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## Do turtle roadkill hotspots shift from year to year?

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#### Abstract

Freshwater turtles face many threats but roadkill is one of the most serious for many species. Roadkill of turtles is not uniformly distributed across roads but aggregated in certain areas, termed hotspots. A key question in identifying hotspots is whether they are fixed locations or if they shift from year to year because of changes in movement patterns. We compared how one, two, and three years of road survey data compared with the pooled data from four years of surveys. We found 254 turtles during 73 surveys during four years along a 15.5 km road section in Ottawa, Ontario, Canada. The four years of pooled data produced four hotspots ("pooled hotspots") while each year or combination of years produced from three to five hotspots, four of which approximately corresponded to the pooled hotspots. The average percentage overlap of hotspots between one, two, or three years of survey data and the pooled hotspots ranged from 58.7% to 88.9%. Just one year of surveys sometimes missed one of the pooled hotspots, underestimated the spatial extent of the pooled hotspots, and also sometimes produced an additional "temporary" hotspot. Two years of surveys generally produced better approximations of the pooled hotspots, and better identified the spatial extent of those hotspots.

Key words: Mitigation; reptiles; road ecology; survey methods; turtles

#### Introduction

Turtles are one of the most endangered groups of species in the world, with more than half of the 360 species threatened with extinction (Stanford et al. 2020). While turtles face many threats, roadkill is a major cause of mortality for many species (Gibbs and Shriver 2002; Steen and Gibbs 2004; Aresco 2005; Dupuis-Désormeaux et al. 2017). Turtle life history strategies are typified by high rates of egg and hatchling mortality offset by extremely low rates of adult mortality (Congdon et al. 1993, 1994; Heppell et al. 1996). Even a small increase in adult mortality rates can lead to population declines (Congdon et al. 1993, 1994; Steen and Robinson 2017). Turtle populations are also extremely slow to rebound from declines (Keevil et al. 2018). Roadkill, which affects adults moving among wetlands, dispersing juveniles, and adult females seeking nesting locations, can lead to population declines (Gibbs and Shriver 2002; Piczak et al. 2019; Nicholson et al. 2020) or extinctions (Howell and Seigel 2019).

Roadkill affects a wide range of freshwater turtle species (Ashley and Robinson 1996; Langen *et al.* 2012; Carstairs *et al.* 2018). Turtles are found on roads throughout the active season, however, peak mortality tends to occur during the nesting season (Beaudry et al. 2010; Cureton and Deaton 2012; Carstairs et al. 2018). In areas with high road density, turtle populations have been found to be strongly male biased (Steen and Gibbs 2004; Piczak et al. 2019) and this could be a result of females being more prone to roadkill during nesting forays. While adult females are more apt to be hit by cars during the nesting season, male turtles have been found on roads throughout the active season, and overall there was no significant difference in the sex ratio of most turtle species found on roads in Ontario (Carstairs et al. 2018). Some species that rarely leave the water except for nesting do show strong female bias in road mortality (Crawford et al. 2014a).

Roadkill of turtles is not uniformly distributed across roads but often aggregated in certain areas, termed hotspots (Langen *et al.* 2007; Crawford *et al.* 2014b). Turtle hotspots often occur along road segments with wetland habitat on both sides of the road, have relatively high traffic volumes, and high forest cover (Haxton 2000; Aresco 2005; Langen *et al.* 2012). Determining hotspot locations is typically

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accomplished by conducting multiple surveys of the road or roads of interest (Choquette *et al.* 2016; Boyle *et al.* 2017). Collecting such data is labour intensive as it requires multiple surveys (weekly or more frequently) and driving potentially thousands of kilometres (Langen *et al.* 2012; Santos *et al.* 2017).

Once hotspots have been identified, road mitigation in the form of wildlife fencing and some form of crossing structure under the road can be installed. Such mitigation structures have demonstrated reduced turtle mortality when properly installed (Aresco 2005; Baxter-Gilbert *et al.* 2015; Read and Thompson 2021). Effective road mitigation can reduce road mortalities of freshwater turtles by more than 90% (Heaven *et al.* 2019) although mortalities at fence ends can remain a problem (Markle *et al.* 2017; Read and Thompson 2021).

A central question in any survey work is how many surveys are sufficient? Are surveys from one year sufficient to determine the location of roadkill hotspots or do the hotspots shift from one year to the next as a result of differences in weather, wildlife movement patterns, or other factors? The location of bat roadkill hotspots varied from one year to the next but appeared to be correlated with yearly variation in plant productivity (Medinas et al. 2021). In contrast, turtle hotspots may be more spatially consistent, as previous turtle studies have found major roadkill hotspots to occur in the same location over time (e.g., Aresco 2005). However, many studies have been for only short periods or have pooled two years of survey data to determine a more robust measure of hotspot locations (e.g., Cureton and Deaton 2012; Langen et al. 2012). One four-year study found that turtle hotspots often re-occurred in subsequent years but none of the hotspots occurred in all years (Garrah et al. 2015). Given the expense of road mitigation, it is important to know how to identify the location and spatial extent of major hotspots where roadkill most commonly occurs. To help assess these issues, we conducted four years of surveys along a road with known high levels of turtle mortality to determine how hotspots varied from year to year when compared with hotspots determined from pooling four years of surveys ("pooled hotspots"). We focussed on turtles because all species in our study area are listed as species at risk by the federal government (Government of Canada 2022) and listed species are more likely to be the focus of road mitigation projects. We hypothesized that one year of surveys would not be sufficient to confidently determine the locations and extent of pooled hotspots but that two or more years would be required.

#### Methods

We selected a 15.5 km section of Roger Stevens Drive in rural Ottawa, Ontario (45.0728°N, 75.8192°W) because it was known as an area of high turtle mortality based on previous surveys by D.C.S. The surveyed section was a paved, two-lane road with a posted speed limit of 80 km/h. The road is technically within the City of Ottawa but is in an area of few houses and adjacent to a large, city-owned, natural area with extensive forest and wetland habitat. Over 4000 vehicles per day were reported along this road in 2019 (City of Ottawa 2021). Road surveys were conducted for four consecutive years starting in 2016. Surveys started in mid- to late-May and finished from August to October depending on the year (Table 1). Surveys after August were typically less productive. For example, only 16% of observations in 2016 were made after August in the year when the most surveys were conducted in September and October. Surveys were conducted by car travelling at ~30-50 km/h, typically with at least two people in the vehicle, and usually between 0900 and 1600. Roadside walking surveys in wetland areas were also occasionally undertaken, in association with finding a dead turtle. This introduced a bias in data collection of sometimes finding additional dead turtles near where a turtle was found from driving surveys. Some of these turtles would likely have not been detected through just driving surveys, which can underestimate total roadkill (Langen et al. 2007). Walking surveys were generally spatially restricted and did not produce a large number of dead turtles, so the overall bias to our data is likely limited.

During surveys, the road surface and road shoulders were scanned for live and dead turtles. For the first three years (2016–2018), the location of turtle observations was recorded with a handheld global positioning system (GPS) unit (various models, Garmin Ltd., Olathe, Kansas, USA). Starting in 2019, most observations were recorded using the iNaturalist app for mobile phones (https://iNaturalist.ca),

TABLE 1. Number of road surveys and number of turtles
found per month along a 15.5 km survey route along Roger
Stevens Drive, Ottawa, Ontario conducted from 2016 to 2019.
Conducted from 2016 to 2016 to 2019.
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	Number of surveys (number of turtles)			
_	2016	2017	2018	2019
May	1 (1)	6 (32)	2 (8)	3 (10)
June	4 (34)	6 (30)	6 (20)	5 (11)
July	4 (14)	6 (23)	6 (11)	2 (6)
August	5 (18)	4 (11)	3 (6)	1 (1)
September	4 (10)	1 (2)	1 (1)	0
October	1 (1)	2 (4)	0	0

using the phone's internal GPS. Most observations had a spatial accuracy of 5–10 m. All turtles were removed from the road or road shoulder to prevent double counting of carcasses on a subsequent survey or to ensure the safety of the animal if alive. We included both live and dead turtles (excluding hatchlings) in the analyses as live turtles would frequently have been killed if we had not removed them from the road and our goal was to determine the main crossing areas along the road, information that is independent of whether the turtle was found alive or dead. Road surveys were conducted approximately weekly or more often during spring and summer.

We used Siriema 2.0 software (Coelho et al. 2014) to analyze the spatial pattern of hotspots as it has been widely used in road ecology (e.g., Gunson and Teixeira 2015; Choquette et al. 2016; Boyle et al. 2017; Arango-Lozano and Patiño-Siro 2020). The data were analyzed as single years, as two- and three-year combinations, and as all four years pooled together, for a total of 15 datasets. All species were given equal weight in the analyses. To determine if there were significant spatial aggregations, a Linear Ripley's K test was performed using a 250 m initial radius, a 200 m radius step, 100 simulations, and a CL of 95%. This was then followed by a Linear Hotspot Analysis using a radius of 200 m, 1000 simulations, 500 road divisions, and a CL of 95%. The radius lengths selected for the Ripley's K test and the Linear Hotspot Analysis were chosen based on the length of the surveyed road (15 km), the fact that turtles can move hundreds or thousands of metres (Obbard and Brooks 1980; Grgurovic and Sievert 2005), and that typical road mitigation fencing for turtles will be in the hundreds of metres (e.g., Aresco 2005; Baxter-Gilbert et al. 2015; Markle et al. 2017; Boyle et al. 2021). Shorter radius lengths typically produce more and shorter hotspots than longer lengths (Spanowicz et al. 2020). Mitigation fencing limited to these shorter hotspot locations increases the risk of mortality at fence ends and hence longer radius lengths should produce more effective guidance for mitigation locations and lengths.

The process was repeated for each turtle dataset. Hotspots were identified as locations where observed values fell above the upper 95% CL. If there were sections within hotspots where observed values equalled but did not dip below the upper CL, then the hotspot was considered continuous. To determine how well each dataset matched the four-year pooled data, we calculated the percentage overlap:

#### % overlap = $O / (L1 + L2 - O) \times 100$

where O = length of sample hotspots that overlaps with pooled hotspot length, LI = total length of sample hotspots, and L2 = total length of pooled hotspots. Differences in hotspot overlap of one, two, and three years of data with the four years of pooled data were compared using the non-parametric Kruskal-Wallis *H* test. No *post-hoc* comparison test was performed given the small sample sizes of each group.

#### Results

We found 254 turtles during 73 surveys from 2016 to 2019. We conducted an average of 18.25 surveys per year (range 11–25) and the number of turtles observed in a given year (mean = 63.5, range 28–102) was positively correlated with the number of surveys ( $r^2$  = 0.88). Painted Turtle (*Chrysemys picta*) made up 55.5% of all observations, Blanding's Turtle (*Emy-doidea blandingii*) 24.8% of observations, Snapping Turtle (*Chelydra serpentina*) 13.0% of observations, and 6.7% of turtle carcasses could not be identified because of their poor condition.

The Linear Ripley's K test indicated that the data for each year and each combination of years were significantly aggregated at all spatial scales from 0 to 11 km. The pooled data from all four years of road surveys resulted in four well-defined hotspots along the first 8 km of the road (Figure 1). The hotspots averaged 0.6 km in length (range 0.4–1.2 km) for a total length of 2.8 km. We found 198 of the 254 turtles (78%) in these four hotspots (Figure 2). Hotspot 3 consistently had a large number of turtles, being ranked first or second in three of the four years, and five out of six two-year datasets.

The number of hotspots in each year or combination of years ranged from three to five, with all but one of those hotspots approximately corresponding to the four pooled hotspots (Figures 1, 3, 4). Most years or combination of years resulted in four hotspots (11 of 15, 73.3%). All years or combination of years that yielded only three hotspots involved the year 2017 (2017, 2016/2017, 2017/2019; Figures 1, 3, 4).

The average percentage hotspot overlap between individual years or combination of years and the pooled hotspots ranged from 58.7% to 88.9% (Figure 5) and the overlap varied significantly among one, two, and three years of data (H = 9.257, P < 0.01). Even with two years of survey data, the percentage overlap with the pooled hotspots was as low as 56.9%. Considering just the major hotspot (hotspot 3; Figure 2), results from single years of surveys resulted in an average overlap with the four years of pooled data of only 58.7%, while two years of surveys produced a mean overlap of 78.5%, and three years of surveys produced a mean overlap of 91.5%.

#### Discussion

The number and location of the hotspots varied from year to year (Figure 1). Considering results



FIGURE 1. Hotspot locations along a 15.5 km survey route along Roger Stevens Drive, Ottawa, determined from survey results from 2016 to 2019, along with hotspots determined from all four years of data pooled together. Hotspots are arranged from west to east and no hotspots were found beyond km 9.



FIGURE 2. Number of turtles found in each of four pooled hotspots from road surveys conducted along Roger Stevens Drive, Ottawa, from 2016 to 2019. Hotspots are arranged from west to east, corresponding to pooled hotspots in Figure 1.

from individual years, each of the four survey years resulted in hotspots that approximately corresponded to the pooled hotspots (Figure 1). Only one of the four years (2018) produced a hotspot that did not correspond to one of the pooled hotspots, and only one year (2017) missed any of the pooled hotspots. This also means that half of all years produced either a "temporary" hotspot, or missed a pooled hotspot.

Considering results from two years of combined data, all of the datasets produced three or four hotspots, which approximately corresponded with the pooled hotspots (Figure 3). Two of the datasets missed one pooled hotspot, but there were no "temporary" hotspots produced. Datasets from three years of pooled data all produced four hotspots that approximately corresponded to the pooled hotspots (Figure 4). By definition, the three-year datasets contain most of the data in the four years of pooled data. However, if hotspot location was highly variable from year to year, even three years of data might be insufficient to identify the approximate locations of the pooled hotspots.

Hotspot 1 was the only hotspot that was not always identified by a single year of data or two years of combined data (Figure 1, 3). This hotspot also had the fewest total number of turtles (Figure 2). Overall, the results from each individual year produced hotspots that approximately corresponded with three of the four pooled hotspots.



FIGURE 3. Hotspot locations along Roger Stevens Drive, Ottawa, determined from pooling two years of survey results using data from 2016 to 2019, along with hotspots determined from all four years of data pooled together. Hotspots are arranged from west to east and no hotspots were found beyond km 8.



**FIGURE 4.** Hotspot locations along Roger Stevens Drive, Ottawa, determined from pooling three years of survey results using data from 2016 to 2019, along with hotspots determined from all four years of data pooled together. Hotspots are arranged from west to east and no hotspots were found beyond km 8.

The question of how much turtle hotspots shift from one year to another has not been explored in great detail. Amphibian and reptile hotspots were found to be generally consistent over a two-year period in New York state (Langen *et al.* 2007). Similarly, Diamondback Terrapin (*Malaclemys terrapin*) hotspots during nesting season were spatially consistent between two years in Georgia, USA (Crawford *et al.* 2014b). In contrast, a four-year survey along a 37 km road in eastern Ontario found that while many turtle hotspots were consistent across some years, none were consistent across all years (Garrah *et al.* 2015).

Hotspot mitigation typically focusses not just on those hotspots that are statistically significant, but on those with the greatest number of turtles, as road mitigation is expensive and budgets are limited. Is one year of data collection sufficient to identify which are the major hotspots? From our data, in three of the four years, the hotspot with the most turtles overall (hotspot 3; Figure 2), was also identified as the road section with the most or second-most turtles. The year with the fewest surveys (2019) also produced no clear major hotspot. Considering our data with two years of pooled surveys, five of the six datasets agreed that hotspot 3 had the most turtles, and in the sixth dataset hotspot 3 tied for first place. Hotspot 3 was also the longest in length indicating an above average number of turtles over a sustained length of road. The hotspot corresponded with large wetland on both sides of the road suggesting turtles were crossing the road at multiple locations in that section of road.



FIGURE 5. Mean percentage overlap of turtle hotspots by number of years of combined data compared with the hotspots from the four years of pooled data. Hotspots are from turtles found along Roger Stevens Drive, Ottawa, from 2016 to 2019. The mean percentage overlap is graphed along with the minimum and maximum overlap from each dataset.

Our data have limitations, beginning with the assumption that four years of surveys are adequate to determine the location and spatial extent of the hotspots. Given that hotspots defined by three or four years of surveys showed great similarity, it is unlikely that more years of surveys would greatly change the location or spatial extent of the hotspots. Although the number of surveys we conducted varied from year to year, even the year with the fewest surveys (with surveys ending in August), and fewest turtles (2019) produced hotspots in general agreement with the pooled hotspots (Figure 1). Our surveys were also conducted over a fairly short distance (15.5 km) and longer road sections may result in greater hotspot variation across time (e.g., Garrah et al. 2015). Overall, our results suggest that one year of intensive survey effort can identify the approximate location of major hotspots with reasonable confidence. Small variations in hotspot locations should be considered of minor importance as wildlife fencing must span a longer distance than the hotspot, as increased roadkill at fence-ends (i.e., where mitigation barrier structures terminate) is a common issue in road mitigation projects (e.g., Huijser et al. 2016; Markle et al. 2017). A more important problem would be if the hotspot from a single year's data greatly underestimated the spatial extent of the pooled hotspot. In our single year survey results, the length of the major hotspot (hotspot 3) averaged less than 60% of the length of the pooled hotspot. Fencing based on one survey year would likely have been inadequate. In contrast, the length of hotspot 3 based on two years of survey results was almost 80% of the pooled hotspot length. In this case,

if wildlife fencing was installed based on the two-year hotspot results and the fencing included a generous extension beyond each end of the hotspot, then the fencing would likely be adequate to reduce or eliminate roadkill. We suggest that road surveys to identify turtle hotspots be conducted across a minimum of two years to reduce the risk of misidentifying the location and spatial extent of hotspots. Considering the expense of permanent wildlife fencing, two years of data collection is not a substantial cost and additional survey years ahead of mitigation planning and installation will only increase the accuracy of where these actions are needed.

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