Effectiveness of wildlife mitigation measures for turtles on Highway 69 from 2015 to 2016

Report for public dissemination

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Snapping Turtle swimming through Reptile Tunnel, W. Kowbasniuk

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Videos available on YouTube at:

https://youtu.be/yrDYJg_Kx9k
https://youtu.be/ak5-f6inII0

Note: All Blanding's Turtle specific locations and maps were removed from this report.

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1 Summary

1. This report documents the results of monitoring effectiveness of road mitigation measures that include three twinned concrete box culverts (2.4 m high by 3.3 m wide, and 24.1 m long), and associated reptile exclusion fencing (4.3 km to 5.5 km both sides of the highway) for turtles, primarily Blanding's Turtle, as part of a larger highway expansion project on a new highway alignment of Highway 69. Effectiveness was evaluated by a measure of reduced highway mortality and by use of crossing structures by turtles.

2. Monitoring consisted of daily walking transects in 2015 and 2016 between May 15th and July 15th. The walking transects took place along two 3.5 km sections of highway, one mitigated (Sheppard Lake) and one unmitigated (Clear Lake). Both transects had similar adjacent wildlife habitat and were approximately 40 km apart.

3. Camera monitoring was also conducted in 2015 and 2016 between June and the beginning of October. The camera monitoring took place in three reptile tunnels intended for use by turtles and other animals along the new highway alignment.

4. In order to ensure full functionality of the reptile exclusionary fencing an assessment and maintenance phase was completed in early May 2015 and 2016. In 2015, fence maintenance focused on improving fence at drainage culvert entrances and medians and took 36 hours. In 2016, minor repairs to fence rips and tears were required and took 3.5 hours. In 2015, three one-way gates were closed and in 2016 two more additional gates were closed as part of the fence extension.

5. Based on recommendations in 2015, a reptile fence extension was completed at the north-west end so the fencing was coincident with the east fence end and joined drainage systems 2 and 3. In 2016, the south-west, and south-east fence ends were extended to the large animal underpass approximately 300 m south to provide an exclusionary buffer beyond the wetland habitat at Reptile Tunnel 3 and to provide another crossing opportunity for small animals. The reptile fencing was 4.8 km in 2015 and increased to 5.6 km in 2016.

6. During the 2015 and 2016 walking transects, a total of seven turtles were observed along the Sheppard Lake transect and 33 turtles were observed along the Clear Lake transect. Eighty-four percent of the turtles found were dead on the road. Sixteen percent of the turtles observed were Blanding's Turtles, 29% were Snapping Turtles and 55% were Painted Turtles.

7. In a previous study conducted in 2012 and 2013, there was a significant 20% increase in turtles when comparing a before fence completion scenario with an after fence completion scenario in a before-after-control impact design. In this study there was a significant 90.5% reduction in the number of turtles on the road in 2016 relative to 2012 when fencing was being implemented. For the most part, this discrepancy in fence effectiveness is a result of the fence improvements completed in 2015 and 2016. Other reasons for contrasting fence effectiveness between the two studies are also discussed.

8. The difference in turtles found between the mitigated Sheppard Lake transect with the unmitigated Clear Lake transect was significant. Adjacent habitat at both sites consisted of an open water lake, and several wetland systems lending support to the fence being the main reason fewer turtles were found at Sheppard Lake. However, the only way to fully evaluate whether the difference between sites is attributed to the mitigation is to conduct a Before-After-Control-Impact study which was not possible for this study.

9. The fence material currently used is predicted to last up to five more years. However, a routine maintenance plan is required to maintain functionality of the fence. Maintenance requirements entail the use of a shovel, some pliable wire and a pair of plyers and requires approximately 1 person, 1 day each year in May.

10. Future fence designs require selection of materials that are robust to withstand ultraviolet degradation, and extreme climatic conditions during winter freeze and spring thaw. Furthermore, fencing needs to be improved and adapted with changes to hydrology that are occurring in and around the new Highway Alignment that bisects considerable wetland and open water habitat.

11. A total of 20 turtles were captured using the Reptile Tunnels in both 2015 and 2016, 10 in each year. Of these 12 were Painted Turtles and eight were Snapping Turtles and none were Blanding's Turtles. It is believed that the camera monitoring captured the majority of adult turtles moving into the three tunnels in the active season. A lack of Blanding's Turtles is likely attributed to an adaptation period or low abundance rather than the size or location of the crossing structures.

12. Monitoring small animals, especially cold-blooded reptiles with available camera technologies is challenging. This is because smaller cold-blooded animals that are typically found in water or at wet sites, do not trigger motion-activated cameras that require temperature differentials between the animal and ambient environment.

13. It is recommended to continue monitoring the tunnels that are also being monitored for large animals during the turtle nesting migrations in June using supplementary techniques.

These techniques include using motion activated cameras with external ports that allow additional triggering systems such as vibration mats and/or active beams. Additional monitoring in 2017 and beyond can explore monitoring techniques as well as assess whether Blanding's Turtles will use the tunnels after a possible habituation period to the new highway alignment.

2 Introduction

Road mortality is a leading cause of decline for many reptile species (Gibbons et al. 2000) and is a well-documented threat in Ontario (Ashley & Robinson 1996; Haxton 2000; MacKinnon et al. 2005). Seven of Ontario's eight turtle species are listed as Species at Risk (SAR) under the Endangered Species Act (2007) and road mortality is a leading cause of decline for five of these species. Turtles are particularly vulnerable to road mortality because their life history strategy is characterized by long life spans, very high adult survivorship, low reproductive recruitment rates, and delayed sexual maturity (up to 25 years). Consequently, even small, but ongoing increases in adult mortality can lead to population declines (Congdon et al. 1993) and slow recovery (Brooks et al. 1991).

There are several mitigation measures that may be used to lessen the risk of turtle road mortality (Ontario Ministry of Natural Resources and Forestry 2016). The measures range from the use of turtle road signs (Gunson & Schueler 2012) to integration of crossing structures and fencing into transportation planning (Dodd et al. 2009). Exclusion fencing along roads, in combination with crossing structures, allows wildlife to continue accessing resources and habitats safely. These mitigation measures are recommended for species that are vulnerable to road mortality and that do not show road avoidance, such as Ontario's turtle populations (Jaeger & Fahrig 2004).

Turtles are particularly vulnerable to road mortality because female turtles often use open roadsides for nesting (Obbard & Brooks 1980; Beaudry et al. 2010; Steen et al. 2012). In most cases, unaware motorists unintentionally run over turtles that have entered the roadway or right-of-way, and in some areas these strikes are deliberate (Ashley et al. 2007). Further, when turtles are on a road and are threatened by vehicle traffic, they respond by retracting into their shell, a behaviour that inevitably leads to road mortality.

The objective of a crossing structure is to allow wildlife to move safely over or under the road in order to access habitat. Crossing structures can be used in conjunction with exclusion fencing to keep wildlife off of the road while funneling animals towards the crossing structures. This concept is generally understood by practitioners who implement wildlife mitigation infrastructure in transportation planning, however design details are still being tested and explored that will maximize effectiveness. The following list contains considerations for effective fence and crossing design that will reduce turtle mortality on roads:

- Materials selected; must be able to meet feasible budgets and low-maintenance requirements, as well as withstand site specific environmental variation;
- Design, location, and number of crossing structures;

- Design, location, and length of fencing installation;
- Detail design specifications and installation must be communicated effectively between the road agency and the contractors;
- Flexibility is required for installation that integrates road and landscape features, such as drainage pathways along right-of-way;
- A maintenance plan is required to perform monthly (soon after installation) and annual routine checks for fence and crossing structures.

The purpose of this study was to assess, maintain and improve a wildlife crossing structure and fencing system built for reptiles, primarily Blanding's turtles, on Highway 69 over a two year period. Three crossing structures (reptile tunnels) were initially installed on a new highway alignment in September 2011. Subsequently, 4.8 km of reptile exclusionary fencing was installed from June to October 2012 that was integrated with the three tunnels and monitoring and evaluation was completed in 2012 and 2013 (Baxter-Gilbert 2014; Baxter-Gilbert et al. 2015) and again as part of this study in 2015 and 2016. This report documents findings from both 2015 and 2016 and compares results between the 2012-2013 and 2015-2016 monitoring periods.

3 Background Information and Study Area

The study area is along two 3.5 km sections of Highway 69 (Figure 1, inset). The first section is called Sheppard Lake transect and is approximately 40 km south of Sudbury near the unincorporated town of Estaire and the community of Burwash in northeastern Ontario. The second section is called Clear Lake transect and is adjacent to Grundy Lake Provincial Park. This section is situated approximately 40 km south of Sheppard Lake (Figure 1, inset). Both areas have very few residential inhabitants surrounding the highway, as the area is characterized as a recreational cottage country region. Weather in the area is characterized by a humid continental climate with warm, and often hot, summers and long, cold, snowy winters.

The terrain is dictated by Canadian Shield rock and the highway bisects large expanses of rock cuts, extensive wetlands, lakes, and several river gorges. The abundant wetland and undeveloped habitat is home to a large diversity of both small and large animals. The primary mid-sized to large carnivores present in the study area are Eastern Wolf (*Canis lycaon*), and Black Bear (*Ursus americanus*). Large ungulates include White-tailed Deer (*Odocoileus virginianus*), Moose (*Alces alces*), and a reintroduced Elk herd (*Cervus elaphus*). Common smaller mammals include Fisher (*Martes pennanti*), River Otter (*Lontra canadensis*), Mink (*Neovison vison*), Muskrat (*Ondatra zibethicus*), Racoon (*Procyon lotor*), and Rabbit (*Sylvilagus floridanus*). More common reptiles and amphibians include the Midland Painted Turtle (*Chrysemys picta*), Northern Watersnake (*Nerodia sipedon*), and Eastern Gartersnake (T*hamnophis sirtalis*); Spring Peepers (*Pseudacris crucifer*), and Green Frog (*Rana clamitans*). Species at Risk reptiles include Blanding's Turtles (*Emydoidea blandingii,* Threatened), Massasauga Rattlesnake (*Sistrurus catenatus*, Threatened), and Snapping Turtle (*Chelydra serpentine,* Special Concern).

As part of a larger expansion project on Highway 69, approximately 10 km of highway was expanded from two to four lanes near the community of Burwash. This phase was comprised of a 6.8 km highway re-alignment, where reptile tunnels and exclusion fencing were integrated (Figure 1). The new alignment was opened to traffic in phases in the summer and fall of 2012. Beginning on June 6th, 2012, two lanes of traffic (currently the northbound lanes) were opened for vehicle use on the new alignment, diverting vehicles away from what is now termed 'Old Highway 69' (Figure 1). On August 8th, 2012, all lanes of traffic were opened for vehicle use.

During the detail design phase, wildlife mitigation measures were integrated into the new highway alignment project. This consisted of several measures for large animals including 10 km of large animal fencing, 27 one-way gates, one 30 m wide wildlife overpass, one large wildlife underpass (twin 5m x 5m culverts) and one wildlife creek-bridge pathway under the Lovering Creek Bridge. For smaller animals, specifically the Blanding's turtle, mitigation

measures comprised of three twinned concrete box culverts (2.4 m high by 3.3 m wide, and 24.1 m long) and 5.5 km of reptile fencing (both sides of highway; Figure 1). The three reptile tunnels were installed in 2011 and the fencing was installed in 2012.

The reptile fencing is made up of heavy-gauge plastic textile extending 0.8 m above and 0.2 m below-ground with a 0.1 m wide lip running perpendicular underground. The fence was affixed to the base of the 2.4 m tall large animal mesh wire fencing (Photo 17). The mesh in the large animal fencing gradually increases with the height of the fence. At ground level it is 5 cm high and 16 cm wide and at the height of the reptile exclusion fencing it is 7 cm high and 16 cm wide. One main continuous section of 4.3 km of reptile fence, or 90% of the entire reptile fence, spanned the three reptile tunnels on both sides of the highway in 2015. In 2016 the reptile fence was extended resulting in 5.5 km of continuous fencing on the new alignment. Five additional drainage systems were located within the study area. Four of five of these drainage culverts were connected with continuous fencing in 2016 fence extensions.

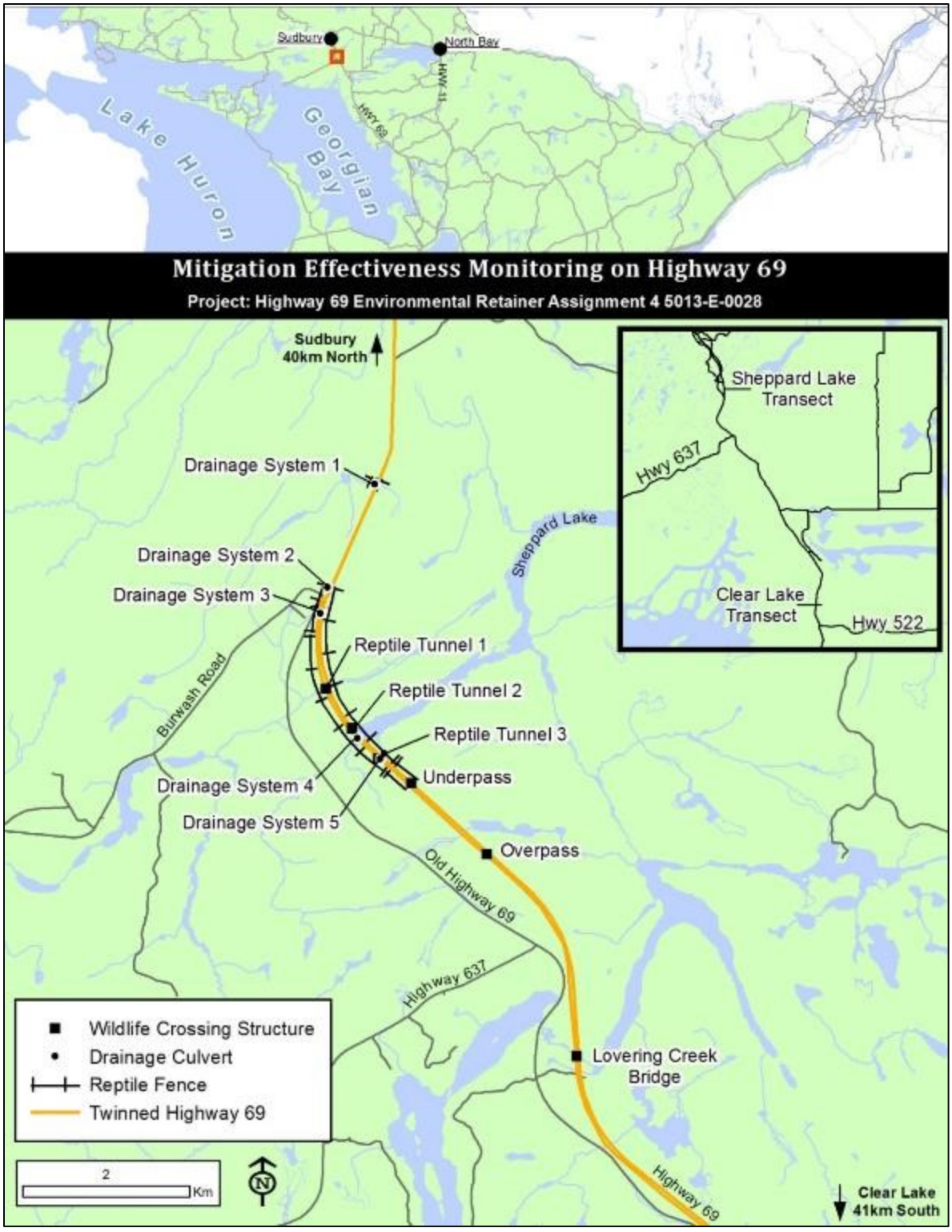

Figure 1: Overview of the study area (inset), with a zoom-in of the mitigation measures and highway configurations along the Sheppard Lake Transect on Highway 69.

4 Fence Assessment and Maintenance

Prior to monitoring in 2015 and in 2016, the reptile exclusion fencing was surveyed and repaired. Maintenance entailed ensuring the fence was buried, no holes existed in material, material was adequately attached to fence, and no gaps or holes associated with drainage culverts, reptile tunnels, and large animal one-way gates or along the fence itself were evident that allowed turtles to breach the barrier.

In 2015, fence maintenance occurred between May 5th and May 14th and in 2016, between May 2nd and May 3rd. See Appendix H for a detailed summary of the maintenance. There were few holes and tears in 2015. In 2016 there were three vertical tears or gaps that were patched to avoid further deterioration (Photo 7; Photo 8). In 2015, the majority of the maintenance entailed elevating the fence higher than existing water levels at each drainage system (DS) entrance and stabilizing posts in the medians and using large rocks to ensure fencing is flush to the ground (Appendix H). In both 2015 and 2016, routine maintenance entailed filling in a reoccurring wash-out at DS 1 (Photo 1; Photo 2), ensuring the fence was buried, closing all one-way gates along or at fence ends (Photo 5; Photo 6), reattaching sections of reptile fence to the large animal fence (30 to 100 m), as well as ensuring fencing securely abutted all culvert and reptile tunnel entrances (Photo 13, Photo 14, Appendix H).

In 2015 a Geographic Positioning System (GPS) inventory was completed for all fence ends, drainage culverts, and reptile tunnels and this was updated in 2016. A map was then created for the Sheppard Lake fenced area (Figure 1). In both years there were five drainage systems, as well as three larger reptile tunnels that were associated with the wildlife exclusion fencing. In 2016, the fencing was extended approximately 300 m south to connect to the large animal underpass (See Appendix H). In 2016, the continuous section of the fence at the northwest side was also extended approximately 500 m to abut DS 2 (Photo 18; Photo 20). This modification created equivalent lengths of exclusion fencing (5.6 km) on both sides of the highway that extended from the large animal underpass to DS 2 (Figure 1). DS 1 remained an isolated structure from the other potential crossing structures: 126 m of fencing on both sides of highway that was not functional because the east culvert entrance ended on the highway side of the fencing.

5 Road Survey Assessment

5.1 Methods

In 2015 and 2016, roadside walking surveys were completed once per day between 8 AM and 5 PM along two transects on Highway 69: Sheppard Lake and Clear Lake from May 15th to July 15th (Figure 1). In both years, the transects along Sheppard and Clear Lake were equivalent in length, and the survey transect lengthened 500 m in 2016 to accommodate a new fence extension at the south end of Sheppard Lake. Subsequently, the Clear Lake transect was also extended 500 m, to end at the Highway 522 interchange. In 2015, these transects were 3 km long, and in 2016 they were both 3.5 km. In both years the transects extended approximately 300 m beyond the exclusion fencing to account for fence end effects.

On average, each transect took one hour to complete. One field technician would drive to the end of each transect and walk along the shoulder of the northbound lane until reaching the end. At the end, the technician would then turn around and walk back to the vehicle along the southbound lane. While walking, the technician would examine the road, shoulder, and ditches to look for any signs of wildlife.

If a reptile was observed during a walking survey, or while driving to and from the Clear Lake and Sheppard Lake transect sites, the species, sex, alive or dead, nesting behaviour, plastron mid-line length and width, age (hatchling, juvenile, adult) and location (Universal Transverse Mercator NAD 83, Zone 17N) were recorded. The size measurements were only taken for carcasses that were intact. Sex was only identified for intact dead carcasses or live adults. Detailed images of all SAR reptile observations were also captured using a digital camera. The daily weather conditions, and temperature high and lows, were also recorded.

For the duration of the roadside walking surveys, the Sheppard Lake transect was classified as the mitigated section of Highway 69. The Clear Lake transect was categorized as the unmitigated section because there are no wildlife mitigation measures. Both Sheppard and Clear Lake transects are similar with open, small lakes on the east side of the highway, and interconnected wetland forested area on both sides of the highway (Photo 55; Photo 56:). The habitat adjacent to both highway transects are known to have Blanding's Turtles and other reptiles present (Natural Heritage Information Centre, unpublished data).

5.1.1 Statistical Analyses

Two complimentary sets of analyses were used to evaluate the effectiveness of the reptile exclusion fence at reducing turtle presence along Highway 69. At the Sheppard Lake site the

number of turtles found over four years (2012, 2013, 2015 and 2016) with each year representing a different status of the reptile exclusion fence installation was used to evaluate mitigation effectiveness (Table 1). The 2012, 2013 data was a subset of data obtained from previous monitoring efforts (Baxter-Gilbert et al. 2015). This analysis was supplemented with a comparison of the number of turtles detected at the mitigated Sheppard Lake site with the number of turtles detected at the unmitigated Clear Lake site over the two years (2015 and 2016) when both sites were monitored as part of this study. Effects of both site and year were tested. For all analyses both dead and alive turtles were used, as the purpose of the reptile exclusion fence is to prevent turtles from accessing the road, any turtle found on the road represents a breach of the mitigation system.

All turtle species were pooled together and the data was summarized into eight full seven day weeks per year. The calendar days for each year were as follows: May 19 – July 13 in 2012 and 2016 and from May 20 to July 14 in 2013 and 2015.

Table 1: Integration of reptile fencing (installation, maintenance, and improvements) and crossing structures on Highway 69 from 2011 to 2016.

Year	Installation
2011	• June to October • Three reptile tunnels built
2012	• June to October • 4.8 km replacement of temporary to permanent reptile exclusion fencing along Sheppard's Lake Transect on the new highway 69 alignment
2015	• May • Reptile exclusion fencing maintenance: closed gaps and one-way gates, 50 m extension, increased fence height at drainage culverts
2016	• May • Reptile exclusion fencing maintenance and approximately 800 m fencing added to the north and south ends

5.1.1.1 Sheppard Lake Analysis

A Poisson Generalized Linear Model (GLM) was used to evaluate the effect of mitigation status on the number of turtles found on the road over four years (2012, 2013, 2015 and 2016) at Sheppard Lake.

The model is as follows:

Count Turtles ~ Mean Temp + Mean Daily Rainfall + Nesting + Year

Where count of turtles, mean temperature, and mean rain are all weekly means. Nesting is whether or not it is turtle nesting season (defined as the month of June) and year is the status of the mitigation system (Table 2). The year 2012 was used as the reference level meaning the effect of each of the three other years on the weekly turtle counts were compared to 2012. Daily temperature and rainfall data was obtained for Sudbury, Ontario and was used to represent conditions at Sheppard Lake (Environment Canada, 2016). While many factors such as water temperature influence the precise timing of nesting (Obbard and Brooks 1987), the majority of turtles found on roads in Ontario occur in June (Gunson et al. 2014) and this month generally captures peak nesting season.

Robust standard errors and p-values were generated to account for potential effects of over-dispersion in the data (UCLA: Statistical Consulting Group, 2016). Further, the residual variance was used to estimate how well the data fit the model, also known as a goodness of fit test. Finally, an analysis of deviance was used to test for an overall effect of mitigation status (year). This was done by comparing the deviance of the full model with that of a model that excludes the year parameter (UCLA: Statistical Consulting Group, 2016).

5.1.1.2 Clear Lake and Sheppard Lake Comparison

A paired t-test (mean counts paired by sampling week) was used to evaluate differences in turtle presence between the mitigated Sheppard Lake site and the unmitigated Clear Lake site using data collected in the 2015 and 2016 turtle surveys. Temperature and rainfall were not integrated into a model because the available meteorological data was the same for both sites. Additionally, no major differences in the timing of nesting season between the two sites is expected. Therefore, the paired t-test design controls for the majority of the variability in temporal factors. Results are reported as the mean ± 1 standard error unless otherwise indicated.

5.2 Results

In 2015, a total of 29 turtles (Midland Painted Turtle, Common Snapping Turtle, Blanding's Turtle) and four snakes (Northern Watersnake, Eastern Gartersnake, Red-bellied Snake) were found while driving and walking the two transects on Highway 69 (Table 2; Figure 3). There were 23 turtles found along the Clear Lake transect, and six found along the Sheppard Lake transect (Table 2). In 2015, 27 (90%) of the turtles that were observed were found dead on the road (DOR), whereas three of the turtles were found alive on the road (AOR). Of the turtles where sex could be determined, 6 (67%) were female (Table 2).

During the 2016 monitoring period, fewer turtles were found (11 turtles: Midland Painted Turtle, Common Snapping Turtle, Blanding's Turtle) and four snakes (Northern Watersnake, Eastern Gartersnake, Massasauga Rattlesnake) were found during the walking transects on Highway 69 (Table 2). A total of 10 turtles were found along the Clear Lake transect, and one found along the Sheppard Lake transect (Table 2). In 2016, 7 (64%) of the turtles that were observed were found DOR, whereas three (27%) of the turtles were found AOR. Of the turtles where sex could be determined, five (71%) were female whereas two (28%) were male (Table 2).

In both years 41 turtles were found on the walking transects and only 27% of these were found in 2016. This was mainly from the fewer number of Painted Turtles found at the Clear Lake transect. In 2015, 14 (13 DOR) Painted Turtles were observed along the Clear Lake transect, whereas only six (four DOR) where observed in 2016. In both 2015 and 2016, all but one of the Blanding's Turtles were observed along the Clear Lake transect (**Figure 2**; Figure 3). The one Blanding's Turtle carcass observed along the Sheppard Lake transect was old and dried, and was likely died on the road prior to the commencement of the 2015 surveys (**Figure 2**).

Of the six turtles found on the Sheppard Lake transect in 2015, three (one Blanding's, one Painted and one Snapping Turtle) likely breached the southwest end of the reptile exclusion fence as all were found within 150 m of the fence end (**Figure 2**). The other three turtles found at Sheppard transect were three juvenile Snapping Turtles that likely entered the highway on the west side where the reptile exclusion fence was no longer continuous with the primary fenced section (**Figure 2**). Only large animal fencing had been installed along these open sections, which permitted the juvenile turtles to breach the fence through the mesh material. In addition, the two northernmost Snapping Turtles were found at Drainage System 3, where a one-way gate had not been closed with reptile exclusion fencing (Photo 5).

In 2016, no turtles were found within the fenced highway section at the Sheppard Lake transect but one adult Snapping Turtle was observed crossing the highway, 10 m south of the large

animal underpass and reptile exclusion fencing (**Figure 2**). The turtle likely breached the large animal fencing on either side of the highway as it was a juvenile (4 cm plastron width). This turtle was included in the statistical analysis because it occurred with the fence end buffer limit of the transect length.

At the Clear Lake transect, four of the five total snake observations were in 2016 (Table 2, Figure 3). This included three species: Northern Watersnake (1), Eastern Gartersnake (2) and a single Massasauga Rattlesnake (Photo 30:). In 2015, a single Red-bellied Snake was observed at the Clear Lake transect (Table 2; Figure 3). At the Sheppard Lake transect, a total of three snakes were found in 2015 and none in 2016 (Table 2, Figure 3). This included two species: Eastern Gartersnake (2) and a Northern Watersnake (1), which was found alive during the walking transect.

The dead on road turtles observed along the Clear Lake transect in 2015 and 2016 were significantly clustered in three distinct sites along the highway (Figure 3). The first northerly hotspot was comprised of Painted Turtles (5), one Massasauga Rattlesnake, one Gartersnake, and a Blanding's Turtle just south of the cluster. The second hotspot at Clear Lake was comprised of Painted Turtles, Snapping Turtles (3), Blanding's Turtles and two Eastern Gartersnakes. One of the Snapping Turtles observed in 2016 was found nesting at Clear Lake in early June (Photo 26:). The most southerly hotspot at the southernmost drainage culvert was comprised of a Blanding's Turtle (1), and Painted Turtles (4). In 2016, a Painted turtle hatchling was found at the location in early May (Photo 28:).

Table 2: A summary of reptiles found on Clear Lake, and Sheppard Lake transects in 2015 and 2016.

Species	Year	On road	Age	Sex	DOR/AOR
Clear Lake Transect					
Blanding's Turtle	2015	4	2 Ad; 2 Unk	4 Unk	4 DOR
	2016	2	2 Ad	1 Fem , 1 Unk	2 DOR
Painted Turtle	2015	14	8 Ad; 1 Juv; 5 Unk	4 Fem, 2 Mal, 8 Unk	13 DOR, 1 AOR
	2016	6	5 Ad; 1 Hat	3 Fem, 2 Mal, 1 Unk	4 DOR, 2 AOR
Snapping Turtle	2015	5	1 Ad, 4 Juv	1 Fem, 4 Unk	5 DOR
	2016	2	1 Ad, 1 Juv	1 Fem, 1 Unk	1 DOR, 1AOR
Total Turtles		**33**	**19 Ad; 6 Juv,1 Hat, 7 Unk**	**10 Fem, 4 Mal, 19 Unk**	**29 DOR, 4 AOR**
Eastern Gartersnake	2015	0	0	0	
	2016	2	2 Ad	2 Unk	2 DOR
Red-bellied snake	2015	1	1 Ad	1 Unk	1 DOR
	2016	0	0	0	
Massasauga Rattlesnake	2015	0	0	0	
	2016	1	1 Ad	1 Male	1 DOR
Northern Watersnake	2015	0	0	0	0
	2016	1	1 Ad	1 Unk	1 AOR
Total Snakes		**5**	**5 Ad**	**1 Mal, 4 Unk**	**4 DOR, 1 AOR**
Sheppard Lake Transect					
Blanding's Turtle	2015	1	1 Unk	1 Unk	1 DOR (likely from last year)
	2016	0	0	0	
Painted Turtle	2015	1	1 Ad	1 Fem	1 DOR
	2016	0	0	0	
Snapping Turtle	2015	4	1 Ad, 3 Juv	4 Unk	3 DOR, 1 AOR
	2016	1	1 Ad	1 Unk	1 AOR
Total Turtles		**7**	**3 Ad, 3 Juv, 1 Unk**	**1 Fem, 6 Unk**	**5 DOR, 2 AOR**
Eastern Gartersnake	2015	2	2 Ad	2 Unk	2 DOR
	2016	0	0	0	

Species	Year	On road	Age	Sex	DOR/AOR
Northern Watersnake	2015	1	1 Ad	1 Unk	1 AOR
	2016	0	0	0	
Total Snakes		**3**	**3 Ad**	**3 Unk**	**2 DOR, 1 AOR**
Grand Total Turtles	**2015-2016**	**40**	**22 Ad, 9 Juv, 1 Hat, 8 Unk**	**11 Fem, 4 Mal, 25 Unk**	**34 DOR, 6 AOR**
Grand Total Snakes	**2015-2016**	**8**	**8 Ad**	**1 Mal, 7 Unk**	**6 DOR, 2 AOR**
Total Reptiles	**2015-2016**	**48**	**30 Ad; 9 Juv; 1 Hat, 8 Unk**	**11 Fem, 5 Mal, 32 Unk**	**40 DOR, 8 AOR**

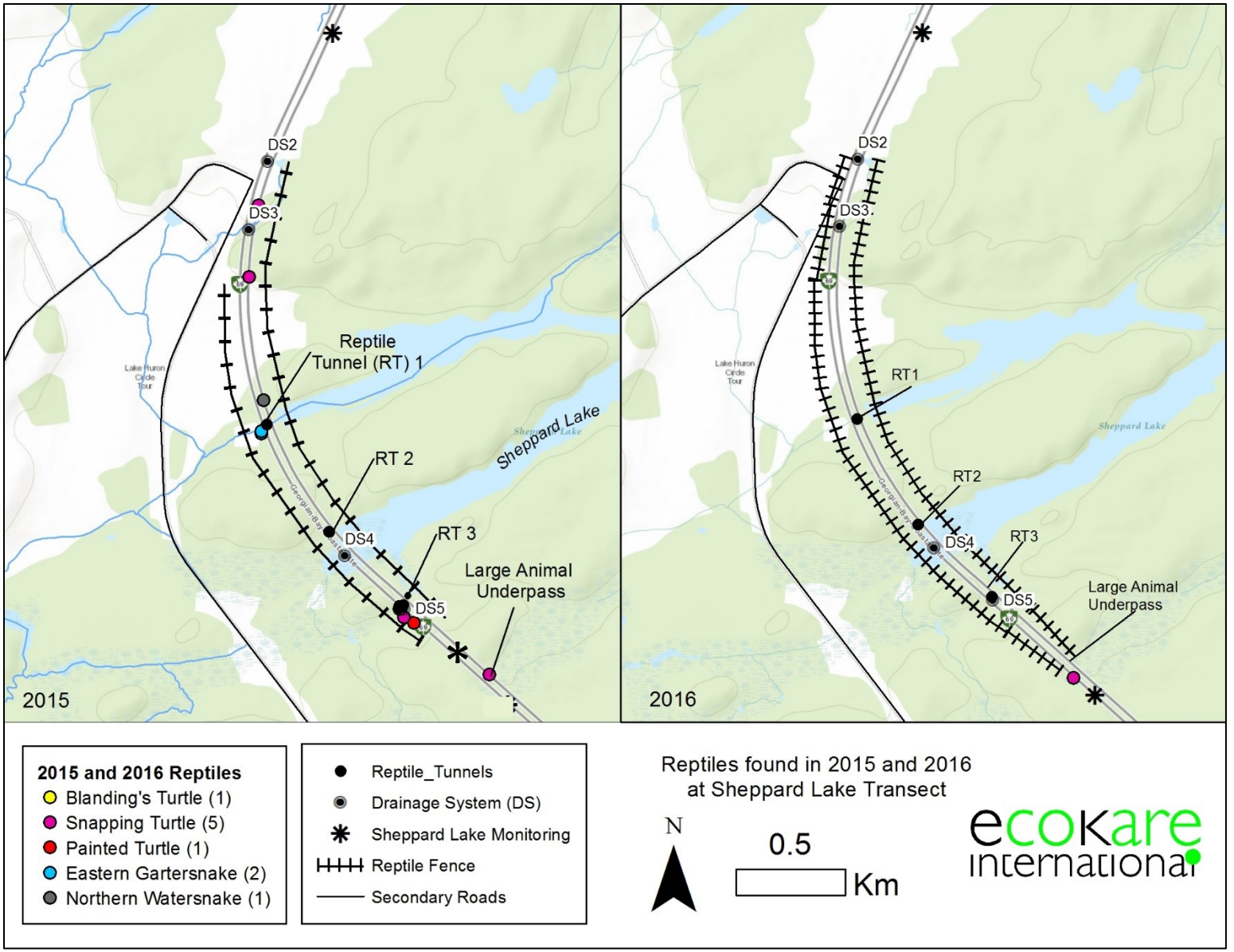

Figure 2: Reptiles found at the Sheppard Lake Transect in 2015 and 2016. Note reptile fence extension in 2016.

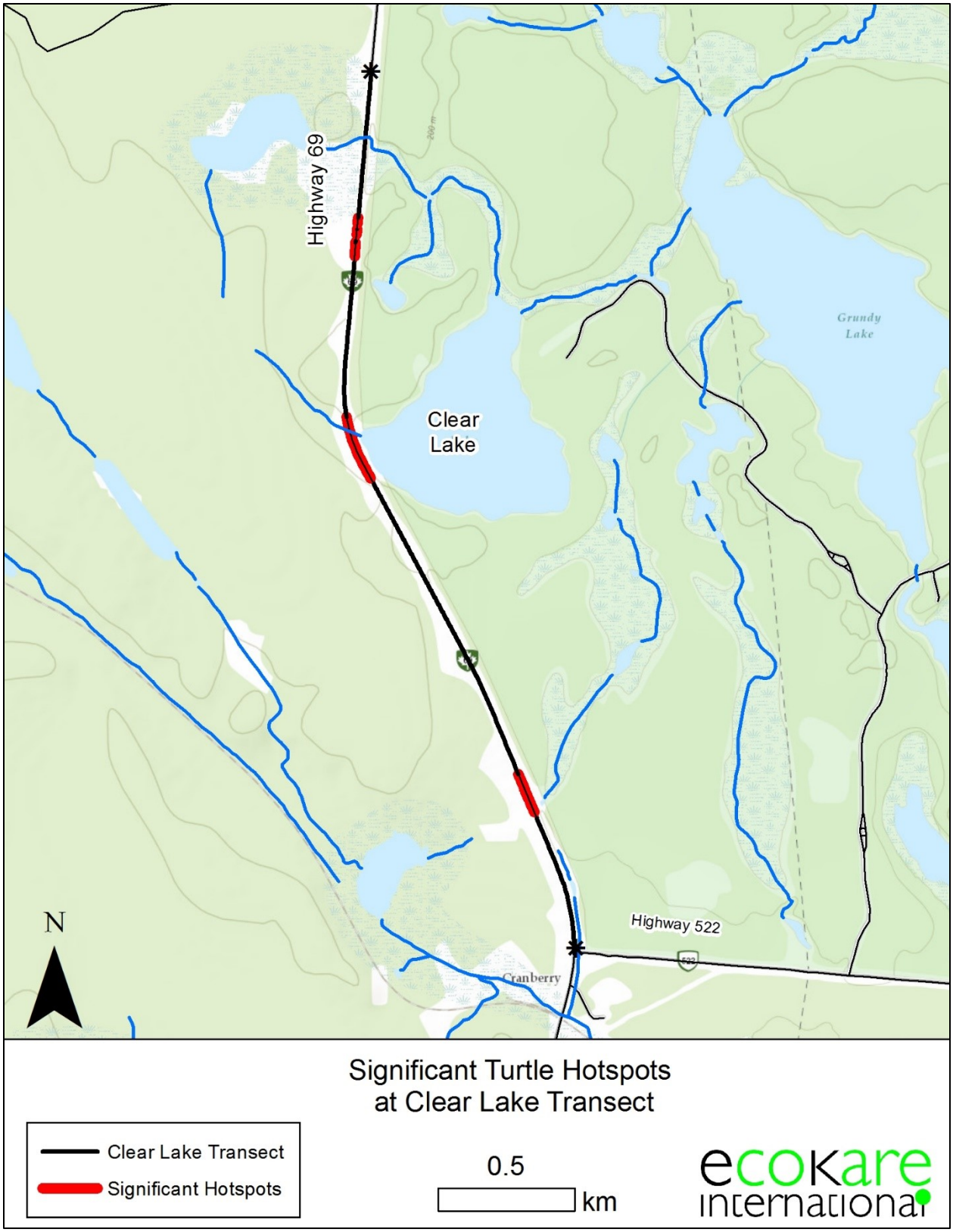

Figure 3: Snakes and turtles found in 2015 and 2016 along Clear Lake (omitted for public report) and significant hotspots for turtles where mitigation measures should be prioritized

5.2.1 Statistical Analyses

5.2.1.1 Sheppard Lake

From 2012 to 2016, 37 turtles were detected within the mitigated section of Hwy 69 at the Sheppard Lake site during the eight-week monitoring period (Figure 4). The highest peak of turtles were found in 2013 (17), followed by 2012 (13), 2015 (6) and 1 turtle at the southern-most fence end in 2016.

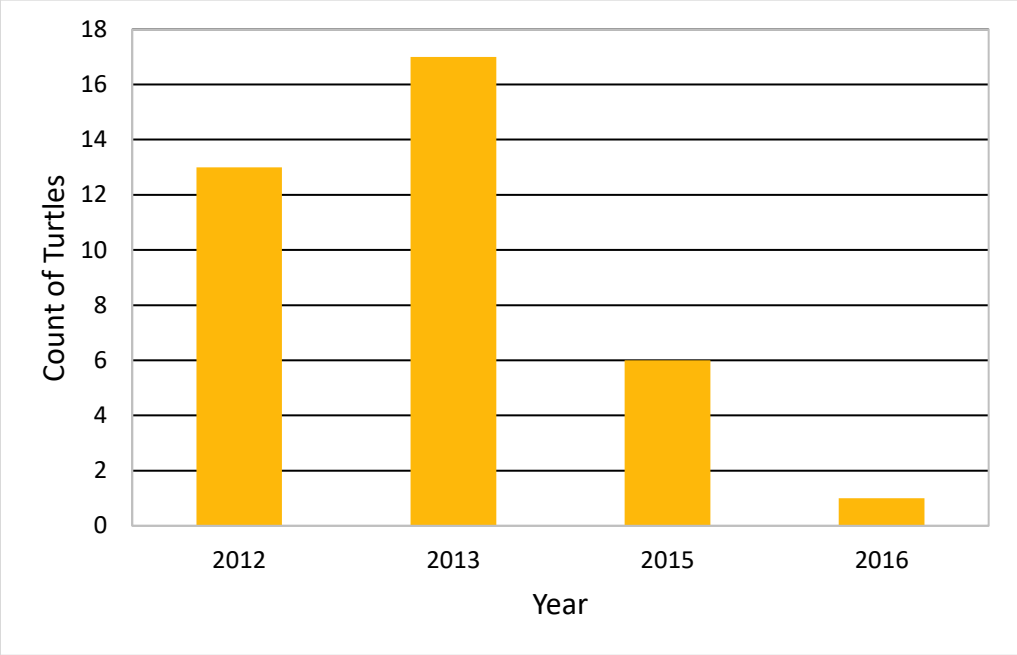

Figure 4. Count of turtles detected during eight-week monitoring period at Sheppard Lake on Highway 69 over four years (2012, 2013, 2015 and 2016).

The goodness of fit test was nonsignificant ($\chi 2 = 34.04$, df = 25, $p = 0.107$) indicating that the Poisson GLM fit the weekly turtle count data well. The analysis of deviance test found that Year had a significant overall effect on the number of turtles found on the road ($\chi 2 = 34.04$, df = 3, $p < 0.001$). Specifically, this result showed that the improved reptile fence in 2016 resulted in a predicted 90.5% reduction in the number of turtles on the road relative to 2012 (95% confidence interval: 30.7% - 98.7%, robust $p = 0.020$) if there is no change in the other variables. Neither 2013 nor 2015 had a significant effect on the number of turtles on the road relative to 2012. Mean rain, which varied considerably across the four study years (Figure 5) positively influenced the occurrence of turtles on the road, with each millimeter increase in the weekly mean rainfall resulting in a 15% increase in the number of turtles found on the road (95% confidence interval: 2.0% - 30.0%, robust $p = 0.023$).

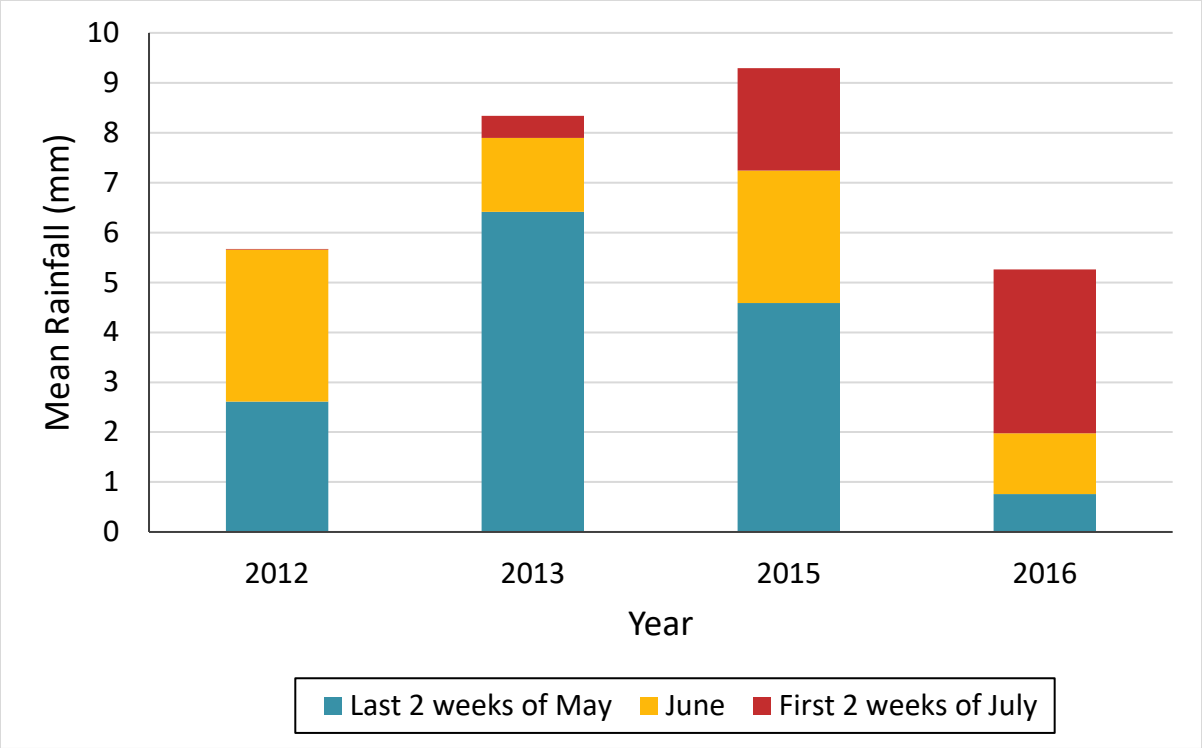

Figure 5: Mean seasonal rainfall at Sheppard Lake site for the four study years (2012, 2013, 2015 and 2016). Meteorological data was obtained from the Environment Canada weather station in Sudbury, Ontario.

5.2.1.2 Clear and Sheppard Lake Comparison

During the eight-week monitoring period in 2015 and 2016 six turtles were detected within the mitigated section of Highway 69 at Sheppard Lake and 33 turtles were detected at the unmitigated section at Clear Lake used for statistical analysis (Figure 6). The paired t-test showed that the mean weekly turtle counts differed significantly between the Sheppard Lake (0.35 ± 0.13) and Clear Lake (1.75 ± 0.51) sites ($t = 2.848$, $df = 15$, $p = 0.013$) which supports the overall effectiveness of the reptile exclusion fencing at reducing the number of turtles accessing the road.

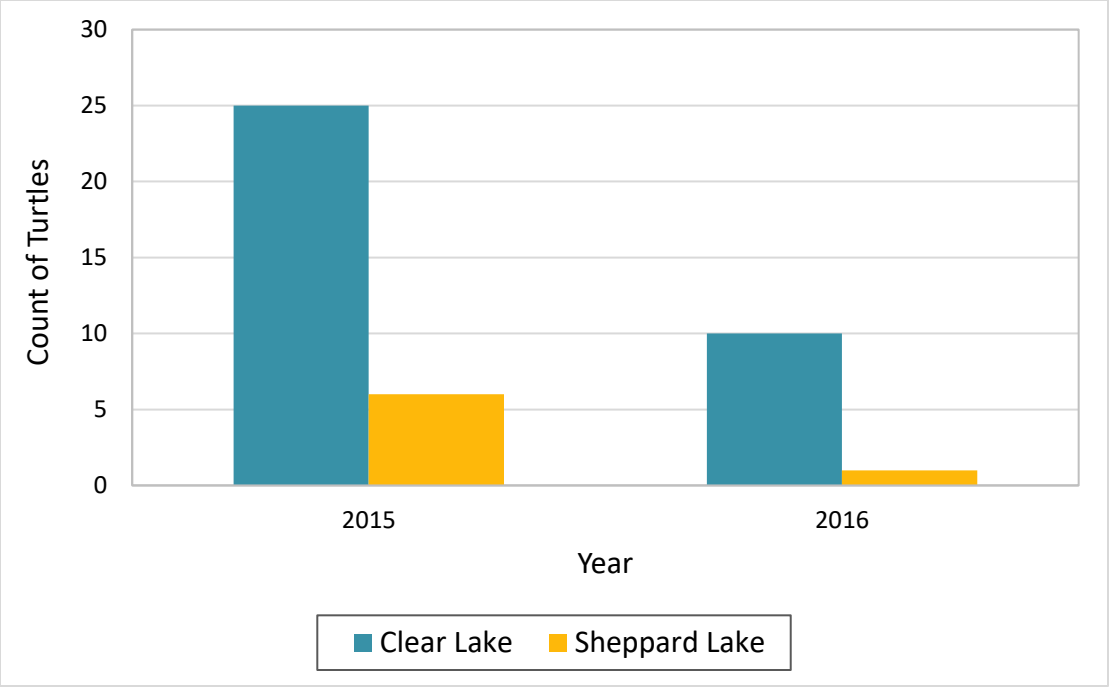

Figure 6: Count of turtles detected during road surveys conducted at the Sheppard Lake and Clear Lake sites on Hwy 69 over two years (2015 and 2016).

6 Camera Monitoring

6.1 Methods

Camera monitoring was completed in the active season: first week of June to end of September in 2015 and 2016. The objective of the monitoring was to assess whether turtles were using the crossing structures and what species. A secondary objective of the monitoring was to provide insight on the best placement and set-up of camera systems to capture cold-blooded animals. Standardized techniques are not yet established for monitoring cold-blooded reptiles in crossing structures, especially where water is present. Another challenge is the size of the tunnels that span 2.4 m high by 3.3 m wide. Therefore, several techniques were used that optimized camera resources as well as consideration for site-specific conditions, i.e. changes in water levels. These included:

a. Three camera trigger set-ups were employed: time lapse and motion (Reconyx model; http://www.reconyx.com/), motion only (Reconyx and Bushnell models; http://bushnell.com/), and active beam activated (JTS model; Table 3);

b. Two camera placements were employed with time lapse and motion cameras: camera placed on top-middle of tunnel facing downwards, and camera placed at bottom-side of tunnel spanning along bottom of tunnel;

    i. The active beam camera set-up was more flexible, in that the beam and camera could be placed at different locations in the tunnel. All systems had the active beam at the entrance of the tunnel and cameras set back at middle-side of tunnel to capture animals moving into or out of tunnel;

c. A barrier system built out of rocks and/or sticks was used to funnel animals within the camera field of view for cameras placed at the bottom side wall or on-top of the tunnel (Table 3)

d. A ramp was built out of rocks and/or sticks and was used to slow down turtles so motion detection was activated, or time lapse capture was initiated; rocks were used in water environments and were built up to the height of the water (Table 3).

In 2015, four active beam triggered cameras that were comprised of an infrared beam transmitter and receiver wired to a digital camera (JTS active camera system) were used. These cameras were custom made by JTS cameras in the United States. When the beam is broken, the

digital camera is triggered, continuously capturing images for 30-second intervals. In addition, one Reconyx time lapse/motion camera was used at Reptile Tunnel 2.

Due to several technical difficulties with the JTS active camera system in 2015, in addition to the ease of set-up of the time lapse/motion systems, only Reconyx and Bushnell cameras were used for the 2016 monitoring period from June 2nd, 2016 to October 8th, 2016. The Reconyx cameras (http://www.reconyx.com) are able to use a motion-activated setting, a time-lapse setting, or both in order to trigger image capture. Once the camera is triggered, there is an almost instantaneous (0.2 second) delay between the trigger and image capture (Table 3).

A 10 or 15 second interval was selected for the time lapse function on the Reconyx cameras. A 10 second interval was assumed to capture all animals using the tunnels. Limitation with using the time lapse function included changing camera SD cards and batteries on average every 3 days. Due to this logistical and budgetary constraint, cameras were only set to time lapse during peak movement periods of the target species. For turtles, this was during the month of June.

In June 2016, two cameras were set-up at each entrance of the three Reptile Tunnels. To compare, effectiveness of motion and time lapse settings at capturing turtles, one camera was set to motion and the other with time lapse. After an initial investigation of the data and a lack of turtle observations, the time lapse function was selected for the tunnel entrance that had more water on June 14th, 2016 (Table 3). This was because turtles would likely be at the same temperature as the surrounding environment and would likely not trigger the motion activated cameras. By July 2nd, all of the cameras were programmed for motion detection and only one camera was used at each tunnel.

Table 3: An overview of the wildlife camera detection systems used at the three reptile tunnels (RT) during the active reptile season (June to September 2015 and 2016). Camera settings: TL = Time lapse; M = Motion

Structure	Year	Date of Camera Set Up and Removal	Camera Set-Up	Average Water Depth (cm)	Comments
RT 1 West side	2015	June 6 –Oct. 1	JTS active camera system	5.1 (dry Aug)	Rock ramp spanning entire entrance of tunnel to trigger beam as turtles climb over.
RT 1 West side	2016	June 2-14 (M); June 14-Jul 2 (TL/M); July 2-July 4 (M)	Reconyx camera installed on side wall	5.1 (dry July)	1 foot wide rock ramp adjacent to the camera; grassy vegetation at entrance, and sand substrate.
RT 1 East side	2015	June 6 – Oct 1	JTS active camera system	7.6 (dry Aug)	Partially full with water, rock barrier with stick ramp built to trigger active beam
RT 1 East side	2016	June 2-14 (TL/M); June 14-Oct. 1 (M)	Reconyx camera installed on side wall	5.1 (dry in June)	Rock barrier with stick ramp built; Large Snapping Turtle observed in tunnel on June 2nd; dry gravel substrate, grassy vegetation at entrance.
RT 2 West side	2015	N/A	No camera	3.8 (dry Aug)	Water levels too high for camera installation.
RT 2 West side	2016	June 2 –Sep. 9 (M)	Bushnell camera installed on side wall	5.1 (dry in mid-June; water in median)	Rock barrier with stick ramp; camera removed on Sep. 9 due to flooding; sand substrate.
RT 2 East side	2015	June 6 –Aug. 3 (TL/M)	Reconyx camera	0	Stick exclusion wing walls built that funneled animals to a ramp (made from sticks and woody debris).

Structure	Year	Date of Camera Set Up and Removal	Camera Set-Up	Average Water Depth (cm)	Comments
RT 2 East side at Sheppard Lake	2016	June 2-July 2 (TL/M); July 2-5 (M); Sep 9 – ongoing (M)	Reconyx camera installed on roof	0	Stick exclusion wing walls to funnel animals to wood dirt ramp; aquatic vegetation 10 m from entrance, sand substrate.
RT 3 West side	2015	N/A	No camera	22.9 (not dry)	Water levels too high for camera installation.
RT 3 West side	2016	June 2 –Oct. 1 (M)	Reconyx camera installed on side wall	16.9 (not dry)	Rock barrier and wood ramp built; 10.2 cm water on Sep 9 visit; aquatic vegetation at entrance; sand substrate.
RT 3 East side median	2015	June 6 –July 15	JTS active camera system	7.3 (not dry)	Rock barrier / ramp built that spanned the entire length of the entrance, camera installed on the median side entrance of the east structure.
RT 3 East side median	2016	June 2-July 2 (TL/M); July 2-July 4 (M)	Reconyx camera installed on side wall	28.8 at entrance, dry in median end of June, wet again in September	Rock barrier; 10.2 cm water on Sep 9 visit; grass aquatic vegetation in median, sand substrate.

6.2 Results

Water levels in the tunnels fluctuated throughout the monitoring period and ranged from 0 (dry) to 28.8 cm (Table 3). Reptile Tunnel 2 was situated at Sheppard Lake where water levels were lowest in the tunnel in both 2015 and 2016 when compared to the other two tunnels. Water levels receded in Reptile Tunnels 1 and 2 in June and July and water levels were not dry in Reptile Tunnel 3 in both years. Water moving through the tunnels provided good aquatic habitat vegetation, e.g. Common Cattail (*Typha latifolia*) in the open medians as well as habitat for turtles. In 2015, a live Painted Turtle was found in the water at the entrance to Reptile

Tunnel 3, a frog in the median, and in 2016, a Snapping Turtle was observed in the east side of Reptile Tunnel 1 (Photo 35; Photo 36).

A total of 20 turtles were captured on cameras in both 2015 and 2016. Of these 20 turtles, the same Snapping Turtle was captured on two different cameras, and the same Painted Turtle was captured entering and turning around so a total of 18 independent turtles were captured on camera inside the structures (Table 4). Of these 18 independent turtles, 11 were Painted Turtle and seven were Snapping Turtles, no Blanding's Turtles have been reported.

An additional Painted Turtle and a Snapping Turtle were observed in the tunnels by a field technician, therefore a total of 20 turtles were observed in the tunnels in both years (Figure 7). Of these, 16 turtles were assumed to cross because they were not observed turning around, and 4 were observed turning around.

The most turtles were found in Reptile Tunnel 1 (10/20 or 50%), followed by Reptile Tunnel 2 (7/20 or 35%) and Reptile Tunnel 3 (3/20 or 15%) (Figure 7). These numbers are believed to reflect the majority of turtle passages especially at Reptile Tunnel 1 where sampling effort was highest with two cameras operational from June 6 to Oct 1 in both 2015 and 2016 and at Reptile Tunnel 2 where time lapse imagery was used in June in both years.

A total of 1182 sightings of large and small mammals, birds, reptiles and amphibians were all captured on camera at the Reptile Tunnels in 2015 and 2016 (Table 4). Small mammals were inside the tunnels the most (49%), followed by birds (42%), then amphibians (5%), then turtles (2%), then large animals (1%), and snakes (1%). The most animals were captured at Reptile Tunnel 1, followed by RT2 and RT3. Two unique bird species were the Spotted Sandpiper, and the American Bittern. Large and mid sized animals included Black Bear, Deer and Coyotes. Higher water levels at Reptile Tunnel 3 west entrance in both years likely deterred some small and large mammal from using the tunnel when water was present (Table 3).

Of the different camera systems used in the two years, the Reconyx time lapse had no operational malfunctions, unlike the active beam system that required repositioning and trouble-shooting on several occasions. Eleven of the 18 turtles were captured with Reconyx cameras. Of these 17 were captured with time lapse and only one time a Snapping Turtle was captured with the motion setting. The active beam system captured five turtle events in 2015. The Bushnell cameras captured three turtles on motion setting.

Due to small sample size and continual manipulation of the camera set-up to accommodate changing water conditions, it is difficult to tease apart the most optimal camera set-up for capturing turtles in this study or any study. Generally speaking, an operational time lapse system set at an interval to capture the moving animal would capture most turtles, however

there is a trade-off with maintaining batteries, and changing cards routinely when full. In addition, there are a large amount of pictures to process.

It is recommended to tailor camera monitoring set-ups to each specific study. Further research should manipulate conditions in a controlled setting to trial and test camera set-ups that can optimize captue of cold-blooded animals under several environmental conditions. Additional testing of camera equipment will occur in the 2017 active season.

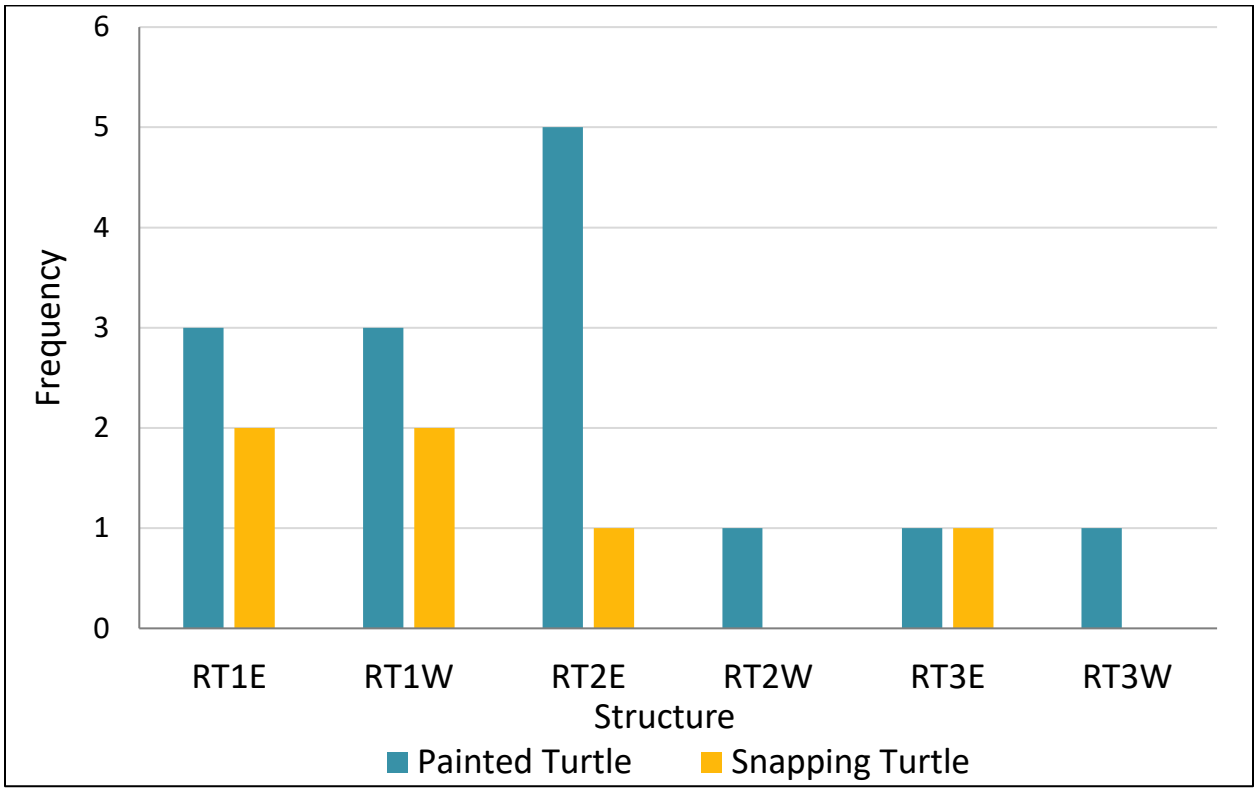

Figure 7 : Summary of frequency of turtles captured on all cameras and observed at reptile tunnels in 2015 and 2016.

Table 4: The number of animals and percentage by animal group captured on four wildlife cameras systems set up at three reptile tunnels.

Structure	Year	Birds	Small mammals	Large mammals	Snakes	Turtles	Amphi-bians	Total (%)
Reptile Tunnel 1	2015	106	86	4	0	6	0	202
	2016	59	164	1	0	4	27	255
Total		**165**	**250**	**5**	**0**	**10**	**27**	**457 (39)**
Reptile Tunnel 2	2015	133	73	2	4	3	23	238
	2016	26	141	2	1	4	1	175
Total		**159**	**214**	**4**	**5**	**7**	**24**	**413 (35)**
Reptile Tunnel 3	2015	119	1	0	0	1	0	121
	2016	55	121	0	0	2	13	191
Total		**174**	**122**	**0**	**0**	**3**	**13**	**312 (26)**
Grand Total		**498 (42)**	**586 (50)**	**9 (1)**	**5 (0)**	**20 (2)**	**64 (5)**	**1182**

7 Discussions and Recommendations

7.1 Reptile Fence Effectiveness

This study provided new insight and built on results from previous work conducted by Baxter-Gilbert et al. (2015) in the same study area. Baxter-Gilbert et al. (2015) showed that the percentage of dead turtles detected along a 13 km highway transect that included the Sheppard Lake mitigated transect, significantly increased by 20% between a defined Before fencing (2012) and After fencing (2013) period when compared to a 2% increase of turtles found at an unmitigated Control site.

In contrast, this study showed that the fence reduced turtle mortality to one juvenile Snapping Turtle 10 m south of the fence end in 2016 and this was a predicted 90.5% reduction in the number of turtles on the road in 2016 relative to 2012 when the exclusion fence was being built. Further, there was a significant difference between turtles found between the mitigated Sheppard Lake transect (6 turtles) and at the unmitigated Clear Lake transect (33 turtles).

The difference in fence effectiveness between the two studies can be attributed to modifications in the reptile fence that improved its functionality to exclude the ability of turtles and other smaller animals such as snakes to access the highway. These modifications included:

- Closing of all gaps with exclusion fencing, e.g. at large animal one-way gates;
- Extending fence above the high water mark at drainage culverts;
- Ensuring the fence was stable and buried at all medians between culvert and tunnel structures;
- Ensuring no gaps existed between the fence and ground substrate from wash-outs;
- Repair of all fence holes from wear and tear, vandalism and wild animals;
- Extending the southern fence end to the large animal underpass and extending the northwest end so it was coincident with the south east end;
    - Ensuring exclusion fencing is continuous adjacent to wetland habitat used by turtles.

In addition to modifications in fence design, there are some differences between study designs in the Baxter-Gilbert (2015) study and this study that should be discussed.

The Baxter-Gilbert (2015) study used a Before-After-Control-Impact (BACI) study design that controls for other interacting variables and isolates the mitigation effect for a between site comparison (Baxter-Gilbert et al. 2015). In other words, the study design controls for whether there are changes in relative turtle population abundance from one year to the next or

between each site. A BACI design was not completed in this study because there was no before data collected at both the Clear Lake and the Sheppard transect (also discussed below).

It is highly likely that there were significantly fewer turtles observed at Clear Lake than at Sheppard Lake due to the presence of fencing and crossing structures and not from other factors such as changes in turtle abundance or highway characteristics, e.g. 2 versus 4 lanes alone. Turtle abundance is unknown at both sites, however Blanding's Turtles were observed at Clear Lake in this study, and Baxter-Gilbert et al. (2015) observed Blanding's Turtle at Sheppard Lake. Further the habitat is very similar among the two sites. Both sites have an open water lake east of the highway with several drainage systems north and south of the lake.

Other differences in study designs are defined transect lengths. The Baxter-Gilbert et al. (2015) study compared a 13 km length of road at both the unmitigated control and mitigated site which included the 4.8 km of reptile exclusion fencing. This study only included the length of road equivalent to where reptile exclusion fencing occurred and approximately 300 m fence end buffers (up to 3.5 km). The latter road survey length ensured there was not a confounding effect of including turtles observed on the road where mitigation was not implemented.

Comparisons between turtle highway observations in the 2012-2013 and the 2015-2016 period may be biased due to sampling effort. Both studies conducted one walking survey per day; however the number of driving surveys per day differed. The Baxter-Gilbert et al. (2015) study completed three systematic driving surveys per day while this study completed one driving survey per day in 2015 and approximately two driving surveys per week in 2016.

This sampling effort bias is thought to be minimal. This is because it is estimated that only a few turtles were likely missed from lack of driving surveys in 2015 and 2016. This is based on finding only one additional turtle during all the driving surveys conducted in both years. Further, it is likely that the daily surveys found the majority of the turtles killed on the road because turtles can persist for up to 3 days (Santos et al. 2015). In addition, Stinnissen (2015) found that 75% of turtles persisted on the road for up to 24 hours.

It is important to consider the implication of different construction phases of roadside fencing and vehicle traffic use with the number of turtles observed along the Sheppard Lake mitigated transect from 2012-2016. On June 6th, 2012 two lanes of traffic (now northbound lanes) were opened for vehicle use along Sheppard Lake. Then, on August 8th, 2012 all lanes of traffic (SB and NB) at Sheppard Lake transect were open for vehicle use (MTO unpublished data).

In the Baxter-Gilbert et al. (2015) study the turtles observed in 2012 were found on two different highways. All turtles found prior to June 6th, 2012 were on the neighbouring old Highway 69 (0.5 km west of the new Highway 69) and all turtles found after this date were on

the southbound (SB) lanes of the new Highway 69 alignment (Baxter-Gilbert 2014). Temporary silt fencing existed on both the SB and NB lanes on the new highway alignment during the 2012 study period. In addition, the permanent reptile exclusion fencing was being installed from June 2012 to October 2012 (Baxter-Gilbert 2014).

The above scenario is important, because in essence a baseline before fencing scenario (no exclusion fencing) was never measured. So turtles observed in 2013, 2015 and 2016 (after exclusion fencing was installed) are compared to a 'during' mitigation construction phase in 2012. A true before fencing evaluation may have found more turtle mortality than that actually observed in 2012 when fencing was being built. Therefore in a true before fencing evaluation an increase in turtle mortality after fencing may have been more difficult to detect. In contrast, a significant decrease in turtles observed in 2015 and 2016 would have been easier to detect.

In addition, the circumstance of traffic volume and highway configuration are also important factors to consider when collecting animal road mortality locations. In 2012, turtle observations on both the neighbouring highway and the Sheppard Lake transect were found where vehicle traffic was only on two lanes of highway. In 2013, observations were on four lanes of highway. Previous research has shown that as the number of road lanes or width of a road increase and traffic volumes remain the same, the probability of turtles being killed on roads also increases (Gibbs & Shriver 2002; Aresco 2005). This increased probability in turtle mortality, positively biases (although slightly) the turtle observations from 2013, 2015, and 2016 when compared to 2012 observations. This circumstance would increase the likelihood that a significant increase in turtle observations is realized after exclusion fencing is installed. Therefore, a significant decrease in turtles after fencing is installed when compared to the during phase of construction would be more difficult to detect.

7.2 Crossing Structure Effectiveness

Similar to Baxter Gilbert et al. (2015) this study found a wide diversity of animals using the reptile tunnels under the highway. The tunnel entrances are situated directly adjacent to wetland habitat, and the structures and median are providing ideal conditions for many species of waterfowl, and several species of amphibians and reptiles. Furthermore, large animals such as White-tailed deer, Black Bear, Wolves, Coyotes, and even Moose have also used the tunnels (MTO, unpublished data; Eco-Kare International 2017).

A total of 20 turtles (10 in 2015 and 10 in 2016) were found inside the tunnels in two years of monitoring. Of these, 12 were Painted Turtles and eight were Snapping Turtles and none were Blanding's Turtles. From May 1st to August 31st 2013, Baxter-Gilbert et al. (2015) found that Painted Turtles were inside the tunnels at least six times along with one Snapping Turtle, and

additionally Snapping Turtle tracks not associated with camera pictures were recorded. Monitoring efforts used different techniques in each year, however the number of turtles seen at all tunnels per annum are similar. Collectively, these results suggest that fence quality is not compromising the ability for turtles to find the tunnels.

The absence of Blanding's Turtle in the structures may be a combination of factors. It is unlikely, that the lack of Blanding's Turtle use is due to the location of the structures. The structures are situated within wetland habitat on both sides of the road, and Baxter-Gilbert et al. (2015) captured 15 Blanding's Turtles within 1 km of the highway new alignment for radio-telemetry research in 2012.

Further, it is unlikely, that the lack of use is due to structural specifications because the reptile tunnels are approximately 5 times larger (9.24 m^2 vs 1.8 m^2) at the tunnel entrance in this study then that recommended for box tunnels (1.8 m high x 1.0 m wide for structures less than 25 m long) for Blanding's Turtles (Ontario Ministry of Natural Resources and Forestry 2016). Futher, Caverhill et al. (2011) showed that 19 individual Blanding's Turtles used a 1.8 m diameter (2.5 m^2) Corrugtaed Steel Pipe drainage culvert that was partially submerged with water up to 17 times each.

Some possible explanations for lack of Blanding's Turtle movements under the highway may be that suitable nesting and overwintering habitat exists on the both sides of the highway (Baxter-Gilbert et al. 2015) and there was not an immediate seasonal need to access additional resources. Or possibly, Blanding's Turtles exist at such smaller numbers than more common Snapping and Painted Turtles that the probability of detection is much less. Or, Blanding's Turtles may take longer to habituate to the new highway alignment and associated traffic volumes. The habituation period to new mitigation measures and highway expansion is unknown for turtles and this may vary for each species. Clevenger and Barrueto (2014) found that adaptation periods for more sensitive carnivore species such as Grizzly Bears using crossing structures was up to 5 years and minimal for deer in Banff National Park.

All the turtles observed at the tunnels in this study appeared to be adults, although Baxter-Gilbert et al. (2015) did find 2 Painted Turtle hatchlings. The absence of juveniles is likely because smaller animals may not trigger the active beam or motion detector, or the rock and woody ramps may have been too steep for the turtles to climb. It is also possible that the juveniles moved in late spring and fall when the cameras were not set up. The camera monitoring period targeted June and July when adult females move during nesting migrations.

7.3 Recommendations

In this study, anecdotal evidence showed that the large animal exclusion fencing that is flush with the ground has some benefit for excluding turtles that were larger in size than the wire mesh (turtle plastron width greater than 16 cm). Only juvenile turtles, less than 9 cm plastron width, were found along the Sheppard transect where reptile exclusion fencing was not present but large animal fencing was.

Wildlife diversity and suitable habitat bisected by the highway 69 corridor are vast. There are more than 30 km of highway being fenced for large animals during two to four lanes highway expansion projects. These same highway sections bisect known reptile habitat and 10's of kilometres are also being fenced to exclude reptiles from the highway. Where both large animal and reptile fencing is prioritized it is recommended to use an additional tight mesh apron, heavy geotextile, or thick plastic material that is buried into the ground with large animal mesh fencing. Where reptile fencing is not prioritized but large animal fencing is then fence should be snug to ground, and where this is not the case, rocks or boulders or other solid debris can be used to impede movement of larger turtles through or under the fence.

With 1-2 days maintenance each year it is predicted that the current reptile exclusion fencing will likely last an additional five years. After this period, the fencing material will likely deteriorate beyond a simple fence maintenance protocol. Long-term plans should be to replace the current geotextile materials with more durable materials especially due to the intense water movements that occur in the area every spring. Fortunately, the existence of a long-term large animal fence already provides a framework to enable an efficient fence installation process.

The reptile tunnels should continue to be monitored with cameras in the month of June to target the turtle nesting migration. The data collection can be conducted in unison to concurrent large animal monitoring by placing cameras on the roof of the structures. New monitoring techniques also need to be investigated that supplement passive and time lapse infra-red technologies. Resources required to maintain and process cameras on time lapse exceed practical budgets. Using rock barriers to elevate turtles out of water may be impeding movement into tunnel. More rigorous and practical techniques are required that work in wet conditions.

The monitoring at Clear Lake not only informed mitigation effectiveness at Sheppard Lake but the information is useful for mitigation planning in upcoming highway upgrades. There were reoccurring clusters of dead turtles that occurred on the highway at Clear Lake and these would be precise locations for turtle crossing structures. Furthermore, turtle observations along this

transect are useful as before construction information that can be compared to turtle observations after mitigation (exclusion fencing and crossing structures) are implemented during upcoming road upgrades.

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Appendix A List of Photos

Appendix B Photo Library: Fence Assessment and Maintenance

A picture inventory of the major fence repairs completed at all drainage systems, reptile tunnels, and other sections of reptile exclusion fencing in 2015 and 2016.

Photo 1: Drainage System 1 west end washout prior to repairs in 2016. This section of fencing also experienced a washout in 2015.

Photo 2: Drainage System 1 washout after repairs in 2016.

Photo 3: Gap under the large animal fencing between Drainage System 2 and Drainage System 3 in 2015.

Photo 4: A five meter piece of geotextile material installed in 2016 to close the gap under the fencing between Drainage System 2 and Drainage System 3.

Photo 5: Open one-way gate on the west side of Highway 69, between Drainage System 2 and Drainage System 3 (2015).

Photo 6: Closing the one-way gate between Drainage System 2 and Drainage System 3 with geotextile material (2016).

Photo 7: A rip in the reptile exclusion fencing on the west side of Highway 69 between Reptile Tunnel 1 and the northwest fence end (2016).

Photo 8: Repaired rip in the fencing between Reptile Tunnel 1 and the northwest fence end (2016).

Photo 9: A second rip in the reptile exclusion fencing on the west side of Highway 69, between Reptile Tunnel 1 and the northwest fence end.

Photo 10: Second rip repaired between Reptile Tunnel 1 and the northwest fence end (2016).

Photo 11: Change in hydrology at the Reptile Tunnel 1 east entrance. Water had flattened the reptile exclusion fencing in 2015. Fence was cut and rocks were used as barrier to exclude turtles but allow water flow, this was maintained in 2016.

Photo 12: Zoom-in of rock wall that permits water flow built in 2015 and repaired in 2016, at the east entrance of Reptile Tunnel 1.

Photo 13: Fallen exclusion fencing due to rip rap erosion at the east entrance of Reptile Tunnel 2 (2016).

Photo 14: Repaired exclusion fencing at the east entrance of Reptile Tunnel 2 (2016).

Photo 15: Non-functional drainage culvert (DS1) for wildlife passage because east end not extending through wildlife fencing.

Photo 16: Water levels higher than reptile exclusion fencing at wetland habitat 700 m south of NE fence end.

Appendix C Photo Library: Fence Extension

Photo 17: Installing reptile exclusion fencing at the south-west side of Sheppard Lake transect (9-May-16).

Photo 18: Installing reptile exclusion fencing at the south-west side of Sheppard Lake transect (10-May-16).

Photo 19: Installing reptile exclusion fencing on the east side of Highway 69, at the south end of the Sheppard Lake transect (13-May-16).

Photo 20: Completed reptile exclusion fencing at the east side of the large animal underpass; this location was the end of the Sheppard Lake transect in 2016.

Photo 21: Large rocks stacked along the bottom of the fence to close gaps in the large animal underpass median (2016).

Photo 22: Reptile exclusion fencing extending to the west side of the large animal underpass (2016).

Photo 23: Rocks and dirt used to fill gaps at bottom of fence at northwest fence extension (16-May-2016).

Photo 24: Reptile exclusion fencing extension on the northwest side of the highway in 2016 (19-May-2016).

Photo 25: Smooth Green Snake found on the east side of the highway during the reptile exclusion fence installation.

Appendix D Photo Library: Road Survey Assessment

Photo 26: Snapping Turtle nesting on the shoulder of Highway 69 in the northbound lanes. Observed at the Clear Lake transect (11-June-16).

Photo 27: Blanding's Turtle found dead north of Sheppard Lake on Highway 69 (16-June-16).

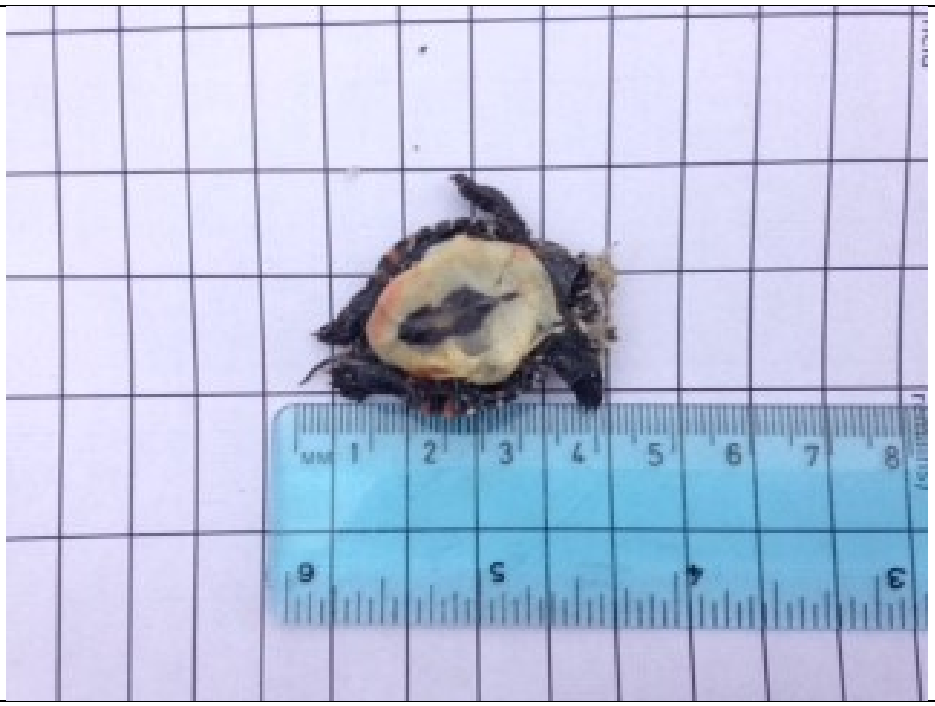

Photo 28: Painted Turtle hatchling found dead at the southernmost drainage culvert along the Clear Lake transect on Highway 69 (16-May-16).

Photo 29: Adult Blanding's Turtle basking on a log in Clear lake (2-July-16).

Photo 30: Massasauga Rattlesnake found dead on Highway 69 along the Clear Lake transect (12-July-16).

Photo 31: Eastern Gartersnake found dead on Highway 69 along the Clear Lake transect (5-July-16).

Appendix E Photo Library: Camera Monitoring

Photo 32: Painted Turtle using the east entrance of Reptile Tunnel 2 (24-Jun-15).

Photo 33: Painted Turtle using the east entrance of Reptile Tunnel 2 (15-June-15).

Photo 34: Snapping Turtle using the east entrance of Reptile Tunnel 2 (1-July-15).

Photo 35: Large adult Snapping Turtle traveling through Reptile Tunnel 1 on 2-Jun-16.

Photo 36: Large adult Snapping Turtle traveling through Reptile Tunnel 1 west (2-Jun-16).

Photo 37: Painted Turtle entering Reptile Tunnel 2 (16-June-16)

Photo 38: Painted Turtle climbing ramp into Reptile Tunnel 2 (16-June-16)

Photo 39: Painted Turtle climbing rock barrier to exit Reptile Tunnel 3E (17-June-16)

Photo 40: Painted Turtle entering Reptile Tunnel (16-June-16)

Photo 41: Painted Turtle climbing ramp into Reptile Tunnel 2 (16-June-16)

Photo 42: Painted Turtle climbing rock barrier exiting Reptil Tunnel 2 West (17-June-16)

Photo 43: Large Snapping Turtle climbing rock barrier entering Reptile Tunnel 3 Eeast (11-June-16)

Photo 44: Painted Turtle climbing rock barrier at Reptile Tunnel 1 East (4-June-2016)

Photo 45: Spotted Sandpiper moving through Reptile Tunnel 1 East (2-June-2016)

Photo 46: Porcupine moving through Reptile Tunnel 1 Eeast (21-June-2016)

Photo 47: Black Bear exiting Reptile Tunnel 2 East (23-June-2016)

Photo 48: Beaver swimming through Reptile Tunnel 1 West (14-June-2016)

Photo 49: Beaver entering Reptile Tunnel 1 West (28-June-2016)

Photo 50: Mink exiting Reptile Tunnel 1 East (07-June-2016)

Appendix F Photo Library: Reptile Tunnels

Photo 51: Reptile tunnel entrance with no water

Photo 52: Reptile Tunnel in median

Photo 53: Wetland habitat adjacent to Reptile Tunnel 1 east entrance (06-Jun-15).

Photo 54: Reptile fencing attached to large animal fencing

Appendix G Google Earth Images of Transects

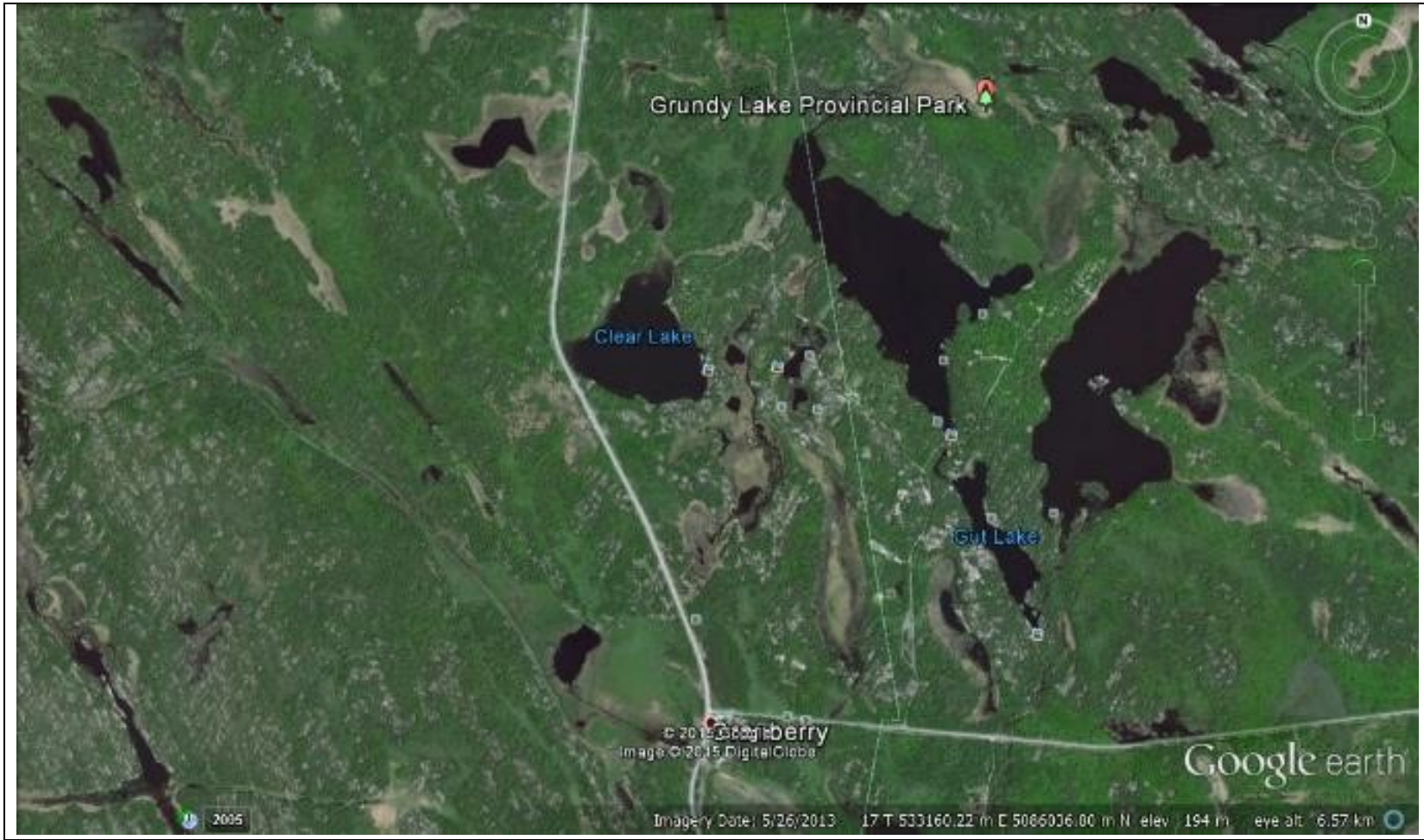

Photo 55: Satellite imagery of the Clear Lake transect and surrounding environment (Google Earth).

Photo 56: Satellite imagery of the Sheppard Lake transect and surrounding environment (Google Earth).

Appendix H Details of fence assessment, maintenance and extensions completed during 2015 and 2016 at Sheppard Lake transect on Highway 69.

Summary of fence extension in 2015 and 2016

In 2015, geotextile material was added to extend the reptile fencing an additional 50 m at the southwest fence end (Photo 19). At the southeast fence end, for approximately 100 m, rocks were stacked in order to close the gaps between the bottom of the large animal fencing and the earthen ground. This technique was also used along the rock cliff on the west side of the highway for approximately 100 m.

On May 6th, 2016, a meeting was coordinated between K. Gunson, W. Kowbasniuk and C. Green from Eco-Kare International, with A. Healy and T. Rogers from the Ministry of Transportation, Northeastern Region. During this meeting, it was decided that a northern extension of the reptile fence on the west side of the highway was required to coincide with the northern limit of the fencing on the east side of the highway. An additional southward extension of the reptile fencing on both the west and east sides of the highway, towards the large animal underpass, was also required to connect additional crossing structures with fencing along potential turtle highway crossing areas.

From May 9th to May 18th, 2016, the geotextile reptile fence was extended at the north and southern ends. The southern extension entailed approximately 300 m extension on both the east and west sides to abut the large animal wildlife underpass (Photo 22; Photo 21). The northern extension was completed on the northwest side to abut drainage culvert 3 and to extend further to drainage culvert 2 and to coincide with the same length of fencing on the east side of the highway (Photo 23:).

For the southern extension, a 1.8 m wide section of 200 woven polypropylene geotextile fabric was doubled over and attached to the large animal fencing (Photo 17). The fence height was approximately 70 cm. The fence was buried into the ground and attached to the large animal fencing with bendable wire clips. At the large animal wildlife underpass large rocks were used to fill gaps between the large animal fence and the ground (Photo 21:). The rocks were piled both inside and outside of the fence to barricade any movement by reptiles when using the large animal underpass.

For the northwest extension, a combination of geotextile fabric (150 m), and rocks and clay soil (85 m) were used to create a barrier for reptile movement. Soils and rock were used when the

ground was extremely rocky and a trench could not be dug. The clay soil piled at the base of the large animal fence hardened and was not washed away by rain (Photo 23). The use of large animal fencing with a rock and soil barrier on the west side of the highway and a flooded section on the east of the highway will allow evaluation of these techniques used in combination with large animal fencing for excluding reptiles from the highway.

Summary of fence maintenance

All sections of fencing that would require maintenance were assessed with field notes and photos in both years (Appendix C). In 2015, fence maintenance was completed by one field technician (24 hours) and one project manager (12 hours) (see Appendix G). In 2016, the maintenance took one field technician 12 hours to complete. In 2015, fence maintenance occurred between May 5th and May 14th and in 2016, between May 2nd and May 3rd.

In both years, there was little vegetation growth along the fence, and for the most part, the fence was buried along its length. There were few holes and tears in 2015 and in 2016 there were three vertical tears or gaps that were patched to avoid further deterioration (Photo 7; Photo 8). In 2015, the majority of the maintenance entailed elevating the fence higher than existing water levels at each drainage system (DS) entrance and stabilizing posts using rocks to bury the fence in each median (Table below Appendix H). In both 2015 and 2016, routine maintenance entailed filling in a reoccurring wash-out at DS 1 (Photo 1; Photo 2), ensuring the fence was buried, closing all one-way gates along or at fence ends (Photo 5; Photo 6), reattaching sections of reptile fence to the large animal fence (30 to 100 m), as well as ensuring fencing securely abutted all culvert and reptile tunnel entrances (Photo 13, Photo 14, Appendix H).

In both 2015 and 2016, the hydrology had changed on the eastern side of the highway and the water had begun flowing over the northeast end of the exclusion fencing that abutted the northern-most reptile tunnel. This water flow tore the reptile fence off of the large animal fencing (Photo 11). As a solution, the fence was cut approximately 8 m to allow water flow and rocks were stacked along the bottom. This created a barrier for animal movement, but allowed for the permeability of water flow (Photo 12). This set-up was maintained in 2016.

At the onset of the project, there were nine large animal one-way gates along the continuous section of fence. The continuous fencing only extended across the base of the gate at each of the four structures. The remaining five gates were closed off, three in 2015 and an additional two in 2016 (Photo 6).

In 2015 a Geographic Positioning System (GPS) inventory was completed for all fence ends, drainage culverts, and reptile tunnels and this was updated in 2016. A map was then created for the Sheppard Lake fenced area (Figure 1). In both years there were five drainage systems, as well as three larger reptile tunnels that were associated with the wildlife exclusion fencing. In 2016, the fencing was connected to the large animal underpass (See **Figure 2**). In 2016, the continuous section of the fence at the northwest side was also extended approximately 500 m to abut DS 2 (Photo 18; Photo 20). This modification created equivalent lengths of exclusion fencing (2,600 m) on both sides of the highway that extended from the large animal underpass to DS 2 (Figure 1). DS 1 remained an isolated structure from the other potential crossing structures: 126 m of fencing on both sides of highway that was not functional because the east culvert entrance ended on the highway side of the fencing.

In 2015 and 2016, it was noted that there were several hundred metres of fence lower than the water levels approximately 680 m south of the northeast section of continuous fence (Photo 16). In 2015, field technicians attempted to increase the reptile exclusion fence height by reattaching it to a higher wire on the large animal fencing but the water was murky that restricted visibility. Water levels did not recede in 2015. In the MTO-Eco-Kare 2016 meeting, it was decided that one more year of monitoring would provide insight about whether turtles were able to breach the flooded section and whether the large animal fence provided exclusion for turtles. If fence repairs were necessary, then the reptile exclusion fencing should be moved away from the large animal fence closer to the highway, where higher ground occurs and flooding is minimal.

Summary of fence maintenance conducted on onset of monitoring in 2015 and again in 2016

Location	Year Completed	Fencing description	Tunnel description	Assessment	Maintenance
Drainage System (DS) 1 (Northernmost; start 5124999 to end 5124817, 1 PSB					
West side; isolated from other fencing and DS	2015, 2016	196 m	1 PSB black drainage culvert (160 cm)	1 major washout, culvert entrance extends to inside of fence	Wash-out reoccurred and repaired in 2015 and 2016 (Photo 1, Photo 2); recommended to close off west side of culvert
Middle	NA	None	Unfenced 2 m gap in median between NB and SB structure	No fencing in median	No fencing added
East side; isolated from other fencing and DS	2015	168 m	1 PSB black drainage culvert (160 cm)	Large animal and reptile fence does not connect to structure (Photo 15).	Added soil at fence bottom; culvert entrance ends on highway side of fence
Drainage System 2					
West side	2015	27 m	1 concrete box tunnel (1.2 m x 1.8 m)	Fence down at entrance to culvert	Fence reattached at culvert entrance and rocks used to stabilize; fence extended southerly to DS 3 (2016)
Middle	2015	Yes	Fenced 15 m gap in median between NB and SB structure	15 cm gap between fence and concrete box culvert	Used rocks to pile up and fill gap
East side	2015, 2016	NE continuos section fence end	1 concrete box tunnel (1.2 m x 1.8 m)	No fencing at turtle habitat 10m north of NE fence end, large animal fencing not on ground	8 m of geotextile material added to NE fence end, fence buried and rocks piled; fence extended southerly to DS 3

Location	Year Completed	Fencing description	Tunnel description	Assessment	Maintenance
Drainage System 3					
West side	2015, 2016	144 m	1 concrete box tunnel (1.2 m x 1.8 m)	Washout causing fencing collapse at entrance to culvert reoccurring in 2015 and 2016	Reattached and added rocks
Middle	2015	Yes	Fenced 15 m gap in median between NB and SB structure	Fence down on south side	Replaced with wood posts, and buried sections of fence
East side	2015	Continuous with south section	1 Concrete Box tunnel (1.2 m x 1.8 m)	Small gap between concrete box tunnel and fence	Added rocks to fence abutment
Reptile Tunnel 1 North (Sheppard Lake)					
West side	NA	2000 m	Concrete Box Culvert (2.4 m high by 3.3 m wide, and 24.1 m long)	Fence abutting tunnel adequately	No maintenance required
Middle	NA	Yes	Fenced 15 m gap in median between NB and SB structure	Fence abutting tunnel adequately	No maintenance required
East side	2015	2300 m	Concrete Box Culvert	Water flow on NE entrance pushing fence down reoccurring in 2016	Piled rocks along fence to allow drainage and added more geotextile; Photo 11; Photo 12
Reptile Tunnel 2					

Location	Year Completed	Fencing description	Tunnel description	Assessment	Maintenance
West side		2000 m	Concrete Box Culvert (2.4 m high by 3.3 m wide, and 24.1 m long)	Fence abutting tunnel adequately	No maintenance required
Middle		Yes	Fenced 15 m gap in median between NB and SB structure	Fencing in good condition	No maintenance required
East side	2015, 2016	2300 m	Concrete Box Culvert	Small gap between concrete box tunnel and fence (2015); Rock slide caused fence to collapse (2016)	Added rocks to fence abutment in 2015 and reattached fence in 2016 (Photo 14)
Reptile Tunnel 3					
West side	2015, 2016	2000 m	Concrete Box Culvert (2.4 m high by 3.3 m wide, and 24.1 m long)	Fence abutting tunnel adequately	No maintenance required (2015 & 2016)
Middle	2015	Yes	Fenced 15 m gap in median between NB and SB structure	Fence sagging and not at full height	Cut wire clips and reattached to large animal fencing to maximize height
East side	2015, 2016	2300 m	Concrete Box Culvert	Fence abutting tunnel adequately	No maintenance completed
Drainage System 4					

Location	Year Completed	Fencing description	Tunnel description	Assessment	Maintenance
West side	2015, 2016	2000 m	1.5 m diameter concrete round culvert	No fencing material around entrance of culvert	Added material around top of culvert that attached to existing fencing (2015); Fence down in 2016, reattached and stabilized with rocks
Middle	2015	Yes	Fenced 7 m gap in median between NB and SB structure	Water levels above fence height	Posts stabilized, and rocks used to keep fence flush with ground
East side	2015	2300 m	1.5 m diameter concrete round culvert	Water levels above fence height; no fencing around top of culvert	Added another layer of geotextile above water levels and around top of culvert
Drainage System 5 (Southernmost culvert)					
West side	NA	2000 m	1.5 m diameter concrete round culvert	Large animal and reptile fence does not connect to structure	No maintenance required; recommended to close off culvert
Middle	NA	Yes	Unfenced 1.5 m gap between NB and SB structures in median	Gap between culverts in median with no fencing	No maintenance completed
East side	NA	2300 m	1.5 m diameter concrete round culvert	Large animal and reptile fence does not connect to structure	No maintenance required because DS is not functional
Northeast flooded section (680 m south of northeast continuous section fence end)					

Location	Year Completed	Fencing description	Tunnel description	Assessment	Maintenance
East side	2015, 2016	Several hundred metres	NA	Fence flooded for 200 m; gap at one-way gate; wash-out at bottom	Added layer of geotextile to one-way gate and fixed wash-out by adding soil and rocks at fence bottom
Southeast and southwest fence end					
East side	2015, 2016	50 m extended (2015); 300 m extension (2016)	NA	No reptile exclusion fencing at wetland habitat	Used rock and geotextile to fill small gaps at bottom of large animal fencing and fence extension to large animal underpass
Cliff gap west side					
West side	2015	Several hundred metres		No fencing for approximately 250 m along cliff	Used rock and soil to fill small gaps at bottom of large animal fencing for 75 m up South side of cliff
One-way gates					
West side between DS 2 & 3	2016	None	NA	Turtles able to navigate gap without fencing	Closed gate with fencing in 2016
East side 130 m north of large animal underpass	2016	None	NA	Turtles able to navigate gap without fencing	Closed gate with geotextile fencing
East side continuous section at flooded section	2015	None	NA	Turtles able to navigate gap without fencing	Closed gate with geotextile fencing in 2015

Location	Year Completed	Fencing description	Tunnel description	Assessment	Maintenance
West side, continuous section between RT 2 and 3	2015	None	NA	Turtles able to navigate gap without fencing	Closed gate with geotextile fencing
47 m south of SW continuous section fence end	2015	None	NA	Turtles able to navigate gap without fencing	Closed gate with geotextile fencing
Fencing between Reptile Tunnel 1 and 2 (west side)					
West side	2015	10 m	NA	Black Bear destroyed section of reptile fencing in mid-June 2015	Inserted 10 m additional piece and 3 metre piece of geotextile fencing to close gap