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Research Article

Road mortality mitigation for red wolves requires a landscape approach

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In 1980, the red wolf *Canis rufus* was declared extinct in the wild. However, starting in 1987, a captive breeding program allowed for a reintroduction effort in and around Alligator River National Wildlife Refuge (Alligator River NWR), North Carolina, USA. In 2012 the wild red wolf population peaked at about 120 individuals. After no new individuals were released from 2010 through 2019, a decline set in, with only an estimated 17–20 individuals remaining in 2021. We explored temporal and spatial patterns in red wolf mortality to formulate recommendations for reducing road mortality. Of all reported mortalities between 1987 and 2022, gunshot (25.90%) and vehicle strike (19.32%) were the most frequently reported known causes of death. For the red wolf population to become independent of intensive management, both sources of unnatural mortality need to be addressed. Mitigating US Highway 64 through Alligator River NWR for road mortality is an immediate priority for red wolf conservation as it would help protect the last remaining wild breeding pair and their offspring (situation 2022–2023). It would also address 24.36% of the overall historic red wolf road mortality in the area. In addition, mitigating this road section is relatively efficient as this is where the highest number of red wolf road mortalities can be addressed for the shortest mitigated road length. However, to help recover the population in the wider area, low volume roads also need to be mitigated as most of the historic road mortalities (92.31%) occurred on road sections, including certain sections of US Highway 64, with only 101–5000 vehicles per day. In addition, many of the historic red wolf birth location concentrations are close to low volume roads. Therefore, this study illustrates that effective conservation for the red wolf requires a landscape approach to mitigation that includes both high and low volume roads.

Keywords: habitat connectivity, population size, population viability, road ecology, road mortality, wildlife crossing structures, wildlife fences

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Introduction

The red wolf *Canis rufus* is listed as 'Endangered' under the Endangered Species Act (USFWS 2025). Before settlement by Europeans, the red wolf was common throughout the eastern and south-central United States. However, intentional killing and habitat loss caused a severe population decline. By 1972, the red wolf only remained present in a coastal area near the Texas–Louisiana border (USFWS 2018). Between 1973 and 1980, the remaining red wolves were captured and screened for hybridization with coyotes *Canis latrans*. In 1980, the species was declared extinct in the wild.

After successful captive breeding efforts and trial releases (USFWS 2022), a mainland reintroduction effort to establish an Eastern North Carolina Red Wolf Population (ENC RWP) was initiated in and around Alligator River NWR in North Carolina (USFWS 2018). The red wolves in this refuge and surrounding areas are categorized as a Nonessential Experimental Population (NEP). Over 60 adults were released between 1987 and 1994 and by the mid-1990s wild red wolves had established territories, operated in packs, and reared young successfully. Breeding pairs reared their natural-born young as well as foster pups that were born in captivity and placed in dens by USFWS personnel. Red wolf packs almost exclusively consist of a breeding pair and their natural-born young of that year (or foster pups) and those from prior years. Red wolves do not typically adopt older individuals as pack members if they were born to another pack (Joe Madison, NC Project Manager, Red Wolf Recovery Program, pers. comm.). Once a breeding male or female dies, this results in a disruption of reproduction, and a replacement mate may not be readily available at low population density (Hinton et al. 2017a; Joe Madison, NC Project Manager, Red Wolf Recovery Program, pers. comm.).

In 2012 the reintroduced red wolf population peaked and was estimated at about 120 individuals (USFWS 2025). After 2012 a decline set in, with only 17–20 individuals estimated in the wild in 2021 (USFWS 2025). The most important causes for this decline were not releasing new individuals between 2010 through 2019, combined with high human-caused mortality. Without substantial intervention (i.e. the release of new individuals and the management of introgression with coyotes), a red wolf population with three breeding pairs and a total population size of 44 individuals was estimated to disappear within eight years (USFWS 2018). Illegal killing of red wolves through gunshots and vehicle collisions amounted to 29.3 and 24.8% of the known causes of death, respectively (Hinton et al. 2017b). The risk for a marked adult red wolf to die because of gunshots or a vehicle collision was estimated at 51.1–63.5 and 15.2–21.4%, respectively (Agan et al. 2021a). Other threats include poisoning, private trapping, hybridization with coyotes, management activities, and stochastic events due to small population size (Roth et al. 2008, Hinton et al. 2017b, USFWS 2018). The release efforts were restarted in 2020 and have continued since (USFWS 2025). Since 2021, the estimated wild population size increased slightly to 20–23 individuals at the end of 2023, and 28–31 individuals in August 2025 (USFWS 2025). The captive population of red wolves was 280 individuals in August 2025 (USFWS 2025).

Since road mortality is one of the main causes of unnatural deaths for wild red wolves, particularly for captive-born red wolves that have been released into the wild, there is great interest in exploring measures that reduce road mortality (Joe Madison, NC Project Manager, Red Wolf Recovery Program, pers. comm.). We explored temporal and spatial patterns in wild red wolf mortality, especially road mortality, for the ENC RWP. We then used the results to formulate recommendations for reducing the impacts of roads and traffic on red wolves.

While contributing to the conservation of the wild population of the red wolf was our main concern, our study also aimed to illustrate the consequences of biases in some road ecology practices. In North America, most road ecology mitigation measures are primarily aimed at improving human safety through reducing collisions with common large wild mammals (e.g. white-tailed deer *Odocoileus virginianus*) along relatively busy highways (Clevenger et al. 2001, Ford et al. 2011, Gagnon et al. 2015). However, if the biological conservation of a specific species is the main objective, we should probably consider implementing mitigation measures in the landscape where the species occurs, rather than only along our busiest highways that may be of greatest concern to human safety (Frair et al. 2008, Matos et al. 2012, Serieys et al. 2021). The red wolf is a suitable species to explore this concept with, as it is an endangered species with a very limited range in the wild, and detailed records are kept on almost every wild red wolf. This means that animals that are killed by vehicles are likely to be recorded anywhere, regardless of the road type, the traffic volume, or who is responsible for road maintenance and carcass removal.

While we did not have access to movement data or successful road crossings by red wolves, red wolf road mortalities indicate unsuccessful crossings and a barrier, at least for the individuals that were killed in the collision. We do know that gray wolves *Canis lupus* tend to avoid busier highways (Musiani et al. 2010, De Feudis et al. 2025). The prevailing theory is that road mortality for wildlife increases with growing traffic volume, but only up to a certain point (Seiler 2003). When traffic volume gets high enough, animals increasingly choose to no longer cross the highway. As fewer animals attempt to cross, road mortality declines. Note that there are profound differences between species regarding what traffic volume levels affect their willingness to approach and cross a highway (Blanco et al. 2005, Waller and Servheen 2005, Coe et al. 2015, Huijser and Bell 2025). We explored how traffic volume along different roads and road sections in the area is associated with red wolf road mortality. Combined with the road length that falls within a certain traffic volume category, it allowed us to identify road sections with a relatively high concentration of red wolf road mortality, and where the implementation of mitigation measures may be most efficient in terms of reducing most road mortality for the shortest road length mitigated.

We not only explored temporal and spatial patterns in red wolf road mortality, but we also identified road sections that are in the vicinity of breeding pairs and their offspring. If the breeding male or female is killed when the juveniles still depend on the parents, it puts the entire litter in jeopardy. Furthermore, if juveniles do not at least survive until they disperse (on average at around 15 months of age; Karlin and Chadwick 2012), they do not contribute to improving the viability or expanding the occupied range of the population. Of course, the survival of the red wolves is also important after the juveniles have dispersed. However, dispersing juveniles can disperse over long distances (e.g. multiple dozens of kilometers; Karlin and Chadwick 2012) and are at risk along just about any road or road section in a very large area. On the other hand, the breeding male and female are likely to stay in their home range centered around the den. Therefore, reducing the likelihood of road mortality near birth locations is also likely to contribute to the long-term survival of the breeding pair, allowing them to produce additional litters in later years.

Material and methods

Study area

The ENC RWP area includes federal, state, and private lands in Beaufort, Dare, Hyde, Tyrrell, and Washington Counties, on the Albemarle Peninsula of North Carolina (Fig. 1). The main highways include US Highway 64, US Highway 264, Highway 94, and Highway 45 (Fig. 1). We also named selected low volume roads on the map because of their proximity to red wolf road mortality locations or red wolf birth locations.

Mortality causes

We analyzed red wolf mortality data, provided by USFWS, in the ENC RWP area. The oldest mortality record in the data was from 29 April 1987, and the most recent mortality record was from 27 June 2022. The data include records of all red wolves known to have died, their cause of death, including 'unknown cause', and coordinates for where the death occurred. Almost every red wolf in the wild population is

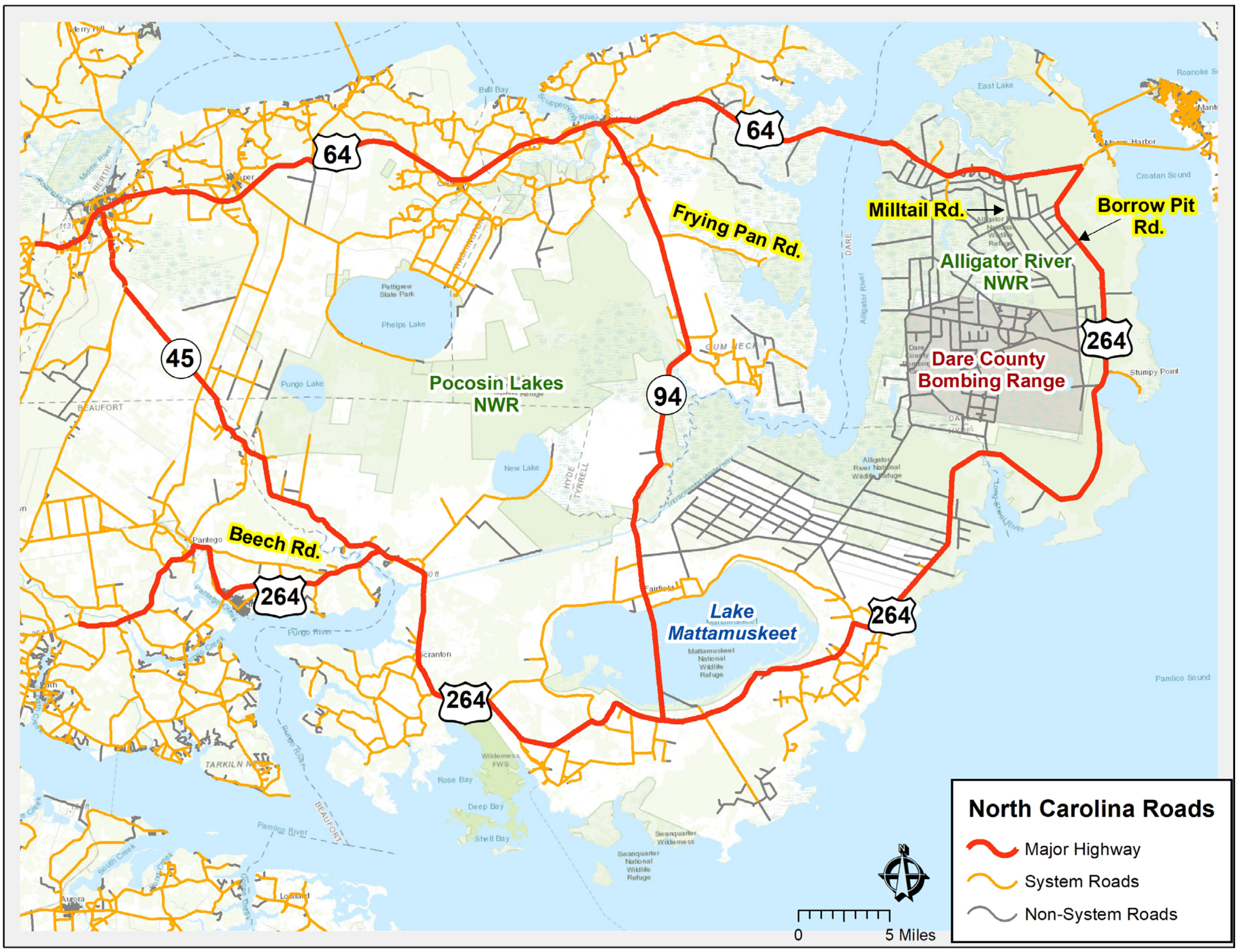

Figure 1. The study area on the Albemarle Peninsula of North Carolina, USA, with wildlife refuges, the main highways, and the most important minor roads and other areas referred to in this paper.

known and kept records for, including whether it is still alive or where and when and how it died (e.g. based on GPS collars or direct observations by USFWS personnel).

There were two parameters in the data file that related to the cause of mortality: 1) cause of mortality as assessed in the field; and 2) cause of mortality based on a formal necropsy. However, not all carcasses were sent to the lab for formal necropsy. We combined these two parameters into one mortality parameter according to the following protocol: if there was a result of a formal necropsy, then we used this cause of mortality. If there was no result of a formal necropsy listed, then we used the cause of mortality as assessed in the field. The new mortality parameter was analyzed for number of red wolves by cause of mortality, mortality numbers by month, and mortality in relation to population size. We summarized the mortality numbers for different types of human-caused mortalities and natural causes of mortality. For the most common mortality causes, we investigated potential differences in mortality numbers over the months of a year through a χ^2 test. To investigate the number of mortalities from different causes corrected for population size, we first summarized the red wolf population size of the ENC RWP area at the end of each calendar year, estimated by USFWS (2025). For some years there was not a single number but a range (minimum–maximum) for the estimated population size. When only a range was available, the average of the minimum and maximum population size estimate was used. We used an analysis of covariance to investigate the effect of the most common mortality types (factor variable) on the mortality numbers, corrected for the calendar year and the population size (both covariates).

Road mortality hotspots

There were 91 reported red wolf–vehicle collisions in total between 29 April 1987 and 27 June 2022. We plotted all road mortality records with coordinates (89 out of the 91 records) to identify the road sections that had the highest road mortality concentrations of red wolf–vehicle collisions, regardless of what type of road or what road surface. To identify the road mortality hotspots, we conducted a kernel density analysis using ArcGIS 10.6.1 (ESRI 2018) for point features of red wolf–vehicle collision locations using a 25 m cell size. The 25 m cell size is relatively fine scale but still accommodates for some spatial inaccuracies in GPS coordinates. The kernel density analysis calculates the density of road mortality in a neighborhood around each cell and is based on the quartic kernel function described by Silverman (1986). We set the neighborhood search radius at 1000 m. On a straight road this means that red wolf road mortalities that are up to 1000 m away are included in the density analysis for each cell. To help interpret the results of the kernel density analyses and identify hotspots, we displayed the raster output using a heat map classification with varying densities of red wolf road mortalities. We used percentage breaks to create five categories (< 5, 5–< 25, 25–< 50, 50–< 75, and 75–100%) that display the areas with the highest densities of red wolf road mortality (< 5) to areas with the lowest densities (75–100%).

Traffic volume in relation to road mortality

Data on traffic volume (average annual daily traffic, AADT) were obtained for the main roads in the ENC RWP area (NCDOT 2023). Most of the traffic volume data were from 2021, but for some minor roads the most recent data were from earlier years (the oldest data were from 2012) (NCDOT 2023). All paved roads maintained by the North Carolina Department of Transportation (NCDOT) (i.e. 'system roads') that were closest to recorded road mortalities of the red wolf and closest to the historic range of breeding pairs in the ENC RWP area were selected for more extensive analyses into where red wolf road mortalities occur in relation to traffic volume, regardless of when these mortalities occurred or when breeding pairs were present since the reintroduction started. A caveat for this analysis is that the red wolves were never homogeneously distributed across the area and there were also substantial fluctuations in population size. Thus, not every road section included in the analysis had equal exposure to red wolves. However, the lifespan of mitigation measures is several decades up to many decades (lifespan for wildlife fences is 25 years, for crossing structures 75 years) (Huijser et al. 2009). Therefore, it is important to use an extensive dataset over a long period (here 1987–2022) to project the possible presence and abundance of road mortality well into the future, rather than only base the mitigation recommendations on what the current situation is. Of course, this is only valid if there have been no major changes in land use in the meantime. The study area includes large areas that are part of multiple wildlife refuges, and there is also a bombing range. The land use in these areas has been stable for many decades. The towns and villages across most of the study area are very small and do not appear to have grown much over the last decades, if they have grown at all. Most development in the wider region has been further to the west along US Highway 64 and further to the east along the Atlantic coast.

Two of the 91 road mortality records did not have coordinates, and 11 road mortalities were on non-system roads, such as gravel or dirt roads. This left 78 recorded red wolf road mortalities that had coordinates associated with system roads. For the selected system roads, each road section was divided into 0.1 mile (160.9 m) road segments and each road segment was linked to its AADT. The number of red wolf road mortalities on the system roads was tallied for different traffic volume categories. In addition, the number of 0.1 mile road segments for each traffic volume category were tallied, differentiating between road segments that had recorded red wolf road mortalities, and road segments that did not have recorded red wolf road mortalities. This allowed for the calculation of the proportion of road-killed red wolves for each traffic volume category compared to what would be expected if traffic volume has no impact on red wolf road mortality.

Birth locations in relation to roads

Highway sections that bisect or are near home ranges of breeding pairs are of high importance for mitigation. For the red wolf population to recover, the survival of breeding individuals and their offspring is essential. For this analysis, we investigated where the highest concentrations of birth locations were for red wolves between 28 April 1988 and 8 April 2016. There were 707 reported birth locations of red wolves in total (data provided by USFWS). As almost every red wolf and red wolf breeding pair in the wild population is known (e.g. based on GPS collars or direct observations by USFWS personnel), the breeding sites and the offspring of breeding pairs were also meticulously recorded by USFWS personnel. Similar to the hotspot analysis for road mortality hotspots, we conducted a kernel density analysis to generate a hotspot map for birth locations. Since there was a substantial decline in the red wolf population between 2014 and 2022, we can expect most of the reported birth location hotspots to be historic rather than current. Nonetheless, birth location hotspots indicate habitat quality where dispersing red wolves were able to find mates, establish territories, and breed successfully.

To examine which system road sections were closest to birth locations, the selected road sections were divided into 0.1 mile (160.9 m) road segments. Each birth location was projected on the closest 0.1 mile road segment of a system road. Three search distances were used for birth locations, regardless of the side of the road the birth location was on: 1) up to 500 m, 2) up to 1000 m, and 3) up to 1500 m. All three distances are well within the average radius of the home range of red wolf (4.7 km for resident red wolves) (Hinton et al. 2016). Birth locations within shorter search distances indicate a greater danger of traffic to breeding red wolves and their offspring. The number of birth locations projected on each 0.1 mile road segment was tallied. The 0.1 mile road segments with the highest numbers of birth locations close to the road can be considered of great concern to breeding red wolves and their offspring, regardless of whether red wolf road mortality has occurred there in the past. The maps can also be interpreted beyond the 0.1 mile road segments. If different 0.1 mile road segments close together have a high number of projected birth locations in total, then these road sections may represent a similar danger to breeding red wolves and their offspring.

Results

Mortality causes

Between 29 April 1987 and 27 June 2022, the total number of reported and selected red wolf mortalities in the wild was 471. Human-caused mortalities (61.15%) were far more frequently reported than natural mortalities (16.14%) (Table 1). Unknown causes of mortality, including cases where it was unclear whether the cause of mortality was human-caused or natural, amounted to 22.72%. Gunshot (25.90%) and vehicle strike (19.32%) were by far the most frequently reported known causes of death (Table 1).

Excluding 1987 and 2022, which were not full years in the mortality database, the average percentage of gunshot mortalities per year out of the total reported mortalities was 23.96% (SD = 21.42). The average percentage of vehicle strikes per year out of the total reported mortalities was 22.67% (SD = 17.31).

Table 1. The frequency of different mortality causes for red wolves (1987–2022). If a mortality cause amounted to less than 5% of the total mortalities, it was grouped with other mortality causes.

Mortality cause	Mortalities (n)	Mortalities (%)
Human-caused		
Gunshot	122	25.90
Vehicle	91	19.32
Management	33	7.01
Other (private trap, poison, foul play)	42	8.92
Subtotal human-caused	288	61.15
Natural		
Intraspecific	25	5.31
Other (health, natural, drowned, fire)	51	10.83
Subtotal natural	76	16.14
Unknown		
Unknown	94	19.96
Other (euthanized, injuries)	13	2.76
Subtotal unknown	107	22.72
Total	471	100.00

Mortality by month

Excluding 1987 and 2022, which were not full years in the mortality database, the frequency of gunshot mortalities was significantly different between the months of the year (χ^2(df = 11, n = 120) = 55.74, p < 0.001). Gunshot mortalities were highest from October through December (Fig. 2). There was no significant difference between months of the year because of vehicle strikes (χ^2(df = 11, n = 90) = 6.23, p = 0.857) or the combined other causes of mortality (χ^2(df=11, n = 252) = 6.25, p = 0.856).

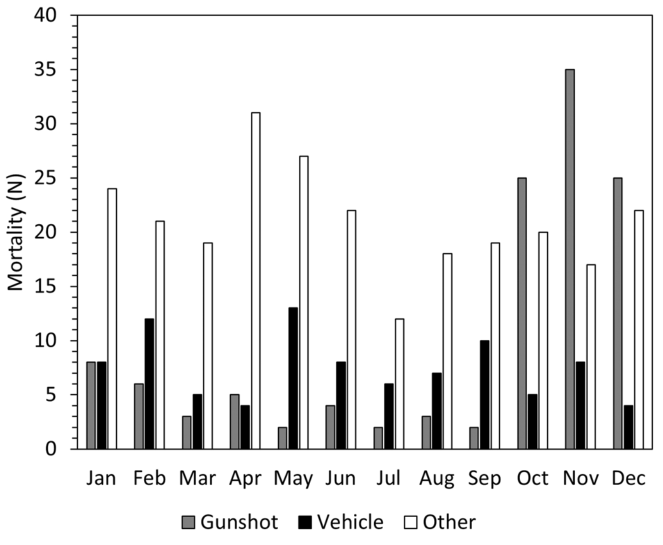

Figure 2. Red wolf mortality in the wild population (1988 through 2021, n_{total} = 462) by month because of gunshots, vehicle strikes, and other causes.

Mortality in relation to population size

In general, the red wolf population size increased between 1987 and 2000, remained relatively stable between 2001 and 2014 (between 81 and 110 individuals at the end of each calendar year), and severely declined afterwards (Fig. 3). While the population size was very low between 2019 and 2022, it seemed to stabilize around 18–20 individuals (Fig. 3). Red wolf mortality numbers were significantly different between the different causes of mortality (gunshot (on average 3.39 per year) versus vehicle strike (on average 2.53 per year) versus other (on average 7.17 per year)) ($F(2,108) = 31.69$, $p < 0.001$) when corrected for the population size ($F(1,108) = 58.89$, $p < 0.001$) (analysis of covariance). The calendar year did not significantly impact red wolf mortality numbers ($F(1,108) = 0.10$, $p = 0.757$) (analysis of covariance). Excluding 1987 and 2022, which were not full years in the mortality database, the average percentage of gunshot mortalities per year out of the estimated population size was 4.95% (SD = 3.58). The average annual percentage of vehicle strikes out of the estimated population size was 5.38% (SD = 5.21).

Road mortality hotspots

While collisions with red wolves are widespread along the major highways as well as smaller roads throughout the ENC RWP area, there are six road sections that have a concentration of collisions in the two highest density categories

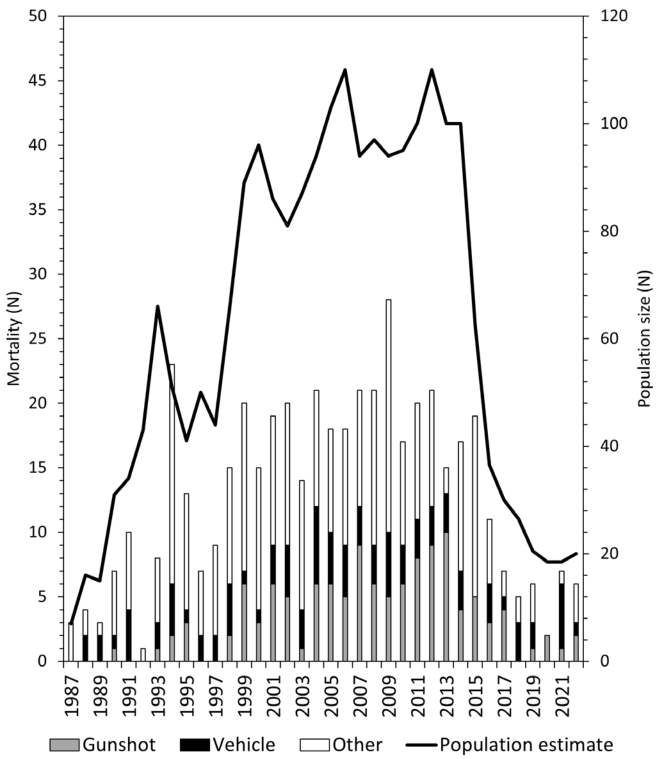

Figure 3. Annual mortality because of gunshots, vehicle strikes, and other causes between 1987 and 2022 (bars in the graph) and the estimated red wolf population size at the end of each year (line in the graph).

(orange and red) (Fig. 4): 1) US Highway 64 through and adjacent to Alligator River NWR (specifically around Lake Neighborhood Rd/River Rd–Buffalo City Rd, around Milltail Rd, just west of the junction with US Highway 264); 2) US Highway 264 around Long Shoal River/Stomper Rd; 3) US Highway 264 north of Engelhard; 4) Highway 94 Columbia–Northwest Fork Alligator River and Frying Pan Rd; 5) Highway 94 north of Fairfield; and 6) Beech Ridge Rd north of Belhaven.

Traffic volume in relation to road mortality

US Highway 64 has the highest traffic volume of the roads in the area, ranging from around 4000 vehicles per day near the coast, up to 8700 vehicles per day towards the west end of the area (Supporting information). Other highways and highway sections in the area had much lower traffic volume, ranging from a few hundred vehicles per day up to about 1000 or 2000 vehicles per day. Red wolf road mortality hotspots occurred along both the busiest highway (US Highway 64, especially through Alligator River NWR) as well as along roads with about 1000 or even just a few hundred vehicles per day. The traffic volume category 101–500 vehicles per day had more red wolf road mortalities (n = 26, 33.33% of all reported road mortality on system roads) than the other traffic volume categories (Fig. 5). Overall, most of the red wolf road mortalities (n = 72, 92.31% of all reported road mortality on system roads) occurred on roads with between 101 and 5000 vehicles per day (Fig. 5).

Proportionally, when considering the length of road segments that fall in the different traffic volume categories, roads with 2501–5000 vehicles per day had the highest observed red wolf road mortality (Fig. 6). There was a factor of 2.34 more red wolf road mortalities for road sections in this traffic volume category than would have been expected if traffic volume did not influence road mortality. This means that implementing mitigation measures along roads with a traffic volume of 2501–5000 vehicles per day would be most efficient with the highest number of red wolf road mortalities addressed for the shortest mitigated road length. Traffic volume categories with a traffic volume between 0 and 2500 vehicles per day had red wolf road mortalities that were roughly proportional to their road length (Fig. 6). The lowest and highest traffic volume categories had proportionally very few road mortalities of red wolves, and this is where mitigation measures would be most inefficient, with the fewest red wolf road mortalities addressed for the road length mitigated.

Birth locations

Road sections that are near high concentration birth locations include: 1) US Highway 64 near Milltail Rd just west of the junction with US Highway 264; 2) (a) US Highway 264 north of Link Rd/Borrow Pit Rd, (b) north of Engelhard, (c) near New Holland and south of Lake Mattamuskeet, and (d) east of Belhaven; 3) Highway 94 north of Frying Pan Rd/ south of Frying Pan Rd, and 4) around Kilkenny (Fig. 7). Note that there are other road sections along lower volume paved roads that are also close to high concentrations of birth

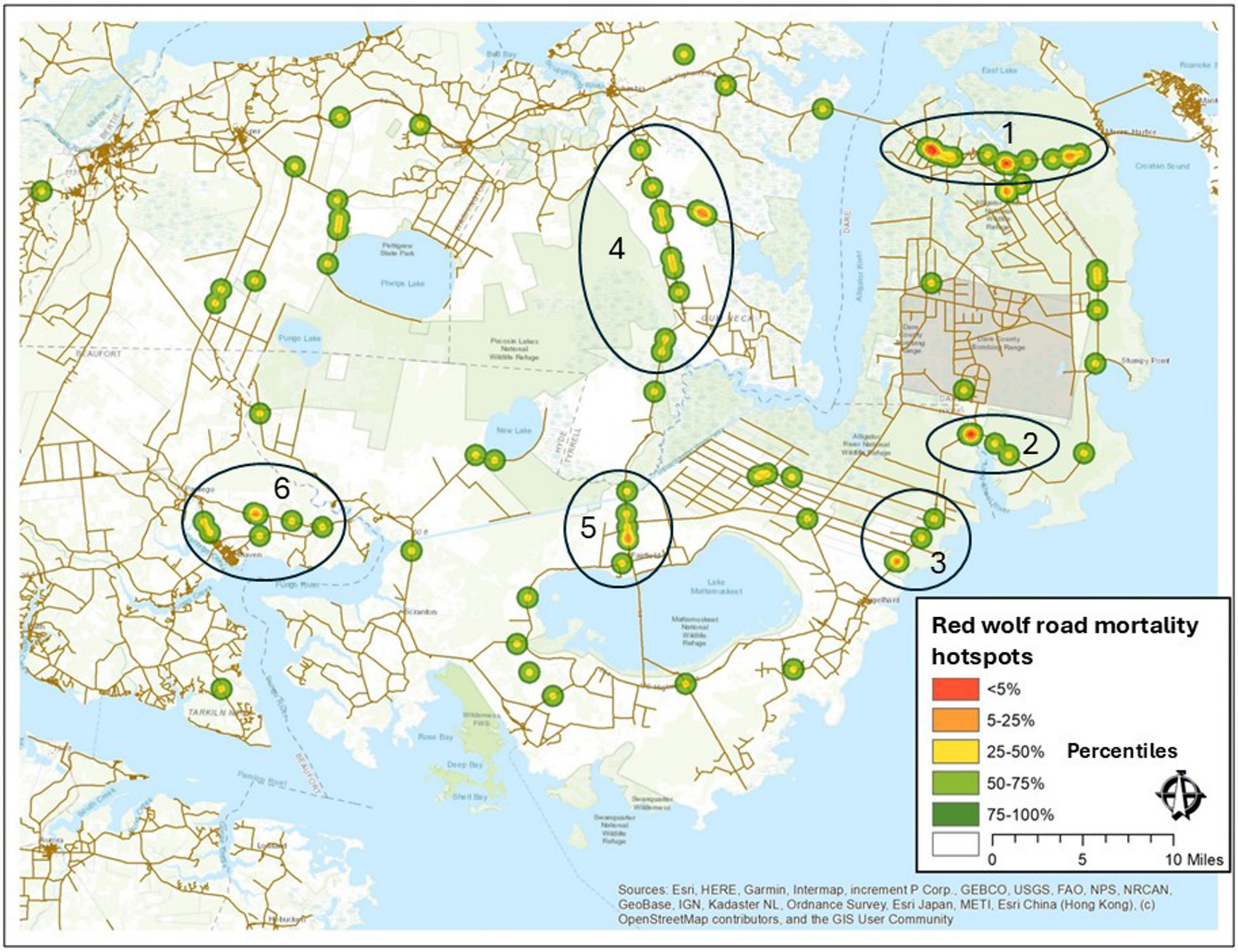

Figure 4. Kernel density hotspot percentiles red wolf–vehicle collisions (1987–2022). The six numbers correspond with the descriptions in the main text.

locations. Examples are: 5) Frying Pan Rd; 6) Highway 45 north of Pungo River; 7) Beech Ridge Rd; 8) Piney Woods Rd along the west shore of Lake Mattamuskeet; and 9) North Lake Rd along north shore of Lake Mattamuskeet (Fig. 7). There are two large contiguous areas without major highways, separated by Highway 94, with the highest number of birth location hotspots that can form the source for colonizing or recolonizing other areas that may be smaller and more isolated: 1) Pocosin Lakes NWR and surroundings (seven birth location hotspots west of Highway 94); and 2) Alligator River NWR and surroundings (six birth location hotspots east of Highway 94) (Fig. 7). Therefore, providing safe crossing opportunities along Highway 94 would connect the greatest number of birth location hotspots between the two sides of a highway in the study area.

The 0.1 mile road segments with the highest numbers of birth locations close to the road (Fig. 8, Table 2) can be considered of great concern to breeding red wolves and their offspring, regardless of whether red wolf road mortality has occurred there in the past. While US Highway 64 around Milltail Rd is close to many birth locations, lower volume roads such as Frying Pan Rd and Beech Ridge Rd present a similar threat to historic breeding pairs and their offspring (Fig. 8, Table 2).

Discussion

The importance of addressing road mortality

Of all reported red wolf mortalities, gunshot and vehicle strike were the most frequently reported causes of death. The average percentage of gunshot mortalities and vehicle strikes per year out of the total reported mortality was very similar. For these two mortality causes, the average annual percentages out of the estimated population size were also very similar. There was no significant seasonal pattern for mortality because of vehicle strikes, but gunshot mortalities were most numerous from October through December which coincides with deer hunting season (black powder and gun) (consistent with Agan et al. 2021a, North Carolina Wildlife Resources Commission, 2026). Both causes of mortality are unnatural and have contributed to the severe population decline in

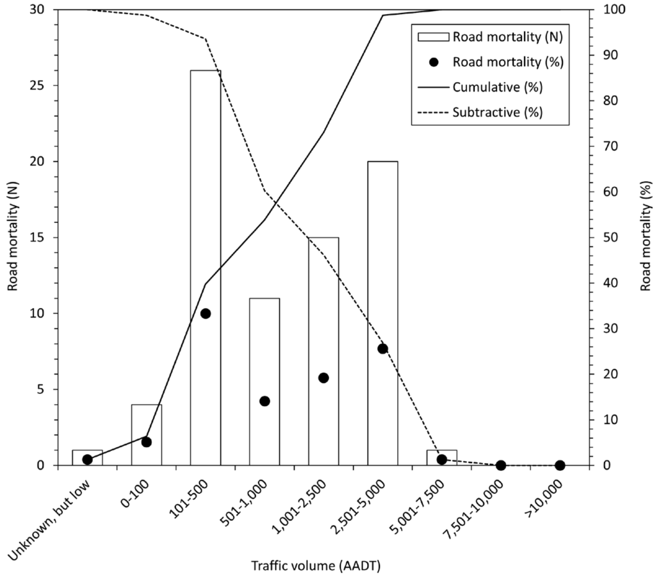

Figure 5. Road mortality counts of red wolves (bars, left y-axis) for the different traffic volume (average annual daily traffic, AADT) categories and the percentage of the total road mortalities on system roads that these counts represent (lines, right y-axis). The two lines illustrate what percentage of mortalities would be addressed if mitigation measures would start with either the highest (subtractive) or lowest (cumulative) volume roads.

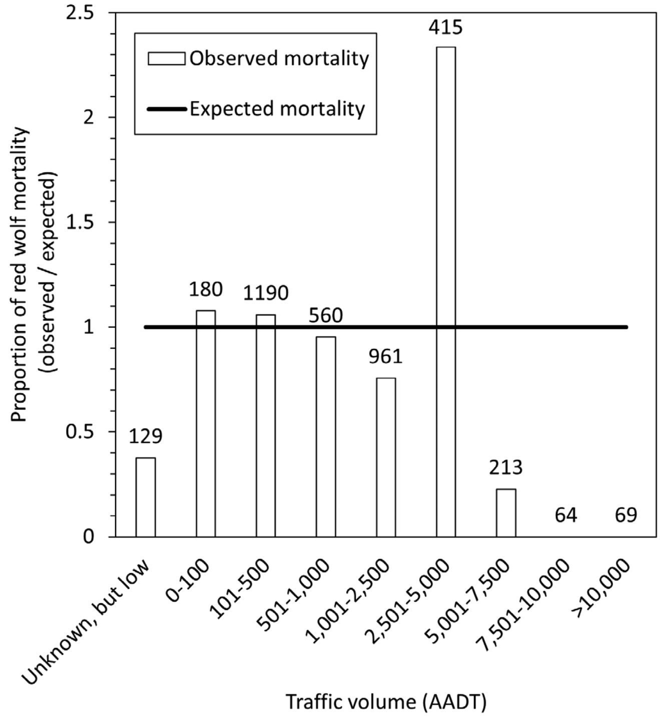

Figure 6. The proportion of observed road mortality of red wolves for each traffic volume category compared to what would be expected if red wolves were hit proportionally among the different traffic volume categories. The total number of 0.1 mile road segments for each traffic volume category, regardless of whether red wolf road mortality was observed in the segments, is shown above the bars. This number is equivalent to the sample size for each traffic volume category.

absence of continuing new releases of red wolves from 2010 through 2019 (USFWS 2023). Together with other causes of mortality this has resulted in a critically low population size and a high risk of extirpation, at least until new releases were reinitiated in 2020. Without new releases, the illegal killing and road mortality have proven too high for the population to remain stable (Hinton et al. 2017b). This suggests that initiatives that substantially reduce both illegal killing and road mortality are essential for the red wolf population to eventually become independent of intensive management. As these mortality causes still only make up just under half of the known mortality, a substantial reduction in illegal killing and road mortality does not necessarily guarantee a self-sustaining population. Nonetheless, a substantial reduction in illegal killing and road mortality is a prerequisite to achieving a more viable red wolf population.

> Reducing mortality by 50%, specifically vehicle strikes and gunshot mortality, and increasing coyote sterilization rate to 10% of the intact coyote population and removal of 5% of coyotes are actions that will be most effective in facilitating an increase in the red wolf population and minimizing coyote hybridization and introgression (USFWS 2023).

Substantially reducing illegal killing of wildlife requires changes in human values, social norms, culture, knowledge, policy, and law enforcement (Serenari and Taub 2019, Agan et al. 2021b, Santiago-Ávila et al. 2022, Toth and Rubino 2024, Ghasemi et al. 2026). These types of societal changes are difficult to accomplish, and they may require a long time, potentially spanning multiple human generations (Treves and Karanth 2003, George et al. 2016, Newsom et al. 2025). Compared to bringing about societal changes, it is relatively simple and fast to plan, design, fund, and construct mitigation measures that can substantially reduce road mortality and that also provide safe crossing opportunities. Whether a substantial reduction in road mortality in absence of a substantial reduction in illegal killing is sufficient for the population to become stable and increase in size is not certain. Therefore, we recommended modeling population size development for a set of scenarios in which road mortality and release numbers are varied, but gunshot mortality is kept at a similar rate. Regardless, if the goal is to eventually achieve a self-sustaining population of red wolves, it is imperative to work towards a substantial reduction in all sources of unnatural mortality. In the meantime, a continuation of new releases is also likely needed to help the population stabilize, increase in size, expand its range, and ensure a sufficiently diverse genetic base.

Where to reduce road mortality first and why?

With critically low population size and ongoing road mortality among the remaining individuals, road mortality should be addressed first along US Highway 64 (especially east of Milltail Rd) and the northern section of US Highway 264

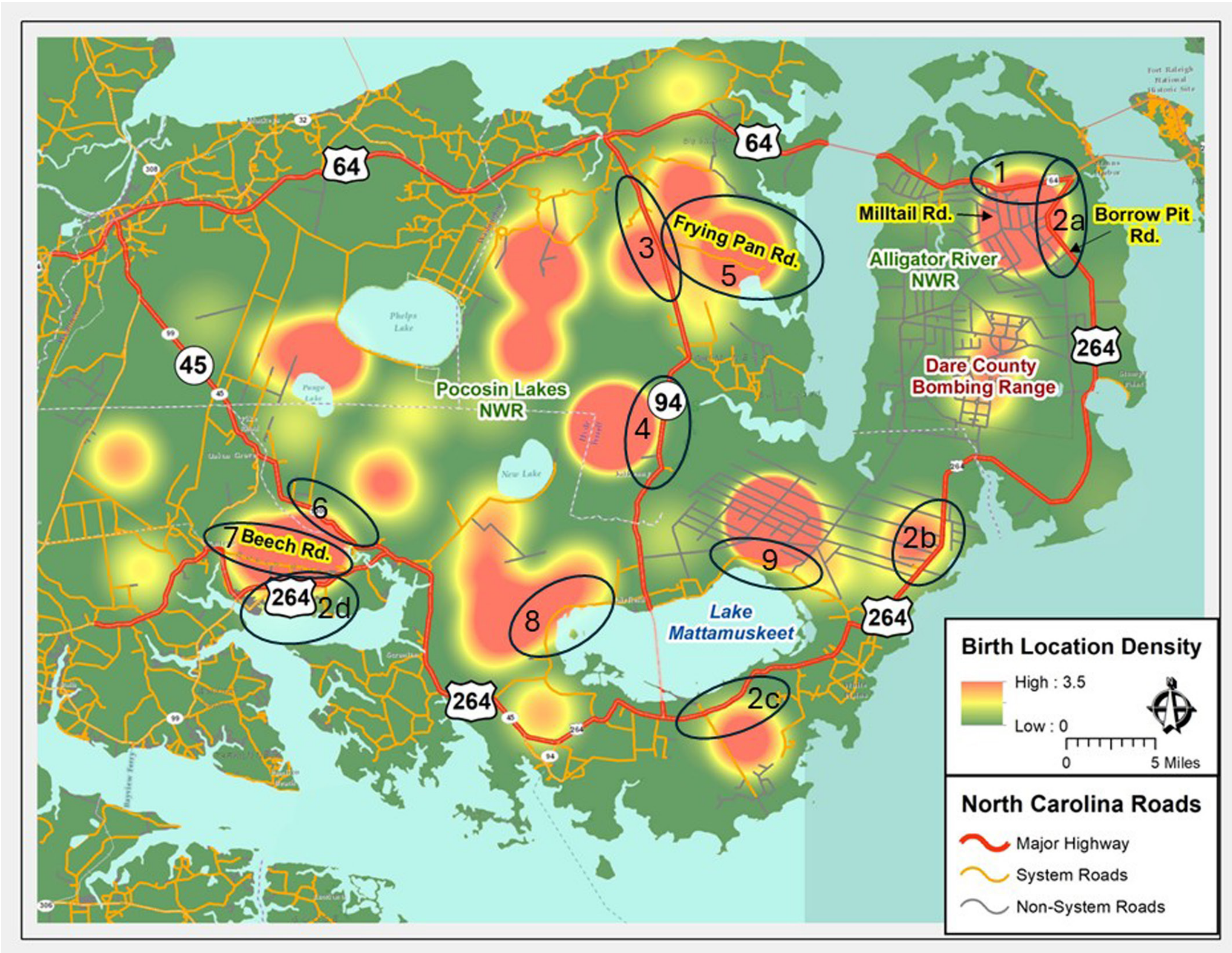

Figure 7. Kernel density hotspot birth locations for red wolves (1987–2022), and the two main areas without major highways (Pocosin Lakes NWR and surroundings and Alligator River NWR and surroundings) with hotspots of red wolf birth locations separated by Highway 94.

(especially the landfill area north of Borrow Pit Rd) through Alligator River NWR. This is the area where the last remaining breeding pair in the wild (i.e. outside of an acclimation pen, i.e. the Milltail family group) has their home range (Situation 2022–2023) (USFWS 2023, 2025). Over the years both adults and juveniles from this family group have been killed on these two highway sections. In addition, both road sections are near concentrations of historic and current birth locations. A substantial reduction in road mortality along these two highway sections is likely to have an immediate conservation benefit for this family group and thus for the entire remaining red wolf population in the area.

While short-term action should be directed at protecting the remaining breeding pair and their offspring, a more strategic long-term approach to reducing red wolf road mortality should be based on locations of historic road mortality and historic concentrations of birth locations close to a road in the wider region. In addition to US Highway 64 through Alligator River NWR, sections of US Highway 264 and Highway 94, Frying Pan Rd, Beech Ridge Rd, and roads just north and west of Lake Mattamuskeet would also be among the priority road sections.

The type of mitigation measures

The most effective and robust measure that can substantially reduce road mortality of large mammals is wildlife fencing. Wildlife fences that are designed for the target species can keep large mammals off the road and reduce road mortality by at least 80% (Huijser et al. 2016, Rytwinski et al. 2016). While wildlife fences along roads can reduce road mortality substantially, they also make a road corridor into a near absolute barrier for any species that cannot cross the fence. Therefore, wildlife fences are typically combined with wildlife crossing structures (i.e. underpasses and overpasses) (Clevenger and Huijser 2011). Wildlife fences can also help guide animals to the crossing structures, thereby increasing their use (Gagnon et al. 2015).

If appropriately designed, a large mammal fence in the ENC RWP area can limit road access for red wolf, black bear *Ursus americanus*, white-tailed deer *Odocoileus virginianus*,

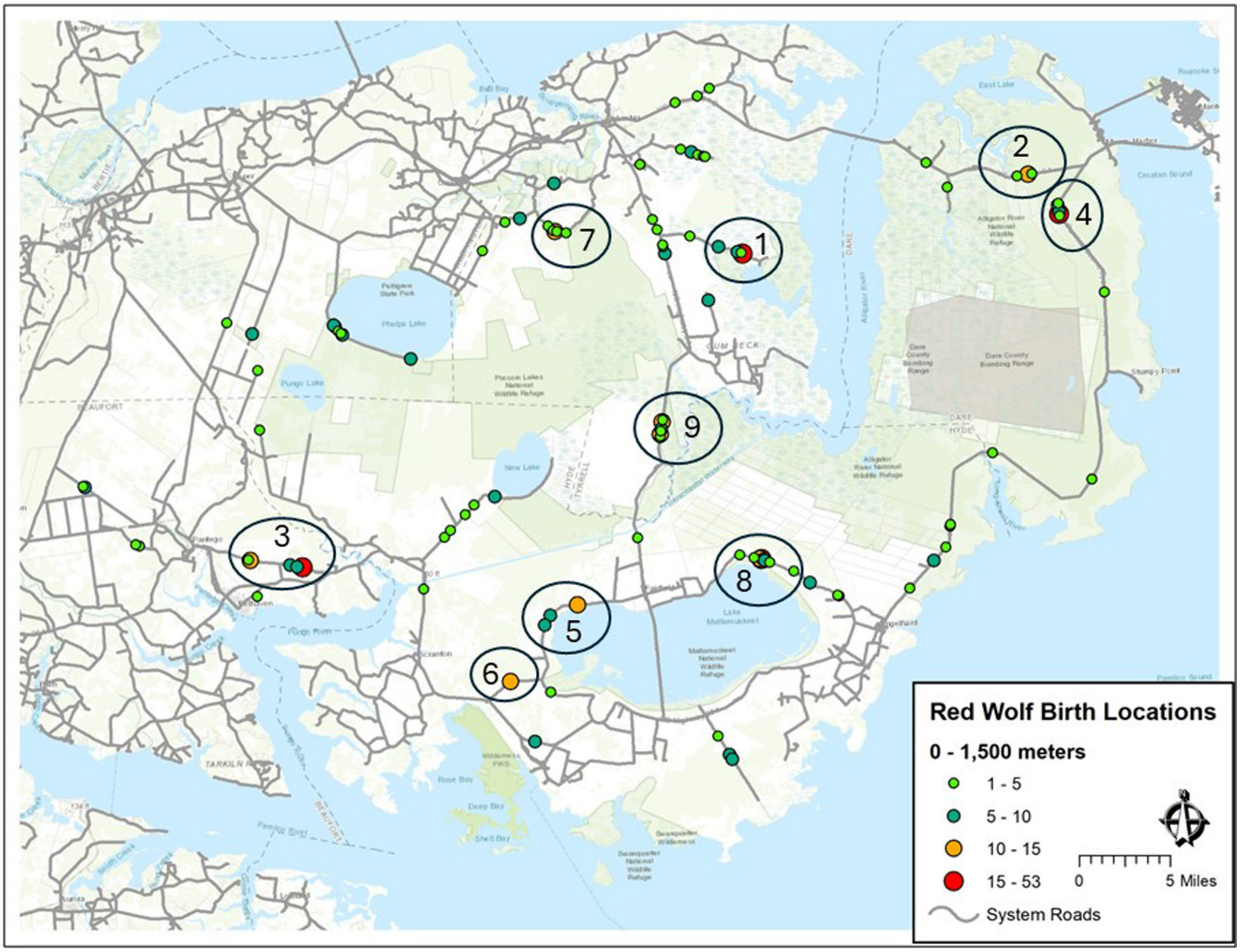

Figure 8. The number of red wolf birth locations projected to the nearest 0.1 mile system road segment, up to 1500 m, regardless of which side of the road. The numbered ovals indicate road sections that had at least 10 red wolf birth locations projected on them (Table 2). Maps for birth locations projected to the nearest road segment up to 500 and 1000 m are included in the Supporting information.

coyote *Canis latrans*, and potentially also cougar *Puma concolor*. Specific fence characteristics for this diversity of species include: 3 m high fence, metal fence posts, chain-link fence material, a dig barrier or apron buried into the soil, an overhang on top of the fence that is directed to the habitat side, potentially electrified wires attached to the main fence on the habitat side of the fence, and a high-tensile wire on top in forested areas (adjust or reconsider in combination with an overhang) (Huijser and Begley 2023).

Assuming red wolves behave similarly to gray wolves and considering the other large mammal species that are present in the area, crossing structures that are most likely suitable include open span bridges (minimum 36 m wide, 3–4 m high) and wildlife overpasses (50–70 m wide) (Foster and

Table 2. Road sections with at least one 0.1 mile road segment that had at least 10 red wolf birth locations projected on it. The order of the locations in the table corresponds to the numbered ovals in Fig. 8.

Location description	≤ 500 m	≤ 1000 m	≤ 1500 m
Frying Pan Rd	X	X	X
US Highway 64, Milltail Rd area	X	X	X
Beech Ridge Rd	X	X	X
US Highway 264, landfill area		X	X
SR 1305, Piney Woods Rd, NW of Lake Mattamuskeet		X	X
SR 1304, Turnpike Rd, W of Lake Mattamuskeet			X
SR 1105, Newlands Rd, SW of Columbia			X
SR 1311, N. Lake Rd, NE of Lake Mattamuskeet			X
Highway 94, N of junction with Kilkenny Rd			X

Humphrey 1995, Clevenger and Huijser 2011, Huijser and Begley 2023). A very gradual approach to an underpass and overpass (10–15% slope at a maximum) is recommended. This may be especially relevant in open and flat landscapes compared to landscapes with lots of cover and topography. Gradual approaches may impact natural vegetation, requiring on-site restoration both to recover the native vegetation and to provide continuous habitat for target species. The structure itself will likely have a lifespan of 75–80 years (Huijser et al. 2009). If structures are replaced, additional disturbance typically affects only the soil and vegetation on top of an overpass or at an underpass rather than on the approaches. Many road sections in the ENC RWP area are adjacent to canals, creating a need to extend crossing structures across canals, similar to some of the structures for the Florida panther *Puma concolor coryi* in south Florida.

The spatial scale of the mitigation measures

A fenced road section should at least cover the entire length of a collision hotspot and include adjacent buffer zones. For large mammals in general, especially large ungulates, this means that the fenced road section should at least be 5 km long (Huijser et al. 2016). However, red wolves tend to have larger home ranges than common large ungulates. The average home range of resident red wolves was 68.4 km^2 based on 95% probability contours (Hinton et al. 2016). For transient red wolves, the average activity area was 319.2 km^2 based on 95% probability contours (Hinton et al. 2016). Assuming a circular shape, this translates to a home range or activity area diameter of 9.3 and 20.2 km, respectively. This means that, for red wolves that have the center of their home range or area of activity on or near the road, fenced road sections would have to be 10–20 km long (excluding buffer zones). Given the scale at which fences for red wolves would have to be implemented, it may be more efficient to extend the fences to neighboring collision hotspots rather than leaving small gaps which can also result in increased fence-end runs and associated collisions (Clevenger et al. 2001, Huijser et al. 2016). It is logical that there should be at least one suitable crossing structure for red wolves within a 10–20 km-long fenced road section, as the fenced road would otherwise be a near-absolute barrier for the red wolves that we aim to keep off the road. A crossing structure should be located where red wolves have shown to be interested in crossing the road (e.g. road mortality hotspots and successful crossings based on data obtained from GPS-collared individuals). The interval between crossing structures that are suitable for red wolves is not necessarily clearcut as it depends on the specific objective(s). The objectives can be related to current or historic crossing locations, proximity to birth locations and core home ranges of different family groups, the presence of suitable (breeding) habitat, or goals associated with recolonization and dispersal. Nonetheless, should a road section be fenced for red wolves for 10–20 km, there should be at least one suitable crossing structure for red wolves in that fenced road section.

How do we reduce red wolf road mortality most efficiently?

It is important to evaluate along what roads and along what road sections to prioritize mitigation measures. Based on our analysis of red wolf road mortality in relation to traffic volume, roads with a traffic volume between 2501 and 5000 vehicles per day had a factor of 2.34 more red wolf road mortalities than would have been expected if traffic volume did not influence road mortality. Traffic volume categories between 0 and 2500 vehicles per day had red wolf road mortalities that were roughly proportional to their road length. The lowest and highest traffic volume categories had proportionally very few road mortalities of red wolves, and this is where mitigation measures would be most inefficient, with the fewest red wolf road mortalities addressed for the road length mitigated. The roads with a traffic volume between 2501 and 5000 vehicles per day predominantly relate to US Highway 64 through Alligator River NWR. Besides protecting the last remaining wild family group (i.e. the Milltail family group, situation 2022–2023), this provides an additional rationale for implementing mitigation measures along this road section first.

However, the road mortality of red wolves is not restricted to US Highway 64. While mitigation measures along US Highway 64 are very likely to substantially reduce road mortality for the few remaining red wolves, additional road mortality of the remaining animals also occurs along US Highway 264 near the landfill area. Provided that the red wolf population increases again, and if its distribution is similar to what it was over the last few decades, mitigation measures are also needed along lower volume roads, including other sections of US Highway 264 and Highway 94. If mitigation is only implemented along all system roads in the ENC RWP area with a traffic volume between 2501 and 5000 vehicles per day, only 20 out of the 78 (25.64%) historic red wolf mortalities on the system roads would be addressed. Specifically for US Highway 64, regardless of where along this highway, only 19 out of 78 (24.36%) red wolf mortalities on the system roads would be addressed. Mitigating US Highway 264 and Highway 94 would address 21 out of 78 (26.92%) and 15 out of 78 (19.23%) historic red wolf mortalities on the system roads, respectively. Mitigating Highway 94 would also connect areas with a relatively high number of historic birth location concentrations on the two sides of the highway. Mitigating Highway 94 for both road mortality and the potential barrier effect is likely to contribute to having a larger and less fragmented wild red wolf population. Nonetheless, substantial historic road mortality also occurred on a road with very low traffic volume (e.g. 60 AADT, Frying Pan Rd). Rather than mitigating roads with very low traffic volume and very high impact on red wolf conservation, one can also reconsider the purpose or existence of such a very low volume road altogether.

Conservation action on the ground

In December 2024, the Federal Highway Administration announced that USD25 million was awarded through the

Wildlife Crossings Pilot Program for the installation of multiple wildlife underpasses and wildlife fencing along a section of US Highway 64 through Alligator River NWR (FHWA 2024). This will not only benefit the red wolf, but also other species (e.g. black bear, white-tailed deer). Ensuring these funds are strategically prioritized can help provide the greatest return on this investment.

Conclusion

We conducted temporal and spatial analyses of red wolf road mortality and birth location concentrations across the landscape to identify strategic approaches for mitigating road mortality to help stabilize and potentially increase the population of reintroduced red wolves in North Carolina. The analysis makes clear that while immediate road mitigation along the major highway is essential to help protect the remaining red wolves, it is insufficient on its own. Effective conservation for the red wolf requires a landscape approach to mitigation that includes both high and low volume roads.

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Author contributions

Marcel P. Huijser: Conceptualization (lead); Data curation (equal); Formal analysis (equal); Methodology (lead); Visualization (equal); Writing – original draft (lead); Writing – review and editing (lead). **James S. Begley**: Conceptualization (supporting); Data curation (equal); Formal analysis (equal); Methodology (supporting); Visualization (equal); Writing – original draft (supporting); Writing – review and editing (supporting).

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Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.63xsj3vhj (Huijser et al. 2026).

Supporting information

The Supporting information associated with this article is available with the online version.

References

Agan, S. W., Treves, A. and Willey, L. L. 2021a. Estimating poaching risk for the critically endangered wild red wolf (*Canis rufus*). – PLoS One 16: e0244261.

Agan, S. W., Treves, A. and Willey, L. L. 2021b. Majority positive attitudes cannot protect red wolves (*Canis rufus*) from a minority willing to kill illegally. – Biol. Conserv. 262: 109321.

Blanco, J. C., Cortés, Y. and Virgós, E. 2005. Wolf response to two kinds of barriers in an agricultural habitat in Spain. – Can. J. Zool. 83: 312–323.

Clevenger, A. P. and Huijser, M. P. 2011. Wildlife crossing structure handbook. Design and evaluation in North America. – Department of Transportation, Federal Highway Administration.

Clevenger, A. P., Chruszcz, B. and Gunson, K. E. 2001. Highway mitigation fencing reduces wildlife–vehicle collisions. – Wildl. Soc. Bull. 29: 646–653.

Coe, P. K., Nielson, R. M., Jackson, D. H., Cupples, J. B., Seidel, N. E., Johnson, B. K., Gregory, S. C., Bjornstrom, G. A., Larkins, A. N. and Speten, D. A. 2015. Identifying migration corridors of mule deer threatened by highway development. – Wildl. Soc. Bull. 39: 256–267.

De Feudis, C., Torretta, E., Orioli, V., Tirozzi, P., Bani, L., Meriggi, A. and Dondina, O. 2025. Dispersal and settlement dynamics of wolves in a lowland ecological corridor in northern Italy: effects of resource availability and human disturbance. – Biol. Conserv. 302: 110936.

ESRI. 2018. ArcGIS desktop: release 10.6.1. – Environmental Systems Research Institute, https://desktop.arcgis.com/en/quick-start-guides/10.6/arcgis-desktop-quick-start-guide.htm.

FHWA. 2024. Wildlife Crossings Pilot Program FY 2024–2025 selections. – Federal Highway Administration, U.S. Department of Transportation, https://highways.dot.gov/federal-lands/wildlife-crossings/pilot-program/fy-2024-2025-selections.

Ford, A. T., Clevenger, A. P., Huijser, M. P. and Dibb, A. 2011. Planning and prioritization strategies for phased highway mitigation using wildlife–vehicle collision data. – Wildl. Biol. 17: 253–265.

Foster, M. L. and Humphrey, S. R. 1995. Use of highway underpasses by Florida panthers and other wildlife. – Wildl. Soc. Bull. 23: 95–100.

Frair, J. L., Merrill, E. H., Beyer, H. L. and Morales, J. M. 2008. Thresholds in landscape connectivity and mortality risks in response to growing road networks. – J. Appl. Ecol. 45: 1504–1513.

Gagnon, J. W., Loberger, C. D., Sprague, S. C., Ogren, K. S., Boe, S. L. and Schweinsburg, R. E. 2015. Cost-effective approach to reducing collisions with elk by fencing between existing highway structures. – Hum.–Wildl. Interact. 9: Article 14.

George, K. A., Slagle, K. M., Wilson, R. S., Moeller, S. J. and Bruskotter, J. T. 2016. Changes in attitudes toward animals in the United States from 1978 to 2014. – Biol. Conserv. 201: 237–242.

Ghasemi, B., Bruskotter, J. T., Vucetich, J. A., Elbroch, L. M., Wilson, R. S., Carter, N. H., Moehrenschlager, A., Perry, S. and Hinton, J. W. 2026. Restoring the wild east: predicting public support for cougar and red wolf reintroduction in the eastern United States. – Biol. Conserv. 313: 111532.

Hinton, J. W., Proctor, C., Kelly, M. J., van Manen, F. T., Vaughan, M. R. and Chamberlain, M. J. 2016. Space use and habitat selection by resident and transient red wolves (*Canis rufus*). PLoS One 11: e0167603.

Hinton, J. W., Brzeski, K. E., Rabon Jr, D. R. and Chamberlain, M. J. 2017a. Effects of anthropogenic mortality on critically endangered red wolf *Canis rufus* breeding pairs: implications for red wolf recovery. – Oryx 51: 174–181.

Hinton, J. W., White, G. C., Rabon Jr, D. R. and Chamberlain, M. J. 2017b. Survival and population size estimates of the red wolf. – J. Wildl. Manage. 81: 417–428.

Huijser, M. P. and Begley, J. S. 2023. Impacts of roads and traffic on the red wolf: potential avoidance, mitigation and compensation strategies. – Report 4W9830. Western Transportation Institute, Montana State University.

Huijser, M. P. and Begley, J. S. 2026. Data from: Road mortality mitigation for red wolves requires a landscape approach. – Dryad Digital Repository, https://doi.org/10.5061/dryad.63xsj3vhj.

Huijser, M. P. and Bell, M. A. 2025. Wildlife crossing hotspot analyses for major highways in Wisconsin, USA. – Report no. 4WB112-A. Western Transportation Institute, Montana State University.

Huijser, M. P., Duffield, J. W., Clevenger, A. P., Ament R. J. and McGowen, P. T. 2009. Cost–benefit analyses of mitigation measures aimed at reducing collisions with large ungulates in the United States and Canada; a decision support tool. – Ecol. Soc. 14(2): 15.

Huijser, M. P., Fairbank, E. R., Camel-Means, W., Graham, J., Watson, V., Basting, P. and Becker, D. 2016. Effectiveness of short sections of wildlife fencing and crossing structures along highways in reducing wildlife–vehicle collisions and providing safe crossing opportunities for large mammals. – Biol. Conserv. 197: 61–68.

Karlin, M. and Chadwick, J. 2012. Red wolf natal dispersal characteristics: comparing periods of population increase and stability. – J. Zool. 286: 266–276.

Matos, C., Sillero, N. and Argaña, E. 2012. Spatial analysis of amphibian road mortality levels in northern Portugal country roads. – Amphib. Reptil. 33: 469–483.

Musiani, M., Anwar, S. M., McDermid, G. J., Hebblewhite, M. and Marceau, D. J. 2010. How humans shape wolf behavior in Banff and Kootenay National Parks, Canada. – Ecol. Model. 221: 2374–2387.

NCDOT. 2023. NCDOT annual average daily traffic (AADT) mapping application. – https://ncdot.maps.arcgis.com/apps/webappviewer/index.html?id=964881960f0549de8c3583bf46ef5ed4.

Newsom, A., Lozano, J. and Martín-López, B. 2025. Social perceptions of carnivores across the globe: a literature review. – Hum. Dimens. Wildl. 31: 97–120.

North Carolina Wildlife Resources Commission. 2026. 2025–2026 season dates. – www.ncwildlife.gov/2025-2026-season-dates/download?attachment.

Roth, J. D., Murray, D. L. and Steury, T. D. 2008. Spatial dynamics of sympatric canids: modeling the impact of coyotes on red wolf recovery. – Ecol. Model. 214: 391–403.

Rytwinski T., Soanes, K., Jaeger, J. A. G., Fahrig, L., Findlay, C. S., Houlahan, J. van der Ree, R. and van der Grift, E. A. 2016. How effective is road mitigation at reducing road-kill? A meta-analysis. – PLoS One 11: e0166941.35.

Santiago-Ávila, F. J., Agan, S., Hinton, J. W. and Treves, A. 2022. Evaluating how management policies affect red wolf mortality and disappearance. – R. Soc. Open Sci. 9: 210400.

Seiler, A. 2003. The toll of the automobile: wildlife and roads in Sweden. – PhD thesis, Swedish Univ. of Agricultural Sciences, Sweden.

Serenari, C. and Taub, M. 2019. Predicting the legitimacy of wolf recovery. – Wildl. Biol. 2019: wlb.00454.

Serieys, L. E. K., Rogan, M. S., Matsushima, S. S. and Wilmers, C. C. 2021. Road-crossings, vegetative cover, land use and poisons interact to influence corridor effectiveness. – Biol. Conserv. 253: 108930.

Silverman, B. W. 1986. Density estimation for statistics and data analysis. – Chapman and Hall.

Toth, M. G. and Rubino, E. C. 2024. Assessing local landowner versus non-local public support for a red wolf reintroduction using varying message treatments. – Wildl. Res. 51: WR23096.

Treves, A. and Karanth, K. U. 2003. Human–carnivore conflict and perspectives on carnivore management worldwide. – Conserv. Biol. 17: 1491–1499.

USFWS. 2018. Red wolf species assessment. Report 19 April 2018. – USFWS, https://ecos.fws.gov/ServCat/DownloadFile/161384.

USFWS. 2022. Cape Romain National Wildlife Refuge. South Carolina. – www.fws.gov/refuge/Cape_Romain/wildlife_and_habitat/red_wolves.html.

USFWS. 2023. Revised recovery plan for the red wolf (*Canis rufus*). – U.S. Fish and Wildlife Service, www.fws.gov/sites/default/files/documents/20230928_fRP_RW_508%20compliant_FINAL.pdf.

USFWS. 2025. Red wolf. *Canis rufus*. – https://www.fws.gov/southeast/wildlife/mammals/red-wolf/.

Waller, J. S. and Servheen, C. 2005. Effects of transportation infrastructure on grizzly bears in northwestern Montana. – J. Wildl. Manage. 69: 985–1000.