Foraging and Spatial Ecology of Red Wolves (*Canis rufus*) in Northeastern North Carolina

by

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Abstract

Red wolves (*Canis rufus*) are critically endangered, with <150 individuals in the only wild population. I collected scats (fecal deposits) of red wolves from that population for 2 consecutive pup-rearing seasons. Packs consumed most species of mammals that were available to them. Generally, packs selected adults and fawns of white-tailed deer. Packs with pups exhibited greater dietary diversity and evenness than packs without pups. Packs appear to be foraging on naturally occurring prey items within a human-altered landscape. I developed resource-selection functions using data obtained from radio-collared red wolves to examine selection of resources. Generally, packs selected agricultural and fallow fields relative to forested, pocosin, and wetland habitat types, and areas near dirt roads, away from water, and with few humans. Factors used to analyze selection of habitat were scale dependent in red wolves.

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Chapter 1

Historical Overview, Recovery, and Conservation of the Red Wolf (*Canis rufus*)

The red wolf (*Canis rufus*) is one of the most endangered species of canid in the

world (Stoskopf et al. 2005). Shaw (1975) recognized three subspecies of red wolves:

Florida red wolf *Canis rufus floridanus*, Mississippi Valley red wolf *Canis rufus*

gregoryi, and Texas red wolf *Canis rufus rufus*. The three subspecies once occupied a

geographic range from central Texas to the Atlantic Ocean and from central Illinois and

Indiana throughout the Mississippi River Valley southward through Florida (Fig. 1.1; Fox

1975). The Florida red wolf was exterminated by the early 1900s and was reportedly the

largest of the three subspecies (Weller 1995). The Mississippi Valley red wolf was

slightly smaller than the Florida red wolf and currently is believed to be the only extant

subspecies (Shaw 1975). The Texas red wolf was the smallest subspecies and is believed

by some researchers to represent a naturally occurring hybridization between red wolves

and coyotes (*Canis latrans*); thus, it is not a true subspecies of red wolf (Shaw 1975).

This conclusion was reached because coyotes were native historically in the midwestern

and southwestern United States (Bekoff and Gese 2003). Prior to the eastward range

expansion of coyotes, the range of the Texas red wolf represented the only overlap in the

distribution of the two species (Shaw 1975). Furthermore, the subspecies of coyote that

is present in the area of overlap is *Canis latrans frustor*, which is one of the largest

subspecies of coyote and is similar to the Texas red wolf in size and appearance (Shaw

1975). According to early naturalists, red wolves occupied coastal prairies, marshes,

bottomland hardwood forests, virgin pine forests, and riverine habitats, preying on small-to-medium-sized mammals (Paradiso and Nowak 1972).

The red wolf almost became extinct in the wild due to habitat fragmentation, intentional eradication efforts by humans, and hybridization with coyotes (Phillips et al. 2003). Hybridization with coyotes is believed to be a result of an eastward range expansion by coyotes, which occurred due to habitat fragmentation and efforts to exterminate carnivores in the eastern United States (Phillips et al. 2003). These efforts greatly reduced the number of large carnivores and allowed coyotes to expand their range eastward to fill the empty niche (Bekoff and Gese 2003). Red wolves and coyotes were believed to naturally hybridize on occasion in the area where their historic ranges overlapped (Shaw 1975). However, with eastward expansion by coyotes and reduced population sizes of red wolves, likelihood of hybridization increased (McCarley 1962). By the 1970s, as few as 100 red wolves were believed to exist in coastal areas of Texas and Louisiana (Phillips et al. 2003).

Following passage of the Endangered Species Act in 1973, a red wolf recovery program was developed (Stoskopf et al. 2005). The United States Fish and Wildlife Service captured >400 canids in and around the area occupied by the remaining population of red wolves (Phillips et al. 2003). Capture of canids, which occurred during 1973-1980, was initiated to begin a captive-breeding program. Through analyses of morphological measurements, vocalizations, and x-rays of skulls, only 43 of the >400 individuals were determined to be red wolves (Stoskopf et al. 2005). These individuals were sent to a breeding facility in Tacoma, Washington. Individuals believed to be Texas red wolves were not used in the breeding program due to speculation surrounding their

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ancestry (Shaw 1975); thus, only Mississippi Valley red wolves were used in the breeding program. Pups produced from individuals that were believed to be hybrids were removed from the breeding program along with their parents; resulting in only 14 of the original >400 individuals remaining in the breeding program (Phillips et al. 2003). The United States Fish and Wildlife Service oversaw the captive-breeding program with intentions to reintroduce red wolves to parts of their historical range (Phillips et al. 2003). In 1987, red wolves were reintroduced into the Alligator River National Wildlife Refuge in northeastern North Carolina (Stoskopf et al. 2005). The reintroduction was successful, with an increase in the population of red wolves from a few to ca. 120 individuals (United States Fish and Wildlife Service Southeast Region 2007), and an increase in the overall geographic area that the population inhabits (Stoskopf et al. 2005), which now includes the entire Albemarle Peninsula and hereafter is referred to as the Red Wolf Recovery Experimental Population Area.

Introgression with coyotes nearly caused extinction of red wolves due to genetic assimilation, and introgression continues between coyotes and the reestablished population of red wolves (Phillips et al. 2003). Biologists from the United States Fish and Wildlife Service are working to limit hybridization of red wolves with coyotes in the Red Wolf Recovery Experimental Population Area by either killing coyotes in the recovery area or sterilizing coyotes via tubal ligations and vasectomies (United States Fish and Wildlife Service Southeast Region 2007). Sterilization is conducted so that the sterilized coyote will act as a place-holder, reducing unoccupied land available to reproductively capable coyotes that disperse into the Red Wolf Recovery Experimental Population Area from surrounding counties (United States Fish and Wildlife Service

Southeast Region 2007). Biologists from the United States Fish and Wildlife Service also

have been working to promote pairings between red wolves by placing unpaired

individuals in a pen together for several months or killing coyotes that have paired with

red wolves (United States Fish and Wildlife Service Southeast Region 2007).

Hybridization with coyotes may be a secondary problem, with the real barrier to

conservation of red wolves being limited accessibility to mates due to a small population

of adults (Chris Lucash pers. comm.). This conclusion is reached because, during the

breeding season, red wolves have killed coyotes to gain access to potential red wolf

mates (United States Fish and Wildlife Service, unpublished data). Further reports

indicate that a red wolf paired with a coyote will leave their mate if the opportunity arises

to pair with a red wolf (United States Fish and Wildlife Service, unpublished data). Thus,

pairings consisting of two red wolves may prove more stable and longer lasting than

pairings consisting of a red wolf and a coyote. These observations have led biologists

with the United States Fish and Wildlife Service to speculate that a red wolf prefers to

mate with a red wolf, but if none is present a red wolf will breed with a coyote (Chris

Lucash pers. comm.).

Although the red wolf now re-occupies a part of its historic range, there are

ongoing debates, primarily concerning issues stemming from hybridization of red wolves

with coyotes (Stoskopf et al. 2005). Some biologists suggest that pure red wolves

became extinct before recovery efforts began due to genetic assimilation and

introgression with coyotes (Wayne and Jenks 1991). However, new evidence suggests

that reintroduced red wolves are genetically representative of the historic genome of red

wolves (Murray and Waits 2007) and that red wolves may be conspecific with Algonquin

wolves (*Canis lycaon*; Kyle et al. 2006). This evidence suggests that *C. rufus/C. lycaon* actually inhabited all of the eastern United States to the eastern edge of the plains and northward surrounding the southern portion of the Great Lakes (Kyle et al. 2006). Further, this evidence would support mating red wolves from the reintroduced population in North Carolina with Algonquin wolves in Ontario to increase heterozygosity and reduce inbreeding in both populations (Kyle et al. 2006).

Studying the ecology of red wolves is important for management of the only population of red wolves and for future reintroduction efforts. Herein, I have examined factors influencing foraging and spatial ecology of red wolves. Understanding what environmental variables influence foraging and spatial patterns is highly desirable. In this thesis, I also describe and recommend improvements to field techniques that would enhance research and management activities that may be conducted with red wolves in the future.

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Fig. 1.1. Historic range of the red wolf (*Canis rufus*).

Florida Red Wolf
(*Canis rufus floridanus*)

Mississippi Valley
Red Wolf
(*Canis rufus gregoryi*)

Texas Red Wolf
(*Canis rufus rufus*)
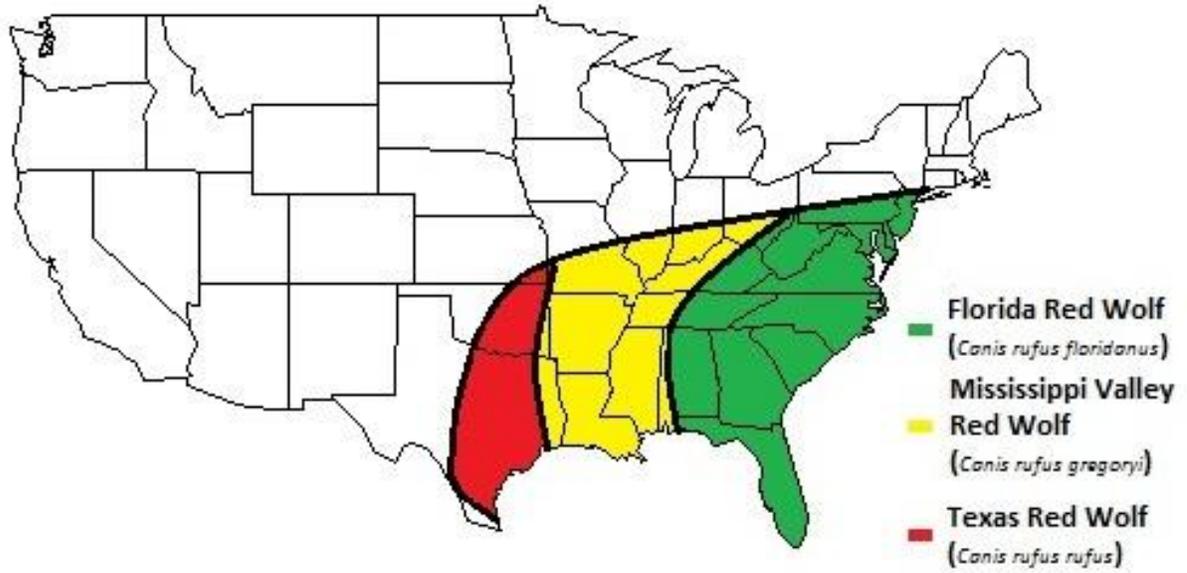
Chapter 2

Foraging Ecology of Red Wolves (*Canis rufus*) in Summer in Northeastern North

Carolina

Abstract

Red wolves (*Canis rufus*) are critically endangered, with <150 individuals in the only wild population. Currently, there is a paucity of information about foraging ecology of this population. I collected scats (fecal deposits) of red wolves for two consecutive pup-rearing seasons from six packs, classified contents into nine food categories and compared diet composition to food availability (as determined by 95% confidence intervals of proportion of foods consumed and proportion of availability of foods consumed). Packs consumed most species of mammalian prey that were available to them. Generally, packs selected adults and fawns of white-tailed deer (*Odocoileus virginianus*), but were indifferent to small-sized prey. Two packs consumed foods associated with humans. Generalized linear modeling indicated that pack identity, prey consumed, and pack-by-prey interactions were the most important variables in predicting diet. Packs with pups had greater dietary diversity and evenness than packs without pups. Packs with pups selectively consumed human-related foods. Consumption and selection for human-related foods could have negative implications by making packs with pups more susceptible to negative interactions with humans. However, red wolves appear to be primarily foraging on naturally occurring prey within a human-altered landscape.

Introduction

Given the continued increase in human population and the substantial habitat

requirements of populations of large carnivores, successful conservation of large

carnivores requires that some populations persist in human-altered landscapes. Reliance

of large carnivores on domestic animals and other human-related foods is likely to only

increase negative human-carnivore interactions and impede conservation efforts.

Prior to reintroduction of red wolves (*Canis rufus*) into part of their native range

in 1987, no large carnivore had been successfully reintroduced (Phillips et al. 2003). For

>20 years the reintroduced population of red wolves has survived and reproduced in a

habitat matrix altered by humans. A better understanding of how red wolves forage in a

human-altered landscape is useful to future reintroductions.

Basic ecological research on red wolves in the wild prior to recovery efforts was

limited due to their small population and difficulty in differentiating adults and juveniles

from hybrids and coyotes (*C. latrans*; Phillips et al. 2003). After reintroduction, the

majority of research concerning red wolves has dealt with resolving identity and studying

hybridization impacts on the species using various genetic techniques (Adams et al. 2007,

Miller et al. 2003). Thus, quantitative information on basic ecology of red wolves,

although vital to recovery and management, is lacking.

Foraging is essential to survival and reproduction. Although few studies have

examined foraging ecology of red wolves, like most canids they are considered

generalists and opportunistic (Mech 1970, Paradiso and Nowak 1972). Studies of diet

conducted on remnant populations of red wolves in Texas and Louisiana concluded that

their diet primarily was small mammals (Paradiso and Nowak 1972, Shaw 1975).

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Paradiso and Nowak (1972) suggested that prior to European settlement of North America, red wolves preyed mostly on small-to-medium-sized mammals. Only Shaw (1975) documented red wolves preying on species larger than raccoons (*Procyon lotor*); however, only 19 scats (fecal deposits) were collected for his study. During European settlement, colonists reported that red wolves in the Carolinas attempted to kill deer (Shaw 1975). Young (1946) also reported that red wolves in Louisiana preyed on wild boars (*Sus scrofa*). Following reintroduction of red wolves to the wild in 1987, red wolves were observed to prey upon adults and fawns of white-tailed deer (*Odocoileus virginianus*) on barrier islands and on the Albemarle Peninsula in North Carolina (Phillips et al. 2003). Red wolves also preyed on wild boars in Great Smoky Mountains National Park (Lucash and Crawford 1993). Although these studies provided insights into diet of red wolves, they were either focused on animals released onto islands with limited diversity of prey, are limited to accounts by early naturalists, or are based on anecdotal information. No study has quantitatively assessed diet of the reintroduced population of red wolves on the Albemarle Peninsula or evaluated whether variation in diet exists among packs, locations, or individuals. My primary goal in this study was to analyze foraging ecology of red wolves. Specific goals were to determine the overall diet during the pup-rearing season, examine variation in diet among packs and years, determine if presence or absence of pups influences diet, and determine if prey are consumed opportunistically or if certain species are favored by red wolves.

Materials and Methods

Study area.—My study area was within the Red Wolf Recovery Experimental Population Area on the Albemarle Peninsula in northeastern North Carolina. At the time

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of my research, this area was home to the only wild population of red wolves in the

world. The study area consisted of >6,000 km^2 of federal, state, and private lands in five

counties (Beaufort, Dare, Hyde, Tyrrell, and Washington). Federal land within the study

area included Alligator River National Wildlife Refuge, Pocosin Lakes National Wildlife

Refuge, Swan Quarter National Wildlife Refuge, Mattamuskeet National Wildlife

Refuge, and a bombing range shared by the United States Navy and Air Force. State

lands included numerous game-management properties, while private lands primarily

were timber plantations and agricultural fields. The study focused on packs in Tyrrell and

Dare counties (Fig. 2.1).

Types of land cover, and their approximate percentages of the study area, were:

agricultural fields (30%); commercial pine (*Pinus*) plantations (15%); pocosin (15%;

dominated by *Pinus serotina* and *Persea palustris*); non-riverine swamp forests (10%;

dominated by *Nyssa*, *Liquidambar styraciflua*, *Acer rubrum,* and *Chamaecyparis*

thyoides); saltwater marsh or open water (10%); and other types of land cover (20%).

Climate was characterized by four full seasons of nearly equal length with annual

precipitation averaging 127 cm (Beck et al. 2009). Temperatures averaged 5°C in winter

and 27°C in summer (Beck et al. 2009). Elevations were from sea level to 50 m (Beck et

al. 2009). Potential prey included white-tailed deer (*Odocoileus virginianus*), rabbits

(*Sylvilagus floridanus* and *Sylvilagus palustris*), raccoons (*Procyon lotor*), wild boars

(*Sus scrofa*), coypus (*Myocastor coypus*), common muskrats (*Ondatra zibethicus*), small

rodents (*Mus musculus, Oryzomys palustris, Reithrodontomys humulis*, and *Sigmodon*

hispidus), quail (*Coturnix*), wild turkeys (*Meleagris gallopavo*), and other ground-

dwelling birds (Phillips et al. 2003). Co-occurring carnivores included gray foxes

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(*Urocyon cinereoargenteus*), red foxes (*Vulpes vulpes*), coyotes, feral dogs (*C. lupus familiaris*), bobcats (*Lynx rufus*), and American black bears (*Ursus americanus*).

 *Survey methods and design.—*Scats were collected during the pup-rearing season, May-July in 2009 and 2010. Because ca. 75% of the red wolves resided on private land, access to private property played a key role in determining which packs were selected for study. In both years, territories of the Milltail, Timberlake, Tyson, and Columbia packs (Fig. 2.1) were surveyed for scats. In 2010, territories of the Northern and Kilkenny packs (Fig. 2.1) also were surveyed for scats. Paved, gravel, and dirt roads, along with game trails, were surveyed on foot within known territories of packs. Territorial boundaries were known based on surveys conducted by biologists of the United States Fish and Wildlife Service. In 2009, Milltail and Tyson packs produced 3 and 4 pups, respectively. In 2010, Milltail, Tyson, Northern, and Kilkenny packs produced 7, 6, 3, and 4 pups, respectively. Columbia and Timberlake packs did not produce pups either year (United States Fish and Wildlife Service, unpublished data). Each territory was surveyed once each week. For these analyses, the sampling unit was the pack; it was assumed that diet of individuals was representative of the pack. To assess if samples were adequate, rarefaction curves were constructed to determine the relationship between number of scats collected for a given pack in a year and number of prey types detected.

 *Identification of scats. —*During the 2009 field season, a sample of fecal matter was taken from all scats and placed in individual 2-ml vials containing 1.4 ml DET buffer solution (Frantzen et al. 1998) to preserve DNA for determination of species of origin. The remainder of each scat was placed in a plastic bag and stored in a freezer at <0°C until DNA analyses were conducted. Fecal matter was extracted from vials using the

Qiagen DNA Stool Kit (Qiagen Inc., Valencia, CA) and a mitochondrial-DNA fragment test was conducted to determine if the animal that produced the scat was a canid (Onorato et al. 2006). Scats that tested positive for mtDNA of *Canis* were screened at nine microsatellite loci (CXX172, CXX173, CXX20, CXX200, CXX109, CXX250, Ostrander et al. 1993; AHT103, AHT121, Holmes et al. 1995; CXX377, Mellersh et al. 1997) for individual identification. These loci were amplified in a single multiplex reaction similar to Adams et al. (2007) using the Qiagen PCR multiplex kit (Qiagen Inc., Valencia, CA). To minimize genotyping errors, I used an approach similar to the reference-genotype method described by Adams et al. (2007). Initially, two PCRs were performed using the nine microsatellite loci above, and scats that failed to amplify at ≥5 loci were removed from further analysis. If genotypes differed between the two PCRs, ≤5 more amplifications were performed as needed. Heterozygous genotypes were accepted if each allele was observed in two independent PCRs; homozygous genotypes were accepted if the genotype was observed in three independent PCRs.

Because there was a possibility of multiple recaptures per individual, the probability of identity for siblings (PID_{Sibs}; Waits et al. 2001) was calculated using Gimlet 1.3.3 (Valiere 2002) to determine minimum number of loci needed to differentiate individual genotypes of closely related individuals. Using genotypes from known red wolves and coyotes, the estimated PID_{Sibs} at six loci was sufficiently low (0.003-0.006) to differentiate individuals.

Genotypes obtained from scats were compared to genotypes of known red wolves and coyotes in the area to match scats to known individuals. When additional resolution was needed, scats were amplified at a second microsatellite multiplex containing eight

additional loci (CXX2054, CXX2062, CXX2001, CXX2004, CXX2010, CXX2145

Mellersh et al. 1997; CXX225, Ostrander et al. 1993; CXX403, Ostrander et al. 1995).

Due to active monitoring by the United States Fish and Wildlife Service, genotypes for all

animals captured within the Red Wolf Recovery Experimental Population Area were

available. Known individuals previously captured by the United States Fish and Wildlife

Service already had been evaluated for genetic ancestry using the red wolf pedigree

(Adams 2006) and a maximum-likelihood-based assignment test (Miller et al. 2003).

Some genotypes from scats did not match any known animal within the Red Wolf

Recovery Experimental Population Area. Because the sampling unit was at the pack

level, scats from unknown individuals were discarded. Scats determined to be from

known red wolves from the target packs were examined for remains of prey.

Most scats (96%) collected in 2009 and identified as red wolf matched genotypes

of individuals belonging to the pack from whose territory the scat was collected within.

Thus identification of scats to individuals was deemed unnecessary. Therefore I used

more cost efficient techniques to identify scats collected during the 2010 field season.

Diameters of scats were measured upon collection for both field seasons. After

generating a normal-distribution probability function for scats collected in 2009 and

identified via faecal genotyping, it was determined that canid scats ≥29 mm in diameter

had 95% probability of having been deposited by a red wolf (Chapter 4). Any canid scats

<29 mm in diameter collected in 2010 were identified using a scat-detection dog (Long et

al. 2007). Scats collected directly from captured wild red wolves and coyotes were used

to train scat-detection dogs, as well as scats of co-occurring carnivores (e.g., bobcats and

domestic dogs; Chapter 5).

Identification of prey and descriptive analysis of diet.—Scats identified as red wolf were examined for content. Contents of scats (e.g., hair, skulls, and teeth) were identified by comparison to reference materials. Remains of white-tailed deer were classified as either fawn or adult based on color, thickness, length, and coarseness of hair (Adorjan and Kolenosky 1969). Contents in scats were designated as belonging to one of nine food categories: deer (adult), fawns, small rodents (hispid cotton rat, marsh oryzomys, eastern harvest mouse, and house mouse), large rodents (coypu and common muskrat), rabbits, raccoons, wild boars (feral and domestic), anthropogenic material, and 'other' (foods not occurring frequently enough to justify a unique category; e.g., ground-dwelling birds, invertebrates such as grasshoppers and freshwater crustaceans, and egg shells). Remains of feral and domestic pigs were grouped together for analysis, but differentiated from one another based on color, thickness, length, and coarseness of hair (Adorjan and Kolenosky 1969).

Four metrics were used to rank and determine contribution of food categories in scats: percent frequency of occurrence (percentage of total number of occurrences of all prey items), relative volume of remains, relative weight of remains (Ciucci et al. 1996), and biomass ingested (only for mammalian food categories) calculated using the regression equation of Floyd et al. (1978). Various methods for describing diet were used because each is recognized as having biases and comparing them gives a better description of diet than any single method (Ciucci et al. 1996). Items that were <1% of a scat were ignored (Ciucci et al. 1996). Although the biomass model of Floyd et al. (1978) was developed using gray wolves (*Canis lupus*), the slight difference in size of the two predators should not influence interpretation of results. I used average mass of adults

from Silva and Downing (1995) for calculating estimates of biomass with the exception

of fawns and wild boars: weight of fawns was taken from Floyd et al. (1978) whereas

weight of wild boars was averaged between adults and piglets (Silva and Downing 1995)

because remains of domestic pigs dumped in carcass pits consisted mainly of piglets and

dump sites were believed to be the primary source of these remains. The category listed

as 'other' was excluded from rankings of biomass because not all foods included in this

category were mammals (Floyd et al. 1978). Rankings and percentages for

anthropogenic material were included only for percent frequency of occurrence because

digestibility of this category was unclear and would likely bias relative volume and

weight of remains.

 Quantitative analysis of diet.—To compare diversity and evenness of diets of

packs with and without pups, Shannon-Wiener diversity indices were calculated (Merrick

et al. 1997). Calculations of Shannon-Wiener diversity indices were based on counts of

occurrence where presence of prey in a scat was counted as one and absence counted as

zero.

 To determine which variables best accounted for variation in diet based on

differences in occurrence of food in scats, I developed generalized linear models. A

global model with a Poisson distribution and an offset, to account for differences in

number of samples of scats between packs, was first constructed by modeling total

observed counts grouped by pack and year against a four-way interaction between food

category, pack, year, and reproductive status. The most-parsimonious model was

determined using Akaike's Information Criterion corrected for small samples (AICc;

Burnham and Anderson 2002) and was selected from among the global model and all

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possible subsets. Odds ratios were then interpreted with a link function (Manly et al. 2002), using estimates of coefficients of the most-parsimonious model to determine likelihood of one pack consuming a food category versus another pack consuming the same prey or the likelihood of a pack consuming one food over another.

Proportions of samples were derived using counts of samples of a food category in the diet of a pack divided by the sum of counts of samples of all prey for that pack. Available proportions were derived for a food category by dividing estimated availability (size of population; see below) of a food category available within the territory of a pack by the sum of all food categories within the territory of that pack. Estimated size of a population of a food category was weighted by body mass (in kg) of individual food items because ratio was not always 1:1 for one food versus another (i.e., one deer was not equivalent to one small rodent). Selection indices were calculated by dividing proportion of counts in samples by corresponding available proportion of a food category for a given pack (Manly et al. 2002). Chi-squared tests were used to test the null hypothesis that packs were consuming food in proportion to availability. Selection of each food category was determined by evaluating range of the 95% confidence interval surrounding the sample proportion.   If available proportion of a food category was >95% confidence interval then food category was selected. If available proportion of a food category was <95% confidence interval the food category was avoided. Confidence intervals were based on standard errors of sample proportions of food categories in diet of a pack and compared with available proportion of a food category within the territory of the same pack (Manly et al. 2002). Foods occurring ≤5 times in the diet of a pack were deleted from analyses (Manly et al. 2002). Confidence intervals were adjusted using a

Bonferroni adjustment due to multiple statistical comparisons between confidence

intervals (Manly et al. 2002).

Measurements of availability.—Using GPS locations recorded every 5 hours from

collars deployed in 2009 and 2010 by personnel of Virginia Polytechnic Institute and

State University on at least one individual belonging to each of the six packs studied,

95% isopleths of home ranges of packs were derived using adaptive-local-convex-hull

methods (a-LoCoH; Getz et al. 2007). Boundaries of home ranges derived using this

method delineated available habitat from which foods were selected by each pack.

Ninety-five percent isopleths of pack home ranges were overlaid onto GIS maps

developed by McKerrow et al. (2006), which modeled suitable habitat for food categories

of interest within the Red Wolf Recovery Experimental Population Area. This allowed

me to predict the possibility of presence of each type of food. Presence of a food in scats

of a pack verified presence of that species within the territory of that pack and suitability

of habitat within the territory for that food category. Foods absent in scats of a pack were

ignored and their availability not assessed. GIS maps were available for all mammalian

foods in this study. From GIS maps, the percentage of home range of each pack that was

suitable for a given food was determined. This percentage was then multiplied by area of

home range (in ha) resulting in total area within a home range where a food category was

expected to occur. Total area was then multiplied by expected density of a food, taken

from the literature (small rodents: Cameron and McClure 1988, Kaufman and Kaufman

1990; large rodents: Allen and Hoffman 1984, Carter et al. 1999; rabbits: Chapman et al.

1982; wild boars: Hanson et al. 2009; raccoons: Riley et al. 1998; and deer and fawns:

Verme 1969, DeNicola et al. 2008) , to give an estimate of number of individuals of a

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food category present within a home range at any time. I assumed densities of foods were relatively constant from one year to the next. Number of individuals of an available food was standardized according to mass of deer (ca. 57 kg; Silva and Downing 1995). For example 57 kg of rabbit was the equivalent of one deer. This was done to account for large differences in size of one food over another. Available biomass of anthropogenic material was determined by measuring dimensions of garbage dumps within home ranges of packs. Available biomass of the category designated as 'other' was determined by assuming density of food making up this category was equal across home ranges of all packs. Available biomass of wild boars was assumed to account for both domestic pigs and wild boars assuming availability of domestic pigs was low enough that it would not drastically affect overall availability of wild boars. Again, these prey items were standardized according to mass of deer. Only the Tyson and Timberlake packs consumed wild boars (domestic and wild, respectively) enough (>5) to warrant inclusion in analyses of selection. Only the Milltail pack consumed anthropogenic material and 'other' enough to warrant inclusion in analyses of selection Indices of selection and confidence intervals for Milltail and Tyson packs were calculated with and without food categories anthropogenic material and 'other,' and wild boar (domestic), respectively, to better determine robustness of selection indices and confidence intervals for all food categories. This was done because derivation of availability measurements for these food categories differed from the rest of the food categories. Milltail and Tyson were the only packs that consumed anthropogenic material and 'other', and wild boar >5 times, respectively, to warrant inclusion in analyses of selection.

Results

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Scats collected.—In 2009 and 2010, 176 and 279 red wolf scats were collected, respectively, for a total of 455 scats. At least 26 scats were from each pack per year. Rarefaction curves of dietary diversity for each pack per year leveled off at 20 scats regardless of diversity of diet. Thus, samples were sufficient.

Descriptive analyses.—Estimates of consumption of biomass indicated that deer and fawns represented 66% of total biomass of foods consumed. Percent frequency of occurrence, relative volume, and weight of remains indicated deer and fawns represented 37, 49, and 49% (respectively) of total foods consumed by all packs (Fig. 2). The biomass model ranked large-sized foods higher and small-sized foods lower in importance compared to the other three metrics. Percent frequency of occurrence ranked small-sized foods higher, and large-sized foods lower, compared to other metrics. Spearman-rank correlation coefficients showed strong agreement between ranks of importance of food categories between metrics both within and across packs ($r_s > 0.78$).

Indices of diversity and generalized linear modeling.—Indices for Shannon-Wiener diversity and evenness showed that packs with pups had more diverse and even diets ($H_s = 2.15$, $J = 0.98$) than packs without pups ($H_s = 1.68$, $J = 0.76$). The global generalized linear model, developed to examine variation in counts of occurrence of food categories in the diet between packs, had a number of cells with <5 counts. These low-value cells resulted in the global model failing to behave asymptotically and it was excluded from consideration for most-parsimonious model (Burnham and Anderson 2002). Based on AICc rankings, one model was superior to all others and included prey, pack, and prey-by-pack interactions. The Akaike weight of the most-parsimonious model was 0.96. The next best model included reproductive status as a variable and had a

ΔAICc = 170, and Akaike weight = 2e -38. Because one model was far superior to all others, only coefficients for the best model were used to derive odds ratios using the link function e$^{(coefficient)}$ (Table 2.1).

Food categories were consumed in differing amounts between packs. The Milltail pack was most likely to consume anthropogenic material, 'other,' and small rodents. The Northern pack was most likely to consume deer, fawns, and rabbits. The Tyson pack was most likely to consume raccoons and wild boars. The Kilkenny pack was most likely to consume large rodents. Because year was not an important variable in the best model, analyses of selection were conducted with data combined across years.

Selection indices and confidence intervals.—All packs selected deer, fawns, or both, during pup-rearing season; based upon selection indices using counts of occurrence of foods in diet divided by estimated availability of foods standardized by mass of deer (Table 2.2). Remaining food categories were either avoided or consumed in proportion to availability across packs with the exception of the Kilkenny pack, which selected for large rodents, and Tyson pack, which selected for wild boars (domestic). Selection indices and confidence intervals of proportions of samples for Milltail and Tyson packs were robust. There was no difference between selection indices when anthropogenic material and 'other,' and wild boar (domestic), respectively, were included in calculations, versus when they were not included. Thus, I report selection indices and confidence intervals of sample proportions for Milltail and Tyson packs with anthropogenic material and 'other,' and wild boar, respectively, included in analyses (Table 2.2).

Discussion

This study revealed that packs of red wolves consumed most species of mammalian foods that occurred within their territories during pup-rearing season. Packs selected fawns and deer over other foods during pup-rearing season (Table 2.2). Selection of adult deer and fawns was expected, given the size of adult red wolves (male red wolves average 27.5 kg, females 21.5 kg; Paradiso and Nowak 1972), their tendency to hunt in packs, and energetic demands of rearing pups during this time of year. All of these factors likely contribute to selection of large-sized foods by red wolves.

All proportions of availability and selection indices, of each food category during pup-rearing season, were considered accurate relative to one another. Only proportions of availability and selection indices for the categories anthropogenic material, other, and wild boar (for Tyson pack only due to consumption of domestic pigs) are suspect due to an inability to accurately quantify availability of these food categories. Milltail pack was the only pack that consumed the categories anthropogenic material and 'other' in sufficient amounts over both years. The den or rendezvous site of Milltail pack was ca.1 km from a county dump. Category 'other' consisted of a number of prey combined together (e.g., ground-dwelling birds and their eggs, insects). It is likely that estimates of availability for 'other' were conservative relative to other food categories. Increasing availability of 'other' would only favor the conclusion of proportional consumption or avoidance of this food category. The den or rendezvous site of Tyson pack was ca. 1.5 km from a pig farm, which routinely dumped remains of pigs into a carcass pit accessible to Tyson pack. I was unable to quantify availability of domestic pigs for Tyson pack via the carcass dumping site. Because carcasses were only added to the site periodically (every 1-2 weeks), the small size of the site (5 by 8 m), and because carcasses were

ground up and mixed with wood chips to aid in decomposition, it is likely that the carcass

pit did not significantly alter proportion of availability of pigs relative to the rest of the

foods consumed by Tyson pack. This method of calculating availability of prey is

inferior to on-the-ground trapping of animals for capture-mark-recapture studies to

determine density of a given species. However, such methods are labor, time, and cost

intensive, and were not feasible for my study.

The most-parsimonious generalized linear model suggested that year and

reproductive status were relatively unimportant in predicting occurrence of foods in diet

of packs. However, arrival of pups did correspond with an increase in diversity of diet of

packs. Packs with pups also consumed all relevant foods in more equal amounts to one

another. Such a response to an increase in energy demands due to arrival of pups is

expected in large carnivores (Mech 1970).

Although, overall, packs selected and relied primarily on adult deer, fawns, or

both, during pup-rearing season (Fig. 2.2, Table 2.2), packs differed from one another in

consumption of foods. It is likely that this prey-by-pack interaction was related to

consumption of secondary or tertiary foods (foods consumed but not consumed as much

as much as deer or fawns, e.g., small rodents, rabbits, and large rodents) and not primary

foods (deer and fawns). This could be the result of an increase in abundance of a given

prey within the territory of a pack compared to territories of adjacent packs, or an

increase in selection for a given food by a particular pack relative to adjacent packs.

Variation in diet between groups of social carnivores could be the result of differential

foraging skills and habits transmitted along lines of kinship (Mech 1970). Variation in

diet due to differences in habitat occupied by each pack is unlikely given the similarity in

habitat within territories of packs in my study (Beck et al. 2009). Variation in diet of red wolves appears to be related primarily to consumption of secondary and tertiary foods, not primary prey, which were adult deer and fawns.

Consumption of anthropogenic material and domestic pig was not surprising given that home ranges of Milltail and Tyson packs contained an active garbage dump and carcass pit, respectively. Ciucci et al. (1997) reported a pack of gray wolves in Italy relied almost entirely on anthropogenic material from garbage dumps and remains of domestic animals from carcass dumps, although wild medium-to-large-sized mammals were present within territories of packs they studied. Hairs of wild boars were in scats of animals belonging to all six packs, but only Timberlake pack consumed wild boars ≥ 5 times (Manly et al. 2002). This agrees with reports by Lucash and Crawford (1993) and Phillips et al. (2003) that packs of red wolves can hunt and bring down wild boars.

These results could raise concern about the reliance of wild red wolves on foods associated with humans. However, given that only two of the six packs consumed, and only one selected, easily obtainable foods associated with humans, reliance of wild red wolves on foods associated with humans does not seem to be widespread. Consumption and selection for these foods can be explained by presence of these items in territories of packs. However, biologists might consider actions to reduce reliance of these packs on foods associated with humans to reduce potential of red wolf-human interactions. Preliminary methods concerning mitigation of human-carnivore interactions suggest reliance of large carnivores on foods associated with humans invariably leads to negative human-carnivore interactions (Barlow et al. 2010). Barlow et al. (2010) suggest that, in the face of little scientific data, fencing placed around sources of human-derived foods is

generally a good method for reducing reliance of large carnivores on such sources.

It is unclear whether Tyson and Milltail packs require human-related foods to maintain current size and reproductive rate of packs, or if they would compensate for loss of access to human-related foods by increasing consumption of natural prey if they were prevented from accessing human-related foods. Currently, these packs are two of the largest, with the highest reproductive rates, in the Red Wolf Recovery Experimental Population Area (Art Beyer pers. comm.). I suspect that, given the ability of these two packs to capture and consume natural prey (Fig. 2; Table 1), eliminating their access to human-related foods would not affect their long-term survival. However, reproductive rate and pack size might decrease due to a reduction of easily obtainable, high-calorie foods. Furthermore, the Milltail and Tyson packs likely would increase home range size to compensate for reduction of available food. Thus, it is possible that such concentrated high calorie foods influence use of habitat within the home range and size of home range. Size of home range of wolves is partly determined by energetic needs of the pack, and packs will increase size of home range if densities of primary prey decrease (Mech 1970).

In conclusion, reintroduction of red wolves to part of their historic range is a success for biologists and conservationists, especially given the historically cautious and sometimes negative public perceptions of carnivores (Mech 1970). My study is the first to quantitatively analyze foraging ecology of red wolves in the Red Wolf Recovery Experimental Population Area following initial reintroduction and can aid in determining suitability of future sites for reintroduction of red wolves within their historic range. Results of my study show that after >20 years since red wolves were first released into the Red Wolf Recovery Experimental Population Area, most packs are surviving

completely by consuming wild prey, at least during pup-rearing season, within a human-altered landscape. The ability of red wolves to forage on naturally occurring prey in a human-altered landscape demonstrates large carnivores are capable of surviving and reproducing in close proximity to humans. This is important because prior to reintroduction of red wolves, no large carnivore had been successfully reintroduced from captivity into the wild (Phillips et al. 2003).

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Table 2.1. Odds-ratios for number of occurrences in diet of red wolves (*Canis rufus*) in the Red Wolf Recovery Experimental Population Area in northeastern North Carolina, 2009-2010. Odds-ratios were derived from estimates of coefficients of the most-parsimonious, generalized linear model using link function, $e^{(coefficient)}$.

Pack	Food category								
	Anthropogenic material	Deer	Fawns	Large rodents	Other	Wild boars	Rabbits	Raccoons	Small rodents
Columbia	0	1.00[a]	5.81	0.43	0.14	0	1.42	0.28	1.28
Milltail	5.26	2.72	1.14	0.71	4.95	0	0.57	1.84	5.70
Timberlake	0.14	4.01	6.11	1.99	0.14	1.99	3.71	0.28	0.43
Tyson	0.14	0.71	4.14	1.00	0.43	4.55	2.27	2.56	4.14
Northern	0.28	5.99	8.17	2.27	0.85	0.28	5.10	1.02	0.28
Kilkenny	0	0.86	5.81	6.27	0	0.28	0.79	0.28	2.00

[a]Reference odds-ratio

Table 2.2. Estimation of selection indices and 95% confidence intervals for consumption of food by each pack of red wolves (*Canis rufus*) in the Red Wolf Recovery Experimental Population Area in northeastern North Carolina, 2009-2010 (data from both years combined). An * indicates foods selected, a [#] indicates foods avoided, and no symbol indicates foods consumed in proportion to availability. If available proportion is less than 95% confidence interval then food item was selected. If available proportion is greater than 95% confidence interval then food item was avoided.

Pack	Food category	Proportion of availability of food category	Number of occurrences of food category	Proportion of occurrences of food category	Selection index of food category	Confidence intervals	
						Lower	Upper
Columbia	Deer	0.06	7	0.10	1.67	0.01	0.20
	Fawns	0.02	41	0.60	60	0.45	0.75*
	Rabbits	0.63	11	0.17	0.27	0.05	0.27[#]
	Small rodents	0.29	9	0.13	0.45	0.03	0.24[#]
Milltail	Deer	0.04	19	0.13	3.25	0.05	0.20*
	Fawns	0.01	8	0.05	5	0.01	0.10
	Raccoons	0.12	13	0.09	0.75	0.02	0.15
	Small rodents	0.49	40	0.26	0.53	0.17	0.36[#]
	Other	0.17	35	0.23	1.35	0.14	0.32
	Anthropogenic material	0.17	37	0.24	1.41	0.15	0.34
Timberlake	Deer	0.07	28	0.23	3.29	0.13	0.33*
	Fawns	0.02	43	0.36	18	0.24	0.47*
	Rabbits	0.65	26	0.21	0.32	0.12	0.31[#]
	Large rodents	0.24	14	0.12	0.50	0.04	0.19[#]
	Wild boars	0.02	10	0.08	4	0.02	0.15

Table 2.2. Continued

Pack	Food category	Proportion of availability of food category	Number of occurrences of food category	Proportion of occurrences of food category	Selection index of food category	Confidence intervals	
						Lower	Upper
Tyson	Fawns	0.01	11	0.10	10	0.02	0.17*
	Rabbits	0.15	16	0.14	0.93	0.05	0.23
	Raccoons	0.13	18	0.16	1.23	0.06	0.25
	Small rodents	0.65	29	0.26	0.40	0.15	0.37#
	Large rodents	0.05	7	0.06	1.20	0.01	0.12
	Wild boars	0.01	32	0.28	28	0.17	0.40*
Northern	Deer	0.07	21	0.28	4	0.15	0.40*
	Fawns	0.02	29	0.37	19	0.24	0.52*
	Rabbits	0.51	18	0.24	0.47	0.11	0.36#
	Large rodents	0.40	8	0.11	0.28	0.02	0.19#
Kilkenny	Fawns	0.01	22	0.37	37	0.21	0.52*
	Rabbits	0.11	9	0.15	1.36	0.03	0.27
	Small rodents	0.79	7	0.11	0.14	0.01	0.22#
	Large rodents	0.09	22	0.37	4.11	0.21	0.52*

Fig. 2.1. Map of Red Wolf Recovery Experimental Population Area in northeastern North Carolina and location of packs (*Canis rufus*) studied, 2009-2010. Map shows boundaries of counties, management zones of the Red Wolf Recovery Experimental Population Area (RWREPA), and federal and commercial lands.
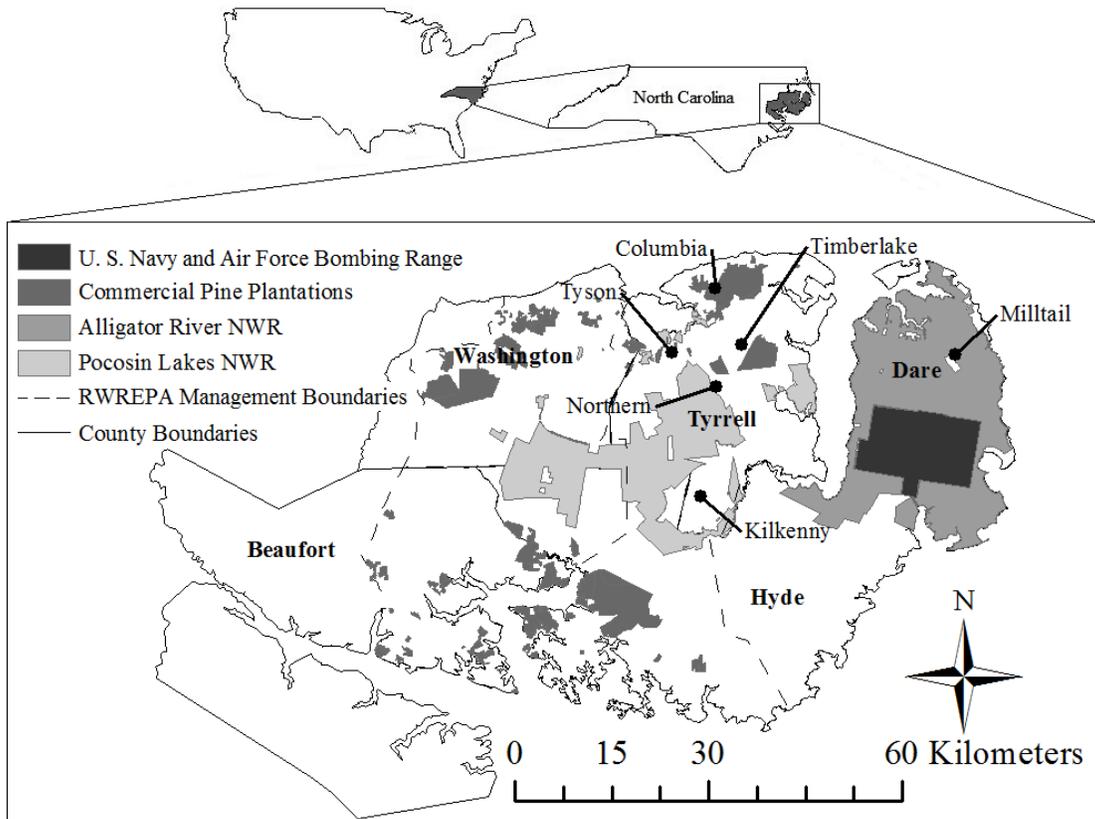
Fig. 2.2. Percentage composition of diet of packs of red wolves (*Canis rufus*) in the Red Wolf Recovery Experimental Population Area in northeastern North Carolina, 2009-2010, using four approaches to classifying diet components. All percentages for each pack sum to 100. Percentages show data from both years combined.
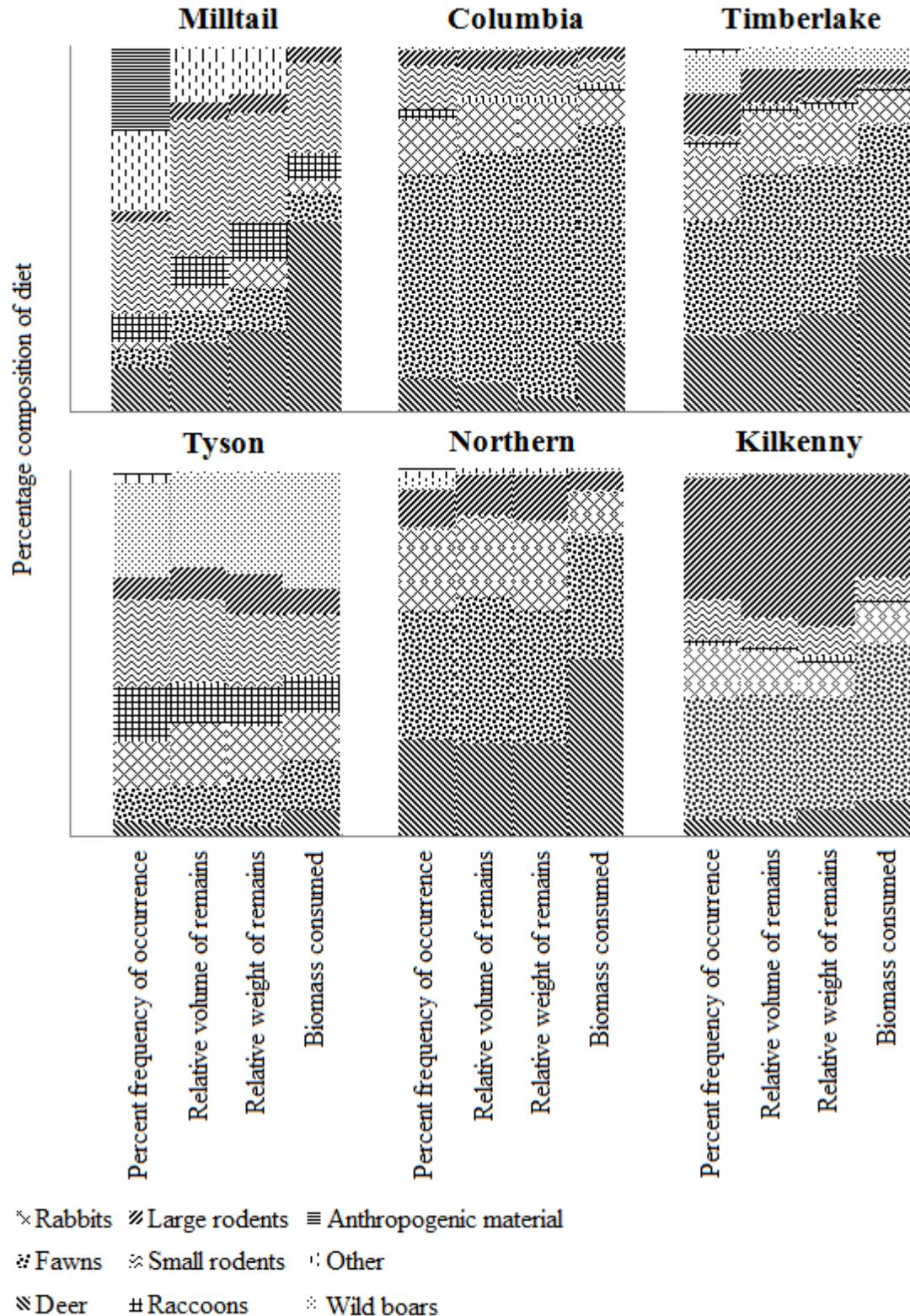
Chapter 3

Spatial Ecology of Red Wolves (*Canis rufus*) in Northeastern North Carolina

Abstract

Red wolves (*Canis rufus*) are endangered, with <150 individuals in the only wild population. Currently, little is known about selection of and habitats within this population. I developed resource-selection functions using locations obtained from radio-collared red wolves to examine patterns in selection of resources. At courser spatial scales (selection at the home range level), generalized linear models containing habitat, distance to roads and water, and population densities of humans were capable of predicting ($r^2 = 0.919$ and 21% improvement over generalized linear model of random selection according to AICc) occurrence of red wolves within the study area. Generally, all packs selected agricultural and early succession fields, areas close to dirt roads and away from water, and areas with few humans. At finer spatial scales (selection within the home range), these factors were unable to accurately predict ($r^2 = 0.921$ and 1.5% improvement over generalized linear model of random selection according to AICc) occurrence of red wolves within a home range. Factors used to analyze selection of habitat were scale dependent in red wolves

Introduction

The first step in conservation and management of endangered species usually involves gathering information about habitat use and environmental factors that influence selection of resources. Most studies combine data from animals fitted with Global Positioning System (GPS) or Very High Frequency (VHF) radio-collars (Millspaugh et al.

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2006) with Geographic Information System (GIS) analyses to provide information on movements and habitat features, respectively (Manly et al. 2002). Such studies can help identify important resources that need protecting, map potential distribution of animals, direct management actions, and mitigate limiting factors such as human-caused mortality (Johnson et al. 2004).

Animals require resources along a continuous scale from landscape to microhabitat (Johnson 1980). The importance of environmental factors in selection of resources can change if the fitness value of such factors is dependent on scale (McLoughlin et al. 2004). Rettie and Messier (2000) suggested that factors most able to limit fitness should be more important at larger scales of selection of resources, while factors less capable of limiting fitness are important only at smaller scales. Thus, studies can have varying results depending on the scale at which data are collected and analyzed, as well as the factors that are selected for examination (Orians and Wittenberger 1991).

Studies of selection of resources by large carnivores in human-altered landscapes will become more important to conservation and management as populations of humans increase. Prior to reintroduction of red wolves (*Canis rufus*) into part of their native range in 1987, no large carnivore had been successfully reintroduced (Phillips et al. 2003). For >20 years, the reintroduced population of red wolves has survived and reproduced in a habitat matrix altered by humans (Phillips et al. 2003). A better understanding of how red wolves select resources in a human-altered landscape is useful to future efforts to reintroduce red wolves into other parts of their historic range.

Red wolves once ranged across the eastern United States from Maine to Florida and central Texas to the Atlantic Ocean (Phillips et al. 2003). Research on selection of resources by red wolves in the wild prior to recovery efforts was limited due to small

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population size and difficulties in differentiating the few remaining adults and juveniles

from hybrids and coyotes (*C. latrans*; Carley 1975). Paradiso and Nowak (1972) and

Phillips et al. (2003) suggested that red wolves occupied warm, moist, and densely

vegetated habitats, including virgin pine and lowland hardwood forests, coastal prairies,

and marshes. Although these reports provided insight into selection of resources by red

wolves, they primarily derived from anecdotal accounts by early naturalists. No study

has quantitatively assessed selection of habitat by red wolves. My primary goal was to

describe the spatial ecology of red wolves. Specific goals were to examine selection of

habitat by red wolves and to determine whether selection varies with spatial scale.

<div align="center">Materials and Methods</div>

Study area.—My study area was within the Red Wolf Recovery Experimental

Population Area on the Albemarle Peninsula in northeastern North Carolina (Fig. 3.1). At

the time of my research, this was home to the only wild population of red wolves in the

world. The study area was described in Chapter 2. Types of land-cover and approximate

percentage of area were agricultural fields (30%), commercial pine (*Pinus*) plantations

(15%), pocosin (15%; *Pinus serotina* and *Persea palustris*), non-riverine swamp forests

(10%; *Nyssa*, *Liquidambar styraciflua*, *Acer rubrum,* and *Chamaecyparis thyoides*),

saltwater marsh or open water (10%), and other types of land-cover (20%). Climate was

characterized by four seasons of nearly equal length with annual precipitation averaging

127 cm. Temperatures averaged 5°C in winter and 27°C in summer. Elevation was from

sea level to 50 m (Beck et al. 2009).

Capturing and radio-collaring animals.—During January-October 2009, adult and

juvenile red wolves were captured by biologists with the United States Fish and Wildlife

Service using padded, number 3, foot-hold traps and fitted with mortality sensitive, Lotek

GPS 4400S radio-collars (Lotek Wireless, Inc., Newmarket, Ontario, Canada). Red

wolves >2 years old were classified as adults, <2 years old as juveniles, and <9 months

old as pups. Pups were not fitted with radio-collars because they were too small to safely

wear them. Following deployment, radio-collars recorded locations every 5 hours. Each

collar emitted a VHF beacon each day during 0900-1200 h, which allowed animals to be

located every 4-8 weeks for retrieval of data. To obtain data, radio-collared animals were

tracked from the air to determine their general location and data were downloaded from

50-500 m away, depending on vegetation and terrain.

　　　　Independence of movements.—One of my study packs had six animals with radio-

collars. Thus, we used these six animals to examine independence of movements among

individuals within the same pack. Home ranges (Getz et al. 2007) were constructed from

complete datasets for all radio-collared animals in that pack. Locations of an animal

were related to corresponding isopleths within the home range of that animal (Getz et al.

2007). Spearman-rank correlation matrices were calculated to determine similarities of

home ranges and use of habitat among all radio-collared animals within the pack. This

was done by matching each location for one animal with isopleths of each other animal in

the pack. Then I related the isopleth that each location for a given animal was contained

in, with the isopleths of each pack member that those same locations corresponded with.

This was done to determine if animals within a pack should be treated individually, or if

use of habitat, selection of resources, and home range of one animal was representative of

the entire pack.

　　　　Home range.—Rarefaction curves of size of home range were constructed using

datasets for each animal to determine if radio-collars had been deployed long enough so

that the entire home range and variation in use of habitats had been captured. I calculated

size of home range during the first week the collar was deployed, during the second week, and so on, until the complete dataset for that animal was included in calculating size of home range. Rarefaction curves were based on 95 and 50% isopleths of home range using adaptive, nearest-neighbor, convex-hull methods (α-LoCoH; Getz et al. 2007). Animals whose home ranges did not stabilize in size were excluded from subsequent analyses. Given that animals of various ages, dominance, and sex were radio-collared, and that composition of home ranges among packs with radio-collared animals varied, I assumed that all factors influencing stabilization of size of home range with number of locations were accounted for.

Use and selection of habitat.—Resource-selection functions were used to examine patterns of selection of home ranges (2nd-order selection of resources; Johnson 1980) and patterns of selection within home ranges (3rd-order selection of resources; Johnson 1980) following Manly et al. (2002).

For 2nd-order selection of resources, the entire Red Wolf Recovery Experimental Population Area was considered to be available habitat and all locations of each radio-collared animal occurring within its respective home range was habitat that was used (95% α-LoCoH; Getz et al. 2007). For 3rd-order selection, the entire home range of each animal was considered to be available and all locations of each radio-collared animal occurring within its respective home range was habitat that was used (95% α-LoCoH; Getz et al. 2007). Animals were tracked for varying times and numbers of locations. To account for effect of varying representation of each animal on resource-selection functions (due to each animal having a different number of locations), I set number of locations for all animals equal to number of locations obtained for the animal with the fewest locations. To do this, locations of each animal, other than the animal with fewest

locations, were randomly selected for inclusion in the resource-selection function. All locations among individuals were combined to examine 2nd-order and 3rd-order selection of resources for the entire population. An equal number of random points, compared to locations, were generated within the available areas for 2nd–order and 3rd-order selection of resources, respectively. Distance to roads and water, density of humans (US census bureau), and habitat (McKerrow et al. 2006) were determined for used and available locations. Habitats, according to McKerrow et al. (2006), were agricultural and early succession fields, lowland and upland forests, wetlands, and pocosin (an upland wetland composed of thick evergreen vegetation). Of locations that were used and available, 75% were used to build resource-selection functions (training data). The remaining 25% of locations that were used and available were withheld to evaluate the final resource-selection function using cross-validation (test data; Johnson et al. 2006). A resource-selection function, with a binomial distribution, was calculated using used and available locations. Habitat, distance to roads and water, and density of humans, along with all biologically meaningful two-way interactions (habitat by distance to roads, habitat by densities of humans, and distance to roads by density of humans) were associated with use or availability of a location (Manly et al. 2002). Use and availability of habitat were coded as 1 or 0, respectively. Akaike's information criterion corrected for small samples (AICc) was used to choose the most-parsimonious, resource-selection function from the global generalized linear model and all possible subsets of the global generalized linear model (Burnham and Anderson 2002). When ΔAICc among models was <2, the most-parsimonious model was used (Burnham and Anderson 2002; Johnson et al. 2006).

Following estimation of resource-selection functions with training data, estimates of coefficients of the most-parsimonious resource-selection function were projected in a

GIS layer of the Red Wolf Recovery Experimental Population Area using a Poisson

regression formula. This resulted in pixels (30 by 30 m) of GIS layers being given values

(continuous scale from 0-1) based on the most-parsimonious, resource-selection function.

Values were divided into 10 ordinal classes of equal size (e.g., 0.0-0.1, 0.1-0.2) and the

mean for each ordinal class was calculated. Mean values for each ordinal class were used

to calculate utilization values for each ordinal class (Johnson et al. 2006). Then test data

were overlaid onto a projected GIS layer and related to corresponding values of the

projected resource-selection function. Next I determined number of locations in the test

data that fell into each ordinal class. Number of expected locations in each ordinal class

was then calculated using the number of use locations in the test data that fell into each

ordinal class, the average value of each ordinal class, and the area encompassed by each

ordinal class (Johnson et al. 2006). Proportion of expected locations and observed

locations used within each ordinal class were compared using linear regression (Johnson

et al. 2006). Student's *t*-tests were used to determine if the slope of the linear regression

line depicting proportion of observed locations within each ordinal class significantly

differed from 0 (use of habitat in proportion to availability of habitat; random use of

habitat) and 1 (proportional to probability of use of habitat), respectively, and if the y-

intercept significantly differed from 0 (proportional to probability of use of habitat).

Both R^2 of linear regression and a χ^2 goodness-of-fit test were used to assess fit of linear

regression relative to line of proportion of expected locations within each ordinal class

(equals proportional probability of use of habitat with slope of 1 and y-int of 0; Johnson

et al. 2006).

AICc weights of most-parsimonious, 2nd-order and 3rd-order, resource-selection

functions were compared to determine whether habitat, distance to roads and water, and

density of humans were scale dependent for red wolves. The resource-selection function

with the greatest AICc weight demonstrated the scale at which habitat type, distance to

roads and water, and density of humans influenced selection of habitat the most.

<div align="center">Results</div>

During January-October 2009, 15 red wolves from 10 packs were captured, radio-

collared, and monitored for 7-12 months. Spearman-rank correlations demonstrated that

use of habitat and size of home range were correlated among all six animals that had

radio-collars in the same pack ($r_s = 0.87–0.91$). The animal used to represent the pack

(11544M) was randomly chosen from among the six animals. Mean (± 1 *SD*) rate of

success in obtaining scheduled GPS locations from radio-collars was $88 \pm 4.4\%$ (range:

84-97%; Table 3.1).

Rarefaction curves of size of home range (50% and 95% α-LoCoH; Getz et al.

2007) leveled off at 10 (50%) and 24 (95%) weeks. The fewest number of weeks an

animal was monitored was 28 (11539F, 11458M, 11165F, 11469M); thus, samples were

sufficient for all animals. Mean size of home range (95% α-LoCoH; Getz et al. 2007)

was 26 ± 17.8 km^2 (range: 6.5-64.9 km^2), and mean size of core area (50% α-LoCoH;

Getz et al. 2007) was 3 ± 1.8 km^2 (range: 1.2-5.4 km^2; Table 3.1).

I used 6,120 locations of red wolves and an equal number of randomly generated

locations within the Red Wolf Recovery Experimental Population Area, and home ranges,

as data to construct the 2nd-order and 3rd-order, resource-selection functions, respectively

(Table 3.2). The resource-selection function containing variables for habitat, distance to

roads and water, density of humans, an interaction term of habitat by distance to road,

and an interaction term of habitat by density of humans was the most-parsimonious

model for 2nd-order selection. The AICc weight of the most parsimonious 2nd-order

<div align="center">43</div>

resource-selection function was 0.98. The next most parsimonious model included an interaction between density of humans and distance to road, and had a ΔAICc value of 8, with an AICc weight of 0.02. Spearman-rank correlations indicated interaction terms were not collinear ($r_s = 0.31$ and 0.07 for 2nd-order and 3rd-order, respectively). The model containing variables for habitat, distance to road, and interaction terms of habitat by distance to road was the most-parsimonious model for 3rd-order selection. Spearman-rank correlations indicated interaction terms were not collinear (distance to road by upland forest: $r_s = 0.13$; distance to road by pocosin and lowland forest: $r_s = 0.07$). The AICc weight of the most parsimonious 3rd-order resource-selection function was 0.42. The next most parsimonious model included distance to water and had a ΔAICc value of <1 with an AICc weight of 0.29.

I used 2,040 locations of red wolves and an equal number of randomly generated locations within the Red Wolf Recovery Experimental Population Area, and home ranges, for testing data for cross validation and evaluating fit of the 2nd-order and 3rd-order, resource-selection functions, respectively. Student's t-tests demonstrated that slope of the linear regression of proportion of observed versus expected locations that were used in each ordinal class for the 2nd-order resource-selection function was significantly different from 0 ($F_{1, 16} = 11.3$, $P < 0.01$), but not different from 1 ($F_{1, 16} = 1.32$, $P > 0.05$; Fig. 3.2a). The Y-intercept was not different from 0 ($F_{1, 16} = 1.05$, $P > 0.05$). Furthermore, an R^2 (0.919) and χ^2 goodness-of-fit test ($\chi^2 = 0.059$, $P > 0.05$) indicated a good fit between proportion of observed versus expected locations used in each ordinal class for the 2nd-order, resource-selection function. With respect to 2nd-order selection of resources, red wolves avoided pocosin, lowland forest, wetland, and upland forest habitat relative to the reference habitats (agricultural and early succession fields). According to odds ratios

(Manly et al. 2002), reference habitats were 1.9 and 2.6 times as likely to have red wolves than pocosin, lowland forest, and wetland and upland forest, respectively. Red wolves selected areas near roads and avoided areas near water and increased density of humans. For every 100 m closer to a road a habitat patch was, red wolves were 2.7 times as likely to occupy the habitat. For every 100 m further from water a habitat patch was, red wolves were 2.7 times as likely to occupy the habitat. For every 10 person/km^2 decrease in density of humans for a habitat patch, red wolves were 2 times as likely to occupy the habitat. With respect to interaction terms, use of pocosin, lowland forest, and wetland habitats decreased as distance to roads increased; use of upland forest and wetland habitats increased as density of humans increased. For every 100 m closer to a road a patch of pocosin, lowland forest, or wetland habitat was, red wolves were 2.7 times as likely to occupy the habitat. For every 10 person/km^2 increase in density of humans for upland forest and wetland habitat, red wolves were 2.2 times as likely to occupy the habitat. Confidence intervals for these variables did not overlap 0, suggesting good precision and strong inference (Johnson et al. 2004). The 2nd-order, resource-selection function predicts a patchy distribution of red wolves across the Red Wolf Recovery Experimental Population Area (Fig. 3.3a).

Student's *t*-tests demonstrated that slope of the linear regression of proportion of observed versus expected used locations in each ordinal class for the 3rd-order, resource-selection function was significantly different from 0 ($F_{1, 16} = 17.5$, $P < 0.01$), but not different from 1 ($F_{1, 16} = 1.26$, $P > 0.05$; Fig. 2.2b). The Y-intercept was not different from 0 ($F_{1, 16} = 0.59$, $P > 0.05$). Furthermore, an R^2 (0.921) and χ^2 goodness-of-fit test ($\chi^2 = 0.126$, $P > 0.05$) indicated a good fit between proportion of observed versus expected use of locations in each ordinal class. With respect to 3rd-order selection of resources, red

wolves selected reference habitats (agricultural fields and wetlands) relative to early

succession, lowland forest, pocosin, and upland forest habitats. According to odds ratios

(Manly et al. 2002), reference habitats were 1.2 and 1.5 times as likely to be used as early

succession, lowland forest, and pocosin and upland forest habitats, respectively. Red

wolves selected areas near roads. For every 100 m closer to a road a habitat patch was,

red wolves were 2.7 times as likely to occupy the habitat. With respect to interaction

terms, use of upland forest increased as distance to roads increased, while use of pocosin

and lowland forest decreased as distance to roads increased. For every 100 m further

from a road a patch of upland forest habitat was, red wolves were 2.7 times as likely to

occupy the habitat. For every 100m closer to a road a patch of pocosin or lowland forest

habitat was, red wolves were 2.7 times as likely to occupy the habitat. The 3rd-order

resource-selection function predicts a relatively equal probability of use of habitat by red

wolves across a given home range, with most of the area within a given home range

having a relative probability of use of 40-60% (Fig. 3.3b).

The most parsimonious 2nd-order resource-selection function had an AICc weight

of 0.98, with the next most parsimonious resource-selection function having an AICc

weight of 0.02. The most parsimonious 3rd-order resource-selection function had an

AICc weight of 0.42, with the next most parsimonious resource-selection function having

an AICc weight of 0.29. Habitat, distance to roads and water, and density of humans

influenced selection of habitat more at coarser spatial scales.

Discussion

Theory dealing with selection of resources indicates that animals should avoid

factors that limit fitness (Orians and Wittenberger 1991). McLoughlin et al. (2004)

suggested that patterns of resource selection that allow for avoidance of such limiting

factors should be strongest at larger scales of selection. Less important limiting factors would become more important at smaller scales of selection of resources. For example, in woodland caribou (*Rangifer tarandus*), risk of predation has a strong influence on selection of resources at large spatial scales, while quality of forage is more important at smaller spatial scales, thus predation is seen as the more important limiting factor (Rettie and Messier 2000).

My resource-selection functions suggested that variables of interest in my study (habitat, distance to roads and water, and density of humans) appeared to influence selection of habitat more at coarser spatial scales (Figs. 3.3a and 3.3b). Habitat likely is related to abundance of prey. Red wolves selectively prey upon white-tailed deer (Chapter 2), which occupy habitats selected by red wolves, i.e., agricultural land and early successional fields (Vercauteren and Hygnstrom 1998; Table 3.2).

Distance to roads possibly influences access to favored prey. Most roads in the Red Wolf Recovery Experimental Population Area are unpaved gravel or dirt roads used for agricultural purposes (C. Lucash pers. comm.). Thus, red wolves likely use these low-traffic roads for hunting because of greater visibility and mobility than in other areas. Much of the Red Wolf Recovery Experimental Population Area is subject to rising water levels due to proximity to the ocean, resulting in many areas being inundated frequently. Areas subject to regular inundation are likely avoided by primary prey (deer and fawns) and, thus, a good reason why red wolves avoid areas near water. The 2nd-order, resource-selection function suggests that areas with much water are used only when bisected by roads (Table 3.2). Thus, red wolves only use such habitat as travel corridors when roads are present. Such an interaction between distance to road and high-water habitats could allow for packs to persist in areas where habitats are highly interspersed and large parcels

of quality habitats are few.

Avoidance of areas with more humans does not mean that red wolves cannot persist in human-altered landscapes. However, given the choice between areas of high and low density of humans, it is reasonable that red wolves select areas with low density of humans; especially given that red wolves experience high mortality due to humans (Fazio 2007). Thus, most red wolves occur in areas with low density of humans. Large carnivores can persist in areas with many humans as long as animals learn behavioral measures for decreasing human interaction, such as caution and when to travel (Ciucci et al. 1997). It is unclear what effect such behaviors might have on fitness of large carnivores. The 2nd-order, resource-selection function demonstrated that upland forests and wetlands were less likely to be occupied by red wolves compared to agricultural and early successional fields. As density of humans increased, red wolves were more likely to occupy upland forests and wetlands when density of humans was low (Table 3.2). At this time it does not appear that occupation of these originally avoided habitats by red wolves negatively influences fitness. A pack of red wolves whose home range consisted primarily of upland forests (60%) surrounded by areas with relatively higher densities of humans, raised a litter of pups during the time of this study (C. Lucash pers. comm.), while he inhabited primarily (60%) upland forests surrounded by areas with higher density of humans. Upland forests were avoided in areas with fewer humans.

My 3rd-order, resource-selection function predicted a relatively equal probability of use of habitat by red wolves across a given home range (Fig. 3.3b), suggesting that at finer spatial scales red wolves select habitat in proportion to availability. However, red wolves did not select habitat at random or in proportion to availability. This is verified by persistent 50% isopleths within home ranges (Table 3.1). Thus, red wolves are using

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some parts of their home range more than others. It is evident that different factors influence 3rd-order selection of habitat compared to 2nd-order selection of habitat. McLoughlin et al. (2004) suggested that gray wolves (*Canis lupus*) selected habitats within their home range based on areas with localized plant communities favored by important prey and areas favorable for sheltering pups.

Within my study area, plant communities were coarse-grained with little variation over small spatial scales (Beck et al. 2009). Thus, important prey probably were not concentrated in a few specific locales within home ranges. However, concentrated high-calorie foraging sites existed in the form of garbage dumps and carcass pits containing remains of wild (associated with hunting outfitters) and domestic (associated with livestock operations) animals (C. Lucash pers. comm.). Upon discovery of such sites within their home range, a pack is likely to visit these sites regularly, making such sites a focal part of the home range. The home ranges of Milltail and Tyson packs contained such concentrated high-calorie sites. These sites were contained within core areas (50% α-LoCoH; Getz et al. 2007) of each animal. Furthermore, diets of these packs contained proportionally large amounts of items from these sites (23% garbage items for Milltail, and 26% remains of domestic pigs for Tyson pack, respectively; Chapter 2). However, not all packs had such sites within their home ranges (Timberlake, Columbia, and Northern), nor did dietary analyses show evidence of consumption of such food items by those packs (Chapter 2). Timberlake, Columbia, and Northern packs had proportionally larger core areas (50% α-LoCoH; Getz et al. 2007) in home ranges (Table 3.1) than Milltail and Tyson packs and, thus, used space within their respective home ranges more evenly.

Little is known about denning ecology of red wolves and factors associated with

selection of den sites. Den sites of red wolves in my study were known. Den sites where pups were born usually were in areas with thick vegetation and low visibility, possibly providing concealment of pups. Preliminary analyses have shown that isopleths of core areas (50% α-LoCoH; Getz et al. 2007) of Milltail and Tyson packs contained known den sites (other animals in my study had pups, but den sites were not located). Future work is needed to associate den sites with various characteristics of habitats to determine what constitutes favorable denning habitat in an attempt to better understand 3rd-order selection of habitat. However, once pups became mobile, members of packs routinely moved pups to various rendezvous sites within the home range. Potentially, this reduces the effect that suitable denning habitat would have on predicting 3rd-order selection of resources through time. This would require consideration of a temporal element in examining 3rd-order selection of resources.

My study highlights the importance of scale in attempting to predict selection of resources by wild animals. Furthermore, given that roughly half of the packs of red wolves in RWREPA during the time of this study reproduced (C. Lucash pers. comm.), packs appear to be selecting habitat within a human-altered landscape that can support reproduction. This is important because, prior to reintroduction of red wolves, no large carnivore had been reintroduced successfully (Phillips et al. 2003). My study is the first to quantitatively analyze spatial ecology of red wolves in the Red Wolf Recovery Experimental Population Area following initial reintroduction and it can serve as an important aid in determining criteria to be evaluated at sites for future reintroductions.

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Table 3.1. Identification number, age and sex of red wolves (*Canis rufus*) monitored, month released in 2009, duration of monitoring, number of GPS locations retrieved, percentage of GPS locations retrieved, and mean sizes of home range (95% and 50% α-LoCoH; Getz et al. 2007) for my study conducted on the Red Wolf Recovery Experimental Population Area in northeastern North Carolina, 2009-2010.

Animal	Month released in 2009	Duration (months)	Number of GPS locations retrieved	Percentage of GPS locations retrieved	Size of home range (95%; km^2)	Size of home range (50%; km^2)
11544M Adult male[a]	January	12	1,603	91	22.3	3.8
11300F Adult female	March	10	1,428	97	30.3	4.6
11657M Adult male	April	11	1,459	90	64.9	6
11577F Adult female	February	12	1,488	85	27	4.1
11539F Adult female	August	7	902	88	39.5	7.5
11628M Juvenile male[b]	February	9	1,108	84	15.7	3.2
11682M Juvenile male	January	12	1,489	85	11.3	1.14
11458M Adult male	October	7	927	91	8.5	0.9
11165F Adult female	April	7	953	93	6.5	1.8
11469M Adult male[c]	June	7	859	84	36.4	7.5

[a]Animal represents 5 other animals within its pack that also were radio-collared.
[b]Juvenile when radio-collared but grew to adulthood.
[c]Animal with fewest locations.

Table 3.2. Most-parsimonious, resource-selection functions according to Akaike information criterion corrected for small samples. Resource-selection functions are for 2nd and 3rd-order selection of resources by red wolves (*Canis rufus*). Resource-selection functions pertain to red wolves in the Red Wolf Recovery Experimental Population Area in northeastern North Carolina, 2009-2010.

Variables	Estimate	*SE*	Confidence intervals	
			Lower	Upper
2nd-order function				
Intercept	0.54	0.04	0.46	0.62
Pocosin	-0.65	0.06	-1.04	-0.49
+Lowland Forest				
+Wetland				
Upland Forest	-0.96	0.06	-1.11	-0.79
Human Density	-0.07	0.06	-0.08	-0.06
Dist. to Road	<-0.01	<0.01	<-0.01	<-0.01
Dist. to H20	<0.01	<0.01	<0.01	<0.01
Dist. to Road x	<-0.01	<0.01	<-0.01	<-0.01
(Pocosin				
+Lowland Forest				
+Wetland)				
Human Density x	0.08	0.01	0.07	0.10
(Upland Forest				
+Wetland)				
3rd-order function				
Intercept	0.13	0.03	0.07	0.23
Early Successional	-0.18	0.05	-0.46	-0.12
+Lowland Forest				
+Pocosin				
Upland Forest	-0.38	0.09	-0.70	-0.29
Dist. to Road	<-0.01	<0.01	<-0.01	<-0.01
Dist. to Road x	<0.01	<0.01	<0.01	<0.01
Upland Forest				
Dist. to Road x	<-0.01	<0.01	<-0.01	<-0.01
(Pocosin				
+Lowland Forest)				

Fig. 3.1. Map of Red Wolf Recovery Experimental Population Area (RWREPA) in northeastern North Carolina and location of packs of red wolves (*Canis rufus*) containing radio-collared animals, 2009-2010. Map shows boundaries of counties, management zones of the RWREPA, federal and commercial lands, and locations where each red wolf was captured, radio-collared, and released.
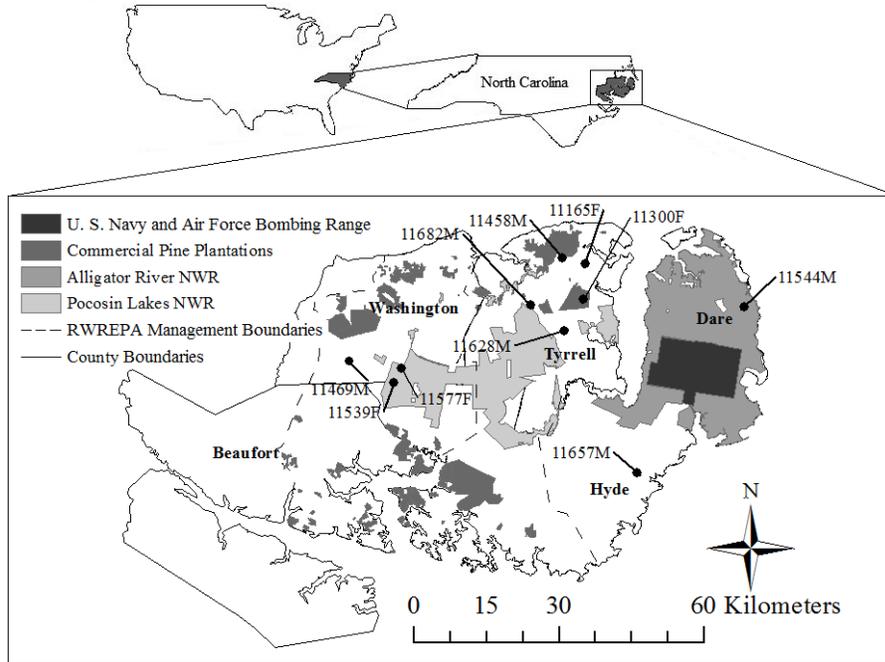
Fig. 3.2. Expected versus observed proportion of GPS fixes (in 10 ordinal classes) for an independent sample ($n = 2,040$) of locations of red wolves (*Canis rufus*) for the most-parsimonious: a) 2nd-order and b) 3rd-order, resource-selection functions. Random selection of resources would be displayed as observed values set to 0.1 (dotted line), while selection of resources proportional to probability of use (observed = expected) would occur along a line with slope = 1 and y-intercept of 0 (dashed line). Linear regression results are shown by a solid line. Locations are from radio-collared red wolves in the Red Wolf Recovery Experimental Population Area in northeastern North Carolina, 2009-2010.
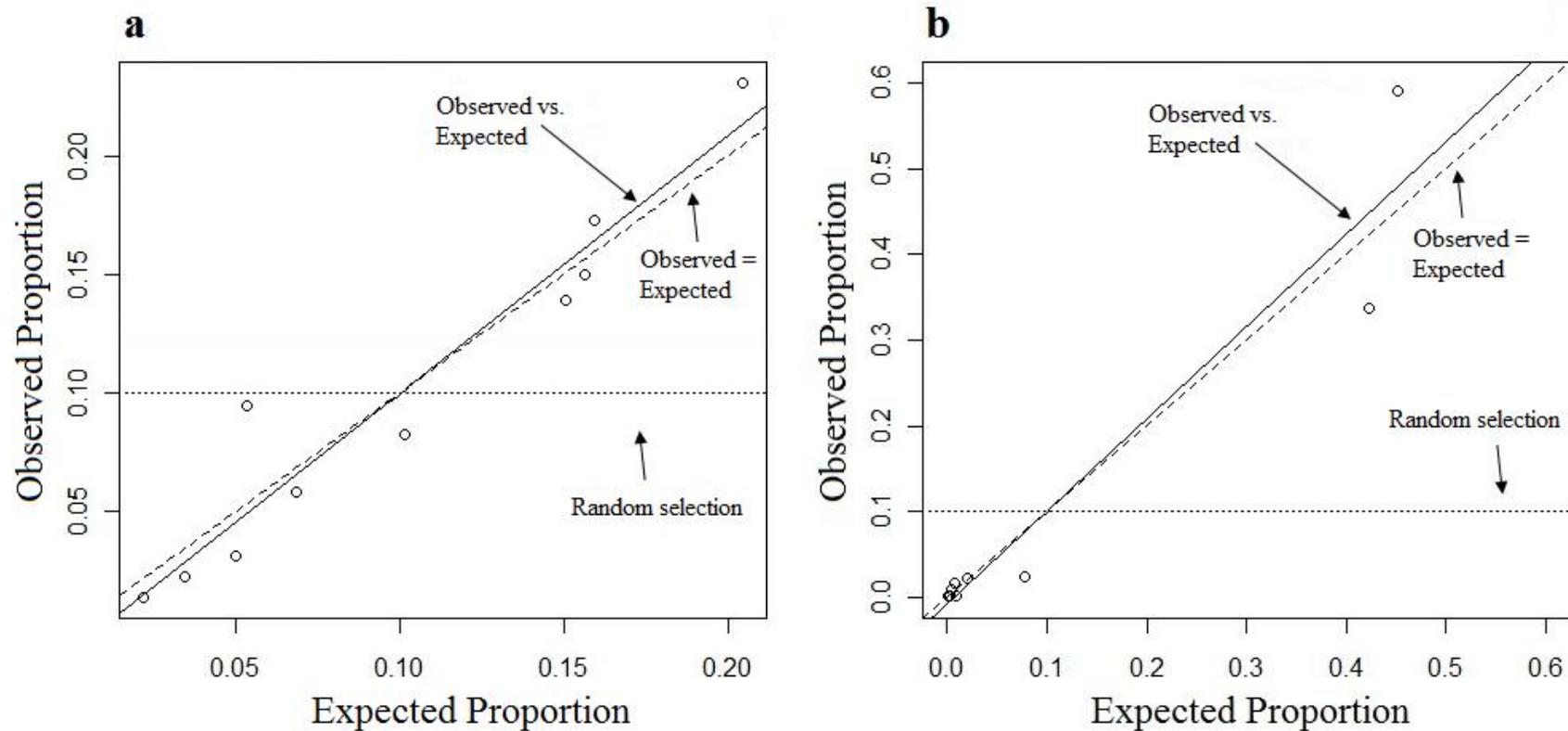
Fig. 3.3. Relative probability of occurrence of red wolves (*Canis rufus*) across the Red Wolf Recovery Experimental Population Area in northeastern North Carolina, 2009-2010. Map represents the product of a) 2nd-order and b) 3rd-order, resource selection functions.
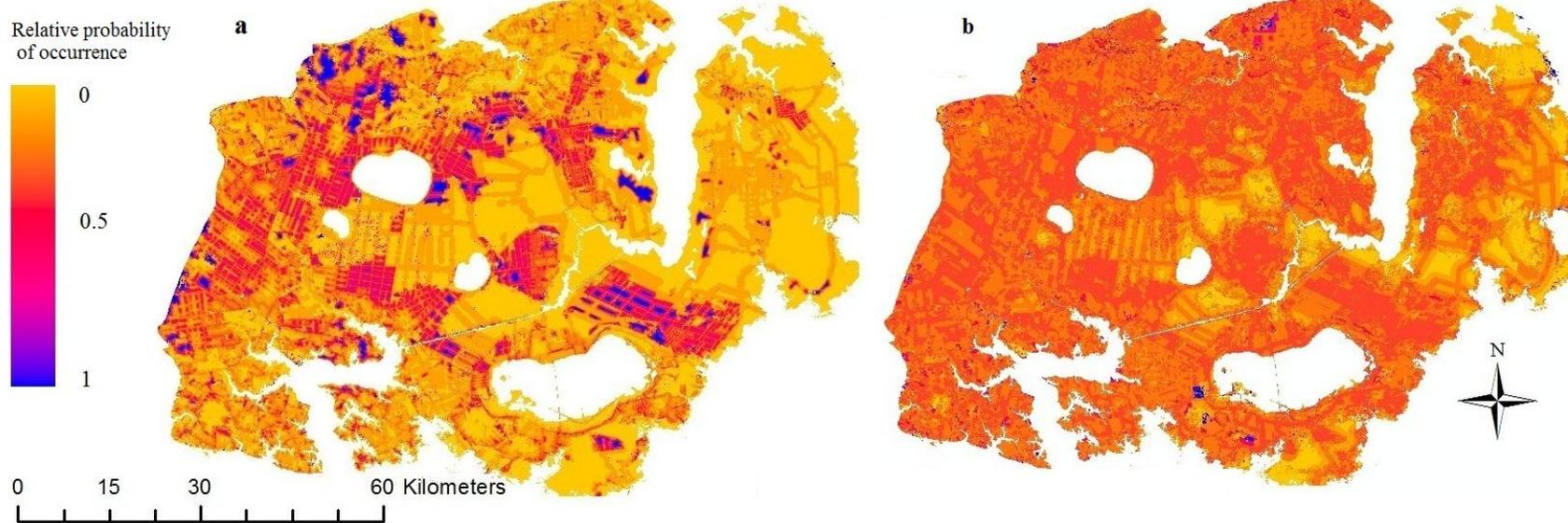
Chapter 4

Diameter Comparison of Scats of Coyotes (*Canis latrans*) and

Red Wolves (*Canis rufus*)

Abstract

Differentiating between scats (fecal deposits) of sympatric canid species is important for determining the presence and movements of individuals, but identification in the field is difficult. Upper and lower thresholds of diameters of scats were calculated to determine whether diameter of scats can be used to distinguish among scats of red wolves (*Canis rufus*), and scats of coyotes (*Canis latrans*) and hybrids in the field. DNA genotyping was used to identify scats collected in the field, and I measured diameters of those scats. Scats of coyote or hybrid origin ≥29 mm in diameter occurred in <5% of samples. Conversely, scats of red wolf origin ≤14 mm in diameter occurred in <5% of samples. Scats >14 mm and <29 mm in diameter cannot be classified to species by diameter alone. These upper and lower ranges of diameters can be used in concert with other methods (e.g., DNA genotyping) to identify scats from red wolves, coyotes, and hybrids.

Introduction

Since 1987, the United States Fish and Wildlife Service has managed the only wild population of red wolves (*Canis rufus*). A significant threat to this endangered species is hybridization with coyotes (*Canis latrans*; Phillips et al. 2003). Biologists routinely monitor location and movement of packs of red wolves (as well as co-occurring

coyotes) within the recovery area to attempt to reduce hybridization between these two canids.

Current monitoring techniques include tracking animals fitted with GPS and VHF radiocollars and identification of scats using faecal DNA genotyping (Adams and Waits 2007, Chadwick et al. 2010). While faecal DNA genotyping generally is reliable, it has drawbacks. These include a high cost (ca. $60/sample), and the necessity of high-quality DNA from fresh scats (Adams et al. 2003). Direct identification of scats in the field would aid in monitoring presence and movement of red wolves, but morphological criteria that distinguish scats of coyotes, hybrids, and red wolves are unknown. Herein, I describe guidelines for distinguishing scats of coyotes and hybrids from red wolves based on morphology.

Materials and Methods

My study area was within the Red Wolf Recovery Experimental Population Area on the Albemarle Peninsula in North Carolina. The study area was >6,000 km^2 of federal, state, and private lands in five counties (Beaufort, Dare, Hyde, Tyrrell, and Washington) and was divided into three management zones. Federal lands included Alligator River National Wildlife Refuge, Pocosin Lakes National Wildlife Refuge, and a bombing range shared by the United States Navy and Air Force. State lands included numerous game-management properties, while private lands primarily were timber plantations and agricultural fields. Types of land cover, and their approximate percentages within the Red Wolf Recovery Experimental Population Area,were: agricultural fields (30%), commercial pine (*Pinus*) plantations (15%), pocosin (15%; *Pinus serotina* and *Persea palustris),* non-riverine swamp forests (10%; *Nyssa*,

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Liquidambar styraciflua, *Acer rubrum,* and *Chamaecyparis thyoides*), saltwater marsh or

open water (10%), and other types of land cover (10%). Climate was characterized by

four seasons of nearly equal length with annual precipitation averaging 127 cm (Beck et

al. 2009). Temperatures averaged 5°C in winter and 27°C in summer (Beck et al. 2009).

Elevation was from sea level to 50 m (Beck et al. 2009). Potential prey included white-

tailed deer (*Odocoileus virginianus*), rabbits (*Sylvilagus floridanus* and *S. palustris*),

raccoons (*Procyon lotor*), wild boars (*Sus scrofa*), coypus (*Myocastor coypus*), common

muskrats (*Ondatra zibethicus*), small rodents (*Mus musculus, Oryzomys palustris,*

Reithrodontomys humulis, and *Sigmodon hispidus*), quail (*Coturnix*), wild turkeys

(*Meleagris gallopavo*), and other ground-dwelling birds (Phillips et al. 2003). Co-

occurring carnivores included gray foxes (*Urocyon cinereoargenteus*), red foxes (*Vulpes*

vulpes), coyotes, feral dogs (*C. lupus familiaris*), bobcats (*Lynx rufus*), and American

black bears (*Ursus americanus*).

During February 2009-March 2010, scats were collected along game trails and

unpaved roads within the Red Wolf Recovery Experimental Population Area. Maximum

diameter of scats at the widest point was measured to the nearest 1 mm using calipers.

Following collection of scats, a sample was removed from each scat and stored in a

buffer solution for DNA genotyping (Adams et al. 2003). Scats were stored in a freezer

for ≥6 weeks until results of DNA genotyping were available. Scats with ≥85%

probability of being a red wolf or coyote based on program Structure 2.3.3 (Pritchard et

al. 2000) were labeled accordingly; otherwise scats were labeled as hybrid. Once DNA

genotyping was completed, all comparative analyses involved two groupings; scats of red

wolves and scats of coyotes/hybrids combined.

Because foods in scats could potentially influence diameters, composition was determined by comparing foods with reference keys. Only the most common food in each scat was identified. Sample sizes of diameters of scats, with respect to foods in scats, were unequal. Therefore I used a Kruskal-Wallis test (R Development Core Team 2010), to assess influence of foods in scats on diameters of scats. This was done by grouping diameters of scats by food in each scat and determining if there was a difference in median diameters of scats composed of different foods.

Sample sizes of diameters of scats of red wolves, and scats of coyotes/hybrids were unequal. Therefore a Mann-Whitney U-test (R Development Core Team 2010) was used to determine if median diameters of scats of red wolves, and coyotes/hybrids differed. A normal-distribution, probability function (R Development Core Team 2010) was constructed to estimate an upper threshold of diameter of scats from coyotes/hybrids, above which classifying the scat as coyote/hybrid would be erroneous 95% of the time; and classifying the scat as red wolf would be erroneous 5% of the time. Similarly, a normal-distribution, probability function was constructed to estimate a lower threshold of diameter of scats from red wolves, below which classifying the scat as red wolf would be erroneous 95% of the time; and classifying the scat as coyote/hybrid would be erroneous 5% of the time. All normal-distribution, probability functions were based on means and standard deviations of scats.

<center>Results</center>

Of 1,377 scats collected, faecal DNA genotyping identified 254 as red wolves, 57 as coyotes, and 54 as hybrids (hybrids were animals with a genetic ancestry of coyote and red wolf combined, and where neither ancestral type made up >85% of the animals

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genetic background). Remaining scats could not be identified using faecal DNA genotyping and were eliminated from the study. Diameters of scats of the two groups overlapped (Fig. 4.1). Mean ($\pm$ 1 *SD*) maximum diameter of scats of coyotes/hybrids was 19 $\pm$ 6 mm (range: 10-35 mm). Mean ($\pm$ 1 *SD*) maximum diameter of scats of red wolves was 24 $\pm$ 6 mm (range: 10-43 mm). Median diameters of scats of red wolves and scats of coyotes/hybrids differed significantly ($P < 0.01$).

Analysis of scats of red wolves revealed seven food categories, while analysis of scats of coyotes/hybrids revealed five food categories (Table 4.1). Dominant foods in scats had no effect on median diameters of scats of red wolves ($P = 0.28$) or median diameters of scats of coyotes and hybrids ($P = 0.32$).

Normal-distribution, probability functions for coyote/hybrid and red wolf scats resulted in upper and lower 95% confidence intervals of 29 and 14 mm, respectively. Scats $\geq$29 mm in diameter were <5% likely to be from coyotes/hybrids. Conversely, scats $\leq$14 mm in diameter were <5% likely to be from red wolves. Scats with diameters >14 mm and <29 mm could not be assigned to group based on diameter alone. The largest diameter of scats from a coyote/hybrid was 35 mm. Conversely, 24% of scats of red wolves were $\geq$35 mm. The scat of a red wolf with the smallest diameter was 10 mm, which equaled the smallest diameter for scats of a coyote/hybrid. For scats of coyotes/hybrids, 24% were $\leq$10 mm.

Discussion

Diameters and ranges in size of scats in my study were similar to those reported by Weaver and Fritts (1979): they reported mean diameters of 21 and 27 mm (range = 7-

34 and 13-47 mm) for coyotes and gray wolves (*Canis lupus*), respectively. Diameters and ranges of scats also were similar to those of Reed (2004) who reported mean diameters of 23 and 26 mm (range = 17-28 and 16-36 mm) for coyotes and Mexican gray wolves (*Canis lupus baileyi*), respectively. Results of my study agree with Weaver and Fritts (1979) that the dominant food has no effect on median diameters of scats of large canids. Diameters and ranges from these studies have been used to study and compare diets and movements of both Mexican and gray wolves with those of coyotes where they co-occur (Arjo et al. 2002, Carrera et al. 2008). Thus, diameters and ranges from my study can be used to differentiate between scats of coyotes and red wolves where the species are sympatric.

Probability of misidentifying a scat of a coyote or hybrid ≥29 mm in diameter within the Red Wolf Recovery Experimental Population Area was calculated to be <5%. Feral domestic dogs (*Canis lupus familiaris*) also are present, but in low numbers and experience low survival (C. Lucash pers. comm.). Thus, scats of canids ≥29 mm in diameter found within the Red Wolf Recovery Experimental Population Area are likely to be from red wolves. I suggest 29 mm as an upper threshold for distinguishing scats of red wolves from scats of coyotes/hybrids within the Red Wolf Recovery Experimental Population Area. Additionally, the probability of misidentifying a scat of a red wolf ≤14 mm in diameter in my study area was calculated to be <5%.

Use of these measurements alone is likely to lead to loss of information due to exclusion of scats of red wolves <29 mm in diameter. In my study, 76% of scats of red wolves collected could not be distinguished from scats of coyotes and hybrids based on diameter, and vice versa. Scats of canids with diameters of 15-28 mm will not be

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identifiable based on diameter alone, so other techniques such as DNA genotyping will be required (Adams and Waits 2007, Adams et al. 2003). The above method allows for identification of ca. 25% of scats, this method provides a starting point from which biologists can begin to more cost-efficiently monitor red wolves and co-occurring canids.

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Table 4.1. Diameters of scats of red wolves (*Canis rufus*), and coyotes (*Canis latrans*) and hybrids grouped by primary food category detected in scats collected within the Red Wolf Recovery Experimental Population Area, North Carolina, 2009-2010.

Food category	Red wolves		Coyotes and hybrids	
	n	Median diameter (mm)	*n*	Median diameter (mm)
White-tailed deer (*Odocoileus virginianus*)	97	25	36	20
Coypus (*Myocastor coypus*) and common muskrats (*Ondatra zibethicus*)	13	25	2	24
House mice (*Mus musculus*) and hispid cotton rats (*Sigmodon hispidus*)	32	23	22	20
Eastern cottontails (*Sylvilagus floridanus*) and marsh rabbits (*Sylvilagus palustris*)	84	23	49	16
Wild boars (*Sus scrofa*; feral and domestic)	11	23	2	26
Raccoons (*Procyon lotor*)	12	28		n/a
Insects (primarily grasshoppers, family Acrididae)	5	22		n/a

Fig. 4.1. Diameters of scats of a) coyotes (*Canis latrans*) and hybrids (*n* = 111) and b) red wolves (*Canis rufus*; *n* = 254) in the Red Wolf Recovery Experimental Population Area on the Albemarle Peninsula in North Carolina, 2009-2010.
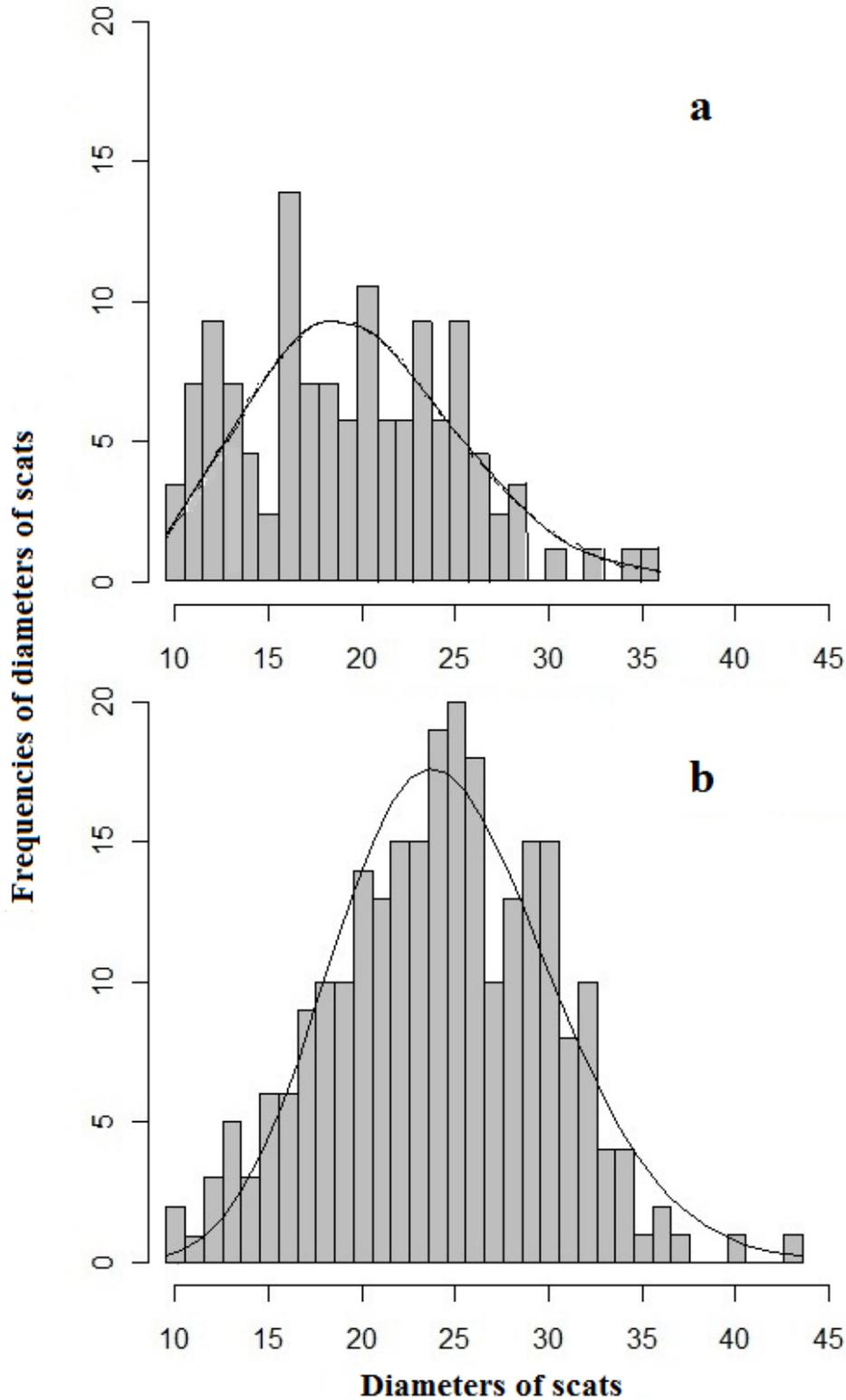
Chapter 5

Concerns about Training Scat-Detection Dogs and Their Applications to Field Research

Abstract

Scat-detection dogs are a useful tool for conservation and research. I compared performance of a scat-detection dog when using scats obtained from captured wild animals v. scats collected in the field and identified via faecal genotyping. I also compared performance of a scat-detection dog when using v. not using non-target scents during training. The scat-detection dog performed better when trained using scats collected from captured wild animals versus scats identified via faecal genotyping ($P = 0.01$). After having been trained without scent of non-target species, performance of scat-detectiond dog decreased when introduced to scent of non-target species ($P = 0.04$). Based on this pilot study, we recommend future studies using scat-detection dogs consider the implications of using or not using scats from non-target species in the training process. We also recommend future studies consider how scats used in training were collected and identified and how variation in collection and identification of scats might impact performance of scat-detection dogs. This pilot study has demonstrated that performance of scat-detection dogs for wildlife research can be influenced during training.

Introduction

Use of detection dogs for studying rare species, as well as other conservation-oriented purposes, is becoming widespread (Long et al. 2007). Detection dogs can locate

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a wide variety of target scents (Smith et al. 2001) and can distinguish among odors of species, sexes, and individuals (Gutzwiller 1990). In studies of wildlife, scat-detection dogs are more cost- and time-efficient than other noninvasive methods, such as hair snares, game cameras, scent stations, and faecal genotyping of scats (Smith et al. 2001, Wasser et al. 2004, Harrison 2006, Long et al. 2007). Scat-detection dogs typically provide larger samples, higher rates of detection, and a more thorough survey of a study area (Harrison 2006). However, as with any technique, shortfalls exist, but such shortfalls can be accounted for and circumvented by properly training scat-detection dogs.

Scat-detection dogs should only respond to scats of species they were trained to find. However, scat-detection dogs may generalize scent of the target species to include scents of non-target species. This is likely if dogs were never introduced to scats or scents of non-target species, or trained to ignore such scats. For example, Long et al. (2007a) were interested in using scat-detection dogs to study American black bears (*Ursus americanus*), fishers (*Martes pennanti*), and bobcats (*Lynx rufus*). They trained dogs using only scats from these species and their dogs accurately located scats from target species. However, their dogs also alerted on scats of co-occurring coyotes (*Canis latrans*), red foxes (*Vulpes vulpes*), raccoons (*Procyon lotor*), house cats (*Felis catus*), and long-tailed weasels (*Mustela frenata*). Performance of scat-detection dogs likely can be improved by including scats from co-occurring species, as well as scats of target species, during the training process. This would help train dogs what to detect and what to ignore.

Obtaining scats to train scat-detection dogs can be difficult. This is because most

scat-detection dogs are used to detect rare and elusive species (Smith et al. 2001), for which scats are not readily available. Researchers may attempt to obtain scats by collecting scats in the field and using faecal genotyping to determine species of origin. Faecal genotyping is a reliable technique (Adams et al. 2003), but Knapp et al. (2009) highlighted errors associated with genotyping DNA from non-invasive sources (e.g., hair and scats). Using too few, or insufficiently variable, loci can result in individuals or species being misidentified: this is known as the shadow effect (Knapp et al. 2009). This source of error becomes more problematic depending upon relatedness of individuals or species (Mills et al. 2000, Waits and Leberg 2000). Using scats collected from captured wild individuals, instead of scats identified via faecal genotyping, which can lead to errors (Creel et al. 2003, Bonin et al. 2004, Buchan et al. 2005), could circumvent the shadow effect (Knapp et al. 2009) and increase accuracy of scat-detection dogs.

A substantial body of research has detailed use of scat-detection dogs for studying rare and elusive species (Wasser et al. 2004, Cablk and Heaton 2006, Harrison 2006, Long et al. 2007). Proper training is vital to improving effectiveness and performance, but no research has addressed ways to improve performance of scat-detection dogs. My study seeks to highlight shortfalls and discuss ways to improve or circumvent these shortfalls. I hypothesize that source of scats (from captured wild animals or samples identified to species by faecal genotyping) and use of scats of co-occurring animals influences performance of scat-detection dogs.

Materials and Methods

Training was conducted on the grounds of Teal Run Retrievers, a retriever-training facility in Camp Hill, Chambers County, Alabama, during 2 July-5 August 2010.

Training was conducted during 0500-0800 h CDT every other morning for a total of 18 days to avoid high summer temperatures and humidity, which can negatively affect performance of detection dogs (Gutzwiller 1990). Average temperature and humidity during times of the study were 29°C and 90%, respectively; wind speed was <5 km/hour with partial or no cloud cover.

Scat-detection dogs were trained to distinguish between scats of red wolves (*Canis rufus*) and co-occurring carnivores. Standard protocols for training detection dogs (Reindl 2004) were employed to train dogs to passively alert or sit (Cablk and Heaton 2006) upon detecting the target scent, i.e., scats of red wolves. Three, 2-year old, black Labrador retrievers, two males and one female, were selected for training. Dogs were first introduced to scats of red wolves under controlled conditions. During this time, a professional dog trainer evaluated the dogs to determine if they had appropriate temperament, strong orientation toward objects, and high drive to search for scents (Wasser et al. 2004, Cablk and Heaton 2006). Temperament is important to ensure that dog and handler work well together. Orientation toward objects was essential due to use of visible scent stations, which the dogs had to visit and inspect for presence of target species. Drive is the desire to search for, chase, capture, and retrieve prey or scents. In my study, high drive to search for scents was associated with chasing, capturing, and retrieving a tennis ball; the reward for correctly responding to a scat of a red wolf. Two dogs, the female and one male, proved inadequate and were removed from the study. Trials using these dogs were excluded from analyses. Gutzwiller (1990) noted that training two or more dogs for detection purposes and cross validating results from one dog with other similarly trained dogs is ideal for minimizing biases between studies. I

acknowledge that one dog is not representative of capabilities of all dogs, but mine is a pilot study to establish protocols for improving accuracy of scat-detection dogs.

Eight scent stations were established 10 m apart in a mowed field. Locations of scent stations were staggered to avoid mixing scents from multiple scent stations due to wind. Scent stations were constructed using PVC pipe that was 10 cm in diameter, 10 cm long, and mounted open-end upward onto plywood platforms about 30 cm in length and width. Scats were placed into 0.25-L containers, which reduced any residual scent left by a scat previously placed at a scent station (Cablk and Heaton 2006). Use of the 0.25-L containers also allowed easy transfer of a sample from one scent station to another and preserved the scent of the scat during training by reducing exposure to the atmosphere. Each scat was kept in a separate container for the duration of the project. It was important that containers fitted securely in the PVC pipe and that tops of containers were flush with those of the PVC pipes. This prevented the dog from ingesting or degrading samples and it also allowed the dog to adequately smell the sample. All samples were stored individually in containers at -2°C when not being used in training exercises. Six scats from red wolves and six from coyotes were used for training. These scats were collected in the field and identified via faecal genotyping. In addition, six scats from red wolves and six from coyotes were collected from wild animals captured in foot-hold traps. Four scats were collected from domestic dogs and four were collected from bobcats (*Lynx rufus*). All scats were from animals on the Albemarle Peninsula in northeastern North Carolina. Using multiple scats from each species helped ensure that dogs were trained to distinguish target species from those of other species, rather than specific individuals of the target species.

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The first phase of the study consisted of inserting two containers with scats of captured red wolves into two randomly selected scent stations, with the exception that no adjacent scent stations had containers with scats of captured red wolves. This exception was to prevent the dog from becoming desensitized to the target scent. The remaining six scent stations were empty. During each trial, dogs were walked past all scent stations. Dogs were limited to 21 trials/day. After a set of three trials the dog was allowed to rest for 5-10 minutes; this helped reduce fatigue and maintained high drive to locate the prey (i.e., target scent). When the handler walked the dog by stations containing scat of a captured red wolf, close attention was paid to the body language of the dog (Cablk and Heaton 2006). Upon interpreting that the dog had perceived the scent of the scat, the handler instructed the dog to sit. After correctly alerting on a scat of a captured red wolf, the dog was given a reward (chasing, capturing, and retrieving a tennis ball). After a trial, the two containers of scats were removed and two new containers were inserted at random. In these trials, the handler was informed as to which stations had scats of red wolves. This was done to aid the handler in assessing the initial reaction of the dog to the target scent. This aided in subsequent trials when the dog was presented with more than one type of scat. Three days of training for a total of 63 trials were spent in completing this first phase of training.

Once the dog consistently demonstrated the ability to perform the correct alert at occupied scent stations when all other scent stations were empty, the second phase of the study began. Containers with scats of captured coyotes or domestic dogs were placed in scent stations next to containers with scats of captured red wolves. At this point, all eight stations contained scats, two from captured red wolves and six with a random

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combination of scats of captured coyotes and dogs. Scats collected from wild animals of both species were used in this phase. The dog was allowed to smell each station and the handler was not informed about the location of the target scent. If the dog alerted incorrectly on a station containing scat of a captured coyote or dog, the handler was informed of the mistake immediately. The handler then negatively reinforced the exhibition of the false alert using vocal reprimands and removing the dog from that station. A correct alert at a station containing the target scent was acknowledged by a member of the research team and the handler then proceeded to reward the correct alert as before. If a dog did not detect the target scent, the trainer was informed and the dog was made to sit at the correct station, then it received the reward. After each trial, containers were replaced and those with the target scent were replaced with non-target scent. Two containers with target scent also were inserted randomly into two new scent stations. Six days of training totaling 126 trials were spent in this second phase. For the last half of this phase, the dog was considered trained and able to distinguish target and non-target scents. Thus, the last half of this phase was the reference for comparing performance of the scat-detection dog under variations in origin of scats and presence of non-target scents, respectively.

Upon demonstrating the ability to consistently distinguish between scats of captured red wolves and those of captured coyotes and dogs, the third phase began. Scats identified via faecal genotyping were included at stations instead of scats from trapped individuals. Following the same protocol as the second phase of study, the handler was uninformed of the identity of scats placed at scent stations, dogs were negatively reinforced if they misidentified or missed a scat, and containers with scats were randomly

placed in stations and changed after each trial. It was assumed that scats identified using faecal genotyping were correctly identified. Three days of training, totaling 63 trials, were spent in the third phase.

Following the third phase of study, scats identified via faecal genotyping were no longer used. Following removal of these scats, a day was spent re-training the dog in accordance with phase two using only scats from captured individuals. This was done to account for scats that potentially were misidentified via faecal genotyping, which could have confused the dog and negatively influenced the dog's ability to distinguish target and non-target scents. After re-training, scats of bobcats were placed at random in scent stations, no more than one scent station contained scats of a bobcat during each trial. In addition, two scent stations contained scats of captured red wolves and five scent stations contained a random combination of scats of captured coyotes and dogs. The handler remained uninformed of the identity of scats in each station. This fourth phase of study, using scats of bobcats, lasted 3 days, for a total of 63 trials.

Errors were recorded for each trial. Errors were numbers of miscues per trial, i.e., failing to identify scats of red wolves, failing to sit after detecting scats of red wolves, sitting at a scat other than that of a red wolf, or exhibiting body language indicating that scat of a red wolf were detected when it was not. A Student's t-test (R Development Core Team 2010) was conducted to test for differences in the daily mean rate of error between the last half of the second phase of study and third phase of study. A Student's t-test also was conducted to test for differences in the daily mean rate of error between the last half of the second phase of study and the fourth phase of study. Student's t-tests were evaluated at $\alpha = 0.05$.

Results

Following initial training (phase one and 1st-half of phase two), the scat-detection dog accurately identified 1,514 of 1,680 scats for an overall accuracy of 90% (8 stations/trial, 21 trials/day for 10 days; Table 5.1). Errors were cyclical, peaking at the beginning of each phase of study and decreasing over time, with the exception of phase three (Fig. 1). Student's *t*-test showed a significant difference between mean rate of error of the last half of phase two (mean = 0.24) and phase three (mean = 1.41; *P* = 0.01, *df* = 4; 95% *CI* = 0.54, 1.8). Student's *t*-test also revealed a significant difference between mean rate of error of the last half of phase two and phase four (mean = 0.89; *P* = 0.04, *df* = 4; 95% *CI* = 0.06, 1.24). It should be noted that the dog only exhibited the trained alert on scats of dogs twice, both on the first day of the second phase of training.

Discussion

Shortfalls can arise in training scat-detection dogs and can lead to high rates of error and poor performance of dogs in the field. This diminishes usefulness of dogs as tools for conservation and management of wildlife. This study highlights areas of improvement in training scat-detection dogs.

My results demonstrate that origin of scats can potentially influence performance of scat-detection dogs. In my study, the scat-detection dog had a higher daily mean rate of error when trained using scats collected in the field and identified via faecal genotyping compared to scats collected directly from captured wild animals. Future studies should consider the origin of scats used to train scat-detection dogs and attempt to discern if there is a difference in performance when using scats collected from captured wild animals and scats collected in the field and identified via faecal genotyping. Scats

collected from captive animals may be insufficient because captive animals typically have diets that are different from wild counterparts. Long et al. (2007) suggested that diet of target species can affect scat-detection dogs. Long et al. (2007) accounted for variation in diet of species of interest when obtaining scats to train scat-detection dogs by using several scats from multiple individuals. Obtaining scats from wild animals, although time consuming, could potentially increase performance of scat-detection dogs and result in more accurate performance.

I believe that faecal genotyping is an accurate method for identifying scats collected in the field. Numerous studies in the past decade alone show the usefulness of faecal genotyping for identifying scats collected in the field (Adams et al. 2007, Farrell et al. 2000, Frantz et al. 2003, Piggott et al. 2006, Prugh et al. 2005). There are, however, errors associated with faecal genotyping, such as the shadow effect (Knapp et al. 2009), age of scats collected, and season of collection of scats (Piggott 2004). Errors associated with faecal genotyping could compound errors associated with using scat-detection dogs (handler, dog, training-related errors, or a combination of these). In my study, the scat-detection dog had difficulty identifying three scats in particular identified via faecal genotyping, intermittently alerting on two scats identified as coyote and one as red wolf. These scats were collected during summer. DNA of these scats could have been heavily degraded due to high temperatures and desiccation, leading to errors in identifying origin of scats. It is also possible that our results could be due to differences in the way certain scats smelled to the dog relative to the scats of captured red wolves, captured coyotes, and domestic dogs that the scat-detection dog was trained on.

Typically, scat-detection dogs are trained to alert on a target scent. My study

suggests that inclusion of non-target scents in training of scat-detection dogs could potentially improve performance by training dogs what to alert on and what to ignore. This also would prevent generalization of target scent to non-target scents. Use of non-target scents could be especially important if scat-detection dogs are likely to come across closely related species and species with similar diets. We acknowledge our results could also have been influenced by the fact that this study did not replicate field conditions, and only involved the use of one dog.

Ability of scat-detection dogs to detect scats in field conditions can be diminished due to environmental conditions, terrain, and other factors. Cablk and Heaton (2006) suggested that training and errors by the handler can have a significant influence on performance of scat-detection dogs. A handler may misread behavior at a non-target scent and erroneously provide a reward for that detection; thus, making the dog more likely to alert on non-target scats in the future (Long et al. 2007). Future studies should assess effects of varying training techniques and level of training and error of the handler on performance of dogs under field conditions.

We acknowledge that attempting to distinguish scats of red wolves from those of co-occurring carnivores using scat-detection dogs is difficult. The close relatedness of red wolves to species such as coyotes and feral dogs increases the difficulty in training scat-detection dogs to distinguish between species. Research seeking to identify scats of species where no other congeners occur would be easier. However, difficulties in this study have revealed areas needing improvement for training scat-detection dogs.

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Table 5.1. Performance of the scat-detection dog at a training facility in Camp Hill, Chambers County, Alabama, 2010. Values correspond to number of times the dog correctly alerted on the target scent (2 is the maximum number of times the dog could correctly alert during a run). Numbers in parentheses are the number of false positive alerts during each run (6 is the maximum number of times the dog could incorrectly alert during a run).

Phase	Day	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	Run 10	Run 11
	1	0	0	0	0	0	0	0	0	0	1	1
1	2	0	0	0	0	1	1	2	2	2	2	2
	3	1	1	2	2	2	2	2	2	2	2	2
	4	2(6)	2(6)	1(6)	2(6)	1(6)	1(5)	2(5)	2(3)	2(4)	2(3)	2(4)
	5	2(4)	1(4)	1(3)	2(2)	2(3)	2(1)	1(2)	2(1)	2(1)	2(2)	2(2)
2	6	2(3)	1(3)	2(2)	2(1)	1	1(2)	2(2)	2(3)	2(1)	1(2)	2(1)
	7	2(2)	2(2)	2(1)	2(2)	2(1)	2	2(1)	2	2	2(1)	2
	8	2(1)	2(1)	2	2	2	2	2(1)	2	2	2	2
	9	2	2(1)	2	2	2	2	2	2	2	2	2
	10	2(3)	2(1)	0	2(2)	2(1)	2(2)	2(1)	1(3)	1(1)	1(3)	2(1)
3	11	2(2)	2(1)	1(1)	1(2)	2(1)	2(1)	1	2(1)	2(3)	2	2
	12	2(3)	2(2)	2(2)	1(3)	1	2(1)	2(2)	2	2	2(1)	1(2)
Retraining	13	2	2(1)	2	2	2	2	1	2	2	2(1)	2
	14	2(1)	2(1)	2(1)	2(1)	2(1)	2(2)	1(1)	2(1)	2(2)	2(1)	2(1)
4	15	2(1)	2(1)	2(2)	2	2(1)	1	2	2(1)	2(1)	2	2(1)
	16	2	2(2)	2(1)	2(1)	2(1)	2	2(1)	2(1)	2(1)	2	2(1)

Table 5.1. Continued.

Phase	Day	Run 12	Run 13	Run 14	Run 15	Run 16	Run 17	Run 18	Run 19	Run 20	Run 21
	1	1	2	2	2	2	2	2	2	2	2
1	2	2	2	2	2	2	2	2	2	2	2
	3	2	2	2	2	2	2	2	2	2	2
	4	1(3)	2(3)	2(3)	2(2)	2(2)	2(1)	2	2(1)	2	2(1)
	5	2(1)	2(1)	1	2	2(1)	1	2	2(1)	2	2
2	6	2	2(2)	2(1)	2	2(1)	2	2(1)	1	2	2(1)
	7	2	2(1)	1	2	2	2	2	2	2	2
	8	2	2	2	2	2	2	2	2	2	2
	9	2	2	2	2	2	2	2	2	2	2
	10	2(1)	2	1(1)	2	2(1)	2	2	2(1)	2(1)	2
3	11	1(2)	2(1)	2	2(2)	2(1)	2	2(2)	0	2(1)	2(1)
	12	2(2)	2(3)	2(1)	2	2(1)	2(1)	2(1)	2(2)	1(1)	2
Retraining	13	2(1)	2	2(1)	2	2(1)	2	2	2	2	2
	14	1(1)	2(1)	2	2(1)	2	2	2(2)	2(1)	2(1)	2
4	15	2(2)	2(1)	2	2(1)	1(1)	2	2(1)	2	2(1)	2(1)
	16	2	2	2(2)	2	2(1)	2(2)	2	2(1)	2(1)	2

Fig. 5.1. Number of errors per run per day by a scat-detection dog at Camp Hill, Chambers County, Alabama, 2010; o = phase 1, □ = phase 2, ◊ = phase 3, and Δ = phase 4.