# Evaluating potential effects of widening US 64 on red wolves in Washington, Tyrrell, and Dare Counties, North Carolina


Final Report

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16. Abstract

We used data from 16 red wolves fitted with GPS-collars between January 2009 and April 2011 to evaluate home range size and habitat selection, road permeability, and identify significant red wolf highway crossing locations. Home range size for red wolves averaged 13.7 $mi^2$ with no significant difference between males and females. Although we found no significant difference in home range size among age classes, dispersers tended to have larger home ranges than adults and juveniles. Red wolf home ranges were larger during winter than during other seasons. Red wolves avoided wetter habitats such as pocosins, wetlands, and lowland forests, leaving agriculture the best predictor of red wolf presence. Red wolves also selected for the presence of agriculture/forest road systems for travel. Road permeability, calculated using GPS-collar data, was 100%, thus the current 2-lane highway does not impose a barrier effect on the red wolf population. This increases the risk of road mortality events. Using a 3281 ft. (1 km) buffer, construction north of the current US 64 in Tyrrell County has the potential to remove up to 0.16 $mi^2$ of red wolf habitat and 6% of the home range area used by a current red wolf pack while construction to the south will impact only 0.09 $mi^2$ of red wolf habitat and will not displace any current red wolf packs. East of Alligator River in Dare County, a widening of the current highway to the south has the potential to remove up to of 0.07 $mi^2$ of red wolf habitat and 20% of the home range used by the only existing red wolf pack in Alligator River National Wildlife Refuge if construction disturbs out to 3281 ft. (1 km) from the current road. Construction to the north of US 64 in Dare County has the potential to remove up to 0.04 $mi^2$ of red wolf habitat and will not overlap with any current packs, based on 95% home ranges. Through the use of GPS-collars and remote camera traps, we identified 5 important red wolf crossing locations, 4 in Tyrrell County west of Alligator River and 1 in Dare County east of Alligator River. The presence of agricultural fields, successional fields, and/or upland forests 328 to 492 ft. from the road provided the most parsimonious explanation for the location of crossing sites identified using GPS-collar locations; trail/road width provided the best explanation for the location of crossing sites identified by remote camera traps. The presence of agricultural fields, successional fields, and upland forests as well as proximity to maintained agricultural/forest roads at crossing sites corresponds to habitat selection results. Four of the 5 red wolf crossing locations we identified are suitable for crossing structures. The most western crossing site is located within the town of Colombia, NC where retro-fitting a wildlife underpass is not practical. Well maintained trails at least 26.24 ft. (8 m) in width leading to and from underpasses, which connect habitats selected for by red wolves (e.g. agriculture, successional fields, and upland forests), is suggested to optimize efficacy.

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# Executive Summary

Information reported here is the result of a 2-year study evaluating red wolf (*Canis rufus*) habitat use and crossing patterns along US 64 from Columbia, NC east to the 64/264 intersection in Manns Harbor, NC. This report includes a problem statement and background information, evaluation of red wolf home range size and habitat selection, an analysis of probable effects to red wolf home range and habitat availability in the event of a highway widening, identification of current red wolf crossing locations, and suggestions for the location and type of crossing structures to mitigate potential adverse effects of a highway widening of US 64.

We used data from 16 red wolves fitted with GPS-collars between January 2009 and April 2011 to evaluate home range size and habitat selection. Home range size for red wolves averaged 13.7 $mi^2$ with no significant difference between males and females. Although we found no significant difference in home range size among age classes, dispersers tended to have larger home ranges than adults and juveniles. Red wolf home ranges were larger during winter than during other seasons. Red wolves avoided wetter habitats such as pocosins, wetlands, and lowland forests, leaving agriculture the best predictor of red wolf presence. Red wolves also selected for the presence of agriculture/forest road systems for travel.

Road permeability, calculated using GPS-collar data, was 100%, thus the current 2-lane highway does not impose a barrier effect on the red wolf population. This increases the risk of road mortality events. A decrease in the red wolf population to the west of Columbia, NC, prevented collaring of red wolves where widening to a 4-lane highway was completed. Therefore, we were not able to compare highway permeability between 2- and 4-lane highways. Using a 3281 ft. (1 km) buffer, construction north of the current US 64 in Tyrrell County has the potential to remove up to 0.16 $mi^2$ of red wolf habitat and 6% of the home range area used by a current red wolf pack while construction to the south will impact only 0.09 $mi^2$ of red wolf habitat and will not displace any current red wolf packs. East of Alligator River in Dare County, a widening of the current highway to the south has the potential to remove up to of 0.07 $mi^2$ of red wolf habitat and 20% of the home range used by the only existing red wolf pack in Alligator River National Wildlife Refuge if construction disturbs out to 3281 ft. (1 km) from the current road. Construction to the north of US 64 in Dare County has the potential to remove up to 0.04 $mi^2$ of red wolf habitat and will not overlap with any current packs, based on 95% home ranges.

Through the use of GPS-collars and remote camera traps, we identified 5 important red wolf crossing locations, 4 in Tyrrell County west of Alligator River and 1 in Dare County east of Alligator River. The presence of agricultural fields, successional fields, and/or upland forests 328 to 492 ft. from the road provided the most parsimonious explanation for the location of crossing sites identified using GPS-collar locations; trail/road width provided the best explanation for the location of crossing sites identified by remote camera traps. The presence of agricultural fields, successional fields, and upland forests as well as proximity to maintained agricultural/forest roads at crossing sites corresponds to habitat selection results.

Four of the 5 red wolf crossing locations we identified are suitable for crossing structures. The most western crossing site is located within the town of Colombia, NC where retro-fitting a wildlife underpass is not practical. Well maintained trails at least 26.24 ft. (8 m) in width leading to and from underpasses, which connect habitats selected for by red wolves (e.g. agriculture, successional fields, and upland forests), is suggested to optimize efficacy.
Detailed results and discussion are provided in the report below.

# Evaluating potential effects of widening US 64 on red wolves in Washington, Tyrrell, and Dare Counties, North Carolina

## Final Report

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## Introduction

Roads have profound effects, both direct and indirect, on natural ecosystems (see reviews by Forman et al. 2003 and Coffin, 2007). Forman and Alexander (1998) estimated that the approximately 10.9 million hectares of public roads in the US and their related habitat loss and degradation has affected >20% of land area in the United States. In a 43-year period (1960 – 2003), the number of registered cars increased from 74 to 231 million nation-wide and the annual distance traveled by car in the US grew from approximately 720,000 to 2.8 million miles (Ouren and Watts, 2005). As transportation needs increase, a rise in the amount of habitat lost and degraded due to road construction can be expected. Indeed, the link between economic development and transportation expansion was well documented by the mid 1960's (Kansky, 1963; Taaffe et al., 1963; Haggett 1965). Such large-scale and multifaceted changes to ecosystems have many detrimental impacts on wildlife (Jackson, 1999), including direct mortality (Lalo, 1987; Harris and Scheck, 1991; Schwabe and Schuhmann, 2002), habitat destruction (Theobald et al., 1997; Angelsen and Kaimowitz, 1999), barrier effects (Forman et al, 2003), and increased human land use activities (Bjurlin and Cypher, 2003; Coffin, 2007).

There is perhaps no other human impact as transportation infrastructure whose far-reaching, cumulative effects on wildlife are so devastating and demanding of attention, yet so commonly underestimated. Direct wildlife mortality results from construction injury and vehicular collisions (Trombulak and Frissell, 2000) and has now surpassed hunting as the leading direct human cause of vetebrate mortality on land (Forman and Alexander, 1998), with an estimated 720,000 to 1.5-million deer-vehicle crashes reported annually (Conover et al., 1995; Forman et al., 2003). While the number of common species killed along roads is staggering, in most cases road mortality of wildlife does not translate into population level effect. However, road related impacts to threatened and endangered species are of particular concern. Road kill is the primary cause of mortality in Florida for Florida panthers, black bears, key deer, and crocodiles (Harris and Scheck, 1991) and accounts for a high percentage of deaths in Iberian lynx (Ferreras et al., 1992). In addition, road impacts have been found in gray wolves (Thiel, 1985; Paquet and Callaghan, 1996), desert tortoises (Boarman, 1996), and some populations of San Joaquin kit foxes (Bjurlin and Cypher, 2003). For the declining copperbelly water snake in Indiana, road mortality accounts for approximately 17% of all deaths (Roe et. al, 2006). Road mortality was the second highest cause of death for red wolves in North Carolina within the 5 county recovery zone between 1999 and 2006, accounting for 14% of mortality overall (USFWS, 2007). When broken down by age class, vehicle strikes were the leading cause of death in dispersing red wolves, accounting for 19% of mortality (USFWS, 2007). The increasing incidence of wildlife-vehicle collisions also presents a real issue for humans, as they claim hundreds of lives, cause tens of thousands of injuries, and inflict an enormous monetary cost for medical treatment and vehicle repair each year nationwide (Forman et al., 2003). For example, in 1993 1.5 million deer-vehicle crashes were reported in the US leading to $1.1 billion in vehicle damages (Durbin, 2004). Deer-vehicle collisions have been reported to cause 29,000 human injuries and claim 211 lives (Conover et al., 1995).

Beyond direct mortality, roads can negatively affect wildlife populations by degrading habitat quality (Theobald et al., 1997; Carr et al., 2002), fragmenting habitat and populations (Oxley et al., 1974; Trombulak and Frissell, 2000; Nellermann et al., 2001), hindering gene flow (Gerlach and Musolf, 2000; Epps et al., 2005; Riley et al., 2006), skewing sex ratios (Gibbs and Steen, 2005), and limiting dispersal (Beier, 1995). Most roads exhibit a distinct trade-off between permeability and road kill (Forman and Alexander, 1998). A highly permeable road might result in a high level of wildlife/vehicular collisions, whereas an impermeable road might have few road kill events. Yet this decrease in road kill comes at the expense of habitat connectivity. As individuals lose their mobility and gene flow is reduced, portions of the population become isolated. In the event of local extinction due to some stochastic event, fragmentation can make recolonization of previously-occupied habitat impossible (Theobald et al., 1997). These affects are of particular concern in small populations. Small populations have an increased risk of extinction due to demographic stochasticity, decreased heterozygosity, genetic drift, inbreeding, and low effective population size (Caughley, 1994), all of which can be exacerbated though road construction and expansion-related barrier effects. Social organization may also be affected by spatial change leading to population instability (Krausman et al., 2004).

Low population densities and large home ranges make carnivores particularly vulnerable to the effects of habitat fragmentation by roads (Whittington et al., 2005). A highway was found to restrict gene flow in a Cleveland, Ohio coyote population and direct the movements of migrants towards urbanizing centers (Rashleigh et al., 2008). Riley et al. (2006) found that coyote and bobcat populations in southern California separated by a major freeway exhibited genetic differentiation, suggesting that the freeway is a barrier to dispersal. Even when they do not constitute an absolute physical barrier, high-use roads can lead to avoidance behavior in canids affecting their ability to move across a landscape (Kaartinen et al., 2005, Paquet and Callaghan, 1996, Whittington et al., 2004). For those that do cross, heightened territorial behavior along roadways can discourage reproductive success, again limiting gene flow (Riley et al., 2006). The degree to which a road affects canid survival is dependent on the specific situation, and sometimes no detrimental effects are observed, as is the case with a California population of San Joaquin kit foxes (Cypher et al., 2009). Some documentation exists regarding wolves in the vicinity of highways. A study tracking gray wolf dispersal in Minnesota found that wolves were willing to cross major highways to colonize areas in Wisconsin and Michigan (Mech et al., 1995). For gray wolves (*Canis lupus*), a 4-lane unfenced highway in Wisconsin seemed to not influence wolf movements (Kohn et al., 1999). In contrast, a 4-lane fenced highway in Banff National Park in Alberta, Canada, appears to hinder wolf movements (Paquet and Callaghan, 1996), although crossing structures mitigated its barrier effect to some degree (Clevenger and Waltho, 2000, 2005). In Spain, wolves whose home ranges were greater than 5 km from the highway crossed a 4-lane, fenced highway via vehicle bridges (Blanco et al., 2005), however those living in close proximity to the highway (<5 km) crossed the highway only after severe habitat disturbance. Gray wolves in Canada and Spain seem to prefer large, open wildlife overpasses (Forman et al., 2003). In general, a large void exists in addressing factors that affect how large carnivores use passages. Major transportation corridors bisect and potentially fragment most of the major ecosystems that still support wide-ranging carnivores. Increased concerns expressed by transportation and natural resources agencies regarding mitigation planning for large carnivores highlight the need for more information and research in this area (Forman et al., 2003).

The use of wildlife crossing structures can mitigate some of the negative effects associated with highways (Forman and Alexander, 1998). The appropriate type of crossing structure to mitigate road effects varies with species (Mata et al., 2008). A study monitoring the success of multi-species highway underpasses following the highway-widening just west of this current study area found that bobcats, black bears, and foxes utilized underpasses (McCollister and van Manen, 2010), however there were no confirmed detections of coyotes or red wolves using the underpasses. Because the red wolf population of both this study and the one cited above is the only wild red wolf population, there does not exist literature on the preference of red wolves for particular crossing structures. However several studies have found that while gray wolves will use wide tunnels and underpasses (Clevenger and Waltho, 2000, Kusak et al., 2009), they prefer open overpasses (Forman et al., 2003, Kusak et al., 2009). In Banff, wolves select for taller underpasses close to town (Clevenger and Waltho, 2000). Coyotes on the other hand have been found to use underpasses of a wide variety of sizes, from pipe culverts to wide underpasses, as long as they did not connect developed areas (Ng et al., 2004). Likewise, a study in Virginia found that coyotes readily used a variety of underpasses (Donaldson, 2007). The Wildlife Crossing Structure Handbook published in 2011 by the Federal Highway Administration recommends that underpasses geared towards large mammals (deer, bears, and wolves) and high mobility medium sized mammals (coyote, fox, and likely the category red wolves would be placed) should be greater than 32 ft. in width and greater than 13 ft. in height and that overpasses be at least 50 ft. wide (Clevenger and Huijer, 2011). If designing mitigating structures solely for high mobility medium sized mammals, underpasses and culverts with a diameter of 4ft. have been effective (Clevenger and Huijer, 2011). However, it appears that the structural components of crossing structures play a larger role in determining success for ungulates (Clevenger and Waltho, 2005; Gagnon et al., 2011) while habitat connectivity plays a larger role in the successful use of crossing structures for carnivores (White and Ernst, 2004; Singleton et al., 2005; Riley et al, 2006; Kindall and van Manen, 2007).

Underpasses can function as effective crossing structures for wolves, but high variability in use indicates that consideration of social interactions, placement, construction specifications and distance between crossings is essential for success (Paquet and Callaghan, 1996). Animals do not treat all sections of a roadway indiscriminately, so crossing funnel areas and natural habitat linkages at the landscape level must be identified. White and Ernst (2004), Singleton et al. (2005), and Kindall and van Manen (2007) all stress the need to identify habitat linkages across barriers to properly place crossings. In addition, Roger and Ramp (2009) discuss the importance of species-specific habitat use data in determining roadway impacts. Thus, it is imperative not only that wildlife underpasses be constructed in areas identified as high use for crossings (Scheick and Jones, 1999), but habitat variables at crossing locations be collected as well to model what landscape factors predict crossings. Often times it is also necessary to install exclusionary fencing in addition to a crossing structure to guide animals to the crossing point and discourage crossing at road-level (Baker, 2005).

Indeed, one study in Portugal found that fencing had a funneling effect, directing larger animals towards culverts (Ascensao and Mira, 2006). Similarly, fencing along a highway reduced elk-vehicle collisions by 97% and other wildlife-vehicle collisions by 64% in Arizona (Gagnon et al., 2010) and likely lead to a decease in white-tailed deer-vehicle collisions in North Carolina (Jones et al., 2010). A study in Germany found that fencing successfully reduced wildcat road mortality by 83% (Klar et al., 20009) and fencing along with culverts lowered wildlife-vehicle collisions by 93.5% in Paynes Prairie State Preserve (Dodd et al., 2004). Ungulate-vehicle collisions were reduced by 80% following the installation of roadside fencing in Banff National Park, Canada (Clevenger et al., 2001). Jaeger and Fahrig (2004) developed a model to look at the trade-off between reductions of road kill and increased barrier effect due to fencing installation. They found that below a certain traffic volume, the barrier effect of fencing is harmful to a population and therefore they only recommend the use of roadside fencing when traffic volume is high (e.g. high risk of road mortality) and the target species does not show behavioral avoidance of roads (Jaeger and Fahrig, 2004). Both Clevenger et al. (2001) and McCollister and van Manen (2010) found that while fencing reduced wildlife-vehicle collisions close to underpasses, wildlife-vehicle collisions increase approaching fence ends. McCollister and van Manen (2010) found that road mortality was higher in fenced highway segments as compared to unfenced segments due to the increased mortality where roadside fencing ends. Therefore, if non-continuous fencing is used, it may be necessary to modify fence ends to direct wildlife away from the highway (Clevenger et al., 2001). Ungulates are the focal species for most studies that successfully demonstrate the effectiveness of roadside fencing. Fencing may be less effective for carnivores as they often go over (e.g. black bears) or under (e.g. coyotes) fencing (Clevenger et al., 2001). Indeed, the use of fencing did not increase culvert use by bobcats in Texas (Cain et al., 2003) and actually lead to an increase in wolf road mortality in Spain (Colino-Rabinal et al., 2011). Burying roadside fencing can help to discourage some species from digging under the fence (Clevenger et al., 2001).

Clearly, an understanding of red wolf activity patterns, movements, and habitat use are all needed in the vicinity of US 64 and across the Albemarle Peninsula. This study assessed red wolf home range, habitat selection, and highway crossing patterns along the US 64 corridor with the use of GPS collars and remote cameras to determine important red wolf habitat and to identify significant red wolf highway crossing locations. In addition, this research examined which landscape attributes promote red wolf use of crossing locations to increase the success of mitigating structures.

## Background Information

The North Carolina Department of Transportation (NCDOT) is planning a highway improvement project for US 64 in Tyrrell and Dare Counties North Carolina, which will extend across the full length of the Albemarle Peninsula when completed, separating the northern section of the 5 county (Washington, Tyrrell, Dare, Hyde, and Beaufort counties) red wolf recovery zone. The effects of the highway widening on red wolf recovery and conservation could be substantial, potentially creating a barrier to movement and gene flow of red wolves and other wildlife from one side of the highway to the other. In addition, the habitat loss associated with a highway widening likely will disrupt red wolves living adjacent to the existing highway causing a shift in current home ranges. Any shifts in home ranges have the potential to affect social order. Even in the absence of a barrier effect, the project may lead to an increase in vehicle related deaths as wolves attempt to cross a wider highway with increased speed limits. In addition, there is a potential to concentrate prey and herbaceous food sources at highway edges, attracting wolves, coyotes, black bears, white-tailed deer and other wildlife, increasing the risk of vehicle collisions. Highway barrier effects, habitat loss, social disruptions, and road mortality resulting from the highway widening may culminate in reduced red wolf population viability.

Problem Need/Definition

Viable populations of wildlife depend, in part, on dispersal to maintain genetic diversity. Whether natural or man-made, barriers to dispersal are of concern to wildlife managers. For restored or recovering populations, potential barriers such as highways or large fenced areas magnify in importance because of their potential to restrict or retard growth and genetic diversity in small wildlife populations. Roads, in particular, recently received attention with respect to large carnivore population dynamics related to increased direct (vehicle collisions) and indirect (changes in behavior that affect food acquisition) mortality (Trombulak and Frissell, 2000). Forced spatial change also may affect area-wide social organization and thus population stability, and increased noise or activity levels may initially affect wildlife behavior (Krausman et al., 2004).

For the past several years the North Carolina Department of Transportation (NCDOT) has been planning a proposed project to widen US 64 from 2 to 4 lanes from Raleigh to Manteo, North Carolina. With respect to the segment of US 64 already widened and elevated between Plymouth and Columbia by 2005, preliminary data collected by the U.S. Fish and Wildlife Service indicates red wolf (*Canis rufus*) movements and gene flow, including dispersal and home range size, may already be restricted by that highway segment. Remaining sections of US 64 planned for widening are the approximate 15.5-mile section from Columbia to Alligator River, and 11.8-mile section that runs through Alligator River National Wildlife Refuge.

The nature of the US 64 widening project calls into question important ecological and regulatory considerations that, together, mean data collection is needed to assist with science-based decisions and project design. Red wolves will be involved in two federal regulatory processes pertinent to widening of US 64, namely, project consultation under Section 7 of the U.S. Endangered Species Act of 1973, and assessment of "refuge compatibility" under the National Wildlife Refuge System Improvement Act of 1997 (Public Law 105-57), along with the National Wildlife Refuge System Administration Act of 1966 (16 U.S.C. 668dd-668ee), as amended. These processes allow cooperation toward achieving a project that takes into account human safety, traffic management, and wildlife concerns that include passage, mortality, large-sized animals, and multiple wildlife refuge values. Refuge considerations include endangered species conservation, waterfowl management, wildlife habitat with associated species, hydrology, wetlands, reptiles and amphibians, public use, fire management, exotic species management, etc.

The effects of US 64 widening on red wolf recovery and conservation could be substantial. Widening US 64 may be accompanied by increased speed limits, and likely will create a barrier to movement of red wolves and other wildlife from one side of the highway to the other. Thus, it is imperative that wildlife crossing structures be constructed in areas identified as high use crossings by red wolves, bears, deer, and other species (Scheick and Jones, 1999). Completed and planned phases of the US 64 widening project extend across the full length of the Albemarle Peninsula, separating and otherwise affecting the entire northern quarter of the 5-county red wolf experimental population area.

Construction of the highway itself most likely will directly disrupt the red wolf population, along with other wildlife populations (e.g., black bear, white-tailed deer) living adjacent to the existing highway during the 1-2 year construction period. These disruptions may cause red wolves to shift out of their current home ranges or territories during the construction phase and move into areas already occupied by other red wolves, causing social disruptions and ripple effects across the Albemarle Peninsula. While the disruption due directly to construction will be short-term, effects on the red wolf population may be long lasting and even permanent. Habitat loss, social disruptions, and ripple effects, as a result of direct, indirect, and cumulative effects of a highway widening, may result in a reduction of the red wolf population, its gene flow, and gene diversity, by some unknown quantity.

Vehicle strike mortality significantly impacts the wild red wolf population on the Albemarle Peninsula in North Carolina (USFWS, 2007). Of 166 known adult red wolf loses since 1999, 23 were killed in vehicle strikes. Vehicle strikes are three times higher in non-breeder (19%) vs. breeder (6%) red wolves in the designated experimental population area. This is partly explained by single red wolves dispersing or roaming over large distances.

Studies are needed to assess how the red wolf population has utilized the area since restoration began. An examination of which landscape attributes promote red wolf use would be helpful, along with a thorough assessment of site-specific habitat availability.

More specifically, the potential problems or benefits examined for red wolves in association with US 64 widening should include the following concerns.

1. Vehicle mortality of red wolves and associated human safety.
2. Reproduction and survival.
3. Considerations of placement of underpasses or overpasses.
4. Changes in red wolf habitat, prey, home range size, dynamics, and associated landscape fragmentation.
5. Effects upon red wolf activity, movements, gene flow, dispersal, territory dynamics, social organization, pack integrity, habitat use, and land occupancy.
6. Ripple effects throughout the red wolf population, across the Albemarle Peninsula.
7. Changes in red wolf numbers pre-project, during project, and post-project.
8. Influences on eastern coyotes, a competitor and threat to red wolves.
9. Effects upon monitoring of red wolves and eastern coyotes.

It is important to understand the effect of canid activity and movements out of or into the experimental area along the expanded highway in western portions of the Albemarle Peninsula. Possible study topics include coyote/red wolf interactions and retrospective examination of adaptability of coyote/hybrids vs. red wolves in the face of significant habitat change and/or significant project construction.

Research Objectives

The objectives of this research project are to:

1. Evaluate wolf habitat use along the entire US 64 corridor from Plymouth to the US 64/264 intersection

2. Evaluate the significance of red wolf habitat changes anticipated from the proposed highway project from Columbia to the US 64/264 intersection in terms of movements, survival, reproduction, home range shifts, and social organization.

3. Identify significant red wolf crossing areas to determine where wildlife crossing structures or other design features could be placed to minimize adverse project effects on red wolves.

# Study Area

The only wild population of red wolves occurs on more than 2,567 $mi^2$ of federal, state, and private lands in 5 counties (Beaufort, Dare, Hyde, Tyrrell, and Washington) in northeastern North Carolina (Figure 1), known as the Red Wolf Recovery Zone (RWRZ). Two of the northern counties within the RWRZ, Tyrrell and Dare Counties, were the focal point of this study because they contain the remaining 27.34 mi of US 64 to be widened. Federal lands within the study area include Pocosin Lakes National Wildlife Refuge, Alligator River National Wildlife Refuge, and a bombing range shared by the Navy and Air Force. State land consists of numerous game management properties, while private lands are primarily made-up of timber plantations, agricultural fields, and a few developed residential and commercial properties.

The most prevalent land cover types within the study area, as identified by the North Carolina Gap database (2009), are agricultural fields (~30%) planted primarily with wheat, corn, soybean, cotton, and potatoes; commercial pine plantations (~15%); pocosin (~15%); non-riverine swamp forests (~10%); and saltwater marsh or open water (~10%). Climate within the study area is characterized by 4 full seasons of nearly equal length with annual precipitation averaging 50 in. Temperatures range from a mean of 41°F in winter to 80.6°F in summer. Elevation ranges from sea level to 164 ft. (Beck et al., 2009). Carnivores that co-occur with red wolves within the study area include gray foxes (*Urocyon cineroargenteus*), red foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), feral dogs (*Canis lupus familiaris*), bobcats (*Lynx rufus*), black bears (*Ursus americanus*), and various mustelids.

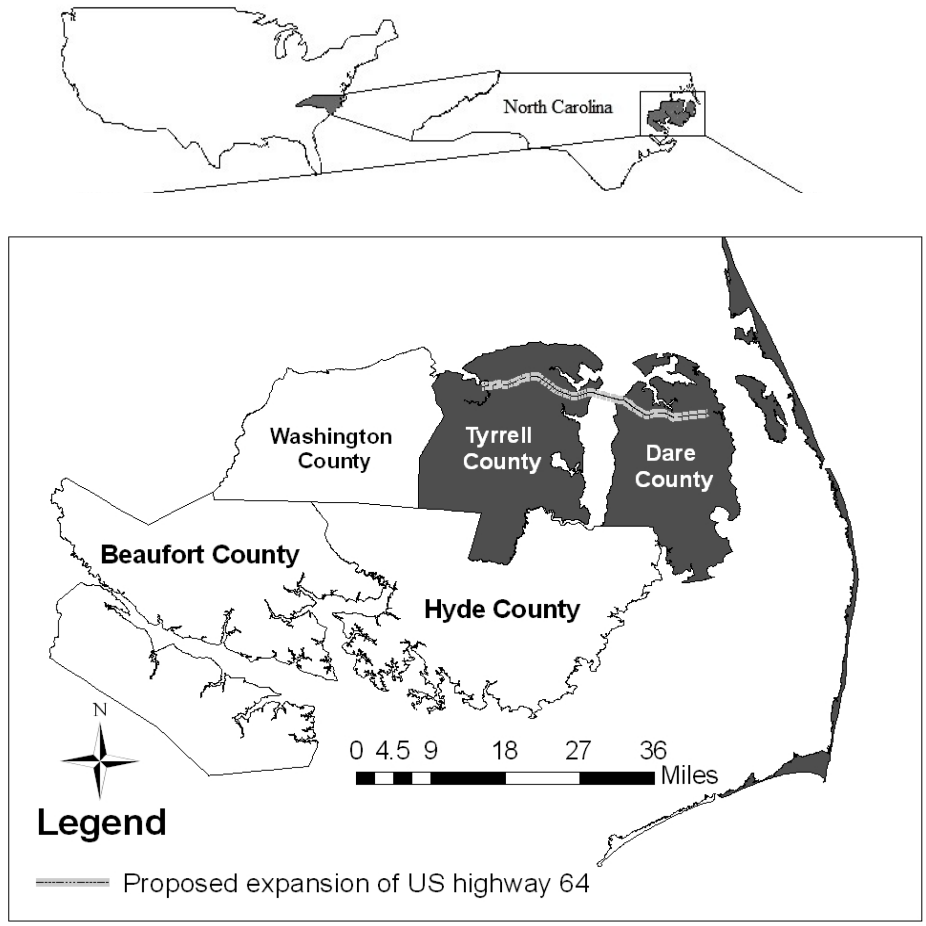


**Figure 1**. The study area (highlighted in gray) is located within 2 of the northern counties, Tyrrell and Dare, of the 5 county red wolf recovery zone in northeastern North Carolina. The study area focuses on the remaining 27.3 mi section of US 64, between Columbia, NC and the US 64/264 intersection, to be expanded from a 2- to 4-lane highway.

# Methods

***Capturing and collaring of animals:*** From January 2009 to April 2011, adult and juvenile red wolves were captured by USFWS biologists and fitted with mortality-sensitive Lotek GPS 4400S collars (Lotek Wireless, Inc., Ontario, Canada). Red wolves > 2 years old were classified as adults, < 2 years old as juveniles, and < 9 months old as pups. Pups were not fitted with GPS collars because typically they were too small to safely wear collars. Prior to deployment, GPS collars were remotely programmed to record locations every 5 hours with a nested program to collect a position every 30-minutes for a 5 hour period daily. The nested 30-minute program was scheduled to rotate around the 24-hour clock to capture detailed movements. Each collar emitted a VHF locator beacon each day from 0900 – 1200, allowing us to locate collared animals every 12 weeks on the ground and remotely download stored data.

## Objective 1: Evaluate wolf habitat use along the entire US 64 corridor from Plymouth NC to the US 64/264 intersection

***Independence of animal movements*:** To address the issue of correlation of GPS location data between pack mates, we calculated home ranges (Getz et al., 2007) for all collared wolves in a single pack then associated locations for each animal with the corresponding isopleth. Next, Spearman correlation matrices were used to determine the similarity of home ranges and habitat use among all collared animals within the pack. This determined if animals within a pack should be treated separately or if habitat use, selection, and home range of one collared animal was representative of the entire pack.

***Home range analyses*:** Following the conclusion of field work, rarefaction curves of cumulative weekly home ranges were calculated on all complete data sets for each collared animal to determine the relationship between length of time collar was deployed and when size of home range stabilized (Bekoff and Mech, 1984). Starting by calculating size of home range of an animal during the first week of collar deployment, we calculated size of home range of the animal during the second week of collar deployment and so on until the complete data set for that animal was included in calculating size of home range. Ninety-five percent home range isopleths were constructed using adaptive nearest neighbor convex hull methods (Getz et al., 2007). Animals whose home ranges did not stabilize in size were excluded from subsequent analyses. Given the varying age, dominance, and sex of the animals that were collared, and that home range composition between packs with collared animals may vary; we assumed that all factors influencing stabilization of size of home range were captured sufficiently. For individuals whose home range stabilized, monthly home ranges were constructed according to Getz et al. (2007) to examine short-term and seasonal variations in home range composition and size.

Overall and monthly home ranges were overlaid onto habitat maps developed by NC GAP to determine percent composition of home ranges. Habitat types included agricultural fields, wetlands, upland forests, lowland forests, successional fields, and pocosin (areas covered with evergreen vegetation and inundated with water). We used one-way ANOVA to test for differences in overall home range size among age classes and Student's *t*-tests to test for a difference in home range size between sexes. Student's *t*-tests were also used to determine if seasonal variation in monthly home range size and composition for each habitat type were significantly different. Following previous studies (Phillips et al., 2003; Chadwick et al., 2010; Hinton et al., 2010), we recognized a summer (April – September) and winter (October – March) season. Significance was set at $\alpha \leq 0.05$.

***Habitat use and selection analyses*:** Resource selection functions (RSFs; Manly et al., 2002) were used to examine 2nd order (home range) and 3rd order (within home range) habitat use by red wolves (Johnson, 1980). Resource selection functions were developed using use/availability data with a binomial distribution (Manly et al., 2002).

For 2nd order habitat use, we considered the entire 5 county red wolf recovery area as available habitat and all locations of each GPS-collared animal occurring within its respective 95% home range (Getz et al., 2007) as used habitat. For 3rd order habitat use, the entire 95% home range (Getz et al., 2007) was considered to be available. All locations of each animal contained within its respective 95% home range were combined to examine 2nd and 3rd order habitat use for the entire population. An equal number of random points, compared to locations, were generated within the available areas for 2nd and 3rd order habitat use, respectively. Distance to road and water, human density (people per square mile), and habitat type were determined for all used and random locations. Habitat types were the same as those for determining home range composition. After combining used and random locations for each order of habitat use, RSFs were developed for each order of habitat use which contained habitat type, distance to roads and water, human density, and all biologically meaningful interactions (habitat type by distance to roads, habitat type by human density, and distance to roads by human density). Animals were monitored for varying lengths of time, had different numbers of locations, were of different age classes, and different sexes. Therefore each animal could have potentially influenced the RSFs more or less than another animal. Thus to make sure that no animal biased the RSFs, preliminary 2nd and 3rd order RSFs were developed using a sampling with replacement method in which each animal was excluded once from calculation of a RSF while all other animals were included. For the 3rd order RSF, a random effect for animal was included in the RSFs to account for differences in habitats available to each animal. Akaike's information criterion corrected for small sample sizes (AICc) was used to choose the most parsimonious RSF from the global (all possible variables included) RSF and all possible subsets for each order of habitat use (Burnham and Anderson, 2002). Twenty-five percent of used and random locations for each order of habitat use were not used in developing all RSFs to evaluate fit of most parsimonious RSFs using cross-validation (Johnson et al., 2006). The most parsimonious RSFs that were shown to have a good fit to the data were projected in a GIS to create habitat suitability maps depicting areas of high, medium, and low quality habitat and probability of occurrence of red wolves.

AICc weights of most parsimonious 2nd and 3rd order RSFs were compared to determine whether habitat type, distance to roads and water, and density of humans were scale dependent for red wolves. The RSF with the greatest AICc weight demonstrated the scale at which the variables of interest and associated interactions influenced habitat use the most. Statistical analyses were conducted in R 2.11.1 (R Development Core Team 2010) and spatial analyses using ArcGIS 10 (ESRI® ArcMap™ 10, Copyright © 1999-2010 ESRI Inc.) and Geospatial Modeling Environment 0.5.3 (Beyer, H. L., Copyright © 2001-2010 Spatial Ecology LLC).

**Objective 2: Evaluate the significance of red wolf habitat changes anticipated from the proposed highway project from Columbia NC to the US 64/264 intersection.**

***Quantifying barrier effects using passage rates*:** Following Dodd et al. (2007), we quantified the barrier effects of US 64 by calculating a permeability index. A permeability index is a passage rate measuring an individuals willingness to attempt a road crossing and is calculated by using the following equation: #crossings/(#crossings + #approaches), where an approach is defined as a red wolf entering into a 164 ft. buffer zone around the highway without crossing (see Figure 2). The 164 ft. (50 m) buffer zone was determined by measuring the distance between US 64 and the boundary of the closest red wolf home range (95% MCP) to the highway. This was done to exclude movements within a home range from being counted as an approach. The permeability index ranges from 0 to 1, with 0 indicating an impermeable road and 1 indicating 100% permeability. Permeability indices were calculated for the 30-minute and 5-hour data sets separately. An overall permeability index (using total number of crosses and approaches from all study animals) for the duration of the study was calculated as well as monthly permeability indices. We used a paired t-test to compare monthly permeability indices calculated using the 30-minute and 5-hour data sets.

Using 30-minute monthly permeability indices, regression analysis was then used to determine if a relationship existed between monthly permeability indices and monthly traffic flow along the existing US 64.

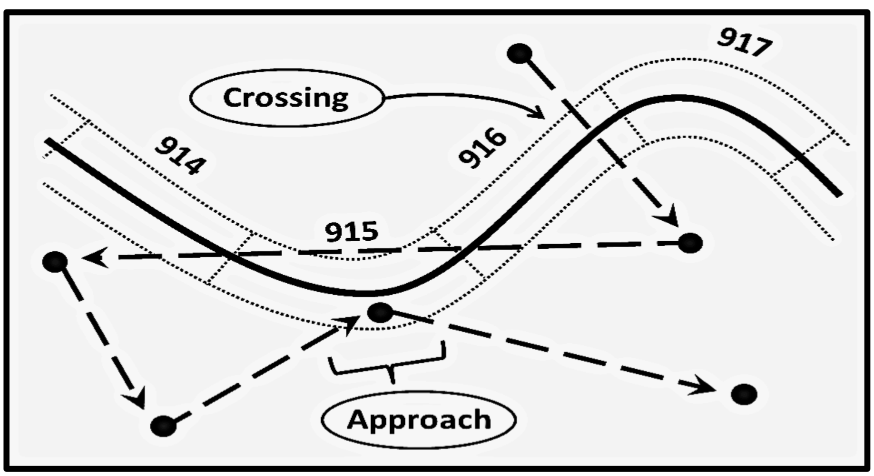


**Figure 2.** A **crossing** was defined as a line connecting two points on opposite sides of a road that intersects the roadway. An **approach** was defined as any excursion from a point further than 164 ft. from the road to a point within 164 ft., and then back, *without* crossing.

***Assessing the effect of the highway widening on current red wolf territories:*** To determine the potential for the highway widening to displace current red wolf packs, buffers at ~ 164 ft. (50 m) intervals were constructed around the current US 64. The buffers were then overlaid on current red wolf home range locations and the number of home ranges intersected by each buffer was counted. Where the buffers intersected home ranges, the percent of total home range intersected was calculated. As with the home range analysis above, only one home range per pack was used when the movements among individuals of a pack were correlated.

***Assessing the effect of the highway widening on important red wolf habitat:*** To determine the potential for the highway widening to affect important red wolf habitat, buffers around the current US 64 at ~164 ft. (50 m) intervals were overlaid on a habitat map. The area of available red wolf habitat was then calculated within each buffer zone.

### Objective 3: Identify significant red wolf crossing areas to determine where wildlife crossing structures or other design features could be placed to minimize adverse project effects on wolves.

***Determining crossing locations and rates using GPS collars*:** Because of the occurrence of different collar schedules, wolf locations were sub-sampled into both 5-hour and 30-minute intervals. The following methods were used to analyze data collected for each frequency, 5-hour and 30-minute, respectively. Using ArcGIS v9.3 (ESRI® ArcMap™ 9.3, Copyright © 1999-2010 ESRI Inc.), we divided the 27.3 mi-section of US 64 into 273 segments, each 0.10 miles long. To determine road crossings, we used the Home Range Tools v9 extension for ArcGIS to calculate the travel path of each individual by connecting consecutive GPS fixes. We then overlaid the travel paths on the segmented highway layer and counted the number of crossings per highway segment for each individual. A crossing was defined as two consecutive fixes on opposite sides of the highway (see Figure 2). Crossing rates for each individual were determined by dividing the number of crossings by the number of days the collar was actively collecting data for each collection frequency sub-sample. Total crossing frequencies per segment were plotted in a histogram to identify the location of key red wolf crossing areas.

***Statistical Analysis*:** To test the hypothesis that the crossing distribution calculated using GPS collar locations was different from a random crossing distribution, an equivalent number of random line segments were drawn between the GPS locations for each red wolf. To approximate actual red wolf movement, random segment lengths were constrained to less than or equal to the maximum distance moved by a red wolf for the 30-minute and 5-hour data sets, respectively. Crossing frequencies for the random segments were calculated for each highway segment following the methods above. The distributions for the GPS crossing frequencies and the random crossing frequencies were compared using a Kolmogorov-Smirnov test (Clevenger et al., 2001). We used a t-test to test for differences in crossing rates between male and female wolves and a one –way ANOVA to test for differences in crossing rates among age classes.

***Determining crossing locations using Camera Traps:*** Even with 30-minute locations, the GPS collars likely did not catch all red wolf crossing events. To capture additional crossing events, remote cameras were placed at canal crossings along the 27.3 mi stretch of US 64 within 328 ft. (100 m) of the roadside. Because drainage canals exist along the entire length of US 64, canal crossings serve as an access point for animals to reach the highway. We used both film and digital remote cameras triggered by laser or heat disturbance. All cameras were active 24-hours per day to maximize the number of crossings captured. Cameras were active from July 2009 to March 2011. However, the number of trap nights varied for each camera station so captures were reported per 100 trap nights. To avoid pseudoreplication, consecutive photos of an individual animal were considered a single event.

Using GPS locations, camera stations were associated with one of the 273 segments along US 64. Not all segments had camera stations. Total crossing frequencies per segment were plotted in a histogram to identify the location of key red wolf crossing areas as identified by cameras.

***Evaluating habitat characteristics at crossing sites identified by GPS collar locations:*** Using the NC GAP habitat map, we extracted the habitat type for each of the 273 highway segments at 164 ft. (50 m) intervals starting at the road to a distance of 656 ft. (200 m) perpendicular to the segment (ArcGIS v9.3). Segments that had at least one crossing were coded with a 1 and segments without crossings were coded with a 0. Logistic regression was used to evaluate 5 *a priori* models developed using site-specific habitat type at different distance intervals and the occurrence of a red wolf crossing. The most parsimonious model was chosen using AIC corrected for small sample size (AICc) (Burnham and Anderson, 2002), with models ranked using ΔAICc.

***Evaluating habitat characteristics at crossing sites identified by camera traps:***
Using the NC GAP habitat map, we extracted the habitat type for each of the camera stations at 164 ft. (50 m) intervals starting at the camera sites to a distance of 656 ft. (200 m) perpendicular to US 64 (ArcGIS v9.3). In addition, the width of the access road/trail was measured at each camera station. Camera sites that captured red wolves were coded with a 1 and camera sites that did not capture red wolves were coded with a 0. Logistic regression was used to evaluate 5 *a priori* models developed using habitat variables and trail width at camera placement. The most parsimonious model was chosen using AIC corrected for small sample size (AICc) (Burnham and Anderson, 2002), with models ranked using ΔAICc.

# Results

***Capturing and collaring of animals:*** Between January 2009 and April 2011, the USFWS Red Wolf Team deployed 32 of 40 collars. Due to a decrease in red wolf population in Washington County, North Carolina, the 8 collars reserved for red wolves living in the vicinity of the previously expanded portion of US 64 could not be deployed. Thirteen of the 32 collars deployed were placed on females (8 adults, 5 juveniles) and 19 on males (8 adults, 11 juveniles). The average collar deployment was 14.8 months (range: 4 to 30 months) and average collar success in obtaining GPS locations was 86.0% (range: 63.6% to 97.5%). In total, 39, 573 successful red wolf locations were collected. We used 6 different collar schedules: 30-minute locations for 5 hours per day, 5-hour locations, 5-hour locations with the nested 30-minute schedule for 5-hours per day, 11-hour locations, 12-hour locations, and 23-hour locations (Table 1).

**Table 1**. Summary of collar statistics for collared red wolves in Dare, Tyrrell, Washington, Beaufort, and Hyde Counties, NC.

| Pack/Area | Collar ID | Sex | Birth Year | Length of Collar Deployment (months) | Reason for Removal | Collar Schedule | Collar Success (%) |
|---|---|---|---|---|---|---|---|
| Milltail | 974 | M | 2005 | 30 | Found Dead | 5hr RO* | 96.20 |
| Milltail | 1779/1883 | M | 2006 | 28 | Died | 30 min w/5hr RO | 90.80 |
| Milltail | 1912/1896 | F | 2004 | 28 | On recovery | 30 min w/5hr RO | |
| Bombming range | 1910 | F | 2007? | 6 | Found Dead | 5hr RO | 79.80 |
| Milltail | 1777/1893 | F | 2008 | 21 | Shot | 30 min w/5hr RO | 91.10 |
| Milltail | 1913/1905 | M | 2008 | 22 | Collar Broken | 30 min | |
| Milltail | 1911/1889 | F | 2008 | 21 | Breeding Female | 30 min | 82.60 |
| Tyson | 1778 | M | 2008 | 21 | Going to be paired w/F | 5hr RO | 80.20 |
| F2 | 1916 | F | 2007 | 22 | Breeding Female/ paired w/M | 5hr RO\23hr RO\30 min w/5hr RO | 85.50 |
| Sunnyside | 1915 | M | 2007 | 23 | Collar in Recovery | 30 min w/5hr RO | 85.40 |
| Timberlake | 1901 | F | 2004 | 18 | Breeding Female | 5hr RO\30 min w/5hr RO | 86.50 |
| Waupaupin | 1894 | M | 2007 | 17 | On recovery | 11hr RO\30 min w/5hr RO | |
| Gator | 1895 | F | 2000 | | Still Deployed | 12hr | |
| Davis Fields | 1880 | F | 2001 | 7 | Shot | 5hr RO | 69.00 |
| Mannings | 1881 | M | 2006 | 7 | Not near any roads | 5hr RO | 63.60 |
| L-Block | 1892 | F | 2006 | 5 | Breeding Female | 30 min w/5hr RO | 80.60 |
| Columbia | 1906 | M | 2005 | 12 | On recovery | 5hr RO\30 min w/5hr RO | |
| Bishops | 1902 | F | 2008 | 9 | Breeding Female/ paired w/M | 30 min w/5hr RO | 94.00 |
| Columbia | 1900 | F | 2007 | 12 | Breeding Female/ paired w/M | 30 min w/5hr RO | 72.20 |
| Lil' Alligator | 1882 | M | 2008 | 10 | Going to be paired w/F | 30 min w/5hr RO | 91.50 |
| Frying Pan | 1909 | F | 2008 | 16 | Breeding Female/ paired w/M | 30 min | 94.50 |
| Tyson | 1884 | M | 2006 | | Still Deployed | 30 min | |
| Milltail | 1891 | M | 2009 | 4 | Hit by Car | 30 min | 97.50 |
| Swindell | 1887 | M | 2009 | | Still Deployed | 30 min w/5hr RO | |
| Milltail | 1904 | M | 2008 | 11 | Died | 30 min | 84.40 |
| Beech Ridge | 1897 | M | 2008 | | Still Deployed | 30 min w/5hr RO | |
| Swindell | 1908 | M | 2009 | | Still Deployed | 30 min w/5hr RO | |
| Beech Ridge | 1903 | F | 2008 | 14 | Breeding Female | 30 min w/5hr RO | 84.40 |
| East Lake | 1777 | M | ? | 9 | On recovery | 30 min w/5hr RO | |
| Camerons | 1898 | M | 2008 | 1 | Shot | 30 min | 95.60 |
| Killkenny | 1907 | M | 2009 | 13 | Management Purposes | 30 min | 94.30 |
| Killkenny | 1888 | M | 2009 | 12 | Management Purposes | 30 min | 92.80 |

* RO = Rollover. A rollover schedule collects locations continuously at a set time interval without resetting at the start of each day.

**Objective 1: Evaluate wolf habitat use along the entire US 64 corridor from Plymouth NC to the US 64/264 intersection**

***Home range analyses*:** Movements of individuals within the same pack were highly correlated ($r_s$ = 0.87–0.91); therefore only 1 animal per pack (chosen randomly) was used in the following analyses. After removing those individuals where multiple animals in a pack were collared, we calculated cumulative weekly home ranges for 21 of 32 animals (Figure 3). Following analysis, we removed 5 (1 juvenile, 2 dispersers, 2 adults) additional individuals from our sample due to an inadequate number of locations to capture a complete home range. Overall home range varied between 2.61 $mi^2$ and 38.19 $mi^2$ with a mean of 12.93 ± 9.50 $mi^2$

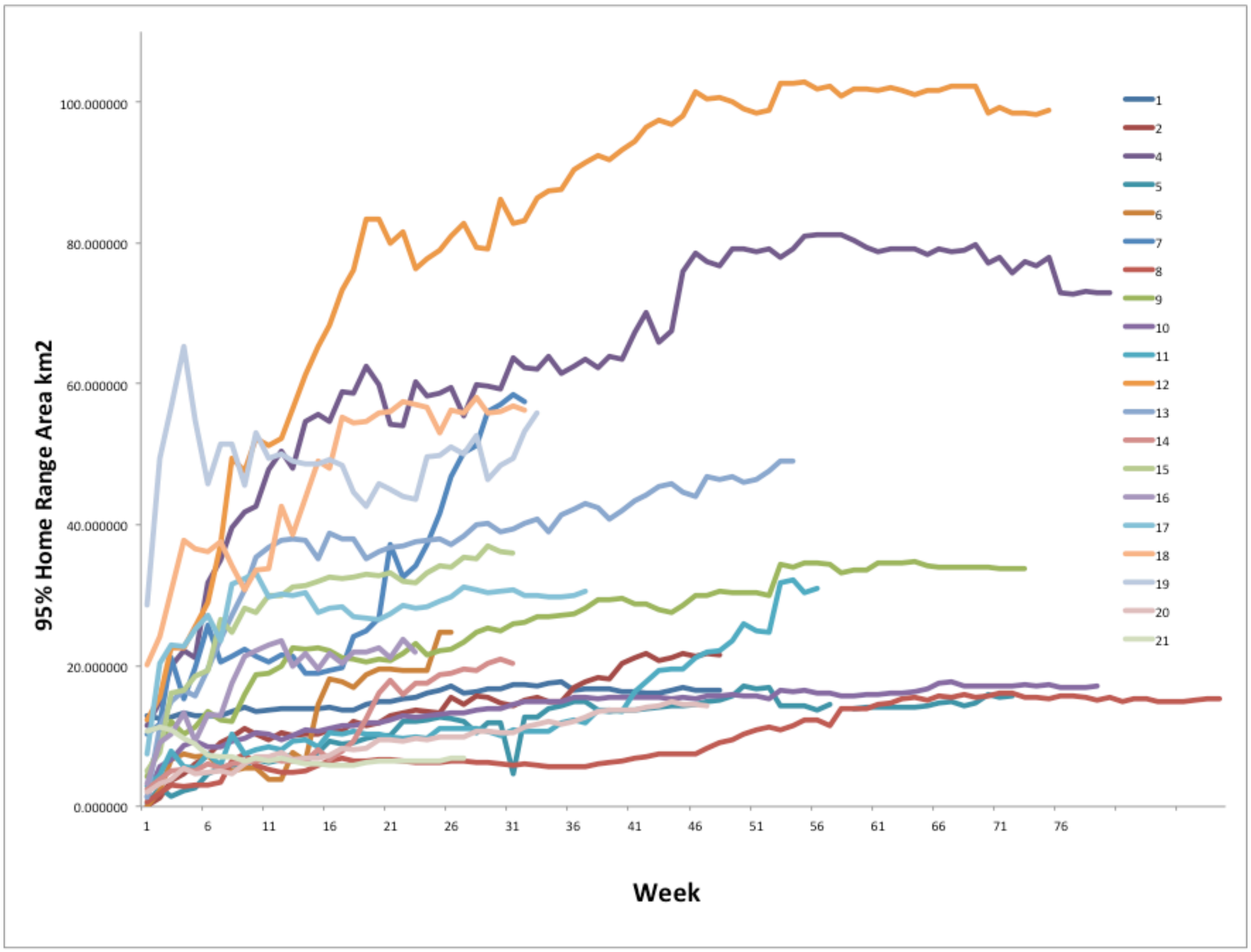


**Figure 3**. To determine if an adequate number of locations were obtained from each wolf to capture home range area, rarefaction curves of cumulative weekly home ranges were calculated for 21 red wolves of different age groups and sexes collared from January 2009 to April 2011. A home range is considered to be at equilibrium at the point that the home range area no longer increases and reaches a plateau (Bekoff and Mech, 1984). Home range did not reach equilibrium for 5 of the 21 wolves in our sample (6, 7, 11, 13, 14), thus they were excluded from further home range analyses. Collared red wolves were located in Tyrell, Dare, Washington, and Hyde Counties, NC.

Although home range size among age classes ($F$ =2.71, $P$ =0.14) and between sexes ($t_{10}$ = 2.10, $P$ = 0.57) did not differ significantly (Tables 2 and 3), the home range size of dispersers tended to be larger than those of juveniles or adults. Five of 8 animals that died while collared were dispersing and 2 additional dispersers were removed from further home range analyses due to inadequate data.

**Table 2**. Average and range of 95% home range areas for three age classes of red wolves. A local convex hull method was used to calculate home range from GPS collar locations. The home range analysis was generated from 16 red wolves collared in Washington, Tyrell, Dare, Hyde, and Beaufort Counties, NC from January 2009 to April 2011.

| | Juvenile | Disperser | Adult |
|---|---|---|---|
| **n** | 3 | 3 | 10 |
| **Average ($mi^2$)** | 9.15 | 21.86 | 15.29 |
| **Min ($mi^2$)** | 5.93 | 21.61 | 2.61 |
| **Max ($mi^2$)** | 11.95 | 22.21 | 38.19 |

**Table 3**. Average and range of 95% home range areas for male and female red wolves. A local convex hull method was used to calculate home range from GPS collar locations. The home range analysis was generated from 16 red wolves collared in Washington, Tyrell, Dare, Hyde, and Beaufort Counties, NC from January 2009 to April 2011.

| | Male | Female |
|---|---|---|
| **n** | 9 | 7 |
| **Average ($mi^2$)** | 14.54 | 12.11 |
| **Min ($mi^2$)** | 2.61 | 5.52 |
| **Max ($mi^2$)** | 38.19 | 22.21 |

Home ranges were composed primarily of agricultural fields with 95% home range isopleths on average containing 55% agricultural fields (Table 4). Summer home ranges were between 0.77 and 3.09 $mi^2$ smaller ($t_{10}$ = -4.84, $P$ < 0.01) than winter home ranges (Table 5). Average monthly home range percent composition was different between summer and winter. Red wolves increased their use of pocosin ($t_{10}$ = -2.65, $P$ =0.03), wetlands ($t_{10}$ = -4.29, $P$ < 0.01), and upland forests ($t_{10}$ = -4.17, $P$ < 0.01) in late winter and increased use of agricultural fields ($t_{10}$ = 3.44, $P$ < 0.01) in summer months (Table 6). Agricultural fields and successional fields account for over 65% of habitat composition regardless of season.

**Table 4.** Average composition of 95% home ranges for 16 red wolves collared in Washington, Tyrell, Dare, Hyde, and Beaufort Counties, NC from January 2009 to April 2011.

| Habitat Type | Average % |
|---|---|
| Pocosin | 6 |
| Lowland Forests | 6 |
| Successional Fields | 13 |
| Upland Forests | 12 |
| Wetlands | 8 |
| Agricultural Fields | 55 |

**Table 5.** Average monthly 95% home range areas for 16 red wolves collared in Washington, Tyrell, Dare, Hyde, and Beaufort Counties, NC from January 2009 to April 2011.

| Month | 95% isopleth ($mi^2$) |
|---|---|
| January | 7.56 |
| February | 5.97 |
| March | 5.71 |
| April | 4.31 |
| May | 4.54 |
| June | 4.98 |
| July | 4.84 |
| August | 4.84 |
| September | 5.73 |
| October | 6.60 |
| November | 6.54 |
| December | 6.36 |

**Table 6.** Average monthly percent habitat composition calculated using a 95% home range for 16 red wolves collared in Washington, Tyrell, Dare, Hyde, and Beaufort Counties, NC from January 2009 to April 2011.

| Month | 95% range isopleth percent habitat composition | | | | | |
|---|---|---|---|---|---|---|
| | Pocosin | Lowland Forests | Successional Fields | Upland Forests | Wetlands | Agricultural Fields |
| January | 10 | 4 | 16 | 5 | 6 | 59 |
| February | 5 | 4 | 15 | 16 | 8 | 51 |
| March | 6 | 7 | 13 | 12 | 8 | 54 |
| April | 3 | 4 | 13 | 11 | 5 | 64 |
| May | 2 | 3 | 14 | 9 | 4 | 67 |
| June | 2 | 3 | 16 | 7 | 3 | 69 |
| July | 1 | 2 | 13 | 5 | 2 | 76 |
| August | 1 | 3 | 14 | 7 | 3 | 71 |
| September | 3 | 5 | 13 | 7 | 5 | 66 |
| October | 3 | 4 | 15 | 8 | 6 | 64 |
| November | 2 | 3 | 17 | 7 | 5 | 66 |
| December | 5 | 4 | 16 | 7 | 7 | 59 |

***Habitat use and selection analyses*:** We used 29,680 locations of red wolves to construct the 2$^{nd}$ and 3$^{rd}$ order resource selection functions (RSFs), respectively (Johnson, 1980). Second order RSF predicted a patchy distribution of red wolves across the 5-county red wolf recovery area (Figure 4). Third order RSF predicted a relatively equal probability of habitat use by red wolves across a given home range (Figure 5). RSFs calculated for each individual wolf included the same variables as the most parsimonious 2$^{nd}$ and 3$^{rd}$ order RSFs. Thus, despite the fact that collars were deployed on wolves of all ages and both sexes over a range of collar deployment periods (2009, 2010, and 2011) and deployment lengths (4 to 30 months), no one animal was considered to bias the RSFs in a unique way different from other animals. The most parsimonious 2$^{nd}$ order RSF contained: habitat type, distance to roads and water, human density, an interaction between distance to road and habitat type, and an interaction between human density and habitat type (Table 7). The AICc weight of the most parsimonious 2$^{nd}$ order RSF was 0.98. The next most parsimonious RSF included an interaction between human density and distance to road, and had a $\Delta$AICc of 8 and an AICc weight of 0.02. Agricultural fields were more likely to be used than all other habitat types. Likelihood of habitat use by red wolves decreased as human density increased, distance to road increased, and distance to water sources (e.g. steams and ponds) decreased. As distance to road increased, lowland forest, pocosin, and wetland habitats were disproportionately less likely to be used by red wolves than other habitat types. As human density increased, upland forests and wetlands were more likely to be used by red wolves than other habitat types.

The most parsimonious 3rd order RSF contained: 5 habitat types, distance to roads, and distance to water sources (e.g. steams and ponds) (Table 8). The AICc weight of the most parsimonious 3rd order RSF was 0.75. The next most parsimonious RSF included habitat type, distance to water, and human density, and had a ΔAICc of 2 and an AICc weight of 0.25. Again, agricultural fields were more likely to be used than all other habitat types. Likelihood of habitat use by red wolves decreased as distance to roads and water increased.

To test the validity of our selected 2nd and 3rd order RSF models, we overlaid 9,893 red wolf locations withheld from the initial analysis on the resulting probability maps (Figures 4 and 5). The GPS-collar locations (observed) overlapped areas identified as high probability of red wolf occurrence (expected) for both 2nd ($t_1 = 0.79$, $P > 0.05$) and 3rd order ($t_1 = 1.06$, $P > 0.05$) RSFs.

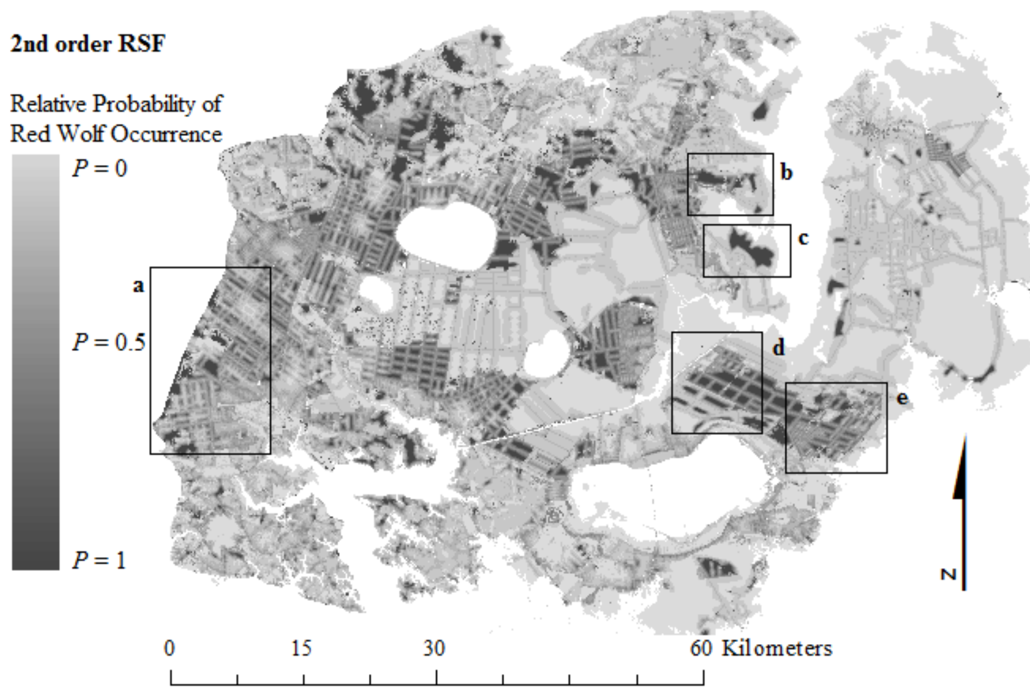


**Figure 4.** Relative probability of occurrence of red wolves (*Canis rufus*) across Washington, Tyrrell, Dare, Hyde, and Beaufort Counties, North Carolina with respect to 2nd order habitat use, 2009-2011. a) Relative location of packs no longer in existence but identified as habitat with high relative probability of occurrence of red wolves; b-e) Relative location of packs not represented in our dataset but in existence at the time of this study.

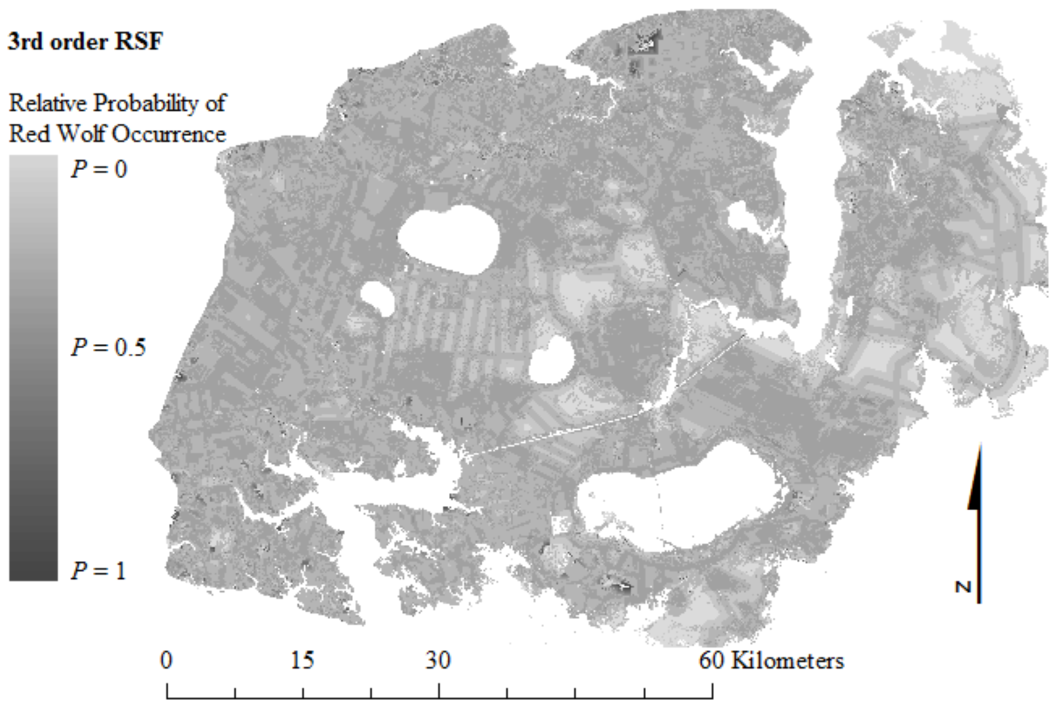


**Figure 5**. Relative probability of occurrence of red wolves (*Canis rufus*) across Washington, Tyrrell, Dare, Hyde, and Beaufort Counties, North Carolina with respect to 3rd order habitat use, 2009-2011.

**Table 7**. Most parsimonious 2nd order RSF, according to AICc, for habitat use of red wolves in Washington, Tyrrell, Dare, Hyde, and Beaufort Counties, North Carolina from 2009-2011.

| 2nd order RSF (AICc weight = 0.98) | | | | |
|---|---|---|---|---|
| Coefficient | Estimate* | SE | Lower CI | Upper CI |
| Intercept | 0.62 | 0.04 | 0.54 | 0.7 |
| Successional Field | -0.21 | 0.09 | 2.68E-03 | 3.02E-03 |
| Lowland Forests | -0.82 | 0.11 | -1.04 | -0.59 |
| Pocosin | -0.67 | 0.09 | -0.84 | -0.5 |
| Upland Forests | -0.95 | 0.08 | -1.11 | -0.79 |
| Wetlands | -0.81 | 0.08 | -0.96 | -0.66 |
| Dist. to Road | -1.29E-03 | 1.10E-04 | -1.50E-03 | -1.10E-03 |
| Dist. to H20 | 2.85E-03 | 0.04 | 2.70E-03 | 3.00E-03 |
| Human Density | -0.08 | 5.00E-03 | -0.09 | -0.07 |
| Dist. To Road x Lowland Forests | -2.70E-03 | 3.40E-04 | -3.37E-03 | -2.04E-03 |
| Dist. To Road x Pocosin | -2.79E-03 | 2.16E-04 | -3.21E-03 | -2.37E-03 |
| Dist. To Road x Wetlands | -2.48E-03 | 2.08E-04 | -2.88E-03 | -2.07E-03 |
| Human Density x Upland Forests | 0.09 | 0.01 | 0.08 | 0.1 |
| Human Density x Wetlands | 0.08 | 0.01 | 0.07 | 0.1 |

*The estimate is the logistic regression coefficient which describes the size and direction of the influence of each variable

**Table 8.** Most parsimonious 3rd order RSF, according to AICc, for habitat use of red wolves in Washington, Tyrrell, Dare, Hyde, and Beaufort Counties, North Carolina from 2009-2011.

| 3rd order RSF (AICc weight = 0.75) | | | | |
|---|---|---|---|---|
| Coefficient | Estimate* | SE | Lower CI | Upper CI |
| Intercept | 0.58 | 0.01 | 0.56 | 0.6 |
| Successional Fields | -0.07 | 0.01 | -0.09 | -0.05 |
| Lowland Forests | -0.1 | 0.01 | -0.13 | -0.08 |
| Pocosin | -0.11 | 0.01 | -0.13 | -0.08 |
| Upland Forests | -0.1 | 0.01 | -0.12 | -0.08 |
| Wetlands | -0.12 | 0.01 | -0.14 | -0.1 |
| Dist. To Road | -4.49E-05 | 2.56E-05 | -9.50E-04 | -1.80E-05 |
| Dist. To Water | -1.28E-04 | 2.25E-05 | -1.72E-04 | -8.37E-06 |

*The estimate is the logistic regression coefficient which describes the size and direction of the influence of each variable

**Objective 2: Evaluate the significance of red wolf habitat changes anticipated from the proposed highway project from Columbia to the US 64/264 intersection.**

***Quantifying barrier effects using passage rates*****:** Though 3 times as many crossings were recorded using the 30-minute collar schedule compared to the 5-hour schedule, the overall permeability for US 64 calculated from both collar schedules was approximately 100% (Table 9). No difference in monthly permeability index was found between the 30-minute and 5-hour collar scheduled ($t_{10}$ = 0.045, $P$ = 0.48). No relationship ($F=0.021, P=0.89, r^2=1.0$) was found between monthly permeability and monthly traffic flow (Figure 6).

**Table 9**. Permeability index for US 64 between Columbia, NC and the US 64/264 intersection in Manns Harbor, NC. The permeability index was calculated by dividing the number of highway crossings by (the number of highway crossings + the number of approaches). Road crossings were determined using red wolf GPS-collar locations collected between January 2009 and April 2011. A permeability index of 1 represents a highly permeable road while an index of zero indicates impermeability.

| Collar Schedule | No. of Wolves | No. of Crossings | No. of Approaches | Permeability Index |
|---|---|---|---|---|
| 5-hour | 6 | 25 | 0 | 1.00 |
| 30-minute | 8 | 76 | 1 | 0.99 |

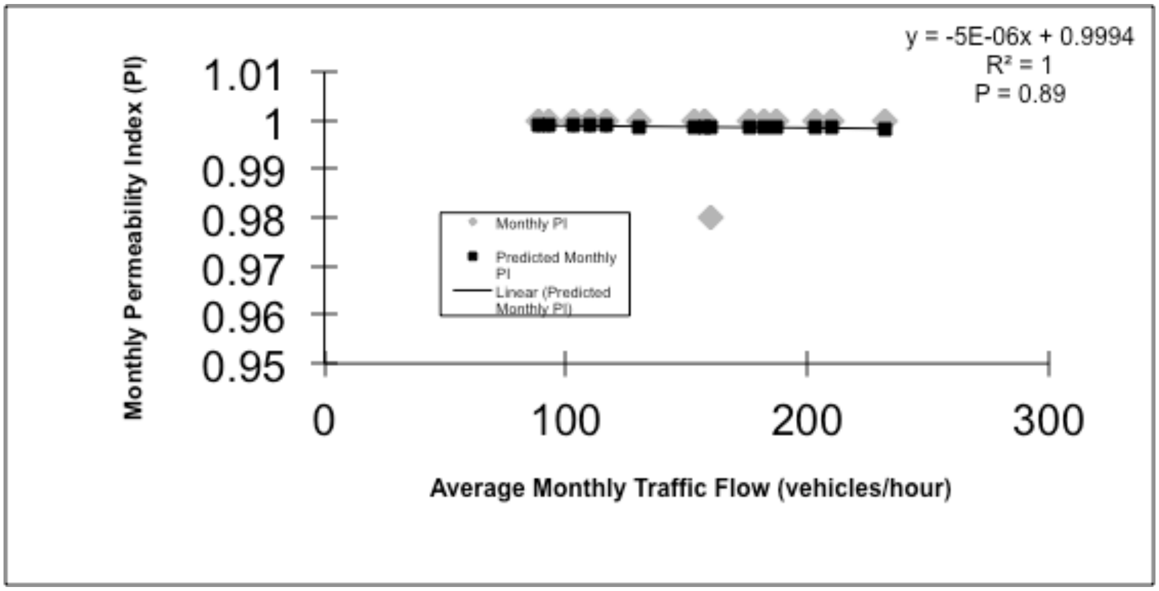


**Figure 6.** Regression analyzing the relationship between monthly permeability index and average monthly traffic flow rates (vehicles/hour). The monthly permeability index was calculated using GPS-collar data on the 5-hour schedule from 6 red wolves between March 2009 and May 2010 in Tyrrell and Dare Counties, NC.

***Assessing the effect of the highway widening on current red wolf territories:*** Buffers around US 64 to a distance of 3281 ft. (1000 m) in 164 ft. (50 m) increments overlaid on a map displaying current red wolf home ranges showed that 2 red wolf packs would be directly affected by a highway widening. One pack is located north of the current 2-lane highway in Tyrrell County and the second is south of the highway in Dare County, the only existing pack in Alligator River National Wildlife Refuge. The proportion of home range to be affected in Tyrell County ranges between 0.11% and 6.63% (Table 10) and is located between 820 ft. (250 m) and 3218 ft. (1000 m) from the existing highway just east of Columbia, NC where the previously widened portion of US 64 narrows to a 2-lane highway (Figure 7). In Dare County, the proportion of home range that would be affected ranges between 0.01% and 20.31% (Table 11) and is located south of US 64 between River Rd. and Bear Rd. on Alligator River National Wildlife Refuge (Figure 8).

***Assessing the effect of the highway widening on important red wolf habitat:*** Important red wolf habitat is defined as, following the results of the resource selection function analysis in objective 1, agricultural fields, successional fields, and upland forests. Buffers at 164 ft. (50 m) increments extending out to a distance of 3281 ft. (1 km) from US 64 overlaid on the NC GAP habitat map revealed that construction to the north of the current US 64 in Tyrrell County would remove more red wolf habitat than construction to the south. The opposite was found in Dare County, with more red wolf habitat at risk south of the current US 64 than north (Figures 9 and 10). If highway construction were to disturb the entire area between the existing US 64 and the 3281 ft. (1 km) buffer, a total of 0.16 $mi^2$ of red wolf habitat will be removed north of the highway vs. 0.09 $mi^2$ south of the highway in Tyrrell County. For Dare County, 0.04 $mi^2$ would be removed north of the highway vs. 0.07 $mi^2$ south of the highway if construction were disturb the whole area between the existing US 64 and the 3281 ft. (1 km) buffer.

**Table 10.** The proportion of a red wolf pack home range that will be directly affected by highway construction in Tyrell County between 820 ft. and 3218 ft. from the existing highway. The red wolf pack is located north of US 64 east of Columbia, NC where the previously widened portion of US 64 narrows to a 2-lane highway.

| Distance from US 64 (ft) | Area of Home Range Intersected ($ft^2$) | Percent of Total Home Range Intersected |
|---|---|---|
| 820 | 195312.38 | 0.11 |
| 984 | 603486.60 | 0.35 |
| 1148 | 1090902.45 | 0.64 |
| 1312 | 1643090.94 | 0.96 |
| 1476 | 2267019.48 | 1.33 |
| 1640 | 3024945.23 | 1.77 |
| 1804 | 3804239.09 | 2.22 |
| 1969 | 4595883.17 | 2.69 |
| 2133 | 5399701.05 | 3.16 |
| 2297 | 6215641.60 | 3.63 |
| 2461 | 7043620.94 | 4.12 |
| 2625 | 7883536.99 | 4.61 |
| 2789 | 8735389.60 | 5.11 |
| 2953 | 9599174.70 | 5.61 |
| 3117 | 10474842.94 | 6.12 |
| 3281 | 11335796.06 | 6.63 |

**Table 11.** The proportion of a red wolf pack home range that will be directly affected by highway construction in Dare County between 264 ft. and 3218 ft. from the existing highway. The home range is located south of US 64 in Alligator River National Wildlife Refuge between River Road and Bear Road.

| Distance from US 64 (ft) | Area of Home Range Intersected ($ft^2$) | Percent of Total Home Range Intersected |
|---|---|---|
| 246 | 13042.46 | 0.01 |
| 328 | 83853.62 | 0.04 |
| 410 | 216085.24 | 0.11 |
| 492 | 437479.56 | 0.23 |
| 574 | 748402.64 | 0.40 |
| 656 | 1263772.11 | 0.67 |
| 820 | 2790219.95 | 1.48 |
| 984 | 4548208.33 | 2.41 |
| 1148 | 6384017.38 | 3.39 |
| 1312 | 8293445.22 | 4.40 |
| 1476 | 10394240.80 | 5.51 |
| 1640 | 12531442.45 | 6.64 |
| 1804 | 14691958.47 | 7.79 |
| 1969 | 16867816.15 | 8.94 |
| 2133 | 19053292.57 | 10.10 |
| 2297 | 21293992.59 | 11.29 |
| 2461 | 23677537.88 | 12.55 |
| 2625 | 26280933.95 | 13.94 |
| 2789 | 28997590.89 | 15.38 |
| 2953 | 31868217.99 | 16.90 |
| 3117 | 34957184.80 | 18.54 |
| 3281 | 38297623.09 | 20.31 |

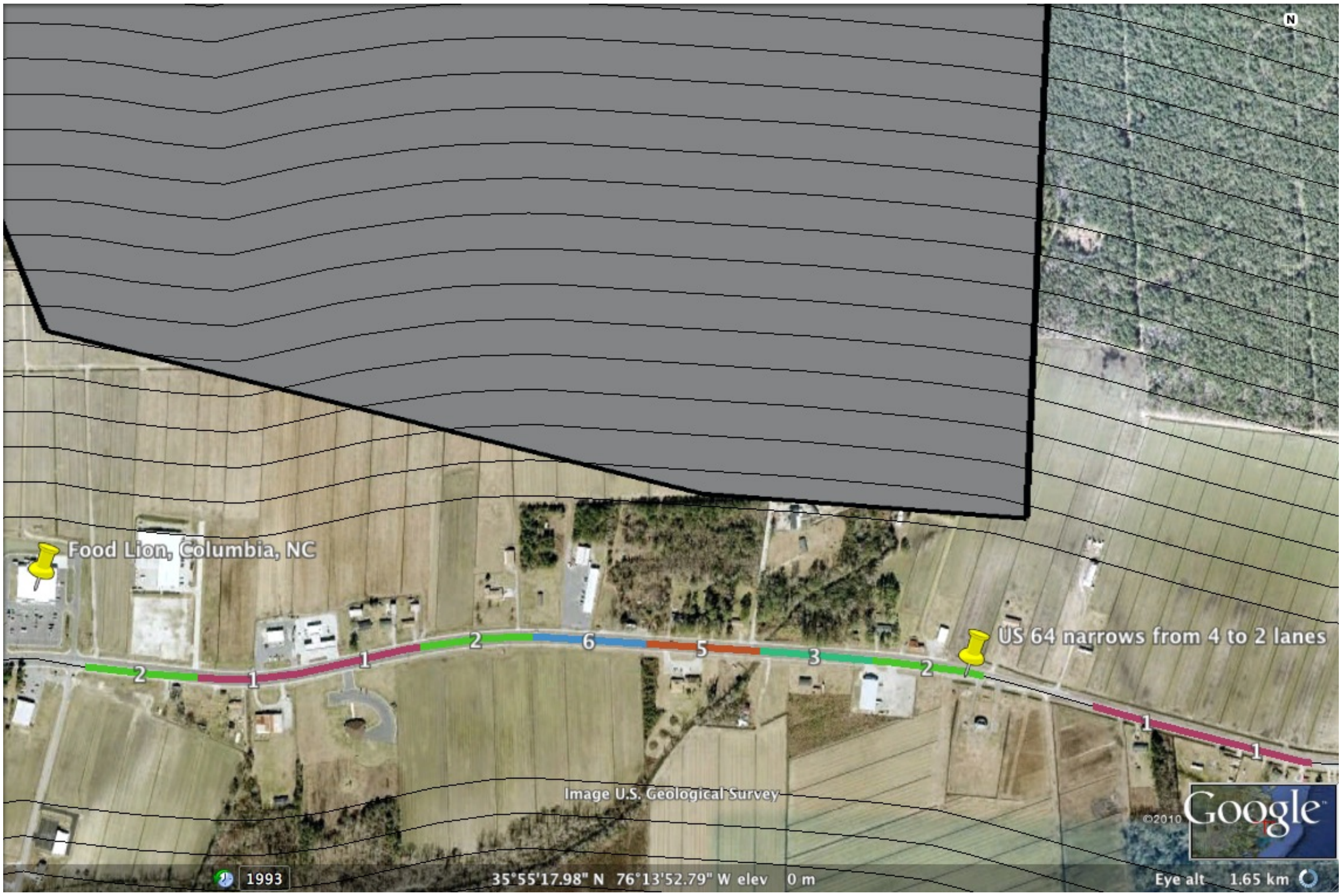


**Figure 7.** Location of the Tyrrell County red wolf pack home range with potential to be directly affected by highway construction. The home range extends to within 820 ft. from the existing highway where 4-lanes merge into 2-lanes just east of Columbia, NC. The highway buffer lines above (black lines) start at 820 ft. from US 64 and end at 3281 ft. in 164 ft. increments. If highway construction were to disturb the area between 820 ft. to 3281 ft. from the existing highway, 6.63% of this pack's home range would be removed. The numbers along US 64 indicate the number of red wolf highway crossings per 0.10 mi. segment captured using GPS-collar data collected between January 2009 and April 2011.

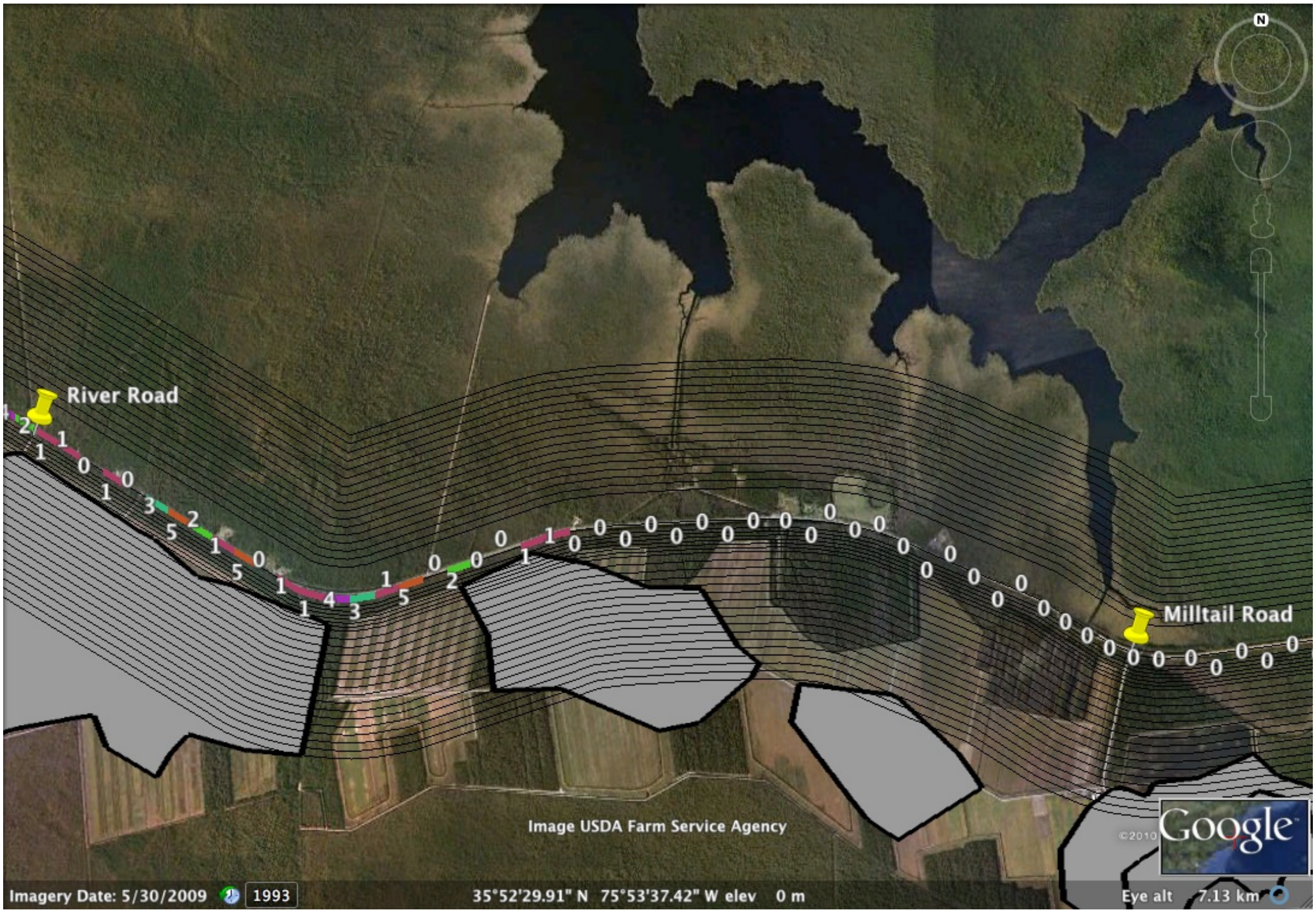


**Figure 8.** Location of the Dare County red wolf pack home range with potential to be directly affected by highway construction. The home range extends to within 264 ft. just south of the existing highway between River Rd. and Bear Rd. (east of Milltail Rd – not pictured) on Alligator River National Wildlife Refuge. This is the only red wolf pack on the refuge. The highway buffer lines above (black lines) start at 264 ft. from US 64 and end at 3281 ft. in 164 ft. increments. If highway construction were to disturb the area between 264 ft. to 3281 ft. from the existing highway, 20.31% of this pack's home range would be removed. The numbers along US 64 indicate the number of red wolf highway crossings per 0.10 mi. segment captured using GPS-collar data collected between January 2009 and April 2011.

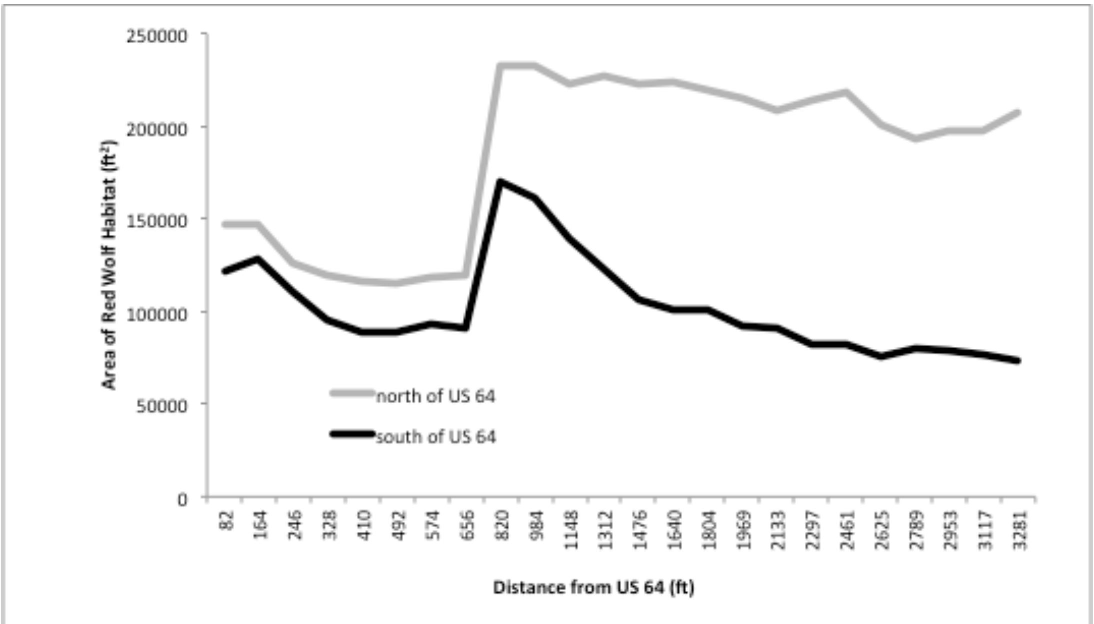


**Figure 9.** The area of important red wolf habitat per 164 ft. buffer for Tyrrell County, NC. Important red wolf habitat for eastern North Carolina includes agricultural land, upland forests, and early successional fields.

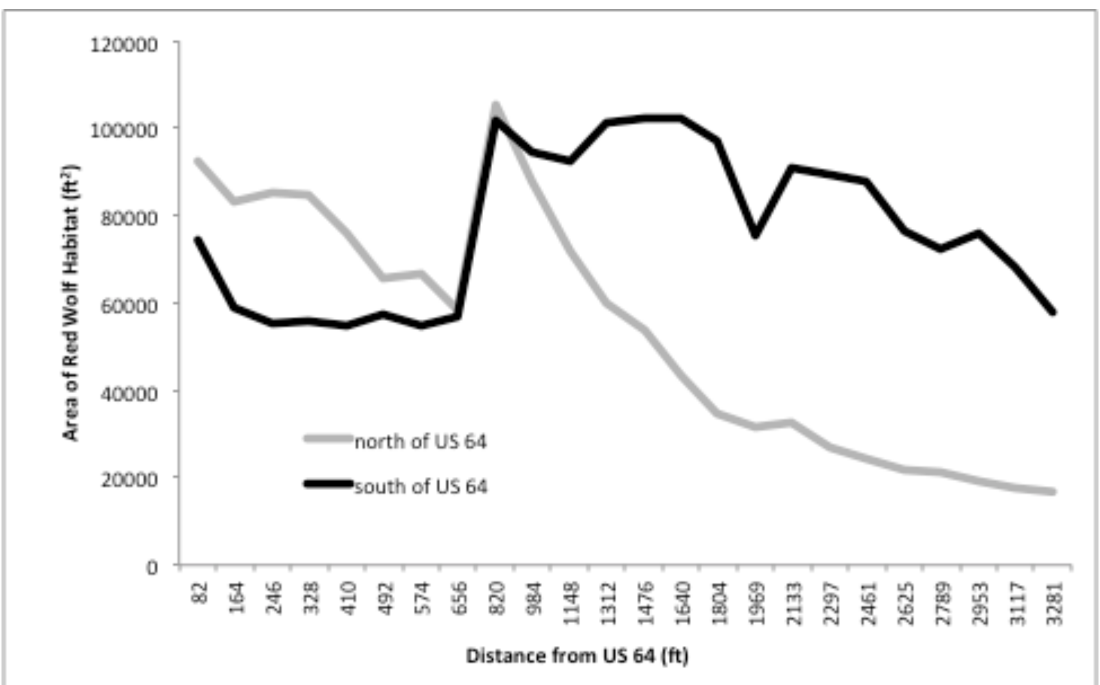


**Figure 10.** The area of important red wolf habitat per 164 ft. buffer for Dare County, NC. Important red wolf habitat for eastern North Carolina includes agricultural land, upland forests, and early successional fields.

**Objective 3: Identify significant red wolf crossing areas to determine where wildlife crossing structures or other design features could be placed to minimize adverse project effects on wolves.**

***Determining crossing locations and rates using GPS collars*****:** Six wolves on the 5-hour schedule (3 M: 3 F) and 8 on the 30-minute schedule (4 M: 4 F), with 3 wolves that started on the 5-hour and were switched to the 30-minute schedule, displayed crossing activity around US 64 out of a total of 32 collars. Only wolves with home ranges along US 64 crossed the highway. Wolves on the 5-hour schedule crossed between 2 and 9 times while wolves on the 30-minute crossed between 2 and 20 times. Five wolves (1 M: 4 F) on the 30-minute schedule, subsampled every 5-hours for a paired t-test, crossed 53 (30-minute) and 19 times (5-hour), respectively (*P=0.030),* showing that the 30-minute schedule captured nearly 3 times the road crossings as compared to the 5-hour rollover.

An additional 5-hour wolf, (8-year-old female #1880; Figure 11), crossed the highway 266 times. On reviewing the distribution of her points, which extended about 11 miles along the highway, it was determined that US 64 bisected the core of her home range (Figure 12). Additionally, her movements in a narrow band surrounding the road increased the likelihood of "false crossings," where the line connecting consecutive points on either side of the highway did not necessarily represent the true crossing location. For these reasons, and because 1880 represented an unusual circumstance that heavily skewed the rest of the data, this wolf was considered an outlier and removed from all further analysis of US 64 GPS-collar data.

**Figure 11**. Photo of wolf #1880 (adult female) obtained by camera trap along US 64 in Tyrrell County, North Carolina in July 2009.

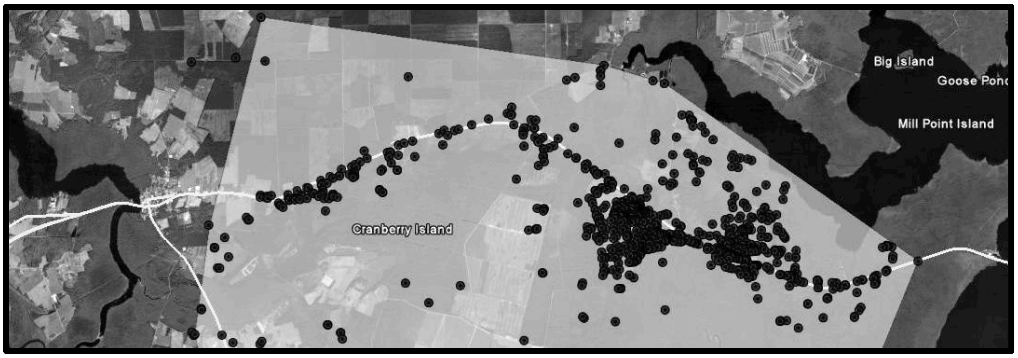


**Figure 12**. GPS-collar locations collected between April 2009 and November 2009 of wolf #1880 along US 64, Tyrrell County, North Carolina within its 95% MCP home range.

Although observed red wolf crossings and randomly generated crossings were both normally distributed, observed red wolf crossings occurred at a significantly lower frequency (*t=1.196, P=0.03*) and were bimodal as compared to the random crossings (Figure 13). Data from both the 5-hour and 30-minute schedules pointed to 2-crossing locations (Figures 14 and 15, respectively), 1 east of Alligator River in Dare County between miles 8 and 10 and 1 to the west in Tyrrell County centered on mile 28. However, the 30-minute data were more tightly concentrated and obvious. The two clusters of crossings identified by the GPS-collar data (Figure 16) coincided with where home ranges approached US 64.

Using the 30-minute collar data, red wolf highway crossing rates did not differ by wolf age ($F=5.14$, $P = 0.13$, $n = 13$; 3 juveniles, 3 dispersers, 7 adults) or sex ($t=0.32$, $P = 0.76$; $n = 13$; 7 males, 6 females).

***Determining crossing locations using Camera Traps:*** Crossing data were collected at 39 camera stations along US 64 accumulated over 8,154 trap nights. The average and median number of trap nights per station was 204 and 160, respectively. The number of trap nights per station ranged from 35 to 617 nights. Four red wolf crossing sites were identified from camera data, 3 west of Alligator River in Tyrrell County at miles 19, 20.5 and 23 - 24 and 1 east of Alligator River in Dare County between miles 9 and 10 (Figure 17). The crossing site in Tyrrell County between miles 23 – 24 and the crossing site in Dare County between miles 9 – 10 were considered one location each due to proximity and habitat continuity.

The combined GPS and camera crossing data indicated 5 important crossing sections along US 64 between Columbia, NC and the US 64/264 intersection, 4 west of Alligator River in Tyrrell County and 1 east of Alligator River in Dare County (see Figures 17 - 19). The crossing site in Dare County identified by the cameras overlaps with the crossing site identified using GPS-collar locations, however that was not the case in Tyrrell County. The 3 crossing sites identified in Tyrrell County using cameras are from crossings made by wolf #1880, the wolf excluded from collar analyses. The crossing site in Tyrrell County identified with the GPS-collar data occurred in an area where no cameras were placed, within the town limits of Columbia, North Carolina.

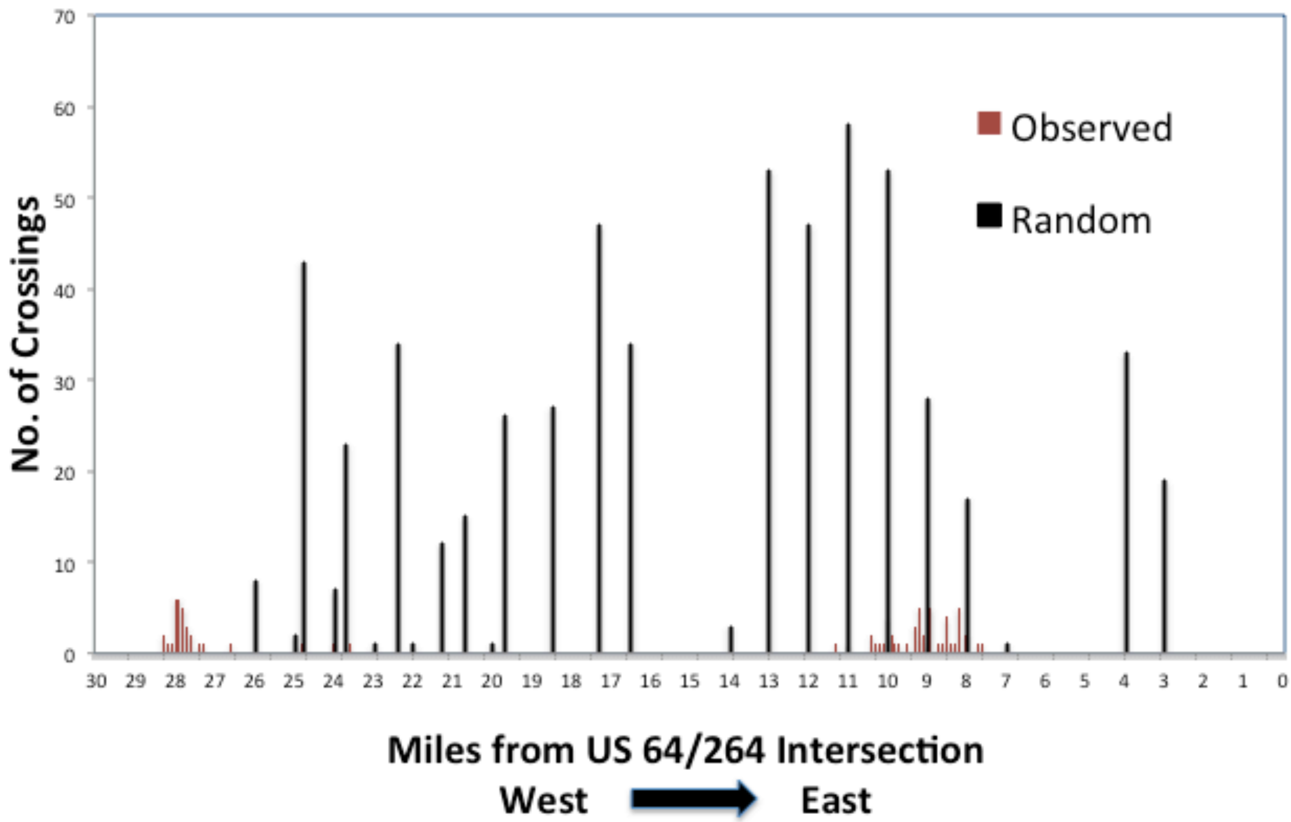


Figure 13. Observed red wolf crossings (red bars) occurred at a significantly lower frequency (*t=1.196, P=0.03*) and were bimodal as compared to the random crossings (black bars). Observed crossings are based on GPS locations taken at 30- minute intervals from red wolves in Washington, Tyrrell, Dare, Hyde, and Beaufort Counties, North Carolina collared between October 2009 and March 2011. An equivalent number of random line segments were drawn between the GPS locations for each red wolf. To approximate actual red wolf movement, random segment lengths were constrained to less than or equal to the maximum distance moved by a red wolf for the 30-minute data sets.

***Determining crossing locations using historical road kill data:*** From May 1988 to February 2009, 58 wolves (31 M, 27 F) died as a result of vehicle collisions in the recovery zone, with an average of nearly 3 wolves per year. Twelve of these occurred on US 64. While not significant, the locations of current known road-kills appear to be generally clustered around crossing sites identified in this analysis, particularly on US 64 (Figures 18 – 19). However many of the historic road kill events highlight the location of packs no longer present.

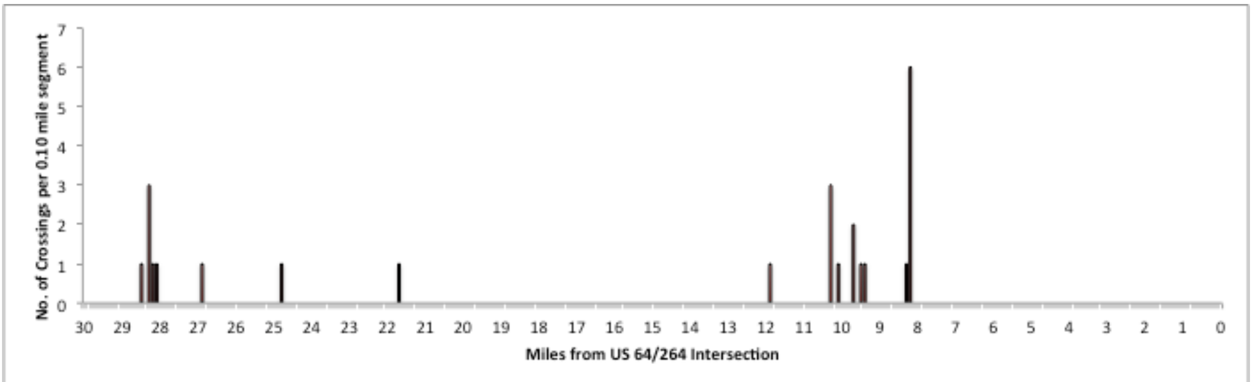


**Figure 14**. The number of red wolf crossings identified by GPS locations per 0.10 mile segments along US64 between Columbia, NC and the US64/US264 intersection. Crossings are based on GPS locations taken at 5- hour intervals between January 2009 and March 2011.

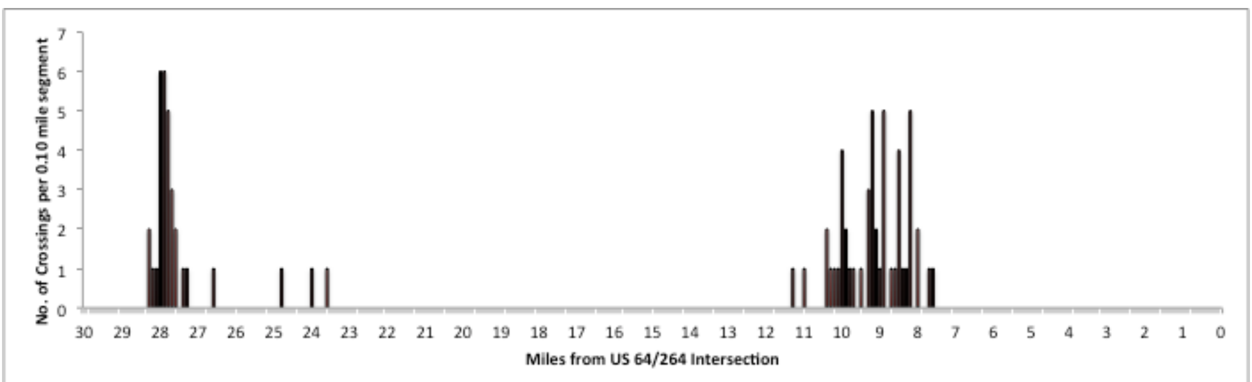


**Figure 15**. The number of red wolf crossings identified by GPS locations per 1 mile segments along US64 between Columbia, NC and the US64/US264 intersection. Crossings are based on GPS locations taken at 30- minute intervals between October 2009 and March 2011.

a)

b)

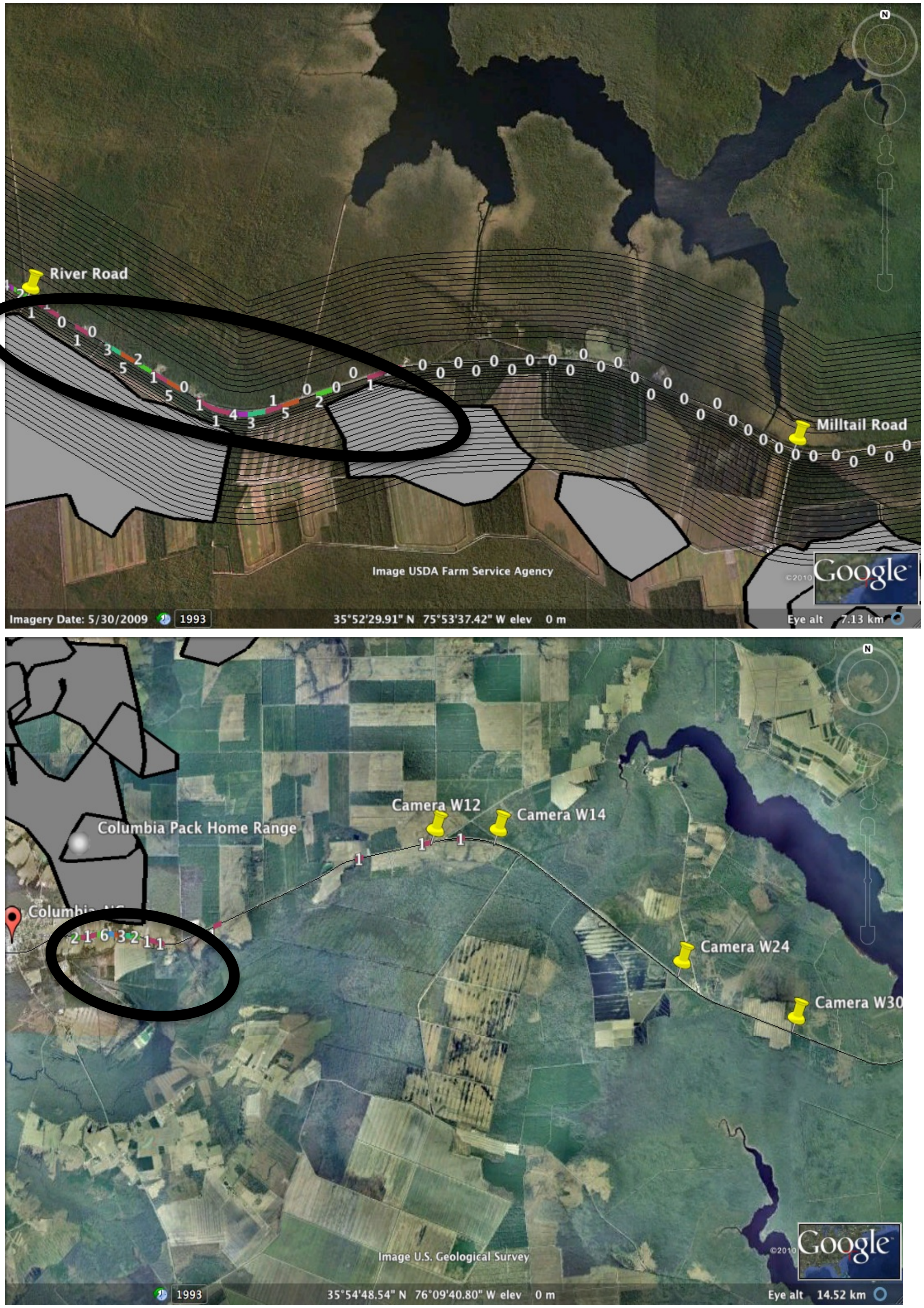


**Figure 16**. The two clusters of crossings identified by the GPS-collar data in both a) Dare and b) Tyrrell Counties, North Carolina coincided with the location where home ranges approached US 64. Crossings are based on GPS locations taken at 30- minute intervals between October 2009 and March 2011.

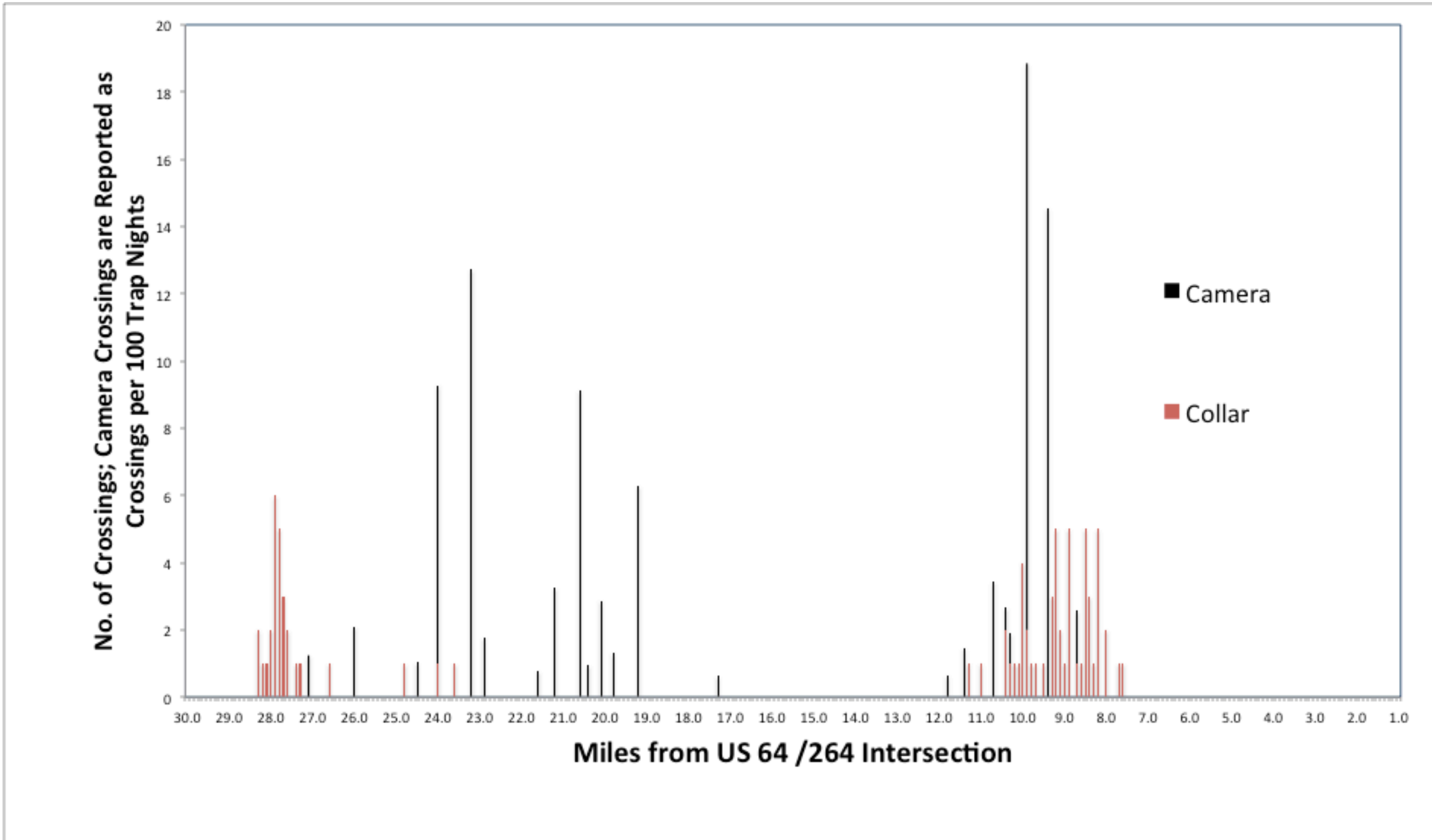


**Figure 17**. Combined GPS- collar (red bars) and camera (black bars) data, collected from 2009 – 2011, revealed 5 areas along US 64 currently used by red wolves as crossing sites, 4 in Tyrrell County west of Alligator River and 1 in Dare County east of Alligator River, North Carolina.  Camera data are presented as number of captures per 100 trap nights. The red wolf crossing site identified by camera data in Dare County (miles 9 – 10) overlaps with the crossing identified by GPS-collar data (miles 8 – 10). However, crossing sites identified by camera data in Tyrrell County (miles 19, 20.5 and 23 – 24) do not overlap with the crossing site identified using GPS-collar data (mile 28). The crossing site in Dare County between miles 9 – 10 and the crossing site in Tyrrell County between miles 23 – 24 were considered one location each due to proximity and habitat continuity.

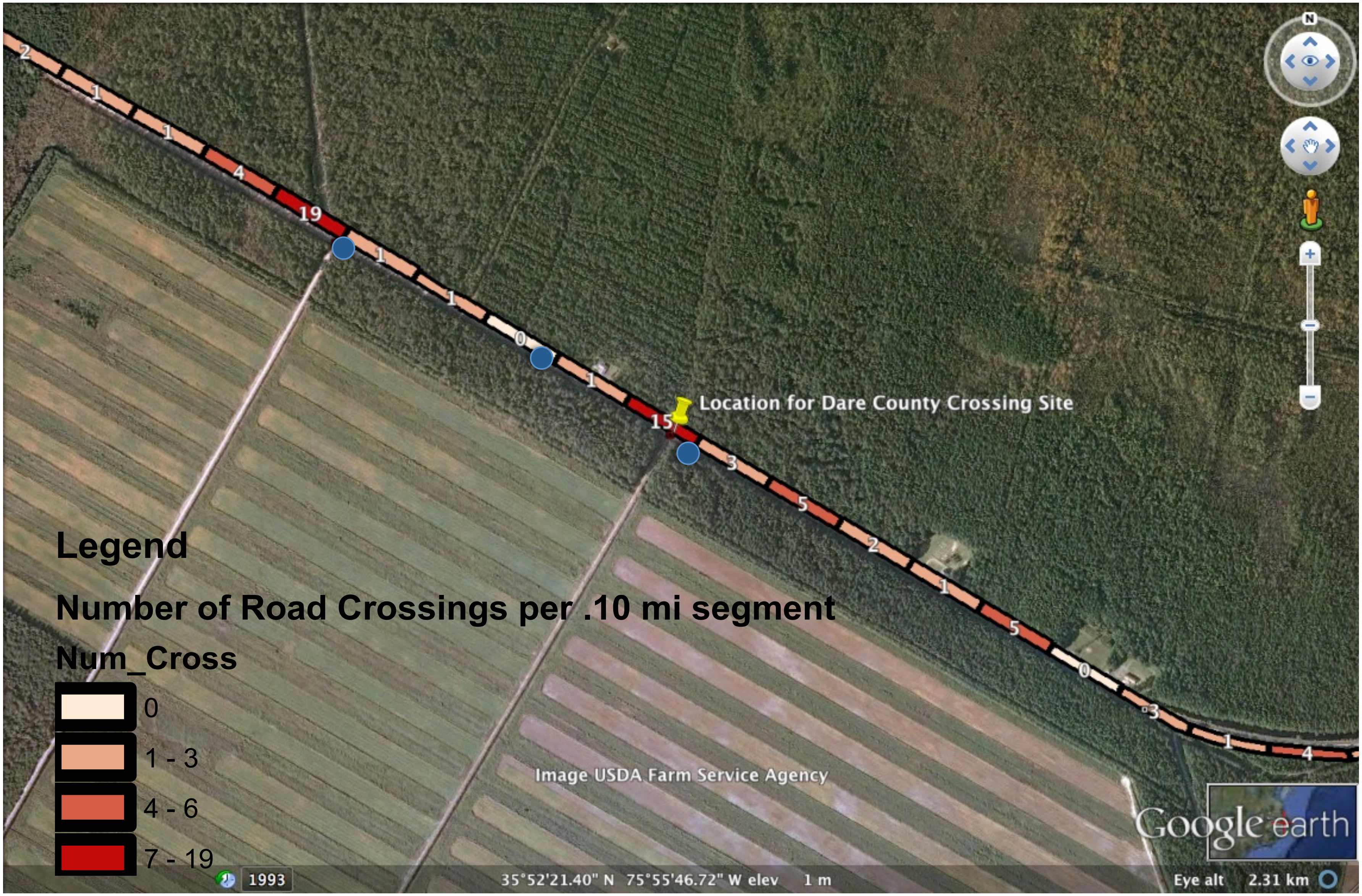


**Figure 18.** Map of red wolf highway crossing locations along US 64 in Dare County, NC. Crossing locations were calculated from GPS collar and remote camera data. The numbers indicate how many crossings per 0.10 mi. segment were recorded. The yellow marker shows the suggested location for a red wolf crossing structure in Dare County. The blue points indicate the location of a red wolf road mortality event.

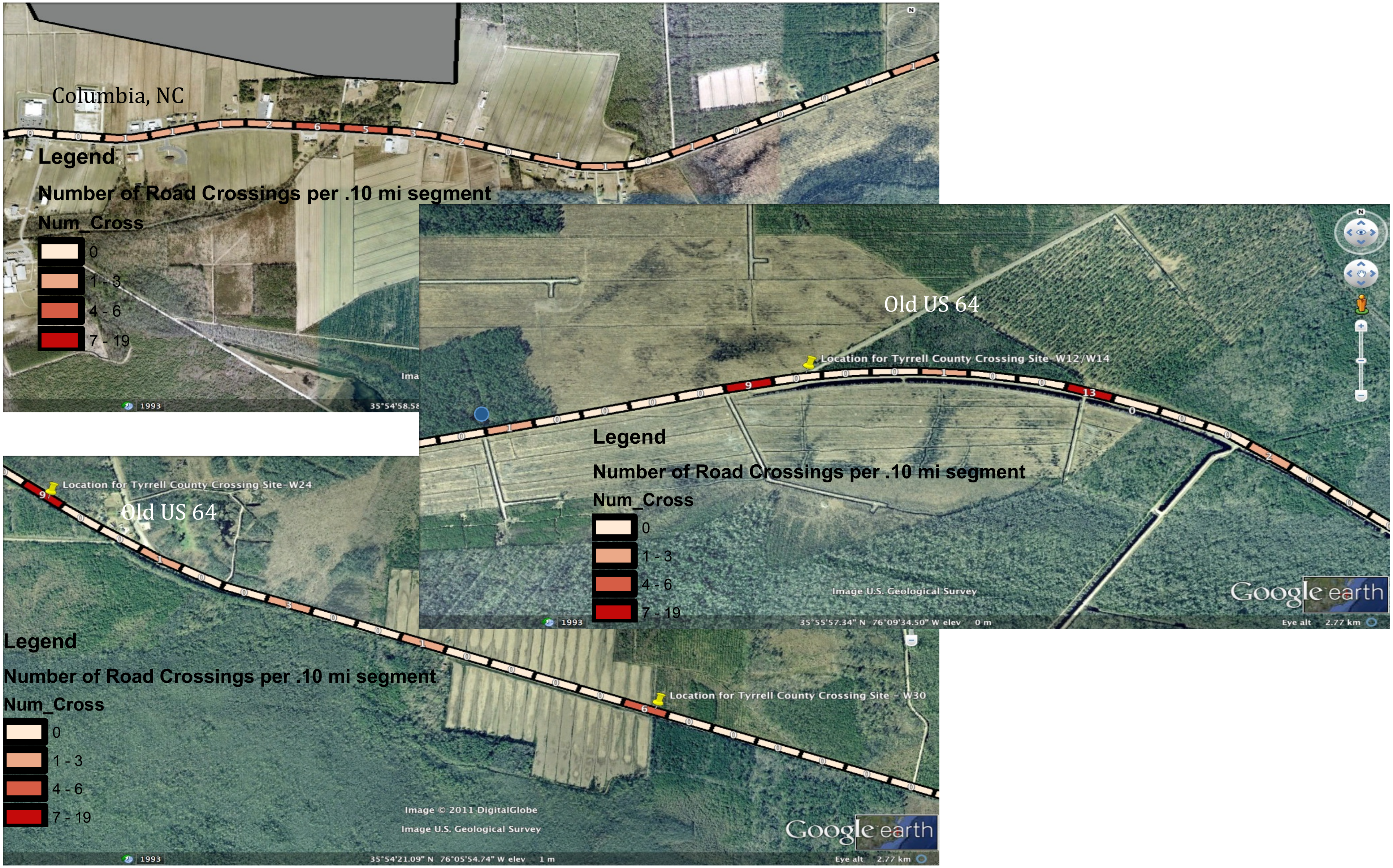


**Figure 19.** Map of red wolf highway crossing locations along US 64 in Tyrrell County, NC. Crossing locations were calculated from GPS collar and remote camera data. The numbers indicate how many crossings per 0.10 mi. segment were recorded. The yellow markers show the location of the 3 suggested locations for red wolf crossing structures in Tyrrell County. The blue point indicates the location of a red wolf road mortality event.

***Evaluating habitat characteristics at crossing sites identified by GPS collar locations:*** The most parsimonious habitat model at crossing locations determined using GPS-collar data included habitat type at distances of 328 ft. and 492 ft. from the crossing site (Table 12). The AICc weight of the most parsimonious habitat model was 0.69. The second most parsimonious model included habitat type at 656 ft. from the crossing site and had a ΔAICc of 3.19 and an AICc weight of 0.07. The habitat types at distances of 328 ft. and 492 ft. from crossing locations correspond to those identified by resource selection functions for red wolves: agriculture, upland forests, and early successional fields.

***Evaluating habitat characteristics at crossing sites identified by camera traps:*** The most parsimonious habitat model at crossing locations determined using camera trap data included width of the road/trail at the camera location (Table 13). The AICc weight of the most parsimonious habitat model was 0.89. The second most parsimonious model included road/trail width and habitat type at 164 ft. from the camera site and had a ΔAICc of 6 and an AICc weight of 0.03. The trail widths (which ranged from 1.64 ft. to 65.6 ft.) at camera trap locations with recorded red wolf crossings were 26.24 ft. or wider.

**Table 12**. Most parsimonious habitat model for red wolf crossing sites in Tyrrell and Dare Counties, NC identified using GPS-collar data collected between January 2009 and April 2011.

| Model | AICc | ΔAICc |
|---|---|---|
| Habitat 329 ft from US 64 + Habitat 492 ft from US 64 | 42.99 | 0.00 |
| Habitat 656 ft from US 64 | 46.18 | 3.19 |
| Habitat 164 ft from US 64 + Habitat 328 ft from US 64 +Habitat 492 ft from US 64 + Habitat 656 ft from US 64 | 46.84 | 3.85 |
| Habitat 164 ft from US 64 + Habitat 328 ft from US 64 | 55.19 | 12.20 |
| Habitat 164 ft from US 64 | 55.42 | 12.43 |

**Table 13**. Most parsimonious habitat model for red wolf crossing sites in Tyrrell and Dare Counties, NC identified using camera trap data collected between March 2009 and April 2011.

| Model | AICc | ΔAICc |
|---|---|---|
| Trail/Access Road Width | 40.08 | 0.00 |
| Trail/Access Road Width + Habitat 164 ft from US 64 | 46.03 | 5.95 |
| Habitat 656 ft from US 64 | 52.95 | 12.87 |
| Trail/Access Road Width + Habitat 328 ft from US 64 + Habitat 492 ft from US 64 | 73.48 | 33.40 |
| Habitat 164 ft from US 64 + Habitat 328 ft from US 64 + Habitat 492 ft from US 64 | 74.32 | 34.24 |

# Discussion

## Objective 1: Evaluate wolf habitat use along the entire US 64 corridor from Plymouth NC to the US 64/264 intersection

Understanding of basic species survival needs is required before completing any wildlife management plan. This study used data from 16 wolves from 16 different packs to estimate home range size and habitat selection of red wolves (*Canis rufus*) in eastern North Carolina. The home range sizes we calculated (2.61 $mi^2$ – 38.19 $mi^2$) were smaller than those reported in 2 earlier studies that followed 3-red wolf packs each (Phillips et al., 2003: 13.40 $mi^2$ - 78.10 $mi^2$; Chadwick et al., 2010: 31.51 $mi^2$ - 57.72 $mi^2$). However, if the red wolf pack with the largest home range size in the Phillips et al. (2003) study is excluded, the home ranges for the remaining 2 packs fall within the range of our findings (13.40 $mi^2$ - 30.00 $mi^2$). In addition, Phillips et al. (2003) used minimum convex polygons to determine home range size where as we used α-NNCH, a more conservative method of home range estimation (Getz et al., 2007), which could account for the discrepancy in home range sizes between the two studies. Chadwick et al. (2010) tracked males 2 – 3-years in age, two of which were brothers, and therefore may have been dispersing individuals. Though not significantly different, our study showed that dispersing animals tended to have larger home ranges than adults or juveniles, which could account for the differences in home range size between our study and the one completed by Chadwick et al. (2010). Small sample size likely accounts for no significant difference in home range size among age classes. Seven dispersers were eliminated from this study, 5 due to death and 2 because of inadequate data. Summer (June – September) home range size (4.84 $mi^2$ – 5.73 $mi^2$) averaged for all 16 packs over 2-years (2009 and 2010) corresponded to summer home ranges reported (1.34 $mi^2$ – 4.72 $mi^2$) for one red wolf pack monitored during the summer of 2005 (Hinton et al., 2010). We did not look at the influence of pack size on home range size, as previous research suggests that a relationship does not exist between pack size and home range size in gray wolves (Jedrzejewski et al., 2007).

Similar to Phillips et al. (2003) and Chadwick et al. (2010), our study revealed that home range size varied with season, being smaller during summer months and larger in winter with monthly home range size peaking in January. Smaller home ranges in summer are likely due to the presence of pups (Phillips et al. 2003; Chadwick et al., 2010). Mating, den preparation and whelping for red wolves typically occurs between February and April (C. Lucash, per. comm.), which coincides with the reduction of monthly home range sizes. This study found that monthly home range continually reduced in size starting in February and continued until reaching the smallest size in April. Monthly home ranges remained small until September when they started a steady increase that peaked in January. Jedrzejewski et al. (2001) showed home range size and movement patterns of gray wolf (*Canis lupus*) packs were also influenced by reproductive cycles.

Habitat and prey availability also may influence seasonal fluctuations in home range size. We found that habitat in the home ranges was primarily composed of agricultural fields year round. However, the percentage of agriculture within home ranges was highest in summer and lowest in winter. Increased use of agricultural fields in summer could be due to increased food resources available to prey species of red wolves such as white-tailed deer. A recent study found that red wolves readily prey on adult white-tailed deer (*Odocoileus virginianus*) and fawns during summer months (Dellinger et al., *In Press*). Growth of crops in agricultural fields in summer could help concentrate prey (Vercauteren and Hygnstrom, 1998). Additionally, the birth of fawns in early summer could provide a source of prey that is easier to catch, thus allowing red wolf packs to gather adequate food in a smaller area. Variation in home range size due to prey availability also has been shown in gray wolves (Ballard et al., 1987).

A decrease in percentage of agricultural fields making up home ranges in winter may be related to the harvesting of crops. Harvesting eliminates food resources available to prey and eliminates potential cover for red wolves. This study found an increase in non-agricultural habitats, such as upland forests, pocosins, and wetlands, during the fall and winter months (Table 6). Chadwick et al. (2010) noted that increased use of non-agricultural habitats corresponded to the harvesting of row crops between September and November and with the onset of the hunting season. Although this study showed that red wolves typically selected against non-agricultural habitats, cover types such as early successional fields, upland forests, and pocosins could be providing essential cover for red wolves after crop harvesting. Also, red wolves tend to prefer cover types with denser ground vegetation for den sites (Phillips et al., 2003), thus leading to a switch in habitat use during late winter and spring months.

Another important habitat finding is the selection for areas closer to roads. Most roads in the red wolf recovery zone are unpaved gravel or dirt roads used for agricultural purposes (C. Lucash pers. comm.). Red wolves likely used the road network as travel corridors, which could allow for packs to persist in areas where habitats are highly interspersed and large parcels of quality habitats are few.

***Conclusion:*** White and Ernst (2004), Singleton et al. (2005), and Kindall and van Manen (2007) all stress the need to identify habitat linkages across barriers to properly place crossings. Thus, it is imperative not only that wildlife underpasses are constructed in areas identified as high use for crossings (Scheick and Jones, 1999), but also that crossings are placed in a manner that connects habitat being selected by the species of concern. This study suggests that red wolf crossing structures should connect agricultural landscapes that are interspersed with upland forests, successional fields and pocosins. In addition, avoiding the aforementioned cover types during construction will minimize direct impacts to the red wolf population.

### Objective 2: Evaluate the significance of red wolf habitat changes anticipated from the proposed highway project from Columbia NC to the US 64/264 intersection.

This was the first study to employ the use of a permeability index to a non-seasonal migrating species. This provided a challenge in determining what could be considered a road “approach”, as we had to be careful not to include normal movements within a home range as an approach. The resulting buffer width of 164 ft., which is similar to the buffer width suggested for gray wolves (Paquet and Callaghan, 1996), illustrates the willingness of red wolves to establish home ranges in close proximity to the current 2-lane highway. The resulting permeability indices calculated for the 2-lane section of US 64 using both 5-hour and 30-minute data were 1.0 and 0.99, respectively. This suggests that the current 2-lane highway is not discouraging the red wolf population from attempting to cross US 64. However, it is important to note that only 14 of the 32 collared red wolves crossed a highway within the 5-county recovery zone, and 8 of those only crossed either once or during dispersal. Just 6 wolves from 3 packs crossed a highway regularly, and all 3 packs had home ranges that were adjacent to or straddled US 64.

The original goal was to compare permeability indices between the previously widened 4-lane section of US 64 in Washington County to the permeability index for the 2–lane section. However, a decrease in and near disappearance of the red wolf population to the west of Columbia, NC, prevented the collaring of red wolves where the widening to a 4-lane highway already was completed.

Although the current 2-lane highway is not discouraging wolves from attempting to cross, it is important to note that most roads exhibit a distinct trade-off between permeability and road kill (Forman and Alexander, 1998). A highly permeable road might result in a high level of wildlife/vehicular collisions, whereas an impermeable road might have few road kill events. Yet this decrease in road kill comes at the expense of habitat connectivity. This trade-off indeed holds true for US 64. Though the 2-lane portion of US 64 may not be hindering attempts to cross, road mortality is the second leading cause of death among red wolves accounting for 14% of mortalities (USFWS, 2007).

Permeability was expected to behave inversely to traffic flow, decreasing during the busy summer months and increasing during the winter. However, due to the high permeability of the highway, no such relationship existed. In addition, time of day may play an instrumental role in the event that crossing times (typically at night) do not coincide with peak traffic hours (midday). Such a pattern could be determined by separating traffic flow and permeability data by time. It should also be noted that while traffic fluctuates heavily on US 64 between summer and winter, the highway experiences relatively low traffic volume (maximum 250 vehicles per hour during the peak season) in comparison to other highways in the vicinity of the Outer Banks outside of the study area (~791 vehicles per hour; Currituck Development Group, 2011). The Federal Highway Administration reports that relatively few animals avoid crossing the road at traffic volumes below 2,500 cars per day and, that while road avoidance increases at moderate volumes (2,500 – 10,000 cars per day), it is not until traffic volume surpasses 10,000 cars per day that a large portion of animals will avoid highway crossing attempts (Clevenger and Huijer et al., 2011). The average daily traffic volume for the study site is 1,995 cars per day with a peak of 6,500 cars per day in July, placing the focal section of US 64 in the low to moderate traffic flow category as defined by the Federal Highway Administration.

Construction north of the current US 64 in Tyrrell County has the potential to remove a maximum of 0.16 $mi^2$ of red wolf habitat and 6% of the home range area used by a current red wolf pack while construction to the south will directly impact only 0.09 $mi^2$ of red wolf habitat and will not displace any current red wolf packs. East of Alligator River in Dare County, a widening of the current highway to the south has the potential to lead to a loss of 0.07 $mi^2$ of red wolf habitat and 20% the home range used by the only existing red wolf pack in Alligator River National Wildlife Refuge. Construction to the north of US 64 in Dare County will only remove up to 0.04 $mi^2$ of red wolf habitat and will not overlap with any current packs. Therefore, limiting construction to the south of the existing US 64 in Tyrrell County and north of the highway in Dare County will avoid direct effects to the current red wolf population.

We highlight that these results quantify only direct effects on current wolf home ranges. Road construction can have many indirect effects through changing hydrology; air, water, noise, and light pollution levels; wind flow; humidity; temperature; vulnerability to invasive species; and habitat continuity (Forman et al., 2003; Coffin, 2007). These indirect effects of the construction can disrupt red wolves living adjacent to the existing highway causing a shift in current home ranges. Any shift in home ranges has the potential to affect social order, mating, and ability to locate prey. At this time we are not able to quantify these effects, but these potential indirect effects may be measured in the "during-" and "post-" construction phases of the project.

***Conclusion:*** Road permeability, calculated using GPS-collar data, was 100%, thus the current 2-lane highway does not discourage the red wolf population from attempting to cross US 64. This does, however, increase the risk of road mortality events. A decrease in the red wolf population to the west of Columbia, NC, prevented collaring of red wolves where widening to a 4-lane highway was completed. Therefore, we were not able to compare highway permeability between 2- and 4-lane highways. To avoid any direct effects to the current red wolf population, highway construction should be limited to the south of the existing US 64 in Tyrrell County and north of the highway in Dare County. Potential indirect effects of highway widening activities were not quantified, as they could not be quantified using GPS or camera data. It is important to note that indirect effects can negatively effect the red wolf population.

### Objective 3: Identify significant red wolf crossing areas to determine where wildlife crossing structures or other design features could be placed to minimize adverse project effects on wolves.

Movement patterns obtained through tracking of GPS locations and remote camera traps clearly demonstrate that red wolves crossed US 64 and two other highways in the red wolf recovery zone, with some frequency. This implies there is potential for one of two outcomes of widening the road from 2 to 4 lanes: (1) either increased traffic or the increased width itself may increase road mortality, or (2) these factors may decrease road permeability. The degree to which these threats are deemed relevant and serious will have a significant bearing on NC DOT's planning and execution of the construction project.

The distribution of GPS-collar locations and camera trap photos along US 64 revealed 5 distinct red wolf crossing sites, 4 west of Alligator River in Tyrrell County and 1 east of the river in Dare County. Though they do not overlap completely, GPS-collar data and camera trap data are in accordance for the location of the crossing site in Dare County. However, that is not the case in Tyrrell County. GPS-collar data revealed 1 crossing site in Tyrrell County, while camera trap data identified the 3 additional crossing sites. Two factors contributed to the 2-methods not overlapping in Tyrrell County. First, due to the number of false crossings, GPS-collar data from wolf #1880 was eliminated from our analysis, thus eliminating GPS-collar data along the section of US 64 coinciding with the cameras. False crossings were obtained from this wolf because US 64 bisected her home range (Figure 12). Secondly, cameras were not set up where GPS-collar data identified a crossing location in Tyrrell County due to increased risk of camera theft within the town limits of Columbia, NC.

The potential for "false crossings," which may suggest that a crossing took place in a different location from where it actually occurred, exists in the remainder of the GPS-collar data as well. However, the presence of such distinct activity clusters, particularly under the 30-minute schedule, suggests that our results captured real movement trends. Although we captured nearly 3 times as many crossing events and the data displayed tighter clustering with better defined locations using the 30-minute collar schedules, the location of crossing sites identified using GPS-collar data was generally consistent between the 2 schedules (5-hour and 30-minute).

The red wolf crossing site identified in Dare County is within the Alligator River Wildlife National Wildlife Refuge and is centered on Hickory Road. This matches the location of an important black bear crossing site (Vaughan et al., 2011), and therefore is a candidate site for the placement of a multi-species crossing structure for large wildlife. Likewise, the 3 red wolf crossing sites in Tyrrell County located via camera trap data overlap with candidate areas for large wildlife crossing structures identified in an earlier study by University of Central Florida (UCF) (Smith, 2011). The red wolf crossing site between miles 23 and 24 (cameras W12 and W14) overlaps with "Area 1" of the UCF study (Smith, 2011), which is centered on the western intersection of Old US 64 and US 64. The red wolf crossing sites at mile 20.5 (camera W24) and mile 19 (camera W30S) overlap with "Area 3"and "Area 5" of the UCF study, respectively (Smith, 2011) and are located near the eastern intersection of Old US 64 and US 64. Using the eastern intersection of Old US 64 and US 64 as a reference point, "Area 3" is 0.31 miles west of the intersection and "Area 5" is 1.16 miles east of the intersection.

The red wolf crossing site in Tyrrell County identified using GPS-collar locations is located within the town limits of Columbia, NC where US 64 narrows from 4 to 2 lanes. Placement of a crossing structure here may not be practical because of proximity to residential areas.

Crossing rates suggest that there is no difference in highway crossing behavior between sexes or among ages. As with the home range analysis, the lack of any significant difference in either sex or age class might simply be a function of low sample size, and it is possible that a real relationship could be hidden by an interaction between the two variables, which we were unable to test for the same reason. This possibility is supported by data on road mortality, which has impacted dispersers hardest among the age classes. Although road mortality accounts for 14% of deaths for the red wolf population over all, when broken down by age class, road mortality accounts for 19% of dispersers but only 6% of breeding adults (USFWS, 2007). Low sample size and high expense per individual is a common hurdle in research involving large carnivores, as was true for this study. In addition, the status of the red wolf as critically endangered puts a major constraint on population size from which to draw a sample.

In addition to identifying the location of important red wolf crossing sites, we also investigated which habitat variables were correlated with those locations. The presence of agricultural fields, successional fields, and/or upland forests 328 ft. to 492 ft. (100 to 150 m) from the road best predicted where a red wolf chose to cross when using GPS-collar data while trail/road greater than 26.24 ft. (8 m) provided the best explanation for the location of crossing sites identified by remote camera traps. The presence of agricultural fields, successional fields, and upland forests as well as proximity to maintained agricultural/forest roads at crossing sites corresponds to habitat selection results.

***Conclusion:*** The distribution of GPS-collar locations and camera trap photos along US 64 revealed 5 distinct red wolf crossing sites, 4 west of Alligator River in Tyrrell County and 1 east of the river in Dare County. Four of the 5 red wolf crossing locations we identified are suitable for crossing structures. The most western crossing site is located within the town of Columbia, NC where retro fitting a wildlife underpass may be impractical. All 4 crossing sites suitable for placement of a crossing structure overlap with large wildlife crossing locations identified in previous studies. The 1 red wolf crossing site located in Dare County is centered on Hickory Road and the 3 crossing sites in Tyrrell County are approximately where US Old 64 intersects with US 64. Although no significant difference in crossing behavior was found during this study, high road mortality among dispersers suggests they may cross the highway more frequently than adults or juveniles. The most parsimonious models looking at the relationship between habitat variables at 164 ft. increments from US 64 and road/trail widths measured at road access points where cameras were placed (e.g. dikes, logging roads, public property access roads) indicates that well maintained trails at least 26.24 ft. (8 m) in width leading to and from underpasses and connect habitats selected for by red wolves (e.g. agriculture, successional fields, and upland forests), will optimize efficacy.

The data presented here are reflective of the current population's behavior. In the event that wildlife crossing structures are deemed necessary, our results identify locations where crossing structures would have the greatest effect on the red wolf population. This project is only one of several examining the use of US 64 by numerous wildlife species. The results of those studies, in addition to this one, should be taken into account in determining the need for mitigation, the type of mitigation to use, and the layout that would be most compatible with all target species. The direct and indirect effects of the road widening project remain difficult to predict, yet the potential for a negative effect on the red wolf must be considered. Careful monitoring of the red wolf population throughout and following the construction process will be crucial to ensuring red wolf survival and will aid management decisions in future road issues.

# Literature Review

## Red Wolves: Past, Present, and Future

Red wolves (*Canis rufus*) were originally described in 1851 by Audubon and Bachman and considered a subspecies of the gray wolf. However, red wolf heritage came under debate in the mid-1900's when Goldman suggested that all of the southeastern wolf subspecies should be combined into the distinct species of *Canis rufus*, separate from gray wolves. Many supported this decision until the advent of genetic methodologies in the 1990's. Genetic studies in the 1990's provided support for the hypothesis that red wolves evolved from a natural hybridization between gray wolves and coyotes (Wayne and Jenks 1991; Wayne 1992; Roy et al. 1994, 1996; Wayne and Gittleman 1995; Wayne et al. 1998; Reich et al. 1999). However, Wilson et al. (2000) suggested that red wolves and Algonquin wolves (*Canis lupus lycaon*) diverged from gray wolves 1.2 million years ago and then diverged from coyotes 150,000 to 300,000 years ago. Work by Hendrick et al. (2000) investigating major histocompatibility complex genetics data indicates that red wolves are more closely related to coyotes than to gray wolves, adding support to the claims made by Wilson et al. (2000).

The current stance that the red wolf is a species in its own right, separate from gray wolves, coyotes, and domestic dogs is based on mtDNA sequencing of 340 base pairs showing a unique sequence for red wolves (Adams, 2002; Adams et al., 2003). However, the debate over red wolf taxonomy is far from over. Both Wilson et al. (2000, 2003) and Kyle et al. (2006, 2007) now suggest that red wolves and Algonquin wolves are genetically similar enough to be combined into one species, the eastern wolf (*Canis lycaon*). In 2007 Murray and Waits, while acknowledging the genetic similarity between red wolves and Algonquin wolves and the plausibility that they are conspecifics, argue that combining the two species would hinder red wolf conservation efforts and the ability to secure conservation funds because red wolf extinction would become an issue of population extinction rather than species extinction. In 2008, Kyle et al. rebutted the article by Murray and Waits stating that taxonomy embracing conservation agendas rather than scientific scrutiny should be avoided. Kyle et al. (2008) go on to say that while they agree with Murray and Waits (2007) that there are instances in which genetically unique populations warrant protection, that the genetic uniqueness of the red wolf population is not supported scientifically. Red wolves and Algonquin wolves are only separated genetically by one mtDNA haplotype differing by one base pair (Wilson et al. (2000, 2003). Kyle et al. (2008) suggest that any difference between red wolves and Algonquin wolves may be an artifact of a low effective population size, a founder effect, a by-product of artificial selection, and/or because of current management strategies that remove individuals that are <80% red wolf from the breeding population (potentially removing important red wolf genes from the population). For now, the taxonomy of red wolves remains under debate.

The historical range of red wolves was originally described as occurring from south central Texas east to Florida and then north to the Ohio River (Nowak, 1979). The historical range was then extended north to Pennsylvania in 1995 (Nowak) and then north again to south central Maine in 2002 (Nowak) in support of the theory that there is one eastern wolf species. Red wolves declined initially with European colonization (USFWS, 2007). Predator control programs and habitat fragmentation in the 1960's dramatically reduced red wolf populations. By the 1970's, red wolves were reduced to remnant populations along the Texas and Louisiana coast. In 1973, the red wolf achieved endangered status with the passing of the Endangered Species Act of 1973. The United States Fish and Wildlife (USFWS) Service worked to capture the remaining wild red wolves between 1974 and 1980 to establish a captive breeding population as a last ditch effort to save the red wolf (USFWS, 2007). The USFWS successfully captured 17 individuals, 14 of which were used as founders for the captive breeding program (USFWS, 2007). As a result of capturing the remaining wild animals, red wolves were declared extinct in the wild in 1980.

Through the establishment of a captive red wolf breeding program with the Association of Zoos and Aquariums (AZA), enough red wolves were bred in captivity to attempt a reintroduction in 1987. The reintroduction began with the release of 4 breeding pairs on the Alligator River National Wildlife Refuge. By 1988, the first pups (2 litters) were born post reintroduction (USFWS, 2007). The USFWS started two additional red wolf reintroduction programs; in 1991 at the Great Smoky Mountains National Park at the Tennessee/North Carolina border and in 1993 at the Pocosin Lakes National Wildlife Refuge in North Carolina just 27 miles west of the original reintroduction site. The reintroduction in the Great Smoky Mountains did not succeed, but the reintroduced populations at Alligator River National Wildlife Refuge and the Pocosin Lakes National Wildlife Refuge continued to expand and merged to form the current, and only, red wolf population in the wild. The current red wolf recovery zone has expanded to 5 counties in North Carolina's Albemarle Peninsula (Dare, Tyrrell, Washington, Beaufort, and Hyde Counties – see current range in Figure 1) and contains between 100 and 130 red wolves forming 20 packs (USFWS, 2007). Red wolves remain listed under the Endangered Species Act of 1973 (USFWS, 2007) and are recognized by IUCN as one of the most endangered canid species in the world (IUCN, 2006). The re-introduced population is designated as non-essential experimental.

The USFWS has a population goal of 220 individuals, yet the population has fluctuated between 100 and 130 individuals over the past 12 years (USFWS, 2007). USFWS biologists with the Red Wolf Recovery team feel that the population can still expand further west allowing population growth to continue. However, non-USFWS researchers on the Red Wolf Implementation Team believe that the red wolf population may have reached carrying capacity within the recovery zone (USFWS, 2007). Models suggest that carrying capacity for red wolves within the current 5 county recovery zone is approximately 138 individuals (Murray, unpublished data). Starting in 2002, to help bolster the wild population, captive-born pups have been fostered to wild parents with similarly aged pups (USFWS). However, a better understanding of habitat requirements is needed to determine the ability of the peninsula to hold more animals.

Management of the red wolf gene pool and genetic fitness are the primary focus of the red wolf recovery and species survival plan due to a low effective population and potential founder effects. Genetic drift and inbreeding depression are of concern with small populations (Caughley, 1994). A study by Kalinowski et al. (1999) reported to find no evidence of inbreeding depression within the captive red wolf population. Long and Waddell (2006) reported that the captive population retained 89.65% of the genetic diversity of the founding captive population. Despite these results, there have been reports of physical anomalies in the captive red wolf population such as progressive retinal atrophy, malocclusion and undescended testicles (USFWS, 2007). Although a study by Miller et al. (2003) showed that only a few individuals per generation were needed to maintain sufficient genetic diversity in a grizzly bear population, further studies are needed to determine if genetic drift and inbreeding depression are impacting the wild red wolf populations.

For now, management of the reintroduced red wolf population focuses on a different genetic problem, the introgression of coyote genetics. Kelly et al. (1999) reported interbreeding between coyotes and red wolves resulting in coyote gene introgression into the wild red wolf population. As a result, an adaptive management plan was developed (Fazio et al., 2005). The plan calls for either the complete removal of coyotes and hybrids or the sterilization of hormonally intact coyotes and hybrids via vasectomy and tubal ligation, depending on the location within the recovery zone. In Zone 1 of the plan, all coyotes and hybrids are removed. In zones 2 and 3, coyotes and hybrids are sterilized and then used as territorial "place-holders" until replaced by wild red wolves. The sterilized coyotes and hybrids cannot interbreed with wild red wolves and they exclude intact coyotes or hybrids from the territory they hold. The idea is that these sterilized animals act as "place-holders" until red wolves replace them either naturally via displacement or through management actions to make room for translocation of a red wolf pair. The effectiveness of the management plan is evaluated via non-invasive genetic monitoring of canid scats (Waits 2004; Waits and Paetkau, 2005; Adams, 2006; Adams and Waits 2007). Through continued genetic monitoring, Adams noted strong evidence that a single hybridization event in 1993 resulted in most introgression of coyote genes into the red wolf population observed to date. From this evidence, Adams (2006) infers that hybridization with coyotes has had less genetic impact on the restored red wolf population than originally thought by Kelly et al. (1999), largely because backcrossing has been rare in the population.

Due to the immediate attention required to address the hybridization of red wolves and coyotes, less is known about red wolf home range, habitat, and diet requirements. Two recent studies examined red wolf home range and habitat use. The first study (Hinton and Chamberlain, 2010) used VHF collars to follow two red wolf packs during summer 2005 (July to September), one with pups and one without pups. This study found that the pack with pups had a smaller average home range size than the pack without pups, 5.74 $km^2$ vs. 9.55 $km^2$ for diurnal home range and 8.24 $km^2$ vs. 9.40 $km^2$ for nocturnal home ranges, respectively (Hinton and Chamberlain, 2010). Although it is important to note that the larger averaged home range calculated for the non-breeding pack is likely driven by one male whose home range was 2-3 times larger than any other wolf in the study. Adults in both packs increased home range size nocturnally (1800-0559 hours) and both packs spent approximately 98% of their time in agricultural fields, defined as corn, soybean, and cotton (Hinton and Chamberlain, 2010).

The second study investigating red wolf home range and habitat use employed GPS collars to monitor 4 male wolves from 3 packs over a period ranging from 11 to 18 months (Chadwick et al., 2010). Chadwick et al. (2010) corroborated the finding that red wolf packs primarily utilize agricultural fields during summer and early fall months, with highest use of agricultural fields occurring July through September. However, they noted a seasonal switch to grass/brush and forested habitats during winter and early spring months, November to May (Chadwick et al., 2010). Though results of both studies showed similar summer habitat preferences, the home range size estimates calculated by Chadwick et al. (2010) were several magnitudes larger than those calculated by Hinton and Chamberlain (2010). Home ranges reported in Hinton et al. (2010) ranged from 3.48 $km^2$ to 12.24 $km^2$ while those calculated by Chadwick et al. (2010) ranged from 81.6 $km^2$ to 148.1 $km^2$. Both studies employed kernel density estimators to estimate home range size. Chadwick et al. (2010) did mention that they found a 40 to 63% reduction in home range size during summer months, but that places their summer home range estimates between 51.4 $km^2$ and 59.24 $km^2$, still considerably larger than those estimated by Hinton and Chamberlain (2010).

Though Hinton and Chamberlain (2010) did calculate home range size for both sexes and all age classes, they only collected point locations for a period of 3 months and the number of daily locations varied. Chadwick et al. (2010), while focusing only on nocturnal movements of males, collected point locations over a period of 11 to 18 months and were able to consistently collect 4 locations per day with the use of GPS collars. This suggests that the discrepancy in home range estimates between the two publications may be the result of Hinton and Chamberlain (2010) not collecting enough locations to accurately capture the entire home range size. Until data on all sexes and age classes collected covering the entire 24-hour period and across all seasons is made available, conclusions concerning red wolf home range and habitat requirements cannot be made.

Phillips et al. (2003) reports that the primary prey species of red wolves include: white-tailed deer, raccoon, rabbits, nutria, and other small rodents. A more recent diet assessment via scat analysis lists white-tailed deer as the primary prey item of red wolves (Dellinger et al. in review). However, packs will increase the amount of small rodents and human-sourced foods (e.g. hog pits) in their diet during periods of increased energy demands such as pup rearing (Dellinger et al., in review).

For red wolf management to move forward, the current gaps in knowledge of red wolf natural history need to be filled. Furthermore, before model building to predict the effect of a highway widening through the red wolf recovery zone starts, base knowledge of home range and habitat selection is required.

## Road Ecology

Accompanying the rapid expansion of our transportation network was a growing concern over the environmental effects of roadways. The emergence of road ecology, coined by Richard T.T. Forman (1998), as a distinct discipline has brought together scientists from many disciplines (e.g. landscape ecology, wildlife biology, toxicology, hydrology, limnology, etc) and engineers to tackle the ecological challenges posed by transportation systems. For several decades now, researchers have studied the effects of roads on both the abiotic and biotic components of ecosystems. As a result, we now know that roads affect hydrology, air, water, noise, light pollution levels, wind flow, humidity, temperature, vulnerability to invasive species, and habitat continuity (Forman et al., 2003; Coffin, 2007). Such large-scale and multifaceted changes to ecosystems have many detrimental effects on wildlife (Jackson, 1999), including direct mortality (Lalo, 1987; Harris and Scheck, 1991; Schwabe and Schuhmann, 2002), habitat destruction (Theobald et al., 1997; Angelsen and Kaimowitz, 1999), barrier effects (Forman et al, 2003), and increased human land use activities (Bjurlin and Cypher, 2003; Coffin, 2007).

Before road mortality can be effectively mitigated, it is important to understand the factors that influence wildlife-vehicle collisions to occur in the first place. Jaarsma et al. (2006) modeled several road, traffic, vehicle, and species characteristics to find which had the greatest influence on the occurrence of a wildlife vehicle collision event. They found that traffic volume and the animal's traversing speed were the greatest predictors in determining a road mortality event, with higher traffic volumes and slower crossing speeds more likely to lead to a collision (Jaarsma et al., 2006). Two separate studies investigating the relationship among road kill events, body size, and diet found that carnivores were less likely to be hit along a road as compared to herbivores and omnivores (Ford and Fahrig, 2007, Barthelmess and Brooks, 2010). Those same two studies found a peaked relationship between road mortality and body size, with small (<1 kg) and large (>10 kg) body animals less like to be killed by vehicles as compared to medium (1 – 10 kg) sized animals (Ford and Fahrig, 2007, Barthelmess and Brooks, 2010). All three of the above cited articles suggest that direct mortality resulting from roads may not have a significant negative impact on carnivore populations as many carnivores are faster moving and larger bodied.

However, a study in southern Texas that looked at the influence of habitat variables on the location of bobcat road mortality events found that suitable habitat adjacent to the highway best explained the location of mortality events (Cain et al., 2003). These results were corroborated by another bobcat study in southern Illinois (Kolowski and Nielsen, 2008). Likewise, red wolves in northeastern North Carolina cross highways at locations adjacent to preferred habitat and established home ranges (Proctor, unpublished data). These results are similar to studies evaluating the use and success of highway crossing structures. The most successful wildlife crossing structures are the ones that connect preferred habitats of the targeted species (Ng et al. 2003, White and Ernst 2004, Singleton et al., 2005, Kindall and van Manen, 2007).

When vehicle strikes do occur, they account for a low percentage of mortality in carnivores and do not translate into population level effects. Even for endangered San Joaquin kit foxes, road mortality rarely accounted for over 10% of mortality, with predators accounting for most mortality events (Bjurlin and Cypher, 2003). In a 3-year study that followed 60 radio collared kit foxes that lived in close proximity to a 2-lane paved highway, only one was lost to a vehicle strike (Cypher et al. 2009). However, prior to mitigation efforts, road mortalities did account for 49% of mortality in Florida panthers (Maehr et al., 1991). The carnivore populations with the highest reports of road kill events in the United States are black bears. In Virginia, black bears and white-tailed deer account for the most frequently recorded road kill events (Donaldson, 2007). Two studies in Florida found increased road mortality of black bears in areas of higher road density (Hostetler et al., 2009, McCown et al., 2009). These results differ from studies focusing on other carnivore populations where an increase in road density lead to increased road avoidance rather than increased mortality events (Dickson et al. 2005, Chetkiewicz and Boyce 2009). However, the studies by Ford and Fahrig (2007) and Barthelmess and Brooks (2010) did find that omnivores are more likely to be stuck by vehicles as compared to carnivores. Although black bears are classified as carnivores, their diet is omnivorous.

A barrier effect blocking access to resources, dispersal, and gene flow is the greatest impact of highways and roads on carnivore population in the United State. A study in southern California found that while cougars often made use of dirt roads, they actively avoided paved roads (Dickson et al., 2005). Similar results were found in another study with cougars negatively associated with roads, particularly during winter months (Chetkiewicz and Boyce, 2009). Riley et al. (2006) found that coyote and bobcat populations in southern California separated by a major freeway exhibited genetic differentiation, suggesting that the freeway is a barrier to dispersal. For those that do cross, heightened territorial behavior along roadways can discourage reproductive success, again limiting gene flow (Riley et al., 2006). Likewise, a study found that a highway in southern Canada is acting as a dispersal barrier for grizzly bears at the US-Canada border, as evidenced through genetic differentiation between the two populations (Proctor et al., 2005). The result is the creation of two vulnerably small populations (Proctor et al., 2005). A highway was found to restrict gene flow in a Cleveland, Ohio coyote population and direct the movements of migrants towards urbanizing centers (Rashleigh et al., 2008). Even when they do not constitute an absolute physical barrier, high-use roads can lead to avoidance behavior in canids affecting their ability to move across a landscape (Kaartinen et al., 2005, Whittington et al., 2004). The degree to which a road impacts canid survival is dependent on the specific situation, and sometimes no detrimental effects are observed, as in the case with San Joaquin kit foxes (Cypher et al., 2009).

The amount to which a road constitutes a movement barrier for black bears is dependent of the level of traffic volume (McCown et al., 2009). A study documenting the movements of two black bear populations along the same highway in Florida found that the population living in the area with lower traffic volume crossed the highway more often (McCown et al., 2009). In Maryland, black bears avoided the larger primary highways, but readily crossed all other road classes (Fecske et al., 2002). In the northern

Rockies, just under 50% of collared black bears were willing to cross a highway at least once were (Lewis et al. 2011). A study in North Carolina found that site occupancy of black bears deceased from 0.81 to 0.35 a highway in the study area was widened from 2-lanes to 4-lanes (Nicholson and van Manen 2009). For black bears, roads appear to exhibit the distinct trade-off between permeability and road kill discussed by Forman and Alexander (1998).

Though the last 10 years has documented many adverse effects of highways and roads on carnivore populations, there have been positive developments as well. Highway crossing structures have been successful at mitigating some negative impacts of highways on carnivore populations. In Texas, bobcats did make use of culverts to cross a highway when the culverts were placed adjacent to suitable habitat (Cain et al., 2003). A study in California found that a large variety of species, including reptiles, small mammals, carnivores, and mule deer use highway underpasses, even underpasses not designed specifically for wildlife (Ng et al., 2004). A study investigating wide variety of structures, including culverts, modified box-culverts, underpasses, and overpasses, found that culverts were the least used preferences between underpasses and overpasses varied with species (Mata et al., 2008). A study in Portugal found that red foxes, badgers, genet, and Egyptian mongooses used underpasses and culverts without preference (Grilo et al., 2008). However, a study of wildlife underpasses in Virginia revealed that while they were effective for foxes and coyotes, they did not find evidence of black bears utilizing highway underpasses (Donaldson, 2007). Likewise, a study monitoring the success of multi-species highway underpasses following a highway-widening project found that bobcats, black bears, and foxes utilized underpasses, but not coyotes or red wolves present in the area (McCollister and van Manen, 2010).

In all documented success of highway crossing structures, the authors noted that the successful structures connected areas of suitable habitat for the target species. The non-detection of all area carnivore species in the multi-species crossing structures may be the result of not being located in an area that contains suitable habitat for all species. Though multi-species structures may be may appear to be more cost effective initially, a lower success rate will decrease the cost effectiveness in the long run.

While the subjects above have gotten considerable coverage in the peer-reviewed literature, relatively little research has been directed at determining the placement of highway underpasses. In may be beneficial to focus future research efforts on determining the effective placement of highway crossing

Of the studies that focused on placing mitigating structures, methodologies have varied widely and range from non-invasive to the capture and handling of target species. Non-invasive techniques include the use of track/trail counts (Van Dyke et al., 1986; Rodriguez et al., 1996; Alexander and Waters, 1999; Scheick and Jones, 1999, 2000; Barnum, 2001, 2003), remote cameras (Scheick and Jones, 1999, 2000), barbed wire hair traps (Wills and Vaughan, 2005), road kill surveys (Clevenger et al., 2003b; Mazerolle, 2004; Smith et al., 2009), and GIS based modeling (Smith et al. 1998; Klein, 1999; Kobler and Adamic, 1999; Sheick and Jones, 1999, 2000; Clevenger et al., 2003a; Lloyd et al., 2005). Non-invasive techniques are relatively inexpensive and can be effective, however all are time intensive. A constraint of track counts is the requirement of an appropriate substrate, the use of sand, or, in some areas, the presence of fresh snow. Remote camera traps provide crossing location, date, time, and work for a wide variety of species. Yet, cameras cannot cover the entire length of the highway simultaneously. Running barbed wire the length of the study area provides crossing location and, with the addition of genetics, crossing frequency on the level of the individual. Drawbacks of using barbed wire include the added cost of genetics and the limited number of mammals this technique is appropriate for. Road-kill surveys to detect crossing hotspots are an excellent method for collecting data on a wide range of species simultaneously. However rate of decay, scavenger activity, and method of survey (driving vs. walking) can affect results and must be considered in planning survey interval times. Road kill surveys may also miss animals that wander away from the collision site before dying. It has also been suggested that while road kill events do represent failed attempts to cross, they do not necessarily indicate important linkage areas. The primary weaknesses of the GIS-based techniques are data availability and data quality. GIS models are most effective when data on habitat use patterns of the subject species are well known and where the habitat is diverse and heterogeneous. A limitation of non-invasive techniques as a whole, with the exception of GIS based models, is inherent bias unless the entire length of the proposed highway construction project is covered. Many of the studies cited here established transects or plots rather than surveying the entire study area, thus missing crossing activity at sites not surveyed.

The more invasive techniques involve capturing and collaring target species in order to track movements. These techniques are especially useful when the study is focused on a particular species as opposed to a generalized group. A few studies employed the use of VHF radio collars to identify road-crossing locations and the influence of highways on animal movements (Beringer et al., 1990; Chruszcz et al., 2003; Riley et al., 2003; Dickson et al., 2005; Wray et al., 2005). Though radio telemetry is a more affordable method of telemetry, it is time intensive and often long time intervals exist between locations. Low-resolution movement data may inaccurately depict crossing sites, or miss crossings altogether. In addition, accessibility to collared animals can be limited due to terrain, road condition, and/or private property. GPS telemetry, though more expensive, allows the tracking of animal movements via satellite and reduces accessibility issues. In addition, GPS collars can be programmed to collect data in short time intervals, improving resolution. High-resolution movement data is essential for pinpointing road-crossing locations. Though GPS collars may cost more up front, they provide more accurate locations, and save money by reducing man and vehicle hours required for data collection (Rodgers et al. 1996, Mech and Barber 2002). GPS collars have successfully been used to identify road-crossings for many large mammals such as grizzly bears (Waller and Servheen, 1999, 2005), black bears (McCoy, 2005), and elk (Dodd et al., 2007).

All of the techniques described above identify cross-locations for the placement of mitigating structures, but do not measure the extent to which a road is acting as a barrier. Most studies use genetic sampling to measure whether or not a road is acting as a barrier to gene flow in a population (Gerlach and Musolf, 2000; Epps et al., 2005; Riley et al., 2006). However, in 2007, Dodd et al. proposed the use of a permeability index to measure the barrier effect of a highway. A permeability index is a passage rate calculated by using the following equation: #crossings/(#crossings + #approaches, where an approach is defined as a red wolf entering into a 164 ft. buffer zone around the highway without crossing (see Figure 2). So far, this methodology has only been used on ungulates with seasonal migration patterns and not on species that remain in smaller defended territories.

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