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Food Habits of Red Wolves During Pup-Rearing Season

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Abstract - *Canis rufus* (Red Wolf) is critically endangered, with the only wild population consisting of <150 individuals. Currently, little is known about the food habits of this population. Such information may be vital to managing for the population's long-term persistence. We collected scats of Red Wolves for two consecutive pup-rearing seasons from six packs, classified contents into prey categories, and assessed diet composition for each pack. Five of the six packs studied consumed only mammalian prey items. Adult *Odocoileus virginianus* (White-tailed Deer) and White-tailed Deer fawns accounted for 37–66% of diet of Red Wolves depending on the metric of diet composition. Adult White-tailed Deer and White-tailed Deer fawns accounted for 21–83% of the diet of individual packs of Red Wolves according to biomass consumed. Two packs regularly consumed foods associated with humans. Generalized linear modeling indicated that diet varied between packs and was not influenced by reproductive status, nor did diet vary between years.

Introduction

Understanding diet of endangered species is necessary for proper management of such species and to determine suitable habitats in which to re-introduce individuals of an endangered species. Prior to the re-introduction of *Canis rufus* Audubon and Bachman (Red Wolf) into part of their native range in 1987, no large carnivore had been successfully re-introduced (Phillips et al. 2003). For >20 years the re-introduced population of Red Wolves has survived and reproduced in a habitat matrix altered by humans. A better understanding of what Red Wolves consume in a human-altered landscape is useful to future re-introductions.

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 *Canis latrans* Say (Coyote) (Phillips et al. 2003). After re-introduction, most research concerning Red Wolves has dealt with resolving the identity of the species and distinguishing hybrids from Red Wolves using various genetic techniques (Adams et al. 2007, Miller et al. 2003). Thus, information on the basic ecology of Red Wolves, although vital to recovery and management, is lacking.

Although few studies have examined food habits of Red Wolves, they are considered generalists and opportunistic like most canids (Mech 1970, Paradiso and Nowak 1972). Studies on remnant Red Wolf populations in Texas and Louisiana concluded that small mammals constituted a large part of the species'

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diet (Paradiso and Nowak 1972, Shaw 1975). Only Shaw (1975) documented Red Wolves preying on species larger than *Procyon lotor* L. (Raccoon); however, only 19 scats were collected for that study. Following re-introduction of Red Wolves to a part of their historic range in 1987, Red Wolves were observed to prey upon *Odocoileus virginianus* Zimmermann (White-tailed Deer) and Raccoons on barrier islands and on the Albemarle Peninsula in North Carolina (Phillips et al. 1995, 2003). Red Wolves also preyed on *Sus scrofa* L. (Wild Boar) in Great Smoky Mountains National Park (Phillips et al. 2003). Although previous studies have provided insights into the diet of Red Wolves, no study has assessed variation in diet of packs of Red Wolves, with the exception of Phillips et al. (1995), which compared diets of two packs, one of which no longer exists (Rabon 2010).

Our objective was to determine food habits of Red Wolves. Our specific goals were to determine overall diet of Red Wolves during the pup-rearing season, examine variation in diet among packs and between years, and determine if pups influence the diet of Red Wolf packs.

Study Site

This study occurred within the Red Wolf Recovery Experimental Population Area (RWREPA) on the Albemarle Peninsula in northeastern North Carolina. At the time of this study, the area was home to the only wild population of Red Wolves in the world. The study area consisted of >6650 km^2 of federal, state, and private lands in five counties (Beaufort, Dare, Hyde, Tyrrell, and Washington). Federal lands 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 were primarily timber plantations and agricultural fields. The study focused on packs in Tyrrell and Dare counties (Fig. 1).

Major land-cover types in the study area were agricultural fields (30%); commercial pine (*Pinus* spp.) plantations (15%); Pocosin (15%; *Pinus serotina* Michx. [Pocosin Pine] and *Persea palustris* (Raf.) Sarg. [Swamp Bay]); nonriverine swamp forests (10%; *Nyssa* spp.[tupelo], *Liquidambar styraciflua* L. [Sweetgum], *Acer rubrum* L. [Red Maple], and *Chamaecyparis thyoides* (L.) B.S.P. [Atlantic White Cedar]); and saltwater marsh or open water (10%). Minor land-cover types comprised the remaining area (20%). Climate was characterized by four full seasons of nearly equal length with annual precipitation averaging 127 cm. Temperatures averaged 5 °C in winter and 27 °C in summer. Elevation ranged from sea level to 50 m (Beck et al. 2009). Potential prey species included White-tailed Deer, *Sylvilagus floridanus* Allen (Eastern Cottontail), *Sylvilagus palustris* Bachman (Marsh Rabbit), Raccoons, Wild Boars, *Myocastor coypus* Molina (Nutria), *Ondatra zibethicus* L. (Muskrat), *Sigmodon hispidus* Say and Ord (Hispid Cotton Rat), *Mus musculus* L. (House Mouse), *Oryzomys palustris* Harlan (Marsh Rice Rat), *Reithrodontomys humulis* Audubon and Bachman

(Eastern Harvest Mouse), *Colinus virginianus* L. (Northern Bobwhite), and *Meleagris gallopavo* L. (Wild Turkey) (Phillips et al. 2003). Co-occurring carnivores included *Urocyon cineroargenteus* Schreber (Gray Fox), *Vulpes vulpes* L. (Red Fox), Coyotes, *Canis lupus familiaris* L. (Domestic Dog), *Lynx rufus* Schreber (Bobcat), and *Ursus americanus* Pallas (American Black Bear).

Methods

Survey methods and design

Scats were collected during the pup-rearing season, May–July in 2009 and 2010. Because about 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. The territories of the Milltail, Timberlake, Tyson, Columbia, Northern, and Kilkenny packs (Fig. 1) were surveyed for scats. Paved, gravel, and dirt roads, and game trails were surveyed on foot within known territories of the packs. Territorial boundaries were known based on surveys conducted by US Fish and Wildlife Service biologists (Chris Lucash, USFWS Red Wolf Recovery Program, Al, Alligator River Wildlife Refuge, Manteo, NC, pers. comm.). In 2009, Milltail and Tyson packs produced 3 and 4 pups, respectively. In 2010, Milltail, Tyson,

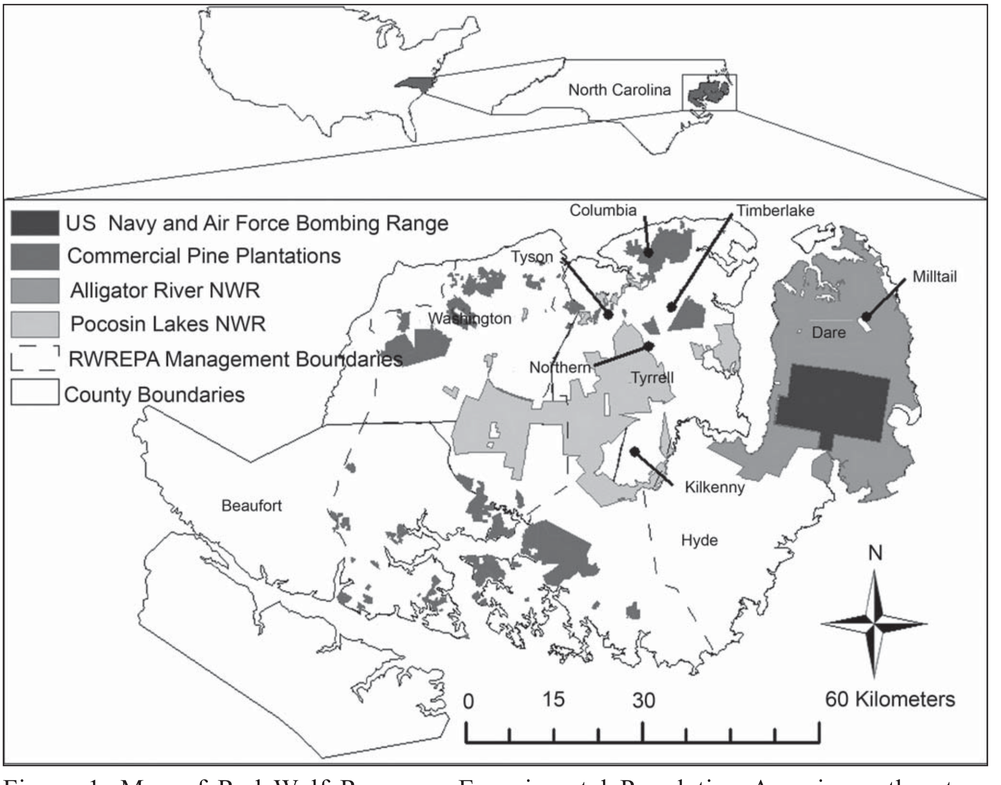

Figure 1. Map of Red Wolf Recovery Experimental Population Area in northeastern North Carolina and locations of packs of Red Wolves (*Canis rufus*) studied in 2009 and 2010. Map shows the boundaries of counties, management zones of the Red Wolf Recovery Experimental Population Area, and federal and commercial lands.

Northern, and Kilkenny packs produced 7, 6, 3, and 4 pups, respectively. Columbia and Timberlake packs did not produce pups either year (Chris Lucash, pers. comm.). Each territory was surveyed at least once per week. For our analysis, the sample unit was the pack: we assumed diet of individuals was representative of the pack. Rarefaction curves were constructed to determine the relationship between number of scats collected for a given pack in a year and the number of prey items detected to assess if sample sizes were adequate.

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. The remainder of each scat was placed in a plastic bag and stored below 0 °C until DNA analyses were completed. Fecal DNA was identified to species and then individual following the methods of Adams (2006) and Adams et al. (2007). For a scat to be identified as Red Wolf or Coyote using fecal DNA genotyping, it also had to be identified to individual animals. Genotypes obtained from scats were compared to genotypes of known Red Wolves and Coyotes in the area to match scats to known individuals. Since our sample unit was the pack, scats from unknown individuals were discarded. Scats determined to be from known Red Wolves from the target packs were analyzed for prey contents.

Ninety-six percent of scats collected in 2009 and identified as Red Wolf matched genotypes of individuals from packs of interest. Thus, we decided it was unnecessary for Red Wolf scats to be related to individuals since most scats collected within territories of packs of interest and identified as Red Wolf could be attributed to an individual of that pack. Because our sample unit was the pack, we only identified scats collected in 2010 to species using more cost efficient techniques. 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 $\geq$29 mm in diameter had <5% probability of having been deposited by a Coyote (Dellinger 2011). Therefore, any canid scats collected in 2010 $\geq$29 mm in diameter were labeled Red Wolf. Scats collected in 2010 <29 mm in diameter were identified using scat dogs (Long et al. 2007). The scats dogs were not used to find scats; rather they were used to distinguish Red Wolf scats collected in 2010 from co-occurring carnivores. The scat dogs were trained using scats collected directly from captured wild Red Wolves and co-occurring carnivores (e.g., Coyotes, Bobcats, and Domestic Dogs), as well as scats collected in 2009 and identified as Red Wolf or Coyote. The scat-detection dog was 96% accurate in training trials at distinguishing scats of Red Wolves from co-occurring carnivores (Dellinger 2011). Thus, we deemed the scat-detection dog was accurate and able to identify Red Wolf scats collected during the 2010 field season.

Identification of prey items and descriptive analysis of diet

Scats identified as Red Wolf were examined for content. Scat contents (e.g., hair, skulls, and teeth) were identified by comparison to reference materials.

Prey items in scats were designated as belonging to one of nine prey categories: adult White-tailed Deer, White-tailed Deer fawns, small rodents (Hispid Cotton Rat, Marsh Rice Rat, Eastern Harvest Mouse, and House Mouse), large rodents (Nutria and Muskrat), rabbits (Marsh Rabbits and Eastern Cottontails), Raccoons, Wild Boars (feral and domestic), anthropogenic material, and other (prey items not occurring frequently enough to justify a unique category, e.g., ground-dwelling birds and terrestrial invertebrates).

We used four metrics to rank and determine percent contribution of prey items in scats: percent frequency of occurrence, relative volume of remains, relative weight of remains (Ciucci et al. 1996), and biomass ingested (only for mammalian prey items) 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). We excluded prey categorized as other from biomass rankings because not all prey species included in this category were mammals (Floyd et al. 1978). Prey category anthropogenic material was only included for percent frequency of occurrence because digestibility of this prey category was unclear and likely biased.

Quantitative analysis of diet

To determine which variables best accounted for variation in diet based on differences in occurrence of prey items in scats, we developed generalized linear models (GLMs). A global GLM with a Poisson distribution and an offset, to account for differences in number of scat samples collected, was first constructed by modeling percent frequency of occurrence of prey items grouped by a four-way interaction between pack, year, prey item, and reproductive status. We determined the most parsimonious model using Akaike's information criterion corrected for small sample sizes from global model and all possible subsets (AICc; Burnham and Anderson 2002). We interpreted odds ratios using the link function $e^{(\text{coefficient})}$, and derived them using coefficient estimates of the most parsimonious model to determine likelihood of consumption of a given prey item (Manly et al. 2002). Odds ratios detail likelihood of consumption of one prey item over another for a given pack as well as the likelihood of one pack consuming a prey item over another pack consuming the same prey item.

Results

In 2009 and 2010, we collected 176 and 279 Red Wolf scats , respectively. Rarefaction curves of diet diversity for each pack per year leveled off at 20 scats regardless of diversity of diet. We found at least 26 scats for each pack per year; thus, there was a low likelihood that we missed any prey items regularly consumed by the Red Wolf packs. Therefore, our sample sizes for determining diet composition of Red Wolf packs were deemed sufficient. We do not suggest 20 scats are sufficient for determining diet of Red Wolves for future studies, rather future studies should use rarefaction curves in their own analyses to assess the adequacy of their sampling efforts.

Estimates of biomass consumption indicated adult White-tailed Deer and White-tailed Deer fawns combined represented 66% of total biomass of prey consumed. Percent frequency of occurrence, relative volume, and relative weight of remains indicated adult White-tailed Deer and White-tailed Deer fawns combined represented 37, 49, and 49%, respectively, of total prey items consumed by all packs (Fig. 2). Spearman rank correlation coefficients showed strong agreement between ranks of importance of prey items between metrics both within and across packs ($r_s > 0.78$).

Based on AICc rankings, the most parsimonious GLM for predicting variation in diet of Red Wolf packs included prey, pack, and prey by pack interaction. Year and reproductive status were not significant variables in predicting variation in diet of Red Wolf packs. The Akaike weight of the most parsimonious GLM was 0.96. The next best GLM included reproductive status as a variable and had a ΔAICc = 170 and Akaike weight < 0.01. Since year was not an important variable in the most parsimonious GLM, diet composition was conducted with data combined across years for all packs. Given that year was not a significant variable, we assume the different methods of identification of scats did not bias our results.

Odds ratios were derived using the link function $e^{(\text{coefficient})}$ and coefficient estimates of the most parsimonious GLM (Table 1). Three of the six packs (Columbia, Timberlake, and Northern) were more likely to consume White-tailed Deer fawns than any other prey item. Milltail, Tyson, and Kilkenny were most likely to consume small rodents, domestic pig, and large rodents, respectively. Compared to all other packs, Northern was most likely to consume both Adult White-tailed Deer and White-tailed Deer fawns.

Table 1. Odds ratios for counts of occurrence in diet of packs of Red Wolves in the Red Wolf Recovery Experimental Population Area in northeastern North Carolina, 2009–2010. Odds-ratios were derived from coefficient estimates of most-parsimonious generalized linear model using link function, $e^{(\text{coefficient})}$.

	Prey item								
	Anthro-pogenic	White-tailed Deer		Large		Wild			Small
Pack	material	Adult	Fawns	rodents	Other	Boar	Rabbit	Raccoon	rodent
Columbia	NC[A]	1.00[B]	5.81	0.43	0.14	NC	1.42	0.28	1.28
Milltail	5.26	2.72	1.14	0.71	4.95	NC	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	NC	0.86	5.81	6.27	NC	0.28	0.79	0.28	2.00

[A]Not consumed.
[B]Reference odds-ratio.

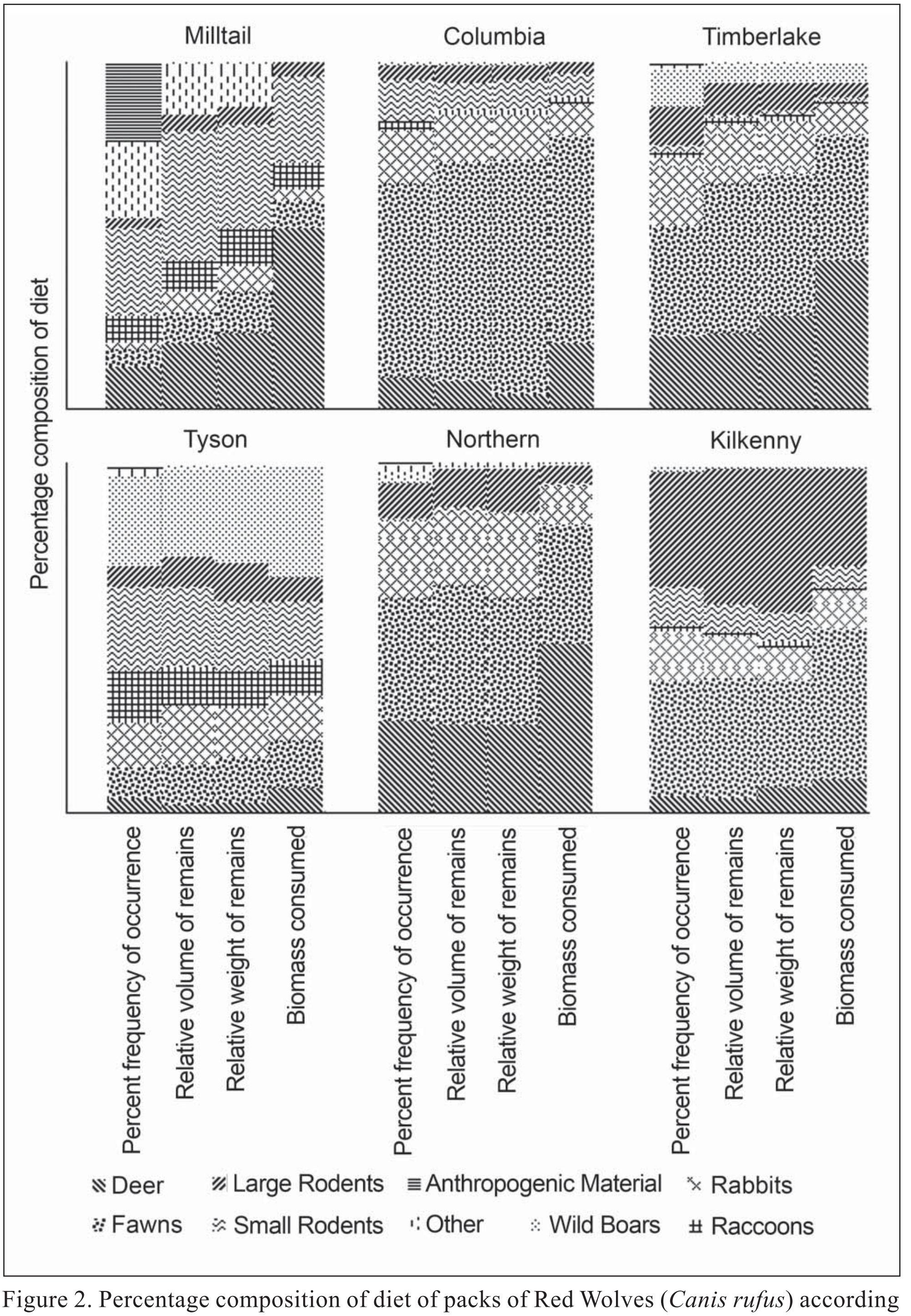

Figure 2. Percentage composition of diet of packs of Red Wolves (*Canis rufus*) according to each metric of diet composition. All percentages for each pack per metric sum to 100. Percentages are given for years combined. Percent frequency of occurrence, relative volume of remains, relative weight of remains, and estimated biomass consumed according to Floyd et al. (1978).

Discussion

Our study revealed that Red Wolf packs primarily consumed mammalian prey species during pup-rearing season. Overall, diets of Red Wolf packs were composed primarily of adult White-tailed Deer and White-tailed Deer fawns during pup-rearing season (Table 1, Fig. 2). Consumption of large-sized mammals such as these 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 the energetic demands of rearing pups during this time of year. The only other large-sized wild mammal available to all Red Wolf packs, feral Wild Boars, was only consumed by Timberlake pack. Phillips et al. (2003) reported Red Wolf packs hunting and bringing down feral Wild Boars.

Although Red Wolf packs consumed primarily adult White-tailed Deer, White-tailed Deer fawns, or both during pup-rearing season (Table 1, Fig. 2), each pack differed from one another in consumption of prey items (Table 1). It is likely that this prey-by-pack interaction is related to consumption of secondary or tertiary prey and not primary prey. 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 prey 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 kinship lines (Mech 1970). Variation in diet due to differences in habitat composition of the territories is unlikely given the low diversity of habitat types across the RWREPA (Beck et al. 2009). Variation in diet of Red Wolf packs during pup-rearing appears to be primarily related to consumption of secondary and tertiary prey, not primary prey which was adult White-tailed Deer and White-tailed Deer fawns. Diets of packs of Red Wolves during pup-rearing did not vary between years; however, a longer study is needed to better assess yearly fluctuations in diets of Red Wolf packs. Diets of Red Wolf packs were not found to vary with reproductive status. This is possible if Red Wolves increase consumption of primary prey items rather than increasing the diversity of prey items consumed.

Consumption of anthropogenic material by the Milltail pack and domestic Wild Boar by the Tyson pack was not surprising given that territories of these packs contained an active garbage dump and carcass pit, respectively. Ciucci et al. (1997) reported a pack of *Canis lupus* L. (Gray Wolf) in Italy relied almost entirely on anthropogenic material from garbage dumps and remains of domestic animals from carcass dump sites. Consumption of human-related foods could raise concern about the reliance of wild Red Wolves on foods associated with humans, particularly pets and domesticated animals. Given that these two packs can catch and consume native prey (Fig. 2), we assume packs of Red Wolves do not seem to rely heavily on foods associated with humans, but will readily consume such foods if given the opportunity. Biologists might consider actions to reduce reliance of these packs on foods associated with humans to reduce potential of Red Wolf-human interactions. Barlow et al. (2010) suggest that, in the face of little scientific data, erection of fencing around sources of human derived foods is generally a good method for reducing reliance of large carnivores on such sources of food.

Our findings suggest that after >20 years since Red Wolves were first re-released to the RWREPA, most packs are surviving completely by consuming wild prey, at least during pup-rearing, within a human-altered landscape. Furthermore, packs of Red Wolves appear capable of catching and consuming a sufficient amount of prey to support reproduction. The ability of Red Wolves to catch and consume natural prey in a human-altered landscape demonstrates their ability to survive and reproduce in close proximity to humans. Future studies should focus on diet of Red Wolves during winter or throughout the year and attempt to assess whether Red Wolves consume prey in proportion to availability or selectively consume prey.

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