RED WOLF (*CANIS RUFUS*) AND COYOTE (*CANIS LATRANS*) ECOLOGY AND

INTERACTIONS IN NORTHEASTERN NORTH CAROLINA

by

JOSEPH WILLIAM HINTON

(Under the Direction of Michael J. Chamberlain)

ABSTRACT

In recent decades, red wolf (*Canis rufus*) hybridization with coyotes (*Canis latrans*) has posed a serious threat to red wolf recovery efforts. Preventing hybridization has become a primary objective requiring intensive management efforts to prevent introgression. As the red wolf population increases, long-term recovery requires general understanding of red wolf and coyote ecology to develop appropriate management strategies for addressing hybridization. The primary objective of this study was to understand the underlying mechanisms that influence red wolf and coyote interactions by examining morphology, diet, and spatial ecology of both species. We examined external morphological characters of red wolves, coyotes, and their hybrids to determine if morphology could be an accurate discriminator among the 3 canid taxa. Using hind foot length, weight, width of head, and tail length, we were able to correctly identify 86% of canids to their *a priori* species groups as identified via genetic analysis. We also assessed factors affecting prey selection of red wolf packs, coyote pairs, and congeneric pairs of red wolves and coyotes and found that all three had similar and overlapping diets. Nevertheless, we detected differential use of prey; difference in diet was associated with body size. Larger individuals within and among different breeding pairs consumed more white-tailed deer, and less rabbits and small mammals. We observed red wolf and coyote preferences for agricultural habitats over forested habitats and space use patterns to be influenced by body size. Coyote home-ranges had an upper limit of approximately 50 km^2, whereas an upper limit for red wolves was

approximately 180 km^2. Home-ranges of congeneric pairs did not exceed 50 km^2 and we suggest the smaller coyote may constrain and limit space use patterns of congeneric pairs. We suggest that similarities in body size of individual red wolves and coyotes may contribute to successful congeneric pairing and hybridization via similar use of space, habitat, and prey. Therefore, lowering hybridization rates between red wolves and coyotes may require increasing the average body size of the red wolf population to facilitate differential use of limiting resources.

INDEX WORDS: coyote, *Canis latrans*, *Canis rufus*, body size, diet, ecology, habitat selection, hybridization, interactions, space use

RED WOLF (*CANIS RUFUS*) AND COYOTE (*CANIS LATRANS*) ECOLOGY AND

INTERACTIONS IN NORTHEASTERN NORTH CAROLINA

by

JOSEPH W. HINTON

B.S., Ferrum College, 2002

M.A., Rice University, 2006

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial

Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2014

RED WOLF (*CANIS RUFUS*) AND COYOTE (*CANIS LATRANS*) ECOLOGY AND

INTERACTIONS IN NORTHEASTERN NORTH CAROLINA

by

JOSEPH W. HINTON

Major Professor:	Michael J. Chamberlain
Committee:	John L. Gittleman
	Nathan P. Nibbellink
	Douglas L. Peterson

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2014

DEDICATION

Dedications are often only offerings from interested baseness to disdainful vanity. –

Voltaire in *Dictionnaire Philosophique*

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

x

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

In recent decades, red wolf (*Canis rufus*) hybridization with coyotes (*Canis latrans*) has become a serious threat to red wolf recovery efforts. During the 1970s, the last remaining red wolves were removed from the wild because the last remnant population began hybridizing with an expanding coyote population. Shortly after reintroducing red wolves onto Alligator River National Wildlife Refuge in northeastern North Carolina, coyotes began expanding their range throughout North Carolina. The current red wolf population co-exists with coyotes and hybridization between the two species occurs. Red Wolf Recovery Team biologists and Red Wolf Recovery Implementation Team scientists identify red wolf-coyote interactions, related resource partitioning, and prevention of coyote gene introgression into the wild red wolf gene pool as critical factors vital to long-term recovery, management, and planning (USFWS 2007). Therefore, preventing hybridization has become a primary management goal (Kelly *et al*. 1999; Stoskopf *et al.* 2005; Rabon *et al.* 2013).

To prevent coyote introgression into the red wolf population, coyotes captured by United States Fish and Wildlife Service (USFWS) personnel within the Red Wolf Recovery Area (Recovery Area) are reproductively sterilized and used as space holders until red wolves move in and occupy those areas. This tactic provides a reproductive advantage to red wolves by reducing coyote reproduction within the Recovery Area (USFWS 2007; Stoskopf *et al*. 2005). More importantly, in the event that a red wolf forms a breeding pair with a sterilized coyote, this prevents introgression because the pair is incapable of successfully breeding. Despite intensive management efforts, red wolf/coyote hybrids are still captured within the Recovery Area indicating that canid management is not ubiquitous. To achieve long-term recovery of red wolves, it is imperative that the Red Wolf Recovery Program improves its general knowledge of red wolf

and coyote ecology to understand mechanisms facilitating red wolf-coyote hybridization. The primary objective of this research is to understand the underlying mechanisms that influence red wolf and coyote interactions in northeastern North Carolina by examining red wolf and coyote morphology, diet, and spatial ecology. Currently, a full understanding of red wolf ecology is lacking, which is fundamental to ensuring recovery and persistence of the species. Furthermore, most research conducted on coyotes has been done in the western United States and Canada and the few studies on coyote behavior in the southeastern United States have been conducted at small scales (Holzman *et al.* 1992; Chamberlain *et al.* 2000; Constible *et al.* 2006).

Among phylogenetically related species that coexist, body size is the most distinguishing feature among those animals (LaBarbera 1989). Within canid communities, competition is strongly asymmetrical with larger species displacing smaller competitors (Paquet 1992; White *et al.* 1994; Arjo and Pletscher 1999; Constible *et al.* 2006; Berger and Gese 2007). Red wolves and coyotes exhibit morphological and niche overlaps, in which red wolves are the larger species. Although competition for space and food resources is common among coexisting species with similar body sizes and ecological needs, competition between red wolves and coyotes extends beyond space and food because both species can use each other as a resource for reproduction when within-species mating opportunities are exhausted. As a result, interactions between the two species are complex because the outcome of red wolf-coyote interactions can range from lethal antagonism to congeneric pair-bonding resulting in hybridization. The functional significance of body size within these interactions remains unclear and the relationship between hybridization and body size is worth examination.

In this dissertation, I propose to examine the influence of body size on red wolf and coyote space and resource use, habitat selection, and interactions. Space use is thought to reflect the ecological requirements of a species and body size is known to have pervasive influence on spatial ecology through consumer-resource interactions (Yodzis and Innes 1992; Basset and DeAngelis 2007). It has been demonstrated that home range sizes of carnivores scales

2

allometrically with individual body size (McNabb 1963; Gittleman and Harvey 1982; Mace and Harvey 1983; Swihart *et al*. 1988) in which larger carnivores have larger home ranges relative to smaller carnivores because larger home ranges provide more food resources for greater energetic demands. Additionally, there is a positive relationship between body size and prey size among carnivores (Gittleman 1985; Radloff and Du Toit 2004). Differing in body size, red wolves (20-38kg) and coyotes (11-18kg) in northeastern North Carolina should have differential energy requirements and different capacities for searching for and processing food resources. This difference in body size may be the primary trait that promotes differential use of space, resources, and habitat between the two species. Therefore, it is likely that selection forces may act on morphological variation in which body size differences create niche partitioning and reduce competitive interactions between red wolves and coyotes.

If body size drives the outcome of competitive interactions between red wolves and coyotes, it is plausible to hypothesize that size may influence the rates of hybridization between the two species. Coyotes are capable of consorting with red wolves that result in the successful formation of congeneric breeding-pairs that are maintained for several years. The over-arching goal of this project is to understand the conditions that facilitate red wolf-coyote hybridization. Red wolf-coyote interactions indicate that both populations in northeastern North Carolina may be limited by the availability of mates and not space and food resources in which congeneric pair-bonding demonstrates weak reproductive barriers between red wolves and coyotes. In the same manner that body size may influence resource partitioning between red wolves and coyotes through differential use of resources, body size may influence reproductive partitioning through assortative mating (Pfennig and Pfennig 2010; Schemske 2010).

If red wolf and coyote mate selection is non-random, there should be a pattern of behavior in which individuals preferentially mate with certain members of the opposite sex. Benefits derived by choosing certain mates rather than others can be difficult to quantify because those benefits can be subtle (Halliday 1983). Successful production of offspring doesn't appear to

influence partner fidelity because sterilized coyotes are capable of maintaining pair-bonds with other coyotes and red wolves for multiple years (USFWS, unpublished data). However, red wolf and coyote mate choice and continued fidelity may result from behavioral mechanisms that result in similar use of space, habitat, and prey in order for both individuals to complement one another. In other words, differences in ecological requirements may serve as barrier to conspecific pair-bonding between red wolves and coyotes. If this hypothesis is correct, body size may create reproductive partitioning, along with resource partitioning, between red wolves and coyotes. For example, if coyotes have a much lower ceiling for home-range sizes then red wolves because of lower energy demands associated with smaller body sizes, then red wolves that exceed those spatial demands are likely to be reproductively isolated from the coyote population. The differences in space use and resource demands will not allow for successful maintenance of monogamous breeding pairs. However, red wolves that are below that ceiling are susceptible to consorting and breeding with coyotes because they can complement the spatial and resource needs of their coyote mates. Therefore, morphological traits, such as body size, that promote dissimilarity in space use and resource needs may create reproductive barriers between red wolves and coyotes.

Chapter 2 summarizes the history of red wolf recovery efforts, provides a synopsis of challenges to red wolf restoration, and suggests future research needed for to pursue full recovery of red wolves.

Chapter 3 examines external morphological characters of red wolves, coyotes, and their hybrids to determine if morphology could be an accurate discriminator among the 3 canid taxa. This would allow us to verify if red wolves and coyotes differed in morphology. To accomplish this we collected mean body measurements from 171 red wolves, 134 coyotes, and 47 hybrids for identifying canid taxa in the wild. We then used polytomous logistic regression analysis of 7 morphometric variables to allocate canids to their *a priori* species groups predetermined via genetic analysis.

Chapter 4 assesses factors affecting prey selection of red wolf packs, coyote pairs, and congeneric pairs. To accomplish this, my team and I collected and analyzed the prey remains in 1754 scats from 13 red wolf packs, 17 coyote pairs, and 8 congeneric pairs. We used several variables (i.e., body weight, social structure, age, etc.) that have not been traditionally used in analyses of scat remains to account for variation in diet. Furthermore, this study is the first to assess dietary behaviors of congeneric canid breeding units that are the source of hybridization. Additionally, this study represents the most comprehensive assessment of canid diet in the eastern United States.

Chapters 6 and 7 assess space use and habitat selection of red wolves and coyotes. Accomplishing this objective required capturing and fitting global positioning system (GPS) radio-collars to red wolves and coyotes. This permitted us to monitor resident and transient animals and assess ways in which both species move, interact, and use several primary habitats in northeastern North Carolina. This study represented the 1st broad scale study (> 5000 km^2) of coyote space use and habitat selection in the southeastern United States. We also provide for the 1st assessment of body weight influence on coyote and red wolf home-range size.

Finally, Chapter 8 examines whether body size is a reproductive barrier between red wolves and coyotes. To accomplish this, we used linear regression to evaluate the influence of body weight on ecological parameters such as home-range size and diet composition. Observed patterns are then used to discuss the effects of energetics on red wolf and coyote hybridization.

Literature Cited

Arjo, W.M., and D.H. Pletscher. 1999. Behavioral response of coyotes to wolf recolonization in northwestern Montana. Canadian Journal of Zoology 77: 1919-1927.

Basset, A., and D.L. DeAngelis. 2007. Body size mediated coexistence of consumers competing for resources in space. Oikos 116: 1363-1377.

Berger, K.M., and E.M. Gese. 2007. Does interference competition with wolves limit the distribution and abundance of coyotes? Journal of Animal Ecology 76: 1075-1085.

Bromley, C., and E.M. Gese. 2001. Surgical sterilization as a method of reducing coyote predation on domestic sheep. Journal of Wildlife Management 65:510–519.

Chamberlain, M.J., C.D. Lovell, and B.D. Leopold. 2000. Spatial-use patterns, movements, and interactions among adult coyotes in central Mississippi. Canadian Journal of Zoology 78: 2087-2095.

Cooper, A.B., and J.J. Millspaugh. 1999. The application of discrete choice models to wildlife resource selection studies. Ecology 80: 566-575.

Cooper, A.B., and J.J. Millspaugh. 2001. Accounting for resource availability and animal behavior in resource selection studies. In radiotelemetry and animal populations. Edited by J.J. Millspaugh and J.M. Marzluff. Academic Press, New York, New York, USA.

Constible, J.M., M.J. Chamberlain and B.D. Leopold. 2006. Relationships between landscape pattern and space use of three mammalian carnivores in central Mississippi. American Midland Naturalist 155: 352-362.

Gier, H.T. 1968. Coyotes in Kansas. Kansas State College, Agricultural Experiment Station Bulletin 393:1 118.

Gittleman, J.L. 1985. Carnivore body size: ecological and taxonomic correlates. Oecologia 67: 540-554.

Gittleman, J.L., and P.H. Harvey. 1982. Carnivore home-range size, metabolic needs and ecology. Behavioral Ecology and Sociobiology 10: 57-63.

Gitzen, R.A., and J.J. Millspaugh. 2003. Comparison of least-square cross-validation bandwidth

        options for kernel home-range estimation. Wildlife Society Bulletin 31:823-831.

Halliday, T.R. 1983. The study of mate choice. In Mate Choice. Edited by Patrick Bateson.

        Cambridge University Press, Cambridge, England.

Holzman, S., M.J. Conroy and J. Pickering. 1992. Home range movements and habitat use of

        coyotes in southcentral Georgia. Journal of Wildlife Management 56: 139-146.

Johnson, D.H. 1980. The comparison of usage and availability measurements for evaluating

        resource preference. Ecology 61: 65-71.

Kelly, B.T., P.S. Miller, and U.S. Seals. 1999. Population and habitat Viability assessment

        workshop for the red wolf (Canis rufus). SSC/IUCNConservation Breeding Specialist

        Group, Apple Valley, Minnesota, USA.

LaBarbera, M. 1989. Analyzing body size as a factor in ecology and evolution. Annual Review of

        Ecology & Systematics 20: 97-117.

McKerrow, A.J., S.G. Williams, and J.A. Collazo. 2006. The North Carolina Gap Analysis

        Project: Final Report. North Carolina Cooperative Fish and Wildlife Research Unit,

        North Carolina State University, Raleigh, North Carolina, USA.

McNab, B.K. 1963. Bioenergetics and the determination of home range size. American Naturalist

        97: 133-140.

Mace, G. M., and P.H. Harvey. 1983. Energetic constraints on home-range size. American

        Naturalist 121: 120-132.

Manly, B. F. J., L. L. McDonald, and D. L. Thomas. 1993. Resource selection by animals:

        statistical design and analysis for field studies. Chapman & Hall, London, England.

Paquet, P.C. 1992. Prey use strategies of sympatric wolves and coyotes in Riding Mountain

        National Park, Manitoba. Journal of Mammalogy 73: 337-343.

Pfennig, D.W., and K.S. Pfennig. 2010. Character displacement and the origins of diversity.

        American Naturalist 176: S26-S44.

Radloff F.G.T., and J.T. Du Toit. 2004. Large predators and their prey in a southern African savanna: a predator's size determines its prey size range. Journal of Animal Ecology 73: 410-423.

Reed, J.E., W.B. Ballard, P.S. Gipson, B.T. Kelly, P.R. Krausman, M.C. Wallace, and D.B. Wester. 2006. Diets of free-ranging Mexican gray wolves in Arizona and New Mexico. Wildlife Society Bulletin 34: 1127-1133.

Riitters, K.H., J.D. Wickham, J.E. Vogelmann, and K.B. Jones. 2000. National land-cover pattern data. Ecology 81:604.

Rodgers, A.R., A.P. Carr, H.L. Beyer, L. Smith, and J.G. Kie. 2007. HRT: Home range tools for ArcGIS. Version 1.1. Ontario Ministry of Natural Resources, Centre for Northern Forest Ecosystem Research, Thunder Bay, Ontario, Canada.

Schemske, D.W. 2010. Adaptation and The Origin of Species. American Naturalist 176: S4-S25.

Seaman, D.E., and R.A. Powell. 1996. An evaluation of the accuracy of kernel density estimators for home range analysis. Ecology 77:2075-2085.

Stoskopf, M.K., K. Beck, B.B. Fazio, T.K. Fuller, E.M. Gese, B.T. Kelly, F.F. Knowlton, D.L. Murray,W.Waddell, and L. Waits. 2005. Implementing recovery of the red wolf – integrating research scientists and managers. Wildlife Society Bulletin 33:1145-1152.

Swihart, R.K., N.A. Slade, and B.J. Bergstrom. 1988. Relating body size to the rate of home range use in mammals. Ecology 69: 393-399.

Telesco, D.J., and F.T. Van Manen. 2006. Do black bears respond to military weapons training? Journal of Wildlife Management 70: 222-230.

U.S. Department of Agriculture (USDA) National Agricultural Statistics Service (NASS) 2010. 2009 and 2010 North Carolina Cropland Data Layer. Available online at http://datagateway.nrcs.usda.gov/. Accessed March 10, 2011.

USFWS. 2005. Revised Red Wolf Recovery Program Adaptive Management Plan. United States Fish and Wildlife Servise, Manteo, NC, USA.

USFWS. 2007. Red wolf (Canis rufus) 5 year status review: Summary and   Evaluation.

    United States Fish and Wildlife Servise, Manteo, NC, USA.

White, P.J., K. Ralls, and R.A. Garrott. 1994. Coyote-kit fox interactions as revealed by

    telemetry. Canadian Journal of Zoology 72: 1831-1836.

Worton, B.J. 1989. Kernel methods for estimating the utilization distribution in home-range

    studies. Ecology 70:164-168.

Yodzis, P., and S. Innes. 1992. Body size and consumer-resource dynamics. American Naturalist

    139: 1151-1175.

CHAPTER 2

RED WOLF (*CANIS RUFUS*) RECOVERY: A REVIEW WITH SUGGESTIONS FOR

FUTURE RESEARCH

_____

Abstract

By the 1970s, government-supported eradication campaigns reduced red wolves to a remnant population of less than 100 individuals on the southern border of Texas and Louisiana. Restoration efforts in the region were deemed unpromising because of predator-control programs and hybridization with coyotes. The U.S. Fish and Wildlife Service (USFWS) removed the last remaining red wolves from the wild and placed them in a captive-breeding program. In 1980, the USFWS declared red wolves extinct in the wild. During 1987, the USFWS, through the Red Wolf Recovery Program, reintroduced red wolves into northeastern North Carolina. Although restoration efforts have established a population of approximately 70-80 red wolves in the wild, issues of hybridization with coyotes, inbreeding, and human-caused mortality continue to hamper red wolf recovery. We explore these three challenges and, within each challenge, we illustrate how research can be used to resolve problems associated with red wolf-coyote interactions, effects of inbreeding, and demographic responses to human-caused mortality. We hope this illustrates the utility of research to advance restoration of red wolves.

Introduction

Perceived threats to human enterprise have historically motivated efforts to exterminate large carnivores such as wolves, bears, and lions. In particular, wolves have been extirpated from much of their historical ranges in North America by government-supported eradication campaigns protecting agricultural and livestock interests. However, changes in American societal beliefs have resulted in profound changes to how wolves are perceived. The passage of the Endangered Species Act of 1973 (ESA) paved the way for restoration of wolf populations that were severely reduced or extirpated during the 19th and early 20th centuries. When the ESA was legislated, gray wolves (*Canis lupus*) and red wolves (*Canis rufus*) existed as declining remnant populations in the contiguous United States. Although gray wolf populations in Alaska and Canada were stable and the species was not threatened with extinction, red wolves were afforded no refuge. Red wolves were likely the first New World wolf species to come in contact with

11

Europeans and, consequently, the first to be persecuted. Prior to European colonization, red wolves were common in the eastern United States and they inhabited an area from the Atlantic coast west to central Texas, with the Ohio River Valley, northern Pennsylvania, and southern New York being its northernmost range and their distribution extending south to the Gulf of Mexico (Figure 1; Nowak 2002, 2003). At the turn of the 20th century, red wolves were extirpated throughout most of their range and approximately 100 individuals occupied coastal habitats of eastern Texas and western Louisiana (McCarley 1959, 1962). Declining because of aggressive predator-control programs and surrounded by an expanding coyote (*Canis latrans*) population, red wolves were incapable of maintaining self-sustaining populations. They began hybridizing with coyotes when they were unable to find conspecific mates and canid populations in the region gradually became genetically admixed (McCarley 1959; Paradiso and Nowak 1972; Carley 1975). This generated concerns that the last remaining red wolves would be genetically assimilated into the coyote genome through hybridization, so the southeast Texas and southwest Louisiana populations were targeted for restoration efforts (Carley 1975).

After the passage of the ESA, the United States Fish & Wildlife Services (USFWS) established the Red Wolf Recovery Program (Recovery Program) with the task of locating and preserving populations of red wolves in southeast Texas and southwest Louisiana (USFWS 1989). However, with rapidly declining red wolf populations and extensive hybridization, the USFWS decided to remove the last red wolves from the wild and place them in captivity. The Recovery Program's objectives soon changed to capture as many red wolves as possible for propagation in captivity, and to re-establish red wolf populations within the species' historic range in the near future (USFWS 1989). To find pure red wolves for the captive-breeding program, the Recovery Program captured as many wild red wolf-like canids as possible in southeast Texas and southwest Louisiana. From 1973 through 1980, approximately 400 canids were captured and 43 met the morphological standards to be considered red wolves. Breeding experiments were then conducted with those 43 individuals and, eventually, 14 individuals met the criteria established to

12

define the species. These individuals were used as the founders to begin the captive-breeding program (USFWS 1989). The red wolf was declared extinct in the wild in 1980, becoming the first species to be purposely extirpated in the wild to save it from extinction.

The captive-breeding program safeguarded the last remaining red wolves and served as the last repository of the red wolf genome. The primary objectives of the captive-breeding program were to certify the genetic purity of wild-caught red wolves, increase the number of red wolves in captivity, and maintain a captive red wolf population for re-establishment of the species in the wild (USFWS 1989). Red wolves readily reproduced in captivity with the first captive-born litters produced during 1977. Early efforts in the captive-breeding program then focused on developing procedures and protocols to ship, handle, and breed red wolves within a network of zoo facilities (Carley 2000). To maintain integrity within the captive-breeding program, the USFWS developed a Species Survival Plan® (SSP) that was accepted by the Association of Zoos & Aquariums (AZA; USFWS 1989). This ensured that the species would be preserved in captivity until a strategy was developed for reintroducing red wolves in the wild. To acclimate captive red wolves to wild conditions, the Recovery Program began conducting experimental releases of captive-born red wolves on island propagation sites such as Bulls Island of the Cape Romain National Wildlife Refuge in South Carolina. Bulls Island became one of three island propagation sites that allowed the Recovery Program to develop restoration techniques.

During 1984, Alligator River National Wildlife Refuge (ARNWR) was established on the Albemarle Peninsula of northeastern North Carolina (NENC) when the Prudential Insurance Company donated approximately 480 km² (48,000 ha) of land to the federal government (USFWS 1989). This area was identified as the future reintroduction site for red wolves because the refuge contained suitable prey for red wolves, coyotes were absent on the landscape, no livestock were present, and the presence of humans was low. In 1987, the USFWS released eight captive-born red wolves (four male-female pairs) onto ARNWR to begin reintroduction efforts. Initially, mortality rates were high as captive-born wolves were hit by cars, drowned, succumbed

to disease, or were attracted to townships (Phillips et al. 2003). As a result, early attempts to established red wolves on ARNWR were aggressive and resulted in the release of more than 60 red wolves from 1987 through 1994 (Phillips et al. 2003). Eventually, the NENC population transitioned from captive-born individuals to wild-born individuals and the release of captive-born adult wolves to augment the NENC population ceased. Currently, almost all red wolves in NENC are wild born. Periodically, island-born juveniles and captive-born pups fostered into wild litters are used to maintain genetic diversity and health of the wild population. By the mid-1990s, red wolves in the wild formed packs, maintained territories, and successfully bred, and the reintroduction marked the first successful reintroduction of a wolf species. It also marked the first successful attempt to reintroduce a large predator that was completely extirpated from the wild.

The USFWS initiated a second reintroduction in the Great Smoky Mountains National Park (GSMNP) of the southern Appalachians (USFWS 1990). During 1991, the initial stage of the GSMNP reintroduction was implemented to gather information on interactions of red wolves and coyotes, livestock, and humans (Lucash et al. 1998). Initial efforts appeared successful when a mated adult pair and two pups established a territory in Cades Cove of the GSMNP, so the USFWS proceeded with a full-scale reintroduction. However, most of the 37 red wolves released were unable to establish and maintain territories within the park boundaries and left for better habitat on surrounding lower-elevation agricultural land (Henry 1998). Additionally, red wolves that maintained territories on GSMNP had low pup survival as a result of Parvovirus, malnutrition, and parasites (Henry 1998). After repeated introduction attempts and low pup survival, it was determined that the red wolf population on GSMNP would have to be perpetually managed within the park and the GSMNP red wolf reintroduction was terminated in 1998. Red wolves that remained in the park were subsequently captured and relocated to ARNWR.

Although nearly 25 years have elapsed since red wolves were reintroduced into the wild, more than half of the red wolf population still exists in captivity. The captive-breeding program safeguards approximately 200 red wolves in more than 40 captive facilities around the United

States while the reintroduced red wolf population has expanded throughout the Albemarle Peninsula to about 70-80 animals in approximately 15 packs (USFWS 2013). Since 1987, the Recovery Area has expanded to accommodate the growing population from approximately 480 km² to approximately 6800 km² of federal, state, and private lands (Figure 2). Although red wolf restoration has experienced success in many ways, efforts to maintain the NENC population and to find future reintroduction sites continually face challenges. For instance, the red wolf species continues to be plagued by taxonomic controversy regarding its origin and arguments against the systematic validity of the red wolf have been used to oppose red wolf restoration (Phillips and Henry 1992; Nowak and Federoff 1998). Red wolves still remain a remnant population and experience a series of ecological threats such as hybridization with coyotes and inbreeding (Kelly et al. 1999; Stoskopf et al. 2005; USFWS 2007). Without management of coyotes in the Recovery Area, it is likely that the red wolf population would be genetically assimilated into the eastern coyote population (Kelly et al. 1999). Additionally, the small number of red wolves makes the population in NENC susceptible to genetic drift and inbreeding depression (Rabon and Waddell 2010). To prevent inbreeding and maintain genetic diversity in the wild population, captive-born and island-born individuals are periodically released into the Recovery Area. Additionally, quixotic fervor within the hunting community to suppress predators continues to hamper red wolf population growth in NENC. Increased mortality by gunshot during the hunting season has reduced the number of red wolf packs, lowered red wolf survival, and has facilitated coyote expansion into the Recovery Area (USFWS 2007).

In the progress of overcoming these challenges to restoring red wolves to the wild, there is a need to consolidate knowledge and contemplate those experiences as recovery efforts move forward. Therefore, our objective is to provide a synopsis of the challenges to restoration of the red wolf and suggest future research needed to pursue full recovery of the species.

Red Wolf Taxonomy

Currently, scientists find themselves in a contentious debate regarding the taxonomy of New World wolves and its implications on the evolution, ecology, and conservation of *Canis* species in eastern North America (Chambers et al. 2012). The origin of the red wolf is central to this debate (Nowak 1979, 2002; Wayne and Jenks 1991; Wilson et al. 2000). Although scientific synthesis has led to new insights into the evolution and ecology of New World wolves, massive loss of historic and geographic genetic data and recent genetic introgression by coyotes continues to hinder consensus on red wolf origin (Chambers et al. 2012). Despite significant voids in data to adequately characterize the historic red wolf populations in the Southeast, limited and anecdotal data does exist to indicate the existence of a large canid in the southeastern United States.

The unique presence of a southeastern wolf was noted during the 18th century (Bartram 1791; Harper 1942; Nowak 1992) and, by 1851, the red wolf was given a valid scientific name (Audubon and Bachman 1851). During the turn of the 20th century, several authors recognized structural differences between gray and red wolves and initiated revisions of the red wolf's taxonomic status (Bangs 1898; Baily 1905; Miller 1912). Eventually, Goldman (1937, 1944) described red wolves as distinct from gray wolves and coyotes based on cranial and dental characters and consigned all wolves of the Southeast to one species, *C. rufus*. By the 1960s, federal and state agencies generally assumed that viable populations of red wolves existed in the Southeast despite a great deal of confusion about the species status. McCarley's (1962) taxonomic study of red wolves concluded that red wolves had been replaced by coyotes and red wolf/coyote hybrids in most areas of eastern Texas, Arkansas, Louisiana, and Oklahoma. His work indicated that a few red wolf populations still existed in parts of Louisiana. After examining a number of *Canis* specimens from the Southeast, Paradiso (1965, 1968) and Pimlott and Joslin (1968) confirmed McCarley's (1962) findings and brought attention to what were believed to be the last surviving red wolf populations on the Gulf Coast in southeast Texas and southwest Louisiana (Carley 2000).

16

Nowak (Nowak 1979, 2002) investigated the taxonomy of *Canis* species of eastern North America using discriminant function analysis to evaluate the characteristics of modern and paleontological *Canis* skulls (Nowak 1979, 2002) and dentition (Nowak 2002). In doing so, he was able to differentiate gray wolves, red wolves, coyotes, and domestic dogs (*Canis familiaris*) into separate groups and postulated that red wolves evolved from a transitional form (i.e., *Canis mosbachensis*) between a wolf-like coyote ancestor and the gray wolf. Nowak (1979) found no evidence that gray wolves existed in the southeastern United States. Despite widespread occurrence of domestic dog in the Southeast, Nowak (1979) found no evidence of introgression from domestic dogs into the red wolf and coyote populations. The earliest red wolf specimens showed no statistical overlap with gray wolves, coyotes, or domestic dogs and had similar multivariate distribution as the red wolf specimens from the Pleistocene era. Specimens collected before 1930 indicated hybridization between red wolves and coyotes was uncommon where their ranges approached. However, specimens from the 1930s until the 1950s indicated hybridization with coyotes was occurring over large areas of the red wolf's southern range where coyotes were replacing red wolves. Nowak (1979, 2002) suggested that hybridization between red wolves and coyotes began at the turn of the 20th century when anthropogenic factors destroyed ecological and behavioral isolation. Despite coyote introgression into the red wolf genome during the 20th century, Nowak (2002) reported that the morphology of modern red wolves is predominately like *C. rufus* that persisted in the eastern United States 10,000 years ago.

Although it had been suggested that red wolves were the result of coyote and gray wolf hybridization (Mech 1970), the hypothesis of a hybrid origin did not receive much attention until applied molecular techniques became the primary means of evaluating red wolf taxonomy. Analyzing mitochondrial DNA (mtDNA), Wayne and Jenks (1991) evaluated the genetic integrity of red wolves in the captive-breeding program and reported no unique genetic markers in red wolves that were distinct from gray wolves and coyotes. Therefore, they concluded that the red wolf is a hybrid form derived from gray wolves and coyotes. Similar conclusions were

17

reached by a series of genetic papers examining red wolf mtDNA and nuclear DNA (nDNA) that accepted the premise that red wolves originated from hybridization events occurring between 250 to 13,000 years ago (Roy et al. 1996; Reich et al. 1999; vonHoldt et al. 2011). However, these conclusions have been contested in morphological (Nowak and Federoff 1996; Nowak 2002, 2003) and molecular (Bertorelle and Excoffier 1998; Wilson et al. 2000; Hedrick et al. 2002; Adams et al. 2003; Wilson et al. 2003; Kyle et al. 2006; Hailer et al. 2008; Chambers et al. 2012; Rutledge et al. 2012) studies.

Examining the origin and taxonomy of wolves in eastern Canada, Wilson et al. (2000, 2003) reported that captive red wolves and eastern wolves (*Canis lycaon*) have mtDNA control sequences more closely related with coyotes, while exhibiting unique haplotypes not found in gray wolves and coyotes. Although these mtDNA sequences don't occur in western coyotes, they cluster among western coyote populations and Wilson et al. (2000) attributed this as evidence that red wolves, eastern wolves, and coyotes share a recent common ancestor in the New World independent of gray wolves. Other studies have supported these conclusions (Wilson et al. 2003; Wheeldon et al. 2010; Chambers et al. 2012) and these results appear to reconcile early observations that red wolves and coyotes approached one another in morphology (Nowak 1979; Goldman 1937). Although the results of these studies indicate that red wolves are not of hybrid origin, Wilson et al. 2000 proposed that the red wolf and eastern wolf are genetically close enough to be considered a single species under *C. lycaon*. The disagreement among these genetic studies stems in part from differing assumptions about the nature of the coyote-like mtDNA found in eastern and red wolves. Those that support a hybrid origin interpret the coyote-like mtDNA as being from coyotes, whereas those that support the hypothesis that red wolves, eastern wolves, and coyotes share a common ancestry interpret the coyote-like mtDNA as being eastern wolf in origin and a result of incomplete lineage sorting.

Significant gaps in the historic and geographic genetic data and recent hybridization makes it difficult to sort out the evolutionary history of red wolves. As a result, the taxonomy of

18

North American wolves is complex and not without debate. Prior to and during European colonization of the Southeast, there appears to have been a small wolf species present and its modern equivalent may be the red wolf. It is also possible that red wolves are morphologically and genetically similar to coyotes because they fall within the species limits of the coyote clade (Chambers et al. 20012). Although the door is open for future taxonomic revision, the hybrid origin of red wolves is difficult to reconcile because gray wolves have historically been absent from the southeastern United States and, until the mid-20th century, coyotes were absent from the region for over 10,000 years (Nowak 2002). Additionally, there is no evidence of ongoing hybridization between gray wolves and coyotes that are currently sympatric (Kyle et al. 2006; Wheeldon et al. 2010; Rutledge et al. 2012). Modern hybridization among *Canis* species in the East makes it difficult to sort out the evolutionary history of red wolves. Recent and developing studies demonstrate that the taxonomy of red wolves is complex and morphological and molecular studies of fossilized wolves from the southeast are essential to settling the debate over red wolf origin.

Ecological Challenges

Red Wolf and Coyote Hybridization

Red wolves and coyotes exist as a panmictic population in NENC and hybridization provides an exceptionally tough set of problems for red wolf recovery. Understanding how red wolves interact with coyotes is an important issue which could dictate the success of the reintroduction project. During 1999, the USFWS re-evaluated the red wolf recovery effort by organizing a Population and Habitat Viability Assessment workshop (PHVA; Kelly et al. 1999). Introgression of coyote genes into the red wolf population was considered the principal threat to recovery efforts when it was discovered that hybridization could render the wild red wolf population unrecognizable within several generations (Kelly et al. 2006; Stoskopf et al. 2005). As a result, priorities were identified and the PHVA called for approaches that would prevent hybridization and promote the growth of a self-sustaining population of red wolves in NENC. An

adaptive management plan (Rabon et al. 2013) was designed during the PHVA with the intent to provide the Recovery Program flexibility to modify management schemes and scientific studies as conditions and threats to red wolf recovery change.

As history has proven, coyote populations are too resilient to state and federal eradication programs and clearing the Albemarle Peninsula of coyotes poses an overwhelming challenge. Two management techniques were developed during the PHVA to prevent hybridization. Coyotes and hybrids captured by USFWS personnel within the Recovery Area are reproductively sterilized (hereafter sterilized) and used as space holders until red wolves move in and occupy those areas. Coyotes and hybrids are taken to a local veterinary clinic in which females and males are sterilized by tubal ligation and vasectomy, respectively. This process keeps the hormonal system intact and avoids disrupting breeding and territorial behavior. Sterilized animals are fitted with mortality-sensitive radio-collars, released, and monitored for the duration of their life. This allows the Recovery Program to collect relevant information on coyote space use, habitat selection, and interaction with red wolves while suppressing coyote reproduction. In the event that a red wolf pairs with a sterilized coyote, the pair cannot produce hybrid litters. Additionally, sterilized coyotes that maintain territories keep those spaces occupied and prevent fertile coyotes from establishing breeding pairs on the landscape.

As recommended during the PHVA, the Recovery Area was divided into three management zones in which management efforts varied in intensity to minimize hybridization on the landscape (Figure 2). The ultimate management goal is to ensure that all *Canis* breeding pairs within the Recovery Area are red wolves. To implement this, Recovery Program biologists began eradicating coyotes and hybrids from Zone 1 while selectively using sterilized coyotes as space holders in Zone 2. When objectives in Zone 1 were completed, management efforts shifted west to Zone 2 in which sterilized space holders were removed to create space for red wolves. Once coyotes and hybrids were removed from Zone 1 and 2, management efforts would be undertaken in Zone 3. Implementing management goals in order of priority allowed the Recovery Program to

minimize hybridization by monitoring red wolf and coyote packs throughout the Recovery Area and replacing coyotes and hybrids with red wolves when opportunities arose.

Prior to the PHVA, the Recovery Program assumed all canids captured within the Recovery Area were wolves unless animals were unusually small and coyote-like in appearance (Stoskopf et al. 2005). Once hybridization was considered the primary threat to recovery efforts, molecular techniques were developed to identify coyotes and hybrids and quantify introgression into the red wolf population. Using microsatellite markers from the 14 founding individuals and other captive red wolves to generate allele frequencies, a pedigree of the red wolf population was developed (Miller et al. 2003; Adams 2006). Animals are now blood sampled upon capture and identified as red wolves, coyotes, or hybrids using 17 microsatellite markers. As these methods were developed, a hybridization event that occurred during 1993 between a female red wolf and a male coyote was detected (Adams 2006; USFWS 2007). Individuals in the wild population considered red wolves were then correctly identified as 2nd and 3rd generation backcrosses from the male hybrid offspring of the 1993 hybridization event. When it was realized that removing all red wolves with introgression would essentially extirpate the wild red wolf population, the Recovery Program opted to allow wild reproduction among red wolves to slowly breed the coyote genetics out. To accelerate purging of coyote genetics, the Recovery Program selectively culled animals they thought were not red wolves. Over time, selective management of backcrosses and minimizing hybridization has been successful in limiting coyote introgression in the wild red wolf population to less than 5% in 2006 (Adams 2006) and has continue to facilitate a decrease since then (USFWS unpublished).

Scientific research is essential to understanding hybridization and the interplay between research and management offers an interesting opportunity to examine this process over the long-term. Initial scientific inquiries after the PHVA were to establish studies to measure, monitor, and manage hybridization in the Recovery Area. In doing so, a complete reconstruction of a red wolf pedigree has been established and this most likely represents the most complete database for any

wild population. It's now understood that hybridization between red wolves and coyotes is not directional in terms of the wolves' sex and hybrids backcross with both species (Adams 2006; Rabon 2009; Bohling 2011). Furthermore, current research has identified young, inexperienced red wolves with coyote ancestry to be more likely to breed with coyotes (Bohling 2011). Despite these successes in measuring and monitoring hybridization, ecological explanations for hybridization have been lacking. In other words, little quantitative information exists on mate selection and possible reproductive barriers between red wolves and coyotes (Bohling 2006; Rabon 2009), and future research efforts should focus on discovering possible reproductive isolating mechanisms that exist between red wolves and coyotes.

Hybridization between red wolves and coyotes implies the obvious break down of reproductive barriers and the two species consort and breed with one another when situations favor opportunities to mate with congenerics. Currently, no extrinsic reproductive barriers (i.e., geographic barriers) exist between red wolves and coyotes because coyotes are ubiquitous throughout the red wolf's historic range. Hybridization occurs between the two species when a red wolf and a coyote form a breeding pair that will defend a territory together until the death or displacement of a mate. Consequently, the red wolf-coyote pair will produce hybrid offspring and maintain pack dynamics similar to gray wolves (Jordan et al. 1967; Mech 1970, 1999), red wolves (Phillips et al. 2003; Hinton and Chamberlain 2010; Sparkman et al. 2011), and coyotes (Gese et al. 1996a, 1996b; Bekoff and Gese 2003). This should be expected because monogamous breeding appears to be a phylogenetic component that operates at the family level and group living is common within *Canis* (Gittleman 1989; Geffen et al. 1996). Therefore, if an isolating mechanism exists, it's most likely to be an intrinsic isolating factor (i.e., behavior) that would prevent pair formation between red wolves and coyotes. Understanding the ecology of red wolf-coyote interactions is crucial to define species traits that serve as isolating mechanisms, describe how these traits prevent hybridization, and identify what selection forces in nature favor the maintenance of red wolves and coyotes as separate species.

22

If intrinsic isolating factors do exist between red wolves and coyotes, then behaviors that promote sexual isolation of individual red wolves and coyotes should be associated with phenotypes that promote divergence in behavior and genetic discontinuity between the two species. Red wolves and coyotes share the same body plan but do not overlap in body size in which red wolves are the larger species (Hinton et al. In review). As a result, body size is the primary trait that distinguishes red wolves from coyotes and it most likely facilitates differential use of resources between the two species. It is well established that body size has a major effect on inter- and intraspecific interactions of mammalian carnivore species in which competitive interactions are strongly asymmetrical with larger species displacing smaller competitors (Rosenzweig 1966, 1968; Gittleman 1985; Palomares and Caro 1999). Furthermore, body size is a key predictor of life history traits, population growth rates, density, space use, and predator-prey dynamics (Huxley 1936; McNab 1963; Gittleman 1985; Brown and Nicoletto 1991; Brown et al. 2004; White et al. 2007). It is logical that red wolves and coyotes are not exempt from the broad influences that body-size allometries have at individual-, population-, and community-level processes. Understanding how body size differences lead to differences in red wolf and coyote resource demands, demographics, diet, and space use will lead to more comprehensive understanding of red wolf-coyote interactions and identify what behaviors facilitate genetic discontinuity between the two species.

Recent research has allowed the Recovery Program to measure, monitor, and manage hybridization in NENC. However, preventing hybridization using reproductive sterilization techniques is heavy handed and a short-term strategy to jump start red wolf colonization. There are other important biological considerations to be addressed and research objectives regarding hybridization should shift in the direction of studying the relationship between phenotypic traits and hybridization. For instance, when choosing a mate, do red wolves and coyotes use a criterion of mate quality as a predictor of benefits that potential mates offer and, if so, how does choosing for mate complementarity effect partner fidelity and breeding pair stability? These types of

23

research objectives could associate specific traits with hybridization and breeding success and, eventually, allow biologists to detect selection processes within the red wolf and eastern coyote populations. Reproductive barriers are maintained through ecological, demographic, and developmental conditions (Mayr 1941) and understanding how sexual isolation operates is crucial to the restoration of red wolves.

Inbreeding Effects

Inbreeding can increase the risk of extinction for small populations by decreasing reproductive rates and increasing susceptibility to environmental change and disease (Charlesworth and Charlesworth 1987; Crnokrak and Roff 1999; Hedrick and Kalinowski 2000; Charlesworth and Willis 2009). A primary goal of many conservation programs is to minimize inbreeding depression, the deleterious effects of inbreeding, because of the link between increased inbreeding and loss of population viability (Lande 1988; Allendorf et al. 2010; Frankham 2010). As a small population pushed to the brink of extinction, the red wolf suffered considerable loss of genetic diversity and obviating the potential effects of inbreeding depression and further loss of genetic diversity on red wolf fitness is a conservation goal (USFWS 1989). Given inbreeding depression may occur when red wolves mate with closely related kin, and as a population founded by few individuals, managing the overall relatedness of captive and wild populations poses challenges for restoration efforts.

Captive breeding of red wolves began three decades ago to preserve the species and provide demographic security. Preservation of genetic diversity in captivity requires using a red wolf Population Analysis and Breeding and Transfer Plan to select sires and dams for artificial breeding (Waddell and Long 2010). The long-term goal is to preserve 80-90% of the genetic diversity for 150 years (USFWS 1989) and, currently, the captive red wolf population has retained 89.5% of the genetic diversity that existed in the 14 founders (USFWS 2007; Waddell and Long 2010). Although heritable defects, such as progressive retinal atrophy, malocclusion, and undescended testicles, were observed in a small number of captive red wolves, early studies

24

that examined juvenile survival and litter size reported no observable inbreeding depression in the red wolf captive program (Kalinowski et al. 1999; USFWS 2007; Hedrick and Fredrickson 2008). Subsequent studies found increased levels of inbreeding in the captive population were correlated with decreased litter size, but overall, inbreeding depression was minimal (Rabon and Waddell 2010). Rabon and Waddell (2010) concluded that improvements in husbandry, veterinary care, and nutrition positively contribute to pup survival and offset the negative effects of inbreeding in the captive population. However, these services are not extended to red wolves in the wild and understanding the effects of inbreeding in the wild population requires further study.

Red wolves are social carnivores in which intraspecific aggression and delayed dispersal play an important role in pack dynamics. The small size of the wild population and the high level of relatedness among individuals increase the risk for incestuous mating to occur. The influence of mate choice and inbreeding avoidance behavior on population dynamics remains poorly understood. It's been shown that dispersal is an important inbreeding avoidance behavior in other canid species that results in few inbred matings (Smith et al. 1997; Jankovic et al. 2010; Geffen et al. 2011). Similarly, Sparkman et al. (2012) found few instances of breeding between 1st degree relatives in wild red wolves and concluded that dispersal behaviors reduced the risk of inbreeding. Red wolf behaviors associated with inbreeding avoidance suggest that inbreeding has a negative effect on fitness and may influence population dynamics.

Inbreeding levels of wild red wolf populations may be high and the effect of inbreeding avoidance on hybridization with coyotes remains unknown. Inbreeding avoidance may cause red wolves to outbreed with a closely related species, such as coyotes, when inbreeding leads to severe fitness consequences. During the mid-2000s, Recovery Program biologists observed dispersing red wolves passing through territories of potentially available red wolf mates and pair-bonding with coyotes. They speculated inbreeding avoidance may influence red wolf mate choice and facilitate hybridization. The premise behind this observation is a hypothesis that assumes when red wolves cannot locate red wolf mates unrelated to them they will opt to breed with

unrelated coyotes to avoid incest (Beyer and Lucash personal communication). Therefore, understanding how inbreeding depression influences hybridization between red wolves and coyotes has become a concern for managing the wild red wolf population.

Research on the effects of inbreeding should involve both ecological and genetic analyses to investigate red wolf and coyote pair formation and how inbreeding avoidance influences hybridization and red wolf fitness. One particular area of promise is sequencing major histocompatibility complex (MHC) genes to examine red wolf kin recognition and mate choice. MHC genes were originally identified in inbred mice during skin graft experiments in which MHC molecules of the host recognized graft tissue as foreign antigens and attacked them (Snell and Higgins 1951). Since then, MHC genes have been discovered to play a critical role in cellular immune response and correlations between MHC alleles, haplotypes, or heterozygosity and pathogen resistance have been shown for a number of species (Apanius et al 1997; Sommer 2005). Given that MHC variation affects disease resistance, there may be an advantage to avoid kin and other mates with similar MHC alleles or haplotypes (Milinski 2006; Huchard et al. 2010). Studies have found MHC-dependent mate choice in both captive and wild species where individuals preferred MHC dissimilar mates (Piertney and Oliver 2006). Cooperative group living is a primary adaptive characteristic of red wolves and individuals are likely to recognize kin. How MHC variation influences kin recognition and, subsequently, inbreeding avoidance and hybridization in red wolves is unknown. Red wolves are known to have fewer MHC alleles than other wild canid populations (Hedrick et al. 2002) and future research should evaluate how MHC variation may influence mate selection, and hybridization.

Red Wolf Demographics

It's well established that variation in survival and reproduction are responsible for the dynamics of populations (Leslie 1945; Ginzburg 1986; Gotelli 2001; Watts et al. 2009; Stahler et al. 2013). Accurate estimates of survival and reproductive rates are essential for conservation programs to minimize extinction risks and promote conditions enhancing the persistence of small,

vulnerable populations (Willams et al. 2002; Norris 2004). Population viability analysis (PVA) has traditionally been used to project population trajectories into the future based on ecological and demographic parameters (Akçakaya 2000a, 2000b). The red wolf population currently exists as a small, vulnerable population with a high risk of extinction to demographic and environmental stochasticity. Understanding how the red wolf population is expected to change in response to environmental conditions is dependent on accurate estimates of vital rates and realistic population estimates from quantitative models.

A primary goal of red wolf recovery is to establish and maintain a red wolf population of 220 individuals in three disjunct populations within the species' historical range (USFWS 1989). To evaluate the red wolf population and its viability in the presence of a ubiquitous coyote population, a PVA model was developed at the PHVA to predict population trends and the effect of hybridization on red wolf persistence (Kelly et al. 1999). The 1999 PVA predicted that red wolves would increase 20% each year for about 10 years before reaching a carrying capacity limit of 140 individuals. Low mortality for wild wolves was assumed to drive the rate of population growth and, despite not reaching 220 individuals, no immediate risk of extinction was suspected given this scenario. When hybridization was incorporated into the 1999 PVA, increased loss of female red wolf breeders to coyote encroachment was predicted to suppress reproductive rates of red wolves to a level too low to offset natural and human-mediated mortality. Therefore, increasing levels of hybridization increases the risk of extinction not only through red wolf assimilation into the coyote population but, also through an inability to replace red wolves lost to mortality.

The 2007 5-year status review (hereafter 2007 Review) of red wolves indicated the NENC population had fluctuated between 80-130 individuals per year since 1999 (USFWS 2007). With an estimated carrying capacity (K) of 140 individuals that was reached in 2001, it was assumed that the red wolf population would continue to expand in subsequent years because red wolves occupied approximately 60% or less of the Albermarle Peninsula land area (USFWS

27

2007). However, the red wolf population did not expand but, rather, gradually declined to approximately 100 individuals since peaking in 2001 (see Red Wolf Recovery Program Quarterly Reports). Preliminary analysis of red wolf demographics from 1999 until 2007 indicated overall annual red wolf survival rate was 78.2% and anthropogenic sources of mortality (e.g. gunshots, trapping, and vehicle strikes) accounted for 58% of red wolf deaths (USFWS 2007). The 2007 Review reported the high proportion of red wolf deaths by anthropogenic factors was additive to other mortality sources and that red wolf fatalities resulting from gunshots remains the most problematic to red wolf persistence.

Red wolves were seven times more likely to be killed during the North Carolina white-tailed deer (*Odocoileus virginianus*) hunting season (October 15 – December 15) than during the non-hunting season (USFWS 2007; Bohling 2011). Illegal take of red wolves is believed to hamper red wolf population growth because it disrupts natural behavioral dynamics that effect demographic processes (Packer et al. 2009; Rutledge et al. 2012b). Furthermore, reduction of red wolves increases coyote presence in the Recovery Area by breaking up packs and destabilizing social dynamics, which reduces the red wolf's ability to hold and defend territories against coyotes. The breeding season for red wolves occurs during white-tailed deer and American black bear (*Ursus americanus*) hunting seasons and increased mortality rates during this time forces red wolf breeders to quickly replace lost mates. When red wolves lost mates to gunshots during the hunting seasons they were more likely to pair with coyotes or fail to replace their mates than to pair with red wolves (USFWS 2007). Evaluating the breeding records and individual histories of red wolves involved in hybridization events, Bohling (2011) found most hybridization events occurred after red wolves lost mates to gunshots and suggested that social structure and stability play a critical role in preventing hybridization. Similarly, Rutledge et al. (2012b) found intense harvest of eastern wolves around Algonquin Provincial Park (APP) during the 1960s to have exacerbated hybridization with coyotes. Therefore, it is prudent for red wolf conservation that managers better understand how high mortality caused by illegal killing of red wolves during the

hunting season may disrupt social structures, influence population dynamics, and promote hybridization with coyotes.

The PHVA warned that human-caused mortality that is additive would facilitate hybridization and increase the risk of extinction for red wolves. Therefore, research on red wolf demographics should focus on elucidating mechanisms that influence persistence of wolves on the landscape. This requires use of the Recovery Program's long-term monitoring data of the NENC red wolf population. Long-term monitoring involves annual trapping of red wolves during the fall to radio-tag juvenile and adult red wolves and inspection of dens during the spring to count and transponder pups (Rabon et al. 2013). These efforts allow the Recovery Program to identify individual red wolves at birth and monitor them until death to collect baseline data on survival and reproduction. Demographic parameters such as survival and population size can be estimated from capture-recapture data (Nichols et al. 1994; Burnham et al. 1995; Ivan et al. 2013) and research efforts should incorporate red wolf monitoring data to develop accurate parameters. Research objectives should estimate annual rates of population change and age-specific survival and reproductive rates of the red wolf population. Additionally, effects of natural and anthropogenic sources of mortality on red wolf persistence should be examined to understand how environmental conditions affect population dynamics over the short- and long-term. This type of research would provide accurate estimates of population parameters for PVAs and assist in developing a valuable framework to evaluate important ecological questions related to red wolf population dynamics.

Conclusions

Created in the wake of new societal values, the Red Wolf Recovery Program was tasked by the USFWS with the responsibility of restoring red wolves within their historic range. Along the road to saving the red wolf from extinction, the Recovery Program extirpated the species from the wild to prevent its genetic assimilation into the expanding coyote population. The Recovery Program established a captive-breeding program, and despite starting with 14 founders, grew a

captive population of red wolves used for future reintroductions into the wild. During fall of 1987, the red wolf became the first carnivore completely extirpated from the wild to be successfully reintroduced back into its historic range. Today, the Recovery Program manages the only wild population of red wolves on the Albemarle Peninsula of North Carolina. However one views the merits of this effort to restore red wolves, it is a story with challenges and one worth contemplation.

Disagreements about the nature of coyote-like DNA found in red wolves have created controversy in red wolf taxonomy and conservation. The initial discovery of coyote-like haplotypes in red wolves spurred conclusions that the species originated through modern hybridization between gray wolves and coyotes (Wayne and Jenks 1991; Roy et al. 1996). As a result, academic debates during the 1990s focused on the role of modern hybridization in red wolves and its implications for red wolf conservation (Gittleman and Pimm 1991; Nowak 1992; Wayne and Gittleman 1995; Nowak and Federoff 1998). However, later research reported the coyote-like DNA found in red wolves indicated a shared ancestry with eastern wolves and coyotes, and concluded that all three species evolved in a New World canid lineage independent of gray wolves (Wilson et al. 2000; Wheeldon et al 2010; Chambers et al. 2012). Predictably, the academic debate has begun shifting towards resolving whether eastern and red wolves are conspecific (Murray and Waits 2007; Kyle et al. 2008; Wheeldon et al. 2010; Chambers et al. 2012). Taxonomy is fluid because species evolve and competing concepts over species statuses are not uncommon. In the case of the red wolf, the lack of historic and geographic specimens coupled with modern hybridization between red wolves and coyotes facilitate conflicting conclusions with regards to the species origin. Therefore, it's realistic to expect scientific debate over the taxonomic status of red wolves and, as future studies provide new information, revisions to competing hypotheses regarding species origin should be expected.

Any discussion of red wolf recovery must occur against the backdrop of current ecological and anthropogenic challenges. Although hybridization, inbreeding, and demographics

were discussed separately earlier, these three issues are intrinsically related because they are influenced by the presence and management of coyotes. Therefore, these issues are complex and controversial causing management plans to promote recovery efforts to be more difficult than planned. Prior to the mid-1990s, coyotes were rare but increasing in NENC and Recovery Program biologists anticipated eventual colonization of the Peninsula by coyotes. The use of sterilized coyotes as space holders allowed the Recovery Program to saturate the Recovery Area with territories of red wolf packs and sterile coyote pairs. During the early 2000s, most coyotes captured, sterilized, and released with radio-collars failed to establish territories or pair with a space holder. In other words, the Recovery Program effectively saturated the Peninsula with canid territories and coyotes dispersing into the Recovery Area failed to find available space or mates and eventually left. However, legislation (NCGS § 113 273) passed by the NC General Assembly allowing owners of fox pens to buy live coyotes from licensed trappers and hunt them within their fox pens (NCWRC 2013) may negatively affect these efforts by disrupting red wolf packs and sterilized coyote space holders. Fox pens are enclosures averaging 250 ha in which gray foxes (*Urocyon cinereoargenteus*), red foxes (*Vulpes vulpes*), and coyotes are hunted with hound dogs for sport in approximately 20 states (Davidson et al. 1992; Baker 1993; Lee et al. 1993). Fox pen operations create legal and illegal markets for the importation and release of coyotes for hunting opportunities, and those markets may supplement local coyote populations through accidental or intentional releases of coyotes into the wild. The number of coyotes trapped in the Recovery Area increased each year after legal trafficking of live coyotes was permitted in 2003 (USFWS unpublished data). Although the number of red wolves captured and hunted in fox pens is unknown, disappearance and illegal take of red wolves has increased since the passage of the law. Increased efforts by trappers to capture coyotes and increased vigilance of deer hunters to shoot coyotes have stagnated red wolf population growth by breaking up red wolf packs and removing sterilized coyote space holders from the landscape through accidental and purposeful killing of red wolves and sterilized coyotes (USFWS unpublished data).

Evident by the widespread persistence and range expansion of coyotes, current policies to control their populations are ineffective and have failed to significantly reduce coyote populations. Laws promoting trafficking and nighttime hunting of coyotes increase the chances that red wolves will be accidentally or purposefully killed by hunters, and attempts to remove these hunting laws are constrained by organized hunting and trapping groups. Increased killing of red wolves by predator-control programs during the early 20th century facilitated the decline of red wolves and promoted their hybridization with expanding coyotes. Recent research showed intense harvest of eastern wolves also facilitated hybridization with coyotes by disrupting the population's social structure (Rutledge et al. 2012b). Today, increased killing of red wolves by humans appears to be disrupting red wolf packs and facilitating hybridization with coyotes (Bohling 2011). Increased relatedness of red wolves through discriminant killing opportunities will eventually lead to inbreeding depression in the wild population. Use of MHC genes to evaluate red wolf mate choice could lead to insights of how red wolves respond reproductively to anthropogenic changes and how MHC variation affects resistance to potential diseases that can be introduced through trafficking coyotes. Therefore, controlling hybridization and inbreeding requires understanding how anthropogenic sources of mortality facilitate conditions favorable to hybridization.

Increased mortality rates of red wolves and coyotes promote high turn-over rates of territories and erode the effectiveness of sterilization methods. Despite this, continued use of sterilization and efforts to increase the number of red wolves on the landscape will likely fail to prevent hybridization if reproductive barriers do not exist in the first place (Fredrickson and Hedrick 2006). Key to developing effective management that prevents the hybridization of sympatric red wolf and coyote populations is to identify unique traits of both species that promote sexual isolation. Within the Recovery Area, some individual red wolves and sterile coyotes appear to always prefer conspecifics as mates while others show random preferences, and assortative mating within both populations may indicate an intrinsic reproductive barrier. In the

hybridization section of this paper, we discussed evaluating the effects of body size on mate complementarity between red wolves and coyotes because body size is the most characteristically distinct trait between the two species. Life history traits (i.e., rates of individuals growth, reproduction, and mortality), population-level processes (i.e., abundance and space use), and community-level interactions (i.e., predator-prey dynamics and interspecific interactions) are known to correlate with body-size allometries regardless of taxonomic status (LaBarbera 1989; Brown et al. 1993, 2004; Capellini et al. 2010). Therefore, there are compelling reasons to study how phenotypes facilitate the outcomes of red wolf-coyote interactions because phenotypes are the direct interface between the two species. Selection acts directly on phenotypes with genetic change occurring as an indirect consequence and phenotypes have ecological effects on population dynamics and community structure (Agrawal 2001; Schluter 2001; Price et al. 2003; Kingsolver and Pfennig 2008; Siepielski et al. 2009; Crispo et al. 2010). If certain phenotypes serve as reproductive barriers between red wolves and coyotes, management can manipulate selection to achieve desired demographic effects and reduce hybridization.

A major impediment to red wolf restoration is the limited knowledge about traits that facilitate behavioral and ecological differences between red wolves and coyotes. This is critical to red wolf restoration because expanding our knowledge about mechanisms that facilitate stable and reproductively isolated red wolf populations will allow us to recognize responses of red wolves to changing environments. This knowledge guides research to make accurate inferences and predictions about the future and promotes implementation of appropriate management. The reality of incomplete reproductive isolation may present challenges to red wolf restoration but evolution is ongoing and management efforts should promote conditions that allow for the gradual evolution of reproductive barriers. Although much work remains to be done, information and experiences gained from more than 25 years of restoration efforts have made crucial contributions to the future of the red wolf. They also allow us to formulate areas of investigation that are of direct relevance to the restoration of red wolves.

Acknowledgements

Literature Cited

Adams, J.R. 2006. A multi-faceted molecular approach to red wolf (*Canis rufus*) conservation and management. Dissertation, University of Idaho, Moscow, ID, USA.

Adams, J.R., B.T. Kelly, and L.P. Waits. 2003. Using faecal DNA sampling and GIS to monitor hybridization between red wolves (*Canis rufus*) and coyotes (*Canis latrans*). Molecular Ecology 12: 2175-2186.

Agrawal, A.A. 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294: 321-326.

Akçakaya, H.R. 2000a. Population viability analyses with demographically and spatially structured models. Ecological Bulletins 48: 23-38.

Akçakaya, H.R. 2000b. Conservation and management for multiple species: integrating field research and modeling into management decisions. Environmental Management 26: S75-S83.

Allendorf, F.W., P.A. Hohenlohe, and G. Luikart. 2010. Genomics and the future of conservation genetics. Nature Reviews Genetics 11: 697-709.

Apanius, V., D. Penn, P. Slev, L.R. Ruff, and W.K. Potts. 1997. The nature of selection on the major histocompatibility complex. Critical Reviews in Immunology 17: 179-224.

Audubon, J.J., and J. Bachman. 1851. Viviparous quadrupeds of North America: volume 2. J.J. Audubon, New York, NY, USA.

Baily, V. 1905. Biological survey of Texas. North American Fauna 25: 1-255.

Baker, O.E. 1998. Status of fox and coyote hunting enclosures in the southeastern United States. Proceedings of the 52nd Annual Conference of Southeastern Association of Fish and Wildlife Agencies 52: 367-376.

Bangs, O. 1898. The land mammals of peninsular Florida and the east coast region of Georgia. Proceedings of the Boston Society of Natural History 28: 157-235.

Bartram, W. 1791. Travels through North and South Carolina, Georgia, east and west Florida, the Cherokee country, the extensive territories of the Muscogulges or Creek Confederacy, and the country of the Chactaws. Containing an account of the soil and natural productions of those regions; together with observations on the manners of the Indians. James & Johnson, Philadelphia, PA, USA.

Bekoff, M., and E.M. Gese. 2003. Coyote (*Canis latrans*). Pages 467-481 *in* G.A. Feldhamer, B.C. Thompson, and J.A. Chapman, editors. Wild mammals of North America, 2nd edition. John Hopkins University Press, Baltimore, MD, USA.

Bertorelle, G., and L. Excoffier. 1998. Inferring admixture proportions from molecular data. Molecular Biology and Evolution 15: 1298-1311.

Bohling, J.H. 2011. Exploring patterns and mechanisms of red wolf (*Canis rufus*) hybridization in North Carolina. Dissertation, University of Idaho, Moscow, ID, USA.

Brown, J.H., and P.F. Nicoletto. 1991. Spatial scaling of species composition: body masses of North American land mammals. American Naturalist 138: 1478-1512.

Brown, J.H., P.A. Marquet, and M.L. Taper. 1993. Evolution of body size: consequences of an energetic definition of fitness. American Naturalist 142: 573-584.

Brown, J.H., J.F. Gillooly, A.P. Allen, V.M. Savage, and G.B. West. 2004. Toward a metabolic theory of ecology. Ecology 85: 1771-1789.

Burnham, K.P., G.C. White, and D.R. Anderson. 1995. Model selection strategy in the analysis of capture-recapture data. Biometrics 51: 888-898.

Capellini, I., C. Venditti, and R.A. Barton. 2010. Phylogeny and metabolic scaling in mammals. Ecology 91: 2783-2793.

Carley, C.J. 1975. Activities and findings of the Red Wolf Recovery Program from late 1973 to July 1, 1975. United States Fish and Wildlife Service, Albuquerque, NM, USA.

Carly, C.J. 2000. The Red Wolf (*Canis rufus*) Recovery Program: things they didn't tell me in school. Pages 125-141 *in* J.R. Choate, editor. Reflections of a naturalist: papers honoring Professor Eugene D. Fleharty. Fort Hays State University, Hays, KS, USA.

Chambers, S.M., S.R. Fain, B. Fazio, and M. Amaral. 2012. An account of the taxonomy of North American wolves from morphological and genetic analyses. North American Fauna 77: 1-67.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18: 237-268.

Charlesworth, D., and J.H. Willis. 2009. The genetics of inbreeding depression. Nature Reviews Genetics 10: 783-789.

Crispo, E., J.D. DiBattista, C. Correa, X. Thibert-Plante, A.E. McKellar, A.K. Schwartz, D. Berner, L.F. De Leòn, and A.P. Hendry. 2010. Evolution and Ecology Research 12: 47-66.

Crnokrak, P., and D.A. Roff. 1999. Inbreeding depression in the wild. Heredity 83: 260-270.

Davidson, W.R., M.J. Appel, G.L. Doster, O.E. Baker, and J.F. Brown. 1992. Disease and parasites of red foxes, gray foxes, and coyotes from commercial sources selling to fox-chasing enclosures. Journal of Wildlife Disease 28: 581-589.

Frankham, R. 2010. Where are we in conservation genetics and where do we need to go? Conservation Genetics 11: 661-663.

Fredrickson, R.J., and P.W. Hedrick. 2006. Dynamics of hybridization and introgression in red wolves and coyotes. Conservation Biology 20: 1272-1283.

Geffen, E., M.E. Gompper, J.L. Gittleman, L. Hang-Kwang, D.W. Macdonald, and R.K. Wayne. 1996. Size, life-history traits and social organization in the Canidae: a reevaluation. American Naturalist 147: 140-160.

Geffen, E., M. Kam, R. Hefner, P. Hersteinsson, A. Angerbjörn, L. Dalèn, E. Fuglei, K. Norèn, J.R. Adams, J. Vucetich, T.J. Meier, L.D. Mech, B.M. vonHoldt, D.R. Stahler, and R.K.

Wayne. Kin encounter rate and inbreeding avoidance in canids. Molecular Ecology 20: 5348-5358.

Gese, E.M., R.L. Ruff, and R.L. Crabtree. 1996a. Social and nutritional factors influencing dispersal of resident coyotes. Animal Behavior 52: 1025-1043.

Gese, E.M., R.L. Ruff, and R.L. Crabtree. 1996b. Foraging ecology of coyotes (*Canis latrans*): the influence of extrinsic factors and a dominance hierarchy. Canadian Journal of Zoology 74: 769-783.

Ginzburg, L.R. 1986. The theory of population dynamics: I. Back to first principles. Journal of Theoretical Biology 122: 385-399.

Gittleman, J.L. 1985. Carnivore body size: ecology and taxonomic correlates. Oecologia 67: 540-554.

Gittleman, J.L. 1989. Carnivore group living: comparative trends. Pages 183-207 *in* J.L. Gittleman, editor. Carnivore behavior, ecology, and evolution: volume 1. Cornell University Press, Ithaca, NY, USA.

Gittleman, J.L., and S.L. Pimm. 1991. Crying wolf in North America. Nature 351:524-525.

Goldman, E.A. 1937. The wolves of North America. Journal of Mammalogy 18: 37-45.

Goldman, E.A. 1944. Classification of wolves. Pages 218-319 *in* S.P. Young, and E.A. Goldman, editors. The wolves of North America: volume 2. American Wildlife Institute, Washington, DC, USA.

Gotelli, N.J. 2001. A primer of ecology. 3rd edition. Oxford University Press, New York, NY, USA.

Hailer, F., and J.A. Leonard. 2008. Hybridization among three native North American *Canis* species in a region of natural sympatry. PLoS One 3: e3333, doi:10.1371/journal.pone.0003333

Harper, F. 1942. The name of the Florida wolf. Journal of Mammalogy 23: 339.

Hedrick, P.W., and S.T. Kalinowski. 2000. Inbreeding depression in conservation biology. Annual Review of Ecology and Systematics 31: 139-162.

Hedrick, P.W., R.N. Lee, and D. Garrigan. 2002. Major histocompatibility complex variation in red wolves: evidence for common ancestry with coyotes and balancing selection. Molecular Ecology 11: 1905-1913.

Hedrick, P.W., and J. Fredrickson. 2008. Captive breeding and the reintroduction of Mexican and red wolves. Molecular Ecology 17: 344-350.

Henry, V.G. 1998. Notice of termination of the red wolf reintroduction project in the Great Smoky Mountains National Park. Federal Register 63: 54152-54153.

Hinton, J.W., and M.J. Chamberlain. 2010. Space and habitat use by a red wolf pack and their pups during pup-rearing. Journal of Wildlife Management 74: 55-58.

Hinton, J.W., and M.J. Chamberlain. In review. Morphometrical discrimination of red wolves (*Canis rufus*), coyotes (*Canis latrans*), and their hybrids in North Carolina. Journal of Mammalogy.

Huchard, E., L.A. Knapp, J. Wand, M. Raymond, and G. Cowlishaw. 2010. MHC, mate choice and heterozygote advantage in a wild social primate. Molecular Ecology 19: 2545-2561.

Huxley, J.S., and G. Tessier. 1936. Terminology of relative growth. Nature 137: 780-781.

Ivan, J., G.C. White, and T.M. Shenk. 2013. Using auxiliary telemetry information to estimate animal density from capture-recapture data. Ecology 94: 809-816.

Jankovic, I., B.M. vonHoldt, and N.A. Rosenberg. 2010. Heterozygosity of the Yellowstone wolves. Molecular Ecology 19: 3246-3249.

Jordan, P.A., P.C. Shelton, and D.L. Allen. 1967. Numbers, turnover, and social structure of the Isle Royale wolf population. American Zoologist 7: 233-252.

Kalinowski, S.T., P.W. Hedrick, and P.S. Miller. 1999. No evidence for inbreeding depression in Mexican and red wolf captive breeding programs. Conservation Biology 13: 1371-1377.

Kelly, B.T., P.S. Miller, and U.S. Seal (eds). 1999. Population and habitat viability assessment workshop for the red wolf (*Canis rufus*). Conservation Breeding Specialist Group (SSC/IUCN), Apple Valley, MN, USA.

Kingsolver, J.G., and D.W. Pfenning. 2008. Patterns and power of phenotypic selection in nature. BioScience 57: 561-572.

Kyle, C.J., A.R. Johnson, B.R. Patterson, P.J. Wilson, K. Shami, S.K. Grewal, and B.N. White. 2006. Genetic nature of eastern wolves: past, present and future. Conservation Genetics 7: 273-287.

Kyle, C.J., A.R. Johnson, B.R. Patterson, P.J. Wilson, and B.N. White. 2008.The conspecific nature of eastern and red wolves: conservation and management implications. Conservation Genetics 9: 699-701.

LaBarbera, M. 1989. Analyzing body size as a factor in ecology and evolution. Annual Review of Ecology and Systematics 20: 97-117.

Lande, R. 1988. Genetics and demography in biological conservation. Science 241: 1455-1460.

Lee, G.W., K.A. Lee, and W.R. Davidson. 1993. Evaluation of fox-chasing enclosures at sites of potential introduction and establishment of *Echinococcus multilocularis*. Journal of Wildlife Disease 29: 498-501.

Leslie, P.H. 1945. On the use of matrices in certain population mathematics. Biometrika 33: 183-212.

Lucash, C.F., B. Crawford, and J.D. Clark. 1998. Species repatriation: red wolf. Pages 225-246 *in* J.D. Piene, editor. Ecosystem management for sustainability. Lewis Publishing, Boca Raton, FL, USA.

Mayr, E.W. 1941. Systematics and the origin of species from the viewpoint of a zoologist. Columbia University Press, New York, NY, USA.

McCarley, H. 1959. The mammals of eastern Texas. Journal of Science 11: 385-426.

McCarley, H. 1962. The taxonomic status of wild *Canis* (Canidae) in the south central United States. Southwestern Naturalist 7: 227-235.

McNab, B.K. 1963. Bioenergetics and the determination of home range size. American Naturalist 97: 133-140.

Mech, L.D. 1970. The wolf: the ecology and behavior of an endangered species. University of Minnesota Press, Minneapolis, MN, USA.

Mech, L.D. 1999. Alpha status, dominance, and division of labor in wolf packs. Canadian Journal of Zoology 77: 1196-1203.

Milinski, M. 2006. The major histocompatibility complex, sexual selection, and mate choice. Annual Review of Ecology, Evolution, and Systematics 37: 159-186.

Miller, G.S. 1912. The names of two North American wolves. Proceedings of the Biological Society of Washington 25: 95.

Miller, C., J. Adams, and L. Waits. 2003. Pedigree based assignment tests for reversing coyote (*Canis latrans*) introgression into the wild red wolf (*Canis rufus*) population. Molecular Ecology 12: 3287-3301.

Murray, D.L., and L.P. Waits. 2007. Taxonomic status and conservation strategy of the endangered red wolf: a response to Kyle *et al* (2006). Conservation Genetics 8: 1483-1485.

NCWRC. 2012. Fox and coyote populations study final report. North Carolina Wildlife Resources Commission, Raleigh, NC, USA. Available at the following website: http://www.ncwildlife.org/Portals/0/Learning/documents/Species/Fox_CoyotePopulationsReport.pdf, accessed June 10th, 2013.

Nichols, J.D., J.E. Hines, K.H. Pollock, R.L. Hinz, and W.A. Link. 1994. Estimating breeding proportions and testing hypotheses about costs of reproduction with capture-recapture data. Ecology 75: 2052-2065.

Norris, K. 2004. Managing threatening species: the ecological toolbox, evolutionary theory and declining-population paradigm. Journal of Applied Ecology 41: 413-426.

Nowak, R.M. 1979. North American quaternary *Canis*. Monograph of the Museum of Natural History, the University of Kansas 6: 1-154.

Nowak, R.M. 1992. The red wolf is not a hybrid. Conservation Biology 6; 593-595.

Nowak, R.M. 1995. Another look at wolf taxonomy. Pages 375-397 *in* L.N. Carbyn, S.H. Fritts, and D.R. Seip, editors. Ecology and conservation of wolves in a changing world: proceedings of the second North American symposium of wolves, Edmonton, Alberta, 25-27 August 1992. Canadian Circumpolar Institute, University of Alberta, Edmonton, AB, Canada.

Nowak, R.M. 2002. The original status of wolves in eastern North America. Southeastern Naturalist 1: 95-130.

Nowak, R.M. 2003. Wolf evolution and taxonomy. Pages 239-271 *in* L.D. Mech, and L. Boitani, editors. Wolves: behavior, ecology, and conservation. University of Chicago Press, Chicago, IL, USA.

Nowak, R.M., and N.E. Federoff. 1996. Systematics of wolves in eastern North America. Pages 187-203 *in* N. Fascione, and M. Cecil, editors. Wolves of America Conference Proceedings, November 1996. Defenders of Wildlife: Albany, NY USA.

Nowak, R.M., and N.E. Federoff. 1998. Validity of the red wolf: response to Roy *et al*. Conservation Biology 12: 722-725.

Packer, C., M. Kosmala, H.S. Cooley, H. Brink, L. Pintea, and D. Garshelis. 2009. Sport hunting, predator control and conservation of large carnivores. PLoS One 4: doi: 10.1371/journal.pone.0005941.

Palomares, F., and T.M. Caro. 1999. Interspecific killing among mammalian carnivores. American Naturalist 153: 492-508.

Paradiso, J.L. 1965. Recent records of red wolves from the Gulf Coast of Texas. Southwestern
Naturalist 10: 218-319.

Paradiso, J.L. 1968. Canids recently collected in east Texas, with comments on the taxonomy of
the red wolf. American Midland Naturalist 80: 529-534.

Paradiso, J.L., and R.M. Nowak. 1972. *Canis rufus*. Mammal Species 22: 1-4.

Phillips, M.K., and V.G. Henry. 1992. Comments on red wolf taxonomy. Conservation Biology
6: 596-599.

Phillips, M.K., V.G. Henry, and B.T. Kelly. 2003. Restoration of the red wolf. Pages 272-288 *in*
L.D. Mech, and L. Boitani, editors. Wolves: behavior, ecology, and conservation.
University of Chicago Press, Chicago, IL, USA.

Piertney, S.B., and M.K. Oliver. 2006. The evolutionary ecology of the major histocompatibility
complex. Heredity 96: 7-21.

Pimlott, D.H., and P.W. Joslin. 1968. The status and distribution of the red wolf. Transactions of
the North American Wildlife and Natural Resources Conferences 33: 373-389.

Price, T.D., A. Qvarnström, and D.E. Irwin. 2003. The role of phenotypic plasticity in driving
genetic evolution. Proceedings of the Royal Society B 270: 1433-1440.

Rabon, D.R. 2009. Factors affecting reproduction in the red wolf (*Canis rufus*). Dissertation,
North Carolina State University, Raleigh, NC, USA.

Rabon, D.R., and W. Waddell. 2010. Effects of inbreeding on reproductive success, performance,
litter size, and survival in captive red wolves (*Canis rufus*). Zoo Biology 29: 36-49.

Rabon, D.R., R. Bartel, and A. Beyer. 2013. Red wolf adaptive management plan FY13-FY15.
United States Fish and Wildlife Service, Manteo, NC, USA.

Reich, D.E., R.K. Wayne, and D.B. Goldstein. 1999. Genetic evidence for a recent origin by
hybridization of red wolves. Molecular Ecology 8: 139-144.

Rosenzweig, M.L. 1966. Community structure in sympatric Carnivora. Journal of Mammalogy
47: 602-612.

Rosenzweig, M.L. 1968. The strategy of body size in mammalian carnivores. American Midland
   Naturalist 80: 299-315.

Roy, M.S., E. Geffen, D. Ostrander, and R.K. Wayne. 1996. Patterns of differentiation and
   hybridization in North America wolflike canids, revealed by analysis of microsatellite
   loci. Molecular Biology and Evolution 11: 533-570.

Roy, M.S., E. Geffen, E. Smith, and R.K. Wayne. 1996. Molecular genetics of pre-1940 red
   wolves. Conservation Biology 10: 1413-1424.

Rutledge, L.Y., P.J. Wilson, F.C. Klütsch, B.R. Patterson, and B.N. White. 2012a. Conservation
   genomics in perspective: a holistic approach to understanding *Canis* evolution in North
   America. Biological Conservation 155: 186-192.

Rutledge, L.Y., B.N. White, J.R. Row, and B.R. Patterson. 2012b. Intense harvesting of eastern
   wolves facilitated hybridization with coyotes. Ecology and Evolution 2: 19-33.

Schluter, D. 2001. Ecology and the origin of species. Trends in Ecology and Evolution 16: 327-
   380.

Siepielski, A.M., J.D. DiBattista, and S.M. Carlson. 2009. It's about time: the temporal dynamics
   of phenotypic selection in the wild. Ecology Letters 12: 1261-1276.

Smith, D., T. Meier, E. Geffen, L.D. Mech, J.W. Burch, L.G. Adams, and R.K. Wayne. 1997. Is
   incest common in gray wolf packs? Behavioral Ecology 8: 384-391.

Snell, G.D., and G.F. Higgins. 1951. Alleles at the histocompatibility-2 locus in the mouse as
   determined by tumor transplantation. Genetics 36: 306-310.

Sommer, S. 2005. The importance of immune gene variability (MHC) in evolutionary ecology
   and conservation. Frontiers in Zoology 2: 1-16.

Sparkman, A.M., J. Adams, A. Beyer, T.D. Steury, L. Waits, and D.L. Murray. 2011. Helper
   effects on pup lifetime fitness in the cooperatively breeding red wolf (*Canis rufus*).
   Proceedings of the Royal Society Biological Sciences 278: 1381-1389.

Sparkman, A.M., J.R. Adams, T.D. Steury, L.P. Waits, and D.L. Murray. 2012. Pack social dynamics and inbreeding avoidance in the cooperatively breeding red wolf. Behavioral Ecology 23: 1186-1194.

Stahler, D.R., D.R. MacNulty, R.K. Wayne, B. vonHoldt, and D.W. Smith. 2013. Journal of Animal Ecology 82: 222-234.

Stoskopf, M.K., K. Beck, B.B. Fazio, T.K. Fuller, E.M. Gese, B.T. Kelly, F.F. Knowlton, D.L. Murray, W.T. Waddell, and L.P. Waits. 2005. Implementing recovery of the red wolf: integrating research, scientists, and managers. Wildlife Society Bulletin 33: 1145-1152.

USFWS. 1989. Red wolf recovery/species survival plan. United States Fish and Wildlife Service, Atlanta, GA, USA.

USFWS. 1990. A proposal to reintroduce the red wolf into the Great Smoky Mountains National Park. United States Fish and Wildlife Service, Atlanta, GA, USA.

USFWS. 2007. Red wolf (*Canis rufus*) 5-year review: summary and evaluation. United States Fish and Wildlife Service, Manteo, NC, USA.

USFWS. 2013. Red Wolf Recovery Program 3rd quarter report: April-June 2013. United States Fish and Wildlife Service, Atlanta, GA, USA.

vonHoldt, B.M., J.P. Pollinger, D.A. Earl, J.C. Knowles, A.R. Boyko, H. Parker, E. Geffen, M. Pilot, W. Jedrzejewski, B. Jedrzejewski, V. Sidorovich, C. Creco, R. Ettore, M. Musiani, R. Kays, C.D. Bustamante, E.A. Ostrander, J. Novembre, and R.K. Wayne. 2011. A genome-wide perspective of the evolutionary history of enigmatic wolf-like canids. Genome Research 21: 1294-1305.

Waddell, W., and S. Long. 2010. Population analysis and breeding and transfer plan – red wolf (*Canis rufus gregoryi*) Species Survival Plan® program. Association of Zoos & Aquariums. Available at the following website: http://redwolfssp.org/web/ssp_recommendations/red_wolf_recommendations_2010.pdf, accessed June 15th, 2013.

Watts, H.E., and K.E. Holekamp. 2009. Ecological determinants of survival and reproduction in the spotted hyena. Journal of Mammalogy 90: 461-471.

Wayne, R.K., and S.M. Jenks. 1991. Mitochondrial DNA analysis implying extensive hybridization of the endangered red wolf *Canis rufus*. Nature 351: 565-568.

Wayne, R.K., and J.L. Gittleman. 1995. The problematic red wolf. Scientific American 273: 36-39.

Wheeldon, T., B.R. Patterson, and B.N. White. 2010. Sympatric wolf and coyote populations of the western Great Lakes region are reproductively isolated. Molecular Ecology 19: 4428-4440.

White, E.P., S.K.M. Ernest, A.J. Kerkhoff, and B.J. Enquist. 2007. Relationships between body size and abundance in ecology. Trends in Ecology and Evolution 22: 323-330.

Williams, B.K., J.D. Nichols, and M.J. Conroy. 2002. Analysis and management of animal population. Academic Press, San Diego, CA, USA.

Wilson, P.J., S. Grewal, I.D. Lawford, J.N.M. Heal, A.G. Granacki, D. Pennock, J.B. Theberge, M.T. Theberge, D.R. Voight, W. Waddell, R.E. Chambers, P.C. Paquet, G. Goulet, D. Cluff, and B.N. White. 2000. DNA profiles of the eastern Canadian wolf and the red wolf provide evidence for a common evolutionary history independent of the gray wolf. Canadian Journal of Zoology 78: 2156-2166.

Wilson, P.J., S. Grewal, T. McFadden, R.C. Chamber, and B.N. White. 2003. DNA extract from eastern North American wolves killed in the 1800s is not of gray wolf origin. Canadian Journal of Zoology 81: 936-940.
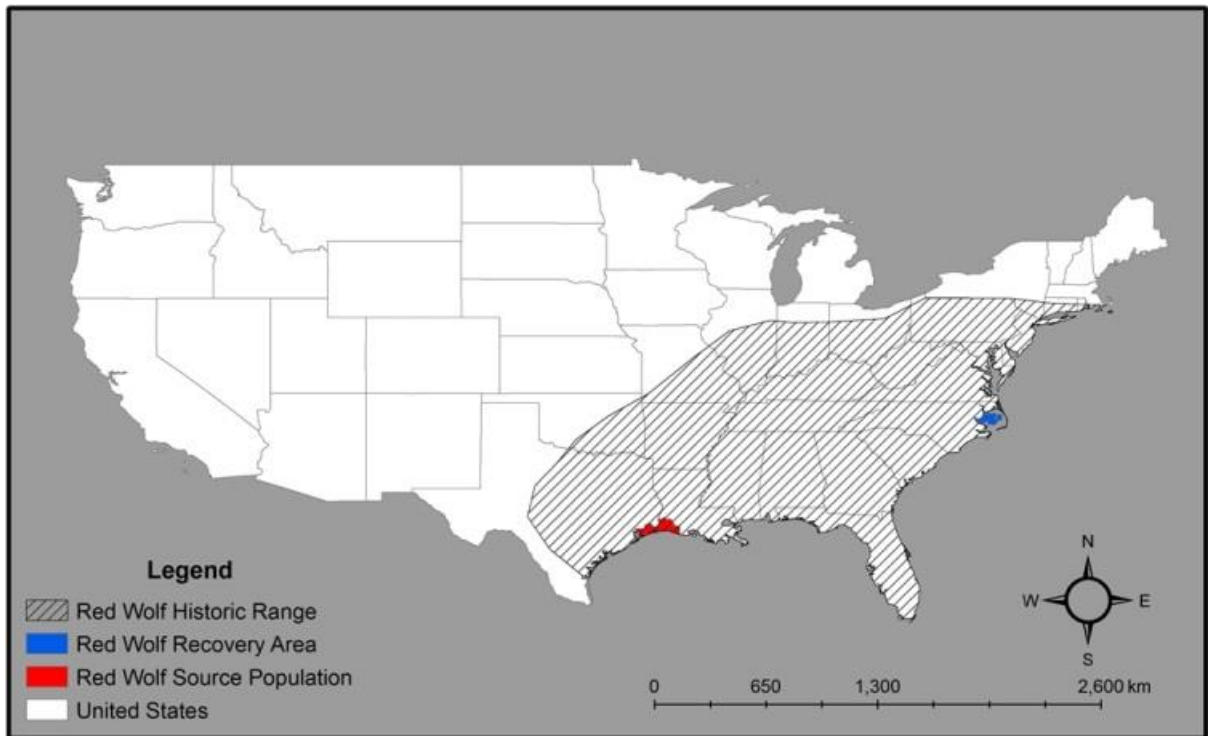
Figure 2.1: Historic and current range of red wolves (*Canis rufus*) in North America.
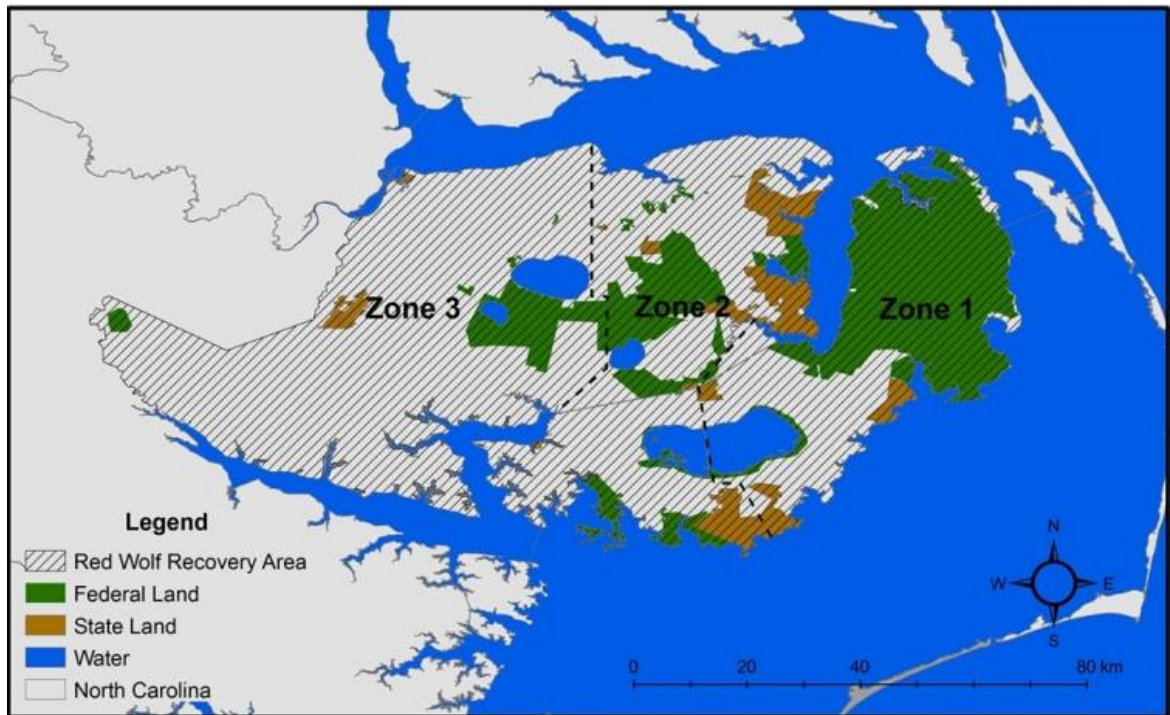
Figure 2.2: Management zone boundaries with the Red Wolf Recovery Area of northeastern

North Carolina.

CHAPTER 3

MORPHOMETRICS OF *CANIS* TAXA IN EASTERN NORTH CAROLINA

_____

Abstract

We describe the external morphological characters of red wolves, coyotes, and their hybrids from

North Carolina and assess if morphology could be an accurate discriminator among the 3 canid

taxa. We used body measurements from 171 red wolves (*Canis rufus*), 134 coyotes (*Canis

latrans*), and 47 hybrids in a polytomous logistic regression analysis to assess if they could be

used to identify canids as red wolves, coyotes, or hybrids. Polytomous logistic regression analysis

of 7 morphometric variables was able to correctly allocate 86% of canids to their a priori taxa

groups. Using Akaike's information criterion, we judged hind foot length, weight, width of head,

and tail length as variables to best separate taxa. Among the 3 sympatric *Canis* taxa in eastern

North Carolina, red wolves are clearly the larger canid with hybrids intermediate to coyotes and

red wolves in body size. Our results suggest that red wolves represent a unique *Canis* phenotype

in the southeastern United States.

Introduction

Recent advances in science and technology have promoted molecular genetics as the

primary tool for inferring the evolutionary and demographic past of North American wolves. In

particular, the role of hybridization has become a predominant and contentious issue in the

evolution and conservation of wolf populations in eastern North America. For example, the use of

molecular markers bolstered the possible role coyotes (*Canis latrans*) played in the ancestry of

wolves in Eastern North America (Chambers et al. 2012; Wayne and Jenks 1991; Wilson et al.

2000). Despite leading to controversies surrounding the taxonomy of New World wolves, genetic

markers have proven invaluable for conservation efforts by providing new insights into the

evolution and ecology of *Canis* species (Chambers et al. 2012). Nevertheless, morphology is a

fundamental component of biology (MacLoed 2004; Nelson 1989) and phylogenetic analysis is

not possible without some method of describing the morphological variation between individuals,

populations, and species (MacLeod and Forey 2004). Therefore, a complete synthesis of *Canis*

species in eastern North America that leads to successful conservation requires studies from

multiple disciplines involving ecological, evolutionary, molecular, and morphological analyses (Rutledge et al. 2012).

The Red Wolf Recovery Program (Recovery Program) of the United States Fish and Wildlife Service (USFWS) oversees the recovery of endangered red wolves (*Canis rufus*) and it currently manages the only wild population of red wolves on the Albemarle Peninsula in eastern North Carolina (Hinton et al. 2013). Since its inception during 1973, the Recovery Program has considered hybridization between red wolves and coyotes to be a primary threat to red wolf recovery efforts because hybridization can render the wild red wolf population unrecognizable within several generations (Kelly et al. 1999; Rabon et al. 2013; USFWS 1989). During the initial stages of red wolf recovery efforts, morphometric data were used to identify individual red wolves captured from hybrid swarms in eastern Texas and western Louisiana, and these individuals were later used as founders for the captive and eastern North Carolina populations (USFWS 1989). Additionally, the use of morphometric measurements alluded to issues of hybridization and coyote introgression into red wolf genetics before modern molecular approaches were developed (Nowak 1979). Therefore, morphometric data provide another method to differentiate among red wolves, coyotes, and red wolf/coyote hybrids (hereafter hybrids) that is essential to determine whether hybridization is occurring. Red wolves and coyotes are sympatric in eastern North Carolina and developing morphometric profiles for red wolves, coyotes, and hybrids in this region is necessary to develop practical approaches to address hybridization and enhance conservation.

An assessment of morphometric data for *Canis* taxa in eastern North Carolina can improve important areas necessary for red wolf recovery efforts. First, it allows tests to determine if morphometric measurements can be used to discriminate among red wolves, coyotes, and hybrids in the absence of molecular markers. If successful, this will permit an evaluation of which measurements are most useful to discriminate among the 3 *Canis* taxa. Such data can be extended to ecological studies to determine the limits of potential resource use and the relative efficiency of

51

red wolves and coyotes to exploit resources within those limits (Wainwright 1996). Second, assessing morphometric measurements will allow the description of phenotypes of the 3 *Canis* taxa. Nowak (2002) observed the morphology of modern red wolves to be consistent with fossilized remains of small wolves in the eastern United States dating back to the Pleistocene. If this is true, regardless of the modern red wolf's evolutionary origins, the eastern North Carolina population may represent a *Canis* phenotype unique to the southeastern United States. Here we provide a systematic analysis of morphometric measurements currently collected by Recovery Program biologists from red wolves, coyotes, and hybrids and assess their reliability to describe and discriminate among the 3 taxa.

Study Area

The Red Wolf Recovery Area (Recovery Area) was established on the Albemarle Peninsula in northeastern North Carolina during 1987. The area included 5 counties (Beaufort, Dare, Hyde, Tyrrell, and Washington) and consisted of approximately 6800 km^2 of federal, state, and private lands (Figure 1). The Albemarle Peninsula was comprised of an intensively farmed agricultural-hardwood bottomland matrix in which approximately 30% of the landscape was driven by agricultural activity.

Methods

Red wolves, coyotes, and hybrids used in this study were captured by the Recovery Program during annual trapping within the designated Recovery Area from 1987 until 2011 (Rabon et al. 2013). Canids were captured using padded foot-hold traps (Victor no.3 Softcatch, Lititz, Pennsylvania, USA) and were sexed, measured, weighed, and aged by tooth wear (Gier 1968), and a blood sample was collected for genetic analysis. Ages of most red wolves were known and tooth wear estimates mostly applied to coyotes and hybrids (Rabon et al. 2013). For this study, only animals captured between the months of November through March were used. This ensured that all pups used in the analysis were at or near full potential body size for the taxa. We aged individuals > 2 years old as adults, < 2 but > 1 years old as juveniles, and > 6 months

but < 12 months old as pups. Microsatellite markers and other genetic information from the 14

founding individuals and other predefined red wolves were used to generate allele frequencies to

reconstruct the pedigree of the red wolf population. Individuals used in this study were genotyped

at 17 microsatellites and genetic analyses of blood samples followed the methods outlined in

Adams (2006), Bohling et al. (2013), and Miller et al. (2003). Individuals were assigned to a

species or hybrid group using the methods developed by Miller et al. (2003) to specifically

identify red wolves, coyotes, and hybrids. During our study, these were the molecular methods

used by the United States Fish and Wildlife Service for monitoring red wolf genetic ancestry

(Bohling et al. 2013).

Morphometric measurements were taken from live animals and measurements were

followed as closely to standard anatomical reference points as possible. Body traits measured

included body weight, ear length (edge of the external auditory canal to the tip of the ear), tail

length (tip of the fleshy part of the tail to the tail base), body length (anterior tip of the nose pad to

the tail base), hind foot length (hock to the tip of the digital pads), shoulder height (tip of the

scapula to the tip of the digital pads), front and hind paw width (width across the cushiony pads at

the widest points), front and hind paw length (base of the metacarpal pad to the tip of the digital

pads), length of head (edge of the premaxillary to the most posterior point of the occipital bone),

and width of head (widest points across the zygomata). All animals measured were later

identified as red wolves, coyotes, and hybrids by the Recovery Program using molecular

methods.

We analyzed measurements of canids using univariate and multivariate statistical

methods in Program R, version 3.0.2 (R Development Core Team 2013). We present statistics of

measurements as the mean ± standard error. To evaluate similarities between pairs of

measurements, we used a correlation analysis. Individuals were included in the analysis only once

to maintain independence. To remove redundancy, we used only one measurement from a set of

strongly correlated measurements to represent that taxon in further analysis.

We used analysis of variance (ANOVA) and *t*-tests to evaluate differences of measurements among and within taxa groups. We then used measurements in a polytomous logistic regression analysis to assess the reliability of morphometrics to identify canid taxa. Polytomous logistical regression is a logical extension of binary logistic regression that allows more than 2 categories of the dependent variable (Hosmer and Lemeshow 2000). The ability of the polytomous logistical regression to identify red wolves, coyotes, and hybrids using morphometric measurements was revealed as the percentage of individuals correctly reallocated to each taxon. The number of misclassified individuals indicated the degree of overlap between the groups. We used the Akaike information criterion (AIC) to compare models of morphometric measurements by calculating the AIC_c for each model and using ΔAIC_c and Akaike weights (*w*i) to select the measurements which best delineated different canid categories (Burnham and Anderson 2002). We performed polytomous logistical regression and model selection using the polytomous and AIC functions from the polytomous and MuIN packages for Program R.

Sample sizes among measurements varied because it was not always possible to measure every variable for each individual. Only individual canids with all measurements were included in the polytomous logistic regression analysis. We used a Kolmogorov-Smirnov one-sample test for each measurement to determine whether measurements for the subset of individuals used in the polytomous logistic regression analysis were biased when compared to the larger sample from which they were drawn.

Results

From 1987 to 2011, 951 canids were captured and measured. These included 528 red wolves (56%), 264 coyotes (28%), and 159 hybrids (17%) that were genetically identified. Measurements differed among red wolves, coyotes, and hybrids (Table 1) and hybrids were intermediate to red wolves and coyotes in all morphometric measurements.

The correlation coefficients of the measurements in the original dataset were strongly correlated with one another ($r = 0.75 - 0.90$). To reduce the number of variables, we used only 7

54

univariate estimates (body weight, ear length, tail length, body weight, hind foot length, shoulder height, and width of head) to characterize overall size because this subset most completely represented the various aspects of canid anatomy needed for analysis. Among red wolves all 7 measurement means increased ($F_{2, 453} \geq 6.79$, $P < 0.001$) with age. Additionally, all 7 measurement means were greater ($t_{528} \geq 6.07$, $P < 0.001$) for males than females. Among coyotes, weight, ear length, body length, hind foot length, shoulder height, and width of head were greater ($t_{264} \geq 2.98$, $P \leq 0.003$) for males than females, but there was no difference in tail length ($t_{264} = 1.60$, $P = 0.11$). Ear length and tail length ($F_{2, 159} \leq 1.72$, $P \geq 0.181$) did not differ among ages, but weight, body length, hind foot length, shoulder height, and width of head ($F_{2, 163} \geq 5.61$, $P \leq 0.004$) increased with age. Among hybrids, tail length and body length ($t_{159} \leq 1.78$, $P \geq 0.077$) did not differ between males and females, whereas weight, ear length, hind foot length, shoulder height, and width of head were larger for males than females ($t_{159} \geq 2.06$, $P \leq 0.041$). Weight, tail length, and shoulder height ($F_{2, 56} \leq 4.77$, $P \geq 0.012$) increased with age, but ear length, body length, hind foot length, and width of head ($F_{2, 56} = 2.95$, $P = 0.060$) did not differ with age.

Included in the polytomous logistical regression analysis were 352 (171 red wolves, 134 coyotes, and 47 hybrids) canids for which all 7 measurements were completed. The subset used in the polytomous logistical regression analysis was not biased when compared to the larger samples of red wolves ($D = 0.10$, $P = 0.094$), coyotes ($D = 0.06$, $P = 0.925$), and hybrids ($D = 0.2$, $P = 0.110$). The polytomous logistical regression model correctly classified 86% of the canids. Coyotes and red wolves were correctly classified 99% and 98% of the time, respectively. Hybrids were correctly classified 13% of the time. Hybrids were more likely to be misclassified as coyotes than red wolves (61% vs. 35%).

When red wolves were separated as pups (> 6 but < 12 months old) and non-pups (adults and juveniles), all non-pups were correctly identified as red wolves and only 2 pups (1.8%) were misclassified as a coyote and a hybrid (Table 2). Only 2 coyotes (1.5%) were misclassified as red wolf pups and none were misclassified as hybrids. Most hybrids were misclassified as coyotes

(53%) and red wolf pups (30%). Hybrids were 29% more likely to be misclassified as red wolf pups than non-pups. This is likely because as red wolf pups, born in the spring, overlap in body size with hybrids during summer and autumn as they approach adult body sizes in the winter.

The most useful measurements for separating red wolves, coyotes, and hybrids were hind foot length, weight, width of head, and tail length (Table 3). Hind foot length was the single most useful measurement. Our findings (Tables 4) suggest a reliable threshold that canids with hind foot lengths > 21.5cm, weights > 21.5kg, width of heads > 10.5cm, and tail lengths > 35cm were most likely to be red wolves. Coyotes typically had hind foot lengths < 19.5cm, weights < 19.5kg, width of heads < 10.5cm, and tail lengths < 35cm. Hybrid values for these 4 measurements would most likely overlap the minimum values for red wolves and maximum values for coyotes.

Discussion

Our results show that body size measurements of red wolves and coyotes are distinct from one another with hybrids representing an ambiguous intermediate size. Using measurements of hind foot length, body weight, width of head, and tail length in a polytomous logistic regression analysis, we were able to correctly classify 86% of 352 canids into their correct taxa category with moderately high (80% to 90%) accuracy. Red wolves and coyotes were correctly classified 98% and 99%, respectfully. On the other hand, hybrids were more difficult to re-assign and only 13% were correctly classified. Hybrids were more likely to be misclassified as either coyotes or red wolf pups. Despite the issue of morphological ambiguity, Recovery Program biologists still correctly classify canids as hybrids prior to genetic confirmation by identifying the morphological ambiguity and breeding status of hybrids. In other words, canids intermediate in size to red wolves and coyotes that have fully developed and active reproductive systems (e.g., males with enlarged testicles and females in estrus) are obviously adult hybrids, whereas those with underdeveloped and inactive reproductive systems are considered red wolf pups and confirmed with genetic analysis.

Of the 153 hybrids measured, only 2% (3 adult males) attained measurements above the minimum threshold values used by the Recovery Program to assign canids as red wolves. Nevertheless, the minimum values for red wolves reported in this study were estimated from the smallest adult females. Adult male red wolves are significantly larger than the minimum threshold reported. Interestingly, 20 of the 25 largest hybrids were captured and measured during 1998 through 2001. Since 2001, hybrids have been more coyote-like in their morphology and rarely exceed 20 kg in body weight. Coyotes did not fully colonize the Albemarle Peninsula until the mid-2000s (USFWS 2007), so hybrids prior to this period may have been more red wolf-like because backcrosses were occurring within the smaller red wolf population. Once introgression was identified and management actions were used to reduce hybridization during the early 2000s (Hinton et al. 2013, Kelly et al. 1999, Rabon et al. 2013), hybrid backcrosses began to occur more often within the larger coyote population.

The Recovery Program uses sterilization of coyotes and hybrids as the primary management tool to prevent coyote introgression into the red wolf genome (Hinton et al. 2013, Rabon et al. 2013). After capture, sterilized canids are released back into the Recovery Area fitted with mortality-sensitive radio-collars and monitored for the duration of their life. In the event that a red wolf pairs with a sterilized canid, the pair cannot produce hybrid litters. Although molecular markers ultimately confirm the taxa of canids captured, Recovery Program biologists routinely use morphometric measurements to pre-screen, process, and re-release individual canids into the wild before receiving genetic confirmation. Morphometric measurements are used to reduce holding times because this lowers the risk that captured canids would lose breeding mates and territories because of absence. Therefore, quick identification during the canid breeding season allows Recovery Program biologists to minimize disruptions to canid packs with excessive holding times while waiting for genetic confirmation. The accuracy of using morphometric measurements we observed confirms that morphological measurements could be used to pre-screen canids for management decisions, while awaiting genetic confirmation. Ultimately, genetic

assessments are necessary to effectively monitor, measure, and manage coyote introgression in the red wolf population.

Regardless of the ambiguity of hybrid measurements, our findings concerning morphometric measurements of red wolves and coyotes are consistent with the results of Nowak (1979, 2002) who demonstrated little to no overlap in red wolf and coyote cranial and dental measurements. Throughout North America, average coyote weights reported in studies rarely exceed 18 kg (Bekoff and Gese 2003; Leopold and Chamberlain 2001; Way 2007). Additionally, our date indicates that F1 and F2 hybrids are incapable of reaching body sizes of adult red wolves. This suggests that the red wolf represents a unique *Canis* phenotype in the southeastern United States. Differences in body size measurements are highly suggestive of differences in ecological requirements, as this is particularly true for carnivores regarding diet (Carbone et al. 2007; Donadio and Buskirk 2006; Gittleman 1985), space use (Gittleman and Harvey 1982; Gompper and Gittleman 1991), and interspecific interactions (Donadio and Buskirk 2006; Palomares and Caro 1999; Rosenzweig 1966). The general relationship between morphology and ecology is well known (Arnold 1983; Hutchinson 1959; Kishida et al. 2010; Wainwright 1996), but effects of body size on the relative ability of red wolves and coyotes to successively hunt prey, acquire mates, and defend territories is not well known.

Among the 3 sympatric *Canis* taxa in eastern North Carolina, red wolves are clearly the larger canid with hybrids intermediate to coyotes and red wolves in body size. Although this is a commonly held opinion, there is no significant literature comparing the morphometrics of red wolves, coyotes, and hyrbrids. Nowak (1979, 2002) assessed measurements from skulls of prehistoric and 20th century red wolves, gray wolves (*Canis lupus*), and coyote specimens and concluded red wolves to be a species intermediate in size to gray wolves and coyotes. USFWS (1989) reported only minimum measurements used to distinguish male and female red wolves from non-red wolf canids in southeastern Texas and southwestern Louisiana during the 1970s. The 1999 Population Habitat and Viability Assessment for red wolves stated the need to develop

a morphological profile for red wolves, coyotes, and hybrids for quick identification in the field (Kelly et al. 1999). Therefore, this study represents the first morphometric comparison of sympatric red wolves, coyotes, and their hybrids. Our analysis has shown morphometrics to be valuable in exploring morphological variation among closely related and sympatric *Canis* taxa. Further examination of morphological characters between red wolves and coyotes could detect patterns of phenotypic discreteness that may highlight opportunities for analysis of traits that may have genetic, evolutionary, and ecological importance. Therefore, we recommend examining the effects of morphology on red wolf and coyote ecology and interactions that may facilitate hybridization between the 2 species.

Acknowledgements

Literature Cited

Adams, J. R. 2006. A multi-facet molecular approach to red wolf (*Canis rufus*) conservation and management. PhD Dissertation, University of Idaho, Moscow.

Arnold, S.J. 1983. Morphology, performance and fitness. American Zoologist 23: 347-361.

Bekoff, M., and E.M. Gese. 2003. Coyote (*Canis latrans*). Pp. 467-481 in Wild Mammals of North America: Biology, Management, and Conservation, 2nd edition (G.A. Feldhamer, B.C. Thompson, and J.A. Chapman, eds.). John Hopkins University Press, Baltimore, Maryland, USA.

Bohling, J. H., J. R. Adams, and L. P. Waits. 2013. Evaluating the ability of Bayesian clustering methods to detect hybridization and introgression using an empirical red wolf data set. Molecular Ecology 22: 74-86.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information theoretic approach. 2nd ed. Springer-Verlag, New York, New York, USA. Available online at http://www.R-project.org.

Carbone, C., A. Teacher, and J.M. Rowcliffe. 2007. The cost of carnivory. PLoS Biology 5: 363-368.

Chambers, S. M., S. R. Fain, B. Fazio, and M. Amaral. 2012. An account of the taxonomy of North American wolves from morphological and genetic analyses. North American Fauna 77: 1-67.

Donadio, E., and S.W. Buskirk. 2006. Diet, morphology, and interspecific killing in Carnivora. American Naturalist 167: 876-885.

Gier, H. T. 1968. Coyotes in Kansas. Kansas State College, Agricultural Experiment Station Bulletin 393:1–118.

Gittleman, J.L. 1985. Carnivore body size: ecological and taxonomic correlates. Oecologia 67: 540-554.

Gittleman, J.L., and P.H. Harvey. 1982. Carnivore home-range size, metabolic needs and
 ecology. Behavioral Ecology and Sociobiology 10: 57-63.

Gompper, M.E., and J.L. Gittleman. 1991. Home range scaling: intraspecific and comparative
 trends. Oecologia 87: 343-348.

Hinton, J. W., M. J. Chamberlain, and D. R. Rabon, Jr. 2013. Red wolf (*Canis rufus*) recovery: a
 review with suggestions for future research. Animals 3: 722-744.

Hosmer, D. W., and S. Lemeshow. 2000. Applied logistical regression. Wiley, New York, New
 York, USA.

Hutchinson, G.E. 1959. Homage to Santa Rosalia or why there are so many kinds of animals?
 American Naturalist 93: 145-159.

Kelly, B. T., P. S. Miller, and U. S. Seal. 1999. Population and habitat viability assessment
 workshop for the red wolf (*Canis rufus*). Conservation Breeding Specialist Group
 (SSC/IUCN), Apple Valley, Minnesota, USA.

Kishida, O., G.C. Trussell, A. Mougi, and K. Nishimura. 2010. Evolutionary ecology of inducible
 morphological plasticity in predator-prey interaction: toward the practical links with
 population ecology. Population Ecology 52: 37-46.

Leopold, B.D., and M.J. Chamberlain. 2001. Carnivorous furbearers. Pp. 248-277 in Wildlife of
 Southern Forests: Habitat and Management (J.D. Dickson, ed.). Hancock House
 Publishers, Blaine, Washington, USA.

Macleod, N. 2004. Phylogenetic signals in morphometric data. Pp. 100-138 in Morphology,
 Shape and Phylogeny (N. MacLeod and P.L. Forey, eds.). Taylor & Francis Group, New
 York, New York, USA.

Macleod, N., and P.L. Forey. 2004. Introduction: morphology, shape, and phylogenetics. Pp. 1-7
 in Morphology, Shape and Phylogeny (N. MacLeod and P.L. Forey, eds.). Taylor &
 Francis Group, New York, New York, USA.

Miller, C. R., J. R. Adams, and L. P. Waits. 2003. Pedigree-based assignment tests for reversing coyote (*Canis latrans*) introgression into the wild red wolf (*Canis rufus*) population. Molecular Ecology 12: 3287-3301.

Nelson, G. 1989. Species and taxa: systematics and evolution. Pp. 63-81 in Speciation and Its Consequences (L.F. Marcus, M. Corti, A. Loy, G.J.P. Naylor, and D.E. Slice, eds.). Plenum Press, New York, New York, USA.

Nowak, R.M. 1979. North American Quaternary *Canis*. University of Kansas Museum of Natural History Monograph 6: 1-154.

Nowak, R.M. 2002. The original status of wolves in eastern North America. Southeastern Naturalist 1: 95-130.

Palomares, F., and T.M. Caro. 1999. Interspecific killing among mammalian carnivores. American Naturalist 153: 492-508.

Piersma, T., and J. Drent. 2003. Phenotypic flexibility and the evolution of organismal design. Trends in Ecology and Evolution 18: 228-233.

R Development Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online at http://www.R-project.org.

Rabon, D. R., R. Bartel, and A. Beyer. 2013. Red wolf adaptive management plan FY13-FY15. United States Fish and Wildlife Service, Manteo, North Carolina.

Rosenzeig, M.L. 1966. Community structure in sympatric Carnivora. Journal of Mammalogy 47: 602-612.

Rutledge, L. Y., P. J. Wilson, F. C. Klütsch, B. R. Patterson, and B. N. White. 2012. Conservation genomics in perspective: A holistic approach to understanding *Canis* evolution in North America. Biological Conservation 155: 186-192.

U.S. Fish and Wildlife Service (USFWS). 1989. Red wolf recovery plan. United States Wildlife Service, Atlanta, Georgia, USA.

U.S. Fish and Wildlife Service (USFWS). 2007. Red wolf (*Canis rufus*) 5-year status review: summary and evaluation. United States Wildlife Service, Atlanta, Georgia, USA.

Wainwright, P.C. 1996. Ecological explanation through functional morphology: the feeding biology of sunfishes. Ecology 77: 1336-1343.

Way, J.G. 2007. A comparison of body mass of *Canis latrans* (coyotes) between eastern and western North America. Northeastern Naturalist 14: 111-124.

Wayne, R. K., and S. M. Jenks. 1991. Mitochondrial DNA analysis implying extensive hybridization of the endangered red wolf *Canis rufus*. Nature 351: 565-568.

Wilson, P. J., et al. 2000. DNA profiles of the eastern Canadian wolf and the red wolf provide evidence for a common evolutionary history independent of the gray wolf. Canadian Journal of Zoology 78: 2156-2166.

Table 3.1. Means (± SE) and results of analysis of variance (ANOVA) for the morphological characters of red wolves, coyotes, and their hybrids in northeastern North Carolina, USA, 1987-2011.

Traits	Red Wolf		Coyote		Hybrid		ANOVA	
	N	Mean ± SE	N	Mean ± SE	N	Mean ± SE	F Ratio	P Value
Weight (kg)	509	23.2 ± 0.23 (7.9 – 38.6)[a]	240	13.4 ± 0.12 (6.9 – 19.1)	147	17.0 ± 0.34 (6.4 – 27.5)	438.97	<0.0001
Ear Length (cm)	458	11.0 ± 0.03 (9.0 – 12.9)	254	9.9 ± 0.04 (8.0 – 12.8)	153	10.5 ± 0.05 (8.5 – 12.5)	236.55	<0.0001
Tail Length (cm)	456	36.4 ± 0.15 (25.8 – 48)	241	33.9 ± 0.20 (20.5 – 44.7)	151	35.7 ± 0.25 (24.5 – 43.5)	48.53	<0.0001
Body Length (cm)	454	106.4 ± 0.33 (75.0 – 125.0)	246	90.0 ± 0.30 (64.0 – 105.0)	136	97.7 ± 0.60 (78.0 – 122.0)	497.72	<0.0001
Hind Foot Length (cm)	460	22.3 ± 0.06 (17.0 – 27.0)	256	18.7 ± 0.06 (16.4 – 22.5)	153	20.4 ± 0.11 (17.0 -25.1)	813.38	<0.0001
Shoulder Height (cm)	455	66.9 ± 0.18 (52.3 – 77.2)	249	57.3 ± 0.02 (47.1 – 68.7)	140	62.2 ± 0.36 (50.3 – 79.9)	563.23	<0.0001
Front Paw Length (cm)	407	7.1 ± 0.02 (5.0 – 8.7)	238	6.0 ± 0.03 (4.44 – 7.73)	148	6.5 ± 0.05 (4.3 – 7.8)	432.24	<0.0001
Front Paw Width (cm)	406	5.0 ± 0.02 (3.4 -6.3)	238	4.1 ± 0.02 (3.0 – 5.5)	148	4.6 ± 0.04 (3.3 – 6.3)	292.59	<0.0001
Hind Paw Length (cm)	381	6.5 ± 0.02 (5.0 – 8.2)	227	5.5 ± 0.03 (4.2 – 6.6)	146	6.0 ± 0.04 (4.9 – 7.5)	364.41	<0.0001
Hind Paw Width (cm)	380	4.5 ± 0.02 (3.0 – 5.9)	227	3.7 ± 0.02 (2.8 – 4.9)	146	4.1 ± 0.03 (3.2 – 5.1)	350.72	<0.0001
Length of Head (cm)	183	22.2 ± 0.11 (19.0 – 26.0)	146	19.9 ± 0.08 (17.5 – 24.0)	50	21.0 ± 0.24 (17.5 – 24.5)	146.91	<0.0001
Width of Head (cm)	182	11.9 ± 0.08 (9.5 – 14.5)	146	10.4 ± 0.05 (9.0 – 12.5)	51	11.1 ± 0.11 (9.5 – 12.5)	108.38	<0.0001

[a] Ranges for trait measurements

Table 3.2. Classification tables obtained from polytomous logistic regression (PLR) for red wolves, coyotes, and hybrids in northeastern North Carolina, USA, 1987-2011.

	Predicted Species				
Actual Species	Red Wolf Pup	Red Wolf Non-Pup	Coyote	Hybrid	Error
Red Wolf Pup (N=112)	94	16	1	1	0.16
Red Wolf Non-Pup (N=59)	23	36	0	0	0.39
Coyote (N=134)	2	0	132	0	0.01
Hybrid (N=47)	14	2	25	6	0.87

Table 3.3. Results of the 10 best models for stepwise analysis of morphological characters for red wolves, coyotes, and hybrids in northeastern North Carolina, USA, 1987-2011.

Model	k^a	AIC_c	ΔAIC_c	w_i
Species ~ HF + WT + WH + TA	6	278.41	0	0.38
Species ~ HF + WT + TA	5	279.91	1.50	0.18
Species ~ HF + WT + BO + TA	7	280.07	1.66	0.17
Species ~ HF + WT + BO + SH + TA	8	281.88	3.47	0.07
Species ~ HF + WT + WH	5	282.20	3.79	0.06
Species ~ HF + WT + BO + WH	5	282.23	3.82	0.05
Species ~ HF + WT + WH + SH	6	283.92	5.51	0.02
Species ~ HF + WT+ BO + SH + WH + TA	9	283.94	5.53	0.02
Species ~ HF + WT + BO + WH	6	284.06	5.65	0.02
Species ~ HF + WT + WH + EA	6	284.20	5.79	0.02

[a] k represents number of parameters for each model

Table 3.4. Means (± SE) and ranges for 4 morphological characters of red wolves, coyotes, and hybrids in northeastern North Carolina, USA, 1987-2011.

Species	Hind Foot Length (cm)			Weight (kg)			Width of Head (cm)			Tail Length (cm)		
	N	Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range
Red Wolf	460	22.3±0.1	17.0 – 27.0	509	23.2±0.2	7.9 – 38.6	182	11.9±0.1	9.5 – 14.5	456	36.4±0.2	25.8 – 48.0
Male	238	22.9±0.1	19.6 – 27.0	260	25.1±0.3	10.2 – 38.6	89	12.3±0.1	10.0 – 14.5	234	37.3±0.2	25.8 – 48.0
Pup	122	22.6±0.1	19.6 – 26.0	128	22.2±0.4	10.2 – 34.0	54	11.9±0.1	12.0 – 14.5	120	36.9±0.3	29.4 – 46.0
Juvenile	47	22.9±0.2	21.0 – 25.4	52	25.9±0.5	18.6 – 31.8	20	12.6±0.2	11.0 – 14.5	46	36.9±0.5	29.0 – 48.0
Adult	69	23.4±0.1	20.7 – 27.0	80	29.1±0.4	21.4 – 38.6	15	13.4±0.2	12.0 – 14.5	68	38.1±0.5	25.8 – 46.0
Female	222	21.7±0.1	17.0 – 24.5	249	21.2±0.3	7.9 – 34.7	93	11.5±0.1	9.5 – 14.4	222	35.4±0.2	28.0 – 44.0
Pup	119	21.5±0.1	18.0 – 24.0	124	18.3±0.4	7.9 – 28.6	63	11.1±0.1	9.5 – 12.8	120	35.1±0.2	28.0 – 43.0
Juvenile	48	21.8±0.2	19.7 – 24.0	54	22.7±0.4	18.2 – 30.0	21	12.2±0.2	11.0 – 14.4	48	35.6±0.3	31.0 – 43.0
Adult	55	22.1±0.2	17.0 – 24.5	71	25.1±0.3	19.9 – 34.7	9	12.1±0.3	11.0 – 13.5	54	36.2±0.4	29.0 – 44.0
Coyote	256	18.7±0.1	16.4 – 22.5	240	13.4±0.1	6.9 – 19.1	146	10.5±0.1	9.0 – 12.5	241	33.9±0.2	20.5 – 44.7
Male	127	19.0±0.1	16.5 – 22.0	122	14.0±0.2	9.0 – 19.1	73	10.7±0.1	9.5 – 12.5	118	34.3±0.3	20.5 – 43.0
Pup	17	18.5±0.2	17.0 – 20.1	15	12.4±0.7	6.9 – 17.0	8	10.3±0.2	10.0 – 11.0	16	34.0±0.9	29.0 – 43.0
Juvenile	28	18.6±0.1	17.3 – 19.9	27	13.7±0.3	11.4 – 18.2	24	10.6±0.1	9.5 – 11.5	28	33.5±0.7	20.5 – 40.0
Adult	36	19.1±0.2	16.5 – 20.5	34	14.5±0.3	10.5 – 18.2	21	10.8±0.2	9.5 – 12.0	34	35.3±0.4	31.0 – 42.5
Unknown*	46	19.2±0.1	17.5 – 22.0	46	14.2±0.2	11.5 – 19.1	20	10.7±0.1	9.5 – 12.5	40	34.0±0.5	25.0 – 38.0
Female	129	18.4±0.1	16.4 – 22.5	118	12.8±0.1	8.9 – 16.5	73	10.2±0.1	9.0 – 11.5	123	33.6±0.3	27.0 – 44.7
Pup	23	17.9±0.2	16.4 – 20.0	23	11.6±0.3	8.9 – 15.0	13	10.0±0.2	9.0 – 11.0	23	34.5±0.8	28.0 – 44.7
Juvenile	35	18.3±0.2	17.2 – 21.5	32	12.8±0.2	10.0 – 15.4	26	10.1±0.1	9.5 – 11.0	35	33.4±0.5	27.0 – 41.2
Adult	27	18.4±0.2	16.8 – 22.5	25	13.1±0.3	9.7 – 16.3	15	10.4±0.1	10.0 – 11.0	26	33.3±0.5	29.4 – 43.0
Unknown*	44	18.6±0.1	17.0 – 21.9	38	13.3±0.2	11.4 – 16.5	19	10.4±0.1	9.5 – 11.5	39	33.5±0.5	28.5 – 40.1

*Age class unknown

Table 3.4. Continued

Species	Hind Foot Length (cm)			Weight (kg)			Width of Head (cm)			Tail Length (cm)		
	N	Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range
Hybrid	153	20.4±0.1	16.4 – 25.1	147	17.0±0.3	6.4 – 27.5	51	11.1±0.1	9.5 – 12.5	151	35.7±0.25	24.5 – 43.5
Male	85	20.6±0.2	17.3 – 25.1	83	17.6±0.5	6.4 – 27.5	32	11.3±0.1	9.5 – 12.5	83	36.0±0.36	24.5 – 43.5
Pup	4	19.5±0.5	18.5 – 21.0	6	9.9±1.2	6.4 – 14.5	1	10.8±N/A	N/A	3	31.5±1.80	29.0 – 35.0
Juvenile	12	19.9±0.3	17.3 – 21.6	10	15.9±1.5	10.1 – 22.4	9	11.3±0.2	10.5 – 12.5	12	35.6±0.72	32.5 – 41.4
Adult	18	21.2±0.2	18.8 – 21.5	18	17.8±0.9	10.2 – 25.9	12	11.4±0.2	9.5 – 12.0	18	36.4±0.54	32.3 – 40.5
Unknown*	51	21.0±0.2	17.8 – 25.1	49	18.9±0.6	12.0 – 27.5	10	11.2±0.2	10.5 – 12.0	50	36.3±0.51	24.5 – 43.5
Female	68	20.2±0.2	17.0 – 22.5	64	16.1±0.4	7.3 – 23.2	19	10.8±0.2	9.5 – 12.5	68	35.2±0.34	27.0 – 41.5
Pup	5	19.3±0.6	16.4 – 20.0	5	13.0±1.9	7.3 – 17.3	13	10.8±1.3	9.5 – 12.0	5	33.1±1.52	29.5 – 37.0
Juvenile	14	19.6±0.4	17.0 – 21.9	13	15.1±0.9	10.2 – 20.9	26	10.8±0.3	9.5 – 12.0	14	35.4±0.93	31.2 – 41.5
Adult	6	19.7±0.5	18.3 – 21.7	6	14.9±0.5	11.8 – 23.2	15	10.0±0.5	9.5 – 11.0	6	35.4±0.89	32.0 – 38.0
Unknown*	43	20.5±0.2	17.8 – 22.5	40	17.0±0.5	11.8 – 23.2	3	11.5±0.6	10.5 – 12.5	43	35.4±0.38	27.0 – 41.0

*Age class unknown

68
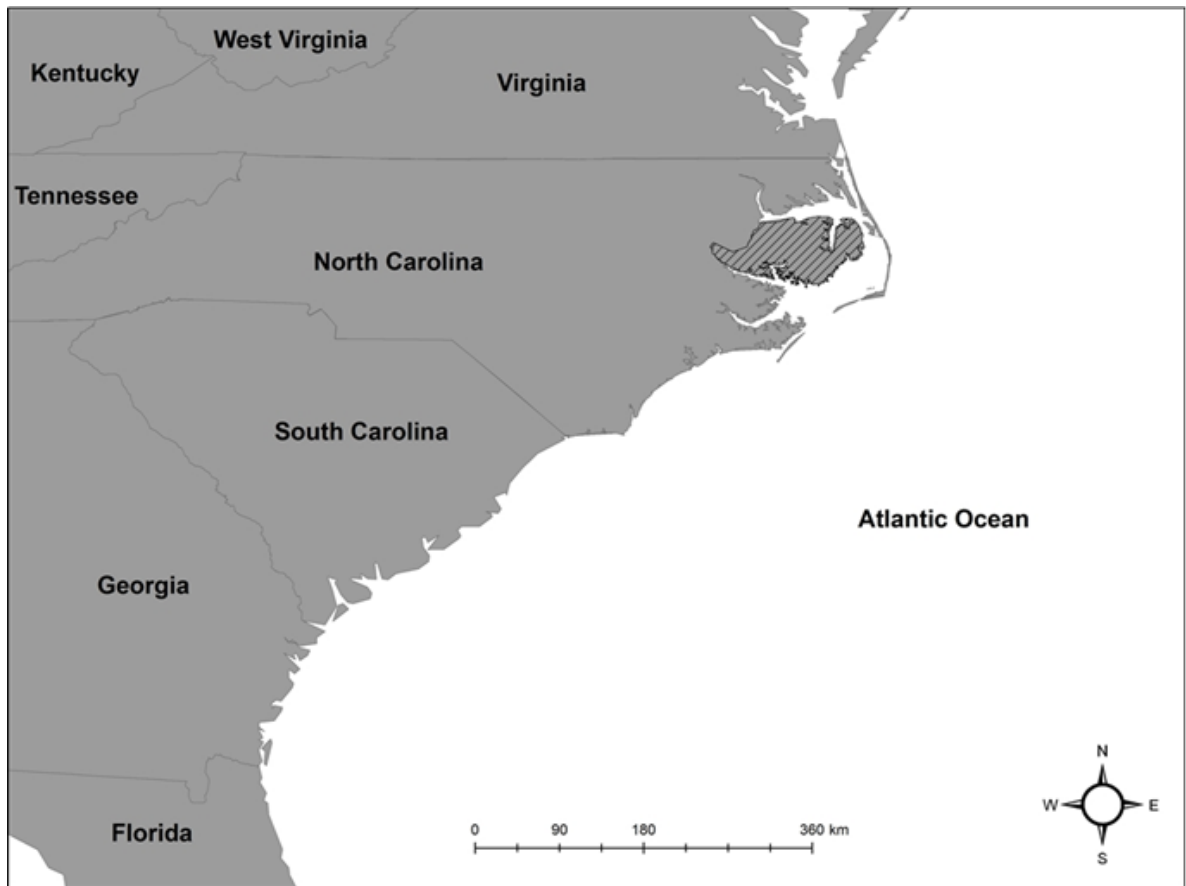
Figure 3.1. Outline map of North Carolina showing the location of the Red Wolf Recovery Area (hatched area) in the northeastern portion of the state.

CHAPTER 4

FACTORS AFFECTING DIETS OF RED WOLVES AND COYOTES

Abstract

Foraging behaviors of red wolves and coyotes are complex and their ability to form congeneric breeding pairs and hybridize adds additional difficulties into understanding factors affecting prey selection. We assessed factors affecting prey selection of red wolf packs, coyote pairs, and congeneric pairs form by red wolves and coyotes and found that all three had similar and overlapping diets. Nevertheless, we detected differential use of prey; red wolf packs consumed more white-tailed deer, fur bearers, and pig than coyote and congeneric pairs. Coyotes that formed pairs with red wolves had 10% more whited-tailed deer in their diet than conspecifics that paired with coyotes. Contrary to many studies on coyote diet in the southeastern United States, we found coyotes in northeastern North Carolina to be largely carnivorous with a narrow dietary breadth. We found breeder weight, pack size, age, white-tailed deer density, and season to be important factors influencing the diet of red wolf packs. Breeder weight and season were important factors influencing diets of coyote pairs, whereas season and white-tailed deer density influenced diets of congeneric pairs. Although prey selection was largely similar among the three groups, differences in diet among different breeding pairs were associated with body size. Larger individuals within and among different breeding pairs consumed more white-tailed deer, and less rabbits and small mammals. Therefore, partitioning of food resources by red wolves and coyotes in northeastern North Carolina is mostly via differences in quantity of similar prey rather than differences in types of prey exploited. We suggest that similarities in energetic demand of individual red wolves and coyotes that approach each other in body size may partially contribute to successful congeneric pairing and hybridization through similar use of prey.

Introduction

Understanding relationships between organisms and their food resources is a central goal in ecology, and describing mechanisms that influence foraging behavior can assist management and conservation of wildlife. In particular, diets of carnivores have always interested ecologists because predation is an essential ecological process that structures communities and influences

ecosystem productivity (Hairston *et al*., 1960; Schmitz *et al*., 2000; Ripple & Bescheta, 2004; Fortin *et al*., 2005). Foraging behavior of carnivores is a fundamental component of their ecology and evolution because it is directly related to behavior, morphology, and physiology (Christiansen & Wroe, 2007; Van Valkenburgh, 2007). A thorough understanding of a carnivore's dietary needs requires identifying primary food resources and describing what factors influence variation in the consumption of those resources. Thereafter, ecologists can use that information to understand how foraging behavior affects life history traits and population processes that will ultimately influence community- and ecosystem-level processes.

Traits affecting foraging behavior are similar between closely related and similarly sized species of Carnivora. This is particularly true for Canidae in which members of this family share similar body plans and monogamous breeding behaviors (Finarelli, 2007; Finarelli, 2008). Recently diverged taxa within Canidae tend to be ecologically similar because there is a direct link between their evolutionary relatedness and the ecological processes that determine their distribution and abundance (Johnson *et al*., 1996). Despite understanding characteristics unique to members of modern Canidae and how those traits influence foraging behaviors (Van Valkenburgh *et al*., 2004; Andersson, 2005; Slater *et al*., 2009), we still know little about factors affecting prey selection of two sympatric *Canis* species in the southeastern United States. Determining what these factors are and how they influence prey selection of red wolves (*Canis rufus*) and coyotes (*Canis latrans*) is essential for enhancing red wolf conservation, understanding mechanisms facilitating coyote expansion into the Southeast, and identifying key components of *Canis* foraging ecology.

Although a common predator of the Southeast prior to European settlement, government-supported eradication campaigns reduced red wolves to a remnant population of approximately 100 individuals by mid-20th century (USFWS, 1989; Hinton *et al*., 2013). As red wolves were eradicated, coyotes expanded east into Arkansas, Louisiana, and other southwestern regions of the red wolf's historic range (McCarley, 1962; Paradiso & Nowak, 1972). With rapidly declining

red wolf populations and extensive hybridization with the expanding coyote population, the United States Fish and Wildlife Service (USFWS) removed the last remaining red wolves from eastern Texas and western Louisiana and later reintroduced their progeny into northeastern North Carolina during the late 20th-century to begin red wolf restoration efforts (USFWS, 1989). Today, red wolves exist as a remnant population of approximately 100 individuals whereas coyotes have become ubiquitous throughout the red wolf's historic range posing a serious ecological threat because of their ability to hybridize with red wolves (Hinton *et al*., 2013).

Red wolves and coyotes share similar canid body shapes with red wolves being the larger of the two species. The effects of body size on red wolf and coyote interactions are currently unknown (Hinton *et al*., 2013). Body size represents a key morphological trait that separates red wolves from coyotes and it may affect differential use of prey between the species. Red wolves, weighing 7-18 kg heavier than the largest coyotes (Chapter 3), should feed at higher trophic levels than coyotes because body size has been shown to predict carnivore interactions with prey (LaBarbera, 1989; Gittleman, 1985; Donadio & Buskirk, 2006). The ability of both species to form congeneric breeding pairs raises an interesting question regarding how differences in body size between congeneric mates determine the efficiency with which those mates acquire and use food resources. Both species face energetic constraints that affect many aspects of their ecology, and small differences in body size between individual red wolves and coyotes may allow for successful formation of congeneric pairs because the effects on the breeder's ability to transfer energy from homeostasis to reproductive efforts is negligible.

Understanding dietary needs of red wolves and coyotes is a primary step to improving management of both species and minimizing hybridization. Our objective was to describe the diets of red wolves and coyotes by identifying the remains of prey found in red wolf and coyote scat. We used several variables (i.e., body weight, social structure, age, etc.) that have not traditionally been used in analyses of scat remains to account for variation in diet. Furthermore, this study is the first to assess dietary behaviors of congeneric canid breeding units that are the

source of hybridization. To accomplish this, we monitored over 35 canid packs and pairs over more than 6,000 km^2. As a result, this study represents the most comprehensive assessment of canid diet in the eastern United States. Assessing red wolf diet and factors influencing its variation will allow us to understand the red wolf's ecological role in the Southeast. Additionally, diets of eastern coyotes have lain at the heart of the species' impact on eastern ecosystems and the need to determine the ecological role of eastern coyotes goes beyond the scope of red wolf conservation.

Study Area

The study was conducted in the Red Wolf Recovery Area (hereafter Recovery Area) on the Albemarle Peninsula of northeastern North Carolina (Beaufort, Dare, Hyde, Tyrrell, and Washington counties). The Recovery Area consisted of approximately 6,800 km² of federal, state, and private lands. The Albemarle Peninsula is an intensively farmed agricultural-hardwood bottomland forest matrix in which approximately 45% of the landscape was driven by agricultural and commercial timber activities. Corn, cotton, soybean, and winter wheat were the primary agricultural crops and comprise approximately 30% of the land cover. Managed pine (*Pinus* spp) plantations comprise approximately 15% of the land cover. The remaining 55% of land cover types were pocosin (15%), bottomland hardwood forests (15%), saltwater marsh (5%), open water (10%), and other land cover types (10%). Potential mammalian prey of red wolves and coyotes in the Recovery Area were white-tailed deer (*Odocoileus virginianus*), raccoon (*Procyon lotor*), eastern cotton-tail rabbit (*Sylvilagus floridanus*), marsh rabbit (*Sylvilagus palustris*), feral pig (*Sus scrofa*), nutria (*Myocastor coypus*), muskrat (*Ondatra zibethicus*), hispid cotton rat (*Sigmodon hispidus*), white-footed mouse (*Peromyscus leucopus*), deer mouse (*Peromyscus maniculatus*), house mouse (*Mus musculus*), eastern harvest mouse (*Reithrodontomys humulis*), voles (*Microtus* spp), and shrews (*Blarina* spp). Primary carnivores sympatric with red wolves and coyotes were gray foxes (*Urocyon cineroargenteus*), red foxes (*Vulpes vulpes*), feral dogs (*Canis lupus familiaris*), bobcats (*Lynx rufus*), and black bears (*Ursus americanus*).

74

Methods

Red wolves and coyotes were captured within the Recovery Area during annual trapping efforts conducted by the United States Fish and Wildlife Service Red Wolf Recovery Program (hereafter Recovery Program). Red wolves and coyotes were sexed, measured, weighed, aged by tooth wear (Gier, 1968), and blood sampled for genetic identification. The Recovery Program categorizes red wolves and coyotes > 2 years old as adults, < 2 but > 1 year old as juveniles, and < 1 year old as pups. Coyotes captured within the Recovery Area by USFWS personnel were reproductively sterilized (hereafter sterilized) and used as space holders until red wolves moved in and occupied those areas (Hinton *et al*., 2013; Rabon *et al*., 2013). Coyotes were taken to a local veterinary clinic for surgical sterilization where males and females were sterilized by vasectomy and tubal ligation, respectively. This process kept hormonal systems intact and avoided disrupting breeding and territorial behavior. Once red wolves and coyotes were fully processed, individuals were fitted with radio-collars, released, and then monitored by the Recovery Program during weekly telemetry flights. Monitoring efforts allowed the Recovery Program to identify red wolf and coyote space use on the landscape.

Territories of red wolf packs, coyote pairs, and red wolf-coyote pairs (hereafter congeneric pairs) were surveyed at least once a month for scat from 2009 until 2011 (Dellinger *et al*., 2011a; McVey *et al*., 2013). Paved, gravel, and dirt roads were surveyed by foot, all-terrain vehicles, and trucks. We also opportunistically collected defecations from red wolves and coyotes captured during annual trapping. Scats collected were bagged, dated, marked with a unique identification number, and stored in a freezer for later dissection and analysis. We identified scats by physical appearance, including size (Dellinger *et al*., 2011b), and the presence of tracks or other predator sign in the immediate area around the scat. Approximately 40% of the scats were identified to species and individual animals using fecal DNA genotyping (Dellinger *et al*., 2011a; McVey *et al*., 2013). We assigned scats collected within known territories to red wolf packs,

coyote pairs, or congeneric pairs but excluded those that could not be associated with known pairs and packs.

We examined scats identified to red wolf packs, coyote pairs, and congeneric pairs for prey remains. Individual scats were placed in nylon panty hose and then secured in small, nylon mesh bags with water-proof labels for washing. Scats were soaked in water for 24 hours before being transferred to a washing machine and washed on the regular cycle with detergent 3 times to separate hair and bone fragments from fecal matter. We allowed scats to air dry for 72 hours prior to examining scat contents. Once dried, prey remains for each scat was analyzed for species composition. We identified the undigested food items microscopically and macroscopically by comparing to reference collections and identification manuals (Moore *et al.*, 1997; Debelica & Theis, 2009). We assigned prey remains in scats to one of 5 categories: white-tailed deer (hereafter deer), rabbits (eastern cotton-tail and marsh rabbit), small mammals (mice, rats, shrews, and voles), fur bearer (muskrat, nutria, and raccoon), pig, and other food items (bird, insect, fruit, and anthropogenic material). We excluded prey items that comprised < 5% of prey found in scat from the analysis. Following identification of prey remains in scats, we estimated the percent of occurrence (PO) for each prey item using the common visual estimation of PO (Dellinger *et al.*, 2011a; McVey *et al.*, 2013).

Red wolf packs, coyote pairs, and congeneric pairs are the only 3 types of *Canis* breeding units monitored by the Recovery Program in northeastern North Carolina. Red wolf packs were comprised of a breeding pair along with juveniles and pups from previous litters. Some red wolves monitored for this study were newly formed breeding pairs and did not have juveniles or pups. Coyote pairs did not have juveniles and pups because at least 1 of the 2 breeders was sterilized as a management technique to suppress coyote reproduction. Congeneric pairs were comprised of a red wolf and a coyote and did not have juveniles and pups because the coyote in the pair was sterilized to prevent hybridization. Therefore, our sampling units were red wolf packs, coyote pairs, and congeneric pairs. We used breeder weight (combined weight of both

breeders), breeder age (mean age of both breeders), pack size (# of individuals in pack), deer density (# deer harvested by hunters/km²), and season (summer and winter) as explanatory variables to account for changes in dietary composition for red wolf packs. However, because coyote pairs and mixed pairs did not have juveniles and pups, we could not include pack size as explanatory variables to account for variation in diet.

We included a measurement of deer abundance as a variable because deer are known to be an important food resource for red wolves (Dellinger *et al*., 2011a, McVey *et al*., 2013) and there is a growing concern that coyotes are negatively affecting deer populations across the Southeast (Kilgo *et al*., 2012). We used county-level hunter harvest data collected by the North Carolina Wildlife Resources Commission as measures of deer abundance in the Recovery Area. Estimates of deer harvests were divided into 3 categories, which likely corresponded with deer population sizes for the 5 counties (Beaufort, Dare, Hyde, Tyrrell, and Washington) in the Recovery Area. Our categorization resulted in areas of low (< 0.75 deer harvested/km²), medium (0.75 – 1.5 deer harvested/km²), and high (> 1.5 deer harvested/km²) deer densities. Although these harvest reports do not reflect true deer densities, these categories do provide a benchmark by which to judge the effect of deer abundance on red wolf and coyote diets. To further aid in univariate comparisons, we pooled scat into 2 seasons (summer and winter) for comparison of prey selection between seasons by breeding pairs. We also collapsed pack size into 2 categories: packs of 4 or fewer individuals (hereafter smaller packs) and packs with 5 or more individuals (hereafter larger packs).

We analyzed the effects of explanatory variables on diet using univariate and multivariate statistical methods in R Statistical Environment, version 2.14.2 (R Development Core Team, 2012). We used analysis of variances (ANOVAs), Tukey tests for multiple comparisons, and *t*-tests to determine if the percentage of prey items consumed differed with respect to breeder weight, breeder age, pack size, deer density, and season. We then developed generalized linear models (GLMs) to model the percentage of a prey category for each sampling unit as a linear

function of the explanatory variables. Changes in coefficient estimates of GLMs represent the change in percent prey consumption following a one-unit change in the explanatory variables. We assessed models using a stepwise procedure by calculating Akaike information criterion (AIC_c) for each model to select which variables best explained diet by selecting the most parsimonious model with the highest weight and rank relative to the entire set of models under consideration (Burnham & Anderson, 2002). With the exception of season, all measurements were continuous data. The GLM analysis was conducted using arcsine-square root transformation to percentage data to correct for non-constant error variance.

Results

From 2009-2011, we collected and analyzed 1754 scats from 13 red wolf packs, 17 coyote pairs, and 8 congeneric pairs. Red wolf packs, coyote pairs, and congeneric pairs accounted for 55.4%, 31.6%, and 13% of the scat, respectively. Breeders of red wolf packs had the heaviest combined weights followed by those in congeneric pairs and coyote pairs and average breeder age was older for red wolves than coyotes and congeneric pairs (Table 1).

Red wolf packs, coyote pairs, and congeneric pairs differentially used deer, rabbits, small mammals, fur bearers, and pigs ($F_{2, 1754} \geq 6.77$, $P < 0.001$); deer and rabbits comprised most prey items found in scat (Table 2). No difference was detected in use of other food items ($F_{2, 1754} = 2.21$, $P = 0.110$). Coyote pairs consumed less deer and more rabbits than red wolf packs and congeneric pairs (Table 2). Coyotes consumed more small mammals than red wolves, but consumption of small mammals by congeneric pairs did not differ from coyote pairs or red wolf packs. Red wolf packs consumed more fur bearers and pig than coyote pairs and congeneric pairs.

Red wolf packs consumed more deer and pig in areas of medium deer density than areas of low and high density ($F_{2,968} \geq 6.43$, $P < 0.001$). Red wolf packs consumed more rabbits in areas of high deer density than in areas of low and medium density ($F_{2,968} = 33.40$, $P < 0.001$). Red wolf consumption of small mammals, fur bearers, and other food items were greater in areas of low deer density than areas of medium and high deer density ($F_{2,968} \geq 8.01$, $P < 0.001$). We only

compare diet between medium and high deer density areas for coyote pairs and congeneric pairs because they were absent in counties with low deer density (Table 3). We detected no difference in coyote consumption of white-tailed deer, rabbit, small mammals and pig between medium and high deer density ($t_{552} \leq 0.86$, $P \geq 0.392$). We did find that coyotes in areas of medium deer density consumed more fur bearers and other food items than in areas of high deer density ($t_{552} \geq 2.00$, $P \leq 0.046$). We detected no difference in consumption of any prey species by congeneric pairs between medium and high deer densities ($t_{227} \leq 1.77$, $P \geq 0.079$).

Older red wolf breeders consumed more rabbits and pig than younger breeders, whereas younger red wolf breeders' consumed more deer, small mammals, and other food items ($t_{969} \geq 2.47$, $P \leq 0.014$) than older breeders (Table 4). No differences were observed in the consumption of fur bearers by older and younger red wolf breeders ($t_{969} = 1.47$, $P = 0.142$). Younger coyote breeders consumed less deer and more rabbits than older breeders ($t_{552} \geq 2.49$, $P \leq 0.014$). No differences were observed between older and younger coyote breeders in their consumption of small mammals, fur bearers, pig and other food items ($t_{552} \leq 1.32$, $P \geq 0.188$). Older breeders in congeneric pairs consumed more small mammals ($t_{227} = 2.22$, $P = 0.027$) than younger breeders. No differences were observed between older and younger breeders in congeneric pairs of their consumption of deer, rabbits, fur bearer, pig, and other food items ($t_{227} \leq 1.83$, $P \geq 0.065$).

No seasonal differences were detected in consumption of deer, small mammals, and other food items ($t_{969} \leq 0.70$, $P \geq 0.484$) by red wolves. Red wolves consumed less fur bearers and pigs and more rabbits during winter than summer (Table 5; $t_{969} \geq 2.24$, $P \leq 0.025$). Coyote pairs consumed more deer and less rabbits during winter than summer ($t_{552} \geq 3.38$, $P \leq 0.001$). No seasonal differences were detected in coyote consumption of small mammals, fur bearers, pig, and other food items ($t_{552} \leq 1.57$, $P \geq 0.060$). Congeneric pairs consumed more deer and less other food items ($t_{227} \geq 2.01$, $P \leq 0.046$) during summer than winter. No seasonal differences were detected in the consumption of rabbits, small mammals, fur bearers, and pig by congeneric pairs ($t_{227} \leq 1.93$, $P \geq 0.060$). Also, smaller red wolf packs consumed more deer than larger packs

whereas larger packs consumed more rabbit, small mammals, fur bearers, pig, and other food items (Figure 1; $t_{969} \geq 2.63$, $P \leq 0.009$).

The most important factors for explaining variation of deer in red wolf diet was breeder weight and pack size (Table 6). Consumption of rabbits of was best explained by breeder age and deer density. All factors were important in explaining red wolf consumption of small mammals and pig. Red wolf consumption of fur bearer was best explained by summer and weight and other food items were influenced by deer density and season. Coyote consumption of deer and rabbits were best explained by weight and season (Table 7). Age, weight, deer density, and season best explained coyote consumption of small mammals and deer density best explained consumption of other food items. Season best explained consumption of deer and other food items by congeneric pairs (Table 8). Deer density, season, and age influenced consumption of rabbits and deer density and season influenced use of small mammals. Consumption of fur bearers and pig by coyote pairs and congeneric pairs was negligible and therefore not modeled.

Discussion

Little is known about the historic diets of red wolves throughout the Southeast because red wolf natural history and ecology was never well-documented. Previous diet assessments of remnant, declining red wolf populations in salt marsh and coastal habitats of Texas and Louisiana during the mid-20th century indicated red wolf diet consisted of nutria, rabbits, and cotton rats (Paradiso & Nowak, 1972; Shaw, 1975). Recent studies conducted after red wolves were reintroduced into NENC indicated greater use of deer and rabbits than the source population (Phillips *et al*., 2003; Dillenger *et al*., 2011a; McVey et al., 2013). Conversely, coyote diet has been studied extensively throughout North America in which they have been labeled as generalists or opportunistic foragers (Henderson, 1930; Korschgen, 1957; Prugh, 2005; Chamberlain & Leopold, 1999; Schecengost *et al*., 2008). Our results demonstrate that red wolves and coyotes in northeastern North Carolina have a strong carnivorous diet and consume primarily mammalian prey, such as deer, rabbits, and small mammals.

Management activities to sterilize coyotes within the Recovery Area influence factors affecting coyote interactions with prey by preventing coyote and congeneric packs from forming packs. For instance, pack size was an important factor influencing red wolf diet in which the diet of packs of fewer than 4 individuals contained greater amounts of deer and less small mammal prey than packs of 4 or more individuals. The negative relationship between pack size and food acquisition has been observed in gray wolves (Nudds, 1978; Thurber & Peterson, 1993; Schmidt & Mech, 1997; MacNulty *et al*., 2011) and our data suggest that red wolves are constrained by similar group dynamics. Although we couldn't assess effects of pack size on coyote diet, we can only speculate that the diets of coyote and congeneric pairs would be similarly constrained by group living dynamics that affect cooperation and conflict within their packs as they increase in size.

Pack size likely caused variation in red wolf diet by effecting pack hunting efficiency and energetic gains acquired from kills. Recently, MacNulty *et al*. (2011) examined hunting efficiency of gray wolf packs and proposed the free-riding hypothesis in which pack members can decrease hunting efficiency by superficially cooperating in hunts to gain access to kills. Although our study was not designed to assess hunting efficiency of red wolves, deer consumption peaked with smaller red wolf packs and that may indicate greater hunting efficiencies at smaller group sizes. It is difficult for our study to discern whether pack hunting efficiency was truly affected by pack size because larger wolf packs had greater percentages of rabbits, fur bearers, pig, and other food items in their diet to compensate for the decrease in use of deer. If hunting efficiency remained the same between small and large red wolf packs, it is likely that deer comprised a lower percentage of the diets of larger packs because kills had to be divided and shared among more individuals. This would cause larger red wolf packs to broaden their diet to include other prey to supplement energetic loses from sharing their deer kills with offspring. Whether large packs experience lower hunting efficiencies or reduce energetic profits from kills through sharing, it is likely that increasing red wolf density within territories has a negative effect on

foraging that may eventually facilitate fragmentation of packs through parent-offspring conflicts over food acquisition (Gese *et al.*, 1996).

Canis species lack adaptations for prey control (i.e., grappling abilities) and increased body weight likely improves their killing ability by allowing individuals to more effectively hold down large prey while other pack members deliver repeated bites to the abdomen and hindquarters. Dentition is also a primary trait used to subdue prey and substantial, prolonged bite forces that are necessary for handling large prey put considerable stress on teeth (Van Valkenburgh, 1991; Slater *et al.*, 2009). MacNulty *et al.* (2009a) observed that the ability of gray wolves in Yellowstone National Park to kill ungulates improved with increased body size. Also, hunting performance of gray wolves was observed to decrease with age because of physiological deterioration caused by senescence (MacNulty *et al.*, 2009b). Similarly, we found body weight and age of red wolf breeders to influence pack diet. Although age was not an important variable predicting red wolf consumption of deer, its positive correlation with consumption of smaller-sized prey indicates that older breeders depended more on rabbits and small mammals for food than did younger breeders. As individuals age, canines and incisors are eventually worn down and fractured because of repetitive use and it's not unusual for red wolves > 4 years of age to have substantially worn and damaged teeth (USFWS, *unpublished data*). As a result, we suspect that deterioration to dentition through aging was a primary reason older breeders relied on smaller-sized prey.

As observed with red wolf packs, larger coyote pairs consumed more deer than smaller conspecifics. Coyote pairs also consumed more deer during winter than summer and our findings are contrary to many studies conducted on coyote diet in the southeastern United States (Chamberlain & Leopold, 1999; Schrecengost *et al.*, 2008; Kilgo *et al.*, 2012). These studies have indicated that coyote predation of deer occurs primarily on fawns during summer and coyote consumption of deer during winter is a result of scavenging of hunter dump sites or deer crippled or un-recovered by hunters (Chamberlain & Leopold, 1999; Thornton *et al.*, 2004; Schrecengost

et al., 2008). We do not believe that scavenging explains increased use of deer during winter because the positive relationship between coyote consumption of deer and coyote body weight implies that body size is an important trait for coyotes to acquire deer in their diet through predation, whereas scavenging is opportunistic and should not be affected by body size. Coyotes in the western United States have been observed hunting and killing white-tailed deer (Patterson & Messier, 2000; Lingle & Pellis, 2002), mule deer (*Odocoileus hemionus*; Bowyer, 1987; Lingle & Pellis, 2002), elk (*Cervus elaphus*; Gese & Grothe, 1995), bighorn sheep (*Ovis Canadensis*; Bleich, 1999), pronghorn (*Antilocapra americana*; Keller *et al*. 2013) and other large prey. Therefore, it is reasonable to assume that coyotes in the southeastern United States are capable of killing deer outside of the fawning season.

The average coyote lifespan in this study was statistically lower than red wolves by almost 2.5 years because many coyotes were removed from the landscape for management reasons related to red wolf recovery or opportunistically by hunters and trappers (Hinton, *unpublished data*). Therefore, it is difficult to insinuate whether the effects of age on coyote and congeneric pair food habits were related to physiological deterioration or experience. Older coyote breeders consumed more white-tailed deer, but this consumption is probably a result of a few coyote breeders approaching their peak performance age of 3-4 years old. If natural senescence had been more common with coyotes in our study area, we believe that age would have had a similar effect on coyote and congeneric pair food habits as it did on red wolf packs.

Although congeneric pairs typically weighed 20-40% more than coyote pairs and consumed 10% more deer, breeder weight was not useful in explaining variation in use of deer in the diets of congeneric pairs. This discrepancy is likely a result of management actions that remove coyotes from congeneric pairs to make resident red wolves available to dispersing red wolves during the breeding season. Therefore, most congeneric pairs form during late winter or early spring when management actions shift to monitoring red wolf denning behavior. Solitary red wolves acquiring a coyote mate likely increase hunting efficiency during spring and summer

83

but, when they lose their coyote mate to management action prior to the breeding season, they hunt solitarily until they find a new mate.

A 15-20 kg threshold was proposed in which carnivores weighing above that threshold would have to switch to larger vertebrate prey that approach their body weight because of higher energetic demands and constraints related their body size (Carbone *et al*., 1999; Carbone *et al*., 2007). Coyotes in northeastern North Carolina approach the 15-20 kg threshold and the smallest red wolves are above it. How these pairs adjust energy budgets associated with changes in breeder body mass and hunting efficiency is unknown. Differences in body size between red wolves and coyotes will affect energy requirements and capacities for searching for and processing prey which, in turn, may affect how they perceive the distribution, abundance, and profitability of prey on the landscape. Therefore, the formation of congeneric pairs and the stability of those pairs to successfully raise hybrid litters may be related to how similar individuals are in their energy demands. Partitioning of food resources by red wolves and coyotes in northeastern North Carolina is mostly via differences in the quantity of similar prey exploited and via differences in types of prey used. This is probably a result of greater energetic demands placed on red wolves from their larger body size to rely more on deer, rabbits, and fur bearers and indicates that the diets of red wolves and coyotes conform to the predictions from existing theory on foraging behavior in which relative body size appear to be important factors governing the overlap of resources and intensity of competition (Rosenzweig, 1966; Gittleman, 1985; Carbone *et al*., 2007). Different use of prey, habitat, and space use by red wolves and coyotes resulting from differences in body sizes may serve as a reproductive barrier by preventing congeneric pairing. Therefore, we hypothesize that hybridization rates between red wolves and coyotes increase as both populations approach each other in body size. Our ability to identify key traits and understand how they facilitate reproductive barriers is crucial for red wolf recovery.

Literature Cited

Andersson, K. (2005). Were there pack-hunting canids in the Tertiary, and how can we know? Paleobiology 31: 56-72.

Bleich, V.C. (1999). Mountain sheep and coyotes: patterns of predator evasion in a mountain ungulate. Journal of Mammalogy 80: 283-289.

Bowyer R.T. (1987). Coyote group size relative to predation on mule deer. Mammalia 51: 515-526.

Burnham, K.P. & Anderson, D.R. (2002). Model selection and multimodel inference: a practical information theoretic approach. 2nd ed. New York: Springer-Verlag.

Carbone, C., Mace, G.M., Roberts, S.C. & Macdonald, D.W. (1999). Energetic constraints on the diet of terrestrial carnivores. Nature 402: 286-288.

Carbone, C., Teacher, A. & Rowcliffe, J.M. (2007). The costs of carnivory. PLoS Biology 5: 363-368.

Chamberlain, M.J. & Leopold, B.D. (1999). Dietary patterns of sympatric bobcats and coyotes in central Mississippi. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 53: 204-219.

Christiansen, P., & Wroe, S. (2007). Bite forces and evolutionary adaptations to feeding ecology in carnivores. Ecology 88: 347-358.

Debelica, A. & Theis, M.L. (2009). Atlas and key to the hair of terrestrial Texas mammals. Baker, R.J. (Ed.). Special publication of the Museum of Texas Tech University, No. 55.

Dellinger, J.A., Ortman, B.L., Steury, T.D., Bohling, J. & Waits, L.P. (2011a). Food habits of red wolves during pup-rearing season. Southeastern Naturalist 10: 731-740.

Dellinger, J.A., McVey, J.M., Cobb, D.T. & Moorman, C.E. (2011b). Diameter threshold for distinguishing between red wolf and other canid scat. Wildlife Society Bulletin 35: 416-420.

Donadio, E. & Buskirk, S.W. (2006). Diet, morphology, and interspecific killing in Carnivora. American Naturalist 167: 524-536.

Finarelli, J.A. (2007). Mechanisms behind active trends in body size evolution of the Canidae (Carnivora:Mammalia). American Naturalist 170: 876-885.

Finarelli, J.A. (2008). Testing hypotheses of the evolution of encephalization in the Canidae (Carnivora:Mammalia). Paleobiology 34: 35-45.

Fortin, D., Beyer, H.L., Boyce, M.S., Smith, D.W., Duchesne, T. & Mao, J.S. (2005). Wolves influence elk movements: behavior shapes a trophic cascade in Yellowstone National Park. Ecology 86: 1320-1330.

Gese, E.M. & Grothe, S. (1995). Analysis of coyote predation on deer and elk during winter in Yellowstone National Park. American Midland Naturalist 133: 36-43.

Gese, E.M., Ruff, R.L. & Crabtree, R.L. (1996). Social and nutritional factors influencing the dispersal of resident coyotes. Animal Behavior 52: 1025-1043.

Gier, H.T. (1968). Coyotes in Kansas. *Kansas State Univ. Agr. Exp. Sta. Bull.* **393**, 1-118.

Gittleman, J.L. (1985). Carnivore body size: ecological and taxonomic correlates. Oecologia 67: 540-554.

Hairston, N.G., Smith, F.E. & Slobodkin, L.B. (1960). Community structure, population control, and competition. American Naturalist 94: 421-425.

Henderson, W.C. (1930). Then control of the coyote. Journal of Mammalogy 11: 336-353.

Hinton, J.W., Chamberlain, M.J. & Rabon, D.R. (2013). Red wolf (*Canis rufus*) recovery: A review with suggestions for future research. Animals 3: 722-744.

Johnson, W.E., Fuller, T.K., & Franklin, W.L. (1996). Sympatry in canids: a review and assessment. In *Carnivore behavior, ecology, and evolution*: 189-218. Gittleman, J.L. (Ed.). Ithaca: Cornell University Press.

Keller, B.J., Millspaugh, J.J., Lehman, C., Brundige, G., & Mong, T.W. (2013). Adult pronghorn (*Antilocapra americana*) survival and cause-specific mortality in Custer State Park, S.D. American Midland Naturalist 170: 311-322.

Kilgo, J.C., Ray, H.S., Vukovich, M., Goode, M.J. & Ruth, C. (2012). Predation by coyotes on white-tailed deer neonates in South Carolina. Journal of Wildlife Management 76: 1420-1430.

LaBarbera, M. (1989). Analyzing body size as a factor in ecology and evolution. Annual Review of Ecology and Systematics 20: 97-117.

Lingle, S. & Pellis, S.M. (2002). Fight and flight? Antipredator behavior and the escalation of coyote encounters with deer. Oecologia 131: 154-164.

MacNulty, D.R., Smith, D.W., Mech, L.D. & Eberly, L.E. (2009a). Body size and predatory performance in wolves: is bigger better? Journal of Animal Ecology 78: 532-539.

MacNulty, D.R., Smith, D.W., Vucetich, J.A., Mech, L.D., Stahler, D.R. & Packer, C. (2009b). Predatory senescence in ageing wolves. Ecology Letters 12: 1-10.

MacNulty, D.R., Smith, D.W., Mech, L.D., Vucetich, J.A. & Packer, C. (2011). Nonlinear effects of group size on the success of wolves hunting elk. Behavioral Ecology 23: 75-82.

McCarley, H. (1962). The taxonomic status of wild *Canis* (Canidae) in the south central United States. Southwestern Naturalist 7: 227-235.

McVey, J.M., Cobb, D.T., Powell, R.A., Stoskopf, M.K., Bohling, J.H., Waits, L.P. & Moorman, C.E. (2013). Diets of sympatric red wolves and coyotes in northeastern North Carolina. Journal of Mammalogy 94: 1141-1148.

Moore, T.D., Spencer, L.E. & Dugnolle, C.E. (1997). Identification of the dorsal guard hairs of some mammals of Wyoming. Wyoming Game and Fish Department Bulletin 14: 1-177.

Nudds, T.D. (1978). Convergence of group size strategies by mammalian social carnivores. American Naturalist 112: 957-960.

Paradiso, J.L. & Nowak, R.M. (1972). *Canis rufus*. Mammalian Species 22: 1-4.

Patterson, B.R. & Messier, F. (2000). Factors influencing killing rates of white-tailed deer by coyotes in eastern Canada. Journal of Wildlife Management 64: 721-732.

Phillips, M.K., Henry, V.G. & Kelly, B.T. (2003). Restoration of the red wolf. In *Wolves: behavior, ecology, and conservation*: 272-288. Mech, L.D., Boitani, L. (Eds.). Chicago: University of Chicago Press.

Prugh, L.R. (2005). Coyote prey selection and community stability during a decline in food supply. Oikos 110: 253-264.

R Development Core Team. 2012. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. Available online at http://www.R-project.org.

Rabon, D.R., Bartel, R. & Beyer, A. (2013). Red wolf adaptive management plan FY13-FY15. U.S. Fish and Wildlife Service.

Ripple, W.J. & Beschta. (2004). Wolves and the ecology of fear: can predation risk structure ecosystems? Bioscience 54: 755-766.

Rosenzweig, M.L. Community structure in sympatric Carnivora. Journal of Mammalogy 47: 602-612.

Schmidt, P.A. & Mech, L.D. (1997). Wolf pack size and food acquisition. American Naturalist 150: 513-517.

Schmitz, O.J., Beckerman, P.A. & Beckerman, A.P. (2000). Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. American Naturalist 155: 141-153.

Schrecengost, J.D., Kilgo, J.C., Mallard, D., Ray, H.S. & Miller, K.V. (2008). Seasonal food habits of the coyote in the South Carolina coastal plain. Southeastern Naturalist 7: 135-144.

Shaw, J.H. (1975). Ecology, behavior, and systematics of the red wolf (*Canis rufus*). PhD Thesis, Yale University.

Slater, G.J., Dumont, E.R. & Van Valkenburgh, B. (2009). Implications of predatory specialization for cranial form and function in canids. Journal of Zoology 278: 181-188.

Thornton, D.H., Sunquist, M.E. & Main, M.B. (2004). Ecological separation within newly

    sympatric populations of coyotes and bobcats in south-central Florida. Journal of

    Mammalogy 85: 973-982.

Thurber, J.M. & Peterson, R.O. (1991). Changes in body size associated with range expansion in

    the coyote (*Canis latrans*). Journal of Mammalogy 72: 750-755.

U.S. Fish and Wildlife Service. (1989). Red wolf recovery plan. Atlanta: U.S. Fish and Wildlife

    Service.

Van Valkenburgh, B. (1991). Iterative evolution of hypercarnivory in Canids

    (Mammalia:Carnivora): evolutionary interactions among sympatric predators. Paleobiology

    17: 340-362.

Van Valkenburgh, B., Wang, X., & Damuth, J. (2004). Cope's rule, hypercarnivory, and

    extinction in North American canids. Science 306: 101-104.

Van Valkenburgh, B. (2007). Déjà vu: the evolution of feeding morphologies in the Carnivora.

    Integrative and Comparative Biology 47: 147-163.

Table 4.1. Average combined weight and age of red wolf breeders, coyote pairs, and congeneric pairs in northeastern North Carolina during 2009-2011.

	Red Wolf		Coyote		Congeneric			
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$F_{2,37}$	P
Weight (kg)	57.2	0.9	29.5	0.7	43.5	1.2	284.65	0.001
Age (yrs)	4.6	0.4	2.8	0.3	3.0	0.3	7.32	0.002

Table 4.2. Percent volume of foods in red wolf and coyote scats in northeastern North Carolina during 2009-2011. Different letters in parentheses next to values represent statistical differences among breeding units ($P < 0.05$, Tukey's test).

	N	% Deer	% Rabbit	% Small Mammal[a]	% Fur Bearer[b]	%Pig	% Other[c]
Red Wolves[d]	972	40.7(A)	26.8(A)	14.4(A)	8.1(A)	4.1(A)	6.0(A)
Congeneric Pairs[e]	228	40.2(A)	29.7(A)	16.8(AB)	3.7(B)	0.4(B)	9.1(A)
Coyotes[f]	554	30.0(B)	38.3(B)	20.5(B)	3.2(B)	0.6(B)	7.6(A)

[a]Rat, mouse, shrew, and vole species

[b]Muskrat, nutria, and raccoon

[c]Insects (i.e., grasshoppers and beetles), grass/seeds, bird species, and human trash

[d]Red wolf pairs and packs

[e]Coyote and red wolf (congeneric) pair bonds

[f]Coyote pairs

Table 4.3. Percent volume of foods in the scats of red wolf packs ($n = 972$), coyote pairs ($n = 554$), and congeneric pairs ($n = 228$) collected in areas of low (< 0.75 deer harvested/km²), medium (0.75-1.5 deer harvested/km²), and high (>1.5 deer harvested/km²) deer density of northeastern North Carolina during 2009-2011.

	Red Wolf Packs			Coyote Pairs			Congeneric Pairs		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
Deer	23.1 ± 4.4	45.7 ± 1.8	35.8 ± 2.9	N/A	27.3 ± 3.6	30.7 ± 1.9	N/A	36.9 ± 4.2	42.3 ± 3.6
Rabbit	6.4 ± 3.8	24.3 ± 16	41.9 ± 2.5	N/A	37.7 ± 3.6	38.4 ± 1.9	N/A	25.3 ± 3.9	32.8 ± 3.4
Small Mammals	30.2 ± 3.0	12.2 ± 1.2	13.3 ± 2.0	N/A	21.5 ± 2.8	20.2 ± 1.5	N/A	20.6 ± 2.8	14.1 ± 2.4
Fur Bearer	12.5 ± 2.5	9.3 ± 1.0	2.8 ± 1.6	N/A	1.3 ± 1.1	3.8 ± 0.6	N/A	6.1 ± 1.6	2.6 ± 1.4
Pig	2.7 ± 1.8	5.6 ± 0.7	0.8 ± 1.2	N/A	0.1 ± 0.6	0.7 ± 0.3	N/A	0 ± 0	0.8 ± 0.6
Other	25.1 ± 1.9	2.9 ± 0.8	5.9 ± 1.2	N/A	12.3 ± 2.0	6.3 ± 1.1	N/A	11.1 ± 2.2	7.4 ± 1.9

Table 4.4. Percent volume of foods in the scats of red wolf packs (n = 972), coyote pairs (n =554), and congeneric pairs (n =228) by age the breeders in northeastern North Carolina during 2009-2011.

	Red Wolf Packs		Coyote Pairs		Mixed Pairs	
	≤ 4 yrs	> 4 yrs	≤ 4 yrs	> 4 yrs	≤ 4 yrs	> 4 yrs
Deer	47.5 ± 2.4	36.8 ± 1.8	28.8 ± 1.7	45.1 ± 6.5	42.6 ± 3.1	30.1 ± 6.0
Rabbits	16.1 ± 2.1	33.2 ± 1.6	39.3 ± 1.7	24.0 ± 6.4	28.4 ± 2.9	34.5 ± 5.7
Small Mammals	17.7 ± 1.6	12.6 ± 1.3	20.2 ± 1.4	24.2 ± 5.0	14.9 ± 2.0	24.8 ± 4.0
Fur Bearer	6.5 ± 1.3	8.9 ± 1.0	3.2 ± 0.6	3.9 ± 2.0	3.7 ± 1.2	5.5 ± 2.3
Pig	2.1 ± 1.0	5.3 ± 0.7	0.6 ± 0.3	0.3 ± 1.1	0.6 ± 0.5	0.0 ± 1.0
Other	10.2 ± 1.1	3.6 ± 0.8	7.9 ± 1.0	3.1 ± 3.6	9.9 ± 1.6	5.3 ± 3.2

Table 4.5. Percent volume of foods in the scats of red wolf packs (n = 972), coyote pairs (n =554), and congeneric pairs (n =228) by season in northeastern North Carolina during 2009-2011.

	Red Wolf Packs		Coyote Pairs		Mixed Pairs	
	Winter	Summer	Winter	Summer	Winter	Summer
Deer	42.3 ± 2.7	40.1 ± 1.7	37.5 ± 2.4	22.7 ± 2.3	32.0 ± 3.4	52.8 ± 4.3
Rabbits	33.9 ± 2.4	23.8 ± 1.5	32.5 ± 2.3	43.8 ± 2.4	33.1 ± 3.3	24.1 ± 4.1
Small Mammals	14.7 ± 1.8	14.3 ± 1.2	18.8 ± 1.9	22.0 ± 1.8	19.7 ± 2.3	12.4 ± 2.9
Fur Bearer	1.4 ± 1.5	10.8 ± 0.9	2.2 ± 0.8	4.2 ± 0.7	3.3 ± 1.3	5.3 ± 1.7
Pig	2.1 ± 1.0	4.9 ± 0.7	0.1 ± 0.4	1.1 ± 0.4	0.7 ± 0.6	0.0 ± 0.7
Other	5.5 ± 1.2	6.2 ± 0.8	9.1 ± 1.4	6.1 ± 1.3	11.3 ± 1.9	5.3 ± 2.3

Table 4.6. Results of the 3 best models for factors influencing selection of 5 food categories by red wolf packs in northeastern North Carolina during 2009-2011.

Model	k^a	$AIC_c{}^b$	$\Delta AIC_c{}^c$	$w_i{}^d$
White-tailed deer				
Weight + pack size	4	5603.73	0.00	0.35
Weight + pack size + age	5	5605.34	1.62	0.16
Weight + pack size + summer	5	5605.63	1.90	0.14
Rabbits				
Age + deer density	4	5468.28	0.00	0.31
Age + deer density + summer	5	5469.37	1.09	0.18
Age + deer density + weight	5	5469.63	1.34	0.16
Small Mammals				
Age + summer + pack size + deer density + weight	7	5085.47	0.00	0.29
Age + summer + pack size + deer density	6	5086.00	0.52	0.23
Age + summer + pack size + weight	6	5086.37	0.89	0.19
Fur bearers				
Summer + weight	4	4626.08	0.00	0.29
Summer + weight + pack size	5	4627.76	1.69	0.13
Summer + weight + age	5	4627.89	1.82	0.12
Pig				
Age + deer density + pack size + weight + summer	7	4012.21	0.00	0.65
Age + deer density + pack size + weight	6	4013.85	1.64	0.29
Age + deer density + pack size + summer	6	4017.50	5.29	0.05
Other				
Deer density + summer	4	4303.20	0.00	0.31
Deer density + summer + pack size	5	4304.95	1.75	0.13
Deer density + summer + pack size + weight	6	4304.96	1.76	0.13

[a]Model parameters
[b]Akaike's Information Criterion
[c]Difference in AIC
[d]Akaike weight

Table 4.7. Results of the 3 best models for factors influencing selection of 5 food categories by coyote pairs in northeastern North Carolina during 2009-2011.

Model	k^a	AIC_c^b	ΔAIC_c^c	w_i^d
White-tailed deer				
Weight + summer	4	3132.08	0.00	0.44
Weight + summer + deer density	5	3132.94	0.86	0.29
Weight + summer + age	5	3134.04	1.96	0.17
Rabbits				
Summer + weight	4	3153.34	0.00	0.34
Summer + weight + age	5	3153.89	0.55	0.26
Summer + weight + deer density	5	3155.37	2.03	0.12
Small Mammals				
Age + weight + deer density + summer	6	2960.97	0.00	0.26
Age + weight + deer density	5	2960.98	0.01	0.26
Age + weight + summer	5	2961.94	0.97	0.16
Other				
Deer density	3	2620.65	0.00	0.18
Null model	2	2621.44	0.79	0.12
Deer density + summer	4	2621.81	1.15	0.10

[a]Model parameters
[b]Akaike's Information Criterion
[c]Difference in AIC
[d]Akaike weight

Table 4.8. Results of the 3 best models for factors influencing selection of 5 food categories by congeneric pairs in northeastern North Carolina during 2009-2011.

Model	k^a	AIC_c^b	ΔAIC_c^c	w_i^d
White-tailed deer				
Summer	3	1312.34	0.00	0.23
Summer + age	4	1312.95	0.61	0.17
Summer + age + deer density	5	1313.55	1.20	0.13
Rabbits				
Deer density + summer + age	5	1294.51	0.00	0.23
Deer density + summer	4	1295.02	0.51	0.18
Deer density + age	4	1295.69	1.18	0.13
Small Mammals				
Deer density + summer	4	1187.97	0.00	0.21
Deer density	3	1188.91	0.94	0.13
Deer density + age	4	1189.09	1.12	0.12
Other				
Summer	3	1108.24	0.00	0.15
Weight	3	1108.85	0.61	0.11
Summer + age	4	1108.97	0.73	0.10

[a]Model parameters
[b]Akaike's Information Criterion
[c]Difference in AIC
[d]Akaike weight

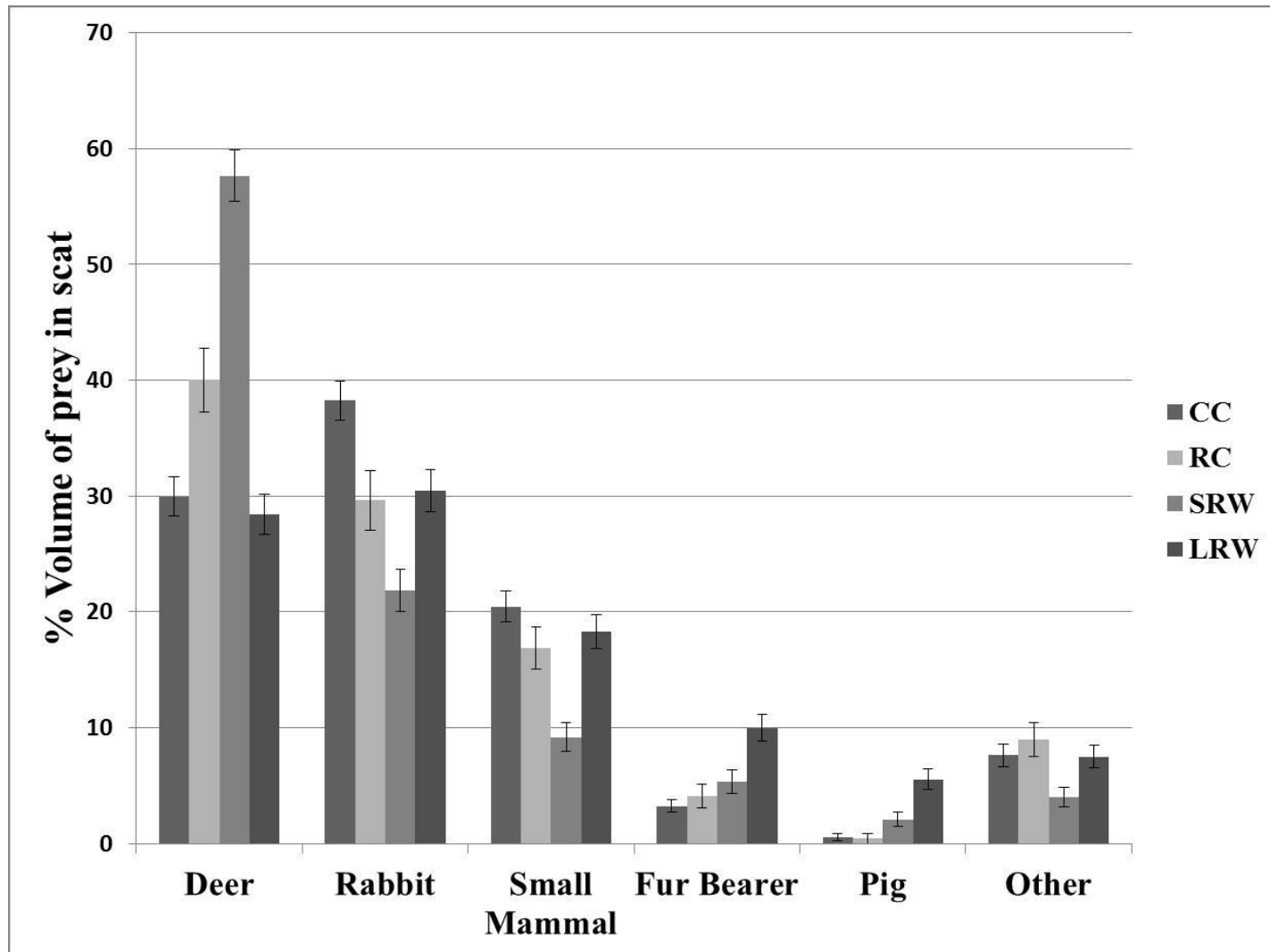Figure 4.1. Percent volume of prey in the scats of coyote pairs (CC), congeneric pairs (RC), red wolf packs with ≤ 4 individuals (SRR), and red wolf packs with > 4 individuals (LRR) in northeastern North Carolina, during 2009-2011.

CHAPTER 5

SPACE USE AND HABITAT SELECTION BY RESIDENT AND TRANSIENT COYOTES

_____

Hinton, J.W., M.J. Chamberlain, and F.T. van Manen. To be submitted to the *Journal of Animal Ecology*.

Abstract

Little information exists on coyote spatial ecology in the southeastern United States and the few studies conducted have been conducted at small scales (e.g., ≤ 500 km2). Studies on coyote ecology in the Southeast have typically been conducted at small scales (e.g., ≤ 500 km²) and often provide conflicting insights regarding coyote ecology. Therefore, studies of coyote spatial ecology at broader geographic areas (e.g., ≥ 2,500 km2) may provide relevant insights as to how coyote populations adjust to various ecological circumstances on the landscape. During 2009-2011, we studied coyote space use and habitat selection on the Albemarle Peninsula (>6,000 km²) of northeastern North Carolina using GPS radio-collars. We quantified home range sizes and 2nd- and 3rd-order habitat selection for resident and transient coyotes to describe space use patterns in response to a dynamic agricultural landscape. We observed an upper limit on coyote home-range sizes to be approximately 50 km² and suggest body size constraints may limit the amount of finite space coyote groups can maintain as a territory. We also observed preferences for agricultural habitats over forested habitats by resident and transient coyotes, and resident coyotes pushed transients into marginal forest habitats. Coyotes had exhibited avoidance of roads during diurnal hours despite exhibiting preferences for them during nocturnal hours. Overall, loss of cover resulting from harvest of agricultural crops facilitated strong shifts to using forested areas for cover by resident coyotes during fall and winter. Consequently, use of forested areas by resident coyotes forced transient coyotes to rely more on road and edge habitats during fall and winter.

Introduction

Coyote colonization of eastern North America has generated much interest from ecologists and the general public (Gompper 2002; Levy 2012) because it occurred in several waves (Parker 1995; Nowak 2002; Bozarth et al. 2011), resulting in noticeable changes in phenotype (i.e., body size and pelt color; Thurber and Peterson 1991; Way 2007) and hybridization with remnant wolf populations (Kays et al. 2010; Wheeldon et al. 2010). Presence

101

in eastern North America during the Pleistocene indicates that coyotes have history of range expansions and contractions that can be attributed to the emergence and loss of other *Canis* competitors on the landscape (Nowak 2002) and changes in climate (Koblmüller et al. 2012). Although successful colonization has been attributed to the coyote's generalist behaviors, phenotypic plasticity, and ability to hybridize with remnant wolf populations, developing a conceptual framework for understanding coyote range expansion remains contentious (Thurber and Peterson 1991; Lariviére and Créte 1993; Kays et al. 2010; Wheeldon et al. 2010).

Coyote colonization of the southeastern United States has received more attention in recent years, but little information exists on large scale spatial and temporal patterns of landscape use by coyotes in the region. Studies on coyote ecology in the Southeast have typically been conducted at small extents (e.g., ≤ 500 km²) and often provide conflicting insights. Fine-scale studies are problematic because they fail to capture broad scale variations in coyote ecology and landscape structure that can be used to explain why coyotes have become established throughout the Southeast. Aspects of coyote spatial ecology that influence variation in size and habitat composition of home ranges have important consequences for processes such as population growth and regulation because differential use of space and habitats allows access to important prey species and reduces risks of mortality, both of which affect survival and reproduction. Therefore, studying the placement, size, and habitat composition of coyote home ranges over broad geographic areas (e.g., $\geq 2,500$ km²) will provide relevant insights as to how coyote populations adjust regionally varying ecological conditions.

In particular, the effects of eastern forested and agricultural landscapes on coyote movements and space use in the Southeast are not well known. While it has been suggested that eastern forested landscapes represent marginal habitat for coyotes (Tremblay et al. 1998; Crête et al. 2001; Richer et al. 2002), other studies have suggested that eastern forests are suitable habitat (Kays et al. 2008). Although these studies were conducted in the Northeast, many regions of the Southeast are characterized by large agriculture-forested mosaics similar to areas colonized by

coyotes in the Northeast and generalities can be drawn by comparing studies conducted in different regions. For instance, coyote home-range sizes typically vary between $2.5 - 70$ km^2 (Bekoff and Gese 2003; Leopold and Chamberlain 2001). Although variability can be attributed to adjustments of space use patterns to local environmental conditions, a central tendency of coyote space use is driven by their metabolic needs, which is known to vary with body mass (McNab 1963; Gompper and Gittleman 1991). Along with body size, locomotor mode is known to be a key parameter for explaining large scale patterns in carnivores (Van Valkenburgh 1999). Coyotes are cursorial carnivores that evolved in the extensive grassland region of central North America. Dense forested areas may make pursuit hunting, and therefore resource acquisition, more difficult for coyotes. On the other hand, agricultural fields and early successional habitats may be analogous to the open, expansive habitats to which coyotes are adapted, and may provide ideal habitat for population centers. Therefore, understanding coyote distributional patterns in response to habitat heterogeneity is fundamental to develop a full understanding of how this species successfully colonized eastern North America.

Coyotes are sympatric with red wolves in northeastern North Carolina and both species are managed and monitored by the United States Fish and Wildlife Service (USFWS) Red Wolf Recovery Program (Recovery Program) on the Albemarle Peninsula of northeastern North Carolina (Hinton et al. 2013; Rabon et al. 2013). Red wolves show strong preferences for agricultural fields over the surrounding forested areas (Chadwick et al. 2010, Hinton and Chamberlain 2010, Dellinger et al. 2013) and very high frequency (VHF) radio-telemetry data indicates that coyotes exhibit similar habitat preferences (USFWS *unpublished*). It is suspected that coastal bottomland forests and wetlands are unsuitable habitat to red wolves and coyotes in northeastern North Carolina because dense understories and periodic inundation hinder or prevent movements. On the other hand, agricultural croplands may provide suitable habitat because they are dry, treeless environments with no understory structure to hinder movements and foraging efforts. Although the effect of roads on coyote movements remains unknown, previous gray wolf

103

(James and Stuart-Smith 2000; Whittington et al. 2005) and red wolf (Dellinger et al. 2013) studies suggested that secondary, low-use roads provided some benefit to wolves by increasing foraging efficiency and lowering movement costs. Coyotes are known to have higher tolerance to human presence than wolves (Grinder and Krausman 2001; Atwood et al. 2004; Way et al. 2004; Gehrt et al. 2009) and studies have documented coyote use of roads in heavily populated urban areas (Tigas et al. 2002; Way 2009; Gehrt et al. 2011; Hinton et al. 2012). This trait may allow them to exploit primary (paved) and secondary (unpaved) roads as travel corridors through barriers (i.e., rivers) and unsuitable habitats regardless of human density.

With *a priori* assumptions that forested areas in northeastern North Carolina represent poor habitat for coyotes, we monitored resident and transient coyotes fitted with global positioning system (GPS) radio-collars to assess ways in which coyotes move, interact, and use several primary habitats on the Albemarle Peninsula. During summer, we expected coyotes to avoid forested areas and increase their use of row crops (i.e., corn, soybean, and winter wheat) because crops provide adequate cover with little understory to impede movements. We also expected coyotes to increase their use of forested areas for cover during winter because crops were harvested and forests provided the only cover during that time of year. Transient coyotes are individuals that have not established residency and display nomadic movement with no fidelity for any one area; they also may display different space use patterns than resident coyotes that maintain home ranges (Gese et al. 1988, Kamler and Gipson 2000; Hinton et al. 2012). Previous work observed localized space use patterns lasting between 2-8 weeks that were analogous to patterns of home range use (Hinton et al. 2012). These space use patterns of transients were called biding areas and studying them may provide important insights into how coyotes move through the landscape searching for unoccupied areas and/or mates to establish residency. This study represents the first broad scaled study (> 5000 km^2) of resident and transient coyotes in the southeastern United States.

Study Area

Our study was conducted on the Albemarle Peninsula in the northeastern region of North Carolina (Figure 1). The study area was approximately 6000 km^2 of federal, state, and private lands comprised of a row-crop agricultural-bottomland matrix with little change in elevation (0-50m). Agricultural crops (i.e., corn, cotton, soybean, and winter wheat) and managed pine (*Pinus* spp) comprised approximately 30% and 15% of the land cover, respectively (Figure 1). Other prominent land cover types were coastal bottomland forests and pocosin (35%), herbaceous wetlands and saltwater marshes (5%), open water (5%), and other minor land cover types (10%). The climate was typical of the mid-Atlantic: 4 full seasons, nearly equal in length, with an annual precipitation averaging between 122-132 cm. Summer climate was typically hot and humid with temperatures ranging from 27°C to over 38°C and winters were relatively cool with temperatures ranging between -4°C to 7°C.

Methods

We captured coyotes using padded foot-hold traps (Victor no.3 Softcatch, Lititz, Pennsylvania, USA) from October through May during 2009-2011. Coyotes were sexed, measured, weighed, aged by tooth wear (Gier, 1968), and a blood sample was collected. We categorized coyotes as > 2 years old as adults, < 2 but > 1 year old as juveniles, and < 1 year old as pups. Coyotes on the Albemarle Peninsula were reproductively sterilized by the USFWS to prevent introgression into the red wolf population (Hinton et al. 2013; Rabon et al. 2013). Coyotes were taken to a local veterinary clinic for surgical sterilization where males and females were reproductively sterilized by vasectomy and tubal ligation, respectively. This process kept hormonal systems intact to avoid disrupting breeding and territorial behavior (Seidler and Gese 2012). Prior to release at the original capture sites, we fitted coyotes with a mortality-sensitive GPS radio-collar (Lotek 3300s, Newmarket, Ontario, Canada) scheduled to record a location every 4 hours (0:00, 04:00, 08:00, etc.) throughout the year. Animal handling methods were

approved by the Louisiana State University Agricultural Center Institutional Animal Care and Use Committee (Protocol Number AE2009-19).

To reflect the anthropogenic effects of agriculture on the landscape, we divided year into 2 6-month seasons based on agricultural activity: planting/growing (1 March – 31 August) and harvest/fallow (1 September – 28 February). We also separated coyote locations for each season into diurnal and nocturnal categories based monthly photoperiods. We estimated space use of resident and transient coyotes by fitting dynamic Brownian bridge movement models (dBBMMs) to the time-specific location data to estimate the probability of use along the full movement track of each coyote (Kranstauber and Smolla 2013) using the R package moveud (Collier 2013) in the R statistical environment (R Core Team 2013). We chose window sizes based on the temporal resolution of each track and our *a priori* assumptions of the time scale of major behavioral shifts (Byrne et al. 2014). For resident coyotes, 95% and 50% contour intervals were considered home ranges and core areas, respectively. For transient coyotes, 95% and 50% contour intervals were considered transient range and biding areas (Hinton et al. 2012), respectively. We used *t*-tests to examine changes in home range and space use sizes between seasons.

Predominant habitat types were estimated from a digitalized landscape map of vegetative communities developed by the North Carolina Gap Analysis Project (McKerrow et al. 2006). We collapsed the vegetative communities estimated by McKerrow et al. (2006) into 7 general habitat types with 30m resolution. For the habitat selection analysis, we divided the landscape into agriculture, coastal bottomland forest, pine forest, open water, upland forest, urban, and wetland. Because roads may serve as corridors for coyote movements, we included road as the 8th habitat type by superimposing a linear feature layer with a 30m buffer around roads onto the final habitat raster map with 30m resolution.

We used analysis of variances (ANOVAs) and *t*-tests to determine if the percentage of habitat composition of home ranges and transient ranges differed between each other and between seasons. We also developed generalized linear models (GLMs) to examine the effects of coyote

body weight and age, white-tailed deer abundance, and habitat composition on coyote home-range size. We used county-level harvest data collected by the North Carolina Wildlife Resources Commission as a measure of deer abundance in the study area. Although harvest reports do not reflect true white-tailed deer densities, hunter harvest/km^2 does provide a benchmark by which to judge the effect of white-tailed deer abundance on coyote home-range size. Habitat composition of home ranges were calculated as % agricultural, % forest (coastal bottomland and pine), and % road. We assessed models using a stepwise procedure by calculating Akaike's information criterion for small sample sizes (AIC$_c$) for each model to select which variables best explained home-range size.

We compared habitat selection of coyotes at two scales: population (2nd-order) and individual (3rd-order). Habitats were converted into continuous variables by quantifying percent of habitat cover for each 30m cell using a moving window with a 150m radius in FRAGSTATS (McGarigal et al. 2012). We also quantified edge density and patch richness across the study area because coyotes are known to forage along edges (Tigas et al. 2002). Information from raster maps were extracted to coyote locations and home ranges using ArcGIS 10.1 (Environmental Systems Research Institute Inc., Redlands, California). We used resource-selection functions (RSFs) to examine the effect of habitat type on where coyotes established home ranges on the landscape (2nd-order selection of resources; Johnson 1980) and examine the effect of habitat type on how coyotes use their home ranges (3rd-order selection of resources; Johnson 1980) following Manly et al. (2002). We assumed seasonal and photoperiod variation in habitat use by residents and transients and, therefore, developed RSFs for residents and transients by season and photoperiod for 3rd-order selection. We used a binomial approach to estimate resource-selection functions by comparing characteristics of known (used) locations to random (available) locations (Manly et al. 2002). We used logistic regression and AIC$_c$ to form RSFs that identified habitats important to individual coyotes. We evaluated the relative importance of coefficients associated with the habitat types by examining Akaike weights (w_i) after adding each of these covariates to a

core model set (e.g., higher w_i and lower AIC_c suggested model improvement; Burnham and Anderson 2002). We also evaluated the predictive performances of the most parsimonious core models using the k-fold cross validation method (Boyce et al. 2002; Johnson et al. 2006). The cross-validation method performs k iterations of training and validation in which a different fold of the data is held out for validation while the remaining $k - 1$ folds are used for learning. For cross-validation, we used 10 folds ($k = 10$) to estimate performance of RSF models.

Results

During 2009-2011, we monitored 28 coyotes fitted with GPS radio-collars. We monitored 22 (79%) resident and 6 (21%) transient coyotes and 8 (29%) coyotes were transients prior to becoming residents. Mean weight and age of coyotes monitored were 14.0 kg ± 0.4 and 2.5 yrs ± 0.2, respectively, in which weights and age of residents were greater than transients (Table 1; $t_{26} \geq 2.23$, $P \leq 0.034$). Home ranges and core areas of residents did not differ between seasons (Table 2; $t_{45} \leq 0.024$, $P \geq 0.800$) and home ranges ranged from 13.4 km² to 47.3 km². We also detected no seasonal difference of transient ranges and biding areas (Table 1; $t_{17} \leq 1.86$, $P \geq 0.080$) and transient areas ranged from 64.5 km² to 633.4 km². Home range size was best explained by the percentage of agricultural fields, white-tailed deer density, and forested areas in which home range size decreased with increasing percentage of agricultural fields ($r^2 = 0.30$, $P = 0.007$) and deer density ($r^2 = 0.40$, $P = 0.001$), but increased with increasing percentage of forests ($r^2 = 0.33$, $P = 0.004$).

Home ranges and core areas were comprised mostly of agricultural fields, coastal bottomland and pine forest, and roads (Figure 2; $F_{6, 161} = 36.69$, $P < 0.001$). Pine and upland forest, urban, road, and wetland composition between home ranges and core areas did not differ ($t_{46} \leq 1.31$, $P \geq 0.195$) but home ranges typically consisted of more coastal bottomland forest and less agricultural fields than did core areas ($t_{46} \geq 2.39$, $P \geq 0.022$). Similarly, transient ranges and biding areas were comprised mostly of agricultural fields, coastal bottomland and pine forests, and roads (Figure 2; $F_{6, 161} = 60.23$, $P < 0.001$). We found no differences in habitat composition

108

between transient ranges and biding areas ($t_{28} \leq 1.67$, $P \geq 0.105$). Home ranges consisted of more

pine forests and roads and less wetlands than transient ranges ($t_{103} \geq 2.07$, $P \leq 0.022$), whereas all

other habitat types were similar ($t_{103} \leq 1.06$, $P \geq 0.293$). Core areas consisted of more pine forests

and less coastal bottomland forests and wetlands than biding areas ($t_{103} \geq 2.30$, $P \leq 0.024$),

whereas all other habitat types were similar ($t_{103} \leq 0.96$, $P \geq 0.337$). We detected no seasonal

differences between habitat types of home ranges and core areas ($t_{45} \leq 1.25$, $P \geq 0.219$) and

transient ranges and biding areas ($t_{17} \leq 1.27$, $P \geq 0.220$).

We excluded wetland, upland forest, and urban habitats from our models because their

occurrence in home ranges and transient ranges were negligible. Therefore, we only included

edge density, patch richness, and agriculture, pine forest, coastal bottomland forest, and road

habitats in our model. Only 1 covariate (edge density) was not retained in the top-ranked model

for 2nd-order selection by residents (Table 2) whereas all 6 covariates were retained for transients

(Tables 3). Roads, coastal bottomland forests, and patch richness were better predictors of

transient occurrence than residents at the landscape level (Table 4). Agriculture, pine forest, and

road habitats were retained in all top-ranked models for 3rd-order selection by residents (Table 2).

Residents appeared to favor agricultural fields and pine forests during both seasons and

photoperiods, whereas roads were avoided during day but used at night (Figures 4&5; Table 4).

Coastal bottomland forest, pine forest, and patch richness were retained in all top-ranked models

for 3rd-order selection by transients (Table 3). Coastal bottomland and pine forests were used by

transients more during the growing season than harvest season, whereas patch rich areas were

preferred during both seasons and photoperiods (Figures 6&7; Table 4). Similar to residents,

transients used roads more at night than during day.

Discussion

The broad front of coyote expansion throughout North America is a result of dynamic

space-use patterns by coyotes that permit them to move across and persist in a variety of

environments. In particular, coyote populations consist of resident and transient individuals

(Andelt et al. 1985, Gese et al. 1988, Kamler and Gipson 2000). Similar to other studies (Gese et al. 1988, Windberg and Knowlton 1988, Chamberlain et al. 2000), our results indicate that approximately 70% of coyotes in northeastern North Carolina are likely residents whereas the remaining 30% are transients.

Home-range sizes of coyotes did not vary between seasons despite an average 35% reduction in winter cover within home ranges resulting from crop harvest (Figure 2). This indicates that coyotes may not adjust home range size to immediate demand, but rather potential demand. In other words, coyotes most likely learn changes in the environment prior to establishing residency so that space acquired for home ranges is adequate to accommodate seasonal fluctuations in resource needs. Home ranges did not exceed 50 km^2 indicating that coyotes may have an upper limit to the amount of space they can effectively exploit and defend. Coyotes must be able to defend a finite area and handle the distribution of prey while maintaining a consumption rate below resource renewal to assure long-term maintenance of their home ranges. Our mean home-range sizes are typical of those reported in other studies (see Table 22.4 in Bekoff and Gese 2003 & Table 21 in Leopold and Chamberlain 2001) and similarity among studies indicates that there is a central tendency in coyote space use that is likely constrained by the effects of body size (McNab 1963; Swihart et al. 1988; LaBarbera 1989). Although we found habitat type and white-tailed deer density to have a stronger effect on variation of home-range size than did body size, it is important to note that body size is an endogenous factor resulting from evolutionary forces that reduce variability, whereas exogenous factors such as habitat and resource density would influence variation around a central tendency in coyote space use. Effects of body size on space use patterns are well established and they should be routinely tested and reported in studies of coyote spatial use regardless of statistical significance. Coyotes exhibit phenotypic variation by regions (Thurber and Peterson 1991; Way 2007) and reporting such information allows ecologists to accurately draw conclusions regarding the effects of habitat and physiology on coyote space use.

Territorial behavior in coyotes assures ideal reproductive possibilities to residents holding space (Gaston 1978). Although this prevents transients from reproducing, transiency is likely an important trait that allows populations to reconstruct themselves rapidly after suffering drastic and extensive mortality. This may be particularly important for coyote populations to persist where they are heavily exploited. Despite their wide-ranging space use patterns, many transients exhibited localized movements that were analogous to home ranges and we referred to them as biding areas (Hinton et al. 2012). Seven of the 8 (88%) residents that were transients established home ranges in or nearby their biding areas. We suggest this behavior may prove beneficial to coyote populations and should be examined in future studies because it increases survivorship of transients via familiarity of areas they roam, allows transients to assess potential areas prior to establishing home ranges, and, when opportunities arise, they can replace residents upon death.

Effects of agriculture and forest habitat on coyote space use in northeastern North Carolina are similar to those reported in other studies in the Northeast and indicate general preferences for open, treeless environments by coyotes (Crête et al. 2002, Gosselink et al. 2003, Van Deelen and Gooselink 2006). The general compositional pattern in coyote home ranges were high percentages of agricultural fields in the interior (i.e., core areas) with forest edges increasing in outer fringes. The forest cover coyotes favored in our study were pine plantations. During harvest season, coyotes typically loafed in forest habitats within 50-300m of edges adjacent to agricultural fields and roads. As winter wheat reached heights of approximately 0.5 meters during the growing season, coyotes abandoned forest habitats to loaf in wheat fields when available and then shifted to corn later in the season as wheat was harvested (Hinton, *personal observation*). Home range size decreased as agricultural fields became the predominant habitat type and vice versa for forested habitats. For example, the home range of a female coyote with the smallest home-range size (13.4 km^2) consisted of approximately 40% and 30% agricultural and forested habitat, respectively. Of her 1987 GPS locations, approximately 87% of them occurred in agricultural fields. On the other hand, the home range of a female coyote with the largest home-

range size (47.3 km^2) consisted of approximately 10% and 70% agricultural and forested habitat, respectively. Of her 2296 GPS locations, approximately 35% of them were in agricultural fields.

Although transients displayed similar attraction to habitat types as residents, residents occupied ideal habitats and most likely push transients into marginal habitats. Resource-selection models were useful in teasing out this effect and demonstrated where patterns of habitat use diverged from those of residents. Models of 2nd-order selection indicated that coastal bottomland forest, roads, and edges were more important to transients than residents. Transients were typically pushed into forested areas and frequent use of roads and edges was likely to reduce the energetic costs of nomadic movements in these highly structured habitats. Conversely, models of 3rd-order selection indicated 2 temporal changes in habitat use by residents that are related to foraging and security. First, they avoided roads during diurnal hours despite relying on them during nocturnal hours when they were foraging and defending their home ranges. This behavior is likely a way to avoid contact with humans, which will occur more during diurnal hours. Second, residents avoided coastal bottomland forest during the growing season and increased use of them during the harvest season, specifically during diurnal hours. Residents ceased use of coastal bottomland forests during nocturnal hours and the diurnal use by residents suggests coastal bottomland forests were used for security. Transient use of roads during nocturnal hours is likely for similar reasons as residents. Nevertheless, their habitat use patterns deviated significantly from residents during the harvest season. Agricultural fields had little influence on transient habitat use and transients avoided forested areas while depending more on roads and edge habitats for foraging and security. Most agricultural fields are barren after harvest and likely provide little foraging or security benefit to transients. Avoidance of forest habitats by transients during the harvest season is likely a result of increased use of these habitats by residents. In other words, shifts in the use of habitats by residents as a result of landscape-wide agricultural activities created significant shifts in habitat use by transients.

Coyote populations appear adept at exploiting human modified landscapes, specifically

road networks, because linear features facilitate travel while providing foraging opportunities.

Although transients were documented using coastal bottomland forests, we suspect those

locations were more associated with the road network than the actual forested habitat (Figure 7).

Roads may provide benefits to coyotes through efficient movements that improve foraging and

territorial defense (i.e., scent marking) and may allow resident coyotes to detect human presence

and assess predictable patterns of human activities to increase security. While facilitating

transient movements on the landscape, roads may also guide coyotes into suitable habitats

modified by humans such as agricultural fields. Interestingly, road networks through unsuitable

forest habitat may decrease intraspecific strife by reducing or masking transient movements

through territories of residents.

Coyotes colonized eastern North America in several waves occurring at different periods

of the 20th century (Nowak 2002; Bozarth et al. 2011). Although it is well documented that

coyotes began colonizing Arkansas, Louisiana, Mississippi, and Alabama during the mid-20th

century after the extirpation of red wolves (McCarley 1962; Nowak 2002), there has been no

parsimonious explanation of how coyotes have colonized the remainder of the Southeast. Large-

scaled construction of the Interstate Highway system facilitated significant changes to the

physical landscape of the United States during the 1960s, 1970s, and 1980s as a result of the

Federal Highway Act of 1956 (Lewis 2013). Additionally, the Watershed Protection and Flood

Prevention Act of 1954 encouraged land drainage and wetland destruction that resulted in

significant loss of bottomland forests in the Southeast (Harris and Gosselink 1990, Dahl and

Allord 1996). Several studies, including this one, have found eastern forests to be relatively poor

habitat for coyotes (Tremblay et al. 1998; Crête et al. 2001; Richer et al. 2002) and that coyotes

are capable of exploiting human altered landscapes and road networks (Tigas et al. 2002; Way

2009; Gehrt et al. 2011; Hinton et al. 2012). Therefore, we propose that, in concomitant with the

extirpation of red wolves, large-scale changes to the landscape through development of extensive

road networks, agriculture, and residential areas during the 1960s-1980s allowed for the eventual colonization of the Southeast by coyotes by the turn of the 21st century.

Acknowledgements

Literature Cited

Andelt, W.F. (1985) Behavioral ecology of coyotes in south Texas. Wildlife Monographs 94: 3-45.

Atwood, T.C., Weeks, H.P. & Gehring, T.M. (2004) Spatial ecology of coyotes along a suburban-to-rural gradient. Journal of Wildlife Management 68: 1000-1009.

Bekoff, M. & Gese, E.M. (2003) Coyote (*Canis latrans*). *Wild Mammals of North America: Biology, Management, and Conservation*, 2nd edn (eds G.A. Feldhamer, B.C. Thompson, & Chapman, J.A.), pp. 467-481. John Hopkins University Press, Baltimore.

Boyce, M.S., Vernier, P.R., Nielsen, S.E. & Schmiegelow, F.K.A. (2002) Evaluating resource selection functions. Ecological Modelling 157: 281-300.

Bozarth, C.A., Hailer, F., Rockwood, L.L., Edwards, C.W. & Maldonado, J.E. (2011) Coyote colonization of northern Virginia and admixture with Great Lakes wolves. Journal of Mammalogy 92: 1070-1080.

Burnham, K.P. & Anderson, D.R. (2002) *Model Selection and Multimodel Inference: A Practical Information Theoretic Approach*, 2nd edn. Springer-Verlag, New York, New York, USA.

Byrne, M., McCoy, J., Hinton, J., Chamberlain, M. & Collier, B. (2014) Using dynamic Brownian bridge movement modeling to measure temporal patterns of habitat selection. *Journal of Animal Ecology* doi: 10.1111/1365-2656.12205

Chadwick, J., Fazio, B. & Karlin, M. (2010) Effectiveness of GPS-based telemetry to determine temporal changes in habitat use and home-range sizes of red wolves. Southeastern Naturalist 9: 303-316.

Chamberlain, M.J., Lovell, C.D. & Leopold, B.D. (2000) Spatial-use patterns, movements, and interactions among adult coyotes in central Mississippi. Canadian Journal of Zoology 78: 2087-2095.

Collier, B. (2013) Moveud: Creates dynamic Brownian bridge movement model individual time-step utilization distributions. R package version 1.0.

Dahl, T.E. & Allord, G.J. (1996) History of wetlands in the conterminous United States. *National Water Summary on Wetland Resources, United States Geological Survey, Water-Supply Paper 2425* (eds J.D. Fretwell, J.S. Williams, & P.J. Redman), pp. 19-26. U.S. Geological Survey, Reston, Virginia, USA.

Dellinger, J.A., Proctor, C., Steury, T.D., Kelly, M.J. & Vaughan, M.R. (2013) Habitat selection of a large carnivore, the red wolf, in a human-altered landscape. Biological Conservation 157: 324-330.

Gaston, A.J. (1978) The evolution of group territorial behavior and cooperative breeding. American Naturalist 112: 1091-1100.

Gehrt, S.D., Anchor, C. & White, L.A. (2009) Home range and landscape use of coyotes in a metropolitan landscape: conflict or coexistence? Journal of Mammalogy 90: 1045-1057.

Gese, E.M., Rongstad, O.J. & Mytton, W.R. (1988) Home range and habitat use of coyotes in southeastern Colorado. Journal of Wildlife Management 52: 640-646.

Gier, H.T. (1968) Coyotes in Kansas. *Kansas State College Agricultural Experiment Station Bulletin* 393. Kansas State University, Manhattan, Kansas, USA.

Gompper, M.E. (2002) Top carnivores in the suburbs? Ecological and conservation issues raised by colonization of north-eastern North America by coyotes. BioScience 52: 185-190.

Gompper, M.E. & Gittleman, J.L. (1991) Home range scaling: intraspecific and comparative trends. Oecologia 87: 343-348.

Gosselink, T.E., Van Deelen, T.R., Warner, R.E. & Joselyn, M.G. (2003) Temporal habitat partitioning and spatial use of coyotes and red foxes in east-central Illinois. Journal of Wildlife Management 67: 90-103.

Grinder, M.I. & Krausman, P.R. (2001) Home range, habitat use, and nocturnal activity of coyotes in an urban environment. Journal of Wildlife Management 65: 887-898.

Harris, L.D. & Gosselink, J.G. (1990) Cumulative impacts of bottomland hardwood forest conversion on hydrology, water quality, and terrestrial wildlife. *Ecological Processes and*

Cumulative Impacts Illustrated by Bottomland Hardwood Wetland Ecolsystems (eds J.G. Gosselink, L.C. Lee & T.A. Muir), pp. 259-322. Lewis Publishers, Chelsea, Michigan, USA.

Hinton, J.W. & Chamberlain, M.J. (2010) Space and habitat use by a red wolf pack and their pups during pup-rearing. Journal of Wildlife Management 74: 55-58.

Hinton, J.W., Chamberlain, M.J. & van Manen, F.T. (2012) Long-distance movements of transient coyotes in eastern North Carolina. American Midland Naturalist 168: 281-288.

Hinton, J.W., Chamberlain, M.J. & Rabon, D.R. (2013) Red wolf (*Canis rufus*) recovery: a review with suggestions for future research. Animals 3: 722-744.

James, A.R.C. & Stuart-Smith, A.K. (2000) Distribution of caribou and wolves in relation to linear corridors. Journal of Wildlife Management 64: 154-159.

Johnson, D.H. (1980) The comparison of usage and availability measurements for evaluating resource preference. Ecology 61: 65-71.

Johnson, C.J., Nielsen, S.E., Merrill, E.H., McDonals, T.L. & Boyce, M.S. (2006) Resource selection functions based on use-availability data: theoretical motivation and evaluation methods. Journal of Wildlife Management 70: 347-357.

Kamler, J.F. and Gipson, P.S. (2000) Space and habitat use by resident and transient coyotes. Canadian Journal of Zoology 78: 2106-2111.

Kays, R., Gompper, M.E. & Ray, J.C. (2008) Landscape ecology of eastern coyotes based on large-scale estimates of abundance. Ecological Applications 18: 1014-1027.

Kays, R., Curtis, A. & Kirchman, J.J. (2010) Rapid adaptive evolution of northeastern coyotes via hybridization with wolves. Biology Letters 6: 89-93.

Koblmüller, S., Wayne, R.K. & Leonard, J.A. (2012) Impact of Quaternary climatic changes and interspecific competition on the demography history of a highly mobile generalist carnivore, the coyote. Biology Letters 8: 644-647.

Kranstauber, B., & Smolla, M. 2013. *Move: Visualizing and analyzing animal track data. R package version 1.1.387.*

LaBarberba, M. (1989) Analyzing body size as a factor in ecology and evolution. Annual Review of Ecology and Systematics 20: 97-117.

Larivière, S. & Crête, M. (1993) The size of eastern coyotes (*Canis latrans*): a comment. Journal of Mammalogy 74: 1072-1074.

Leopold, B.D. & Chamberlain, M.J. (2001) Carnivorous furbearers. *Wildlife of Southern Forests: Habitat and Management* (ed J.G. Dickson), pp. 248-277. Hancock House Publishers, Blaine, Washington, USA.

Levy, S. (2012) Rise of the coyote: the new top dog. Nature 485: 296-297.

Lewis, T. (2013) *Divided Highways: Building the Interstate Highways, Transforming American Life*. Cornell University Press, Ithaca, New York, USA.

Nowak, R.M. (2002) The original status of wolves in eastern North America. Southeastern Naturalist 1: 95-130.

Manly, B.F., McDonald, L.L., Thomas, D.L., McDonald, T.L., & Erickson, W.P. (2002) *Resource selection by animals: statistical analysis and design for field studies*. 2nd edn. Kluwer Academic, Boston, Massachusetts, USA.

McCarley, H. (1962) The taxonomic status of wild Canis (Canidae) in the south central United States. Southwestern Naturalist 7: 227-235.

McGarigal, K., Cushman, S.A. & Ene, E. (2012). FRAGSTATS v4: spatial pattern analysis program for categorical and continuous maps. University of Massachusetts, Amherst, Massachusetts, USA. http://www.umass.edu/landeco/research/fragstats/fragstats.html

McKerrow, A.J., Williams, S.G., & Collazo, S.G. (2006) *The North Carolina Gap Analysis Project: Final report*. North Carolina Cooperative Fish and Wildlife Research Unit, North Carolina State University, Raleigh, North Carolina, USA.

McNab, B.K. (1963) Bioenergetics and the determination of home range size. American Naturalist 97: 133-140.

Parker, G.R. (1995) *Eastern coyote*. Nimbus Publishing, Halifax, Canada.

R Core Team. (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://ww.R-project.org/

Rabon, D.R., Bartel, R. & Beyer, A. (2013) *Red Wolf Adaptive Management Plan FY13-FY15*. U.S. Fish and Wildlife Service, Manteo, North Carolina, USA.

Richer, M., Crête, M. & Ouellet, J. (2002) The low performance of forest versus rural coyotes in northeastern North America: inequality between presence and availability of prey. Ecoscience 9: 44-54.

Seidler, R.G. & Gese, E.M. (2012) Territory fidelity, space use, and survival rates of wild coyotes following surgical sterilization. Journal of Ethology 30: 345-354.

Swihart, R.K., Slade, N.A. & Bergstrom, B.J. (1988) Relating body size to the rate of home range use in mammals. Ecology 69: 393-399.

Thurber, J.M. & Peterson, R.O. (1991) Changes in body size associated with range expansion in the coyote (*Canis latrans*). Journal of Mammalogy 72: 750-755.

Tigas, L.A., Van Vuren, D.H. & Sauvajot, R.M. (2002) Behavioral responses of bobcats and coyotes to habitat fragmentation and corridors in an urban environment. Biological Conservation 108: 299-306.

Tremblay, J., Crête, M. & Huot, J. (1998) Summer foraging behavior of eastern coyotes in rural versus forest landscape: a possible mechanism of source-sink dynamics. Ecoscience 5: 172-182.

Van Deelen, T.R. & Gosselink, T.E. (2006) Coyote survival in a row-crop agricultural landscape. Canadian Journal of Zoology 84: 1630-1636.

Van Valkenburgh, B. (1999) Major patterns in the history of carnivorous mammals. Annual Review of Earth and Planetary Sciences 27: 463-493.

Way, J.G., Ortega, I.M. & Strauss, E.G. (2004) Movement and activity patterns of eastern coyotes in a coastal, suburban environment. Northeastern Naturalist 11: 237-254.

Way, J.G. (2007) A comparison of body mass of *Canis latrans* (coyotes) between eastern and western North America. Northeastern Naturalist 14: 111-124.

Way, J.G. (2009) Observations of coywolves, *Canis latrans* x *lycaon*, crossing bridges and using human structures on Cape Cod, Massachusetts.

Windberg, L.A. & Knowlton, F.F. (1988) Management implications of coyote spacing patterns in southern Texas. Journal of Wildlife Management 52: 632-640.

Wheeldon, T., Patterson, B. & White, B. (2010) Colonization history and ancestry of northeastern coyotes. Biology Letters 6: 246-247.

Whittington, J., St. Clair, C.C. & Mercer, G. (2005) Spatial responses of wolves to roads and trails in mountain valleys. Ecological Applications 15: 543-553.

Table 5.1. Mean body weight, age, and space use of resident and transient coyotes in northeastern North Carolina during 2009-2011.

| Coyote Status | Mean Weight (kg) | Mean Age (yr) | Size of Area Used (km²) | | | | | |
| | | | Growing | | Harvest | | Composite | |
			95%	50%	95%	50%	95%	50%
Resident	14.7 ± 0.4	2.7 ± 0.2	24.1 ± 2.3	4.0 ± 0.5	25.0 ± 2.8	4.0 ± 0.4	27.2 ± 2.0	4.2 ± 0.4
Transient	12.3 ± 0.7	1.6 ± 0.5	212.5 ± 58.0	11.6 ± 4.1	296.9 ± 55.0	21.7 ± 3.9	307.9 ± 44.9	20.6 ± 3.2

Table 5.2. Statistics of the best models selected from the generalized linear model (GLM) analysis for 2nd-order and 3rd-order resource selection of resident coyotes in northeastern North Carolina during 2009-2011.

Season	Order	Model	k	AIC_c	w_i	CV^7
All	2nd	Ag[1], CPB[2], Pine[3], Road[4], PR[5]	6	105853.45	0.62	79.9
Growing (Day)	3rd	Ag, Pine, Road, ED[6], PR	6	31488.75	0.31	77.5
Growing (Night)	3rd	Ag, CPB, Pine, Road	5	25619.80	0.44	78.1
Harvest (Day)	3rd	Global	7	17983.49	0.61	77.4
Harvest (Night)	3rd	Global	7	21470.41	0.75	79.2

[1]Agricultural fields
[2]Coastal bottomland forests
[3]Pine forests
[4]Roads
[5]Patch richness
[6]Edge density
[7]Estimated accuracy of 10-fold cross-validation

Table 5.3. Statistics of the best models selected from the generalized linear model (GLM) analysis for 2nd-order and 3rd-order resource selection of transient coyotes in northeastern North Carolina during 2009-2011.

Season	Order	Model	k	AIC_c	w_i	CV^1
All	2nd	Global[2]	7	33601.99	0.41	79.9
Growing (Day)	3rd	Ag[3], CPB[4], Pine[5], ED[6], PR[7]	6	10279.93	0.38	76.8
Growing (Night)	3rd	Ag, CPB, Pine, Road, PR	6	8426.62	0.54	77.4
Harvest (Day)	3rd	CPB, Pine, ED, PR	5	6918.13	0.49	77.2
Harvest (Night)	3rd	CPB, Pine, Road, ED, PR	6	9366.49	0.69	78.4

[1]Estimated accuracy of 10-fold cross-validation
[2]Global model
[3]Agricultural fields
[4]Coastal bottomland forests
[5]Pine forests
[6]Edge density
[7]Patch richness

Table 5.4. Effects of model averaged habitat selection parameter estimates for 2nd- and 3rd-order habitat selection for resident and transient coyotes in northeastern North Carolina during 2009-2011.

Model	Season	Photoperiod	Agriculture	Coastal Bottomland Forest	Pine Forest	Road	Edge Density	Patch Richness
2nd Order - Resident	All	All	+++[a]	---[a]	+++	++[b]	0[d]	+++
3rd Order - Resident	Growing	Day	+++	0	+++	---[b]	0	--
3rd Order - Resident	Growing	Night	+++	--	+++	+++	0	0
3rd Order - Resident	Harvest	Day	+++	+++	+++	---	-[c]	--
3rd Order - Resident	Harvest	Night	+++	+[c]	+++	+++	---	+++
2nd Order - Transient	All	All	+++	+	-	+++	+	+++
3rd Order - Transient	Growing	Day	+++	+	+++	0	-	+++
3rd Order - Transient	Growing	Night	+++	++	+++	+++	0	+++
3rd Order - Transient	Harvest	Day	0	---	---	0	++	+++
3rd Order - Transient	Harvest	Night	0	---	---	+++	+++	+++

[a] Significance level 0.0001
[b] Significance level 0.001
[c] Significance level 0.01
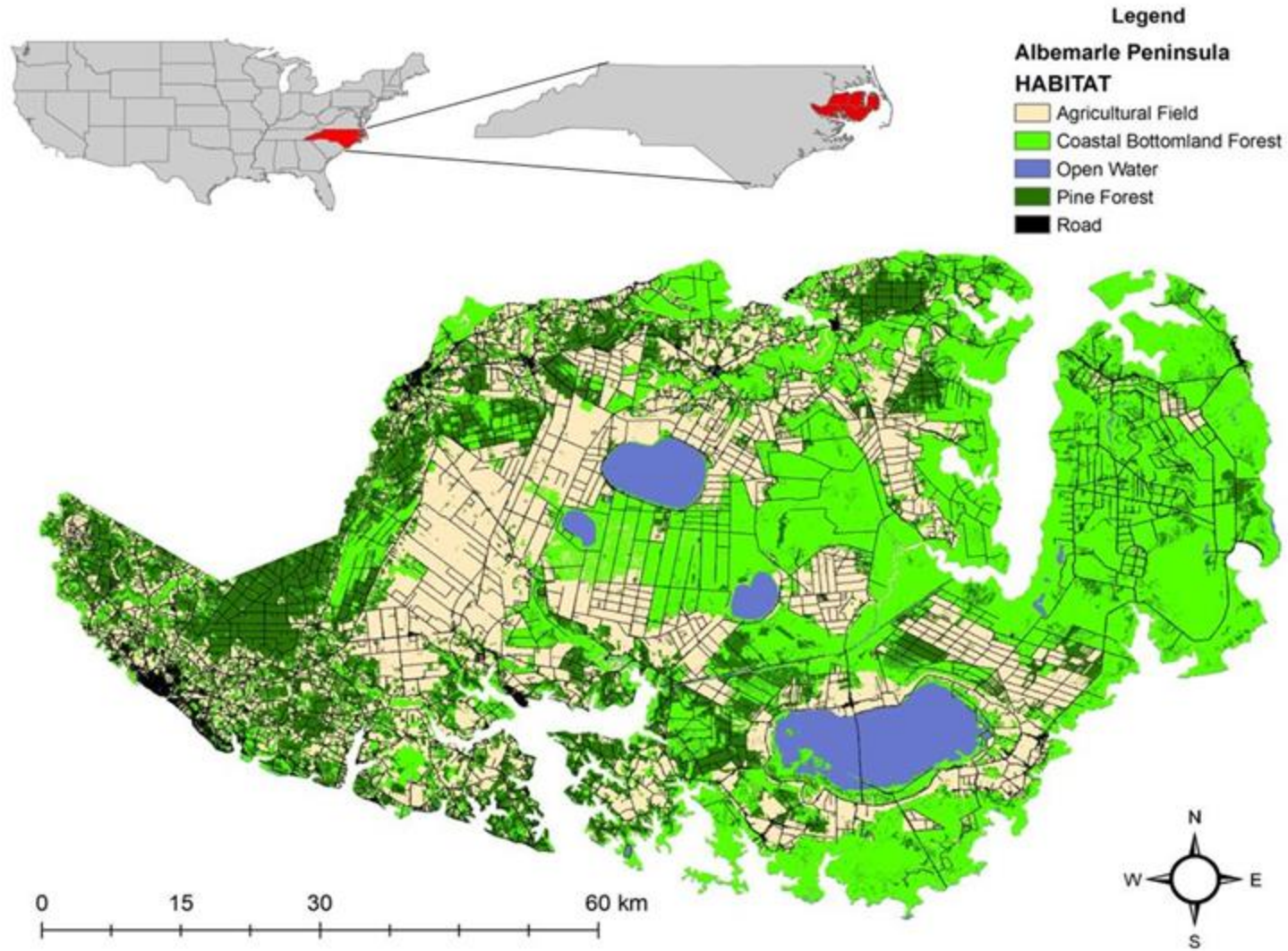[d] Significance level 0.1

Figure 5.1. Map of the Albemarle Peninsula of northeastern North Carolina with primary habitat types during 2009-2011.
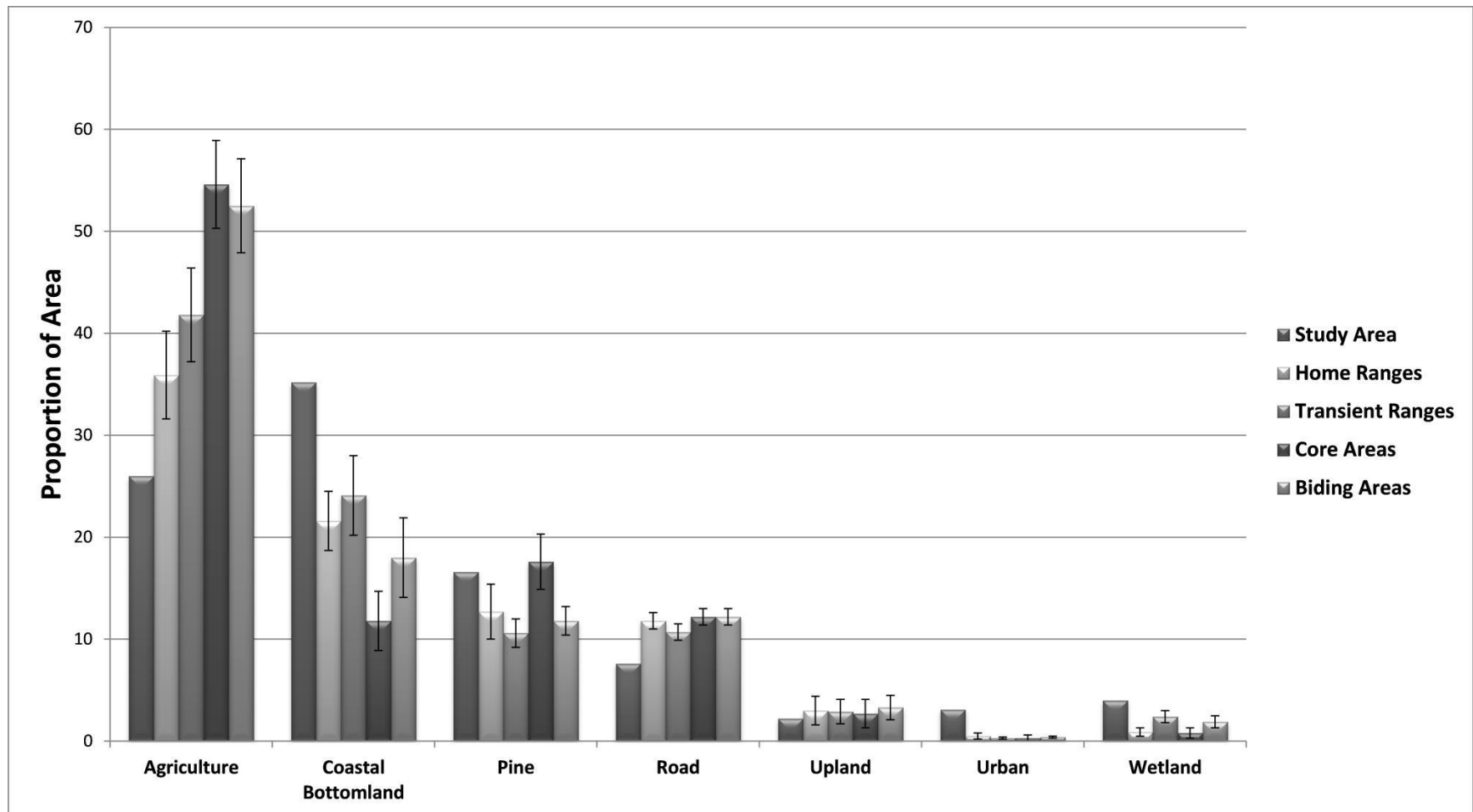
Figure 5.2. Habitat availability and habitat proportions of space used by resident and transient coyotes in northeastern North Carolina during 2009-2011.
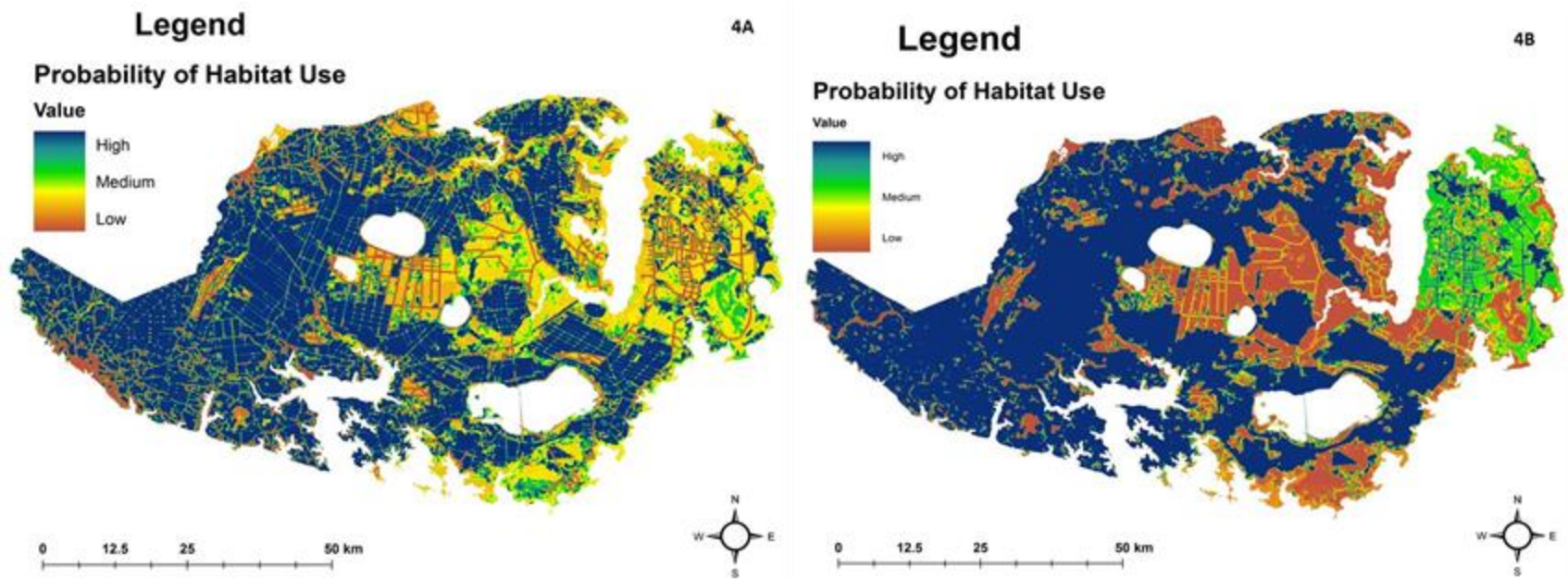
Figure 5.3. Proportional probability of 3rd-order habitat selection by residents during the growing season's diurnal (4A) and nocturnal (4B) hours for coyotes across the Albemarle Peninsula in northeastern North Carolina during 2009-2011.

Figure 5.4. Proportional probability of 3rd-order habitat selection by residents during the harvest season's diurnal (5A) and nocturnal (5B) hours for coyotes across the Albemarle Peninsula in northeastern North Carolina during 2009-2011.
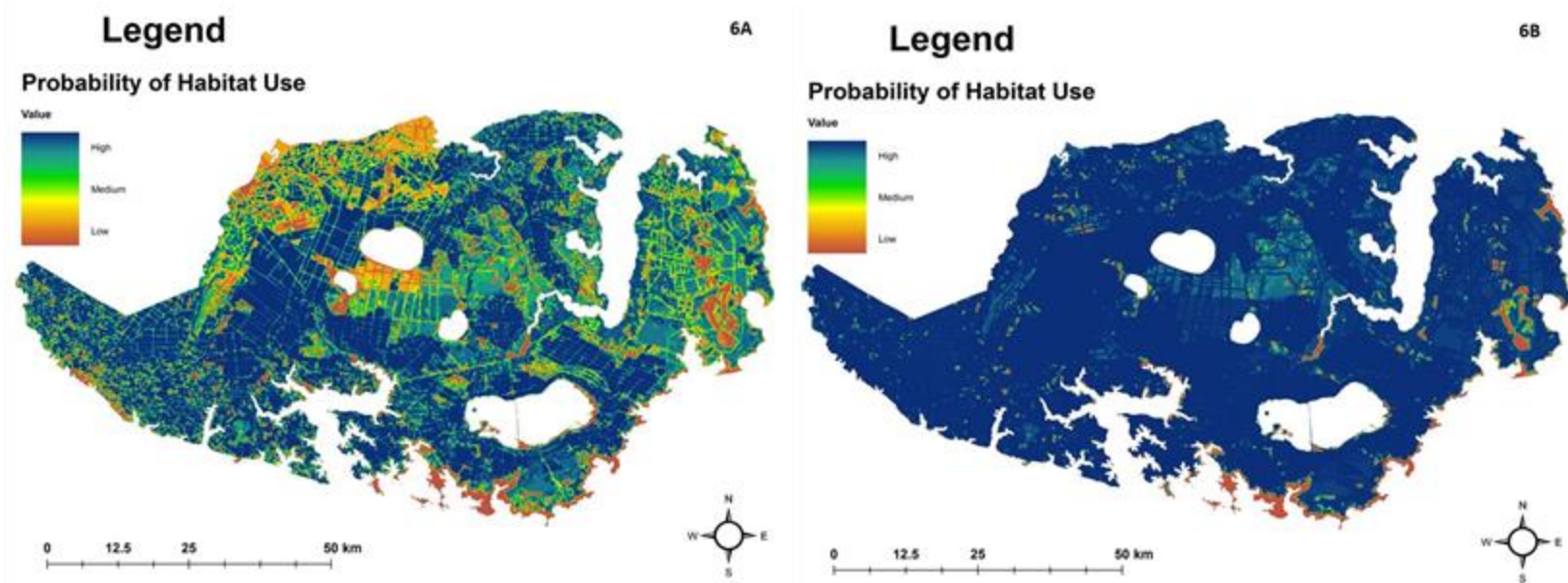
Figure 5.5. Proportional probability of 3rd-order habitat selection by transients during the growing season's diurnal (6A) and nocturnal (6B) hours for coyotes across the Albemarle Peninsula in northeastern North Carolina during 2009-2011.
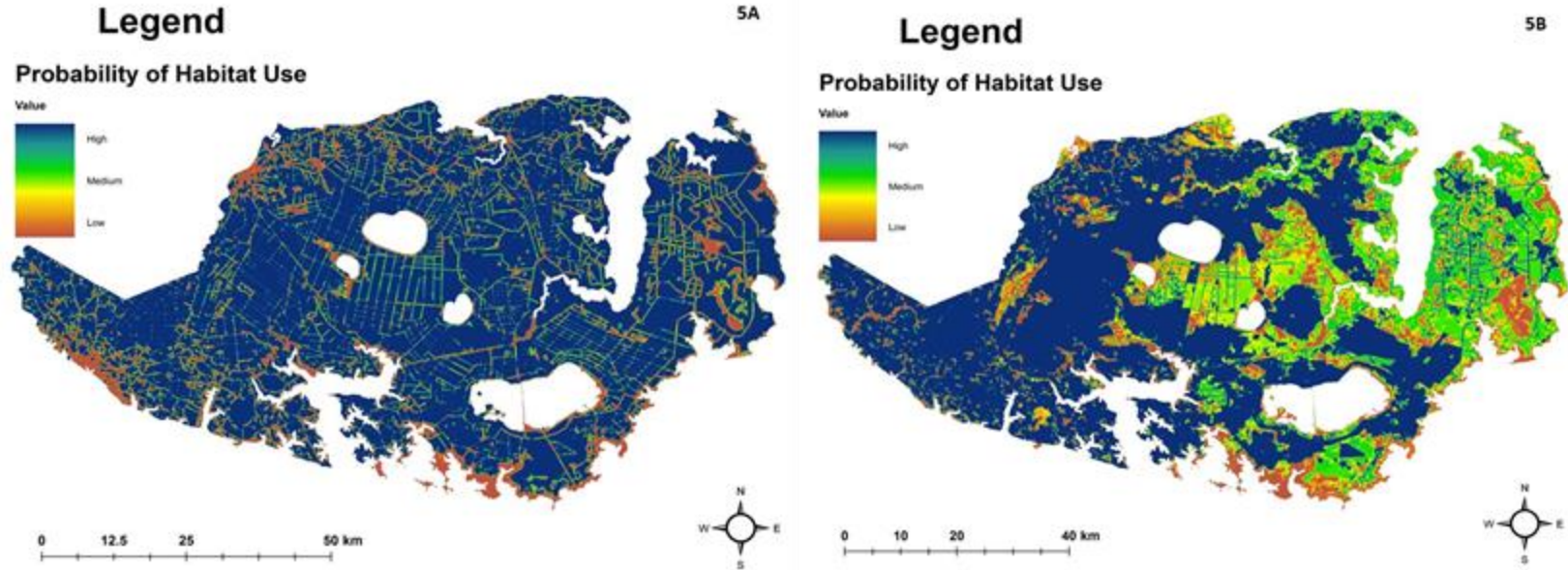
Figure 5.6. Proportional probability of 3rd-order habitat selection by transients during the harvest season's diurnal (7A) and nocturnal (7B) hours for coyotes across the Albemarle Peninsula in northeastern North Carolina during 2009-2011.
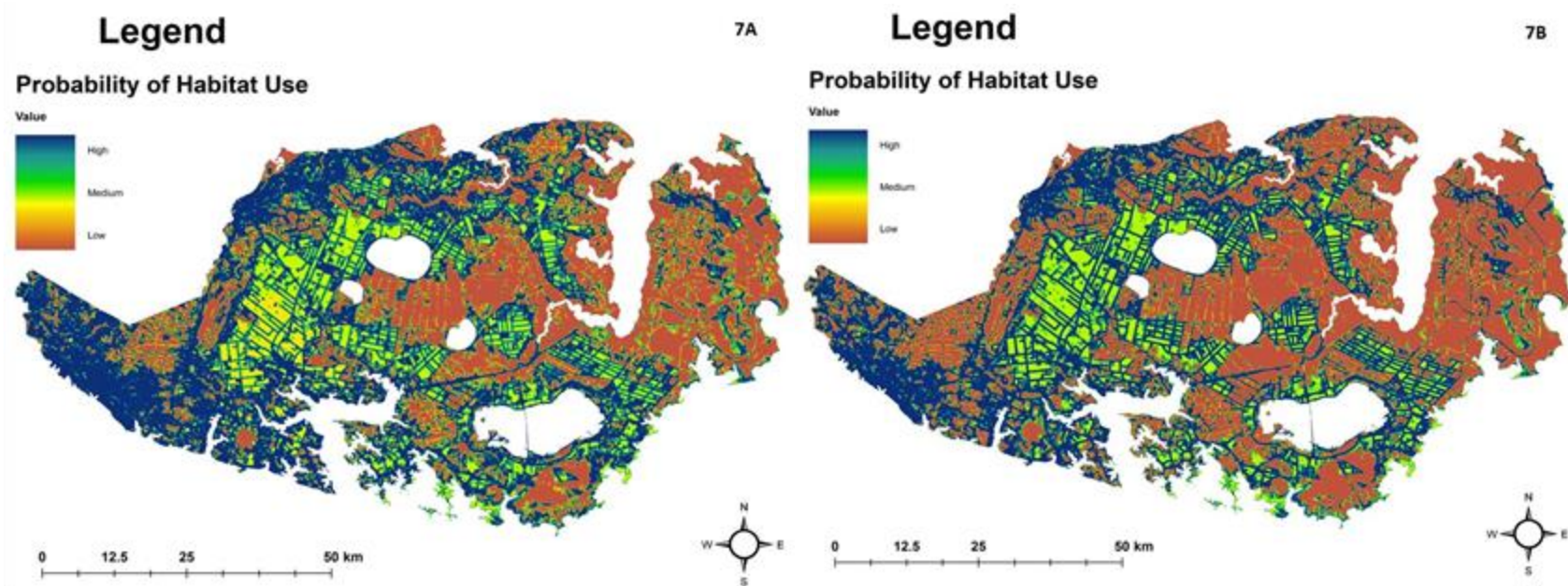
CHAPTER 6

SPACE USE AND HABITAT SELECTION BY RED WOLVES

Abstract

A general lack of knowledge regarding the ecology of our most imperiled carnivores continues to hamper recovery efforts. This is particularly true for red wolves because they were completely extirpated before any ecological profile was developed to assist recovery efforts. Therefore, reintroduction efforts were conducted with little-to-no knowledge of the species ecology. In this study, we radio-collared 34 red wolves in northeastern North Carolina to identify patterns of space use and habitat selection for resident and transient red wolves. Red wolf home range size scales positively with body weight and energetic requirements may influence variation in home range more than vegetation cover. Overall, red wolves prefer open, treeless agricultural fields over the forested habitats in the Recovery Area. Although transients display similar attraction to habitat types as residents do, resource-selection models are useful in defining differences in habitat selection between transients and resident red wolves. Specifically, transients are more likely to use forest cover and rely on road networks for travel. Reduced use of forest habitats and reliance on road networks indicates the importance of open habitats for red wolves. We suggest, prior to European settlement, fire regimes created by Native Americans and natural processes that maintained grasslands and open woodlands may have influenced red wolf abundance and distribution. We also suggest that the use of control burns to create early successional habitats and reduce forest understory may be beneficial to red wolf recovery via creating more suitable habitat.

Introduction

Carnivore reintroductions are extremely difficult and typically result in failure (Reading & Clark 1996; Breitenmoser *et al*. 2001; Gittleman and Gompper 2001). This is because the persistence of large carnivore populations is dependent on specific ecological requirements (i.e., expansive areas, access to large prey, and low mortality rates) that are difficult to achieve. Our general lack of knowledge regarding those requirements hinders conservation strategies because recovery efforts are conducted in the presence of factors responsible for global declines in large

carnivores (Breitenmoser *et al*. 2001; Hayward *et al.* 2007). As a result, recovery of large

carnivores remains a primary challenge to conservation biologists because area-size requirements,

negative public perception, and government-supported campaigns designed to protect livestock

and hunting industries continue to hamper recovery of large carnivores in the United States

(Kellert *et al*. 1996; Ripple *et al.* 2014). In particular, the conservation of red wolves (*Canis

rufus*) has been exceptionally difficult because of their vulnerability to extinction via human-

caused mortality and hybridization with coyotes (*Canis latrans*; Hinton *et al*.2013).

Indigenous to the United States, red wolves were completely extirpated from their

historic range by 1980 and later reintroduced into northeastern North Carolina in 1987 (USFWS

1989). Although the North Carolina reintroduction has been successful, a lesser well known

reintroduction attempt from 1991 until 1998 in Great Smoky Mountains National Park failed

because red wolves were unable to maintain territories within park boundaries and the population

suffered from low pup survivorship (Henry 1998). Similarly, red wolves in northeastern North

Carolina preferred agricultural habitats of private lands over forested habitats provided by federal

lands. To address this constraint, the Red Wolf Recovery Program (Recovery Program)

developed management strategies to include private lands and non-federal stakeholders that

allows for the expansion and management of the red wolf population on private, non-federal

lands (Rabon *et al*. 2013). As a result, red wolves persist in an agricultural-bottomland forest

landscape that is a mosaic of private and public lands. Recovery Program biologists are therefore

faced with potential issues of predicting how the red wolf population will organize itself on the

landscape as it increases, anticipate logistic and social constraints (i.e., conflicts with landowners

and hunters), and understanding red wolf-coyote interactions. Therefore, studying the size and

placement of red wolf home-ranges on the landscape will not only allow us to describe red wolf

space use and habitat selection, but it will also allow us to identify landscape characteristics that

are favorable to red wolf recovery. Additionally, space use patterns can provide information

regarding where red wolves forage, disperse, and interact with coyotes.

Initial research conducted on remnant red wolf populations failed to develop an ecological profile of the species because biologists were constrained by the necessity to locate small pockets of declining populations, develop techniques to distinguish red wolves from coyotes and red wolf/coyote hybrids, and to establish a captive breeding program (McCarely 1962; Shaw 1975; Carley 2000). Although the Recovery Program has monitored and maintained a wild population in northeastern North Carolina for over 2 decades, a general lack of ecological knowledge still hinders developing practical approaches to address issues of hybridization, inbreeding, and stunted demographic trends (Hinton *et al*. 2013). Understanding patterns of red wolf space use and habitat selection is a first step towards identifying key processes responsible for red wolf persistence. Previous studies of red wolf space use and habitat selection reported home ranges that varied between 10-150 km^2 (Phillips *et al*. 2003; Chadwick *et al*. 2010) with strong preferences for agricultural habitats (Chadwick *et al.* 2010; Hinton & Chamberlain 2010; Dellinger e*t al*. 2013); however, these studies lacked testable hypotheses to explain variation observed in red wolf spatial ecology. For instance, home-range size is widely measured to study the relationship between the distribution of animals and their ecological resources and consistent statistical patterns observed in home-range sizes of carnivores are largely influenced by body size (Harestad &Bunnell 1979; Gompper & Gittleman 1991). As a large carnivore (> 20 kg), body size is a critical factor influencing red wolf prey selection (Chapter 3) and it is likely to influence spacing patterns observed in red wolves. Furthermore, *Canis* species are known to rely on open habitats and linear corridors because their locomotor habits are favored in these habitats (Van Valkenburgh 1985; Andersson 2004; McKenzie *et al*. 2012). Aligning empirical data of space use within a larger theoretical framework is essential for identifying key traits and ecological requirements of red wolves that will assist in developing conservation strategies to overcome challenges to recovery efforts.

We monitored resident and transient red wolves fitted with global positioning system (GPS) radio-collars to assess red wolf spatial ecology in northeastern North Carolina. Here we

assess the ways in which red wolves interact and use several primary habitats. We also assess if body weight influences variation observed in red wolf home ranges. Although transient individuals are known to exist in gray wolf (*Canis lupus*) and coyote populations (Thurber & Peterson 1993; Berger & Gese 2007), their general relevance to *Canis* ecology are rarely understood and have never been investigated in red wolves. Transients are nomadic individuals that have not established residency and show little-to-no fidelity for one area (Berger & Gese 2007; Hinton *et al*. 2012). We examined space use and habitat selection patterns of transients and compared them to residents. Using our analyses, we extrapolate reasons for the red wolf's decline after European colonization of North America and offer suggestions for improving red wolf recovery.

Study Area

Our study was conducted on the Albemarle Peninsula in the northeastern region of North Carolina (Figure 1). The study area was approximately 6000 km^2 of federal, state, and private lands and is hereafter referred to as The Red Wolf Recovery Area (Recovery Area). The Recovery Area was comprised of a row-crop agricultural-bottomland matrix in which agricultural crops (i.e., corn, cotton, soybean, and winter wheat) made up approximately 30% of the vegetative cover (Figure 1). Managed pine (*Pinus* spp) comprised approximately 15% of the land cover. Other prominent land cover types were coastal bottomland forests and pocosin (35%), herbaceous wetlands and saltwater marshes (5%), open water (5%), and other minor land cover types (10%). Changes in elevation were minor (0-50m) and the climate was typical of the mid-Atlantic: 4 full seasons, nearly equal in length, with annual precipitation averaging between 122-132 cm. Summer climate was typically hot and humid with temperatures ranging from 27°C to over 38°C and winters were relatively cool with temperatures ranging between -4°C to 7°C.

Methods

We captured red wolves using padded foot-hold traps (Victor no.3 Softcatch, Lititz, Pennsylvania, USA) from October through May during 2007-2011. Red wolves were sexed,

135

measured, and weighed. Ages and genetic confirmation of captured red wolves were known if individuals were carrying a subcutaneous passive integrated transponder (PIT) tags inserted into pups during annual surveys of suspected red wolf dens (Beck *et al*. 2009; Rabon *et al.* 2013). Individuals without PIT tags were aged by tooth wear (Gier 1968) and a blood sample was taken.

We categorized wolves > 2 years old as adults, < 2 but > 1 year old as juveniles, and < 1 year old as pups. Prior to release at the original capture sites, we fitted red wolves with a mortality-sensitive GPS radio-collars (Lotek 4400S, Newmarket, Ontario, Canada) scheduled to record a location every 5 hours on a scheduled to rotate around the 24-hour clock throughout the year. Animal handling methods were approved by the Louisiana State University Agricultural Center Institutional Animal Care and Use Committee (Protocol Number AE2009-19).

To examine the anthropogenic effects of agriculture on the landscape, we divided year into 2 6-month seasons based on agricultural activity: planting/growing (1 March – 31 August) and harvest/fallow (1 September – 28 February). Locations were further separated into diurnal and nocturnal categories based on monthly photoperiods. We estimated space use of resident and transient red wolves by fitting dynamic Brownian bridge movement models (dBBMMs) to the time-specific location data to estimate the probability of use along the full movement track of each red wolf (Kranstauber & Smolla 2013) using the R package moveud (Collier 2013) in the R statistical environment (R Core Team 2013). We chose window sizes based on the temporal resolution of each track and our *a priori* assumptions of the time scale of major behavioral shifts (Byrne *et al*. 2014). For residents, 95% and 50% contour intervals were considered home ranges and core areas, respectively. For transients, 95% and 50% contour intervals were considered transient range and biding areas (Hinton *et al*. 2012), respectively. We used *t*-tests to examine changes in home range and space use sizes between seasons.

Predominant habitat types were estimated from a digitalized landscape map of vegetative communities developed by the North Carolina Gap Analysis Project (McKerrow *et al*. 2006). We collapsed the vegetative communities estimated by McKerrow *et al*. (2006) into 7 general habitat

types with 30m resolution. For the habitat selection analysis, we divided the landscape into agriculture, coastal bottomland forest, pine forest, open water, upland forest, urban, and wetland. Because roads may serve as corridors for red wolf movements (Dellinger *et al*. 2013), we included road as the 8th habitat type by superimposing a linear feature layer with a 30m buffer around roads onto the final habitat raster map with 30m resolution.

We used analysis of variances (ANOVAs) and *t*-tests to determine if the percentage of habitat composition of home ranges and transient ranges differed between each other and between seasons. We also developed generalized linear models (GLMs) to examine the effects of body weight and age, pack size, white-tailed deer abundance, and habitat composition on red wolf home-range size. We used county-level harvest data collected by the North Carolina Wildlife Resources Commission as measures of deer abundance in the study area. Although harvest reports do not reflect true white-tailed deer densities, hunter harvest/km^2 do provide a benchmark by which to judge the effect of white-tailed deer abundance on red wolf home-range size. Habitat composition of home ranges was calculated as the percentages of the 8 habitat types. We assessed models using a stepwise procedure by calculating Akaike's information criterion for small sample sizes (AIC$_c$) for each model to select which variables best explained home-range size.

We compared habitat selection of red wolves at 2 scales: population (2nd-order) and individual (3rd-order). Habitats were converted into continuous variables by quantifying percent of habitat cover for each 30m cell using a moving window with a 150m radius in FRAGSTATS (McGarigal *et al*. 2012). We also quantified edge density and patch richness across the study area because we suspected red wolves of foraging along edges. Information from raster maps were extracted to red wolf locations and home ranges using ArcGIS 10.1 (Environmental Systems Research Institute Inc., Redlands, California). We used resource-selection functions (RSFs) to examine the effect of habitat type on where red wolves established home ranges on the landscape (2nd-order selection of resources; Johnson 1980) and examine the effect of habitat type on how they use their home ranges (3rd-order selection of resources; Johnson 1980) following Manly *et*

al. (2002). We assumed seasonal and photoperiod variation in habitat use by residents and transients and, therefore, developed RSFs for residents and transients by season and photoperiod for 3rd-order selection. We used a binomial approach to estimate resource-selection functions by comparing characteristics of known (used) locations to random (available) locations (Manly *et al*. 2002). We used logistic regression and AIC_c to form RSFs that identified habitats important to individual red wolves and evaluated the relative importance of coefficients associated with the habitat types by examining Akaike weights (w_i) after adding each of these covariates to a core model set (e.g., higher w_i and lower AIC_c suggested model improvement; Burnham and Anderson 2002). We also evaluated the predictive performances of the most parsimonious core models using the *k*-fold cross validation method (Boyce et al. 2002; Johnson et al. 2006). The cross-validation method performs *k* iterations of training and validation in which a different fold of the data is held out for validation while remaining $k - 1$ folds are used for learning. For cross-validation, we used 10 folds ($k = 10$) to estimate performance of RSF models.

Results

During 2007-2011, we monitored 34 red wolves fitted with GPS radio-collars. Of those red wolves, 30 (88%) were residents and 11 (32%) were transients with 7 (63%) of the residents being transients prior to establishing home ranges. Mean weight and age of red wolves monitored were 27.1 kg ± 0.4 and 3.1 yrs ± 0.2, respectively, in which weights and age of residents did not differ from transients (Table 1; $t_{43} \leq 1.41$, $P \geq 0.017$). Home ranges and core areas of residents did not differ between seasons (Table 1; $t_{65} \leq 0.46$, $P \geq 0.657$) and home ranges ranged from 25.4 km² to 183.0 km². We also detected no seasonal difference of transient ranges and biding areas (Table 1; $t_{12} \leq 0.16$, $P \geq 0.878$) and transient areas ranged from 125.1 km² to 709.9 km². Home-range size scaled positively with body weight (Figure 2; r^2=0.37, $P = 0.002$), occurred in the top 5 models (Table 2), and best explained the variation observed in red wolf home-range size.

Home ranges and core areas were comprised mostly of agricultural fields, coastal bottomland and pine forest, and roads (Figure 3; $F_{6, 231} = 111.98$, $P < 0.001$). Pine and upland

forest, urban, and road composition of home ranges and core areas did not differ ($t_{66} \leq 1.78$, $P \geq$ 0.084) but home ranges typically consisted of more coastal bottomland forest and wetland habitat and less agricultural habitat than did core areas ($t_{66} \geq 2.31$, $P \leq 0.024$). Similarly, transient ranges and biding areas comprised mostly of agricultural fields, coastal bottomland and pine forests, and roads (Figure 3; $F_{6, 70} = 25.15$, $P < 0.001$). With the exception of roads ($t_{22} \leq 3.29$, $P = 0.004$), we found no differences in habitat composition between transient ranges and biding areas ($t_{22} \leq 1.40$, $P \geq 0.176$). Home ranges consisted of more roads and less wetland habitat than transient ranges ($t_{43} \geq 2.11$, $P \leq 0.043$), whereas all other habitat types were similar ($t_{43} \leq 1.64$, $P \geq 0.109$). Core areas consisted of more agricultural habitat and less coastal bottomland forest and wetland habitats than biding areas ($t_{43} \geq 2.04$, $P \leq 0.047$), whereas all other habitat types were similar ($t_{43} \leq 0.55$, $P \geq 0.586$). We detected no seasonal differences between habitat types within home ranges ($t_{65} \leq 1.21$, $P \geq 0.230$). Core areas consisted of more agricultural habitat and less coastal bottomland forest and wetland habitat during the growing season ($t_{65} \geq 2.21$, $P \leq 0.031$), whereas all other habitat types did not differ between seasons ($t_{65} \leq 1.17$, $P \geq 0.268$). We detected no seasonal differences between habitat types within transient ranges and biding areas ($t_{12} \leq 1.39$, $P \geq 0.188$).

We excluded wetland, upland forest, and urban habitats from our models because their occurrence in home ranges and transient ranges was little. Therefore, we only included edge density, agriculture, pine forest, coastal bottomland forest, and road habitats in our model. All 5 covariates were retained in the top-ranked model for 2nd-order selection by residents (Table 3), whereas only 1 covariate (edge density) was not retained for transients (Table 4). Agriculture and pine forest habitats were retained in all top-ranked models for 3rd-order selection by residents (Table 3). Residents appeared to favor agricultural fields and pine forests during all seasons and photoperiods (Figures 4 & 5; Table 5). Agriculture and road habitats were retained in all top-ranked models for 3rd-order selection by transients (Table 4). Coastal bottomland and pine forests

were used by transients more during the harvest season than growing season, whereas agriculture and road habitats were preferred during both seasons and photoperiods (Figures 6 & 7; Table 5).

Discussion

In general, measurements of home ranges have proven useful in understanding spacing patterns of carnivore populations and comparative studies have demonstrated that carnivore home-range size scales positively with body size (Gittleman and Harvey 1982; Gompper and Gittleman 1991). Given the red wolf's intermediate body size between gray wolves and coyotes, it's not surprising that our mean home-range sizes fall between those estimated for gray wolves (see Table 6.3 in Fuller *et al*. 2003) and coyotes (see Table 22.4 in Bekoff & Gese 2003) in North America. Our findings appear similar to those observed in comparative studies of home ranges, and suggest that body weight is a primary driver of red wolf space use with larger individuals generally maintaining larger home ranges than smaller individuals. Results of our simple model, using red wolf body weight, pack size, white-tailed deer density, and % agricultural cover , indicated that body weight alone best explained variation in home-range size. This is not surprising because we should expect the energetic requirements of red wolves and the distribution of their food resources to influence the size of home ranges. Although deviations from the regression slope can be attributed to differences in diet, we found no effect of white-tailed deer density on red wolf home-range size. We suspect that our density estimate was too crude and, because white-tailed deer only comprise approximately 40-50% of red wolf diet (Dellinger *et al*. 2011; McVey *et al*. 2012; Chapter 4), a measurement of overall prey productivity might better serve as a variable for prey.

Home-range sizes of red wolves did not vary between seasons despite the loss of agricultural cover resulting from crop harvest during the fall. The size and placement red wolf home-ranges are likely driven by energetic demands and home ranges are large enough to handle seasonal changes in food resources. Although most red wolf home-ranges (50%) fell within the 45-80 km^2 range, 25% of the red wolves maintained home ranges > 85 km^2 and < 185 km^2. Long-

term maintenance of home ranges requires red wolves to defend a finite area and consume resources at a rate low enough to allow prey populations to persist. Therefore, we suggest that 175 km^2 may be an upper limit in which red wolves can efficiently maintain and defend a home range due to body size constraints.

The general compositional pattern in red wolf home ranges were high percentages of agricultural fields in the interior (i.e., core areas) with forest edges increasing in the outer areas. Red wolf home ranges typically had more coastal bottomland forest habitat than pine forest, but this was a result of most pine plantations residing in the western portion of the Recovery Area where red wolf packs are the fewest. Similar to behaviors observed by coyotes in the Recovery Area (Chapter 5), red wolves typically loafed in forest habitats within 50-300m of edges adjacent to agricultural fields during the harvest season. As crop cover reaches heights of approximately 0.5 meters during the growing season, red wolves abandon early successional and forest habitats to loaf under crop cover. Red wolf packs typically abandon their dens and day beds in early successional and forested habitats 4-6 weeks after whelping pups to center their activities, including pup rearing, in corn fields (Hinton & Chamberlain 2010).

Transient individuals lack mates because of their nomadic behaviors and do not contribute reproductively to the population. Despite this, transiency may be an important life history trait for red wolves because they facilitate metapopulation dynamics (Hanski 1998), replace residents upon death, and may allow populations to reconstruct themselves after suffering drastic mortality event. Transient red wolves displayed localized movements similar to those observed by coyotes in northeastern North Carolina (Hinton et al. 2012). These movements were analogous to home ranges and we referred to them as biding areas (Hinton et al. 2012). Seven of the 11 (63%) transients established residency near their biding areas. We suggest this behavior allows red wolves to assess resource availability and become familiar with areas before establishing residency.

Overall, red wolves prefer the open, treeless agricultural fields over the forested habitats in the Recovery Area. For instance, 52% of approximately 55,000 GPS locations recorded were in agricultural fields, whereas 30% were in forested habitats. Although transients displayed similar attraction to habitat types as residents did, resource-selection models were useful in identifying differences in habitat selection between transients and resident red wolves. Models of 2nd-order selection indicated that agricultural fields, forested habitats, and roads were important to transients and residents; however, transients relied more on roads than did residents. Increased use of roads by transients indicates that they likely travel more and forage less than residents and roads reduce energetic costs of movements. Models of 3rd-order selection indicated that residents and transients had temporal changes in habitat use. During the growing season, residents loafed in agricultural fields near roads during the day but included some moderate use of forested habitats adjacent to roads at night, whereas transients decreased their use of forested habitats at night to increase their use of roads and edges in agricultural fields. During the harvest season, residents used agricultural fields and forested habitats near roads and decreased their use of roads and forested habitats at night to forage in the agricultural fields. Similarly, transients used agricultural fields and forest habitat during the day, but relied mostly on roads and agricultural fields during the night. Despite low use of forested habitats, red wolves do benefit from the surrounding forests because those habitats provide security during the fall and winter when agricultural fields are barren after harvest. Similar to coyotes, red wolves take advantage of road networks and other linear features to reduce costs associated with travel and increase foraging opportunities. In fact, red wolf use of forested habitats is mostly associated with the use of roads (Dellinger *et al*. 2013).

The red wolf's low-to-moderate use of forested habitats and preference for agricultural fields indicates that red wolves most likely occupied grassland and early successional habitats preying mostly on white-tailed deer and rabbits (*Sylvilagus* spp.) prior to European settlement. Forested habitats may have offered marginal habitat that was used by dispersing and transient individuals to maintain metapopulation dynamics. Prior to European settlement, fire was

142

widespread and frequent throughout the eastern United States in which Native Americans used fire for forest clearance and maintenance to promote growth of hard mast trees and browse for game species (Delcourt & Delcourt 2004; Nowacki & Abrams 2008). Red wolves likely exploited the mosaic of grasslands, savannas, and open woodlands maintained by natural and Native American fire-regimes. As Europeans colonized the eastern United States, Native American populations were greatly reduced and land-use practices shifted to an agricultural-grazing system. This system created significant shifts in vegetation composition and structure from grasslands, savannas, and open woodlands to closed-canopy forests with dense understories (Nowacki & Abrams 2008) that would have disrupted red wolf metapopulaton dynamics by impeding movements and creating isolated pockets of red wolf populations. Therefore, we suggest that red wolf populations first became isolated through the conversion of open habitats to closed-canopy forests. The extirpation of white-tailed deer likely lowered red wolf recruitment while increased conflict with European and American settlers disrupted pack dynamics and increased mortality rates. Together these factors eventually led to the complete extirpation of red wolves by the mid-20th century. As coyotes began colonizing the eastern United States, hybridization became a primary conservation challenge to red wolf recovery (Kelly *et al*. 1999; Hinton *et al*. 2013).

Nowak (2002) noted that the modern red wolf morphology still shows continuity with fossilized remains of small wolves in the eastern United States dating back to the Pleistocene. Therefore, the morphology of modern red wolves may not have been significantly altered from modern hybridization with coyotes. Results of this study indicate that body size is a primary trait influencing red wolf space use and it is likely that modern red wolves maintain a body size adapted to a vastly more open landscape that existed prior to European settlement of North America. Therefore, it is likely that red wolf populations will require extensive areas of open, early successional habitat with high prey productivity to support red wolf packs and facilitate the movement of dispersers and transients. Currently, red wolves use approximately 50-60% of the Recovery Area and current management strategies may need to use controlled burns to create

early successional habitat and reduce forest understory, thereby increasing the amount of suitable forest habitat available to red wolves. Understanding how red wolf populations structure themselves on the landscape is critical for making accurate inferences and promoting processes and conditions that will not only allow for the gradual expansion of red wolf populations, but permit red wolves to gradually adapt to modern landscapes.

Acknowledgements

Literature Cited

Andersson, K. (2004). Elbow-joint morphology as a guide to forearm function and foraging behavior in mammalian carnivores. Zoological Journal of the Linnean Society 142: 91-104.

Beck, K.B., Lucash, C.F. & Stoskopf, M.K. (2009). Lack of impact of den interference on neonatal red wolves. Southeastern Naturalist 8: 631-638.

Bekoff, M. & Gese, E.M. (2003) Coyote (*Canis latrans*). In *Wild mammals of North America: biology, management, and conservation*: 467-481. Feldhamer, G.A., Thompson, B.C., Chapman, J.A. (Eds.). Baltimore: John Hopkins University Press.

Berger, K.M. & Gese, E.M. (2007). Does interference competition with wolves limit the distribution and abundance of coyotes? Journal of Animal Ecology 76: 1075-1085.

Breitenmoser, U., Breitenmoser-Würsten, C., Carbyn, L.N. & Funk, S.M. (2001). Assessment of carnivore reintroductions. In *Carnivore conservation*: 242-281. Gittleman, J.L., Funk, S.M., Macdonald, D., Wayne, R.K. (Eds.). Cambridge: Cambridge University Press.

Burnham, K.P. & Anderson, D.R. (2002). Model selection and multimodel inference: a practical information theoretic approach. 2nd ed. New York: Springer-Verlag.

Byrne, M., McCoy, J., Hinton, J., Chamberlain, J. & Collier, B. (2014). Using dynamic Brownian bridge movement modeling to measure temporal patterns of habitat selection. *J. Anim. Ecol.* In press.

Carley, C.J. (2000). The Red Wolf (*Canis rufus*) Wolf Recovery Program: things they didn't tell me in school. In *Reflections of a naturalist: papers honoring professor Eugene D. Fleharty*: 125-141. Choate, J.R. (Ed.). Hays: Fort Hays State University

Chadwick, J., Fazio, B. & Karlin, M. (2010) Effectiveness of GPS-based telemetry to determine temporal changes in habitat use and home-range sizes of red wolves. Southeastern Naturalist 9: 303-316.

Collier, B. (2013) Moveud: Creates dynamic Brownian bridge movement model individual time-step utilization distributions. R package version 1.0.

Delcourt, P.A. & Delcourt, H.R. (2004). Prehistoric Native Americans and ecological change: human ecosystems in Eastern North America since the Pleistocene. Cambridge: Cambridge University Press.

Dellinger, J.A., Proctor, C., Steury, T.D., Kelly, M.J. & Vaughan, M.R. (2013) Habitat selection of a large carnivore, the red wolf, in a human-altered landscape. Biological Conservation 157: 324-330.

Fuller, T.K., Mech, L.D. & Cochrane, J.F. (2003). Wolf population dynamics. In *Wolves: behavior, ecology, and conservation*: 272-288. Mech, L.D., Boitani, L. (Eds.). Chicago: University of Chicago Press.

Gese, E.M., Rongstad, O.J. & Mytton, W.R. (1988) Home range and habitat use of coyotes in southeastern Colorado. Journal of Wildlife Management 52: 640-646.

Gier, H.T. (1968). Coyotes in Kansas. Kansas State University Agricultural Experiment Station Bulletin 393: 1-118.

Gittleman, J.L. & Harvey, P.H. (1982). Carnivore home-range size, metabolic needs and ecology. Behavioral Ecology and Sociobiology 10: 57-63.

Gompper, M.E. & Gittleman, J.L. (1991) Home range scaling: intraspecific and comparative trends. Oecologia 87: 343-348.

Hanski, I. (1998). Metapopulation dynamics. Nature 396: 41-49.

Harestad, A.S. & Bunnel, F.L. (1979). Home range and body weight – a reevaluation. Ecology 60: 389-402.

Hayward, M.W., Adendorff, J., O'Brien, J., Sholto-Douglas, A., Bissett, C., Moolman, L.C., Bean, P., Fogarty, A., Howarth, D., Slater, R. & Kerley, G.I.H. (2007). The reintroduction of large carnivores to the Eastern Cape, South Africa: an assessment. Oryx 41: 205-214.

Henry, V.G. (1998). Notice of termination of the red wolf reintroduction project in the Great

    Smoky Mountains National Park. Federal Register 63: 54152-54153.

Hinton, J.W. & Chamberlain, M.J. (2010) Space and habitat use by a red wolf pack and their pups

    during pup-rearing. Journal of Wildlife Management 74: 55-58.

Hinton, J.W., Chamberlain, M.J. & van Manen, F.T. (2012) Long-distance movements of

    transient coyotes in eastern North Carolina. American Midland Naturalist 168: 281-288.

Hinton, J.W., Chamberlain, M.J. & Rabon, D.R. (2013). Red wolf (*Canis rufus*) recovery: A

    review with suggestions for future research. Animals 3: 722-744.

Johnson, D.H. (1980) The comparison of usage and availability measurements for evaluating

    resource preference. Ecology 61: 65-71.

Johnson, C.J., Nielsen, S.E., Merrill, E.H., McDonals, T.L. & Boyce, M.S. (2006) Resource

    selection functions based on use-availability data: theoretical motivation and evaluation

    methods. Journal of Wildlife Management 70: 347-357.

Kamler, J.F. & Gipson, P.S. (2000) Space and habitat use by resident and transient coyotes. *Can.

    J. Zool.* **78**, 2106-2111.

Kellert, S.R., Black, M., Rush, C.R. & Bath, A.J. (1996). Human culture and large carnivore

    conservation in North America. *Conserv. Biol.* **10**, 977-990.

Kranstauber, B., & Smolla, M. (2013). *Move: Visualizing and analyzing animal track data. R*

    package version 1.1.387.

Manly, B.F., McDonald, L.L., Thomas, D.L., McDonald, T.L., & Erickson, W.P. (2002)

    Resource selection by animals: statistical analysis and design for field studies. 2nd edn.

    Boston: Kluwer Academic.

McCarley, H. (1962). The taxonomic status of wild *Canis* (Canidae) in the south central United

    States. Southwestern Naturalist 7: 227-235.

McGarigal, K., Cushman, S.A. & Ene, E. (2012). FRAGSTATS v4: spatial pattern analysis

program for categorical and continuous maps. University of Massachusetts, Amherst,

Massachusetts, USA. http://www.umass.edu/landeco/research/fragstats/fragstats.html

McKenzie, H.W., Merrill, E.V., Spiteri, R.J. & Lewis, M.A. (2012). How linear features alter

predator movement and the functional response. Interface Focus 2: 205-216.

McKerrow, A.J., Williams, S.G., & Collazo, S.G. (2006) *The North Carolina Gap Analysis*

Project: Final report. North Carolina Cooperative Fish and Wildlife Research Unit,

North Carolina State University, Raleigh, North Carolina, USA.

McVey, J.M., Cobb, D.T., Powell, R.A., Stoskopf, M.K., Bohling, J.H., Waits, L.P. & Moorman,

C.E. (2013). Diets of sympatric red wolves and coyotes in northeastern North Carolina.

Journal of Mammalogy 94: 1141-1148.

Nowak, R.M. (2002). The original status of wolves in eastern North America. Southeastern

Naturalist 1: 95-130.

Nowacki, G.J. & Abrams, M.D. (2008). The demise of fire and "mesophication" of forests in the

eastern United States. BioScience 58: 123-138.

Phillips, M.K., Henry, V.G. & Kelly, B.T. (2003). Restoration of the red wolf. In *Wolves:*

behavior, ecology, and conservation: 272-288. Mech, L.D., Boitani, L. (Eds.). Chicago:

University of Chicago Press.

R Development Core Team. 2012. R: a language and environment for statistical computing. R

Foundation for Statistical Computing. Vienna, Austria. Available online at http://www.R-

project.org.

Rabon, D.R., Bartel, R. & Beyer, A. (2013). Red wolf adaptive management plan FY13-FY15.

U.S. Fish and Wildlife Service.

Reading, R.P. & Clark, T.W. (1996). Carnivore reintroductions: an interdisciplinary examination.

In *Carnivore behavior, ecology, and evolution*: 296-336. Gittleman, J.L. (Ed.). Ithaca:

Cornell University Press.

Ripple, W.J., Estes, J.A., Beschta, R.L., Wilmers, C.C., Ritchie, E.G., Hebblewhite, M., Berger, J., Elmhagen, B., Letnic, M, Nelson, M.P., Schmitz, O.J., Smith, D.W., Wallach, A.D. & Wirsing, A.J. (2014). Status and ecological effects of the world's largest carnivores. Science 343: 1241484.

Shaw, J.H. (1975). Ecology, behavior, and systematics of the red wolf (*Canis rufus*). PhD Thesis, Yale University.

Thurber, J.M. & Peterson, R.O. (1993). Effects of population density and pack size on the foraging ecology of gray wolves. Journal of Mammalogy 74: 879-889.

U.S. Fish and Wildlife Service. (1989). Red wolf recovery plan. Atlanta: U.S. Fish and Wildlife Service.

Van Valkenburgh, B. (1985). Locomotor diversity within past and present guilds of large predatory mammals. Paleobiology 11: 406-428.

Table 6.1. Mean body weight, age, and space use of resident and transient red wolves in northeastern North Carolina during 2009-2011.

Coyote Status	Mean Weight (kg)	Mean Age (yr)	Size of Area Used (km²)					
			Growing		Harvest		Composite	
			95%	50%	95%	50%	95%	50%
Resident	27.2 ± 0.5	3.0 ± 0.2	73.3 ± 8.5	9.1 ± 1.4	67.8 ± 8.3	9.0 ± 1.6	68.4 ± 7.5	8.7 ± 1.3
Transient	26.8 ± 0.8	3.5 ± 0.4	277.9 ± 80.7	27.3 ± 14.5	260.7 ± 66.1	29.3 ± 8.6	319.2 ± 57.3	32.8 ± 10.8

Table 6.2. Results of the 5 best models for stepwise analysis of factors influencing home range size of red wolves in northeastern North Carolina during 2009-2011.

Model	k^a	$AIC_c{}^b$	$\Delta AIC_c{}^c$	$w_i{}^d$
Weight, pack size	4	253.52	0.00	0.33
Weight	3	253.72	0.20	0.30
Weight, agriculture	4	256.01	2.49	0.09
Weight, deer density	4	256.06	2.54	0.09
Weight, pack size, deer density	5	256.46	2.94	0.08

[a]Model parameters
[b]Akaike's Information Criterion
[c]Difference in AIC
[d]Akaike weight

Table 6.3. Statistics of the best models selected from the generalized linear model (GLM) analysis for 2nd-order and 3rd-order resource selection of resident red wolves in northeastern North Carolina during 2009-2011.

Season	Order	Model	k	AIC_c	w_i	CV^1
All	2nd	Global[2]	6	117708.4	1.00	80.6
Growing (Day)	3rd	Ag[3], Pine[4], Road, Edge[5]	5	41478.78	0.57	78.1
Growing (Night)	3rd	Global	6	26786.21	0.85	77.6
Harvest (Day)	3rd	Global	6	27084.75	0.67	76.7
Harvest (Night)	3rd	Ag, Edge, Pine	4	32234.26	0.34	76.4

[1]Estimated accuracy of 10-fold cross-validation
[2]Global model
[3]Agricultural fields
[4]Pine forests
[5]Edge density

Table 6.4. Statistics of the best models selected from the generalized linear model (GLM) analysis for 2nd-order and 3rd-order resource selection of transient red wolves in northeastern North Carolina during 2009-2011.

Season	Order	Model	k	AIC_c	w_i	CV^1
All	2nd	Ag[2], CPB[3], Pine[4], Roads	5	12948.76	0.59	78.2
Growing (Day)	3rd	Global[5]	6	3759.36	0.75	78.1
Growing (Night)	3rd	Ag, Edge[6], Roads	4	2808.94	0.51	78.5
Harvest (Day)	3rd	Global	6	3300.29	0.66	76.8
Harvest (Night)	3rd	Ag, CPB, Pine, Roads,	6	9366.49	0.57	77.0

[1]Estimated accuracy of 10-fold cross-validation
[2]Agricultural fields
[3]Coastal plains bottomland forests
[4]Pine forests
[5]Global model
[6]Edge density

Table 6.5. Effects of model averaged habitat selection parameter estimates for 2nd- and 3rd-order habitat selection for resident and transient red wolves in northeastern North Carolina during 2009-2011.

Model	Season	Photoperiod	Agriculture	Coastal Bottomland Forest	Pine Forest	Road	Edge Density
2nd Order - Resident	All	All	+++[a]	+++	+++	+++	---[a]
3rd Order - Resident	Growing	Day	+++	0[d]	+++	+++	+++
3rd Order - Resident	Growing	Night	+++	+[c]	+++	+++	--[b]
3rd Order - Resident	Harvest	Day	+++	+++	+++	+++	+
3rd Order - Resident	Harvest	Night	+++	0	+++	0	+
2nd Order - Transient	All	All	+++	+++	+++	+++	0
3rd Order - Transient	Growing	Day	+++	+	++[b]	+++	++
3rd Order - Transient	Growing	Night	+++	0	0	+++	+++
3rd Order - Transient	Harvest	Day	+++	++	++	+++	+
3rd Order - Transient	Harvest	Night	+	++	++	+++	0

[a] Significance level 0.0001
[b] Significance level 0.001
[c] Significance level 0.01
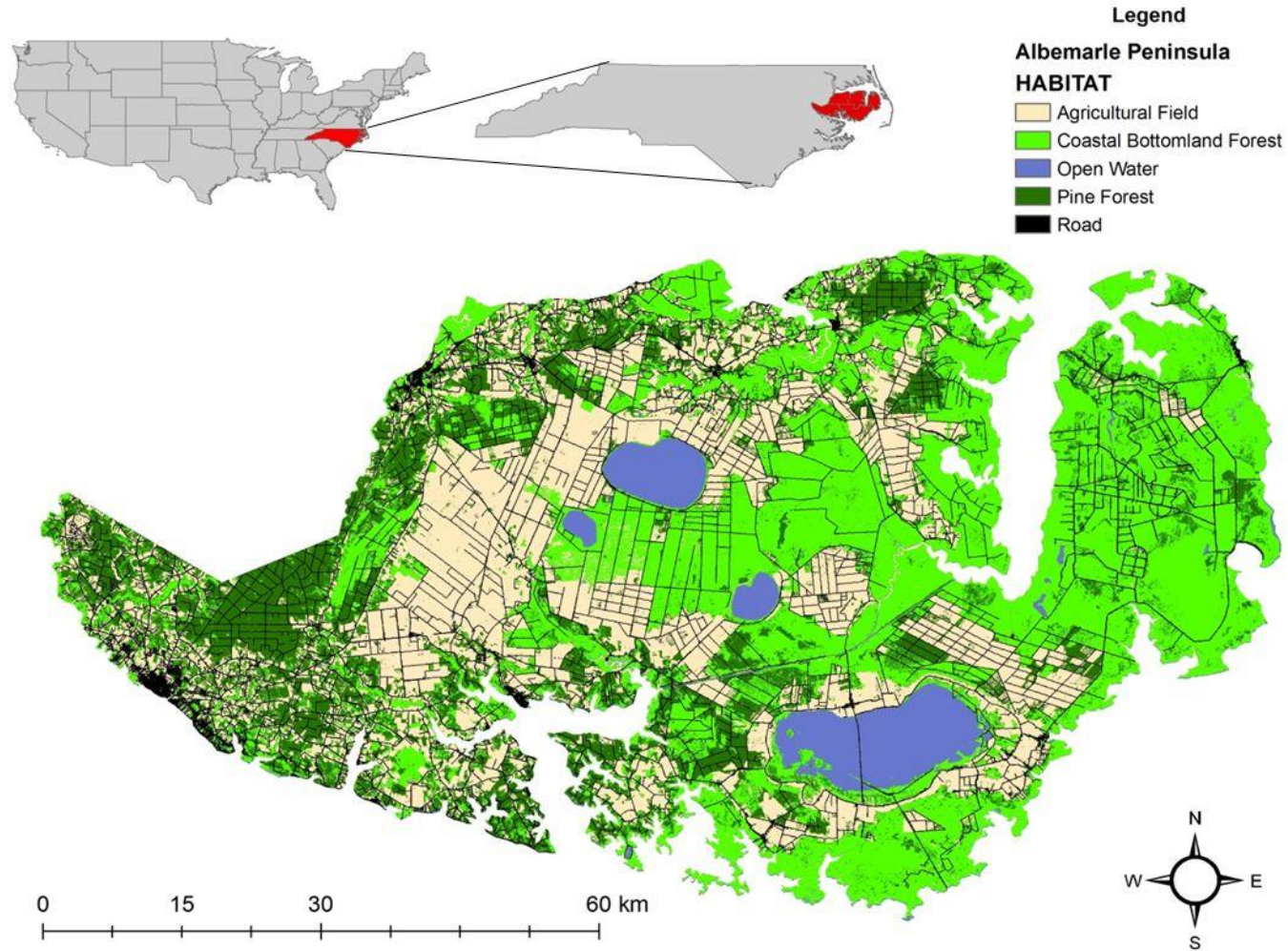[d] Significance level 0.1

Figure 6.1. Map of the Red Wolf Recovery Area in northeastern North Carolina with primary habitat types during 2009-2011.
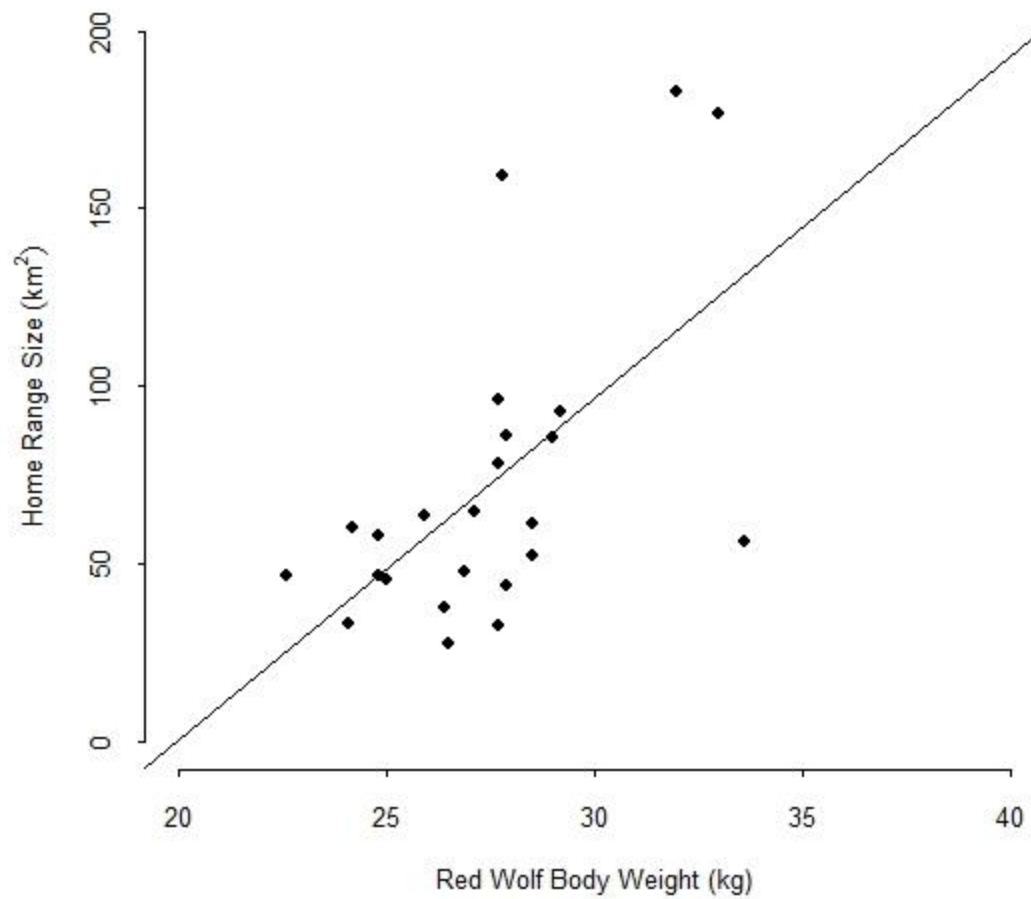
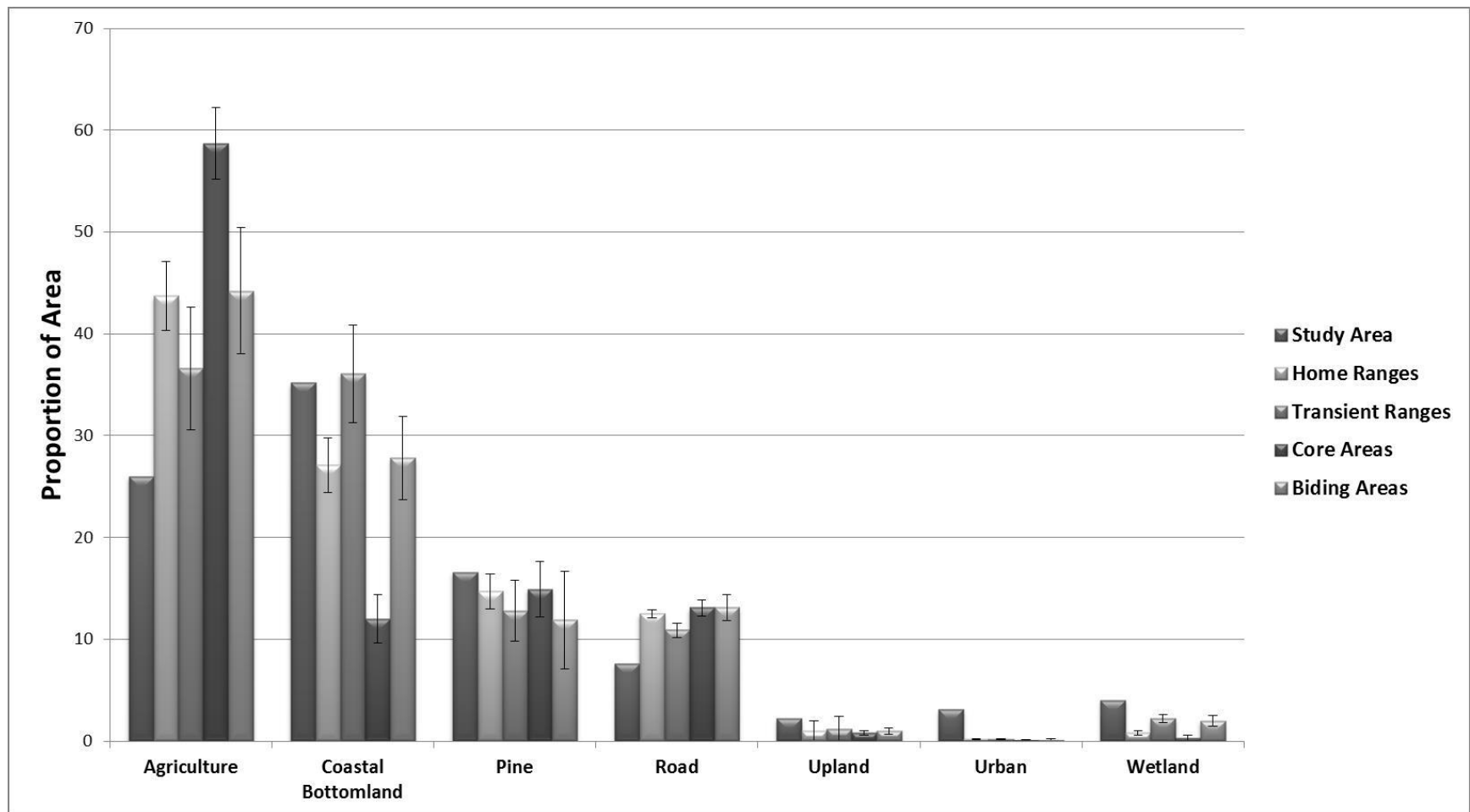Figure 6.2. Correlation between home range size and body weight of red wolves.

Figure 6.3. Habitat availability and habitat proportions of space used by resident and transient coyotes in northeastern North Carolina during 2009-2011.
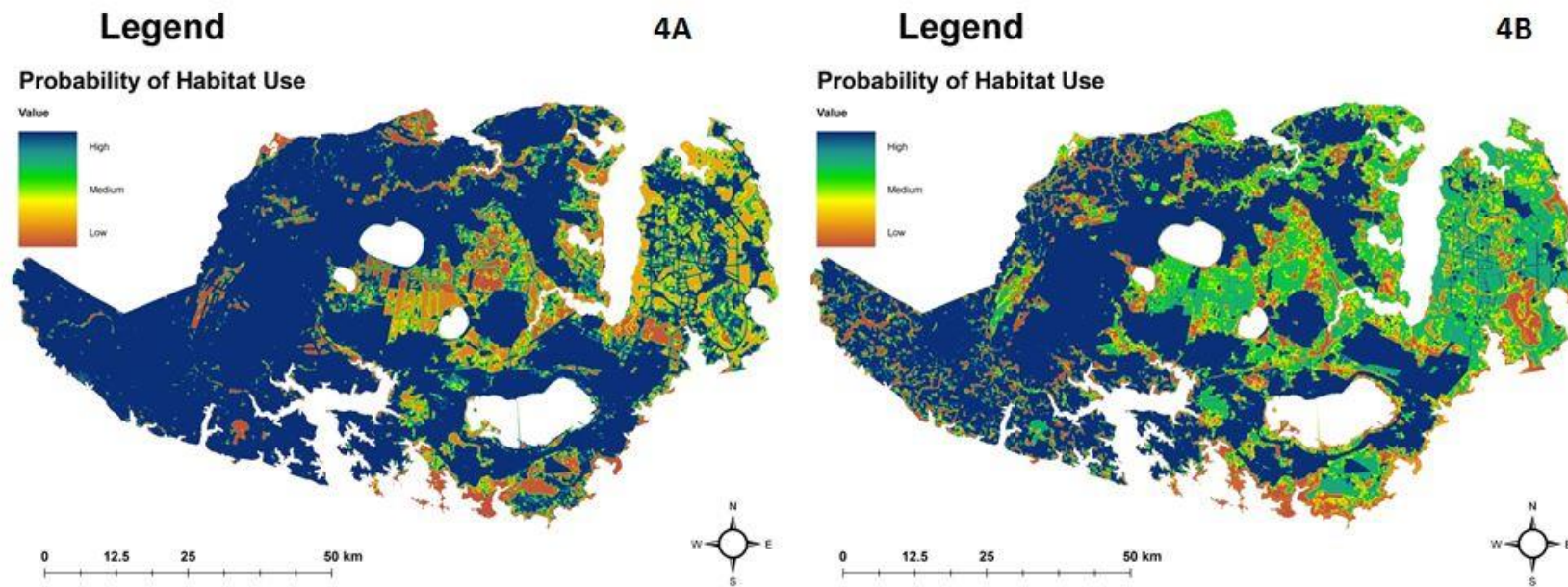
Figure 6.4. Proportional probability of 3rd-order habitat selection by residents during the growing season's diurnal (4A) and nocturnal (4B) hours for red wolves across the Red Wolf Recovery Area in northeastern North Carolina during 2009-2011.
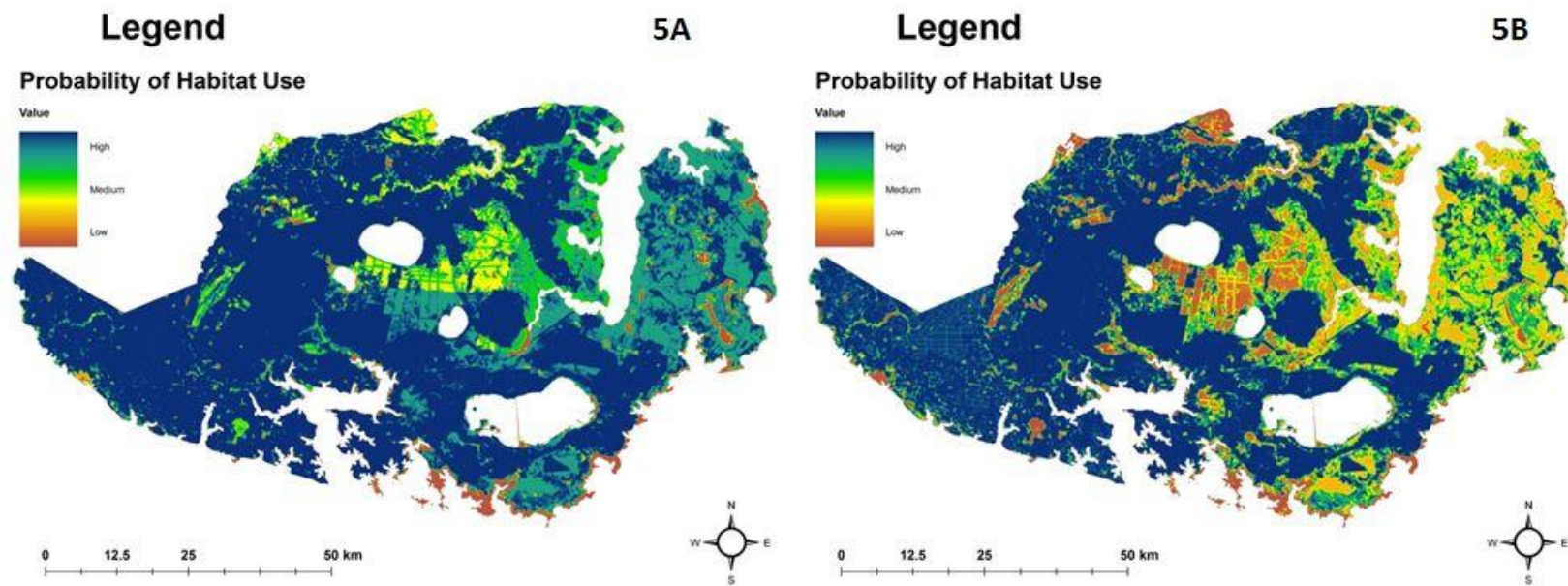
Figure 6.5. Proportional probability of 3rd-order habitat selection by residents during the harvest season's diurnal (5A) and nocturnal (5B) hours for red wolves across the Red Wolf Recovery Area in northeastern North Carolina during 2009-2011.
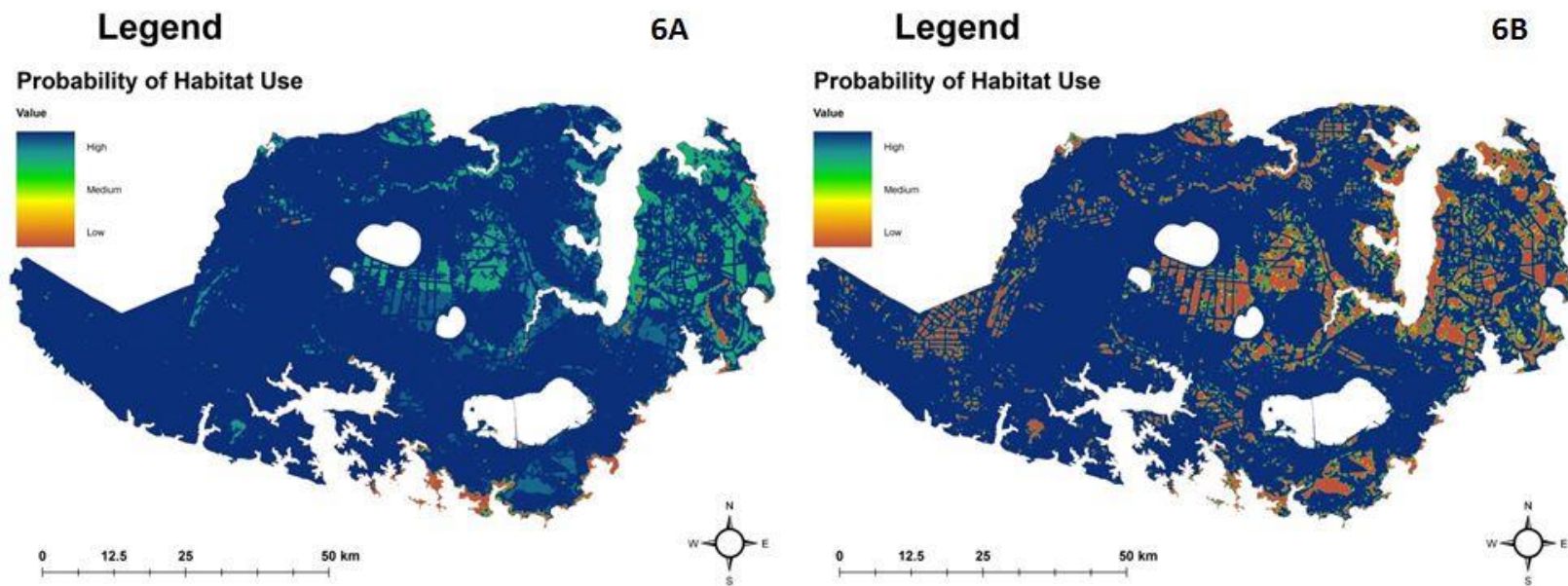
Figure 6.6. Proportional probability of 3rd-order habitat selection by transients during the growing season's diurnal (6A) and nocturnal (6B) hours for red wolves across the Red Wolf Recovery Area in northeastern North Carolina during 2009-2011.
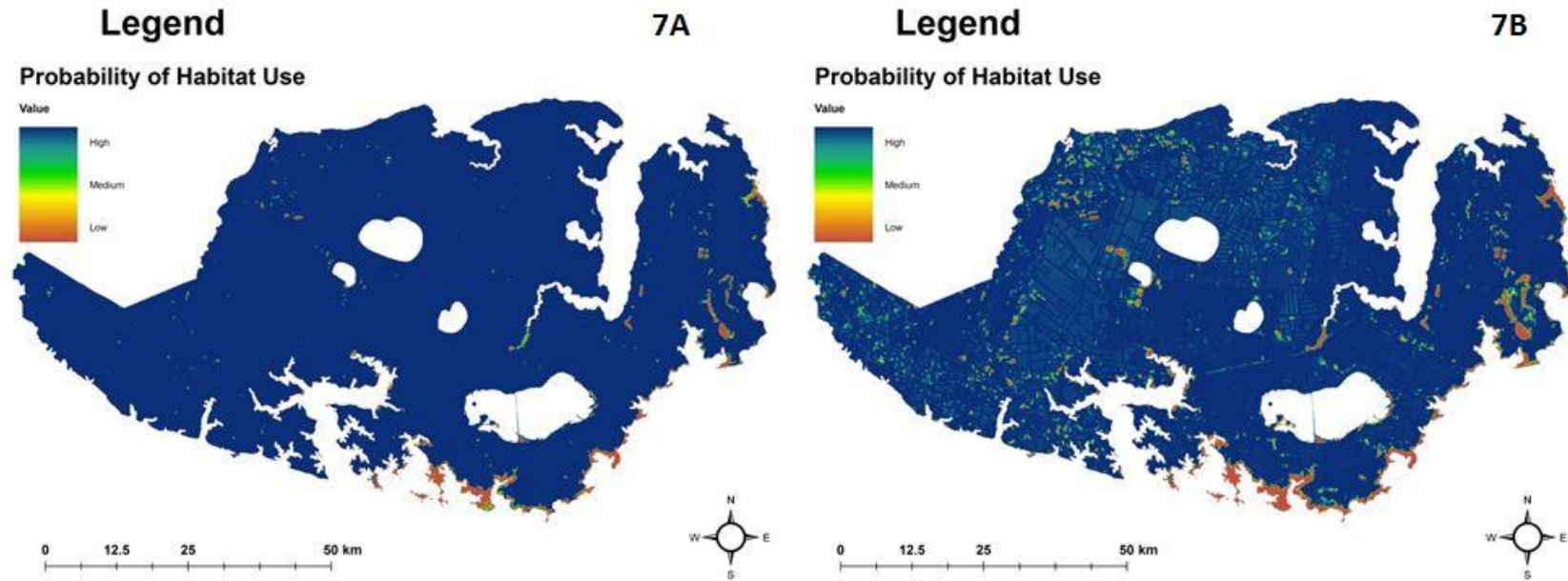
Figure 6.7. Proportional probability of 3rd-order habitat selection by transients during the harvest season's diurnal (7A) and nocturnal (7B) hours for red wolves across the Red Wolf Recovery Area in northeastern North Carolina during 2009-2011.

CHAPTER 7

BODY SIZE AS A REPRODUCTIVE BARRIER IN *CANIS*: A HYPOTHESIS

Abstract

Anthropogenic exploitation of natural resources has a ubiquitous presence across the globe, resulting in the elimination of geographic and biological barriers that prevented secondary contact between closely related, allopatric taxa. This is particularly problematic for endangered carnivores because they can be genetically assimilated into the more common sister species' population. Hybridization has become the primary challenge for red wolf recovery and conservation. Red wolves and coyotes exist as a panmictic population in northeastern North Carolina and hybridization occurs when individuals form congeneric breeding pairs. We hypothesize that differences in individual body size between red wolves and coyotes can serve as a reproductive barrier by promoting differential use of space and resources. Our results indicated that home-range size and consumption of white-tailed deer scaled positively with red wolf and coyote body weight. Additionally, red wolves in congeneric breeding pairs were smaller than red wolves in conspecific breeding pairs. Therefore, we suggest that body size differences between red wolves and coyotes serve as a reproductive barrier.

Introduction

Globally, carnivore species have been extirpated from most of their historic ranges and exist as remnant populations as a result of excessive killing and habitat alteration by humans. Although societal beliefs have resulted in profound changes to how carnivores are perceived, successful recovery and conservation of carnivores remains one of the most difficult ecological challenges facing our society today. What makes this challenge so difficult to overcome is that conservation efforts must be carried out in the presence of factors responsible for the global declines of carnivores (Breitenmoser *et al*. 2001; Hayward *et al*. 2007). Furthermore, anthropogenic exploitation of natural resources has a ubiquitous presence across the globe, resulting in the elimination of geographic and biological barriers that prevented secondary contact between closely related, allopatric taxa. This is particularly problematic for endangered carnivores because they can be genetically assimilated into the more common sister species

population. As a result, hybridization has become a primary conservation challenge for some carnivore species.

In particular, wolves have been extirpated from much of their historic ranges in North America by government-supported eradication campaigns to protect livestock and hunting industries. With the passage of the Endangered Species Act of 1973, several reintroductions of gray (*Canis lupus*) and red (*Canis rufus*) wolves occurred in the United States during the late 20th century. During this same time period, coyotes (*Canis latrans*) expanded their range throughout North America and are currently ubiquitous throughout the United States. Although gray wolves are reproductively isolated from coyotes (Pilgrim *et al*. 1998; Wheeldon *et al*. 2010), ongoing hybridization with coyotes has created unique conservation challenges for eastern wolves (*Canis lycaon*) in eastern Canada and red wolves in the southeastern United States (Chambers et al. 2012; Rutledge et al. 2012; Hinton et al. 2013). Despite suggestions for a more holistic approach to examining *Canis* evolution by using ecology, natural history, and the fossil record in concomitant with genetic analyses (Rutledge *et al*. 2012), research has focused *Canis* taxonomy with little effort to identify traits responsible for reproductive isolation among *Canis* species. Even in the presence of modern hybridization with coyotes by eastern and red wolves, morphological and genetic differences among the populations indicate past isolation and, therefore, the existence of isolating mechanisms. Although the literature pertaining to reproductive isolation and speciation is voluminous, its integration into carnivore conservation has been lacking despite the increasing threat hybridization now plays in the conservation of imperiled species (Rhymer and Simberloff 1996; Simberloff 1996; Genovart 2009; Ellstrand et al. 2010).

Red wolves and coyotes exist as a panmictic population in northeastern North Carolina and the Red Wolf Recovery Program (Recovery Program) intensively manages hybridization via reproductive sterilization of coyotes within the designated Red Wolf Recovery Area (Hinton et al. 2013). Although this technique ensures that hybridization does not occur when red wolves form

breeding pairs with sterilized coyotes, continued use of sterilization and efforts to increase the number of red wolves on the landscape will likely fail to prevent hybridization if reproductive barriers do not exist (Fredrickson and Hedrick 2006). Nevertheless, assortative mating may exist within both populations as some individual red wolves and sterile coyotes appear to always prefer to pair with conspecifics after the loss of a mate, whereas others show random preferences (USFWS unpublished data). A central characteristic of *Canis* is monogamous breeding which manifests in pair-bonding behavior and group living (Gittleman 1989; Geffen et al. 1996). As a result, sexual isolation between red wolves and coyotes likely occurs when both species are incapable of forming congeneric pairs to maintain breeding territories.

Red wolves and coyotes exhibit similarity in morphology and ecology, in which red wolves are the larger species (Chapter 3). Additionally, red wolves and coyotes exhibit similar use of prey and habitat selection and differences in resource use are associated with body size (Chapters 4, 5, and 6). These studies have shown red wolves to rely on larger prey than coyotes and require larger home ranges to fulfill their energetic requirements. This is not surprising given that body size is a major phenotypic trait that commonly differentiates co-occurring species (LaBarbera 1989) and co-occurring guilds of species frequently demonstrate greater morphological disparity than expected by chance (Dayan and Simberloff 2005; Davies *et al*. 2007). Long-term maintenance of monogamous breeding units by *Canis* likely requires similar and synchronized use of space and resources within each breeding pair. Differences in body size between red wolves and coyotes will affect energy requirements and capacities for searching for and processing prey which, in turn, may affect how they perceive the distribution, abundance, and profitability of prey on the landscape. Red wolves typically weigh about 10 kg or more than coyotes and the ability of congeneric pairs to adjust energy budgets associated with breeder body mass, hunting efficiency, and spatial requirements is likely key to maintaining breeding territories and, subsequently, successful hybridization. Therefore, the formation of congeneric pairs and the

stability of those pairs to successfully raise hybrid litters may be related to how similar individuals are in their energy demands via similarity in body size.

The functional significance of body size and *Canis* hybridization is unclear and the relationship between the two is worth examination. We hypothesize that as red wolves and coyotes approach each other in body size, they can complement each other as possible mates whereas larger red wolves and smaller coyotes are incapable of maintaining breeding pairs because of differences in resource needs. We use the interactions of red wolves and coyotes to predict that body size serves as a reproductive barrier for *Canis*.

Methods and Analysis

Our data are derived from red wolves, coyotes, and their breeding pairs monitored in North Carolina by the United States Fish and Wildlife Service. We attained data on known *Canis* breeding pairs during the duration of earlier studies specifically focused on red wolf and coyote morphology, diet, and space use in northeastern North Carolina (Hinton and Chamberlain 2014; Hinton *et al*. 2014a,b,c,). Methods used for estimating body weight, diet, home-range size, and habitat composition of home ranges were consistent in all studies.

Variables

1. Body weight: weight (kg) recorded from individual red wolves and coyotes prior to being released back into the wild (Chapter 3).

2. Breeding pair weight: combined weight (kg) of breeding pairs for coyote pairs, congeneric pairs, and red wolf packs (Chapter 4).

3. Home-range size: the total area (km^2) used by an individual or breeding unit as estimated in Chapters 5 and 6.

4. Percent of white-tailed deer in diet: the average percent (%) of occurrence in the diet as observed in Chapter 4.

5. Percent of rabbit in diet: the average percent (%) of occurrence in diet as observed in Chapter 4.

6. Percent of forest: percent (%) forest composition of home ranges as observed in Chapters 5 and 6.

7. Percent of agriculture: percent (%) agricultural composition of home ranges as observed in Chapters 5 and 6.

We used analysis of variance (ANOVAs) and t-tests to test for differences among breeding units and between red wolves and coyotes. We used simple linear regression to examine the effects of body weight and combined breeder weight on diet, home-range size, and habitat of red wolves and coyotes. We obtain the best-fitting lines between individual weight variables and ecological variables to calculate regression coefficients (r) and levels of significance.

Results

Red wolf packs had substantially larger home-range sizes than coyote and congeneric pairs (Figure 1; $F_{2,45} = 14.78$, $P < 0.001$), whereas no difference was detected in coyote and congeneric home-range sizes. We also detected no difference in habitat use among the breeding units ($F_{2, 45} \leq 0.94$, $P \geq 0.397$). We detected differential use in white-tailed deer and rabbits among the breeding units ($F_{2, 32} \geq 7.04$, $P \leq 0.002$). We found body weight of individual *Canis* positively influenced home-range size (Figure 3; $r^2 = 0.44$, $P < 0.001$) as did combined breeder weight (Figure 3; $r^2 = 0.34$, $P < 0.001$). Consumption of white-tailed deer was positively influenced by combined breeder weight (Figure 4; $r^2 = 0.38$, $P < 0.001$), whereas consumption of rabbit was negatively influenced (Figure 5; $r^2 = 0.28$, $P < 0.001$). No difference was detected in coyote body weight between breeding pairs ($t_{43} = 0.25$, $P = 0.802$). Overall, body weight of red wolves in conspecific pairs was greater than those in congeneric pairs ($t_{72} = 2.43$, $P = 0.017$). We found no difference between body weight of male red wolves in conspecific and congeneric pairs ($t_{40} = 1.22$, $P =$

0.23), whereas female red wolves in conspecific pairs were greater than those in congeneric pairs ($t_{30} = 3.09$, $P = 0.005$).

Discussion

The association between body size and spatial and feeding ecology in carnivores is well documented (Gittleman and Harvey 1982; Gittleman 1985; Gompper and Gittleman 1991; Carbone *et al*. 1999) and our results indicate that body size provides an important indicator of red wolf and coyote diet and space use patterns. Home-range size and use of white-tailed deer scaled positively with red wolf and coyote body weight. This pattern is associated with energetic requirements and foraging strategy because large carnivores have relatively high metabolic rates and increased ranging behavior would be required to fulfill greater energetic needs (Carbone *et al*. 1999; Carbone *et al.* 2007). Furthermore, we found that red wolves that formed congeneric breeding pairs with coyotes were, on average, smaller than red wolves in conspecific breeding pairs. It appears that this trend is largely driven by female red wolves, which are the smaller of the two red wolf sexes (Chapter 3). Small red wolves may approach similar ecological requirements to those of coyotes because of their smaller body size, and this may allow them to form and maintain breeding pairs with coyotes via similar spatial and feeding needs.

Coyote home-ranges in northeastern North Carolina did not exceed 50 km^2 indicating that energetic costs may limit the maximum size of coyote home ranges (Chapter 5). In this study, we observed home-range sizes of congeneric pairs to be under 50 km^2 and statistically similar to those of coyotes, whereas most red wolf home-ranges exceeded this threshold. Although coyote body weight had no effect on coyote home-range size (Chapter 5), it was the primary trait influencing variation in red wolf home-ranges (Chapter 6). Additionally, 41% of coyote pairs monitored in our study had diets in which white-tailed deer comprised < 25%, whereas white-tailed deer comprised > 30% of the diet for all red wolf packs monitored. For both coyotes and red wolves, body weight was the most important factor influencing consumption of white-tailed deer (Chapter 4). Less use of space and white-tailed deer by coyotes suggest that they impose

168

energetic constraints on congeneric pairs and are likely responsible for smaller home-range sizes and less reliance on white-tailed deer by congeneric pairs when compared to red wolf packs.

Intrinsic reproductive barriers are traits that prevent gene flow between populations of closely related taxa and facilitate speciation via ecological divergence (Mayr 1941; Schluter 2000; Coyne and Orr 2004; Pfennig and Pfennig 2010). As previous allopatric species, geography and habitat may have served as the primary reproductive barrier between red wolves and coyotes, but the inability to form monogamous breeding pairs likely serves as the primary barrier to gene flow between red wolves and coyotes during secondary contact. Phylogenetic relatedness and phenotypic similarities permit red wolves and coyotes to compete with each other for mating opportunities and conflict between consorting individuals may be a fundamental phenomenon preventing hybridization. Our study indicates that larger red wolves generally avoid consorting with coyotes and size-assortative mating may arise largely as a result of ecological differences resulting from divergence in body size. Conflict may arise between consorting red wolves and coyotes during energetically stressful situations, such as foraging and defending territories, that prevents successful pair formation. As red wolves and coyotes approach each other in body size, these conflicts may be reduced allowing for long-term maintenance of congeneric breeding pairs through similar use of space use and prey.

Our results highlight the need to better understand factors affecting hybridization in *Canis*, such as body size. As seen in comparative studies of carnivores, our results indicate that body size influences home-range size and prey selection of red wolves and coyotes. Morphological disparity between red wolves and coyotes likely promotes reproductive isolation and ecological divergence through energetic constraints. We believe that the key to understanding how body size affects interactions, and subsequently hybridization, between closely related *Canis* is to understand how energetic requirements scale with *Canis* body size and its effect on *Canis* ecology. For example, it has been observed that gray wolves and coyotes are reproductively isolated where populations are sympatric despite having the potential to hybridize (Pilgrim *et al*.

169

1998; Wheeldon *et al*. 2010). On the other hand, eastern wolves, which are intermediate in body size to gray wolves and coyotes, are capable of hybridizing with both species and are believed to serve as a conduit for genetic material between gray wolves and coyotes (Rutledge *et al*. 2010). Currently, it is unknown whether innate preferences or environmental conditions are responsible for reproductive barriers observed in eastern wolves (Benson *et al*. 2012), but it is believed that prey selection (Rutledge *et al*. 2010) and territorial aggression (Benson *et al*. 2013) play a role in reducing hybridization. Nevertheless, our findings are consistent with invariant trends observed in comparative studies which suggest that our hypothesis is biologically plausible. Our hypothesis is based on allometric relationships and represents a useful predictive framework to understand patterns of interactions and hybridization among *Canis* species. We also suggest more sophisticated comparisons using different methods and evidence are needed to fully evaluate this hypothesis.

Literature Cited

Benson, J.F., and B.R. Patterson. 2013. Inter-specific territoriality in a *Canis* hybrid zone: spatial

    segregation between wolves, coyotes, and hybrids. Oecologia 173: 1539-1550.

Benson, J.F., B.R. Patterson, and T.J. Wheeldon. 2012. Spatial genetic and morphologic structure

    of wolves and coyotes in relation to environmental heterogeneity in a *Canis* hybrid zone.

    Molecular Ecology 21: 5934-5954.

Breitenmoser, U., Breitenmoser-Würsten, C., Carbyn, L.N. & Funk, S.M. (2001). Assessment of

    carnivore reintroductions. In *Carnivore conservation*: 242-281. Gittleman, J.L., Funk,

    S.M., Macdonald, D., Wayne, R.K. (Eds.). Cambridge: Cambridge University Press.

Carbone, C., Mace, G.M., Roberts, S.C. & Macdonald, D.W. (1999). Energetic constraints on the

    diet of terrestrial carnivores. *Nature* **402**, 286-288.

Carbone, C., Teacher, A. & Rowcliffe, J.M. (2007). The costs of carnivory. *PLoS Biol.* **5**, 363-

    368.

Chambers, S.M., S.R. Fain, B. Fazio, and M. Amaral. 2012. An account of the taxonomy of

    North American wolves from morphological and genetic analyses. North American

    Fauna 77: 1-67.

Coyne, J.A., and H.A. Orr. 2004. Speciation. Sinauer, Sunderland, MA, USA.

Davies, T.J., S. Meiri, T.G. Barraclough, and J.L. Gittleman. 2007. Species co-existence and

    character divergence across carnivores. Ecology Letters 10: 146-152.

Dayan, T., and D. Simberloff. 2005. Ecological and community-wide character displacement: the

    next generation. Ecology Letters 8: 875-894.

Ellstrand, N.C., D. Biggs, A. Kaus, P. Lubinsky, L.A. McDade, K. Preston, L.M., Prince, H.M.

    Regan, V. Rorive, O.A. Ryder, and K.A. Schierenbeck. 2010. Got hybridization? A

    multidisciplinary approach for informing science policy. BioScience 60: 384-388.

Fredrickson, R.J., and P.W. Hedrick. 2006. Dynamics of hybridization and introgression in red

    wolves and coyotes. Conservation Biology 20: 1272-1283.

Geffen, E., M.E. Gompper, J.L. Gittleman, L. Hang-Kwang, D.W. Macdonald, and R.K. Wayne.
1996. Size, life-history traits and social organization in the Canidae: a reevaluation.
American Naturalist 147: 140-160.

Genovart, M. 2009. Natural hybridization and conservation. Biodiversity and Conservation 18:
1435-1439.

Gittleman, J.L. 1985. Carnivore body size: ecology and taxonomic correlates. Oecologia 67: 540-
554.

Gittleman, J.L. 1989. Carnivore group living: comparative trends. Pages 183-207 *in* J.L.
Gittleman, editor. Carnivore behavior, ecology, and evolution: volume 1. Cornell
University Press, Ithaca, NY, USA.

Gittleman, J.L. & Harvey, P.H. (1982). Carnivore home-range size, metabolic needs and ecology.
Behav. Ecol. Sociobiol. **10**, 57-63.

Gompper, M.E. & Gittleman, J.L. (1991) Home range scaling: intraspecific and comparative
trends. *Oecologia* **87**, 343-348.

Hayward, M.W., Adendorff, J., O'Brien, J., Sholto-Douglas, A., Bissett, C., Moolman, L.C.,
Bean, P., Fogarty, A., Howarth, D., Slater, R. & Kerley, G.I.H. (2007). The
reintroduction of large carnivores to the Eastern Cape, South Africa: an assessment. *Oryx*
41, 205-214.

Hinton, J.W., Chamberlain, M.J. & Rabon, D.R. (2013). Red wolf (*Canis rufus*) recovery: A
review with suggestions for future research. *Animals* 3, 722-744.

LaBarbera, M. 1989. Analyzing body size as a factor in ecology and evolution. Annual Review of
Ecology and Systematics 20: 97-117.

Mayr, E.W. 1941. Systematics and the origin of species from the viewpoint of a zoologist.
Columbia University Press, New York, NY, USA.

Pfennig, D.W., and K.S. Pfennig. 2010. Character displacement and the origins of diversity.
American Naturalist 176: S26-S44.

Pilgrim, K., D. Boyd, and S. Forbes. 1998. Testing for wolf-coyote hybridization in the Rocky Mountains using mitochondrial DNA. Journal of Wildlife Management 62: 683-689.

Rhymer, J.M., and D. Simberloff. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics 27: 83-109.

Rutledge, L.Y., C.J. Garroway, K.M. Loveless, and B.R. Patterson. 2010. Genetic differentiation of eastern wolves in Algonquin Park despite bridging gene flow between coyotes and grey wolves. Heredity 105: 520-531.

Rutledge, L.Y., P.J. Wilson, F.C. Klütsch, B.R. Patterson, and B.N. White. 2012. Conservation genomics in perspective: a holistic approach to understanding *Canis* evolution in North America. Biological Conservation 155: 186-192.

Schluter, D. 2000. Ecological character displacement in adaptive radiation. American Naturalist 156: S4-S16.

Simberloff, D. 1996. Hybridization between native and introduced wildlife species: importance for conservation. Wildlife Biology 2: 143-150.

Wheeldon, T., B.R. Patterson, and B.N. White. 2010. Sympatric wolf and coyote populations of the western Great Lakes region are reproductively isolated. Molecular Ecology 19: 4428-4440.
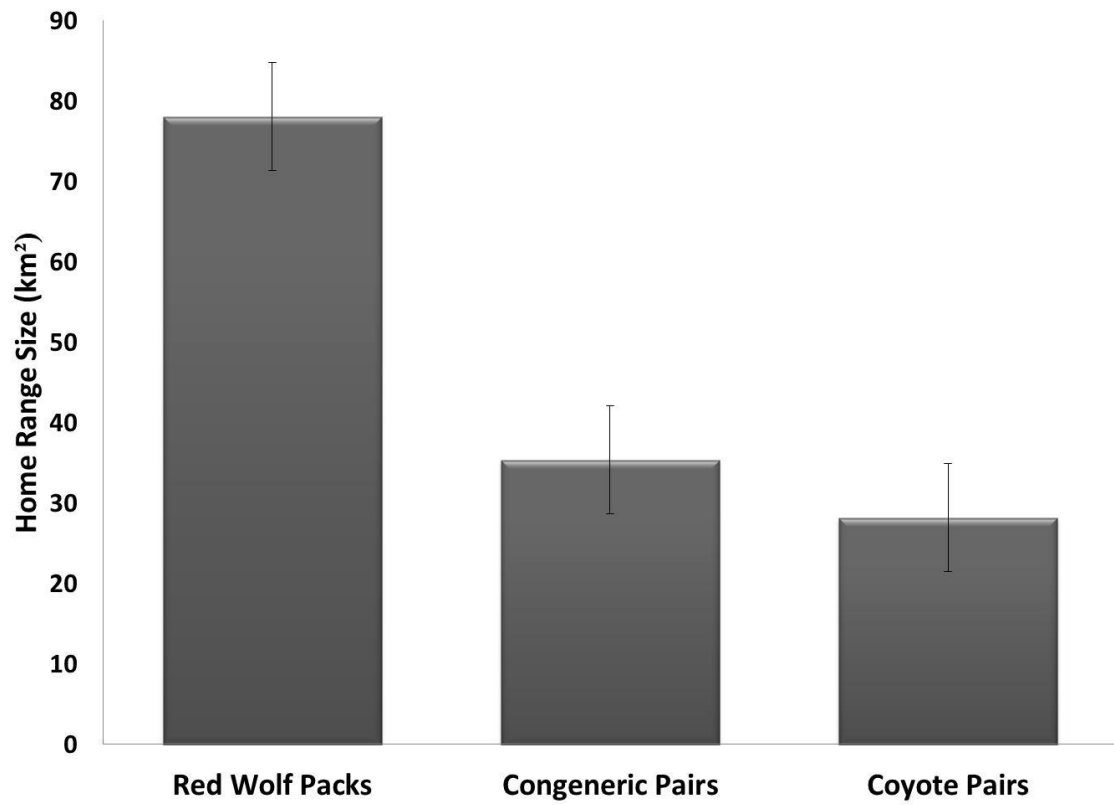
Figure 7.1. Mean home-range sizes of red wolf packs, congeneric pairs, and coyote pairs in northeastern North Carolina.
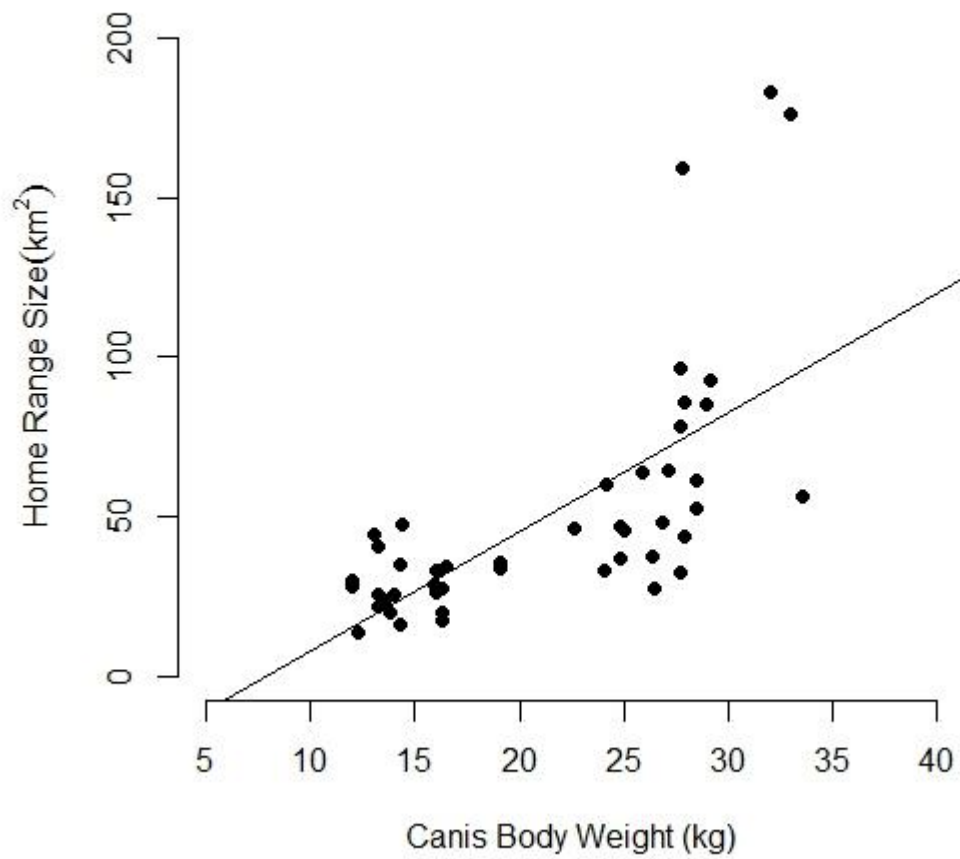
Figure 7.2. Correlation between home range size and body weight of red wolves and coyotes.
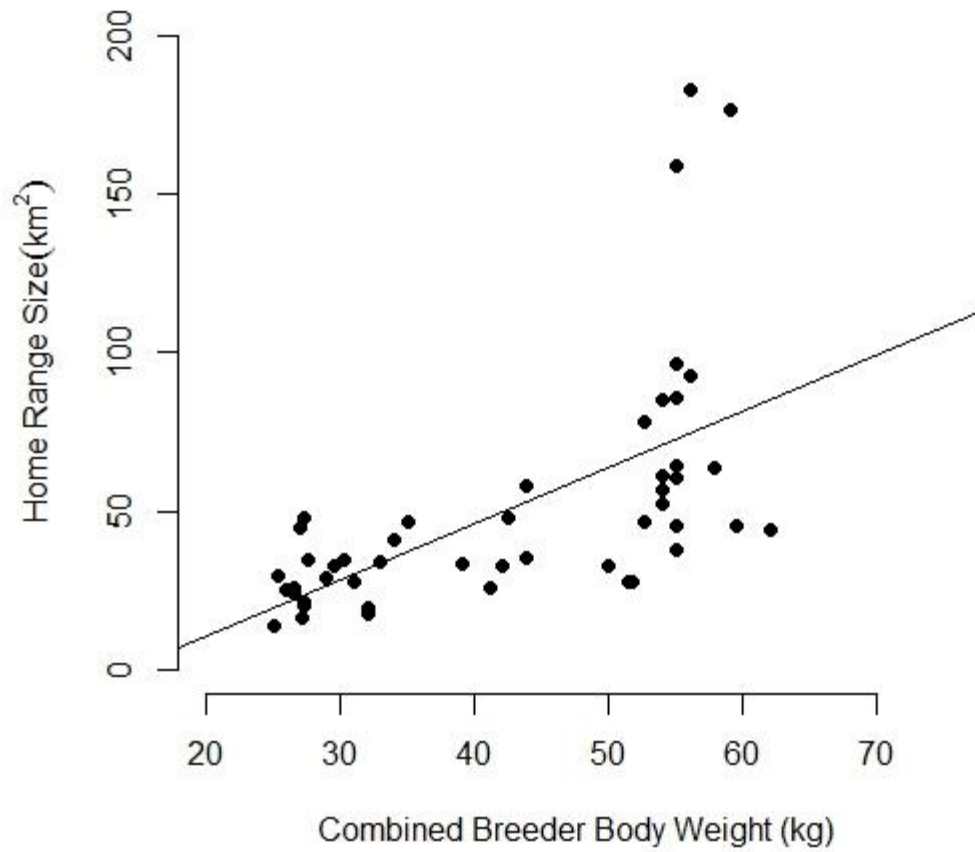
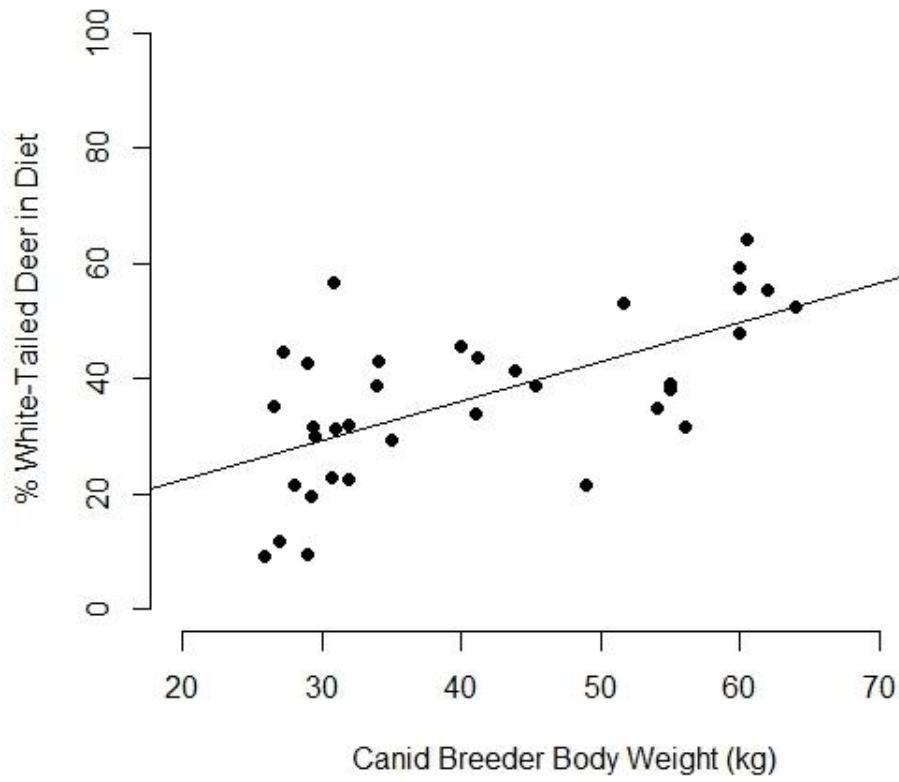Figure 7.3. Correlation between home range size and combined breeder body weight of *Canis* breeding units.

Figure 7.4. Correlation between percent white-tailed deer in diet and body weight of *Canis* breeding units.
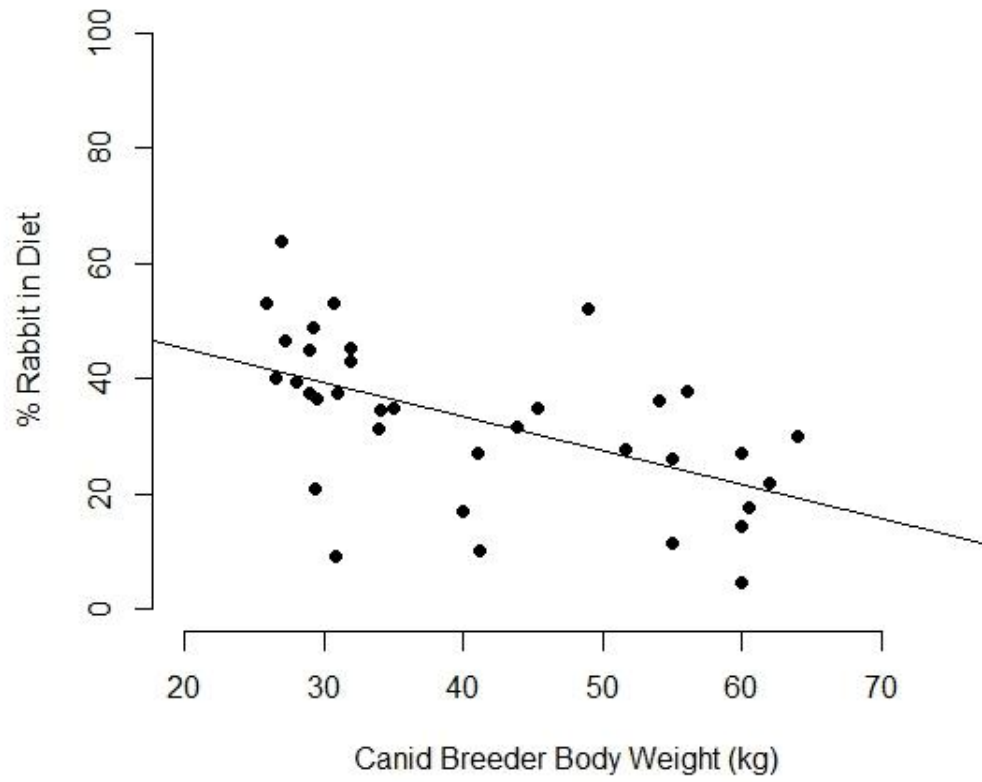
Figure 7.5. Correlation between percent rabbit in diet and body weight of *Canis* breeding units.

CHAPTER 8

CONCLUSIONS

Understanding how red wolves interact with coyotes is an important issue which could

dictate the success of recovery efforts. It also presents an opportunity to explore mechanisms that

facilitate ecological divergence and reproductive isolation between *Canis* species. There has been

limited examination of potential reproductive barriers between sympatric *Canis*, partly due to the

difficulty in monitoring the interactions of large carnivores. Recent studies have suggested

several possible mechanisms responsible for slowing hybridization rates between *Canis* such as

differences in prey selection (Rutledge et al. 2010), stability in social structure (Bohling 2011),

and aggressive interactions related with territoriality (Benson et al. 2013). Noting that

monogamous breeding and group living as a central characteristic of *Canis*, we hypothesized that

difference in body size between interacting *Canis* species prevents congeneric breeding-pair

formation via disparity in diet and spatial needs. In other words, long-term maintenance of

monogamous breeding units by *Canis* likely requires similar and synchronized use of space and

resources within each breeding pair and this is likely to happen when individuals are similar in

body size.

This dissertation found 4 important components of red wolf and coyote ecology that

demonstrate the biological plausibility that body size disparity can serve as a reproductive barrier.

First, we demonstrated that red wolves are substantially larger than coyotes with hybrids

intermediate to both coyotes and red wolves in body size. In fact, red wolves attain a body size

that is not achieved by coyotes or hybrids. Therefore, good concordance was found between

morphometric variables and the molecular methods accepted by the United States Fish and

Wildlife Service for monitoring red wolf genetic ancestry.

Second, we found red wolves and coyotes to have similar and overlapping diets. Nevertheless, consumption of white-tailed deer positively scaled with body weight in which red wolves consumed more deer than did coyotes. Interestingly, our results of coyote diet are contrary to many studies conducted in the southeastern United States because we found coyotes in northeastern North Carolina to be largely carnivorous with a narrow dietary breadth. Partitioning of food resources by red wolves and coyotes is mostly via differences in quantity of similar prey rather than differences in types of prey exploited. This suggests foraging ecology of red wolves and coyotes become similar as they approach each other in body size.

Third, we found body size to have an effect on red wolf and coyote space use. Home-range size in red wolves scaled positively with body weight in which larger red wolves generally require more space than smaller red wolves. Although body size did not influence coyote space use, coyote home ranges did not exceed 50 km^2 indicating that coyotes cannot maintain large home ranges. This constraint of coyote space use is likely a result of their smaller body size because approximately 75% of red wolf home ranges were > 40 km^2. We also found red wolves and coyotes preferred open, treeless agricultural fields over the forested habitats in northeastern North Carolina. Red wolf and coyotes centered their home ranges in open, agricultural habitats and the primary difference in spatial ecology was size of their home ranges which was influenced by body size.

Fourth, we found that red wolves that formed congeneric breeding pairs with coyotes were, on average, smaller than red wolves in conspecific breeding pairs. It appears that this trend is largely driven by female red wolves, which are the smaller of the two red wolf sexes. Small red wolves may approach similar ecological requirements to those of coyotes because of their smaller body size, and this may allow them to form and maintain breeding pairs with coyotes via similar spatial and feeding needs. For instance, we observed home-range sizes of congeneric pairs to be under 50 km^2 and statistically similar to those of coyotes, whereas most red wolf home-ranges exceeded this threshold. For both coyotes and red wolves, body weight was the most important

factor influencing consumption of white-tailed deer. Less use of space and white-tailed deer by coyotes suggest that they impose energetic constraints on congeneric pairs and are likely responsible for smaller home-range sizes and less reliance on white-tailed deer by congeneric pairs when compared to red wolf packs.

In conclusion, we suspect morphological disparity between red wolves and coyotes likely promotes reproductive isolation and ecological divergence through energetic constraints. We believe that the key to understanding how body size affects interactions, and subsequently hybridization, between closely related *Canis* is to understand how energetic requirements scale with *Canis* body size and its effect on *Canis* ecology. Our findings are consistent with invariant trends observed in comparative studies that home-range size and prey size scale positively with carnivore body size and this suggests that our hypothesis is biologically plausible.

Literature Cited

Benson, J.F., and B.R. Patterson. 2013. Inter-specific territoriality in a *Canis* hybrid zone: spatial segregation between wolves, coyotes, and hybrids. Oecologia 173: 1539-1550.

Bohling, J.H. 2011. Exploring patterns and mechanisms of red wolf (*Canis rufus*) hybridization in North Carolina. Dissertation, University of Idaho, Moscow, ID, USA.

Rutledge, L.Y., C.J. Garroway, K.M. Loveless, and B.R. Patterson. 2010. Genetic differentiation of eastern wolves in Algonquin Park despite bridging gene flow between coyotes and grey wolves. Heredity 105: 520-531.