to circuit theory for determining locations for road-effect mitigation, but in situations when funding is limited, circuit theory is still valuable as it provides an estimate of hotspot location, and although less defined, it

can be useful for certain taxa. Together though, circuit theory and road surveys can be used to critically identify and prioritize locations for mitigation structures, advancing both the conservation of focal populations as well as the scientific rigour of future studies.

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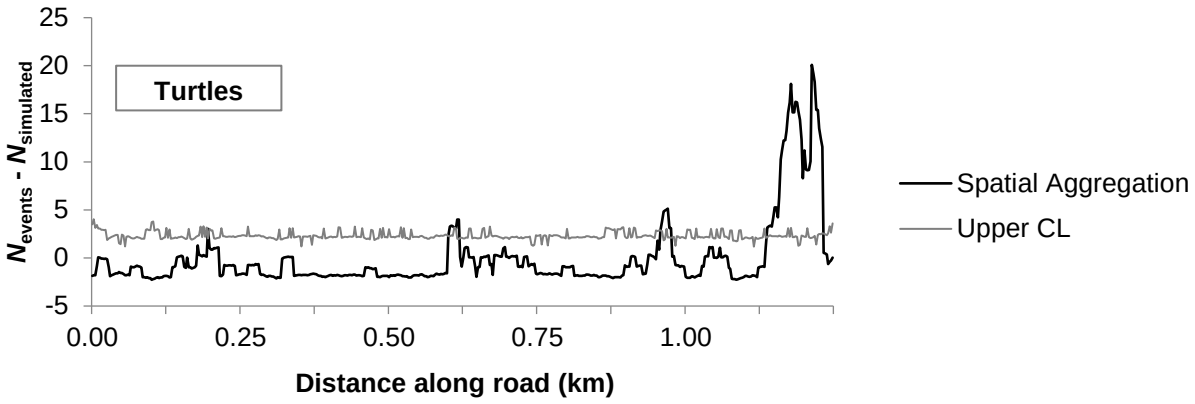
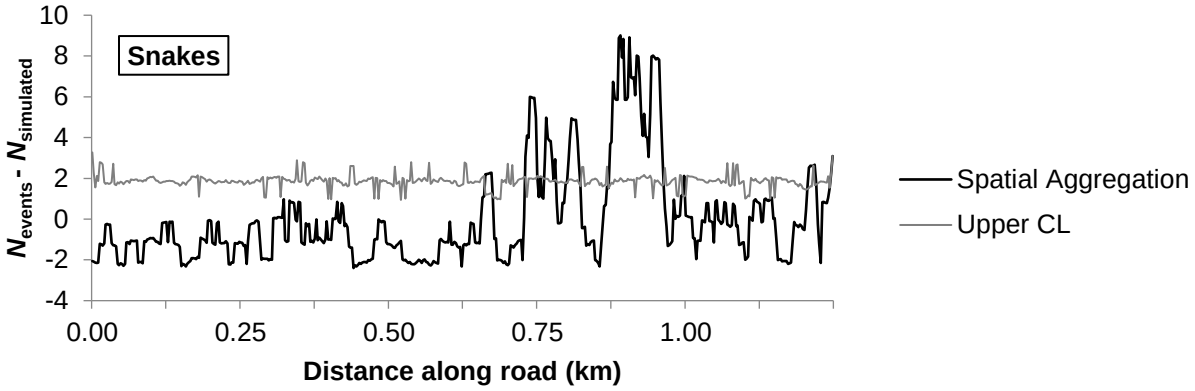
Figures

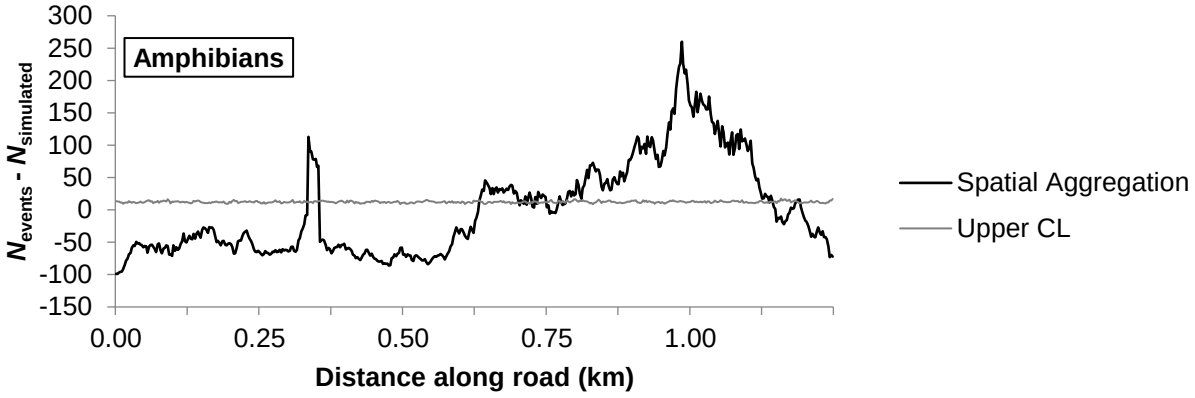
a)


Turtles
N_{events} - $N_{simulated}$
25
20
15
10
5
0
-5
0.00
0.25
0.50
0.75
1.00
Distance along road (km)
Spatial Aggregation
Upper CL

b)


Snakes
N_{events} - $N_{simulated}$
10
8
6
4
2
0
-2
-4
0.00
0.25
0.50
0.75
1.00
Distance along road (km)
Spatial Aggregation
Upper CL

c)


Amphibians
N_{events} - $N_{simulated}$
300
250
200
150
100
50
0
-50
-100
-150
0.00
0.25
0.50
0.75
1.00
Distance along road (km)
Spatial Aggregation
Upper CL

d)

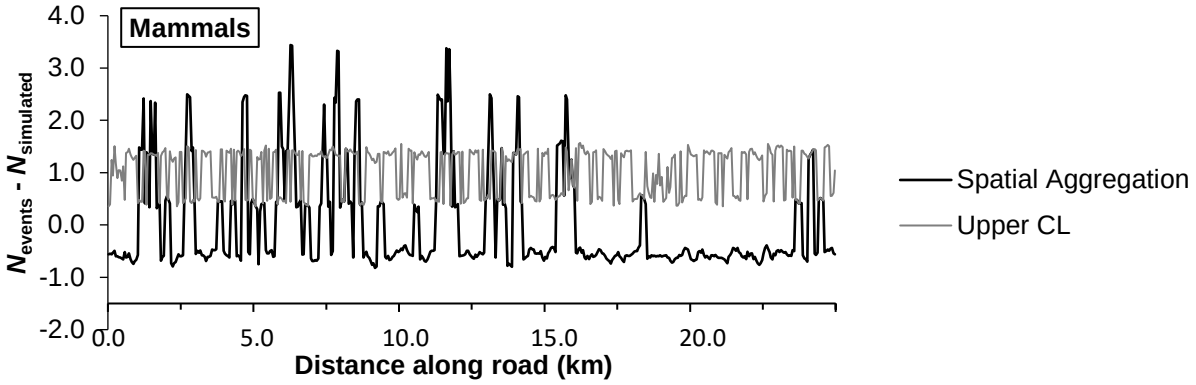


Figure 2.1 a-d. Output from 2-D Hotspot Identification Tool from program *Siriema*® using road survey data. Grey line indicates upper 90% confidence limit, and black line represents the spatial aggregation of real data points from which the randomly distrubuted data points simulated by the program (N_{events}-$N_{simulated}$) are subtracted. Where N_e-N_s exceeds upper confidence limit (i.e., when the black line is above the grey line), the location has an aggregation of real data points significantly greater than expected by random, and is considered a hotspot. Distance along road (x-axis) begins at the northern point for both survey sites.

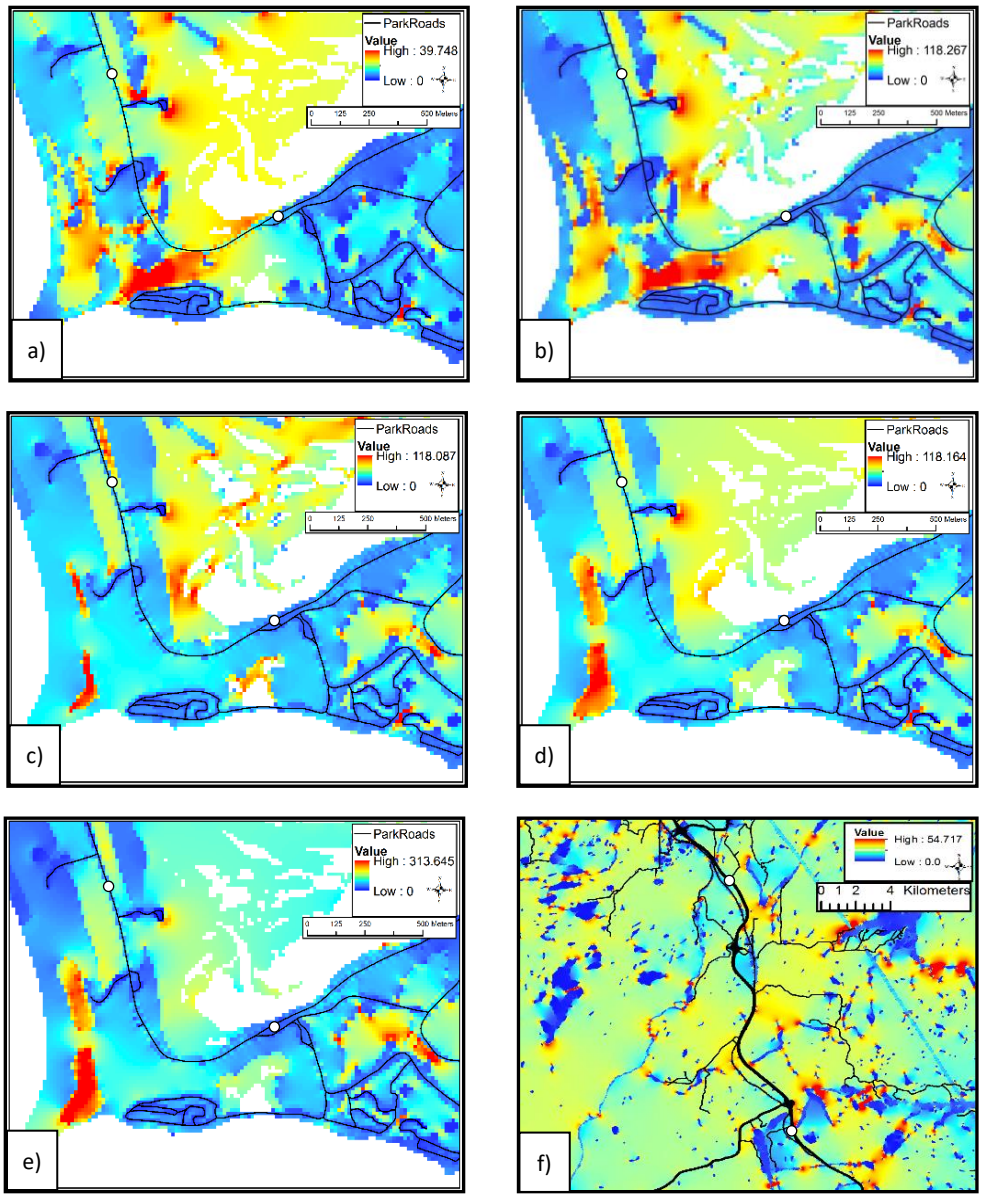


Figure 2.2 a–f. Circuit theory models (see Appendix A2.1), produced by *Circuitscape*, for Presqu'ile Provincial Park Herpetofauna (a–e) and Highway 69 large Mammals (f) depicting animal movement through the landscape. Areas with warmer colours indicate higher predicted movement volume (i.e., hotspots) relative to the rest of the map, and cooler colours indicate lower predicted movement volume (i.e., coldspots). White dots represent survey route boundaries. Resistance values for Lake Ontario (SOLRIS— open water) corresponds to model parameters (a–e); however, the lake has been clipped from the image for visual clarity.

Tables

Table 2.1. Number and width of Turtle, Snake and Amphibian hotspots observed in Presqu'ile Provincial Park, and large Mammal hotspots observed along Highway 69, south of Sudbury. All hotspots were identified using road surveys and Sireima's 2-D hotspot tool, and *Circuitscape* resistance analysis. Boxes outlined with dashed lines represent groups for which individual hotspots fell within 4% of the total length of the survey route (50 m - herpetofauna; 1000 m - large mammals) of at least one other hotspot (total number of groups indicated in brackets). Hotspot numbers organized from North to South.

.	**Road surveys and Sireima - 2D Hotspot Analysis**				**Circuitscape - Circuit Theory Analysis**					
	Turtles	Snakes	Amphibians	Mammals	Herp Model 1	Herp Model 2	Herp Model 3	Herp Model 4	Herp Model 5	Mammals
Total number of Hotspots (Hotspot Groups)	5 (4)	9 (4)	11(2)	18(7)	3(3)	2(2)	1(1)	2(2)	1(1)	9(3)
Total width of Hotspots (m)	127.6	170.2	483.0	2689.9	666.8	545.5	277.9	558.4	276.1	5186.3
Hotspot #1	2.5	10.0	20.0	146.7	120.8	55.1	277.9	319.6	276.1	382.5
Hotspot #2	7.5	20.0	67.6	48.9	55.1	490.4		238.8		4803.8
Hotspot #3	5.0	15.0	2.5	48.9	490.9					399.5
Hotspot #4	17.5	2.5	2.5	48.9						811.1
Hotspot #5	95.1	20.0	5.0	293.5						4781.9
Hotspot #6		90.1	2.5	195.6						356
Hotspot #7		2.5	15.0	195.7						3395.9
Hotspot #8		7.5	5.0	48.9						333.6
Hotspot #9		2.5	352.9	195.6						516.2
Hotspot #10			5.0	48.9						
Hotspot #11			5.0	195.6						
Hotspot #12				146.7						
Hotspot #13				244.6						
Hotspot #14				195.6						
Hotspot #15				146.7						
Hotspot #16				97.8						
Hotspot #17				244.6						
Hotspot #18				146.7						

Table 2.2. Pros and Cons of using road surveys or circuit theory for identification of hotspots during the planning stages for mitigation of road effects.

	Pros	*Cons*
Road Surveys	• Engagement of local stakeholders creates the potential for additional conservation value • Road crossing/kill points are the literal representation of where animals are most likely to cross a road within a given time frame • Can be used for Before-After-Control-Impact evaluation of mitigation • Greater number of narrow hotspots • Addresses local abundances, not specifically associated with general habitat traits	• Often must obtain landowner permission or special research permits • May not represent historical hotspots in areas with depressed populations • Can be time consuming and costly; surveys should occur throughout temporal and climatic variation to be representative • Requires planning to begin multiple years prior to mitigation installation
	Requires greater degree of logistical and monetary commitment; however, it produces fewer hotspots covering less overall distance, providing a stronger basis for mitigation evaluation; appropriate at small and large spatial scales	
Circuit Theory	• Does not rely upon, but can capitalize on, site-specific data • Specifically addresses dispersal or movement patterns of wildlife and may produce a better representation of historical patterns • Flexibility allows customization of maps for specific traits • Less costly, and can be executed much more quickly	• Specific customization of models can decrease usefulness of results • GIS access and competency critical (although free options available) • Fewer wide hotspots make it somewhat less informative in local landscapes • Cannot be used as a precursor to mitigation evaluation, and does not address local abundances
	Reduced logistical support and funding required, but wider hotspots reduce value of results; more appropriate at large spatial scales	

Table 2.3. Costs and considerations prior to initiation of mitigation planning using either road surveys, circuit theory analysis or a combination of both, to ensure the most informative results possible are obtained given resources available.

	Logistics and Funding	*Time Commitment*	*Skills Required*
Road Surveys	• One or more field staff depending on frequency and length of surveys • Vehicle costs for surveys (gas, bicycle) if not done by foot • Additional staff if control sites are to be included for robust future evaluation • Free software (e.g. Sireima) • **Can become very costly depending on # of staff and survey frequency**	• 1-3 surveys per day optimal. Distance, focal species, and data requirements dictate survey length • Multiple years of data collection prior to mitigation installation • Data handling and analysis easy to learn and use • Running analysis, even with several thousand individuals (amphibians), takes ~15 min • **Rigorous analysis requires temporal representation (i.e., full active season, multiple years)**	• Identification of target species, or species signs (i.e., tracks, scat) • Physical fitness if not done by car • Obtaining results from Sireima is relatively simple following user guide • **Relatively easy skills to acquire, simple analysis and interpretation**
Circuit Theory	• Staff with background in GIS • Access to GIS software (e.g. ArcGIS is $1500 for single use licence) • Free GIS options (QGIS, Grass GIS) available • Free software (i.e., Circuitscape) • **Requires short-term staff and free software**	• Dependent on size of study area, and prior GIS experience • GIS data handling, and raster transformation • Circuitscape run time dependant on file size and computer processor power • **Small areas can be analyzed quickly**	• Competent usage of GIS software • Using Circuitscape is relatively straight forward with GIS background and user guide • Expert knowledge of focal species, or pre-existing data to develop models, is required • **GIS and ecological background important; interpretation is intuitive**

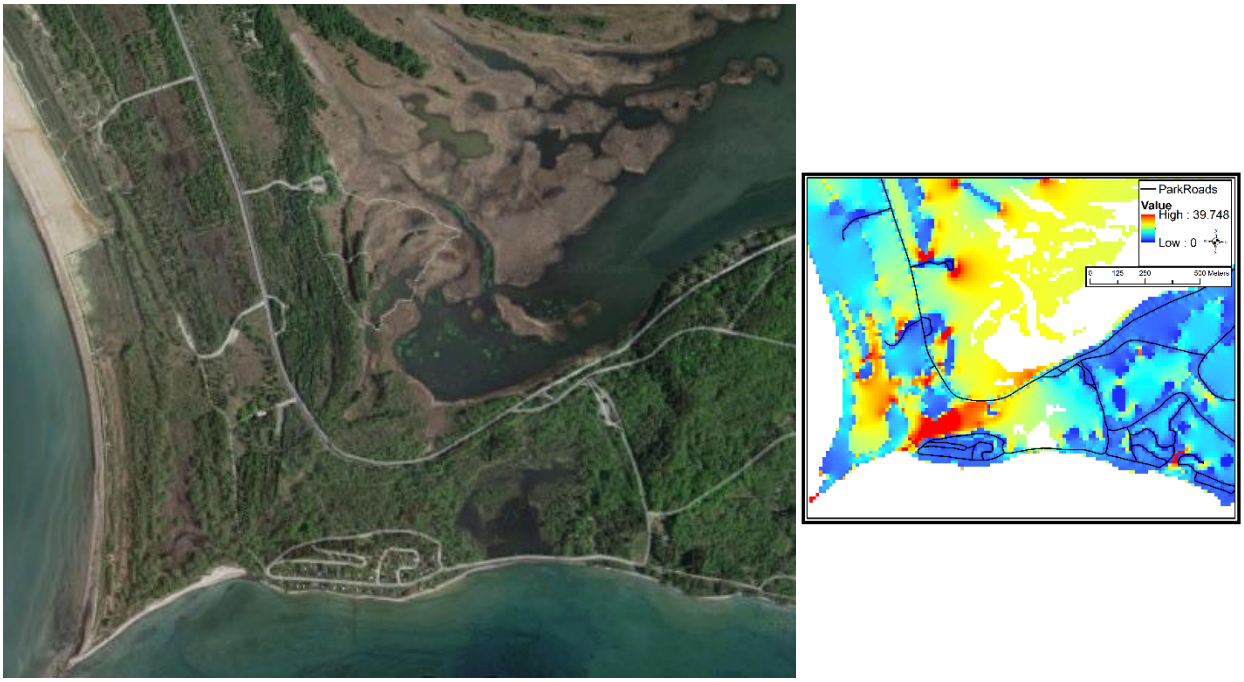
Appendix A2.1

Table A2.1. Resistance parameters used in *Circuitscape* to generate current density maps for herpetofauna to identify movement patterns across the landscape, and potential road crossing locations.

SOLARIS Class	*SOLRIS Code*	*Model 1*	*Model 2*	*Model 3*	*Model 4*	*Model 5*
Open Cliff	CTO	100	100	100	100	100
Alvar	AL	100	100	100	100	100
Open Shore	BBO	10	10	10	10	10
Open Bluff	BLO	100	100	100	100	100
Open Sand Barren and Dune	SBO	10	10	10	10	10
Treed Sand Barren and Dune	SBT	10	10	10	10	10
Open Tallgrass prarie	TPO	25	25	25	25	25
Tallgrass Savannah	TPO	25	25	25	25	25
Tallgrass Woodland	TPW	25	25	25	25	25
Forest	FO	25	25	25	25	25
Coniferous Forest	FOC	25	25	25	25	25
Mixed Forest	FOM	25	25	25	25	25
Deciduous Forest	FOD	25	25	25	25	25
Plantation - tree cultivated	CUP	25	25	25	25	25
Hedge Rows	CUH	100	100	100	100	100
Transportation	COT	100	100	100	100	100
Extraction	COE	100	100	100	100	100
Built up area - pervious	COP	100	100	100	100	100
Built up area - impervious	COI	100	100	100	100	100
Swamp	SW	1	1	1	1	1
Fen	FE	1	1	1	1	1
Bog	BO	1	1	1	1	1
Marsh	MA	1	1	1	1	1
Open Water	OA	1	10	10	10	100
Undifferentiated	UN	25	25	25	25	25

Model 1: Wetlands = low, uplands = medium (10-25), infrastructure = 100
Model 2: Model 1, but Open water = 10
Model 3: Model 2, but all habitat within 250 m of a swamp/fen/bog/marsh have a resistance of 10, road maintained at 100
Model 4: Model 3, but all habitat within 30 m of a swamp/fen/bog/marsh have a resistance of 1 as well as the 250 buffer with a resistance of 10.
Model 5: Same as Model 4, but Open Water resistance = 100

a)



b)

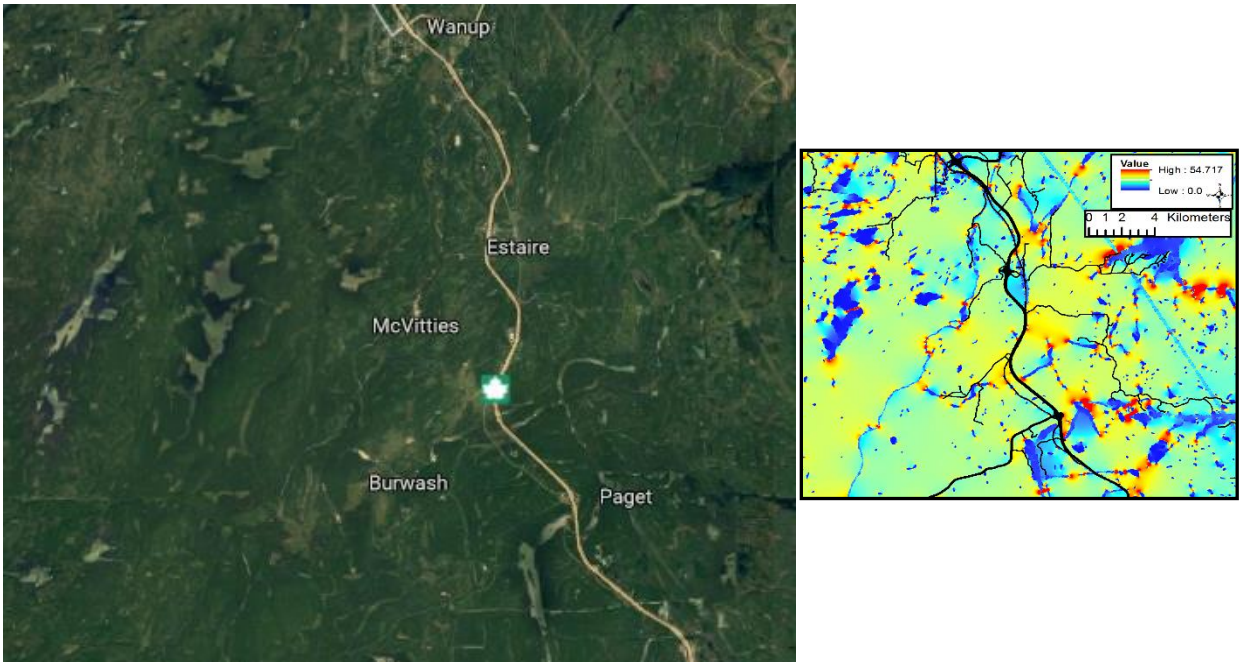


Figure A2.1. Aerial maps of study sites: a) Presqu'ile Provincial Park (reptiles and amphibians) and b) Highway 69 (large mammals)(Google 2019). Example of circuit theory models have been included for perspective.

Chapter 3

Population-level impact of road-effect mitigation: promoting connectivity and reducing mortality

Abstract

Roads are one of the most prevalent threats to reptile and amphibian populations because of their propensity to fragment the landscape and increase mortality. In response to the pervasive and severe threat of roads to population persistence, road-effect mitigation strategies have become increasingly common, typically in the form of exclusion fencing combined with connectivity tunnels. To evaluate the success of these structures, I conducted a six year Before-After-Control-Impact (BACI) experiment in Presqu'ile Provincial Park, Ontario, Canada. I used road surveys, trapping, and monitored the usage of connectivity tunnels with PIT tag scanners and camera traps. I found that exclusion fencing reduced the number of turtles and amphibians on the road, but there was no effect for snakes, possibly due to the design of the fence. Wildlife cameras detected tunnel use by 54 turtles, 72 snakes, 615 frogs and 271 salamanders. In comparison, PIT tag scanners detected tunnel use by turtles 55 times (24 unique individuals) and snakes 69 times (15 unique individuals),which for some species, represented up to 44% of the tagged individuals, by species. I contextualized this crossing rate by estimating the population size of painted and snapping turtles in the surrounding habitats. Through extrapolation, I highlight that for some species, over 15% of turtles likely use crossing structures, enough to ensure that genetic connectivity is maintained despite the road. In addition I determined that tunnel usage was in fact not spatially or demographically biased, indicating that the tunnels were used broadly by the local population. Representing one of the first studies to utilize a BACI design of this magnitude, my results demonstrate the success of exclusion fencing and connectivity tunnels, based on both decreases in road mortality and population-level metrics.

Introduction

Roads are one of the most pervasive threats to wildlife globally. Although the physical footprint of a road is relatively small, the effect-zone surrounding roads can be substantially larger (Forman & Alexander 1998; Forman & Deblinger 2000). As such, roads are considered a main driver of habitat degradation and destruction (Laurance & Balmford 2013) and can have many negative implications for wildlife (Steen & Gibbs 2004; Lengagne 2008; Owen et al. 2014; Shannon et al. 2014; Dananay et al. 2015), including direct mortality from collisions with vehicles which leads to reductions in population persistence and viability (Jaeger & Fahrig 2004; Borda-de-Água et al. 2011). This is especially true for reptiles and amphibians, which are among the fastest declining taxa globally (Gibbons et al. 2000; Böhm et al. 2013; Rhodin et al. 2018). These taxa are especially vulnerable to road-effects because of their small size, slow reproductive rates and limited road avoidance behaviour (Steen & Gibbs 2004; Rytwinski & Fahrig 2012; Andrews et al. 2015; Dodd et al. 2016). Given the vulnerability of herpetofauna to road-effects, mitigation - typically including a combination of exclusion fencing and structures to facilitate habitat connectivity (e.g., ecopassages) - have become an integral part of conservation plans for many species (Glista et al. 2009; Ontario Ministry of Natural Resources and Forestry 2016).

Despite the arguably greater conservation concern, management practices and techniques to mitigate road-effects on herpetofauna have lagged behind those for larger vertebrates (Andrews et al. 2015; Popp & Boyle 2017). Attempts to mitigate the effects of roads on herpetofauna have had mixed results. Although some studies attribute meaningful decreases in road mortality due to exclusion fencing (Aresco 2005; Colley et al. 2017), others indicate limited success or even increased mortality as a result of improper installation (Baxter-Gilbert et al.

2015; Markle et al. 2017). Similarly, connectivity structures are highly dependent on effective exclusion fencing to direct animals towards them (Cunnington et al. 2014; Baxter-Gilbert et al. 2015). Most evaluations of connectivity structures report only detection rates, often failing to quantify unique individual usage or to consider usage with respect to overall population trends (van der Grift et al. 2013; Rytwinski et al. 2015; Heaven et al. 2019; Ottburg & van der Grift 2019). Further compounding this issue, the logistical challenges associated with monitoring expansive road networks limit the ability of researchers to appropriately control their study designs (Lesbarrères & Fahrig 2012; Rytwinski et al. 2015). Despite these challenges, understanding the effectiveness of connectivity structures and exclusion fencing is paramount given their potential value to conservation, especially for rare and declining species, as well as the high economic costs associated with road-effect mitigation.

The goal of my study was to identify the value of road-effect mitigation efforts for multiple taxa using a Before-After-Control-Impact (BACI) design and to contextualize my results at a population-level. To date, no other published studies have combined these approaches to evaluate road-effect mitigation targeting herpetofauna (but see M.Sc. thesis by Colley 2015). I developed hypotheses which addressed each component of the mitigation efforts separately. To evaluate the effectiveness of exclusion fencing, I predicted that the number of herpetofauna found during road surveys would decrease after mitigation fencing was installed along the road, but that this reduction would be taxa-specific because the fencing used was designed specifically for turtles. To elucidate the dynamics of tunnel usage, I questioned if usage was disproportionate either demographically or spatially. I hypothesized that if tunnel usage was demographically skewed, proportions of marked males, females and juveniles detected using the tunnel would differ and not be representative of marked individuals overall. I also hypothesized that

individuals with mean capture locations closer to the tunnels would be more likely to use them. Further, to evaluate the ability of tunnels beneath the road to facilitate landscape connectivity, and to determine if exclusion fencing was increasing the barrier effect of the road, I quantified the total tunnel usage by herpetofauna and the number of unique reptiles using the tunnels.

Methods

Study Site

My study took place inside and outside, but close too, Presqu'ile Provincial Park (PP), Ontario, Canada (43.992328, -77.724742). Presqu'ile PP is a tombolo-formed peninsula extending into Lake Ontario (Figure 3.1). The main road of the park, which at the time of this study, experienced a traffic volume ranging from 161-212 vehicles/hour during my summer field season (Boyle et al. *unpublished data*), extended from the park entrance to the heel of the peninsula, bisecting multiple types of terrestrial (i.e., old panne, mixed wood forest) and wetland habitats (i.e., panne, swamp, marsh). Presqu'ile PP is home to a broad assemblage of herpetofauna, including five species of turtles, five species of snakes, nine species of frogs, and three species of salamanders (Appendix A3.1). The main road of Presqu'ile PP has been identified as a major source of local road mortality, thus providing an opportunity to execute a BACI study to rigorously determine the impact of road-effect mitigation structures along this linear structure.

Experimental Design and Mitigation Structures

My study was six years long (2013-2018), with two field seasons of monitoring prior to the installation of mitigation, one field season while fencing was being erected, and three field

seasons post-installation. In October 2015, between my second and third field seasons, two crossing structures (ACO AT-500; 10 m length x 0.5 m width x 0.32 m height with a slotted top to allow light and rain penetration) were installed beneath the road during resurfacing (Figure 3.1). Beginning late-June 2016, approximately 1000 m of *Animex* 'Vertical Above Ground' exclusion fencing (Hampshire, England; Animex© International) was installed along both sides of the road (hereafter the impact site). The fencing was 0.865 m tall with a 0.15 m perpendicular lip pointing away from the road, and was erected above ground, supported by posts with a second perpendicular lip pointing towards the road for stability. As a cost-saving measure, approximately 0.1 m of gravel was used to bury and secure the fence rather than digging a trench in which to bury it. Because exclusion fencing was installed over the course of 2016, and because previous studies have shown that incomplete fencing can lead to negative outcomes (Baxter-Gilbert et al. 2015), I considered this a 'during' phase in my design, in order to detect any impact of partial/incomplete mitigation. My control site was located immediately outside of the park along a connecting road and had no mitigation features installed along it (Figure 3.1), but had similar adjacent habitat, traffic volume and speed limit.

Road Surveys

Surveys (1250 m in length) were executed three times daily by bicycle along two sites (control and impact sites; Figure 3.1) at 9h00, 18h00 and 22h00 from 2013-2018. Except for 7-10 days of training at the onset of each field season, surveys were performed simultaneously by one member of the field crew at each site to pair my design. Sites were randomly assigned a surveyor daily to limit individual biases. Following the bicycle survey at 9h00, the survey route was walked in order to maximize detections (Baxter-Gilbert et al. 2017). For logistical reasons, in 2018, only one site was surveyed each day. Further, the site surveyed each day was still

selected randomly, but surveyed twice daily instead of three times, contrary to the previous five field seasons. The 18h00 bicycle survey was excluded because it consistently yielded considerably lower capture rates throughout the previous five field seasons.

On surveys, all detections of live and dead herpetofauna were recorded, and processed immediately. In 2013-2017, this included recording a geospatial reference (Kansas, USA; Garmin eTrex 10), species, life stage (adult, juvenile) and sex (if sexually dimorphic), as well as the snout-to-vent length (SVL) for snakes, and mid-carapace length (MCL) for turtles. In 2018 however only species counts were included. For identification purposes, live reptiles over 30 g and captured within 1 km of the impact site were equipped with passive integrated transponder tags (PIT; Idaho, USA; Biomark HPT12 tags) from 2014 onwards. Additionally, all turtles from 2013 onwards and regardless of where they were captured, were marked with a unique notch code (Cagle 1939). After animals were processed, they were released at the site of capture. Animals found dead were removed from the road to prevent recounting.

Tunnel and Population Monitoring

Hoop traps (1 m diameter) were set in the wetlands adjacent to the impact survey site to catch turtles (Figure 3.1). Each year from 2013-2017, between 6-8 traps were set daily (6 in 2013 and 8 in 2014-17). Suitable trapping areas were divided into 43 equally-sized quadrats (85 x 85 m each/ total = 31.1 ha). Every four days, a new trap location was assigned randomly with replacement and traps were re-baited with ~42 g of canned sardines. The wetlands in which I set traps were part of a much larger wetland complex typical of the north shore of Lake Ontario, so I focused on catching individuals within the immediate vicinity of fencing and tunnels to increase the chance of capturing individuals that would interact with mitigation. All turtles captured via hoop trap were also equipped with PIT tags as described above.

Two methods were used to quantify herpetofauna usage of the tunnels installed beneath the road of Presqu'ile PP. First, wildlife cameras (Kansas, USA; Bushnell TrophyMAX) were installed at both ends of each tunnel approximately 2 m from the entrance (Figure 3.1). Cameras were set to use high sensitivity infrared triggers from July 5 – September 30 in 2016. To maximize detection rates, in 2017 cameras were set to take photographs both on infrared triggers and every minute from May 1 - Oct 27, following previous studies (Pagnucco et al. 2011; Baxter-Gilbert et al. 2015; Colley et al. 2017). I tabulated all entrances and exits from the tunnel, and any detections in which animals seemed to pass by tunnels without interacting with them, in order to interpret usage rates. To reduce temporal autocorrelation and double counting individuals, images of the same species captured by the same camera within 10 minutes of each other (assuming they could not be distinguished using distinct markings or size differences) were considered a single occurrence. For snakes and turtles, tunnel interactions detected by trail cameras were cross-referenced to identify full crossings. Second, inside the northern entrance of each tunnel, a PIT tag scanner (Idaho, USA; Biomark IS1001 reader connected to 0.45 x 0.45 m antennae) was buried on the floor of the tunnel, blending in with the substrate, which consisted of gravel, soil, leaves and fine woody debris. PIT tag scanners were active in both tunnels from ~May 1 – October 15 of 2016-2018. When any PIT-tagged animal passed over the scanner, their unique identification code was stored externally to a flash drive.

Analyses

All analyses were conducted using the program R v.3.4.1 (R Development Core Team 2018) and graphs were created using ggplot2 (Wilkinson 2011). To compare the number of herpetofauna detected during road surveys, I created Generalized Additive Models (GAMs) for each taxon (turtles, snakes, amphibians) because they allowed me to tease apart the effect of both

seasonality and yearly variation. I constructed my models based on the daily road survey counts, and included the variables site (impact or control), time period (before, during, or after construction), and an interaction between site and time period. Both within-season date and between-season date were used as smoothing factors, to account for the variation associated with both seasonality (i.e., comparing May 1 2017 to August 1 2017) and inter-yearly differences (i.e., comparing May 1 2015 to May 1 2017), respectively (Zurr et al. 2009)

Trail cameras photos (~1.5 million) were manually reviewed and identified to taxa, and if possible, species. I tabulated all detections of herpetofauna and non-herpetofauna by trail cameras and created frequency distributions to visualize temporal patterns of herpetofauna tunnel usage. To understand the detection probabilities of the wildlife cameras I employed, I used associated data from behavioural trials conducted at the same tunnels from Dillon et al. (*in review*). These trials investigating the tunnel crossing dynamics of garter snakes allowed me to calculate the probability that my trail cameras would detect garter snakes exiting a tunnel by cross-referencing known instances when snakes exited tunnels during behavioural trials against my trail camera detections from the same time (i.e., the proportion of known snake exits captured by my cameras). I similarly tabulated and a visualized tunnel usage patterns by PIT-tagged reptiles.

To identify if tunnel usage was influenced either spatially or demographically, I used binomial link Generalized Linear Models (GLMs) for snakes and turtles separately. Only individuals which were alive and had PIT tags while PIT tag scanners were active were included in this analysis to meet the assumption of equal detectability; however, animals were considered alive unless specifically found dead. Predictor variables for turtle usage included species (Blanding's – *Emydoidea blandingii*, snapping – *Chelydra serpentina*, and painted turtles –

Chrysemys picta), MCL, sex (male, female), life stage (adult, juvenile) and distance of previous captures to tunnel. Because my turtle species variable included three levels, I conducted a likelihood ratio test in order to confirm its effect. For snakes, I included the following predictors: SVL, sex and distance of previous captures to tunnel. I did not PIT-tag juvenile snakes because of their small size and thus excluded them from this analysis. Further, I were unable to compare species differences for snakes because the only two species in Presqu'ile PP large enough to safely implant PIT tags are garter snakes (*Thamnophis sirtalis*) and milksnakes (*Lampropeltis triangulum*). Unfortunately, only one milksnake was tagged and it was subsequently found dead on the road prior to the installation of tag readers. In instances where an individual was captured multiple times, their capture locations were averaged and the distance from this point to either the tunnel used, or the nearest tunnel (for non-users), was measured. The nearest tunnel was chosen for non-users to be conservative.

To determine the population size of painted and snapping turtles, I used an open population Cormack-Jolly-Seber estimate from the R package FSA (Ogle et al. 2018). For my estimate, I considered years as individual trapping occasions. Capture data from all trapping sessions (~100 days, 2013-2017) and impact site surveys (2013-2017) were included in my estimate. Although trapping effort varied from year to year (570 - 864 trap nights, 6 traps in 2013, 8 traps in 2014-2017), capture rates were low, and thus I assumed equal effort to develop a simple estimate of population size. To contextualize the effectiveness of mitigation installed along the road in Presqu'ile PP I used the most up to date estimate I could generate with the data available.

Results

Road mortality

Relative to the control site, the numbers of both turtles (Figure 3.2a; df = 2, X^2 = 27.6, P = 1.02 x 10^{-6}) and amphibians (Figure 3.2b; df = 2, X^2 =13.0, P = 0.015) detected on road surveys were greatly reduced after exclusion fencing was completely installed. Although the number of turtles tended to be lower in the 'during' phase compared to the 'before' phase, I found no significant effect of partial mitigation for either turtles or amphibians. In contrast, the abundance of snakes detected on roads did not differ among the phases of my study, that is, mitigation did not reduce snake abundance on roads (Figure 3.2c; df =2, X^2 = 0.2, P = 0.92).

Tunnel Usage

My trail cameras detected 126 reptiles and 886 amphibians interacting with the tunnel (Table 3.1, Appendix A3.2). An additional 116 reptiles and 1415 amphibians were detected but appeared to ignore the tunnel (Table 3.1). Trail cameras detected complete crossings by eight snapping turtles and three garter snakes. An additional 38 turtles and 66 snakes were detected either entering or exiting the tunnel. The motion activated triggers of my wildlife cameras were most likely to detect turtles (56%) compared to snakes (14%), and especially amphibians (2%). Further, only half of all known garter snake exits (from behavioural observations; Dillon et al. *in review*) were detected by my wildlife cameras (11/22). Detections varied temporally and between species. Turtles were most commonly detected in June with smaller peaks in May and August, snakes in June and September, and salamanders in October (Figure 3.3a-c). Frogs were detected frequently from July - October (Figure 3.3d). A wide assemblage of small (e.g., shrews, mice, chipmunks, and squirrels) and mesomammals (e.g., raccoons (*Procyon lotor*), rabbit

(*Sylvilagus floridanus*), opossum (*Didelphis virginiana*), and ermine (*Mustela erminea*)) were also detected interacting with the tunnels, whereas birds rarely interacted with the tunnels (Table 3.2).

In total, 197 turtles (44 painted turtles, 144 snapping turtles and 9 Blanding's turtles) and 42 snakes (41 garter snakes and 1 milk snake) were PIT tagged, and my PIT tag scanners detected reptile tunnel usage 125 times. These detections corresponded to 43 unique individuals (22 snapping turtles, 2 painted turtles and 1 Blanding's turtle and 18 garter snakes; Table 3.2). Of the 43 individuals which I detected using the tunnels, 39 had GPS locations associated with their captures (i.e., four individuals did not have GPS locations due to GPS failure). Of these 39 individuals, 95% had a mean previous capture location within 700 m of the tunnel they used, and 87% had a mean capture location within 400 m. At the extreme, one adult male snapping turtle had a mean previous capture location ~1600 m from the tunnel it used, nearly three times as far as the next furthest turtle's capture location (Figure 3.4). The greatest mean previous capture location for a snake was ~850 m, though this was much more similar to other snakes compared to the vagrant turtle.

For snakes, I found no evidence of the tunnel usage was disproportionate compared to the overall population with respect to size ($z = -1.39$, $P = 0.15$), distance of previous capture locations from tunnels ($z = 0.03$, $P = 0.98$), sex ($z = 0.83$, $P = 0.41$). Similarly, for turtles, size ($z = 1.63$, $P = 0.10$), distance ($z = -1.00$, $P = 0.32$), sex ($z = -0.28$, $P = 0.78$), life stage ($z = 0.01$, $P = 0.99$), and species ($X^2 = 1.10$, $P = 0.58$) had no effect on likelihood of tunnel usage.

Population-level usage

Despite relatively high trapping effort (~100 survey days and 570-864 trap nights in each season), my annual population size estimates for turtles were highly variable (Table 3.3). Capture rates for snapping turtles were too low to estimate population size in 2017, and too low in both 2016 and 2017 for painted turtles. In 2015, my estimate of the painted turtle population size was 216; however, the confidence limits approached infinity because of limited capture and recapture rates. In 2014, my estimate of the painted turtle population size was 45 ± 109 turtles. My 2016 estimate of the snapping turtle population size was 497 ± 770. Thus, I contextualized tunnel usage to population estimates from 2014 for painted turtles and 2016 for snapping turtles. Relative to these population estimates, a minimum of ~4.5% of each species used the tunnels. Relative to the number of tagged individuals, 15.2% of snapping turtles, 4.5% of painted turtles, 11.1% of Blanding's turtles and 43.9% of garter snakes were detected using tunnels.

Discussion

Road mortality

The exclusion fencing installed in Presqu'ile PP to mitigate herpetofauna road mortality significantly reduced the number of turtles and amphibians on the road compared to my control site. This result is crucial because road mortality is a persistent threat for many herpetofauna, especially freshwater turtles (Steen & Gibbs 2004; Gibbs & Shriver 2005; Dodd et al. 2016). Contrary to my expectations and the trends I observed for other taxa, mitigation structures had no impact on the number of snakes detected on road surveys. This is concerning because although snakes typically have higher natural mortality rates compared to turtles and thus the additive

effect of road mortality would be proportionally smaller (annual survivorship ~68-98% for snakes versus ~90-95% for turtles; Larsen & Gregory 1989; Keevil et al. 2018), this additive mortality would still likely lead to population declines (Andrews et al. 2015). This is possibly because the mitigation in Presqu'ile PP primarily targeted turtles which more than other taxa, are especially at risk from road mortality due to their life history (Steen & Gibbs 2004; Steen et al. 2006; Dodd et al. 2016). This species-specific outcomes of road-effect mitigation emphasize the need for targeted mitigation efforts to maximize success (Lesbarrères et al. 2004).

My road mortality results reinforce the need for multiple years of monitoring in both 'before' and 'after' phases of mitigation. My study did include two years of monitoring post-mitigation; however, in the first year of my 'after' phase (2017), Presqu'ile PP experienced a severe flood. Thus, an alternative explanation for the apparent null effect of exclusion fencing on snake abundance deserves attention. The slope from the road towards the lake was smaller for the majority of the impact site as compared to the control site and thus flooding likely limited terrestrial habitat to a greater extent at the impact site. It is therefore possible that snakes, the most terrestrial of all the taxa I studied, were more abundant along the impact site road surveys because of less available terrestrial basking opportunities or refugia. However, although I suggest there was an effect of the flood, it also appeared that the fencing was much more permeable to snakes compared to turtles or amphibians. Often when snakes were observed on the road or fence, they were able to escape through areas where sections of fencing met (Boyle et al. 2019). The combination of higher permeability and reduced terrestrial habitat during flooding likely explains the limited exclusionary effect of the fencing on snakes, and without multiple years of monitoring before and after mitigation, this may have confounded my results or have been missed entirely.

Tunnel usage

Tunnel usage has been shown to depend on exclusion fencing (Cunnington et al. 2014). Alternatively, exclusion fencing, when not combined with road-crossing structures (e.g., tunnels), has the potential to reduce connectivity, leading to genetic bottlenecks and an eventual reduction in population persistence (Jaeger & Fahrig 2004). I found that the likelihood of tunnel usage was not skewed demographically, spatially, or on a species-specific basis. Although contrary to my expectations, this result is promising, especially considering the number of unique individuals I detected using tunnels to cross the road. Although I could not document instances of successful reproduction and recruitment by tunnel users because of the life history of reptiles, only a few individuals are required per generation to maintain genetic continuity (Mills & Allendorft 1996; Wang 2004). Relative to the study years with my most reliable population size estimates (2014 for painted turtles and 2015 for snapping turtles), I observed approximately 4.5% of both painted and snapping turtle populations using tunnels, which should be a sufficient proportion of the population to maintain genetic connectivity (Mills & Allendorft 1996; Wang 2004).

Overall, both PIT tag scanners and wildlife cameras indicated tunnel usage was high, which is contrary to other studies of that used either similar methods or behavioural trials to quantify tunnel usage (Baxter-Gilbert et al., 2015; Colley et al., 2017; Dillon et al., *in review*.; Hamer et al., 2014, Jarvis et al., 2019; van der Ree, Mahony, & Langton, 2014; Pagnucco et al., 2011). The difference could be due in part to high animal density in my field site; however, the placement of mitigation structures in Presqu'ile PP was uniquely based on both road survey data and habitat resistance modelling (Boyle et al. 2017), potentially increasing their effectiveness. I assume the detection probability for PIT tag readers was high because scanners were optimized

upon installation and scanned very rapidly. By contrast, wildlife cameras only detected approximately 50% of known garter snake crossings, suggesting that a considerable number of snakes (and likely other herpetofauna) went undetected when crossing. This caveat/challenge associated with camera trapping, also observed by Pagnucco et al. (2011), is bolstered by the fact that many individuals appeared to be entering or exiting the tunnel, but a full crossing could not be confirmed because detection rates were low. I set cameras to take photographs both on an infrared motion trigger and on a one-minute timer. Turtle detections were made by infrared scanners only 56% of the time; however, in comparison, only 14% of snake detections were captured using infrared motion triggers. Further, timer trigger detections rely on animals moving slowly enough to be in the frame thus also providing imperfect estimates of usage. Trail cameras are a useful tool for monitoring tunnel usage; however, the ectothermic nature of herpetofauna reduces the effectiveness of infrared triggers. Given the low detection probabilities I observed with each method, my usage estimates should be considered highly conservative.

Population-level usage

Only tagged animals could be detected by tag scanners, and therefore usage rates are better contextualized relative to the tagged population. While for painted turtles, the difference between the total number of tagged painted turtles and my 2014 estimate of their population size is negligible (tagged = 44, population = 45), I only tagged 29.0% of snapping turtles. Through extrapolation, I suggest that in fact, the proportion of snapping turtles using tunnels is closer to 15.5% of the population. The confidence limits of my 2015 painted turtle population size estimate approached infinity, but was much higher compared to the previous year. I suggest that in 2014, I likely underestimated painted turtle population size. Although this may indicate that relatively fewer painted turtles are using crossing structures, the proportion of tagged individuals

remains the same, and overall the usage rates relative to the total population are likely still high enough to successfully promote connectivity. Tunnel usage rates of Blanding's turtles and gartersnakes relative to the number of tagged individuals were 11.1% and 43.9%, respectively, also suggesting substantial population-level usage of the tunnels. In all cases, usage estimates are well within the acceptable range for genetic connectivity, given the generation time of turtles (~30-40 years; COSEWIC 2018), and snakes (~4 years; COSEWIC 2010). Unfortunately, due to the life history of turtles (i.e., slow reproduction, high juvenile mortality) it was impossible to detect shifts in population size within the time scale of this project. However, together my results indicate that a substantial portion of the reptile community remains unaffected by the barrier effect of the fence, suggesting habitat connectivity has been maintained through mitigation.

Conclusions

Proper and thorough planning is critical for effective mitigation of negative road effects. This is especially true for commonly applied conservation techniques such as the exclusion fencing-crossing structure model used to mitigate the impacts of roads on wildlife, especially herpetofauna. Through the combination of a robust BACI study design spanning over 6 years and rigorous field methodology, my results strongly support my prediction that the exclusion fencing and crossing structures in Presqu'ile PP are effectively reducing road mortality rates while maintaining landscape connectivity of local herpetofauna. Further, I contextualized my results by estimating the local population size for two species of turtles, addressing a crucial gap in our understanding of road-effect mitigation dynamics at the population-level (Rytwinski et al. 2015). My approach also allowed me to identify and control for yearly variation, which has confounded many previous studies (Lesbarrères & Fahrig 2012; Rytwinski et al. 2015). Despite

rare examples of rigorous road-effect mitigation research (Sawaya et al. 2013, 2014; Baxter-Gilbert et al. 2015; Ford et al. 2017; Markle et al. 2017; Gilhooly et al. 2019; Jarvis et al. 2019; Ottburg & van der Grift 2019), my study is one of the first to identify that mitigation structures can effectively reduce road mortality while facilitating connectivity for a substantial proportion of the overall population.

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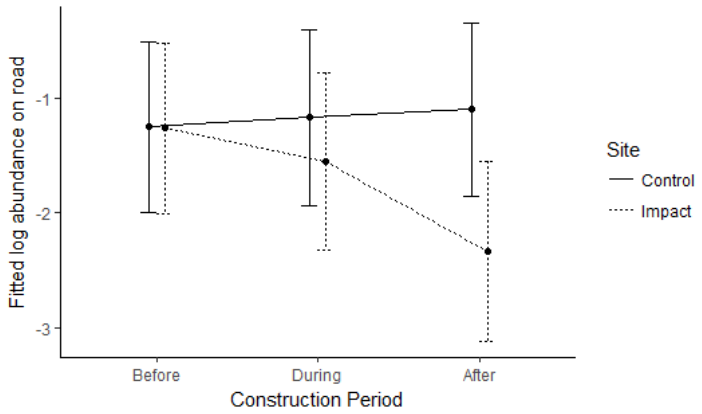
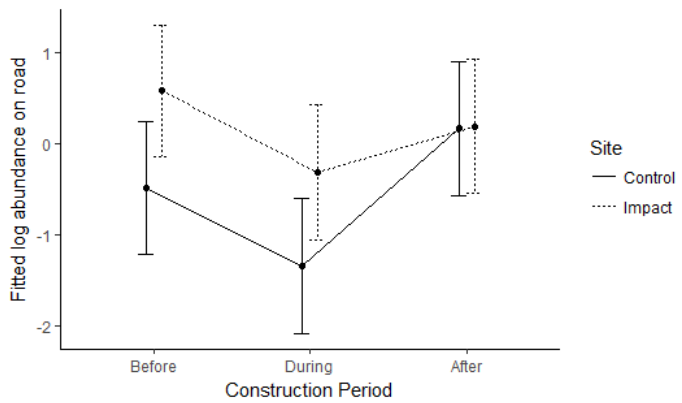
Figures

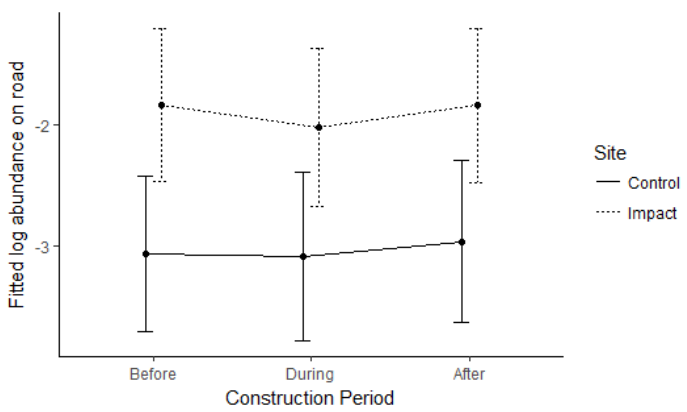
Figure 3.1. Aerial image of study site (Google 2019; https://www.google.com/maps/@44.0049476,-77.7222449,5191m/data=!3m1!1e3). White diamonds (◊) indicate locations of crossing structures and red circles (●) indicate fence ends. Straight lines (▬) indicate survey start and end points, as the survey route does not overlap entirely with the fencing. Impact site symbols are in red, and control site symbols are in yellow. Hoop traps were set out randomly throughout all wetlands within the dashed box (~31 ha of trappable area). Inlaid image is an example of the tunnel and fencing used, with trail camera positioned on post facing tunnel opening.



(a)



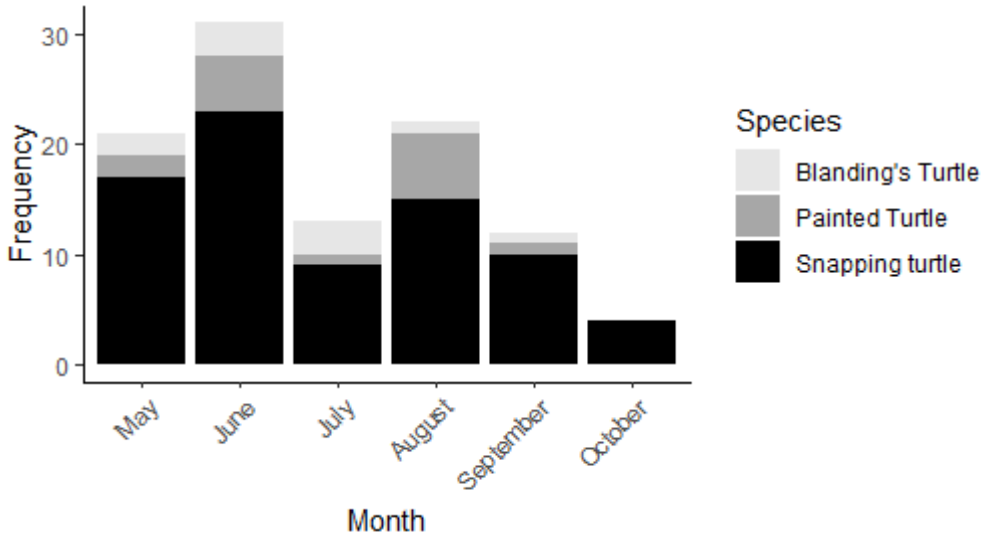
(b)



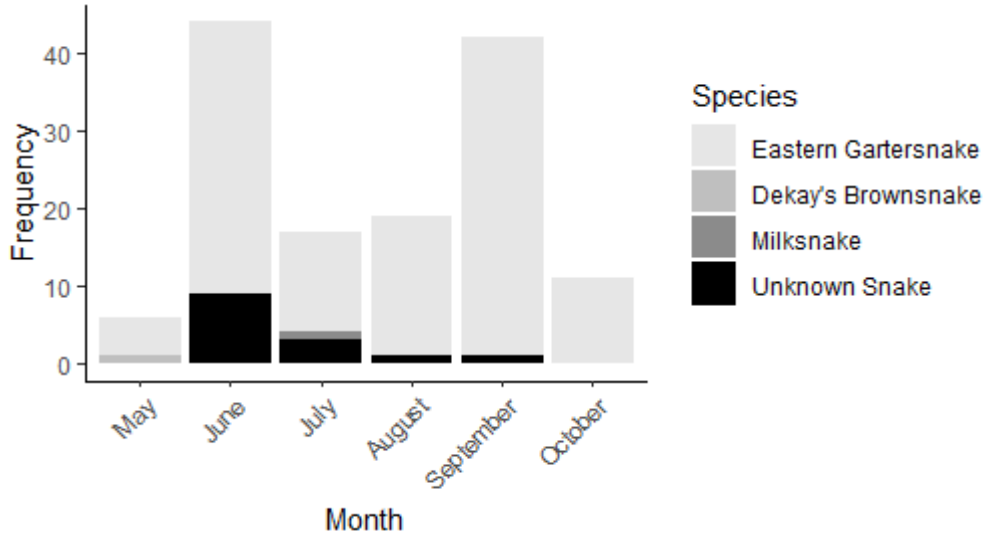
(c)

Figure 3.2. Log abundance of (a) turtles, (b) amphibians and (c) snakes found during standardized road surveys along control and impact sites. Surveys were conducted 'before',

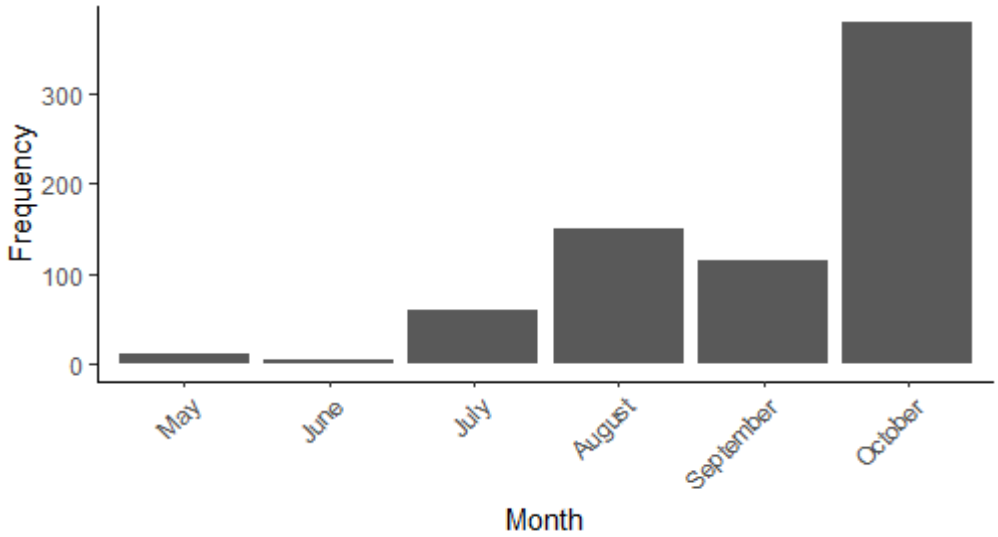
‘during’ and ‘after’ installation of ~1000 m of exclusion fencing at the impact site, along the main road of Presqu’ile Provincial Park, ON, Canada. Within- and between season dates were used to account for seasonal and yearly variation. Note the significant effect of mitigation in ‘after’ phase as compared to previous phases for both turtles and amphibians, but not for snakes.


Frequency
30
20
10
0
May
June
July
August
September
October
Month
Species
Blanding's Turtle
Painted Turtle
Snapping turtle

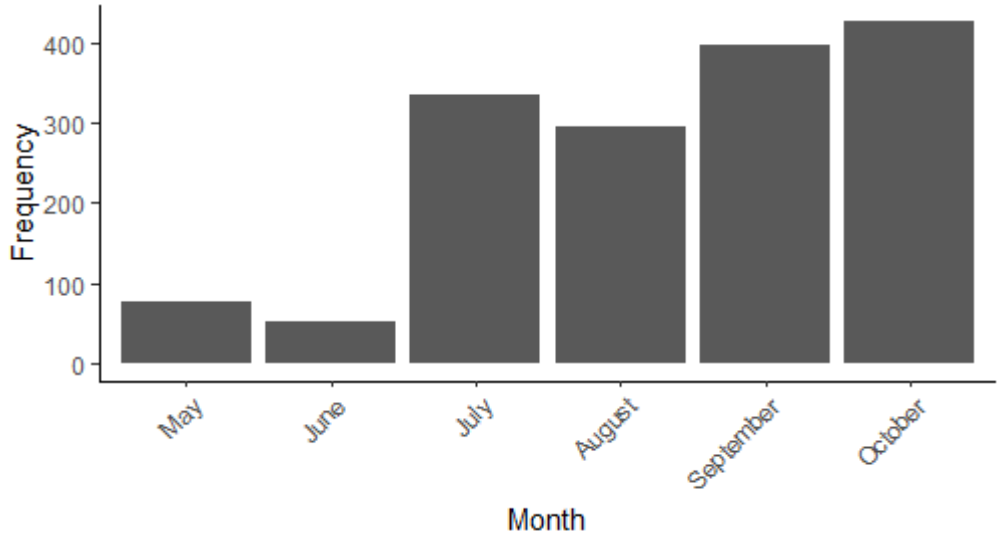
(a)


Frequency
40
30
20
10
0
May
June
July
August
September
October
Month
Species
Eastern Gartersnake
Dekay's Brownsnake
Milksnake
Unknown Snake

(b)



(c)



(d)

Figure 3.3. Monthly counts of (a) turtles, (b) snakes, (c) salamanders, and (d) frogs detected by trail cameras positioned outside of the entrances of two AT-500 tunnels located in Presqu'ile Provincial Park, ON, Canada in 2017. All cameras were set to record images on both a motion trigger and a one-minute timer.

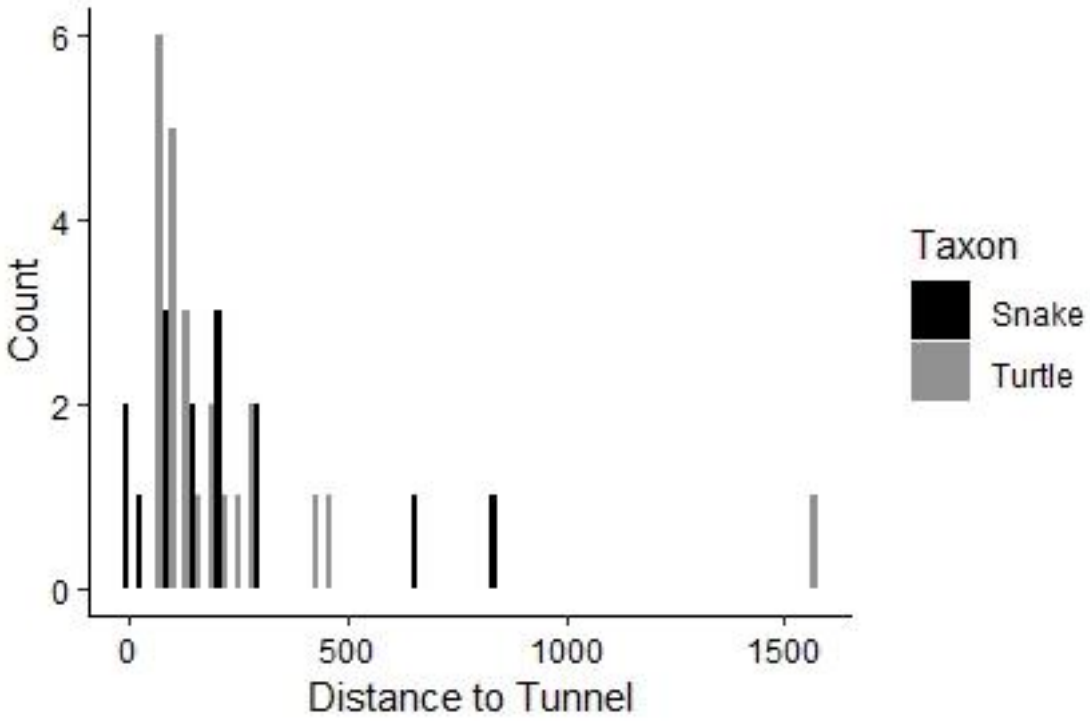


Figure 3.4. Frequency distribution of the average distance from previous capture locations to crossing structures where snakes and turtles were detected by PIT tag scanners. Scanners were inside ACO AT-500 crossing structures located along the main road in Presqu'ile Provincial Park, Ontario, Canada, and operational May-October in 2016 and 2017. The clustered count within 500 m is likely because tunnels were in the center of my capture radius. Note, one turtle was captured both within and beyond the area in which I outfitted turtles with PIT tags, thus had an average capture ~1600 m from the tunnels.

Tables

Table 3.1. Summary of herpetofauna detections by trail cameras positioned at the entrances of two tunnels (one camera at the northern and southern ends of both the eastern and western tunnels) in Presqu'ile Provincial Park, Canada, separated by species for each tunnel in 2017. Use is defined as any instance where an animal either exited or entered a tunnel. Trigger % refers to the proportion of detections using the infrared motion trigger instead of the trigger on a one minute timer. Note only eight snapping turtles and three gartersnakes were confirmed to have crossed completely.

Camera ID:	***Southeastern***			***Northeastern***			***Southwestern***			***Northwestern***			***Total Uses***	***Total Ignores***
Species:	***Use***	***Ignore***	***Trigger %***	***Use***	***Ignore***	***Trigger %***	***Use***	***Ignore***	***Trigger %***	***Use***	***Ignore***	***Trigger %***		
Turtles	*35*	*17*	*38.5%*	*9*	*25*	*38.2%*	*6*	*6*	*75.0%*	*4*	*1*	*20.0%*	54	49
Blanding's Turtle	0	2	0.0%	0	5	40.0%	1	1	100%	1	0	0.0%	2	8
Painted Turtle	5	5	50.0%.	0	4	25.0%	0	1	100%	0	0	n/a	5	10
Snapping Turtle	30	10	37.5%	9	16	40.0%	5	4	66.7%	3	1	25.0%	47	31
Snakes	*23*	*10*	*8.6%*	*6*	*15*	*38.1%*	*21*	*7*	*21.4%*	*22*	*35*	*5.3%*	72	67
Gartersnake	20	9	6.5%	5	13	33.3%	21	7	21.4%	18	30	6.3%	64	59
Milksnake	1	0	100.0%	0	0	n/a	0	0	n/a	0	0	n/a	1	0
Dekay's Brownsnake	0	0	n/a	0	0	n/a	0	0	n/a	0	1	0.0%	0	1
Unknown snake sp.	2	1	0.0%	1	2	66.7%	0	0	n/a	4	4	0.0%	7	7
Amphibians	*412*	*948*	*1.1%*	*21*	*70*	*26.4%*	*372*	*364*	*1.5%*	*81*	*33*	*0.9%*	886	1415
Frog	299	588	1.2%	20	64	26.2%	247	285	0.7%	49	29	1.3%	615	966
Salamander	113	360	0.8%	1	6	28.6%	125	79	2.6%	32	4	0.0%	271	449
Other	393	315	82.0%	79	311	72.6%	497	212	71.0%	412	275	92.0%	1381	1113
Small mammals	310	193	81.5%	44	138	80.8%	414	147	76.3%	318	172	92.7%	1086	650
Mesomammals	65	30	87.4%	27	19	84.8%	58	20	83.3%	80	31	96.4%	230	100
Birds	9	73	87.8%	2	149	60.3%	6	20	75.0%	8	68	84.2%	25	310
Unknown	9	19	50.0%	6	5	54.5%	19	25	7.1%	6	4	70.0%	40	53

Table 3.2. Summary of reptile detections by PIT tag scanners during three summer field seasons (2016 – 2018). PIT tags readers are located inside the northern entrance of two AT-500 tunnels installed beneath the road in Presqu'ile Provincial Park, Ontario, Canada. The corresponding number of unique individuals is denoted in brackets beside the total number of detections.

Species	***Male***	***Female***	***Juvenile***	***Total***
Turtles				
Blanding's Turtle	0 (0)	1 (1)	0 (0)	1 (1)
Snapping Turtle	22 (9)	26 (10)	4 (2)	52 (21)
Painted Turtle	0 (0)	3 (2)	0 (0)	3 (2)
Snake				
Eastern Gartersnake	36 (4)	33 (11)	0 (0)	69 (15)

Table 3.3. Cormack-Jolly-Seber open population estimates for snapping and painted turtle populations in Presqu'ile Provincial Park, Ontario, Canada. The placeholder n/a is used to denote years in which data was insufficient to generate population estimates. Associated confidence intervals (95%) were able to be generated except in 2015 for painted turtles. Note, population estimates represent individuals found within approximately 1 km of the impact site, where mitigation features were installed along the main road of the Park.

	Year				
	2013	2014	2015	2016	2017
Snapping Turtle	n/a	1063 ± 1180	255 ± 223	497 ± 770	n/a
Painted Turtle	n/a	45 ± 109	216 ± infinity	n/a	n/a
Trap Nights	570	688	864	744	784

Appendix A3.1

Table A3.1. Summary of reptile and amphibian assemblage found in Presqu'ile Provincial park, Ontario, Canada and their respective conservation status under federal (Species at Risk Act - SARA) and provincial law (Endangered Species Act - ESA).

Species	**SARA**	**ESA**
Turtles		
Snapping Turtle (*Chelydra serpentina*)	Special Concern	Special Concern
Midland Painted Turtle (*Chrysemys picta marginata*)	Special Concern	Not assessed
Blanding's Turtle (*Emydoidea blandingii*)	Threatened	Endangered
Northern Map Turtle (*Graptemys geographica*)	Special Concern	Special Concern
Eastern Musk Turtle (*Sternotherus odoratus*)	Threatened	Special Concern
Snakes		
Eastern Gartersnake (*Thamnophis sirtalis sirtalis*)	Not assessed	Not assessed
Milksnake (*Lampropeltis triangulum*)	Special Concern	Special Concern
Dekay's Brownsnake (*Storeria dekayi*)	Not assessed	Not assessed
Red-bellied Snake (*Storeria occipitomaculata*)	Not assessed	Not assessed
Frogs		
Green Frog (*Lithobates clamitans*)	Not assessed	Not assessed
American Bullfrog (*Lithobates catesbeianus*)	Not assessed	Not assessed
Wood Frog (*Lithobates sylvaticus*)	Not assessed	Not assessed
Northern Leopard Frog (*Lithobates pipiens*)	Not assessed	Not assessed
Mink Frog (*Lithobates serpentrionalis*)	Not assessed	Not assessed
Spring Peeper (*Pseudacris crucifer*)	Not assessed	Not assessed
Western Chorus Frog (*Pseudacris triseriata*)	Threatened	Not at Risk
Grey Treefrog (*Hyla versicolor*)	Not assessed	Not assessed
American Toad (*Anaxyrus americanus*)	Not assessed	Not assessed
Salamanders		
Blue-spotted Salamander (*Ambystoma laterale*)	Not assessed	Not assessed
Spotted Salamander (*Ambystoma maculatum*)	Not assessed	Not assessed
Eastern Red-backed Salamander (*Plethodon cinereus*)	Not assessed	Not assessed

Appendix A3.2

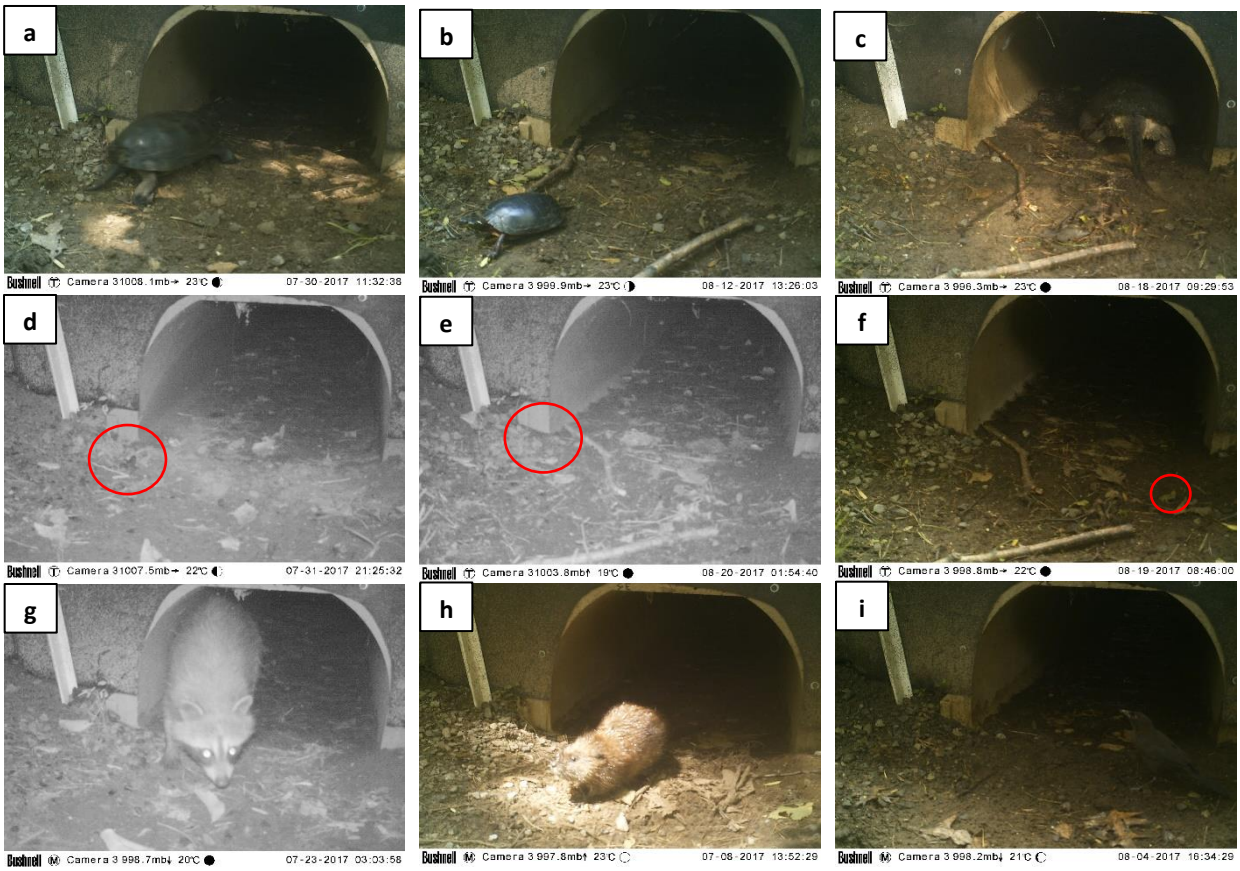


Figure A3.1. Examples of wildlife camera photos. (a) Blanding's Turtle entering tunnel, (b) painted turtle exiting tunnel, (c) snapping turtle entering tunnel, (d) frog about to enter tunnel – highlighted in in red circle, (e) salamander exiting tunnel – highlighted in in red, (f) juvenile gray treefrog ignoring tunnel – highlighted in in red, (g) raccoon exiting tunnel, (h) muskrat exiting tunnel, (i) grackle investigating tunnel entrance, but not entering. Note: all entrances and exits were confirmed using time series photos.

Chapter 4

How outreach increases youth engagement in conservation

Abstract

Despite the utility of science communication, its value for engaging youth in conservation is rarely evaluated experimentally. Using a mixed-methods approach, self-retrospective surveys were administered to discern the impact of conservation outreach on students' self-reported willingness to participate in conservation and what attributes of the presentation most affected them. Presentations significantly increased students' self-reported likelihood to participate in conservation both directly and indirectly, and presenter experience had no effect on this relationship. Novel information and conveying threat significance were the most important presentation elements. Incorporating these elements into presentations, increases the value of outreach in conservation initiatives.

Key words: science communication, road ecology, reptiles and amphibians, environmental education

Introduction

Even the most profound and meaningful scientific discoveries are of little value if not communicated widely and accurately, making science communication integral to any scientific field (McNutt 2013); conservation biology is no exception. Although the many benefits of engaging the public in conservation initiatives have been well documented (Brooks et al. 2006; Pagdee et al. 2006; Berkes 2007; Scyphers et al. 2015), little is known about how to maximize the effectiveness of communication efforts designed to augment conservation engagement (Bennett et al. 2017). Yet, engaging the public is vital to the success of any conservation initiative given that resources (i.e., funding, time, labour, land access) are often limited. Moreover, conservation is intrinsically linked to society (i.e., implications for private and public land use, and access to natural resources, ecosystem services, etc.), highlighting the need for the public to be included in conversations about conservation issues (Mascia et al. 2003; McNutt 2013). Thus, communication efforts must be guided by an understanding of how people interpret and are engaged by science, because, from a conservation perspective, building this connection with people through environmental values is crucial (Ballantyne et al. 2018).

Fortunately, scientists have many communicative tools at their disposal to raise awareness about conservation initiatives and to mobilize the public, including: news (Peters et al. 2008; Sampei & Aoyagi-Usui 2009), social media (Shiffman 2012; Parsons et al. 2014), public forums (e.g. science cafes, naturalist club meetings, etc.; Jensen and Buckley, 2014; Sugimoto et al., 2013), science centres, museums, zoos and aquaria (Ellenbogen et al. 2004; Ballantyne et al. 2007; Visscher et al. 2008; Barriault 2014) and outreach programs. However, it is important to understand the effectiveness of these communication activities and how these efforts affect people’s willingness to participate in specific conservation actions. Some studies have

investigated the impact of outreach programs and found that people are more likely to be engaged by outreach programs if they can relate to the material personally or if the issue is geographically relevant to them (Newton 2001; Shunula 2002). There are also broadly applicable guidelines and best practices for participant engagement and learning in conservation outreach (Jacobson et al. 2015). Still, it can be argued that little is known about outreach effectiveness and its value in mobilizing the public to achieve specific conservation goals (van der Ploeg et al. 2011).

I investigated ways to optimize the effectiveness of conservation outreach by examining the effects of outreach on participants' self-evaluated willingness to take part in direct (participant interacts with nature) or indirect (participant does not interact with nature, but contributes useful information) conservation actions. My study was conducted in the context of road ecology and the negative effects of roads on wildlife. I made several *a priori* hypotheses. In particular, I expected that participants who are more likely to take part in direct conservation action as a result of my outreach would consider themselves more likely to help a turtle cross the road after participating in my outreach program. I also expected that participants who are more likely to take part in indirect conservation as a result of my outreach would consider themselves more likely to take part in citizen science initiatives after my program. Further, I questioned whether presenter experience had an effect on the self-evaluated likelihoods reported by outreach participants. Finally, I qualified participant responses into themes to identify the attributes that most influenced conservation action, to help broadly guide conservation outreach initiatives in the future.

Methods

Outreach Presentations

Science teachers from high schools in southern Ontario, Canada were contacted in the springs of 2014 and 2015 to host outreach presentations on local reptile and amphibian conservation research. Presentations were targeted to grade 9 science classes so that data were not confounded by multiple cohorts and curriculum differences. Presentations were delivered in a classroom, replacing regularly-scheduled lessons and teachers were asked to administer a short survey to students after presentations.

Outreach was delivered to five classes from one school in 2014 by one presenter (P_1) and to a total of seven classes from three schools in 2015 by one of three presenters (P_1, P_2, P_3). All classes were within two adjacent school boards (max distance < 100 km), and all teachers whom responded to my request to present were included in the study. All presenters were biologists with varying levels of research and outreach experience. P_1 was a male graduate student and had several years of experience in both field ecology and outreach. P_2 was female, had completed an undergraduate degree in Biology and had one year of field experience but had little outreach experience. P_3 was female, had one year remaining to complete her undergraduate degree, and had little outreach or field experience. Presentations were identical - incorporating images, graphs and information about focal species – however, they varied in the personal experiences and personalities of the presenters.

Outreach presentations consisted of a 45-minute interactive presentation involving PowerPoint slides, props, opportunities for students to ask questions, and giveaways upon completion (Ontario Species at Risk stickers). Presenters began by introducing the interests and

experiences which led them to become a biologist. During the presentation, participants were introduced to native and non-native herpetofauna of Ontario, Species at Risk protection and laws in Ontario, threats affecting Ontario herpetofauna, a local herpetofauna conservation initiative concerning roads in Presqu'ile Provincial Park, Ontario, and citizen science activities that are easily accessible and directly applicable to herpetofauna conservation in Ontario ('Ontario Turtle Tally' and 'Frog Watch' programs). To maintain engagement, questions were regularly asked both to individuals and to the entire class.

Post-outreach Surveys

Two-to-three weeks after presentations, teachers administered surveys (Appendix A4.1) to determine the effect of outreach presentations on the self-reported willingness of participants to take part in conservation actions (specific examples used: direct participation - helping a turtle across the road, indirect – participating in Frog Watch or the Turtle Tally, citizen science programs), and to elucidate the reasoning for any changes in likelihood to take action. Surveys were anonymous, self-retrospective, and administered while no members of the outreach team were present to minimize social desirability bias (Nederhof 1985), however I recognize that desirability bias could persist for other reasons. Following previously published works, self-retrospective tests were used to determine self-evaluated willingness of participants to contribute to conservation (Kruse & Card 2004). I attempted to minimize response-shift bias by using a single self-retrospective test (Then/Post design) instead of separate Pre- and Post-surveys so that participants could reflect on their own behaviour and accurately assess changes in their attitude, thus providing a better approximation of treatment (i.e., outreach) effect (Howard et al. 1979; Lam & Bengo 2002). Further, my Then/Post design was preferable because of a Pre/Post design's tendency to deliver incomplete data (i.e., if students missed school on either survey day)

(Raidl et al. 2004). While admittedly, reliance solely on self-reported data can limit the usefulness of impact evaluations (Jensen 2014; Mellish et al. 2018), no additional experimental attributes (i.e., longitudinal studies, repeated measures) were incorporated into my design for two reasons. First, the main purposes of my study were to identify cues which most affected participant perceptions of their own willingness to take part in conservation and to determine if presenter experience affected those perceptions, and second, it limited the extra-curricular time commitment required of participants. Of approximately 200 students, 177 participated in the post-outreach survey suggesting a representative estimate of the sample population.

Analyses

Shapiro-Wilk tests confirmed suspected non-normality of my data, so I used non-parametric analyses. Changes in willingness to take part in both direct and indirect conservation actions were compared using Wilcoxon signed-rank tests. Wilcoxon rank-sum tests compared Then/Post-outreach willingness of participants to take part in conservation directly and indirectly, and determined if presenter had an effect on the survey results. All quantitative analyses were completed using R version 3.2.3 (R Development Core Team 2018).

I qualitatively analyzed responses to an open-ended question (Question 5; Appendix A4.1) to identify reoccurring themes. Responses were coded for commonality (i.e., phrases and sentiments), using only the words stated by the respondent, manually and using the qualitative analysis software NVivo Plus (QSR International 2016). Responses were scored and categorized by three people (SPB, and two science communication researchers), then cross referenced to ensure inter-rater reliability in identifying themes across participants. To further identify response trends, word frequency was tallied, excluding common words (e.g. prepositions, ‘the’, ‘as’ and ‘and’). As many students provided multiple statements within their responses, co-

occurrences of categories within the same response were tabulated. In instances where two categories or sub-categories co-occurred more than 10 times, these co-occurrences were noted.

Results

Self-evaluated likelihood

All 177 respondents answered the first four survey questions (those which were not open ended; see Appendix A4.1). After outreach, participants considered themselves significantly more likely to contribute to conservation directly ($V = 435$, $p < 0.0001$, pseudomedian = -1.0; Figure 4.1) and indirectly ($V = 214.5$, $p < 0.0001$, pseudomedian = -1.0; Figure 4.2). Participant self-evaluations demonstrated a 17.6% increase in self-reported likelihood of helping a turtle across the road and a 19.4% increase to contribute to citizen science. Unfortunately, teachers of all classes to which P_2 presented failed to administer surveys to students. However, between the remaining two presenters (P_1 and P_3 - the most divergent in terms of previous experience), there was no difference in the effect of outreach on students' self-perceived willingness to contribute to conservation (direct: $W = 2042.5$, $p = 0.074$, Hodges-Lehmann estimator < -0.01; indirect: $W = 2247$, $p = 0.314$ Hodges-Lehmann estimator < -0.01), indicating that presenter experience did not have an effect on outreach outcomes. Both before and after presentations, students considered themselves more likely to contribute directly than indirectly to conservation (before: $W = 6433$, $p < 0.0001$, Hodges-Lehmann estimator = -1.0; after: $W = 7226.5$, $p < 0.0001$, Hodges-Lehmann estimator = -1.0).

Open ended question responses

Overall, 167 of 177 students submitted responses to the open-ended question ("…what part of the presentation most affected your decision?"). As several students provided more than one reason, the total number of responses provided was 178. Responses were coded into five categories, the most common of which were an increased awareness or introduction to novel information and a general concern for wildlife (Table 4.1). Each category was separately coded into sub-categories, allowing for more detailed analysis. The most common responses included taxon-specific references, such as 'turtle' and 'amphibian', as well as references to specific facts which were included in presentations such as 'extinct', 'endangered', 'children', and references to emotions elicited by the presentation such as 'shocking' and 'sad' or conservation measures discussed such as 'crossing' (Appendix A4.2).

In several instances, categories and sub-categories co-occurred within an individual participant's response to my open-ended question. Concern for the lives of endangered species co-occurred with an increased awareness of endangerment (n = 16), information that affected participants through surprise or sadness (n = 11) and a stated increase in likelihood to take part in conservation (n = 22). Similarly, an increased awareness of endangerment co-occurred with being affected through surprise or sadness (n = 10) and a stated increase in likelihood to take part in conservation (n = 28). Lastly, an increase in likelihood to take part in conservation actions co-occurred with participants stating that they would help a turtle across the road (n = 18). While helping a turtle across the road (my definition of direct participation in conservation) was not specifically one of my categories, I argue the frequency of its co-occurrence with an increased likelihood of participation in conservation warranted its inclusion.

Discussion

Outreach presentations significantly increased students' self-evaluated likelihood to take part in conservation both directly and indirectly, confirming my expectations. Although my study design did not include more rigorous longitudinal methods (i.e., long-term diaries), they are meaningful and informative because the effect of conservation outreach is rarely evaluated experimentally (McNutt 2013; Ries & Oberhauser 2015) and are therefore relevant both in the specific context in which I delivered outreach presentations (road ecology and conservation of reptiles and amphibians), as well as more broadly in the context of science education. Most importantly, my findings demonstrate the increase in self-reported conservation engagement is bolstered by specific cues (i.e., information which is surprising or builds empathy and information about natural history) which play an important role in facilitating engagement. Specifically, the most effective parts of my outreach presentation, as reported by the student participants, were the introduction of novel information and imparting concern for wildlife. Many students responded that they were surprised at the scope and magnitude of the problem and admitted that prior to my outreach, they were unaware of many of the details surrounding the threat of roads to reptiles and amphibians, or the methods of mitigating those threats. This new information to them included specific descriptions of local threats to herpetofauna and broad statements about their conservation status and life-history brought about by student questions. Such dialogue suggests that students were genuinely interested and were more likely to participate in conservation action if they had a better understanding of the species and local conservation issues. Determination of cues such as those I identified is crucial to the successful application of conservation outreach and increasing engagement in conservation activities.

Intuitively, it makes sense that building empathy, surprise, and informing students about natural history would increase their likelihood to take part in conservation. However, the dominance of 'new information' in the responses highlights an important gap in science communication. Prior to outreach, students considered themselves somewhat likely to help a turtle across the road, but in general unlikely to take part in citizen science. Several students were unaware of the seriousness of the threats faced by reptiles and amphibians, nor were they aware of the programs I described or others like them. This lack of prior knowledge pertaining to conservation initiatives, and how to become involved in conservation represents a serious hurdle for biologists. However, many students appeared to have a moderate level of previous knowledge about reptiles and amphibians, and were excited to share personal stories about their experiences, indicating that many were keen to become engaged. Every audience has a different level and diversity of prior experiences and knowledge, and these past experiences play a major role in the likelihood of participating in conservation (Moss et al. 2017). I found that introducing basic information to participants and encouraging audience participation increased engagement, which is important for influencing participant opinions. Interestingly, several of the cues I designed into my presentations did not appear in participants' answers regarding their change in likelihood to contribute to conservation actions. Specifically, I included humour, personal stories, and information for those interested in science and conservation beyond high school; none of these cues were listed as affecting participant responses. Previous research has shown that presentation style positively affects knowledge retention in students (Visscher et al. 2008), and teacher comments about these attributes of the presentation in conversations afterwards suggest that these cues played a role in my survey results; however, they appeared to be too subtle to be specifically stated by students as reasons for changing their self-evaluation.

Outreach targeted at schools may provide access to an underexposed demographic. My data, although collected over a narrow spatial scale, provides a broadly applicable framework for interacting with and engaging youth in conservation. Often, scientists and science communicators reach only those who already engage with nature as part of their jobs or lifestyle (e.g., concerned citizens, amateur naturalists, active campers; Brewer, 2001; Nadkarni, 2004), leaving others unaware of conservation initiatives and ways to become involved. Arguably, reaching people not already involved in conservation is more important than reaffirming those already engaged. This is especially true with respect to youth, as the opinions they form now can be carried forward for many years, and opportunities to pursue scientific interests increases their comfort with, and interest in science later in life (Feinstein et al. 2013). Further, my results demonstrate that the presenter did not significantly affect participant responses, implying that meaningful and enjoyable experiences for students, which are important in increasing engagement (Ainley & Ainley 2011; Swarat et al. 2012), can be delivered regardless of presenter background. This finding is critical because, although many researchers acknowledge the value of conservation outreach (Jensen 2014; Wünschmann et al. 2017), some find it difficult to pursue these opportunities, considering it auxiliary to their other obligations (Andrews et al. 2005). Knowing how to maximize the impact of outreach efforts, especially given that presenter experience has no effect, may motivate independent researchers to seek out opportunities to communicate their work to the public.

Engaging citizens to participate in conservation actions and policy should be considered one of the most important goals in the field of conservation biology (Bickford et al. 2012) and has been shown to positively impact road-related conservation initiatives (Sterrett et al. 2019). Often, scientific research can serve as a first critical step in policy change, but one which has far

less impact than public engagement (Berger & Cain 2014). Conservation outreach, especially when encouraging citizen science initiatives, has major social benefits including an understanding of science, the development of social capital, and a citizenry engaged in issues of local importance (Conrad & Hilchey 2011). Engaged citizens can provide broad-scale data at little cost (Ruiz-Mallen & Corbera 2013) which can in turn act as a warning system for environmental changes and population declines (Conrad & Hilchey 2011; Cosentino et al. 2014). Neglecting the importance of citizen engagement in conservation is an omission that is detrimental to any conservation initiative (Leenhardt et al. 2015), and represents an important hurdle which must be overcome for success to be realized.

My results inform outreach on a broad scale, providing a valuable framework for conservation outreach initiatives. By incorporating the cues and methods I describe, it is possible to increase the effectiveness of outreach activities by affecting the willingness of participants to contribute to conservation both directly and indirectly. Such optimization is especially important given that pro-conservation behaviour after outreach is difficult to sustain (Bueddefeld & Van Winkle 2017). Specifically, presentations should be informative, providing information that is novel to participants while reaffirming prior knowledge in order to maximize engagement. Along with informative value, it is critical to convey the severity of conservation issues to outreach participants honestly, without over sensationalizing. Incorporating these frameworks into the systematic design of conservation outreach can increase its effectiveness (Jacobson et al. 2015). Understanding how people react to and interpret conservation outreach in any setting informs presenters, and as such, experimental evaluations of outreach communications such as this one are important and should be executed more frequently, especially in new settings, to new audiences, and across platforms.

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Figures

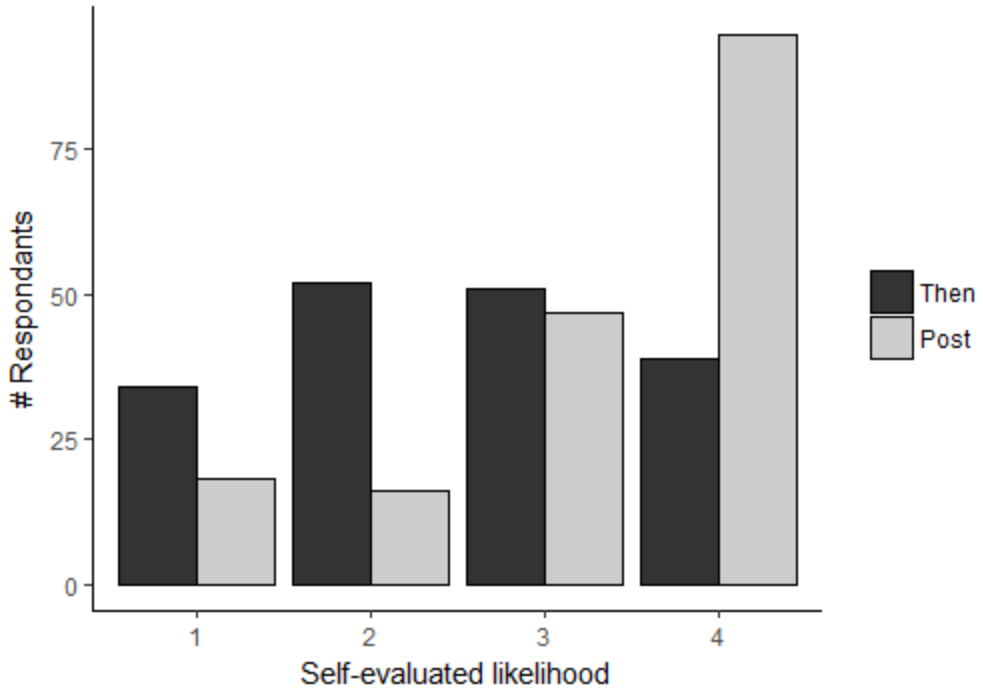

Figure 4.1. Changes in participant self-evaluation of their willingness to help a turtle across the road (x-axis; scale from 1 – 4 representing least to most likely to participate) based on Then/Post retrospective surveys administered approximately two weeks following outreach presentation. Changes represent significant increase in willingness to directly contribute to herpetofauna conservation ($V = 435$, $p < 0.0001$, pseudomedian = -1.0).

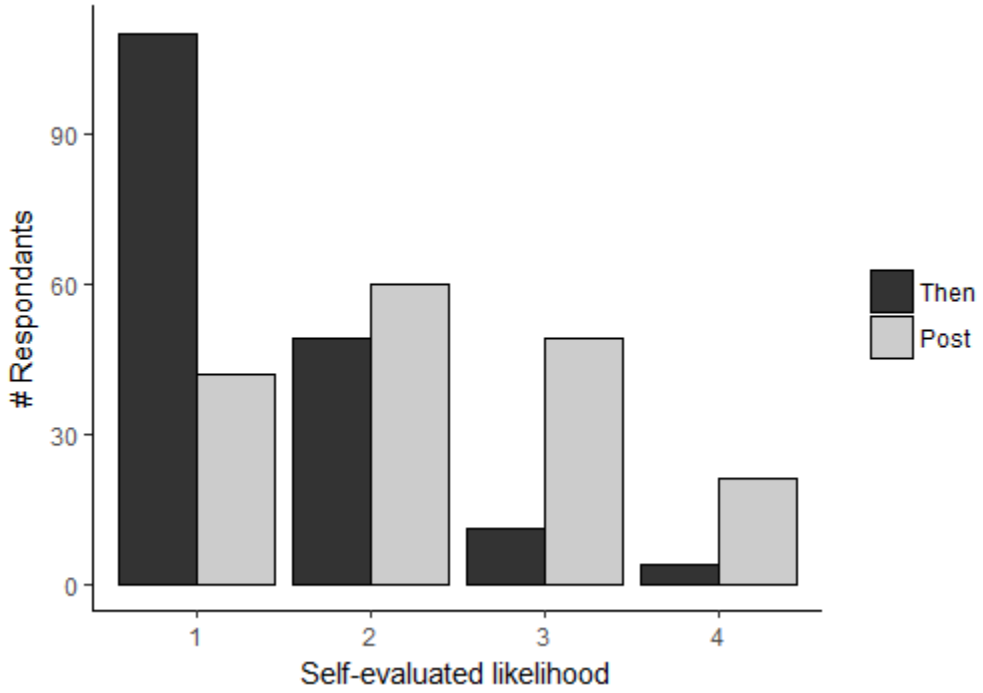

Figure 4.2. Changes in participant self-evaluation of their willingness to participate in citizen science (x-axis; scale from 1 – 4 representing least to most likely to participate) based on Then/Post retrospective surveys delivered approximately two weeks following outreach presentation. Changes represent significant increase in willingness to indirectly contribute to herpetofauna conservation ($V = 214.5$, $p < 0.0001$, pseudomedian = -1.0).

Tables

Table 4.1. Categories and sub-categories with which grade 9 participant responses were coded following outreach presentations. The number of times each category and sub-category was referenced by students is noted, and examples of responses are provided for specific sub-categories. Category and sub-category references were counted independently, as students often specifically referenced each separately. Note my final category (likelihood of action or participation) had no direct references as it was a broad category used to identify changes in self-evaluated likelihood of participation in conservation. All responses were coded and inter-rated into as few categories as possible, then further divided into sub-categories representing similar themes to the parent category. Sub-category counts do not contribute to parent category reference counts. A single student response was not limited to one category, as students often listed multiple reasons for their self-evaluated change in likelihood to participate in conservation.

Category	*# of category references*	*Sub-categories*	*# of sub-category references*	*Examples*
Increased awareness or provided new fact based information	52	Learned about turtle tally and frog watch	10	"The part where the tally of turtles, frogs and snakes killed in a certain area."
		Support for turtle tunnels and fences	8	"…learning about the new thing people are building for turtles…"
		Graph of declining population	6	"…graph on how many turtle, frogs, snakes all die each year in a certain amount of space."
		Reproduction is slow	2	"…turtles have a long time to have babies."
		Places devoted to conserving turtles	2	"…how well the sanctuaries help turtles like fixing their shell."
		Don't take turtles from the wild	1	"…we should never take a turtle from its habitat to make it a home pet or just move it."
Concern for the lives of animals - general sentiment	49	Expressing fondness for amphibians and reptiles	15	"Snakes are adorable, so are salamanders and turtles"
		Picture of a dead turtle	8	"After seeing the images of the dead turtles on the road I realized that someone or something has to be done."
		In the future my children might not see the same wildlife	7	"After knowing that my children or my grandchildren might not see a turtle helped me choose."
Information affected participant via surprise or sadness	25	Greater feeling of consideration for reptiles and amphibians	18	"I felt that everything should be able to live"
		Information provided changed perspective	16	"It got me thinking and showed me I need to help out more"
Understanding of how humans affect animals	5	Understanding of threats associated with roads	19	"When he told us that you can't walk around 2 km without running into a road"
		Loss of habitat	2	"Our actions are killing them, destroying their habitat and much more."
Likelihood of action or participation	0	Increased likelihood	69	"My likelihood increased because of the presentation…"
		Remained likely to help	24	"my decision stayed the same because I knew it was important to help"
		Won't participate due to lack of time or apathy	2	"I still wouldn't participate in the Turtle tally because I don't enjoy them"
		Likelihood decreased	1	"My likelihood decreased because I am not very interested in the project"

Appendix A4.1 - Survey administered to grade nine outreach participants

Survey Instructions:

- Please answer all questions as honestly as possible
- Either circle the number, where applicable, or write a short sentence
- Do not include your name or any information about yourself on this survey

1) On a scale of 1-4, with 1 being *never* and 4 being *always*, how likely would you consider yourself to have helped a turtle across the road **BEFORE** the "*Road to Conservation*" presentation given on May 16, 2014?

   1 2 3 4

2) On a scale of 1-4, with 1 being *never* and 4 being *always*, how likely would you consider yourself to have helped a turtle across the road **AFTER** the "*Road to Conservation*" presentation given on May 16, 2014?

   1 2 3 4

3) On a scale of 1-4, with 1 being *never* and 4 being *always*, how likely would you consider yourself to have participated in a citizen science project like the Toronto Zoo's *'Frog Watch'* or '*Turtle Tally*' programs **BEFORE** the "*Road to Conservation*" presentation given on May 16, 2014?

   1 2 3 4

4) On a scale of 1-4, with 1 being *never* and 4 being *always*, how likely would you consider yourself to have participated in a citizen science project like the Toronto Zoo's '*Frog Watch*' or '*Turtle Tally*' programs **AFTER** the "*Road to Conservation*" presentation given on May 16, 2014?

   1 2 3 4

5) In the previous questions you were asked to describe how likely you were to help a turtle cross the road or to participate in a program such as '*Frog Watch'* or *'Turtle Tally'*. Using the space below, and 1-3 short sentences please describe what part of the presentation most affected your decision (i.e., if you increased, decreased in likelihood, or if your likelihood stayed the same – what caused this?)

Appendix A4.2

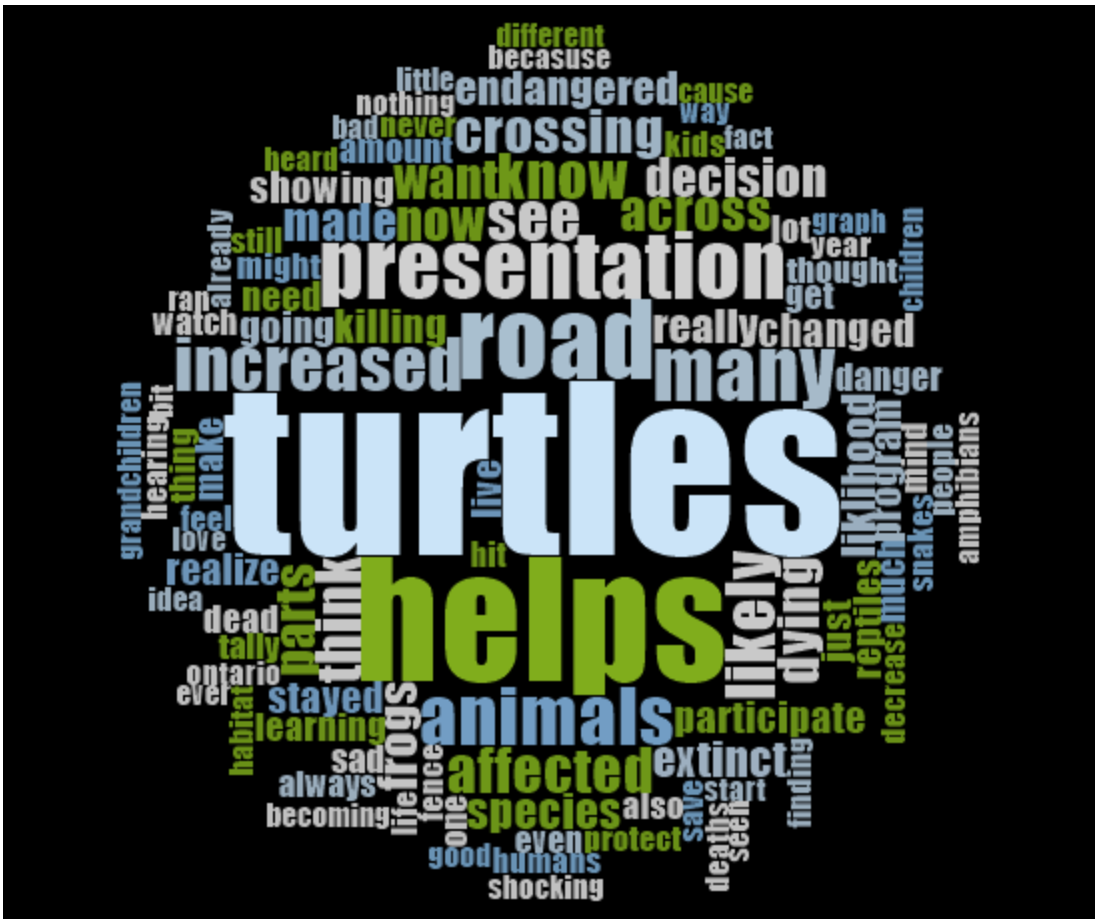

Figure A4.1 Visualization of the 100 most common words in outreach participant responses. Size is directly correlated with the number of times a word was used. Common words such as the, like, and, etc. were excluded.

General Conclusion

The Road Ahead

Roads are one of the most ubiquitous human structures on the planet – they are everywhere. Since its onset, road ecology has grown and our understanding of the impacts of roads on wildlife and how they can be mitigated has dramatically increased. Despite these advances, many critical issues remain unaddressed. The chapters preceding this overview target several of these enigmatic problems that remain at the forefront of road ecology.

In general, the goal of my dissertation was to investigate how roads negatively affect wildlife and determine how best to mitigate these threats in a holistic way. I did this first by shedding light on how highway twinning affects wildlife, demonstrating that it has minimal impact on large mammal population and spatial ecologies. As road networks expand, highway twinning appears to be a viable approach to accommodate increased traffic volume. However, further understanding the responses of wildlife to highway twinning is integral to the development of ecologically friendly infrastructure.

To complement this approach to managing the impact of road expansion, I developed tools to streamline the mitigation planning process. The high cost and time commitment required for ecological monitoring impede the ability of researchers and managers to fully understand landscape connectivity and thus, identify ideal locations to implement road-effect mitigation structures. By combining field data and habitat resistance models, I demonstrated that the process of planning mitigation infrastructure can be optimized by precisely identifying ideal locations of crossing structures and exclusion fencing for herpetofauna and mammals. Further, I also evaluated mitigation effectiveness using a Before-After-Control-Impact study to measure

the reduction in reptile and amphibian road mortality post-mitigation installation. I demonstrated that mitigation structures reduced reptile and amphibian road mortality while maintaining landscape connectivity, and importantly, for turtles, I documented connectivity at a population-level. Continued application of robust study designs and prioritizing questions that address population-level processes will advance our understanding of road-effects and road-effect mitigation, thus facilitating more broadly-effective management practices.

Finally, although mitigation is likely to be widely beneficial, it can be cost-prohibitive in some circumstances. In such cases, education can serve as an important stop-gap in those areas where physical mitigation is not feasible. Outreach is important not only for engaging stakeholders that are crucial to the success of road-effect mitigation (i.e., NGOs, industry, government officials), but also for connecting with the public and thus generating political will. Moreover, outreach facilitates access to audiences who would not otherwise engage with nature, and thus may be less informed or invested in conservation efforts. I demonstrated that outreach changes the attitudes of youth regarding their own participation in conservation actions. Further, I used participant commentary and experiences to create guidelines that optimize outreach effectiveness. The role of effective outreach in the context of road ecology is still developing, and factors such as the demographics targeted, outreach delivery methods, and the breadth of information shared deserve future consideration.

Road ecology is an incredibly diverse field. Successful initiatives, although critical for conservation, are dependent on a network of individuals and organizations working in unison. Collaboration between government agencies, NGOs and research institutes proved essential throughout my dissertation. By combining approaches that increase inferential power with persistent collaborative action, the impact of roads on wildlife can be successfully managed. As

our understanding of the threats that roads present to wildlife develop, effective and efficient tools will greatly increase the value of conservation efforts around roads. I applied rigorous methods to enhance and optimize strategies commonly used to mitigate road-effects, filling critical gaps in our understanding of the dynamics surrounding road-effects and road-effect mitigation. Further, lessons learned here will provide essential frameworks for managing the threats associated with other forms of transportation (e.g., railways). Thus, creating tools and optimizing strategies not only answers questions pertinent today, but provides the basis for effective long-term mitigation strategies in the future.

Additional Research

My dissertation examined road-effects and road-effect mitigation in a holistic way, however by no means does this body of work fill all existing knowledge gaps, nor encompass the entire field of road ecology and its sub-disciplines. Although not officially part of my dissertation research, during my tenure at Laurentian I have collaborated on several related research projects, which have either been published or are currently under review. These contributions can be divided into the following two categories:

Effect of roads and road-effect mitigation on herpetofauna

Various methods have been used to monitor roadsides for wildlife to quantify road mortality rates. Although these methods may yield similar results, little evidence exists to support this assumption. Together with colleagues from Australia (James Baxter-Gilbert and Julia Riley), we compared efficiency and effectiveness of three different methods of surveying roads for turtles: by car, bicycle and by foot (Baxter-Gilbert et al. 2017). We found that while limited differences exist between surveys conducted on foot and by bicycle, surveys done by car detected significantly fewer turtles. While there are advantages to conducting surveys by car (i.e., increased distance travelled over less time), these trade-offs have important implications that must be considered.

While conducting road surveys in Presqu'ile Provincial Park, ON, Canada, we recorded instances of both dead and live herpetofauna. During the field season the exclusion fencing was being installed, we noted on several occasions that reptiles and amphibians appeared to be trapped within the road's right-of-way (Boyle et al. 2019). Further, several snakes and amphibians were found in varying states of desiccation. Many of those amphibians were found

dead, with no signs of having been struck by a vehicle. Exclusion fencing has been linked to several negative impacts on wildlife including inhibiting or modifying movement patterns (Jaeger & Fahrig 2004; Clark et al. 2010), increased sun exposure (Peaden et al. 2017), incidental capture in pit fall traps, (Ferronato et al. 2014), and becoming tangled in fencing material (Ontario Ministry of Natural Resources and Forestry 2016). We highlighted the importance of regular monitoring during road-effect mitigation efforts and caution against assuming only positive effects of conservation initiatives.

In Chapter 3, I use the results of behavioural trials to quantify the false negative rate of my trail cameras for detecting snakes exiting the tunnels installed beneath the road. Those same behavioural trials were part of a willingness to use experiment designed to quantify the likelihood of gartersnakes to use the tunnels. Although willingness to utilize experiments have been used to quantify the likelihood of animals using tunnels before (Lesbarrères et al. 2004; Hamer et al. 2014; Baxter-Gilbert et al. 2015), few have used this approach in the context of snakes (although see Colley et al. 2017). This study, led by Rachel Dillon, found that when presented with the opportunity to enter the tunnel or to leave in either direction along the abutting fence, 50% of gartersnakes chose to enter , demonstrating that snakes show no aversion to using the tunnels (Dillon et al. *in review*). Further, our results indicate that likelihood of tunnel usage increases with familiarity to the tunnels and that perhaps reptiles are learning to use mitigation structures.

Transportation Ecology

Road ecology has developed considerably. Roads, despite being one of the most abundant forms of human infrastructure globally, are not the only form of transportation that negatively impacts wildlife. Increasingly, the impacts of other forms of transportation have been recognized

and quantified (Dorsey 2011; Bennett & Litzgus 2014; Davy et al. 2017), however limited investigation into these impacts hinder effective management. My colleague, Dr. Jesse Popp, and I highlighted this gap by quantifying the scientific literature available concerning the impact of both roads and railways on wildlife (Popp & Boyle 2017). Since we published our review, railway ecology has expanded rapidly, including a book documenting its main effects (Borda-de-água et al. 2017; book review: Boyle 2018).

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