ON THE ROAD AGAIN: MEASURING THE EFFECTIVENESS OF MITIGATION STRUCTURES FOR REDUCING REPTILE ROAD MORTALITY AND MAINTAINING POPULATION CONNECTIVITY

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ABSTRACT

Wildlife is negatively impacted by roads, and reptile populations are no different. Highway 69 connects southern and northern Ontario by running along the eastern Georgian Bay Coast, one of Canada's richest areas of reptile biodiversity and a new 4-lane section has been designed to include mitigation structures intended to lessen the detrimental effects this major roadway poses to numerous Species-at-Risk (SAR) reptiles. We used a Before-After-Control-Impact-Paired (BACIP) study design to compare reptile abundance on the non-mitigated 2-lane highway (1 May to 5 June 2012) to that on the new, mitigated 4-lane highway (1 May to 5 June 2013). In both years of the study, a control site without any mitigation measures was also monitored. Radio telemetry, automated PIT-tag readers, and wildlife cameras were used to monitor reptile movements around road, and examine the use of population connectivity structures (*e.g.*, ecopassages). Additionally, a willingness to utilize (WTU) experiment was conducted to assess turtle responses to the connectivity structures. Our preliminary findings show no difference in the abundance of turtles and snakes found on the highway between the non-mitigated and the mitigated highways, suggesting that the present design of reptile fencing was not effective. Although the ecopassages have been used by reptiles, the crossing rates we observed were much lower than those reported in the literature. We make several recommendations regarding improving the effectiveness and reliability of highway mitigation measures.

INTRODUCTION

Reptiles are declining globally (Gibbons *et al*. 2000; Fahrig & Rytwinski 2009). In Ontario, 19 of the 26 reptile species (73%) are listed as Species at Risk (SAR) (Crowley & Brooks 2005; COSEWIC 2011). Reptile population decline is primarily due to anthropogenic causes, such as: habitat destruction, persecution, and over-harvesting (Gibbons *et al*. 2000). Habitat destruction and fragmentation are by far the leading causes of reptile declines across Ontario. Of the many forms taken by habitat destruction and fragmentation, road construction and traffic may present the longest lasting effect; contributing to the imperilment of many reptile species (McKinney 2002). Unfortunately, this threat is not expected to decrease in the near future. For instance, central Ontario has recently begun the expansion of Highway 69, the main roadway which connects northern and southern Ontario, into a large multi-lane 400 series highway (Pagnucco 2005). It is expected that the traffic volumes will increase to an average of 10,000 vehicles per day annually, in turn causing the further urbanization of central Ontario (Pagnucco 2005). Highway 69 also bisects one of Ontario's richest areas of reptile biodiversity, the Georgian Bay coastline (Hecnar *et al.* 2002) and the expansion of Highway 69 will thus provide a long-lasting and significant threat to SAR reptiles in the area (Rouse & Willson 2001; McKinney 2002).

Ontario has three taxa of reptiles (*i.e.,* turtles, snakes, and a lizard), all of which are negatively affected by roads (Seburn & Seburn 2000; COSEWIC 2007, 2011). Turtles are imperilled by roadways because they have a tendency to make long-distance seasonal movements, and gravid females often select road shoulders as nest sites (Gibbs & Shriver 2002; Marchand & Litvaitis 2004; Steen *et al*. 2006). Adult mortality poses a great threat to turtle populations, because in order to sustain healthy populations it is essential to maintain high adult survivorship to offset low juvenile recruitment and delayed sexual maturity (Heppell 1996; Gibbs & Shriver 2002). Similar to turtles, seasonal movements of snakes can occasionally require them to cross roads, and road mortality has been identified as a direct threat to several Ontario species (Rouse *et al*.

2011). Snakes also are thought to bask on the road surface during the day, and absorb radiant heat at night (Enge & Wood 2002). Use of roads for thermoregulation increases mortality risk due to prolonged exposures to traffic and potential collisions (Rosen & Lowe 1994; Rudolph *et al*. 1998; Enge & Wood 2002). The only lizard species in Ontario, five-lined skink (*Plestidon fasciatus*), is also negatively affected by roads in portions of its global range (COSEWIC 2007); however, little is known about how much roads effect this species in Ontario. The threats roads pose to reptiles are exacerbated by the fact that an average of 2.7% of drivers will intentionally run over reptiles when they are on roads (Ashley *et al*. 2007).

In response to the substantial threat roads pose to SAR reptiles, certain newly constructed, or expanded, 400 series highways through SAR habitat in Ontario are including mitigation measures (Gunson *et al*. 2009). It has been proposed that the most effective means of preventing road-based reptile mortality and fragmentation of populations is to implement a series of exclusion structures in conjunction with population connectivity structures in areas where reptiles frequently encounter traffic (Dodd *et al.* 2004; Aresco 2005; Ashley *et al*. 2007). Up until recently, the effectiveness of these mitigation structures has not been thoroughly quantified (Forman *et al*. 2003; Jochimsen *et al*. 2004; Lesbarrères & Fahrig 2012). Such lack of assessment poses an issue for conservation, as the allocation of funding to protect SAR is finite and must be spent effectively (Engeman *et* al. 2009; Mahoney 2009). It is thus important to test the effectiveness of mitigation measures to both maximize the recovery of SAR, and to develop a framework so that mitigation measures can be regularly implemented into roadways (Roedenbeck *et al*. 2007; van der Ree *et al*. 2007; Glista *et al*. 2009).

To rigorously evaluate the effectiveness of mitigation measures, road ecology studies should include the following five criteria (Forman *et al*. 2003; Jochimsen *et al*. 2004): 1) reduction in the abundance of wildlife on roads, 2) maintenance of habitat connectivity and access to critical habitats (*e.g.* hibernacula, nesting sites), 3) maintenance of dispersal routes, 4) preservation of gene flow throughout the populations, and 5) continuance of metapopulation processes. Our study will address these criteria to evaluate the effectiveness of mitigation structures (ecopassages and fencing) along Highway 69 for multiple SAR. During our study some of the criteria (1-3) will be directly addressed, while the rest (4-5) will be set in motion through data collection and the creation of genetic baselines for future projects. The objectives of our project will be accomplished over a two year period by using six different methods to fully understand the effectiveness of the mitigation measures: i) Before-After-Control-Impact-Paired (BACIP) study to examine change in reptile presence on the roads, ii) the creation of a before-mitigation genetic baseline for future analysis, iii) radio-telemetry study of *Emydoidea blandingii* and *Chelydra serpentina* to determine movements around the road, iv) camera traps in the ecopassages, v) PIT-tagging of reptiles and an automated PIT-tag readers installed in the ecopassages, and vi) a willingness to utilize (WTU) experiment to assess likelihood of ecopassage use.

Our study will be examining the effectiveness of two types of mitigation structures (*i.e.*, fences and ecopassages), with the understanding that there are several ways to classify effectiveness: 1) is a statistically significant effect seen and 2) is that effect biologically significant? If there is a significant reduction in the abundance of reptiles present on the highway post-mitigation compared to that of pre-mitigation, then the exclusion measures will have achieved the desired result and would be considered effective in some capacity. The extent to which the exclusion structures are effective depends upon how much the abundance of reptiles on the roadway was reduced, especially the abundance of SAR reptiles. For example, in snapping turtles (*Chelydra serpentina*), an annual decrease of 1% of the adult population will result in a population crash within 50 years (Congdon *et al.* 1994). Therefore for mitigation to be biologically effective for a snapping turtle population, the abundance of turtles on road needs to be less than 1% of the adult population within that area. The effectiveness of the population connectivity structures will be determined experimentally (WTU utilize test), and quantitatively (monitoring movements patterns around the road, and through culvert with radio telemetry, automated PIT-tag readers, and wildlife cameras). If turtle are willing to use the ecopassages to gain access to the other side of the road, and if reptiles in natural settings are also using the ecopassages, then the ecopassages will be considered to be an effective population connectivity structure.

METHODS

Study Area

Two sites were used for this study, an impact and a control site, located 50 km apart along Highway 69. The impact site is located 40 km south of Sudbury, Ontario along Highway 69 and consists of a 13 km stretch of road (unmitigated 2-lane road during 2012, and 4-laned road with reptile mitigation during 2013). The control site is a 13 km stretch of road in and around the Magnetawan First Nation community, located 90 km south of Sudbury, Ontario. Both of these sites exhibit similar habitat structures (rocky outcrops, mixed upland forests and wetland mosaics). The species assemblage at the impact and control sites include: 6 species of turtle, 11 species of snake, and 1 species of lizard (Hecnar *et al*. 2002). Of the reptiles present in the study area, 5 turtle, 5 snake, and the 1 lizard species are listed as SAR (COSEWIC 2011).

Mitigation Types

The mitigation measures at the impact site were installed during the course of the summer and fall of 2012, and were considered to be complete by 1 October 2012. The reptile fencing consists of a heavy gauge plastic material affixed to a 2.3 m tall steel wire, large mammal fence (Fig. 1). The reptile fence rises 0.8 m out of the ground, and is also buried 0.2 m deep with a 0.1 m wide lip running perpendicular underground. The top of the reptile fence is attached to the large mammal fence using crimped metal D-rings, and the buried bottom of the reptile fence secures it to the base of the large mammal fence and the ground. The purpose of this fence is to prevent reptiles from gaining access to the road surface, and to guide them towards the ecopassages.

FIGURE 1 The reptile fence (0.8 m tall), affixed to a large mammal exclusion fence and buried at the base (0.2 m deep). A female snapping turtle navigates along the fence in an effort to find a gap to reach the wetland-side after becoming trapped on the traffic-side of the fence during a nesting season movement.

There are three ecopassages at the impact site, spaced 300-500 m apart. Each ecopassage consists of two 3.4 m (span) x 2.4 m (rise) x 24.1 m (length) cast, reinforced concrete, box culverts that cross the north-, and south-bound lanes of the highway (Fig. 2). A fenced 15.25 m gap connects the culvert below the north-, and south-bound lanes. This fenced opening in between the two culverts allows for increased light within the ecopassage. The culverts have an openness ratio (0.34 m) thought to be within the range that promotes use by diurnally active reptiles (Gunson 2010; Woltz *et al*. 2008). The placement of the culverts was based on wetland habitats that were believed to contain SAR reptiles.

FIGURE 2 The ecopassage running below the north bound (foreground) and south bound (background) lane of Highway 69 near Shepard Lake, Ontario; a large 15.25 m gap between the culverts allows for increased light with the ecopassage.

BACIP Study

The Before-After-Control-Impacted-Paired (BACIP) portion of the study was used primarily to test the effectiveness of exclusion structures, as suggested by the Rauischholzhausen Agenda (Roedenbeck *et al*. 2007).

Data Collection

Vehicular surveys were conducted on a 13 km section of Highway 69 at each of the impact and control sites at simultaneous time intervals (9:00, 18:00, 22:00) every day from 1 May to 31 August at each site. Roadside transects (RST) were conducted on foot and covered 2 km of roadside and ditch parallel to the highway. The RST were located in specific areas at each site where the highway bisects a large wetland, as well as rocky upland habitat that separates smaller wetlands. The RST was walked after the 9:00 vehicular survey each day (at approx. 10:00). A total of 960 surveys were conducted in 2012, with effort being replicated for the 2013 field season. The analyses presented in this paper use data from the surveys conducted from 1 May to 5 June 2012 (before period; 282 surveys) and 1 May to 5 June 2013 (after period; 282 surveys).

When a reptile was found on the road, alive or dead, data were collected on its location using a handheld GPS receiver. If the reptile was alive, the behaviour was noted (*e.g.*, crossing, basking, nesting). Data were collected on body size (morphometrics), life-stage, and gender, whenever possible, and using the appropriate tools (*e.g.*, Pesola® spring-scales, vernier calipers, tree calipers, probes, etc.). To determine if individual reptiles were being recaptured, each reptile captured on the road was individually marked. Turtles were notched in the marginal scutes (Cagle 1939) using a triangular file. Snakes and lizards were marked using a permanent marker (Radford 2012). During the 2013 field season, living snakes and turtles found at the impact site were also outfitted with a passive integrated transponder (PIT-tag) microchip (HPT12, Biomark®, Boise, ID). After all measurements were taken, the individual was released 2-5 m off to the side of the road in the direction it was heading, to reduce the likelihood of immediate contact with traffic.

Data analysis

Differences between reptile abundances on the highway during before and after periods were examined using a BACI-ANOVA (Smith 2002). The BACI-ANOVA included the fixed main effects of period (Before, After), Location (Control, Impact), and an interaction between these two effects (Smith 2002). Additionally, the difference between reptile (turtle and snake) abundances on the road at the control site and impact site was calculated for each sample period. This difference was then compared between before and after periods using an un-equal variance, two-sample t-test (Smith 2002). For both tests a significant difference was accepted when $p < 0.05$. All data are presented as the mean ± standard error (SE). Analyses were completed using the statistical program R (R Foundation for Statistical Computing, Vienna, Austria).

Genetic Sampling

Data Collection

Genetic samples were taken from living and deceased reptiles (Doyon *et al*. 2003) found on the road and within 1 km of the road at the impact and control sites. Blood was taken from the caudal vein of living, and tissue was excised from freshly killed individuals, after fatal collision with vehicles, using tweezers and scissors. The genetic material was stored on FTA™ genetic storage cards (Smith & Burgoyne 2004), and information on species, date, location, and gender was recorded.

Radio Telemetry

Data Collection

The spatial ecology of two species of turtle were studied using radio-telemetry: the Blanding's turtle (*Emydoidea blandingii*), and snapping turtle (*Chelydra serpentina*). These species are both provincially listed as SAR, and are threatened by road mortality (Seburn 2007). Adults were captured at the impact site using visual surveys, dipnets, and baited hoop traps, within 1 km of the road. Once captured, individuals were processed using the same methods as in the BACIP study (see above). After processing, individuals were outfitted with an Advanced Telemetry Systems (ATS) radio transmitter (Product # R1920, ATS, Isanti, MN). The transmitters were attached using a marine epoxy paste (PC-11™) for adhesion of the base, and a plumbing epoxy putty (PC-Marine™) was used to attach the sides of the transmitter to the shell and contour the transmitter; the package mass was less than 2% of the individual's mass. The individuals were tracked every 2 to 3 days. After the animals were located, GPS locations, physical state (*e.g.,* health, gravid), behaviours (*e.g.,* basking, nesting, hibernating), habitat, and environmental conditions were recorded. Tracking individual turtles prior to the newly constructed highway's opening allowed for critical habitats (*e.g*., overwintering and nesting sites) to be identified during the 'before' phase of the study, as well it provided a larger sample size of turtles to be monitored the following year. Tracking during the 'after' phase of the study will allow us to monitor reptile movements and behaviours around the mitigation measures to give information on use by individual turtles (Kaye *et al.* 2005).

Data Analysis

Location data were uploaded to ArcView (ESRI, Redlands, CA, USA). Home ranges, movement patterns, and the number of road crossings per individual were analysed for the 2012 field season. Individual home range sizes were calculated using 95% minimum convex polygons (Litzgus and Mousseau 2004) to determine if they overlapped with the highway.

Ecopassage Monitoring

Wildlife Cameras

Wildlife cameras have been successfully used to monitor the movement of animals through ecopassages (Barichivich & Dodd 2002; Pagnucco *et al.* 2011). Wildlife cameras (Buschnell Trophy Cam HD Max, 119576C, Overland Park, Mo) were installed on 28 April 2013 at both entrances of each of the three ecopassages to record any reptiles using the ecopassages, as well as the number of potential reptile predators using the ecopassages. The cameras were mounted to the ceiling of the culvert 0.3 m from the mouth of the culvert, and aimed directly at the ground to maximize frame coverage. Because reptiles are ectothermic, and therefore would not activate the infra-red sensor used for night photography, the camera trap was programmed to take a photograph every minute between the hours of 18:30 to 6:30 (Pagnucco *et al.* 2011), while the motion sensor trigger activated the cameras for the other 12 hours per day between 1 May and 30 October 2013.

Automated PIT-tag Readers

During the 2013 field season, all healthy adult turtles (painted turtles; *Chrysemys picta*, Blandings turtles and snapping turtles) and certain snakes (eastern gartersnakes; *Thamnophis sirtalis,* and northern watersnakes; *Nerodia sipedon*) captured during the road surveys, walking surveys, radio-telemetry searches, and haphazard surveys were outfitted with PIT-tags. The PIT-tags were injected subcutaneously. For turtles, the PIT-tags were injected below the skin of the inguinal space in front of the right hind leg (Buhlmann & Tuberville 1998). For snakes, the injection occurred on the right side of the posterior third of the body (anterior to the cloaca) at the junction of the dorsal and ventral scales. Injection sites were sterilized with alcohol before the injection, and the injection site was closed with cyanoacrylate glue after the needle was removed (Rowe & Kelly 2005).

On 3 May 2013, the automated PIT-tag readers (HPR PLUS, Biomark®, Boise, ID) and loop antennas (3.05 m span x 1.22 m rise; Biomark®, Boise, ID) were installed and activated at both entrances of the middle ecopassage located in the impact site. The readers are powered by 12 V deep cycle marine batteries which were replaced and recharged throughout the field season. Once activated, the readers constantly scan and log the PIT-tag number of any animal that passes through the loop antenna. The date and time of each scan was also recorded. The PIT-tag readers provide information on the number of individuals using the ecopassage, the frequency of use per individual, seasonal timing of use, gender bias, time-of-day use, and crossing duration (calculated with the use of readers at either entrance of the ecopassages).

Willingness to Utilize (WTU) Test

Data Collection

These experiments are ongoing, and here we report on progress to-date. From 1 May to 5 June 2013, a total of 14 painted turtles from the Burwash Township, Ontario were captured using dipnets, visual surveys, basking traps, and hoop traps baited with sardines. These turtles were then transported using a vehicle to the southern ecopassage at the impact site. Turtles were taken from outside the local area to ensure that they have not previously interacted with, or become habituated to, the ecopassage. This allowed testing the turtle's unbiased reaction to the culverts. The turtles were introduced to the ecopassage on the east side of the highway, as the Burwash collection site is 2 km west of the highway. This method was used so that the turtles, using the sun to orient themselves (DeRosa & Taylor 1978; Caldwell & Nams 2006), would want to move westward to return to their known home range (Ernst 1970). The ecopassage resides between the individual and favoured habitat, simulating if a turtle was making a seasonal movement to critical habitat (*e.g.*, overwintering or nesting sites).

Prior to the experiment, the turtles were temporarily outfitted with a small radio transmitter (Product # R1920, ATS, Isanti, MN) that was attached with a 10 cm strip of electrical tape to the carapace. The turtle was then placed in an acclimation box, a 0.30 m x 0.30 m x 0.15 m wooden box constructed with a wire mesh lid, an open base, and spring-loaded hinged sides. This acclimation box faced the ecopassage, and was situated 5 m from the entrance. The turtle was left to acclimate to the sun's position, the substrate, and the noise and smell of the highway for 10 minutes. After the acclimation period, the hinged sides of the box were opened by a counter-weight system triggered by a researcher situated behind a blind. A blind was used to reduce the influence of the presence of a potential predator on the animal's behaviour (Andrews *et al*. 2005). Turtle movements were monitored from behind the blind for 20 minutes to assess the individual's interactions with the ecopassage. Similar experiments testing turtle willingness to use ecopassages in artificial arenas set testing duration to 15 minutes (Woltz *et al*. 2008), but because our experiment occurred beside an active highway, we increased the time to 20 minutes.

Each individual turtle's behaviour during the experiment was ranked using a measure of crossing success on a scale from 0-5: 0) not willing to use, walked away; 1) made no choice, sat at entrance; 2) experimenting with use, entered first quarter of culvert; 3) cautiously willing to use, crossed half of culvert; 4) slowly willing to use, crossed three quarters of culvert; and 5) completely willing to use, crossed full length of culvert (Lesbarreres *et al*. 2004). After 20 minutes, or if an individual moved greater than 10 m from ecopassage, the turtle was recaptured and processed. Information on morphometrics and gender was recorded, and the transmitter removed. Turtles were marked on the marginal scutes with a notch code (Cagle 1939) using a triangular file to indicate if an individual has previously been captured. Marking the turtles will help to avoid repeating use of individuals in the experiment. Turtles were released at their site of capture with 6 hours.

PRELIMINARY RESULTS

During the 2012 field season (1 May to 31 August) 960 road surveys were conducted over 143 days; resulting in a total of 485 reptiles (59 turtles and 81 snakes at the impact site; 149 turtles, 170 snakes, and 29 lizards at the control sites) observed interacting with Highway 69. In 2012, 67% of the reptiles seen were found dead on the road (DOR) at the impact site, while 74% were DOR at the control site. Following a similar trend, during the first 5 weeks of the 2013 field season (1 May to 5 June) a total of 176 road surveys have been completed, resulting in 135 reptiles (19 turtles and 16 snakes at impacted site; 57 turtles and 44 snakes at the control site) observed on Highway 69. The mortality rate was high at both sites; 83% of the reptiles were DOR at the impact site, and 81% were DOR at the control site.

BACIP Study

No significant interaction was found between period (After, and Before), and location (Control, and Impact) for the tests of turtle ($F_{1,137} =$ 0.03, P = 0.86; Table 1, Fig. 3) or snake abundances ($F_{1,137} =$ 0.02, P = 0.89; Table 2, Fig. 4). Additionally, the difference in turtle abundance between the control and impact sites did not differ between the before and after periods (T_{69} = 0.17, P = 0.86). At the impact site, during the before period an average of 0.81 ± 0.13 turtles were found on the road daily, and during the after period an average of 0.53 ± 0.09 turtles were found on the road daily (Fig. 3). At the impact site this translates to a difference of 0.28 ± 0.04 turtles between the before and after period. At the control site, during the before period an average of 1.69 ± 0.28 turtles were found on the road daily, and during the after period an average of 1.5 ± 0.25 turtles were found on the road daily (Fig. 3). At the control site this translates to a difference of 0.19 ± 0.03 turtles between the before and after period.

Similarly, the difference in snake abundance between the control and impact sites did not differ between the before and after periods (T_{69} = -0.14, P = 0.89). At the impact site, during the before period an average of 0.81 ± 0.13 snakes were found on the road daily, and during the after period an average of 0.44 ± 0.07 snakes were found on the road daily (Fig. 4). At the impact site this translates to a difference of 0.37 ± 0.06 snakes between the before and after period. At the control site, during the before period an average of 1.58 ± 0.26 snakes were found on the road daily, and during the after period an average of 1.17 ± 0.19 snakes were found on the road daily (Fig. 4). At the control site this translates to a difference of 0.41 ± 0.10 snakes between the before and after period.

TABLE 1 BACI-ANOVA Table for the Test of a Difference in Turtle Abundance on the Highway at the Impact Site Before and After Mitigation, While Controlling for Confounding Environmental Factors Using a Control Site

Source	df	MS	*F*	*P* value
Period (Before,After)	1	2.0069	0.9516	0.3309
Location (Control,Impact)	1	31.1736	14.7817	0.0002
Interaction	1	0.0620	0.0296	0.8636
Error	137	2.1089		
Total	140			

TABLE 2 BACI-ANOVA Table for the Test of a Difference in Snake Abundance on the Highway at the Impact Site Before and After Mitigation, While Controlling for Confounding Environmental Factors Using a Control Site

Source	df	MS	*F*	*P* value
Period (Before,After)	1	5.4444	3.6951	0.0566
Location (Control,Impact)	1	20.2500	13.7436	0.0003
Interaction	1	0.0278	0.0189	0.8910
Error	137	1.4734		
Total	140			

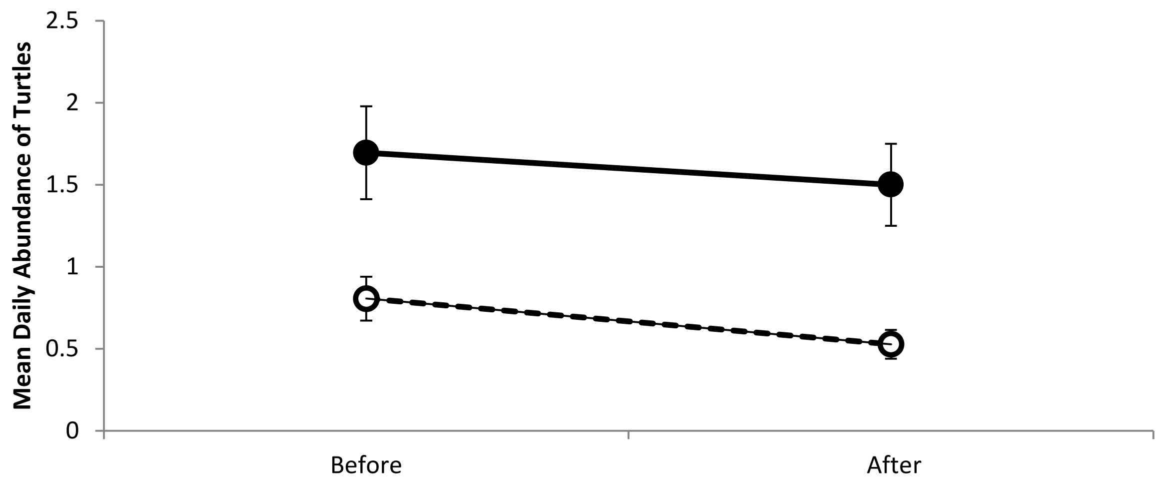

FIGURE 3 Mean daily abundance of turtles on the highway at the control (●) and impact (○) sites before and after the mitigation was installed.

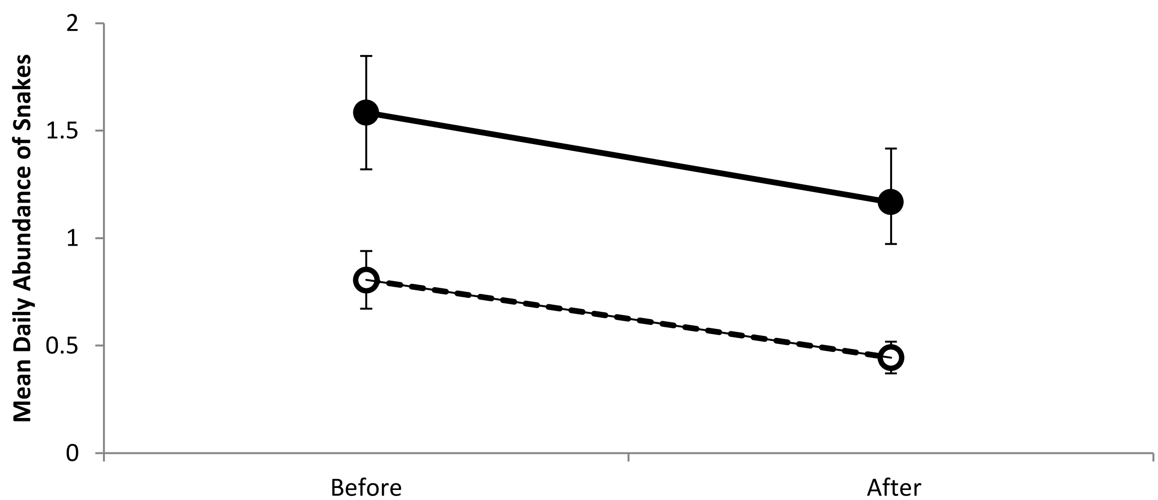

FIGURE 4 Mean daily abundance of snakes on the highway at the control (●) and impact (○) sites before and after the mitigation was installed.

Genetic Sampling

A total of 337 genetic samples (133 impacted site; 204 control site) were collected from 13 reptile species. Collection will continue through the rest of the study in order to obtain samples from as many species as possible, and at least from 30 individuals from each species to ensure an accurate sample of the population's genetic variation.

Radio Telemetry

A total of 22 turtles (10 Blanding's turtles, and 12 snapping turtles) were outfitted with radio transmitters and tracked for various lengths of time during the 2012 and 2013 active seasons. Home ranges from the field season of 2012 varied from 6.2 to 118.0 ha, and 4 individual's home ranges overlapped with the highway (Fig. 5). A total of 7 road crossings occurred using various means. A confirmed crossing through an ecopassage was documented by a female snapping turtle, and 2 road crossings by Blanding's turtles (a male, and a female) are presumed to have occurred via an ecopassage, based on location and proximity to the ecopassage at the time of crossing. A crossing by a male Blanding's turtle occurred using gaps in the fence, and 3 crossings by a male snapping turtle occurred via a 1 m diameter drainage culvert.

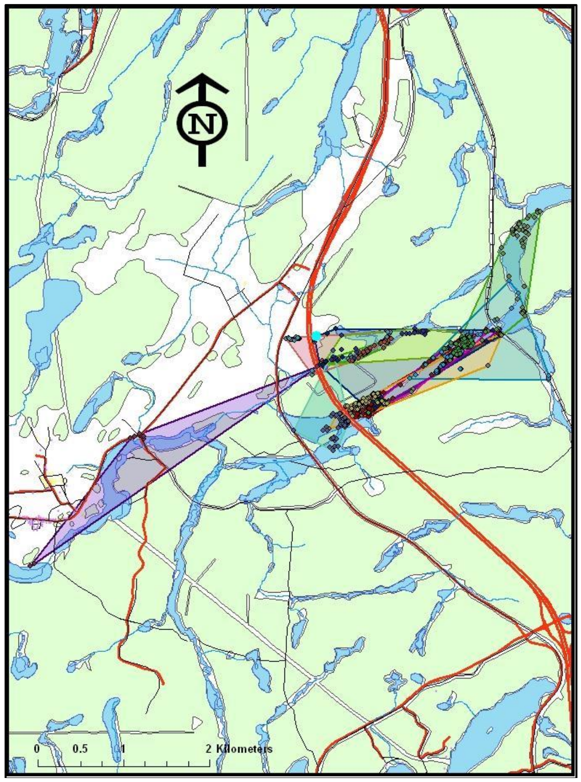

FIGURE 5 Individual home ranges of Blanding's turtles and snapping turtles around Highway 69 (thick double red line).

Ecopassage Monitoring

Wildlife Cameras

Since they have been installed a total of 162 individual animals were photographed in the three ecopassages, consisting of at least 14 non-reptile and 2 reptile species. Canada Geese (*Branta canadensis*; in 25.3% of the photographed wildlife) was the most common species recorded using the ecopassages. Midland painted turtles were observed using the ecopassages on 4 occasions (3 adults and 1 hatchling; 2.5% of photos), and northern watersnakes were observed using the ecopassages on 3 occasions (1.8% of photos). A number of reptile predators were seen using the ecopassages as well; Great Blue Herons (*Ardea herodias*; 19.7% of photos), raccoons (*Procyon lotor*; 3% of photos), American minks (*Neovison vison*; 1.2% of photos), and coyotes (*Canis latrans*; 0.6% of photos).

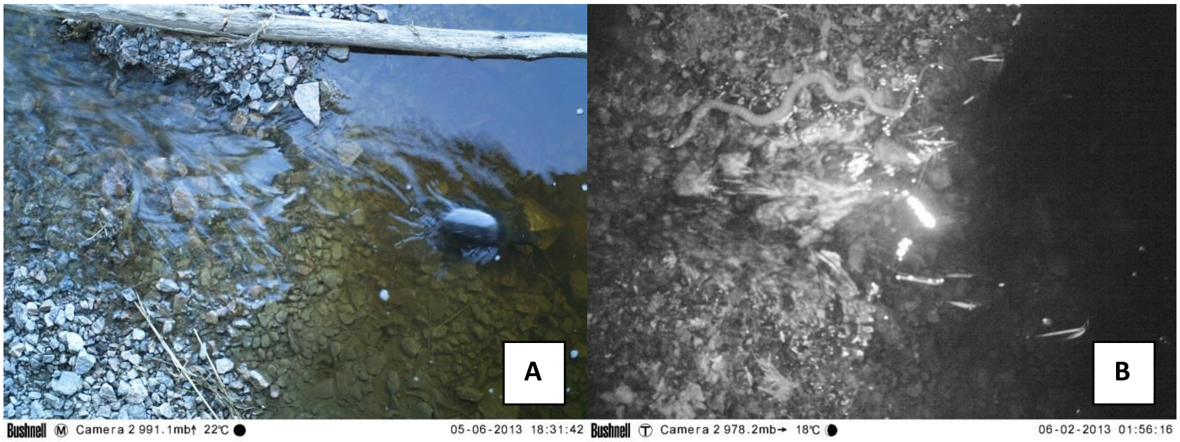

FIGURE 6 A midland painted turtle seen crossing during the day (A) and northern watersnake seen crossing at night (B).

Automated PIT-tag Readers

A total of 34 individuals have been implanted with PIT-tags: 12 Blanding's turtles, 7 snapping turtles, 5 midland painted turtles, 7 northern watersnakes, and 3 eastern gartersnakes. Several validation tests have been conducted for the automated PIT-tag readers using strategically released individuals to ensure the readers are scanning and logging properly. To date, none of the marked individuals have made a crossing through the ecopassage that is outfitted with the automated PIT-tag reader.

Willingness to Utilize (WTU) Test

To date, 14 midland painted turtles have been tested, and our goal is to test 50 individuals. Thus, these are extremely preliminary results. Only a single individual (7.1% of the total tested) has entered, and attempted to cross the ecopassage. Additionally, this individual turtle's attempt to cross the ecopassage was done slowly, and it only successfully crossed ¾ of the ecopassage in the allotted time period. The rest of the individuals either did not react to the culvert or stayed still throughout the entirety of the testing period (64.3% of the total tested,), or walked away from the testing area and ecopassage (28.6% of the total tested).

DISCUSSION

Effectiveness of the Exclusion Structures

Our preliminary findings suggest that the specific exclusion structure tested in our study is not effective at preventing reptiles from gaining access to the road-surface. We did not detect a significant reduction in the abundance of reptiles on the highway after the mitigation was installed, in turn leaving the reptile population around Highway 69 at the same threat level that existed prior to the installation of mitigation measures. This absence of positive results should not necessarily be generalized; as there have been many studies documenting high success rates with various styles of exclusion structures (Barichivich & Dodd 2002; Dodd et al. 2004; Aresco 2005). Although an overall reduction in the abundance of turtles (Fig. 2), and snakes (Fig. 3) were observed at the impact site, it was mirrored by a similar reduction in reptile abundance during the after period at the control site. This indicates that the reduction of abundance seen in the 2013 field season is likely due to environmental variation, or some other stochastic event. Furthermore, the difference between reptile abundance on Highway 69 between the control and test sites, when compared between the before and after periods was not different. As neither statistical test demonstrated a difference in reptile abundance at the impact site between when it was unmitigated to when it was mitigated, it is apparent that the exclusion structure tested in our study is not effective at preventing both snakes and turtles from gaining access to the road. So, why was this particular fence not effective, when other fences have been documented to be effective?

Closer examination of the fence itself has demonstrated a couple of issues that rendered this exclusion structure ineffective. These issues are based on the materials used to build the fence, as well as the construction process, rather than the concept of the exclusion structure itself.
In our study, the construction of the fence was completed by October 2012. Either during the installation of the fence, or from November 2012 to April 2013, a total of 115 reptile-sized (or larger) gaps had formed along the 3 km stretch of fence. Although this specific reptile fence was made out of a heavy gauge plastic mesh, believed to be an improvement on the temporary mitigation fence (drift/slit fence) previously used in areas of Georgian Bay (Followes 2010), rips, tears, and holes were still an issue. Another problem was that the fence was easily washed-out, as large sections were never properly buried in the first place because of the rocky terrain. Future analysis of our data will aim to determine if reptiles that are found on the road are closer than expected to these known gaps in the fence (which have all been documented using GPS), to explore if the reason this fence is ineffective is due to these holes.

A solution to the problems observed with the fencing design tested in our study would be to use more durable materials. Plastic and thin gauge hardware cloth are prone to rips and tears, and quickly degrade over the short-term. Hydrology, high-water levels, and drainage must be taken into consideration so that the threat of washouts and flooding are minimized, as exposure to water will degrade or destroy the fence. Roads are built to be long-lasting structures on our landscapes, and the mitigation measures for roads should be equally long-lasting. An alternative to expensive annual maintenance, and replacement every few years (Aresco 2005), a current necessity to maintain effectiveness of the exclusion fence at our study site, would be to install exclusion structures that are more durable and will persist over the long-term. An exclusion structure such as concrete or steel retaining walls (Barichivich & Dodd 2002; Dodd *et al*. 2004) fitted into the sloped gravel between the shoulder and ditch, or into raised sections of road, would provide a solid barrier that has been seen to have high levels of success (63% reduction in reptile and amphibian abundance on roads, 93% when hylid treefrogs were removed from the analysis; Dodd *et al*. 2004). Retaining walls built into the roadside structures would prevent the issues seen with rips and tears, as well as the issues with the fencing becoming washed-out, un-buried, and not buried in the first place. Although there may be a much higher initial cost for the installation of a more durable, permanent exclusion structure that is incorporated into the road design, over the long-term this mitigation option may be far more cost effective, and biologically effective.

A consideration that could be taken by governmental bodies in charge of either transportation or natural resources would be to make a point of including 'sign-off' authority within construction contracts, especially when the contract includes mitigation measures for SAR. This would provide an opportunity for these governing bodies to ensure that the construction of mitigation is adequate and vetted by an external expert, preferably a biologist specialized on the species for which the road is being mitigated, before the contract is completed. For exclusion structures this will involve walking the length of the fence, and examining it in its entirety, much in the same fashion that the animals using the mitigation measures would. Reptiles, such as snakes and turtles, will move along a fence-line constantly looking for a gap to exploit; if one exists they will find it.

Effectiveness of the Population Connectivity Structures

The ecopassages were seen to be minimally effective, and likely would have been more so if the exclusion structure was functioning properly. The wildlife cameras mounted in the ecopassages were able to detect both snakes and turtles using all three ecopassages. However, the number of reptiles seen in the ecopassages (n = 7) was much less than the number of reptiles observed on the highway (n = 35); nevertheless, documenting use of the ecopassage by reptiles is promising. Conversely, we have not recorded any of the PIT-tagged reptiles crossing the road, and this may simply be because only a small sample size of reptiles have been PIT-tagged to date. Furthermore, the 2013 full field-season has not yet been completed so individuals implanted with PIT-tags may not have attempted to cross the road yet, either over the road or through the ecopassage.

The radio telemetry study of Blanding's and snapping turtles established that a number of radio-tagged individuals used critical habitats (*i.e*. nesting, and overwintering sites) and seasonal habitats (*i.e.*, basking, and foraging sites) on both sides of the highway, further demonstrating the importance of installing population connectivity structures. Highway crossings were made by 18.2% of the radio-tracked individuals only. However, many of the turtles had large home ranges, up to 116 ha, indicating that when mitigating for turtles in this portion of central Ontario considerations must be made over a large area; mitigating only the immediate wetland may not be enough for animals with large home ranges.

Preliminary results from the WTU test demonstrated that painted turtles are willing to use the ecopassage, supporting the results seen with the wildlife cameras; however, they do so in much lower numbers than previously reported in the literature for this size and style of culvert. In our study, we found that only 7.1% of the individuals tested used the culvert. Paulson (2010) had 78.4% crossing success by painted turtles in culverts that were smaller in size, ranging from 0.6 m x 0.6 m to 1.2 m x 1.2 m, in a 120 minute testing period. Similarly, Woltz *et al.* (2008) had 15% crossing success testing a round culvert with a 0.6 m diameter, which was 9.1 m long, within a 15 minute time period. Our crossing success rate was substantially lower than that of Paulson (2010), and less than half that of Woltz (2008). Such differences may be due to the fact that we are testing a culvert that exists beneath a live section of highway, whereas the other two studies tested the culverts in an arena setting. Unlike the arenas, ours has the sights, sounds, and smells of real traffic on a major highway. Testing in situations with live traffic may provide a more accurate understanding of turtle's in-situ willingness to use an ecopassage. Further study will continue to increase our sample size of turtles tested, so that we can increase our certainty that we are currently seeing are representative of actual crossing rates and not just an artifact of low sample size during this preliminary portion of the study.

FUTURE DIRECTIONS OF RESEARCH

This study is only a quarter of the way through the second field season, and so work is currently on-going using all of the above-mentioned methods in order to rigorously evaluate the mitigation structures. It is important that we attempt to increase our understanding of the flaws in the current design of the exclusion structures, and use this knowledge to develop recommendations for future highway mitigation projects.

The genetic samples taken will be used to create baseline from which future research on the effects these mitigation structure will have on reptile population genetics over the long term. Although we try to determine the effectiveness of population connectivity structure it is important to understand that the structure's success will remain debatable until genetic studies can be conducted comparing pre- and post-mitigation population genetics for multiple species. The generational time for each of the species differs, a minimum of 10 years would be needed to test genetic isolation for northern watersnakes (2-3 year generation time, and a 7-9 year life span; King & Lawson 2001; Rowell 2012). While a minimum of 50 years would be necessary to test for alleviation of genetic isolation post-mitigation in snapping turtles (15-25 year generation time, and a 60-100 life span; Congdon *et al.* 1994). These 'snapshots' of the reptile population genetic will provide the require information to one day truly determine the success of these culverts.

CONCLUSION

It is crucial for highway designers and wildlife managers to rigorously and thoroughly test the effectiveness of mitigation measures. As global biodiversity decreases (McKee *et al.* 2004), and threats to animal populations are identified, we must strive to increase our level of protection for rare and imperiled species beyond current norms. We need to understand that mitigation is more than a political mandate satisfying a piece of legislation, it is a means to create infrastructure that directly reduces the negative impact of development on species (Dodd *et al*. 2004). If care is taken to assess the effectiveness of mitigation, and steps are taken to try to mitigate development as best we can, not only are there benefits to SAR, but also to the overall ecosystem and human health alike (Brooks *et al.* 2006).

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Baxter-Gilbert obtained his BSc in Biology (2009) and his GDip in Science Communication (2010) from Laurentian University. He has been working in the field of herpetology and conservation biology for the past 5 years. He is currently completing his MSc at Laurentian University examining the effectiveness of mitigation measures under the co-supervision of Dr. David Lesbarrères and Dr. Jacqueline Litzgus.

David Lesbarrères

Lesbarrères is an associate professor and director of Graduate Studies at Laurentian University. His interest included theoretical and applied questions about the evolution and ecology of amphibian species and communities. For the past 6 years his research program centred on population genetics in human dominated landscapes, focussing on gene flow interruption and its consequences for amphibian populations. Part of his research also investigated the fitness consequences of phenotypic and genetic variation. Amphibian populations are facing various threats in Northern Ontario such as habitat removal, connectivity disruption and EIDs and his research ultimately integrate all these aspects to understand the declines of populations. His work integrates intense field work coupled with molecular approaches for the analysis of parentage and population genetics, and laboratory experiment to estimate measures of fitness. The sum of his research has resulted in 36 publications.

Jacqueline Litzgus

Litzgus obtained her BSc in Wildlife Biology (1993) and her MSc in Ecology (1996) from the University of Guelph, Ontario. She then worked as a research lab coordinator (1996-99) in the Ecophysiological Cryobiology lab at Miami University, Ohio. Litzgus obtained her PhD (2003) in Ecology, Evolution and Organismal Biology from the University of South Carolina, and then began her faculty position at Laurentian University, Ontario in 2004. She has been working on the ecology and conservation of reptiles since 1991. Her research program has been funded by NSERC, CFI, Environment Canada, Parks Canada, National Geographic, WWF, Canadian Wildlife Federation, Ontario Power Generation, and the Ontario Ministry of Natural Resources, among others. The research has resulted in 46 peer-reviewed publications and over 170 presentations at scientific conferences. Litzgus has trained 20 undergraduate researchers, 14 MSc students, 1 PhD student, and 1 post doc. The basic and applied research outcomes from the Litzgus Lab have been used in policy changes and management plans related to species at risk.

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