University of North Carolina Asheville
Journal of Undergraduate Research
Asheville, North Carolina
December 2022

Wildlife Road Mortality on Two Roads Bordering Vernal Wetlands

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Abstract

Habitat fragmentation, caused by roads or other human-made structures, can reduce the abundance of various species by preventing animals from moving normally through their habitat. Populations can be separated from each other, and animals can be blocked from moving between their typical breeding, foraging, and hibernation locations. Amphibians are at especially high risk from fragmentation by roads because they are small, difficult to see, frequently move at night, and occasionally participate in mass migrations. Under-road wildlife passages can reduce incidence of amphibian mortality; however, building effective passages requires knowing where along roads migrations are likely to occur and funneling animals toward the passages. Sandy Bottom Preserve is a protected area in Buncombe County, NC containing several vernal pools, streams, and permanent ponds that host a wide range of amphibians, birds, reptiles, and other small vertebrates. The Preserve is divided by two roads, Parkway Crescent, and Sandy Springs Dr., that are known to have high incidence of road mortality. The purpose of this project was to quantify road mortality in order to inform the placement of a wildlife passage on each road. I surveyed a 350 m stretch of each road and recorded all instances of vertebrate mortality, biweekly for 25 weeks from the late spring to early fall of 2022. The two roads differed greatly in specimen number: Parkway Crescent had 67 specimens while Sandy Spring Rd. had only 21. Salamanders were the most common clade, with *Notophthalmus viridescens* being the most common species. There were several areas with high concentrations of specimens on Parkway Crescent, such that no single location for the under-road passages stood out. If two passages can be built, I suggest that they both be placed under Parkway Crescent, with fences along both sides of the road to direct animals toward the passages.

1. Introduction

One major threat to biodiversity is habitat fragmentation. Habitat fragmentation is the interruption of an organism's natural habitat, often due to the introduction of human made structures such as roads. Evidence shows that habitat loss, fragmentation, and landscape modification are the primary drivers of biodiversity loss[1,2]. Some habitats are made up of patches naturally, but human action has fragmented most landscapes worldwide[2]. Since habitat fragmentation is a leading cause of biodiversity loss and fragmentation is occurring worldwide, this has become a problem for many species of animals in almost every biome.

One of the ways that habitat fragmentation can reduce biodiversity is by decreasing movement of individuals throughout the habitat[2]. Animals that used to be able to travel through a large range to breed or to hibernate can become cut off from important parts of their habitat. This can cause several problems. The first is a lack of genetic diversity. If a larger animal population becomes separated into two separate sub-populations due to habitat fragmentation, they become genetically isolated from one another, leading to decreased genetic diversity within populations, and sometimes an increase in harmful mutations[3]. Due to the presence of habitat fragmentation, animals can be killed in their migration as they cross human-altered landscapes such as farmlands or roads. Many animals have a biological imperative to migrate and will do so whether or not there is an obstacle in their path[4]. Roads not only provide a geographical barrier between two fragments of a habitat, but they provide an added danger to animals that need to migrate through their habitat, as many animals, especially smaller ones, can be easily killed by traffic. Roads separating habitat fragments have shown to greatly decrease genetic diversity[5,6]. This decrease indicates that the organisms cannot safely cross the road to breed.

Among all of the species that have been threatened by habitat loss and fragmentation, amphibians seem to be among the most affected[7,8]. They are declining more rapidly than birds or mammals[7]. Studies from different continents have shown amphibians to be the most frequently killed vertebrates on roads. In central Europe there are between 70 and 88 percent more instances of amphibian road mortality compared to instances of other animals[7]. Local populations of amphibians have gone extinct or have become inbred due to genetic isolation[7]. One of the reasons amphibians are at such a high risk of road mortality is that they are easily overlooked due to their size, and they are very slow[9]. Despite their mass migrations, their small size makes it very difficult for motorists to spot and dodge them[7]. Because their movements are mostly tied to breeding events, road mortality among amphibians tends to be seasonal. In temperate zones there are many incidents in the warmer months, and little to no incidents in the winter[10]. This is due to seasonal migration and hibernation patterns among amphibians. Many salamander species and some frog species migrate in the spring and fall. Additionally, some species of salamanders move within their habitat during the summer due to habitat changes after severe rain or the drying out of vernal pools[11]. They seem to prefer to travel after the rain, likely because a moist environment protects them from drying out as they move around[12]. They also primarily travel at night; during one study, only three percent of salamanders arrived at their breeding site during the day, the rest arrived at night[13]. This makes sense, as many salamanders are nocturnal and traveling during the night might make it easier to avoid predators. Unfortunately, as difficult as it is to see small amphibians on the road during the day, it must be even more difficult to see them at night.

Habitat fragmentation is clearly a tremendous problem for biodiversity, and roads present a special danger, especially to salamanders. Due to this, solutions to habitat fragmentation have been a focus of ecologists and conservationists for some time. One well studied solution is the installation of wildlife passages. Current research shows that they can help to reduce occurrences of vertebrate mortality on the road. One study showed that salamander road mortality decreased from 10% of the population to less than 2% following the installation of tunnels going under the road[13]. Once it was established that wildlife passages help reduce road mortality, researchers attempted to discern what could be used as a wildlife passage. There are several types of passages, and they are typically designed around the animals that use them. There are wildlife bridges that go over roads, tunnels of various sizes that go under roads, and passages that can be carved into roads. Amphibians tend to prefer the passages under, or into the road, while mammals tend to prefer larger under road tunnels or over road bridges[7,14].

The focus of this paper is on the tunnels and passageways that go under roads, as the primary focus is reducing road mortality of small animals such as salamanders. One problem in constructing all wildlife corridors is the need for conservationists to get funding for them. In order to combat this, researchers looked into ways to adapt previously existing structures for the use of wildlife. There is substantial evidence that the metal culverts that are often put into place to direct the flow of rainwater can be used for under-road tunnels for amphibians, and some roads already have them installed to prevent flooding[7]. However, many species may need alterations of the tunnels in order to entice individuals to use them. A series of experiments showed that many amphibian species appeared to select tunnels that appeared more natural, with soil or gravel substrate and greater light permeability[15] (Wolza et al. 2008). These conditions can be met by the passageways carved into roads. Once the passage is carved, the bottom is typically lined with gravel and the top is covered by a grate, which maintains road integrity and provides sufficient natural light. One other important factor of corridor placement is to put the corridor in an area where the animals are naturally inclined to move. It is important to place the corridor in a location that is in the path of the organism's migration, and it can be useful to block sections of the road off with fencing to direct the animals toward the corridor[7].

The research covered in this paper looked at the instances of road mortality at Sandy Bottom Preserve. It has a wide variety of animal diversity, but it is divided by a two lane road. This is problematic for the animals living in the area as many travel for breeding, feeding, and hibernation. Sandy Bottom vernal wetlands is a large area, near the French Broad River[16,17]. It consists of forested areas, vernal wetlands, permanent ponds, rivers, and seasonal ponds. The land is divided by two roads, Parkway Crescent and Sandy Springs Drive, both of which head toward a neighborhood. Since the roads divide a biodiverse habitat, with a large array of microhabitats, animals have to cross them, and there are regular instances of road mortality along them[16,17]. The goal of this study was to determine the patterns of occurrences of vertebrates killed by vehicles and analyze them to see where the animals living in Sandy Bottom tend to cross the road. This analysis will be used to suggest two locations for under-road wildlife passages to be installed, in order to lower instances of road mortality. At the start of the experiment, it was predicted that there would be more instances of vertebrate mortality found on Parkway Crescent than on Sandy Springs Dr., as Parkway Crescent has more shade and seems to dry out less frequently than Sandy Springs Dr. It was also thought that instances of road mortality might be concentrated toward the East end of Parkway Crescent, near Highway 191, as there are several sections of large vernal pools on the North and South side of the road in that area. There were no similar predictions about Sandy Springs, because the habitat to the North is known, but the habitat to the South is not.

2. Methods

2.1. Study Site

The Sandy Bottom Preserve is a vernal wetland located in the floodplain of the French Broad river. It is a 34 acre Unique Wetland situated in the floodplain of the French Broad River[16,17]. It contains areas of seasonal wetlands, temperate forests, and permanent ponds. It also contains two roads, Parkway Crescent and Sandy Springs Dr., which both run westward from a high traffic two lane road, Highway 191. The French Broad River is just to the East of this site. Parkway Crescent is to the North of Sandy Springs Dr. They are separated by a stretch of land with several wetland areas, two permanent pools, and a creek[16,17]. There are steep and rocky slopes on the North and South ends of the property, a road and a river on the East side, and steep slopes to the west. Both roads begin level, and they remain flat for 100 meters or so. After that, Parkway Crescent has a steep elevation for the next 150 meters and then it levels out for the next 100 meters. Sandy Springs simply increases steadily in elevation for the last 250 meters[16,17].

2.2. Sampling methods

In order to determine the best locations for under-road tunnels along Parkway Crescent and Sandy Spring Dr., it was necessary to determine where along the road the mortality was concentrated. Each road was divided into thirty-five 10m sections and were marked with flags. The flags began at zero and ended at thirty five, and were placed on the north side of the road beginning near Hwy 191 and running westward for 350m. The zones were defined as beginning at the first flag, and continuing until the next flag, so that zone one began at flag one and continued until flag two and so on. This allowed researchers to determine which area of the road had the highest mortality rate, and therefore, where the under-road tunnels would be most effective. Twice a week, I surveyed the road, beginning at the east end, walking westward on the north side of the road and then returning eastward on the south side of the road. Each vertebrate found was photographed, and its location was recorded in a lab notebook and a photo library. The specimens were considered a part of the zone they were found in, regardless of where in the zone they were found - e.g., animals found at 28m and at 21m were both in zone 2.

The surveys occurred twice a week, a few days apart, but not on the same days each week. I chose days that worked with my schedules, with a preference toward the days after rainstorms. Based on the earliest samplings I determined that the amphibians tend to move during or directly after a rainstorm. However, it was impractical to survey during a rainstorm, as the rain affected the ability of the researchers to see any samples along the road. The data were collected beginning in May and continuing until October.

All collected data were entered into a spreadsheet, and analyzed in R[18,19,20]. A pipe was used to sort the specimens by the road, and then the summarize function was used to determine how many specimens each road had in total. The total number of specimens found on each road were compared using a X^2 test.

The number of specimens per zone was evaluated for each road in order to show where the road mortality incidences were concentrated. Finally, I determined how many of each species was found across both roads. Once the patterns of road mortality were determined, they could be used to determine where the under-road tunnels would be most helpful for the conservation of the animals living in Sandy Bottom.

3. Results

The analysis revealed several interesting patterns regarding the distribution of road mortality. Parkway Crescent had 67 specimens while Sandy Spring Dr. had 21 (Table 1). The results of the X^2 test indicated that there were significantly more specimens found along Parkway Crescent than were found on Sandy Springs Dr. ($X^2 = 14.246$, df = 1, $p < 0.0002$; Figure 1).

Next, the distribution of the specimens across the zones was analyzed, divided by road so that the precise locations of wildlife corridors could be determined for each road. It was apparent that there are concentrations of specimens at locations 2-9, 16, 19, 23-27, and 33 on Parkway Crescent (Figure 2a). These zones were defined as areas with especially high numbers of specimens, or areas where there were multiple zones with higher numbers of specimens. The lowest number of specimens per zone was zero, which was found in zone 0, 1, 3, 4, 6, 7, 12, 13, 29, and 34. The maximum number of specimens per zone was five, which was found in each of zones 19, 25, 27, and 33. The concentrations of specimens are the areas with five specimens per zone or areas where multiple zones have three to four specimens right next to each other.

The number of specimens distributed along Sandy Springs Dr. was lower overall, and there was one zone with six specimens, zone 13 (Figure 2b). The minimum was also zero, which was seen in zones 1, 3, 4, 6, 7, 8-10, 12, 15-18, 20, 22, 26-28, 30, 31, and 35. There was one concentration of specimens along this road, at zone 13. There were six specimens at zone 13, but there were no other areas that contained five or more specimens. The abundance of each species found on both roads was also analyzed to determine if some species are more susceptible to road mortality during summer months. I was able to identify thirteen taxa to the genus or class; however, there were also two groups of unidentifiable specimens, one for salamanders, and one of all others (Table 2). There were 24 *Notophthalmus viridescens* (eastern newt), making it the most prolific species found. Following this there were Other Salamanders with eighteen individuals and Unknown with thirteen individuals. The category Other Salamanders included all salamanders that we could not identify to their genus and the Unknown category included all specimens that were too damaged by cars to be identified. There were also ten instances of *Pseudotriton ruber* (red salamander). The bulk of individuals found were salamanders, but there was one member of the class Aves, several frogs, four turtles, and one rodent from the genus *Napaeozapus*.

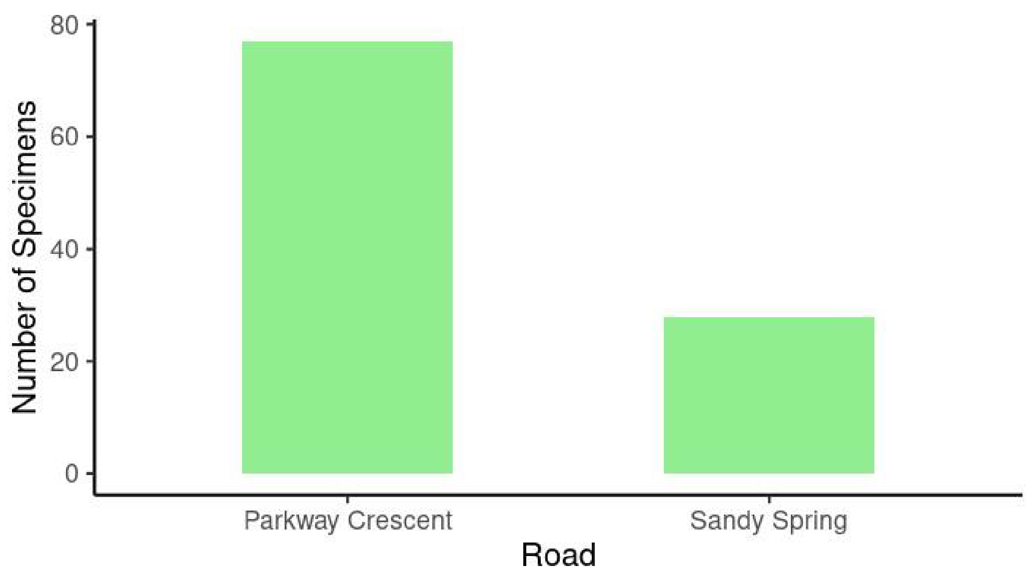

Figure 1. The number of specimens found on each road. Parkway Crescent had significantly more specimens than Sandy Spring Dr. ($X^2 = 14.246$, df = 1, p = 0.0002).

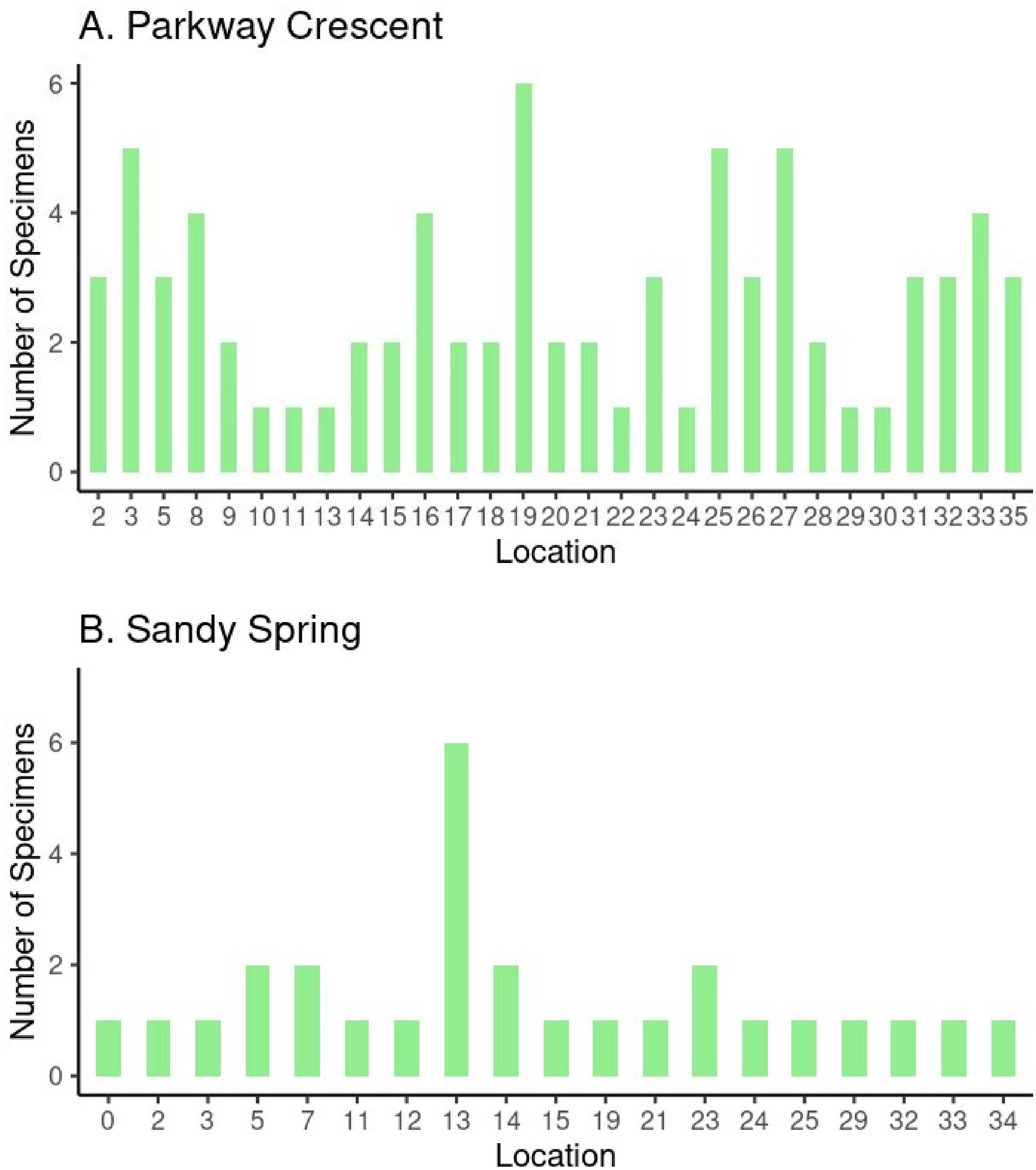

Figure 2. A) The number of specimens present in each 10m zone on Parkway Crescent. There were areas of high road mortality all along the road, with peaks in zones 2-9, 16 -19, 23-27, and 33. B) The number of specimens present in each 10m zone on Sandy Springs Dr. There was one peak of specimens at zone 13.

Table 1. Number of Each Species Found on Both Roads, Combined.

Species	Number of Specimens
Birds	2
Salamanders	
Ambystoma opacum	1
Eurycea wilderae	1
Notophthalmus viridescens	28
Pseudotriton ruber	15
Other Salamanders	20
Snakes	
Carphophis	1
Diadophis punctatus	3
Pantherophis	2
Thamnophis	2
Turtles	
Chelydra	3
Terrapene	2
Frogs	
Dryophytes	1
Pseudacris	7
Mammals	
Napaeozapus insignis	1
Lizards	
Plestiodon	5
Unknown	15

4. Discussion

Overall, I determined that Parkway Crescent had a significantly higher mortality rate than Sandy Springs Dr., that *Notophthalmus viridescens* was the most common organism found, and that there was at least one zone of high mortality on each road. There were multiple areas with large concentrations on Parkway Crescent and while Sandy Springs Dr. showed little mortality overall, there was one zone of concentration. The difference between the number of incidents on two roads has several potential causes. The land on the North side of Parkway Crescent has several vernal pools, which dried out as the summer progressed, while the land on the South side of Parkway Crescent, between it and Sandy Springs Dr., has several vernal pools, a stream, and two permanent ponds. This means that if the vernal pools dry out any amphibians may need to travel to the permanent ponds in order to find soil of the appropriate dampness. Additionally, the south side of Sandy Springs Dr. is very steep, and there are fewer sources of water present. Due to this, there may be fewer desirable habitats present for animals. There is also the possibility that as the soil dries in the vernal pools, the prey that the salamanders feed on may move toward the stream and the ponds, and the salamanders are following them.

The three snakes that were found along the roads were likely following prey. The ring necked snake, *Diadophis punctatus*, and the Eastern Checkered Garter Snake, *Thamnophis sirtalis sirtalis*, both eat salamanders[21,22]. They could have been hit traveling to the area of the wetlands where salamanders were likely to be concentrated. It is possible that salamanders were hatching in the vernal pools and metamorphosing into adults in mid spring to early summer, as several species in the Sandy Bottom area are known to lay eggs in the early spring. These newly metamorphosed adults and efts may have left the area to disperse throughout the vernal wetlands in order to avoid some competition for resources. Since the southmost side of the area, past Sandy Springs Dr., seems to have less available wet habitat overall, it makes sense that few individuals would disperse there. It is also possible that the only desirable breeding locations were in the land between the two roads, and to the North of Parkway Crescent. If no eggs were laid to the South of Sandy Springs Dr., there would be significantly fewer individuals traveling to and from it.

There are also a wide variety of reasons that the number of specimens per zone may differ. It was originally expected that the zones in the area from 14-22 would have few to no incidents of road mortality, as the South side of the road in this area was very steep, and leads directly to a stream. However, there were two areas of high specimen concentration at zone 16 and 19, so that explanation is not currently favored. Many of the breeding areas of the salamanders are further to the East of the site, as that is the area lower in elevation between zones 0 and 15. It is possible that the adults lay their eggs and vacate the area, heading westward and dispersing to avoid competition. This could indicate that many of the individuals seen in the eastward zones were newly morphed individuals, while many at the westward zones were established adults, but there is currently not sufficient evidence to support this. It is possible that the salamanders crossing the road are following the chemical trails left by other salamanders. However, if this were the case, the number of specimens should be even more concentrated. Another possibility is that the salamanders are following prey, and the insects they hunt are traveling through those specific zones. Insects could possibly be less hampered by geographical obstacles such as hills. It is very difficult to find a precise reason why the individuals are choosing the zone they are traveling through, as there are no specific concentrations that would indicate what drives them. If the routes were centered on flat land or land lower in elevation it would be easier to narrow down what drives the salamanders route choices, but given that the concentration is more dispersed, this is a more difficult prospect.

One interesting discovery was that *N. viridescens viridescens*, or the eastern newt, was, by far, the most common organism found on the road. There are several potential explanations for this. The research began in late spring, which is approximately the time that *N. viridescens* larvae would be morphing into efts[23,24]. These efts would be leaving the pools and moving into the terrestrial phase of their life cycle, which can last from three to seven years. This is likely not a complete explanation, as the regular discovery of efts on the road did not stop at any point over the summer. It is also possible there are far more eastern newts in the habitat than there are any other salamanders. There are two hypothesized causes for the eastern newts terrestrial eft stage, both of which could shed light on the reasons that there are so many of them found on the road. It is hypothesized that the terrestrial eft stage allows *N. viridescens* to disperse further throughout a habitat[23,24]. If this is the case then it is possible these newts are traveling in order to find a new range within their habitat. The other potential explanation for the eft phase is that it prevents the juvenile, *N. viridescens* and the mature *N. viridescens* from competing with one another[23,24]. Mature eastern newts are fully aquatic, and their efts are terrestrial, which allows them to occupy different niches in the habitat and eat different food sources. If this hypothesis is true then the terrestrial efts may be more prone to traveling throughout their habitat to find food, as they are less dependent on water.

Based on the data from this study, the current recommendation for the location of the wildlife passages is in zone 5 in Parkway Crescent. This will allow animals crossing between zone 2 and zone 9 to access it, and at zone 26, also in Parkway Crescent in order to allow the animals crossing at both zone 25 and zone 27 to move freely. It is also recommended that fencing be put up along the road in order to direct the animals traveling outside of these two locations to move across the road safely. Current research shows that wildlife passages are much more effective when fences are installed and that their presence decreases road mortality significantly[7]. This is especially important in the case of Sandy Bottom Preserve, since there was such a wide distribution of specimens, it is not possible to choose two locations where all amphibians trying to cross the road will be inclined to use it, unless they are prevented from crossing elsewhere. Without fencing funneling animals to the designated crossing points any individuals crossing at zones 13 or 33 will have no reason to divert from the road, but fences will force them to. The current recommendation based on all data present is to not install any wildlife passages on Sandy Springs Dr. There are significantly fewer individual specimens found on this road, and there was only one distinct concentration of specimens in any of the allotted zones. Based on this, and the multitude of crossing locations on Parkway Crescent, it will be more helpful to have both tunnels along it. If there should be additional funding for more tunnels, it would be recommended to put one at zone 19 on Parkway Crescent to aid the animals crossing at the more steep portion of the road, and at zone 13 on Sandy Springs Dr., as it is the location on that road with the highest concentration of specimens.

There were several potential confounding variables during this study, which are important to acknowledge. The first is that while surveying the roads it could be impossible to guarantee that all specimens were found. In situations where there were falling leaves, flower petals, or intense rain, the view of the road was obscured, and it could be very difficult to spot all available specimens. Additionally, specimens were often incredibly damaged from the impact of the car. When there was a significant amount of rain, it could be very difficult to distinguish specimens from waterlogged debris. Due to all of these factors, it was possible that some individuals were missed during the survey. Finally, research shows that amphibian road kill victims tend to disappear quickly, especially in the presence of scavengers. One study noted that the majority of amphibians killed on a road disappeared within one day of their death[25]. Since it was only possible for the roads to be surveyed twice a week, and specimens could vanish within a day, it is very likely that more individuals were killed on these roads during the course of this investigation, and they were not counted due to the surveys not being performed for several days after the incident.

In order to clarify the conclusions reached in this paper and to gain more understanding of the ecology at Sandy Bottom Preserve, there are several recommendations for further research. It is recommended that a survey is conducted of the salamanders found at Sandy Bottom in order to determine if there are more *N. viridescens* present than other salamanders. This could be done by setting up coverboards near locations where salamanders are likely to feed or breed, and checking them regularly to determine how many of each species of salamanders are present. The number of each species of terrestrial salamanders could be extrapolated from this. It is also recommended to continue the road mortality surveys, but to perform them all year, and to carry them out four or more times a week to attempt to catch more specimens before they decompose. Perhaps the roads could be surveyed every other day. This would allow for the observation of the entire spring migration, which occurs in early spring, and the entirety of the fall migration, which can end in mid autumn[23,24]. The current data is enough to suggest the locations for under road passages that would most benefit the animals at Sandy Bottom, but the aforementioned research would shed more light on the ecology of Sandy Bottom, and the potential reasons why those locations are preferable.

5. Acknowledgements

I would like to thank my research professor, Dr. Rebecca Hale and my research committee Dr. Graham Reynolds and Professor Caroline Kennedy, the staff and students of the Biology Department at UNC Asheville, and the Steve and Frosene Zeis Professorship, which funded my research and provided the basic equipment for the surveys.

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