# US 64 Roadkill Monitoring Survey Year One Interim Report

***Covering August 1, 2024 to July 31, 2025***

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*Clockwise from top left: rainbow snake, spotted turtle, black bear, leopard frog*

**Executive Summary:**

Since August 1, 2024, the Wildlands Network has been conducting daily roadkill surveys on US 64 and 264 in Tyrrell and Dare counties in northeastern North Carolina, with two primary goals: 1. To support NCDOT's application for federal funding to build wildlife crossings on US 64, and 2. To provide updated baseline data that will be essential for evaluating the success of any crossings that are built on the highway. A third goal for the project is to keep the highway clean of road-killed animals that would otherwise potentially attract critically endangered red wolves (*Canis rufus*), given that vehicle strikes have been established as the leading cause of mortality for red wolves in recent years. This report covers our findings from the first year of the surveys.

The roadkill survey route runs along US 64 from Columbia, NC, to the US 64/264 junction, and from there south to the junction of US 264 and Borrow Pit Rd. We tallied, photographed, and geolocated all road-killed vertebrates found during round-trip drives along the full survey route each weekday morning, alternating between 4 starting positions/directions in order to avoid temporal bias.

Over the first year of surveys, we have detected 5,044 instances of road-killed vertebrates along US 64 and US 264, representing a total of 144 vertebrate species. Reptiles and amphibians were more abundant during warmer months, and there were 1,050 snakes, 1,186 turtles, and 1,529 frogs recorded in total as roadkill. The colder months had far more bird roadkill events, and there were 801 road-killed birds recorded in total. Mammal roadkill seemed to remain relatively similar across the months, and there were 450 road-killed mammals recorded in total. We found similar levels of roadkill between counties, with Tyrrell County having 2,601 observations and Dare County having 2,443 observations. ***To our knowledge, no red wolves***

***were killed by vehicles along our survey route while our daily roadkill removal activities were in operation.***

Previous roadkill surveys were conducted along our route during the period 2008-2011, when the highway was being studied for a planned 4-lane expansion (those plans have since been postponed indefinitely). Broad trends in wildlife abundance and distribution on the highways have generally been similar between the two surveys periods, but some differences have stood out, such as the larger number of *Liodytes pygaea*, a species of special concern in NC, that we have detected. Based only on this initial year of our results, it does appear that we are detecting significantly fewer road-killed amphibians than the prior surveys in Dare and Tyrrell Counties.

Looking ahead, we plan to continue the roadkill surveys for one more year, to the end of July, 2026, at which point it is hoped that wildlife crossing construction on the Dare County portion of our study route will begin to get underway due to the large Wildlife Crossing Pilot Program grant received by NCDOT in late 2024. After the completion of the wildlife road crossing structures, we plan to conduct additional surveys to determine the impact of the structures on reducing wildlife vehicle collisions.

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**Introduction:**

Collisions between animals and vehicles on roadways are a constant and prominent concern for wildlife conservation and public safety. Since August 2024, Wildlands Network has been conducting roadkill surveys on US Highways 64 and 264 along a critical survey route for biodiversity and individual species of concern such as the red wolf (*Canis rufus*). This report will cover the first year of this roadkill survey, which is currently planned to continue until the end of July 2026.

A report published by the United States Department of Transportation (USDOT) in 2008 estimated that there are between one and two million cases of vehicles hitting large animals in the US per year (Huijser et al. 2008). The report also indicates this trend is increasing over time (Huijser et al. 2008), and acknowledges this estimate does not account for small or medium-sized animals that are hit by vehicles, meaning the actual total wildlife road mortality events nationwide are much, much higher. The Huijser et al. (2008) study identified 21 vertebrate species where road collisions are a major threat to their survival, most of which were not considered large animals. Based on an estimation of one million deer-vehicle collisions (which account for the majority of large animal collisions on highways), the authors estimate the total annual cost of wildlife road collisions in the United States at $8,388,000,000 (Huijser et al. 2008).

Fortunately, solutions exist for greatly reducing the amount of wildlife being killed by vehicles on particular stretches of highways. In particular, studies have found that wildlife fences with or without wildlife crossing structures reduce wildlife road collisions by 80 percent or more (Huijser et al. 2008). The problem with adding wildlife fencing alone is that fencing causes a barrier effect, increasing habitat fragmentation and decreasing wildlife habitat connectivity.

However, when fences are paired with well-designed wildlife crossing structures such as underpasses or overpasses, the connectivity barrier effect is removed, and wildlife populations are enabled to move safely over or under highways. USDOT found that installing crossing structures with appropriate wildlife fencing to be among the most cost-effective methods of preventing wildlife road collisions (Huijser et al. 2008).

Research is an important step in the construction of wildlife crossing structures. In order to be cost effective, it is imperative to know where hotspots of wildlife road-crossing activity occur so the sites can be chosen that are most effective both in mitigating wildlife road collisions and maintaining habitat connectivity. Without the data provided through research, wildlife crossing structures may not be as effective or may not have the desired effect at all. Given the costs associated with building crossings, it is imperative that the relatively small amounts of funds available for wildlife road mortality mitigation be spent in the most effective ways at the most important locations.

With this in mind, we set out to undertake an intensive daily roadkill survey along US Highway 64 (and an adjacent stretch of US Highway 264). The study route was chosen in light of the active planning efforts by NCDOT, USFWS, and NC Wildlife Resources Commission to develop proposals for installing wildlife crossings and fencing on US 64, with the immediate goal of reducing vehicle strike mortality for critically endangered red wolves (NCDOT 2024). While previous roadkill surveys on US 64 in Dare and Tyrrell Counties had been completed in the 2008-2011 time period, we wanted to provide an updated snapshot of what we predicted would be continued very high levels of road killed vertebrates along this survey route, in support of NCDOT's planned application for crossing funding to the Wildlife Crossing Pilot Program run by Federal Highways. We also realized that providing more recent roadkill data would be

essential as a fresh baseline for evaluating any future wildlife crossings that were installed on the highway.

The target study route along US 64 and US 264 crosses through numerous conservation areas, including the expansive Alligator River National Wildlife Refuge (ARNWR). US 64 serves as a major access route to the world-famous Outer Banks, a popular summer tourist beach destination. According to the Average Annual Daily Traffic counts reported by NCDOT in 2025, 4,815 vehicles are estimated to travel eastbound on US 64 east of Old US 64 each day and 4,640 vehicles are estimated to travel westbound on US 64 west of US 264 each day (NCDOT 2025). However, these are annualized estimates, and the actual traffic levels on busy summer weekends are estimated to be 3 times higher (~15,000 vehicles/day) according to a traffic report commissioned by NCDOT (WSP 2018). In contrast, US 264 receives much less tourist traffic, and only 1,064 vehicles are estimated to travel southbound south of US 64 on US 264 each day (NCDOT 2025).

The section of US 64 in Dare County along Alligator River National Wildlife Refuge stands out as a particularly important area for wildlife crossing structures due to the population of critically endangered red wolves inhabiting the refuge. Since 2014, vehicle strikes have ranked as the highest source of mortality for the wild red wolves (NCDOT 2024). A study commissioned by USFWS found that from 1987 to 2022, there were 91 recorded road mortalities of red wolves (Huijser and Begley 2023). Of these, 78 occurred on paved roads, and of those, 19 were on US 64 (Huijser and Begley 2023). Since the publication of that report, three more red wolf fatalities have occurred on US 64 (NCDOT 2024). This makes the stretch of US 64 through ARNWR a high priority target for crossing structures.

Our study hopes to reinforce the data published by other studies previously conducted in the area. A study conducted from April 2009 through July 2011 by Dan Smith (formerly of the University of Central Florida) had multiple wildlife research components, including a set of driving-based roadkill surveys on US 64 in Tyrrell County from Columbia, NC to the Alligator River (Smith 2011). These surveys were conducted every other day for three days per week at a speed of 10 mph or less (Smith 2011). In total, Smith (2011) documented an incredible 27,886 road-killed animals over the course of the 2+ year study, over 25,000 of which were amphibians. Smith's research team had to resort to area-based sampling methods for estimating the total number of amphibians killed on certain mass-mortality events on US 64. In addition to roadkill surveys, they employed camera trapping, tracking stations, drift fences, and pitfall traps to get a better grasp on the wildlife in the area (Smith 2011).

Another previous study, this one by researchers based at Virginia Tech and conducted from January 2009 through April 2011, looked at red wolves in Dare and Tyrrell counties (Vaughan et al. 2011a). At that time, red wolves had a larger and more dispersed wild population than they do currently. Using GPS data collected from 16 collared red wolves, they estimated the average home range sizes for the wolves, as well as habitat selection and road permeability (Vaughan et al. 2011a). Vaughn et al. (2011a) documented high numbers of wolves crossing US 64, including one female wolf that may have crossed as many as 200 times. At the same time, they observed very low road mortality during their GPS collar study, with only one wolf reported being hit by a vehicle (in contrast to the high levels of red wolf vehicle strikes in more recent years). The study did identify 5 road crossing hotspots for red wolves, including 4 in Tyrrell County and 1 in Dare County.

The same authors published another study in the area that was conducted from March 2009 through March 2011 focusing on American black bears (*Ursus americanus*) and other wildlife in and near Alligator River National Wildlife Refuge (Vaughan et al. 2011b). They used barbed wire along guardrails for hair collection, driving surveys, and camera trapping. In addition to this, GPS collars were deployed on 49 individual bears, showing 15 bears crossing US 64 on 99 occasions (Vaughan et al. 2011b). Roadkill surveys were also deployed, surveying US 64 and part of US 264 on mainland Dare County (Vaughan et al. 2011b). The roadkill surveys were conducted by walking both sides of the route roughly every 7 days from April to August, and roughly every 14 days every other month (Vaughan et al. 2011b). Vaughan et al. (2011b) documented a total of 12,906 road-killed vertebrates during the two years of their surveys, including 7498 amphibians, 4014 reptiles, 1153 birds, and 241 mammals, including 1 red wolf.

## Methods:

### Survey Route

We performed road monitoring surveys on US 64 from Columbia, NC, to the US 64/264 Junction, and on US 264 from the US 64/264 Junction to Borrow Pit Rd on the eastern flank of Alligator River NWR. Surveys were done for one year (8/1/24-7/31/25) every Monday through Friday (except during holidays and inclement weather), starting between 60 and 90 minutes after sunrise (weather permitting). The survey team included two wildlife monitoring technicians, each alternating on a daily basis between serving as the driver and the observer. The driver drove along the survey route and looked for roadkill, pulling over safely and completely into the shoulder of the road when they spotted roadkill or when the observer made a gesture indicating they saw roadkill. The driver also took pictures of each live or dead animal, removing small

roadkill from the road using grill tongs and using a spade to scoop larger roadkill out of the road. Nitrile gloves were also used to drag roadkill out of the road where tongs or a spade did not suffice. Roadkill was tossed or dragged past the tree line or directly into a canal holding water to reduce the risk of scavengers like the red wolf from approaching the roadway. The roadkill was moved far away from the road to ensure it was not recounted. However, there were some exceptions to this practice. For roadkill white-tailed deer and raccoons, USFWS was contacted so that the animals could be collected to feed to captive red wolves. For any canids observed, USFWS was also contacted so they could collect further data on the specimens. For large animals that were difficult to move such as black bears and alligators, NCWRC was contacted with GPS coordinates. Injured and live vertebrates were also photographed when possible and recorded in the roadkill data sheet. Once the survey was over, the driver uploaded the pictures to a computer for backup.

The observer also looked for roadkill (especially on the right side of the road and shoulder) and watched for traffic when the driver was taking pictures and removing roadkill. In addition, the observer recorded GPS coordinates of roadkill using Garmin GPSMAP 64s handheld GPS and noted information about each animal on paper data sheets. Once the survey was over, the observer entered the data into the computer. Vulture soaring time was recorded in the trip log sheet each day that it was detected. Soaring is defined as a turkey vulture or black vulture airborne for a prolonged amount of time (not perched or moving from tree to tree).

Various survey checkpoints have been established and are defined as follows:

- Columbia: Columbia N-Seine Seafood Market, US 64 (35.91895, -76.22780)
- ARM: Alligator River Marina, US 64 (35.90483, -76.02799)
- 64/264 Junction: Junction of US 64 and US 264 (35.87120, -75.78512)

- Borrow Pit Rd: Entrance road to ARNWR, US 264 (35.81126, -75.79143)

In order to reduce temporal bias from scavengers and vehicles removing roadkill from the road, four survey routes with various starting locations and directions were employed, with each route ultimately resulting in one round-trip survey transit of the entire road transect (Figure 1). Route 1 starts in Columbia going east to ARM, ARM east to 64/264 Junction, 64/264 Junction south to Borrow Pit Rd, Borrow Pit Rd north to 264 Junction, 64/264 Junction west to ARM, ARM west to Columbia. Route 2 starts at Borrow Pit Rd going north to 64/264 Junction, 64/264 Junction west to ARM, ARM west to Columbia, Columbia east to ARM, ARM east to 64/264 Junction, 64/264 Junction south to Borrow Pit Rd. Route 3 starts at ARM going east to 64/264 Junction, 64/264 Junction south to Borrow Pit Rd, Borrow Pit Rd north to 64/264 Junction, 64/264 Junction to west to ARM, ARM west to Columbia, Columbia east to ARM. Route 4 starts at ARM going west to Columbia, Columbia east to ARM, ARM east to 64/264 Junction, 64/264 Junction south to Borrow Pit Rd, Borrow Pit Rd north to 64/264 Junction, 64/264 Junction west to ARM. These survey routes were followed according to the following quasi-randomized order: 1, 2, 3, 4, 2, 3, 4, 1, 3, 4, 1, 2, 4, 1, 2, 3, with this cycle repeated every 16 surveys. This approach was easier than full randomization to achieve the balanced level of survey effort we desired across the different routes. The soon-to-be-replaced Lindsay C. Warren Bridge, a 3-mile structure along US 64 that spans across the Alligator River, was not sampled for roadkill due to the lack of a shoulder to safely stop on.

In certain areas along US 64 and US 264, the presence of metal guardrails close to the edge of the highway prevented us from safely stopping for roadkill, as we could not block highway traffic with our survey vehicle without risking serious accidents. In almost all cases, the guardrails were only on one side of the highway, so we were still able to stop for roadkill while

traveling in the other direction on that survey route. We also noted roadkill observed in areas where we could not stop due to the guardrails, and made a point of trying to find those animals on the return trip. But ultimately, the presence of the guardrails meant that some parts of our survey route had less effective sampling than others, a factor we will explore at the end of the 2-year study once we have mapped out the guardrail locations in greater detail and compared them to our roadkill survey results.

**Roadkill Data Collection**

We recorded several types of data when roadkill was encountered. The date and time of each roadkill observation allows us to cross-reference that specimen with pictures for future identification and verification. In addition to decimal latitude and longitude coordinates from the GPS, we recorded the road (US 64 or US 264), the county (Tyrrell or Dare), the direction (which lane the survey vehicle was traveling when the roadkill was encountered), and the position (where on the road or shoulder the roadkill was located). In the field, we made species identifications to the lowest taxonomic level determinable. If an animal was not identified to the genus or species, the best guesses for the ID are written in the notes section, which indicates that photos need to be referenced. We also kept track of other more subjective characteristics of each roadkill, mainly for our own reference to distinguish individual roadkill observations, but also to enable analyses of detection probability based on carcass size, condition, etc. These categories are the age (adult, subadult, juvenile, neonate, unknown), freshness (very fresh [vfresh], fresh, old, very old [vold], live), condition (flat, pieces, gross, average, intact, still moving, live, unknown), sex (male, female, unknown), and observation method (road, incidental). Roadkill classified as “road” meant we intentionally stopped for it after it was spotted from the road, and

roadkill classified as “incidental” meant we only found it because we were walking to inspect a different road-killed animal and would not have seen the incidental animal from the road.

**Water Quality Measurements**

In addition to daily roadkill surveys, we also performed water quality measurements on October 17 and 18, 2024, of the water bodies (primarily canals and ditches) adjacent to the highway. Using ArcGIS Pro, points along the survey route were determined by spacing them one mile from each other (Figure 2). Points located on the Lindsay C. Warren Bridge were not sampled. A YSI meter was used to measure environmental variables including salinity, dissolved oxygen, pH, and water temperature. As each point was visited, we checked both sides of the road and only included those where the YSI meter was properly submerged in water for an accurate reading. In addition, the relative proportions of open water, algae, floating vegetation, submergent vegetation, and emergent vegetation were estimated at each water quality measurement point.

**Canal Mapping**

From November 21, 2024, to December 17, 2024, we also mapped every roadside water body along the survey route. At the start and end points of the canals, a series of 3 photos in different directions were taken to help document vegetation and water conditions. Where roads and driveways existed over the canals, separate points were taken. Another variable we estimated was the width of the canal (in feet). Also, we recorded new points when vegetation structure or width changed. The data from canal mapping have not yet been analyzed.

**Geospatial Analysis**

The roadkill data sheet information was transferred into Microsoft Excel (*Microsoft Excel*, 2025) and saved as a CSV file. Data manipulation was conducted in ArcGIS Pro (*ArcGIS Pro*, 2024) to

incorporate geospatial elements such as road segmentation and water quality measurements. A 150 ft buffer with a flat end type was placed around a road polyline of US 64 from Columbia to the 64/264 Junction and of US 264 from the 64/264 Junction to Borrow Pit Rd. Using the Divide tool in the Modify Features pane, the buffer road was divided into 100 equal segments (of approximately 1620 ft each) The roadkill data CSV file was imported as a standalone table in ArcGIS Pro. Using the XY Table to Point geoprocessing tool, the roadkill data sheet, water quality data sheet, and canal mapping data sheet were all displayed onto the map. Inaccurate roadkill points occurred outside of the buffer and were corrected when necessary by referencing the written data sheets. Using the Spatial Join geoprocessing tool, the segment number of the road buffer was joined with the roadkill data using the Intersect Match Option. A new point feature class was created for the water quality measurement data by selecting only the points located in large canals. Using the Spatial Join geoprocessing tool, the water quality point feature class was joined with the roadkill data using the Closest Match Option. For the canal mapping, the Coordinate Table to 2-Point Line geoprocessing tool was used to connect start and end points of each documented canal. Any resulting line segments that did not follow the contour of the road were manually rerouted to follow the road. This polyline feature class was separated into two feature classes, one for the canals in the eastbound (EB) and southbound (SB) shoulder and one for the canals in the westbound (WB) and northbound (NB) shoulder. Using the Spatial Join geoprocessing tool, each of the EBSB and WBNB canal mapping feature classes were joined with the roadkill data using the Closest Match Option. Maps showing 3D bars of roadkill counts in each road segment were generated in ArcGIS Pro using a buffered mean-centered point extruded by the number of roadkill points in each segment. Graphs, figures, and tables using the finalized roadkill data were produced in RStudio (Posit team, 2025).

**Results:**

The observations from the past one year of roadkill surveys confirm high levels of roadkill along the target stretches of US 64 and US 264. We tallied 5,044 instances of roadkill across 144 species, including 1050 snakes (24 species), 1,186 turtles (9 species), 1,529 frogs (11 species), 801 birds (68 species), 450 mammals (26 species), and 28 others (6 species) (Table 1, Figure 3). The most observed snake species were the black swamp snake (*Liodytes pygaea*, a protected species of special concern in NC) and the banded water snake (*Nerodia fasciata)* (Figure 5a). The most commonly observed turtle species were the spotted turtle (*Clemmys guttata),* eastern mud turtle (*Kinosternon subrubrum),* and stinkpot musk turtle (*Sternotherus odoratus)* (Figure 5b). The vast majority of the frogs we recorded were in the "true frog" genus *Lithobates*, particularly southern leopard frogs (*Lithobates sphenocephalus)* and bullfrogs (*Lithobates catesbeianus)* (Figure 5c). The bird species with the greatest amount of recorded mortality was the yellow-rumped warbler (*Setophaga coronata),* but several other species such as swamp sparrow (*Melospiza georgiana),* American robin (*Turdus migratorius),* and hermit thrush (*Catharus guttatus)* were commonly encountered as roadkill during the winter (Figure 5d). The most observed mammal species was Virginia opossum (*Didelphis virginiana)* followed to a lesser extent by raccoon (*Procyon lotor),* but several medium-large-sized other mammals, such as black bear (*Ursus americanus),* coyote (*Canis latrans),* white-tailed deer (*Odocoileus virginianus),* river otter (*Lontra canadensis),* and American mink (*Neovison vison),* were also documented (Figure 5e). Roadkill in the "other" category largely consisted of Atlantic menhaden (mostly *Brevoortia tyrannus*), which likely dropped from seafood trucks as several dead fish were documented in close proximity during a few of our surveys in the fall of 2024 (Figure 5f).

The two surveyed counties have had similar levels of roadkill. Tyrrell County had 2,601 roadkill observations, and Dare County had 2,443 roadkill observations. However, each county differed in its distribution of animal types as Tyrrell County had more snakes and turtles, while Dare County had more frogs and birds (Table 1).

Through the first year of this study, we have noticed trends start to emerge. Predictably, the overall amount of roadkill detected has been higher in the warmer months due to increased traffic (personal observation) and higher levels of wildlife activity, particularly of reptiles and amphibians. The exception was a period in January after a winter storm, when there was a spike in bird roadkill, particularly of *Setophaga coronata*. Among individual animal types, temporal trends were also clear. In the warmer months (April to September), a higher proportion of reptiles and amphibians were detected, and in the cooler months (October to March), a higher proportion of birds were detected (Figure 4a-b). The detection of mammals remained relatively consistent proportionally throughout the year (Figure 4a-b).

We also observed spatial trends for where each major animal type was concentrated along our survey route (Figures 6a-g). Snakes have been detected in higher concentration in eastern Tyrrell County near Alligator River. Turtles were also concentrated in eastern Tyrrell County, but a little further west near the Alligator Game Land where the canals had lower salinity. Frogs had an area of concentration that overlaps with the turtles, but the main area of concentrated frog detection was on US 264 near a landfill pond. Birds were concentrated in western Dare County near Alligator River and the East Lake community. Mammal detections were relatively evenly spread throughout the study area.

So far, we have detected five NC species of special concern on the survey (Figure 8). Those five NC species of special concern are *Liodytes pygaea, Nerodia sipedon williamengelsi, Crotalus horridus, Corynorhinus rafinesquii macrotis,* and *Condylura cristata*.

**Discussion:**

Reinforcing what previous studies in the area have concluded, we have also detected high amounts of roadkill along US 64 in Tyrrell and Dare Counties, as well as along the surveyed section of US 264 in Dare County. Our finding of thousands of dead vertebrates in a 12-month span confirms the continued high levels of vehicle mortality suffered by a very broad range of species in this area, and emphasizes the continued need for wildlife crossings.

Our roadkill temporal trends were similar to the results from the 2009-2011 Tyrrell County roadkill survey (Smith 2011). However, there were some key differences. While both surveys detected high numbers of *Nerodia fasciata,* the Smith (2011) study did not detect as many *Liodytes pygaea,* which was the most numerous snake in our survey. *Liodytes pygaea* is of particular note because we have detected 267 of them so far and identified what seems to be an activity hotspot between Old US 64 and the Alligator River Marina in eastern Tyrrell County. There is an ongoing bridge construction project at the edge of this hotspot that will incorporate a wildlife underpass, which may reduce the number of snakes being killed in the immediate vicinity of the new bridge.

Similar to our data, the previous Tyrrell County study detected many *Clemmys guttata, Didelphis virginiana, Kinosternon subrubrum,* and *Setophaga coronata*. In total from April 2009 to July 2011, they detected 27,886 road-killed animals, a much higher per-year value than we have had this year, perhaps due to the observation methods – in particular, Smith's roadkill surveys were conducted at 10 mph, a low speed that we ultimately rejected for our study due to

the increased risk of being rear-ended by high-speed beach traffic on US 64. Smith (2011) also used an area estimation approach to quantifying amphibian mass mortality events, which we so far have not documented in the Tyrrell County portion of our study route. Whether the lack of huge numbers of dead amphibians reflects a change in wildlife populations or habitat conditions remains to be further evaluated after we collect an additional year of data under different weather conditions. Smith (2011) detected 134 species compared to our 144 total and 111 in Tyrrell County.

The part of the 2009-2011 Virginia Tech survey in Dare County that focused on wildlife road collisions also had broadly similar data to ours. Like us, they detected a large amount of *Didelphis virginiana* and *Procyon lotor*, but we detected many more *Didelphis virginiana* than *Procyon lotor*, while their numbers were more even (Vaughan et al. 2011b). Their most-detected bird species by far was *Setophaga coronata*, which is echoed by our data (Vaughan et al. 2011b). *Clemmys guttata* and *Kinosternon subrubrum* were the most detected turtle species across both the Dare County survey and our survey; however, the 2011 survey detected more *Kinosternon subrubrum* (Vaughan et al. 2011b). This contrasts with our survey, as we detected an equal amount of both species. Interestingly, Vaughan et al. (2011b) detected 56 road-killed *Anolis carolinensis*, while we have only detected one so far. This is likely because they performed walking surveys, and *Anolis carolinensis* is difficult to detect while driving. As with Tyrrell County, and in contrast to our survey, the Dare County survey did not detect a high amount of road-killed *Liodytes pygaea* (Vaughan et al. 2011b). A snake species that they did detect in large numbers was *Nerodia sipedon* (Vaughan et al. 2011b), a species that we did not detect very often. Other species that were seen at similarly high rates across both of our surveys include *Nerodia fasciata* and *Lithobates sphenocephalus* (Vaughan et al. 2011b). In total from March

2009 to March 2011, they detected 12,906 road-killed animals across 156 species (Vaughan et al. 2011b). Although the species totals are similar (116 for our study vs 156), as with Smith (2011), Vaughn et al. 2011b) documented significantly higher numbers of roadkill per year in Dare County compared to our Dare County results (2443 animals). Again, it is unclear whether these numbers, which were driven by amphibians as in Tyrrell County, represent inter-annual variability or some sort of dramatic shift in local wildlife populations. In the case of Dare County, we suspect that the increased salinity we observed in the canals along US 64 and 264 may have something to do with reduced observations of amphibian and reptile roadkill (see Figure 7, and also the salinity trends apparent in Figure 6a-g), but it is too early to draw that conclusion at this time based on just one year of data.

*Nerodia sipedon williamengelsi* (Carolina watersnake, the distinctively-patterned local subspecies of *N. sipedon*) is an NC species of special concern, but it is a species that we have only detected one of so far in our study. This is in stark contrast to the 2011 Dare County survey, which detected 267 *Nerodia sipedon*, the most numerous snake species in their roadkill survey (Vaughan et al. 2011b). We suspect that there is a chance these snakes may have been misidentified, and were likely members of another *Nerodia* species, particularly the similar *N. fasciata*. We will follow up with the Vaughn et al. researchers to see if any photos or voucher specimens of *N. sipedon williamengelsi* were retained.

As previously stated, we have detected high numbers of *Liodytes pygaea*, an NC species of special concern. The biggest hotspot for these snakes was on US 64 in Tyrrell County near the Alligator River Marina. It is unclear after just one year of study whether the high rates of *L. pygaea* roadkill we observed are normal and/or sustainable for the local population of this species. We speculate that the construction of the new Alligator River bridge in this area may

also be a current stressor to *Liodytes pygaea*, but upon completion, the underpass along the edge of the river should help mitigate the disruption that US 64 causes. The high levels of snake and turtle mortality present in the eastern Tyrrell County portion of our study route do seem to provide strong support for the need for an extensive set of additional wildlife crossing structures in this area, in addition to crossings planned for Dare County.

**Conclusions and Future Research:**

With the goal of providing support to NCDOT's Wildlife Crossing Pilot Program grant application, Wildlands Network began conducting surveys on road-killed vertebrates on US 64 in Tyrrell and Dare counties and a stretch on US 264 in Dare County on August 1, 2024. In September 2024, North Carolina Department of Transportation (NCDOT) applied for a $25 million grant from the Wildlife Crossing Pilot Program to fund wildlife crossing structures, referencing the 1000+ vertebrate roadkill events we had already recorded in the first month of the study. In December 2024, NCDOT learned their proposal had been successful, and they were awarded the full $25 million to build a set of underpasses on one key section of US 64 near Buffalo City Rd (the epicenter of red wolf vehicle collisions, and also a hotspot for bear road mortality as well). In addition to our roadkill research results, the NCDOT proposal was also aided by the $4 million in private funds that Wildlands Network and the Center for Biological Diversity raised to provide most of the 20% non-federal match required by Federal Highways. Our project has thus already accomplished our first goal, of boosting NCDOT's chances of receiving a federal grant for crossings.

For the next steps of our study, we will be conducting the second year of the roadkill survey, effectively doubling the dataset we have collected thus far and providing a crucial

estimate of year-to-year variability that can be used to better evaluate the success of future wildlife crossings that are installed on US 64. We hope to redo our water quality measurements along the route at a finer resolution (<1 mile spacing between points) in the future. With the concern of rising sea levels and evidence of salinity encroachment in the area, such refined water quality measurements can be used to try to explain some of the emerging patterns in wildlife roadkill abundance that we are observing along our survey route.

It may be worth remapping the canals during a season when focal aquatic species are more active so that the vegetation and canal width measurements are more predictive. For some species, such as the aquatic vertebrates, these canals serve as a source or destination to cross the road. For others, the canals may be a barrier to movement, either due to being too wide for terrestrial mammals or not having the right water quality parameters for sensitive amphibians. These roadside canals clearly influence wildlife crossing events, so they warrant further investigation.

We will map the roadside guardrails on US 64 and 264 as well. They are an obstacle that keeps us from stopping for roadkill on the survey on certain stretches of the route (see Methods section above for more explanation), so it will be valuable to see how the guardrails may affect our relative detection rates for portions of the study area.

Additionally, there are likely other geographical features not visible from the road (such as lakes, open fields, timber plantations, etc.) that may harbor certain species and influence crossing events near them on the road. When we have more data accumulated, it will be worthwhile trying to map these features and relate them to the local abundance of roadkill.

After this study is over and the wildlife crossing structures along the route have been installed, we plan on conducting additional roadkill surveys to compare with the baseline data collected in this study.

The first year of our US 64/264 survey has reinforced data collected previously in Tyrrell and Dare Counties. We have identified a major hotspot of herpetofauna road collisions, as well as found temporal trends overall and for different animal types such as the black swamp snake. The data we are collecting is essential for the future construction of fencing and underpasses along US 64, to prevent further damage to wild populations, maintain habitat connectivity, and to improve the safety of this major highway.

*Clockwise from top left: bullfrog, eastern mud turtle, bobcat, black swamp snake*

**Tables and Figures:**


Figure 1. Survey checkpoints

Figure 2. Water quality measurement points

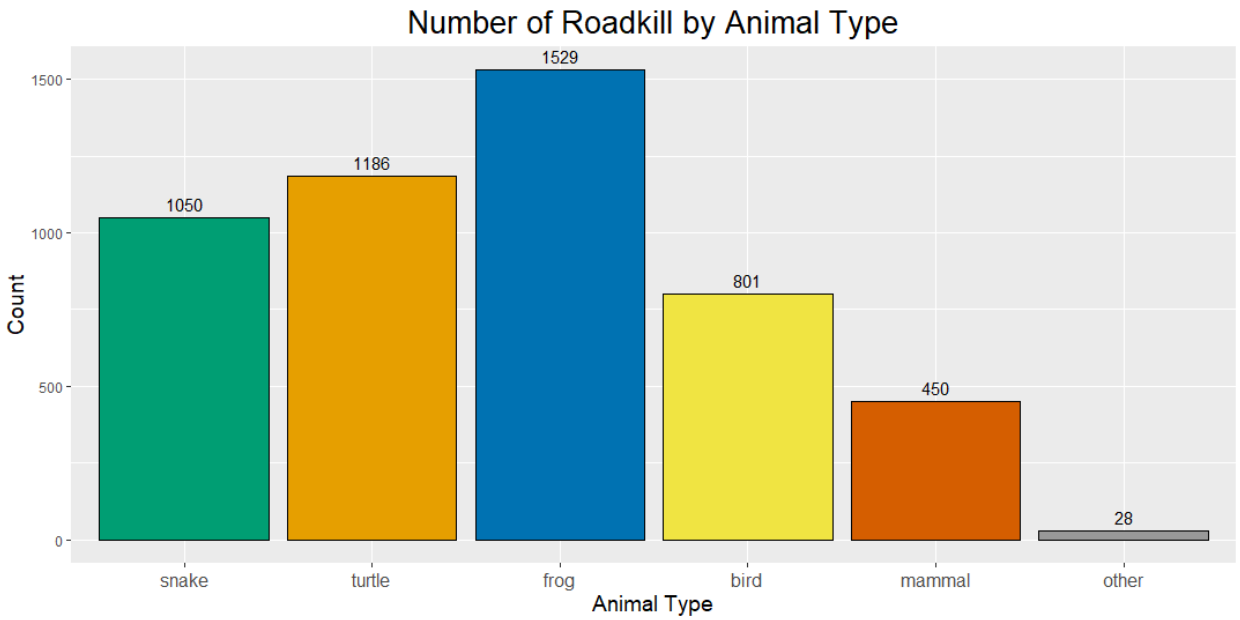


Figure 3. Roadkill counts by animal type

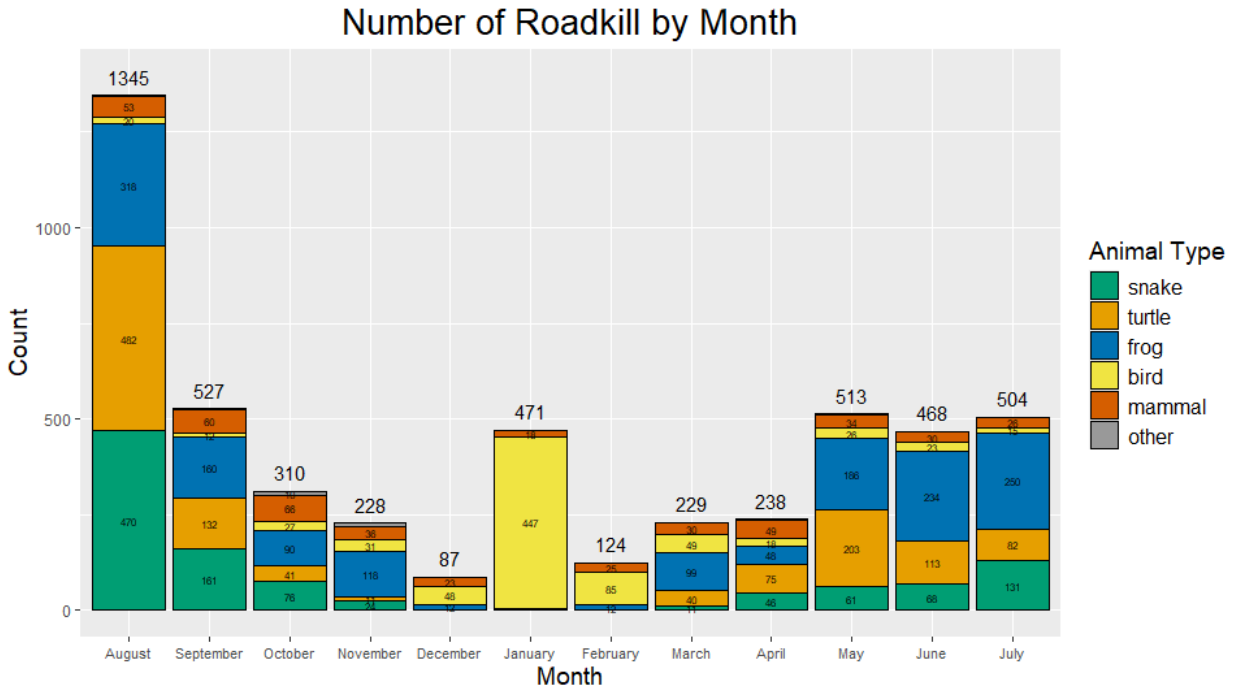


Figure 4a. Roadkill counts by month

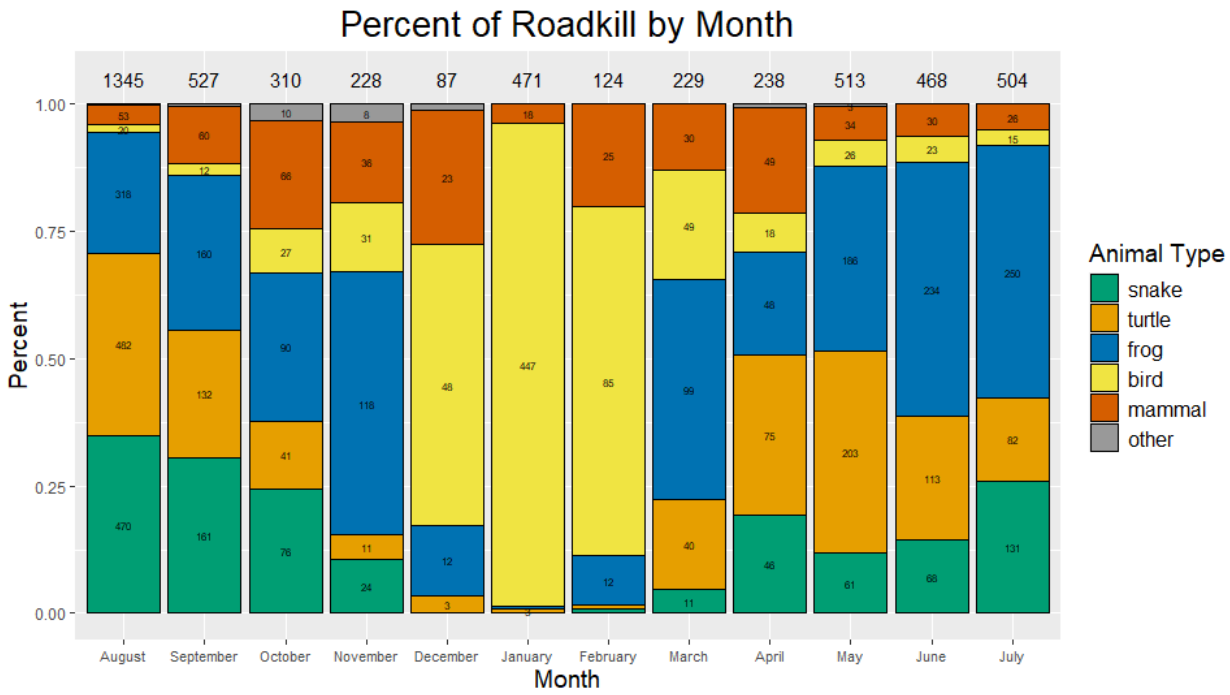


Figure 4b. Roadkill percents by month

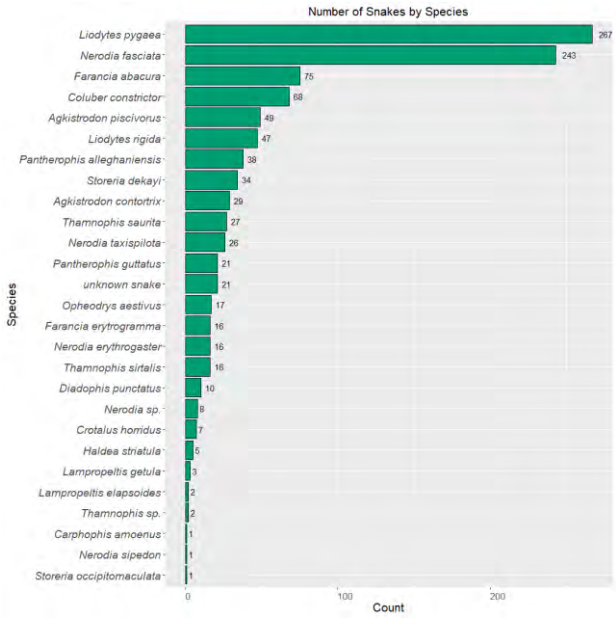


Figure 5a. Snake roadkill counts by species

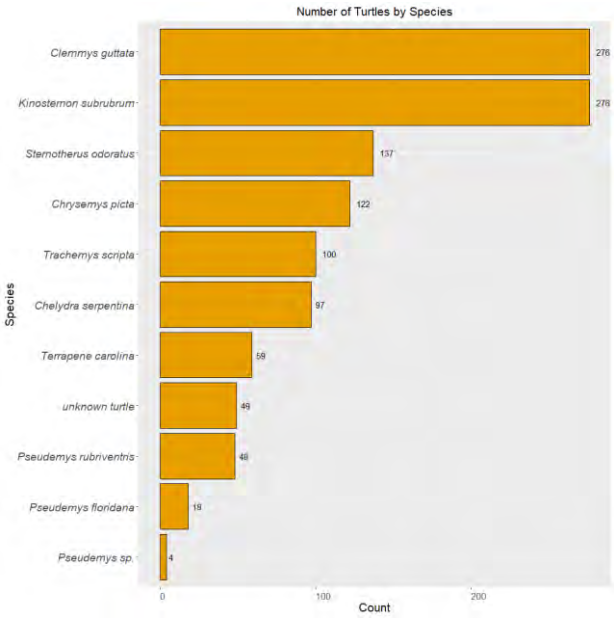


Figure 5b. Turtle roadkill counts by species

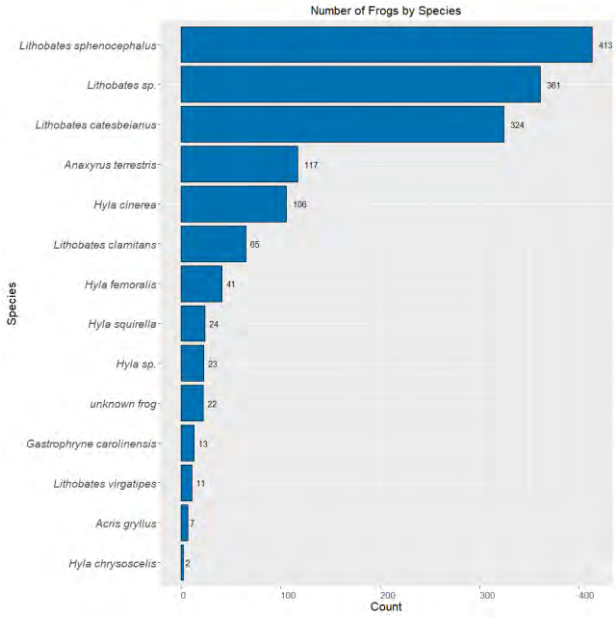


Figure 5c. Frog roadkill counts by species

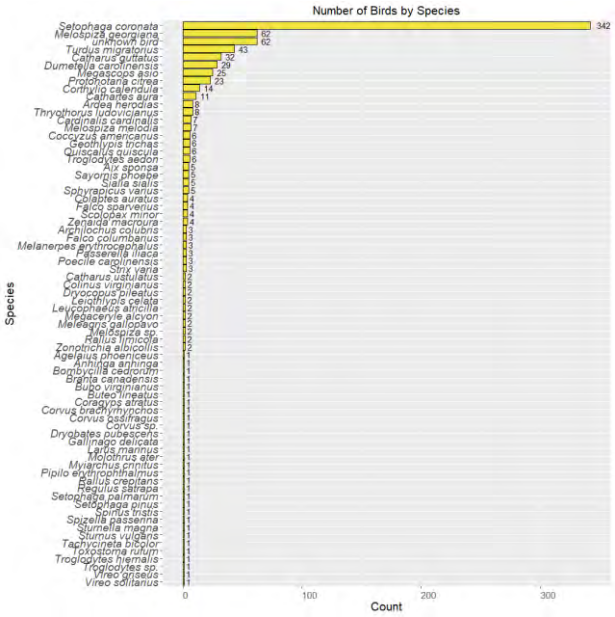


Figure 5d. Bird roadkill counts by species

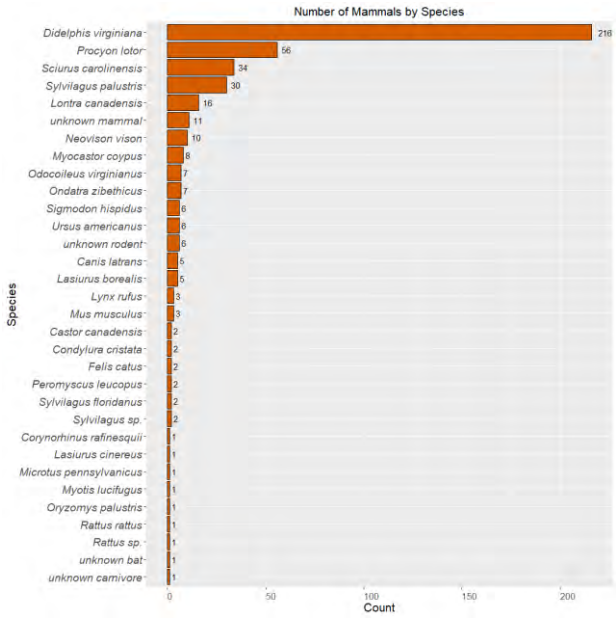


Figure 5e. Mammal roadkill counts by species

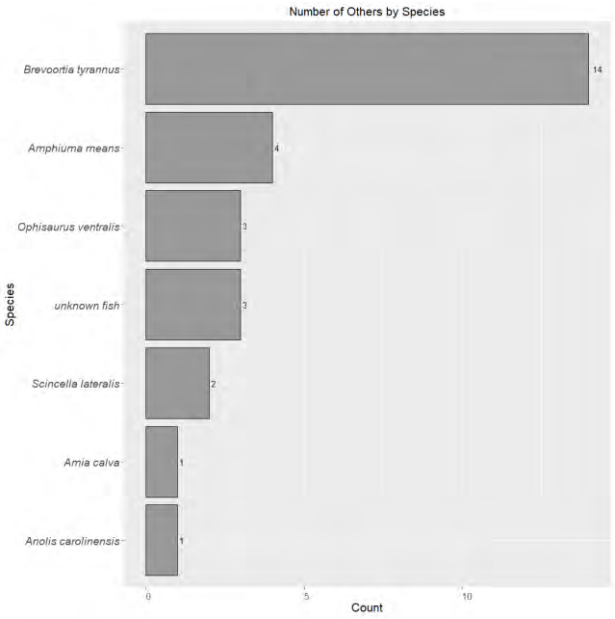


Figure 5f. Other roadkill counts by species

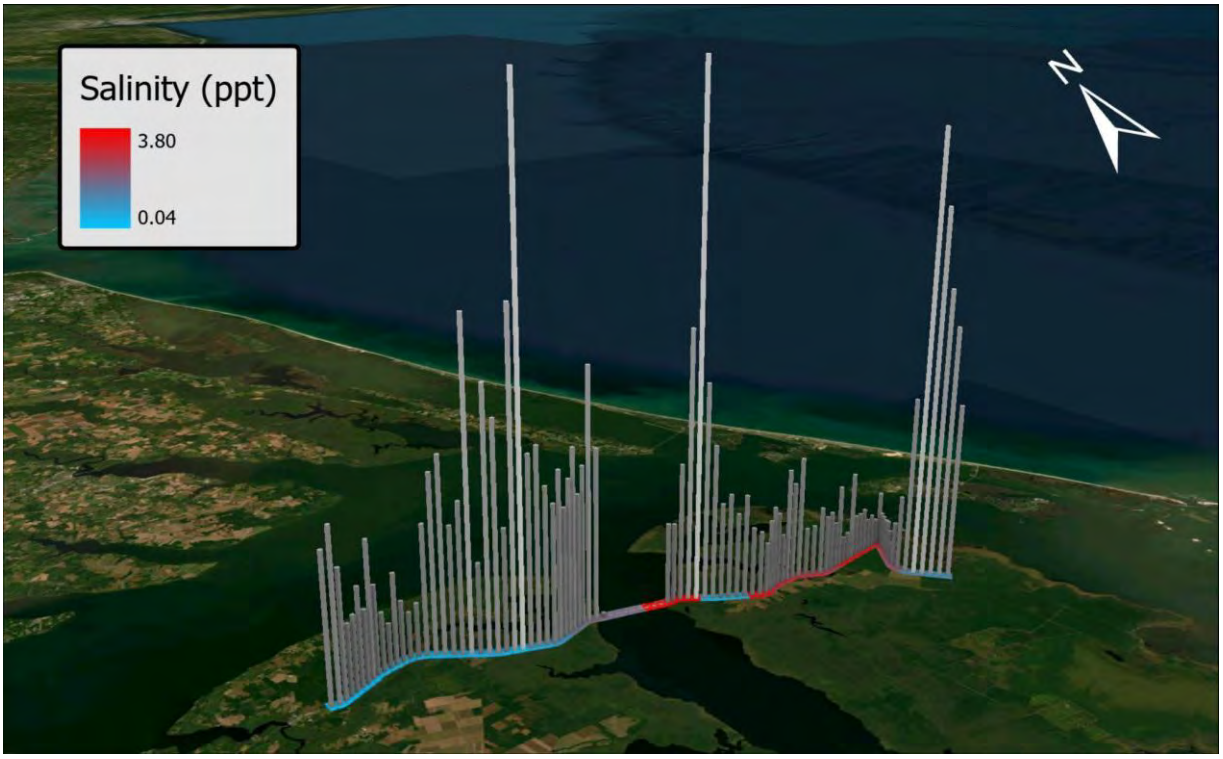


Figure 6a. Total roadkill observations across the survey route

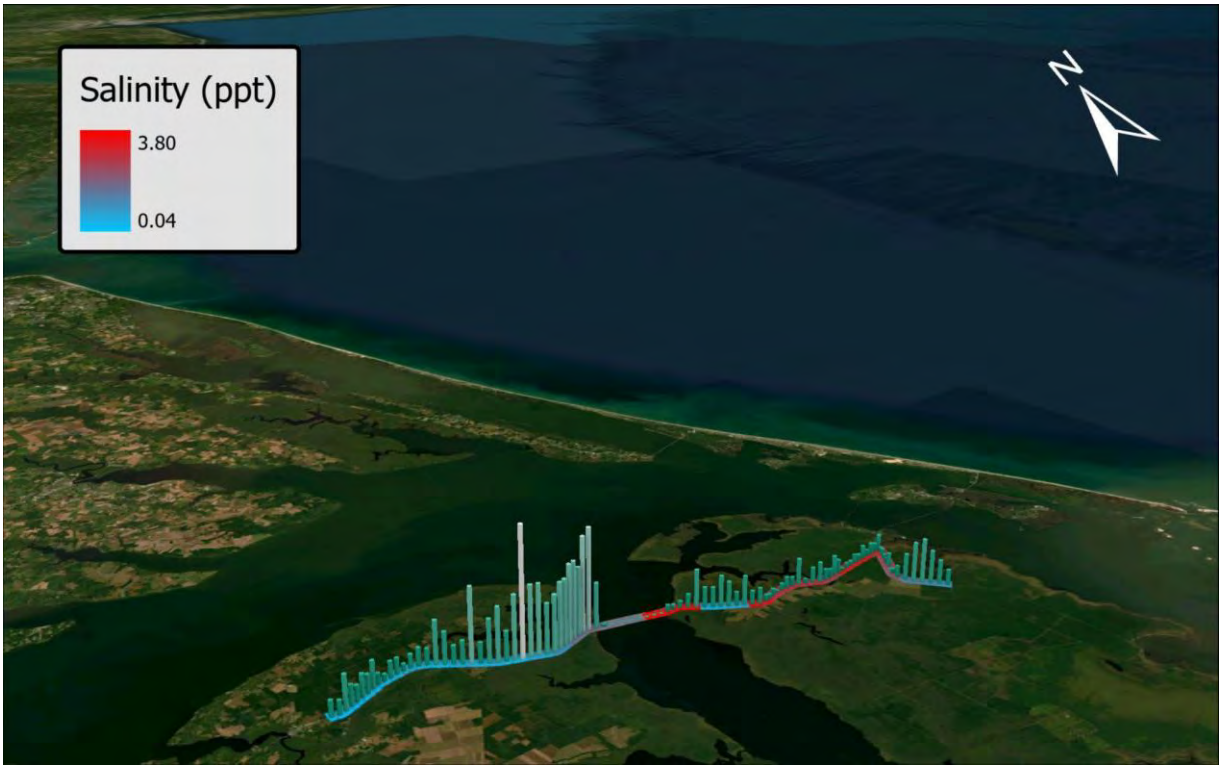


Figure 6b. Snake roadkill observations across the survey route

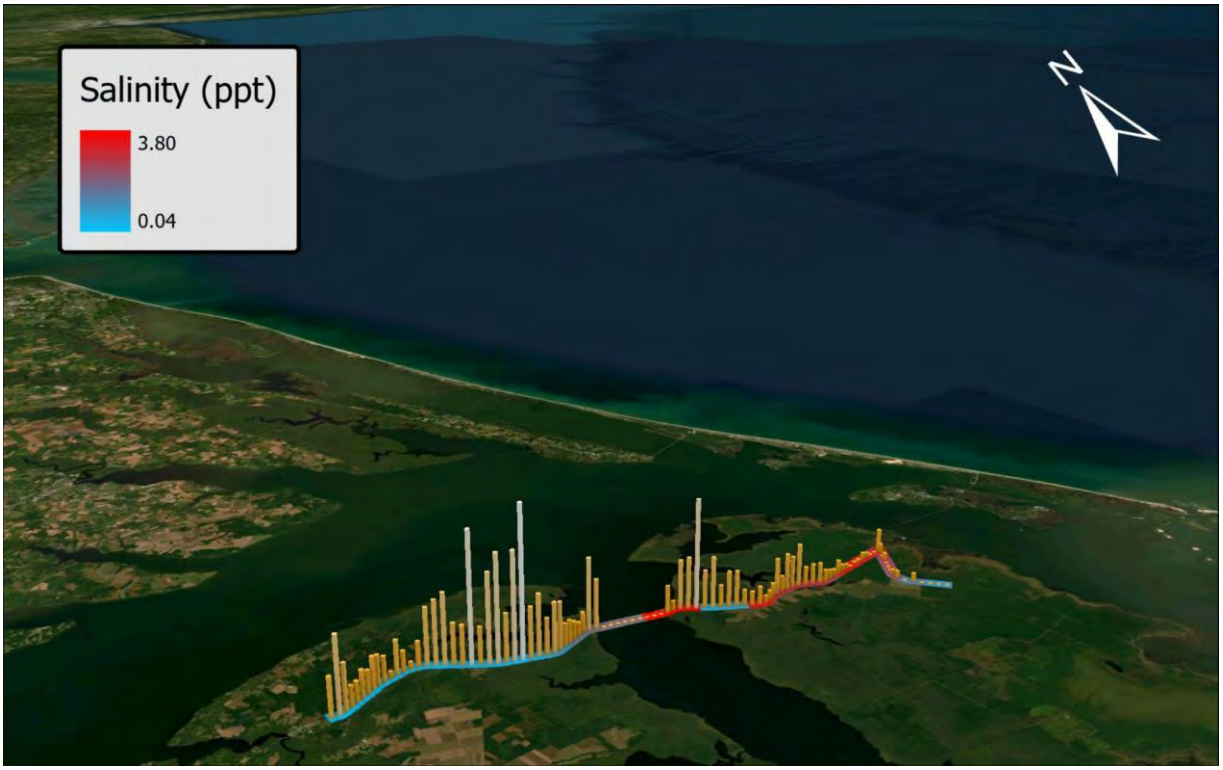


Figure 6c. Turtle roadkill observations across the survey route

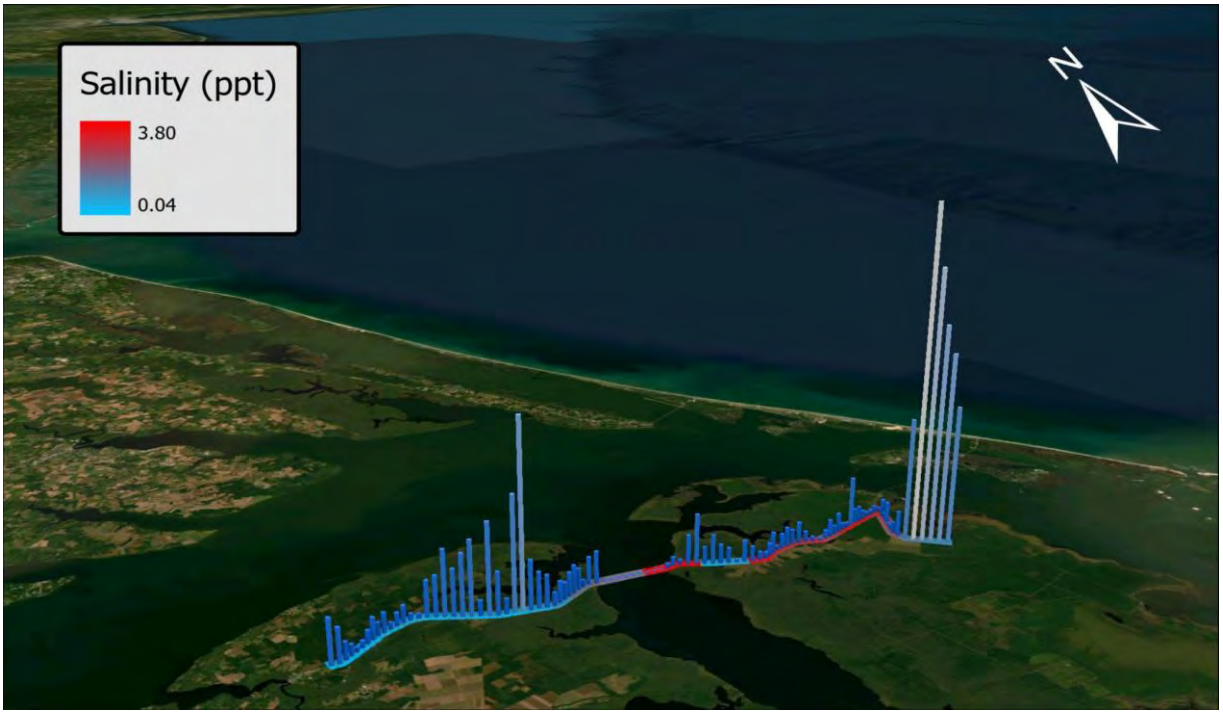


Figure 6d. Frog roadkill observations across the survey route

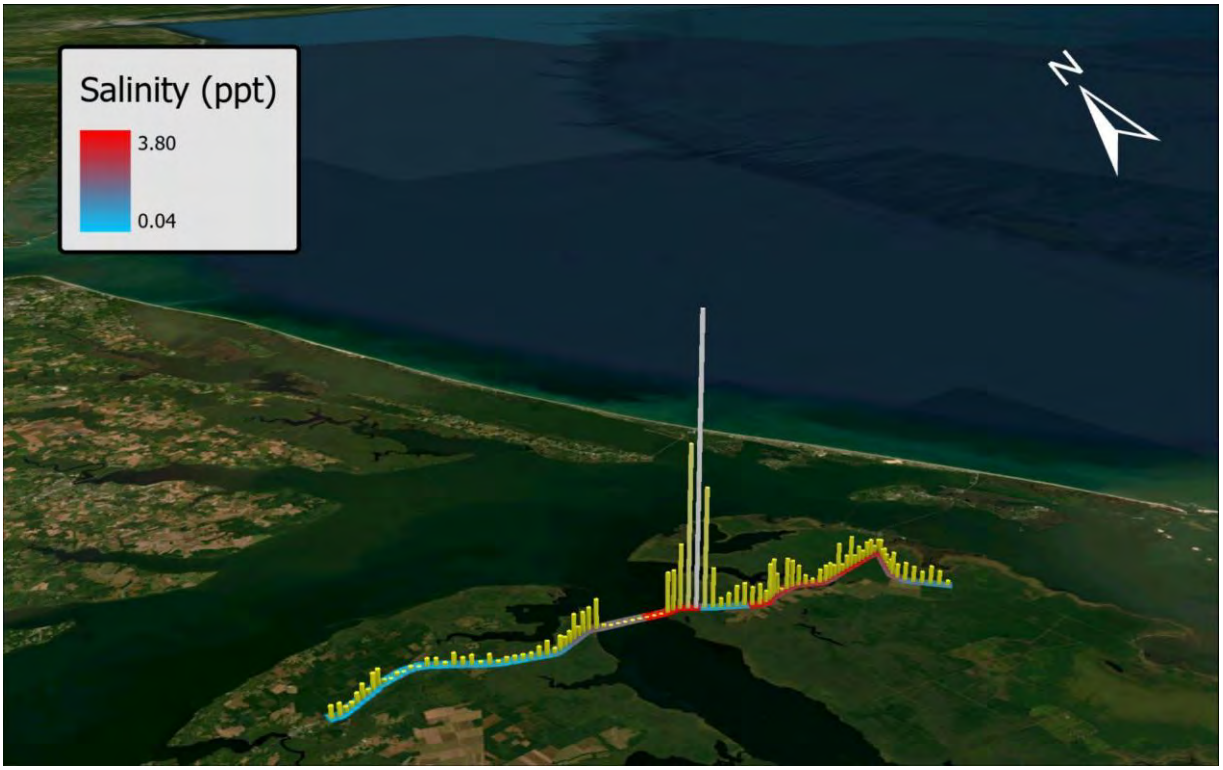


Figure 6e. Bird roadkill observations across the survey route

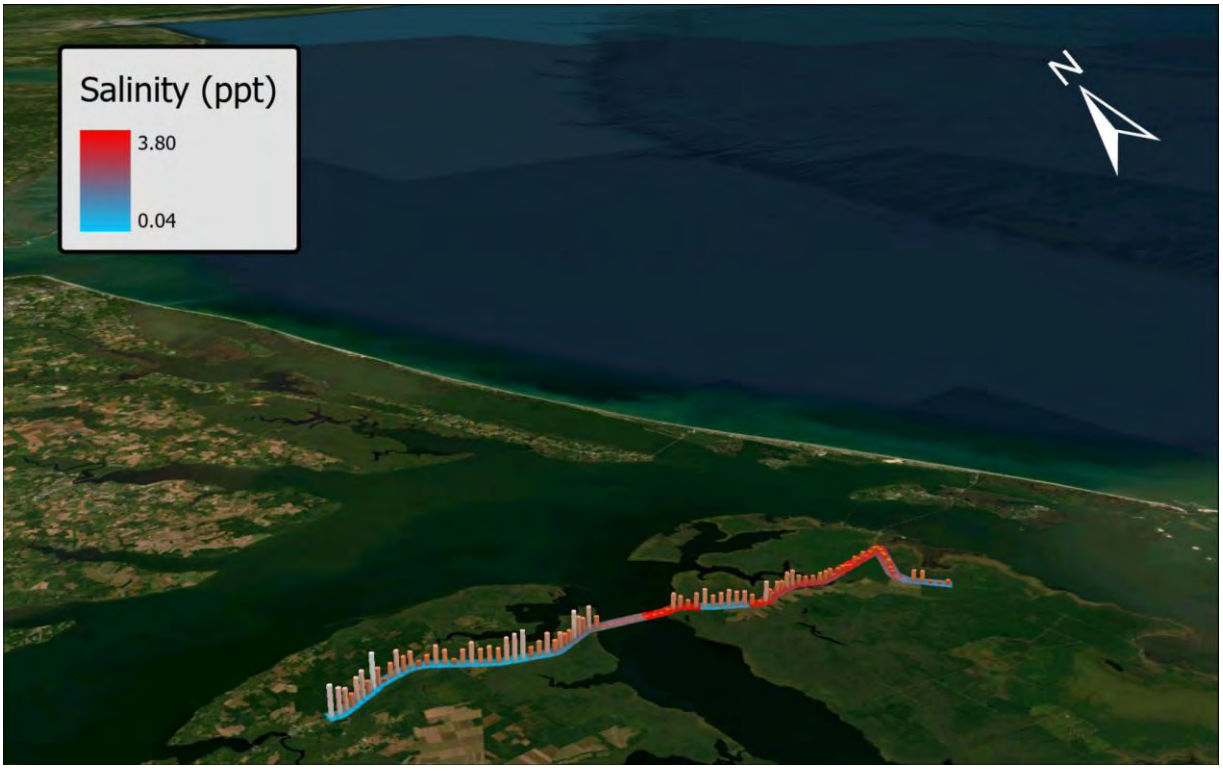


Figure 6f. Mammal roadkill observations across the survey route


Figure 6g. Other roadkill observations across the survey route

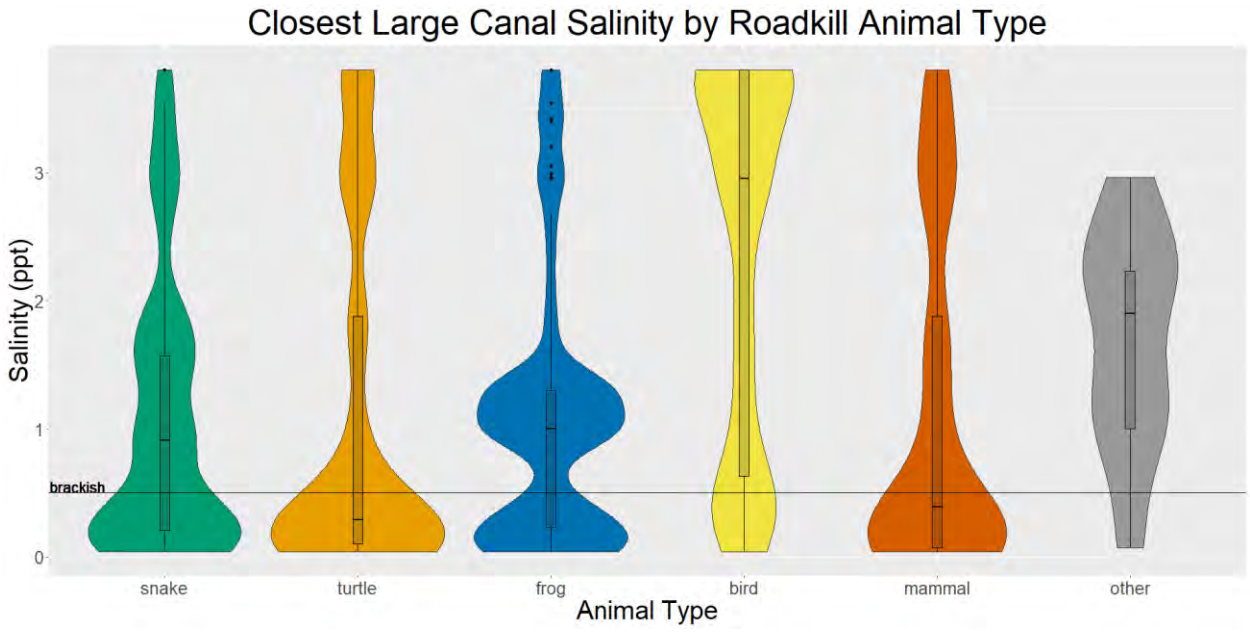


Figure 7. Violin plot and boxplot of closest large canal salinity of roadkill by animal type


Figure 8. Recorded roadkill of NC protected species

Table 1. Number of roadkill counts and species by animal type and county

| Animal Type | Total Counts | Total Species | Tyrrell Counts | Tyrrell Species | Dare Counts | Dare Species |
|---|---|---|---|---|---|---|
| Total | 5044 | 144 | 2601 | 111 | 2443 | 116 |
| Snakes | 1050 | 24 | 727 | 21 | 323 | 22 |
| Turtles | 1186 | 9 | 806 | 9 | 380 | 9 |
| Frogs | 1529 | 11 | 624 | 11 | 905 | 10 |
| Birds | 801 | 68 | 151 | 43 | 650 | 54 |
| Mammals | 450 | 26 | 287 | 24 | 163 | 17 |
| Others | 28 | 6 | 6 | 3 | 22 | 4 |

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