ABSTRACT

RABON, DAVID REID, JR.  Factors Affecting Reproduction in the Red Wolf (*Canis rufus*). (Under the direction of Dr. Harold F. Heatwole).

The endangered red wolf (*Canis rufus*) was preserved in captivity with just 14 founders following its planned extirpation in the wild. Longitudinal reproductive events were investigated to determine whether inbreeding, parental age, and breeding experience were factors in reproductive performance and fitness. A behavioral preference study using olfactory presentations of conspecific and congeneric social odors also was conducted to determine those factors that are important in the selection of mates.

Over 30 years of managed breeding, the level of inbreeding in the captive population has increased, and litter size has declined. Inbreeding levels were lower in sires and dams that reproduced than in those that did not reproduce, but there was no difference in the level of inbreeding of actual and predicted litters. Litter size was negatively affected by offspring and paternal levels of inbreeding, but the effect of inbreeding on offspring survival was restricted to a positive influence.

Younger wolves were more likely to reproduce, and were more likely to produce larger litters, than were older individuals. The age of the dam, but not the sire, had a significant negative effect on pup survival. Sires and dams that had prior experience in the production of offspring were more likely to reproduce again than were individuals without prior reproductive success, but prior sexual experience alone was not a factor in the production of offspring. Parental breeding experience had a significant negative effect on pup survival, but no apparent relationships with size or sex ratio of the litter.
In general, females responded to the presentation of social odors of conspecific males more quickly than did males, and males responded more quickly to social odors of conspecific females than did females. There were no differences in the proportion of time males and females spent proximal to conspecific social odors. Males and females typically responded more quickly to and spent more time investigating social odors during the non-breeding season than during the breeding season. Species-specific responses to social odors were indeterminate.
Factors Affecting Reproduction in the Red Wolf (*Canis rufus*)

by

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A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

Zoology

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DEDICATION

To Tracy.
David Reid Rabon, Jr. was born in Charlotte, North Carolina, to David and Dianne. In his youth, David spent numerous hours exploring urban streams and riparian corridors. After graduating from Independence High School, David started ascending the “corporate ladder,” completing an Associate of Arts degree from Central Piedmont Community College along the way. Although he enjoyed the benefits of a steady paycheck, the corporate life could not satisfy David’s interest in animal behavior and wildlife conservation. David entered the University of North Carolina at Wilmington to “formally” study the natural world, and graduated with Bachelor of Arts degrees in Biology and Psychology, and a Master of Science degree in Biology. Upon completion, David continued his graduate studies in Ontario Veterinary College’s Department of Biomedical Sciences at the University of Guelph before entering the doctoral program in the Department of Biology at North Carolina State University.

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David currently holds memberships in several professional societies, including American Society of Mammalogists and Animal Behavior Society. He also was appointed
membership in PSI CHI, the National Honor Society in Psychology, and Sigma Xi, the International Honor Society for Science and Engineering.
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CHAPTER 1

GENERAL INTRODUCTION

For centuries, the wolf (*Canis lupus* Linnaeus) was regarded with great admiration, and revered as a courageous, intelligent, and wise icon in folklore and mythology. In the Middle Ages, however, it was transformed from an emissary of gods into a symbol of evil. As an object of hostility, the wolf was maligned in folktales, fables, and fairy tales (e.g., Brothers Grimm, *Kinder- und Hausmärchen* (Children's and Household Tales), vol. 1, 1812; vol. 2, 1814) and persecuted in reality. Enmity toward the wolf would not remain confined to Europe, though. Fear and hatred of the wolf, irrationally passed down the generations, was exported to the New World where the beast was victimized with renewed fervor.

The red wolf (*Canis rufus* Audubon and Bachman), the smaller cousin of the gray wolf, became a focus of animosity for Euro-American colonists. Shortly after their arrival at Plymouth, the pilgrims, acting on ignorance and religious prejudice, placed a bounty on the wolf. So effective was the bounty that before the eighteenth century came to a close, the wolf was extirpated from the northeastern United States. Persecution of wolves, however, didn’t end there. Bounties on wolves spread like an epidemic south and west with the growing nation. With the expansion and increasing number of pastoralists, the wolf was seen as much as an ecological competitor that threatened livestock and livelihoods as it was a diabolical and malevolent beast. In the south-central United States, where the last
remaining populations of the red wolf persisted, the conversion of forests to fields and other lumbering practices created new problems for the wolf. Alteration of habitat and the creation of edge habitat facilitated the eastward expansion of the coyote (*Canis latrans* Say) (Young, 1951), and forced the remaining red wolves into suboptimal environs. The eastward resettlement of the coyote also brought the red wolf and coyote closer together and across larger areas than ever before, creating greater opportunities for interaction and hybridization.

By the early 1900s, the United States government took the reigns from hunters in the business of killing wolves, becoming “the executioners of American progress” (Coleman, 2004: 210). As the wolf was being systematically exterminated by hunters and government biologists through extensive predator-control programs, it was also being romanticized in popular literature (e.g., Carhart and Young, 1929; Leopold, 1949). The change in sentiment would eventually reverse the reaction to wolves, or at least the government’s role in their eradication, but not before the red wolf was extirpated east of the Mississippi River.

Widespread extirpation, extensive modification of habitat, and the growing threat of hybridization between the coyote and the surviving red wolves brought the red wolf close to extinction. By the latter part of the twentieth century, however, public attitude had swayed sufficiently to persuade the United States government to create a progressive law to conserve the red wolf and other imperiled animals. With the passage of the Endangered Species Act of 1973, the red wolf attracted immediate attention. However, land modification and efforts at exterminating wolves were highly successful. The preservation
of the red wolf would require drastic measures (see THE DECLINE OF THE SPECIES section), including the planned extirpation of red wolves in the wild to create a captive breeding program, with the hopes of one day restoring a population in the wild. Less than a decade after Congress passed legislation to protect imperiled species, the red wolf was considered biologically extinct in the wild (McCarley and Carley, 1979; USFWS, 1984).

The precarious removal of red wolves from the wild was successful in captive reproduction and, later, the unprecedented reintroduction of captive-born red wolves into a portion of the species’ former range (see RESTORING A SPECIES section). Unfortunately, success in breeding and restoration in the wild has not left the red wolf free from threat. In fact, the threats (e.g., wolf-coyote hybridization, declining reproduction, alteration of habitat, persecution by humans) are as real and numerous today as they were in the mid-1900s when the circumstance of the red wolf was made known. However, decades of breeding the red wolf in captivity and monitoring and managing a restored wild population have presented new and unique opportunities to learn about the species, and find novel approaches to deal with age-old problems.

This dissertation represents an analysis of the effects of captive-breeding a species once driven to the brink of extinction, and an evaluation of behavioral mechanisms important for reproduction in that species when interacting with a competing congener. The goals of this study were to elucidate those factors (e.g., behavioral, environmental, and physical) that have influenced and/or continue to influence reproduction in the red wolf, and to identify strategies that will assist in the development of management tools to increase reproductive potential in the captive and restored populations and abate
hybridization in the reintroduced population. To achieve these goals I had two specific objectives –

1. to conduct a comprehensive, retrospective assessment of the efforts of the captive breeding program to determine those factors that have affected reproductive output, and

2. to conduct a comprehensive, behavioral preference study using olfactory cues and stimuli to determine traits that are important in the selection of a mate among conspecifics and congeners (e.g., sex-specific and species-specific preferences, respectively).

Chapter 1 provides a general introduction to the red wolf, its life history, behavior, and ecology. The chapter also includes a discussion of historical events leading to the decline of the species, the efforts to preserve it as an entity, and its restoration in the wild. I do not include an extensive discussion of the issue of taxonomy, as that subject is beyond the scope of this study.

Chapters 2 and 3 provide an analysis of the factors affecting reproduction of the red wolf in captivity. The effects of inbreeding on reproductive output are the focus of Chapter 2. In Chapter 3, the effects that age and experience have on reproduction are examined. Data from the wild population are used for comparison where relevant.

Studies have shown that canids use excreta as a form of scent-marking, and that excreta might be important in recognizing individuals and in identifying and selecting mates. Understanding how red wolves use excreta in selecting mates among conspecifics and congeners can assist managers in improving opportunities for successful reproduction.
between wolves and in reducing the threat of hybridization between wolves and coyotes. Chapter 4 provides an evaluation of the behavioral responses of wolves to excreta of unrelated conspecifics and congeners to determine which factors, if any, are important in the selection of mates.

Chapters 2, 3, and 4 are prepared as publishable manuscripts; thus, each includes introduction, methods, results, and discussion sections. Chapter 1 is a general introduction to the study, with additional information on the red wolf that is not necessarily pertinent for a publishable manuscript, but which introduces the reader to the broader circumstances of the red wolf. The dissertation concludes with a general discussion (Chapter 5) that synthesizes relevant information not included in the discussion sections of other chapters.

THE SPECIES

There is considerable geographic and individual variation among North American Canis (the genus containing wolves, coyotes, and dogs), and while there are distinctive characters among the species, identification, especially at the subspecific level, generally requires combinations of quantitative values (Goldman, 1944). Typically, museum collections contain preserved skulls, skins, and whole specimens of animals collected across their range, thereby assisting in the process of diagnosis and limits. However, that is not the case with all members of the genus Canis, especially C. rufus. Although countless wolves were killed during decades of unregulated hunting and government-sanctioned predator-eradication programs such that they became extinct in many areas, including the
eastern United States, few specimens were retained in museum collections. Indeed, Goldman (1937: 37) stated that wolves “have remained very imperfectly known” as a result of the limited material available for study. The lack of preserved material and the situation in which the few remaining extant red wolves were discovered have led to much confusion and to a long-running debate on the origins of the red wolf, and have confounded an accurate diagnosis of the species. As such, the following descriptive characters for the red wolf should be considered only qualitative.

**Distribution**

The red wolf is one of two species of so-called “true wolves” from North America (the other being *Canis lupus*) (Goldman, 1944: 397). Red wolves were once distributed throughout much of the eastern and south-central United States, but documentation is so scarce that the precise limits to its geographic distribution are poorly known (Nowak, 1970, 1979, 1995, 2002). The species likely occurred from Maine southward to Florida and thence westward to central Texas (Nowak, 2002). However, recent genetic evidence supports an even greater range extension into southern Ontario and Quebec, Canada, based on genetic similarities with the eastern Canadian wolf (*Canis lupus lycaon*) of Algonquin Provincial Park (Wilson et al., 2000).

Three subspecies of red wolf were recognized by Goldman (1937, 1944), and supported by Nowak (2002) with minor rearrangement of subspecific distribution: *C. r. rufus* Audubon and Bachman, known from coastal Texas and possibly central Texas, and
which may have extended eastward along the Gulf coast (including Louisiana); *C. r. gregoryi* Goldman, known from the lower Mississippi Valley and in the Big Thicket area of eastern Texas; and, *C. r. floridanus* Miller, known from Florida to Maine, and inland as far as Ohio and northern Alabama (Figure 1). *Canis rufus rufus* is presumed to be representative of extant red wolves based on recent morphometric analyses (Nowak, 2002); the subspecies *C. r. gregoryi* and *C. r. floridanus* are presumed extinct.

At present (2009), the red wolf exists in 42 captive-breeding facilities spread throughout the United States (Waddell and Long, 2009), and in a free roaming population extending over a five-county area (Beaufort, Dare, Hyde, Tyrrell, and Washington) in northeastern North Carolina, known as the Red Wolf Recovery Area (RWRA) (Figure 2). Insular propagation sites have also been used to breed and rear wolves; currently, one insular propagation site is active. See RESTORING A SPECIES section below for more detail on the distribution of extant red wolves.

**Taxonomy**

There has been substantial debate on the origins of the red wolf (see Parker, 1989, for an earlier review of red wolf taxonomy). Some authorities have suggested the red wolf might be a subspecies of *C. lupus* (Audubon and Bachman, 1851, as *C. lupus var. rufus*; Lawrence and Bossert, 1967; Phillips and Henry, 1992), or a hybrid resulting from the interbreeding of *C. lupus* and *C. latrans* (e.g., Jenks and Wayne, 1992; Mech, 1970; Reich et al., 1999; Roy et al., 1994, 1996; Wayne and Jenks, 1991). Others have suggested that
the red wolf is a distinct species in which hybridization contributed, in part, to its demise, but not its origin (e.g., Nowak, 1970, 1979, 1992, 1995, 2002; Paradiso, 1968; Paradiso and Nowak, 1973; Phillips and Henry, 1992).

The origin of the red wolf is principally at issue because current policies of the U.S. Fish and Wildlife Service (USFWS) do not permit the protection of hybrids under the Endangered Species Act (USFWS, 1990; see also Protected Status section). Yet, protection of the red wolf as an entity does not appear to be at issue in the taxonomic debate. Even among some of those proposing the concept of a hybrid origin, the red wolf is recognized as an essential component of the fauna that fills an ecological niche and warrants protection (e.g., Roy et al., 1996; Wayne and Jenks, 1991). Indeed, the USFWS, when once petitioned to delist the red wolf (remove from the list of threatened and endangered species) on the basis of a hybrid origin, clarified its position on the red wolf as a distinct species by stating that “the red wolves of today are representative of the canids that roamed the Southeast historically and are morphologically and behaviorally distinct from coyotes and gray wolves” (USFWS, 1992b: 1250). This decision, based on an evaluation of the known behavioral (e.g., Riley and McBride, 1972; Shaw, 1975), morphological (e.g., Atkins and Dillon, 1971; Elder and Hayden, 1977; Nowak, 1979), and biochemical genetic (e.g., Ferrell et al., 1980; Morizot, 1981 in Parker, 1989) data, has been reaffirmed in subsequent behavioral (e.g., Schneider, 1999) and morphological (e.g., Nowak, 1995, 2002; Nowak and Federoff, 1996; Nowak et al., 1995) studies, but intensely contested when molecular genetics are considered (e.g., Brownlow, 1996; Dowling et al., 1992a,b; Nowak, 1992;
Nowak and Federoff, 1998; Reich et al., 1999; Roy et al., 1994, 1996; Wayne and Gittleman, 1995; Wayne et al., 1998).

Recent studies suggest the red wolf and the wolf of eastern Canada (C. lupus lycaon) are distinct from C. lupus and C. latrans and share a common ancestry (Wilson et al., 2000), but developing genetic studies appear to support the assertion that the red wolf is distinct from C. lupus lycaon (L. Waits, Department of Fish and Wildlife Resources, University of Idaho, pers. comm.). Given the genotypic complexities in discerning North American Canis affected by hybridization, the red wolf taxonomic issue is not likely to be resolved soon. Furthermore, concession of a conspecific relationship between C. lupus lycaon and C. rufus would not necessarily imply consensus on the management implications of that relationship (e.g., Kyle et al., 2006, 2008; Murray and Waits, 2007). Nowak (2003) and Wayne and Vilà (2003) provide a thorough review of the uses of morphometric analyses and molecular genetics, respectively, in wolf taxonomy and evolution, and specifically in regard to the origin of the red wolf.

Description

The red wolf is a relatively large canid, intermediate in size between the coyote and gray wolf. Compared to the red wolf, the coyote is smaller and less massive through the head, chest, legs, and feet, and it weighs about one half to two-thirds as much. The red wolf is distinguishable from the gray wolf by its narrower proportions of body and skull, shorter fur, and relatively longer legs and ears.

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Adult red wolves from the last remaining wild population measured 1355 to 1650 mm in total length (Paradiso and Nowak, 1972), males generally being larger than females. Male red wolves from the RWRA measure from 1370 to 1710 mm (mean = 1506 mm) in total length, and females from 1285 to 1641 (mean = 1436 mm) (Kelly et al, 2004). Using minimum morphological standards to differentiate red wolves from coyotes and coyote-like canids, McCarley and Carley (1979) proposed male red wolves stand at least 685.8 mm at the shoulder, and females 673.1 mm (but see Carley, 1979b; female 647.7 mm). Average shoulder height in extant males from the RWRA is 699 mm (range 640 to 772 mm), and females 662 mm (range 590 to 729 mm) (Kelly et al., 2004).

Based on specimens of red wolves collected in Arkansas (prior to 1930), the average weight of adult males was 27.6 kg (range 21 to 41 kg) and of adult females was 21.6 kg (range 16 to 29 kg) (as reported in Paradiso and Nowak, 1972). Riley and McBride (1972) reported on the weights of 14 adult red wolves captured from Chambers County, Texas: males weighed between 20.9 and 28.1 kg (mean 23.7 kg) and females between 20.4 and 24.5 kg (mean 21.2 kg). Shaw (1975), in comparing weights of adult wolves assigned as C. r. rufus (n = 8; 6:2, ♂♂:♀♀) and C. r. gregoryi (n = 7; 5:2) collected from Chambers County, Texas, recorded subspecific means of 18.9 and 24.7 kg, respectively. Differences in weights of specimens from these two general populations (i.e., Arkansas and Texas) may be attributed to subspecific variation (i.e., C. r. gregoryi and C. r. rufus). An east-to-west cline of size was noted in the red wolf, with C. r. floridanus being the largest and C. r. rufus the smallest (Goldman, 1944; Nowak, 2002). Weights of red wolves (especially C. r. floridanus) from the eastern United States prior to extirpation in these areas are not known.
Average weights of male and female red wolves from the RWRA are 28.5 kg (range 22.0 to 34.1 kg) and 24.3 (range 20.1 to 29.7 kg), respectively (Kelly et al., 2004).

The red wolf takes its name from the red element of the pelage, which can be pronounced during winter, but is typically a mixture of cinnamon buff, cinnamon, or tawny, with gray or black (Figure 3a). The dorsal area is generally heavily overlaid with black. The outer portions of the ears, muzzle, and limbs are usually tawny. The belly is generally whitish to pinkish buff, and the tip of the tail is black. A black phase of the pelage was at one time common (Figure 3b), at least in the floridanus subspecies, as evidenced by the name “lupus niger” originally given by Bartram (1791). In fact, black pelage was long considered a distinguishing red wolf characteristic (Halloran, 1958, 1959, 1960; Pimlott and Joslin, 1968). Several black canids were identified during the early 1970s, but were later determined to be rare melanistic coyotes or hybrids and not red wolves (Carley, 1977; Waddell, in litt.). Black pelage is rather common in *C. lupus* (Gipson et al., 2002; Young, 1944), but rare in *C. latrans* (Halloran, 1958, 1959, 1963; Young, 1951). The black phase in the red wolf is now presumed extinct. A single captive-born, female red wolf had a whitish pelage (Figure 3c) that became more pronounced as she aged; no red wolves have been reported in a white phase in the RWRA. White coloration is rare among *C. latrans* (Young, 1951), and among *C. lupus* (Gipson et al., 2002) south of the Arctic. As noted, there is substantial variation in pelage color, and for that reason color is generally not considered a diagnostic feature. There is an annual molt during summer in the red wolf.
Habitat and Ecology

Little is known about red wolf habitat because the species’ range was drastically reduced by the time scientific investigations began. However, given its historical distribution, red wolves likely used a variety of habitats. The last naturally occurring population used the coastal prairie marshes of southeastern Texas and southwestern Louisiana (Carley, 1975; Russell and Shaw, 1972; Shaw, 1975), but this was not likely the preferred habitat. The extant population of wolves in northeastern North Carolina occupies a mosaic of coastal swamps and marshes, canebrakes, forested wetlands, agricultural plots, and small patches of upland forest. This may suggest that red wolves are habitat generalists and can adapt to a variety of habitats as long as sufficient prey is available. Hahn (2000) found low human population density, wetland soils, and greater distances from roads to be the best predictors of potential wolf habitat in the RWRA.

Red wolf home ranges appear to vary with season and age of animal. Red wolf movements are reported greater in winter than in spring and summer during the whelping period, and juvenile red wolves are reported to exhibit larger home ranges than do adults. Winter home ranges of adult red wolves in the remaining wild population in Chambers County, Texas, were reported to average 28.4 km$^2$; those of juveniles were reported to average 56.5 km$^2$ (Shaw, 1975). Summer home ranges averaged 6.2 km$^2$ for adults and 8.2 km$^2$ for juveniles (Shaw, 1975). These average home range sizes are smaller than those reported by Riley and McBride (1975; 64.8 to 129.5 km$^2$) and Shaw (in Riley and McBride, 1975; 90.7 km$^2$) from the same general populations. They also are smaller than home
ranges reported from free-ranging red wolves in the RWRA, which range over 59.0 km$^2$ to 110.6 km$^2$ (Hinton, 2006). Phillips et al. (2003) reported average home ranges of wolves in the RWRA to be about 88.5 km$^2$ for individuals and 123.4 km$^2$ for packs. However, all reported red wolf home ranges appear to be intermediate between those of coyotes, which typically average less than 50 km$^2$ (e.g., Gese et al., 1988; Holzman et al., 1992), and those of gray wolves, that can well exceed 500 km$^2$ (e.g., Mech and Boitani, 2003, and references cited therein). Type of habitat and availability of prey appear to be factors determining the size of home range in red wolves, but pack size may also be an interacting factor (Phillips et al., 2003). Pack size and territory size were found to be related in the coyote (Bowen, 1982; but see Andelt, 1985) and in the gray wolf (e.g., Ballard et al. 1987; 1997; but see Mech and Boitani, 2003, and references cited therein).

The red wolf is mostly nocturnal with crepuscular peaks of activity, and a slight seasonal shift to diurnal activity during winter (Shaw, 1975). Red wolves typically hunt for prey at night or at dawn or dusk, individually and as a group (Kelly et al., 2004). Resource partitioning within a pack sometimes occurs. Phillips et al. (2003) reported that rodents were consumed more frequently by juvenile red wolves than by adults, and that consumption of rodents diminished as juvenile wolves matured. Resource partitioning might also play a role in home range size. Phillips et al. (2003) reported that packs partitioning resources had smaller home ranges than did packs that did not partition resources. The differential use of large vs. small prey also is seen among red wolf packs, and appears to be related to availability and abundance of prey (Knight, 2001; Phillips et al., 2003).
The diet of red wolves varies depending on the area they inhabit and on available prey; red wolf diets in eastern North Carolina mainly include white-tailed deer (*Odocoileus virginianus*), raccoons (*Procyon lotor*), marsh rabbits (*Sylvilagus palustris*), and small rodents (primarily cotton rats [*Sigmodon hispidus*] and house mice [*Mus musculus*]) (Knight, 2001; Phillips, 1995; Phillips et al., 2003). This differs from red wolf diets recorded in Texas and Louisiana, where nutria (*Myocastor coypus*), rabbits (*Sylvilagus* spp.), cotton rats, and muskrats (*Ondatra zibethicus*) were the predominant prey (Riley and McBride, 1972; Russell and Shaw, 1971; Shaw, 1975). The habitats of red wolves from Horn Island, Mississippi, an insular propagation site (Parker, 1987b; see also RESTORING A SPECIES section), included rabbits, raccoons, and nutria as the primary prey; deer were absent from the island (Weller, 1995). Diets of wolves recorded from Texas and Louisiana might not reflect natural choice of prey by the red wolf. Gipson and Sealander (1976) suggested that variations in diets could be the result of changes in the habitat and subsequent changes in abundance and availability of the red wolf’s preferred prey. Indeed, Knight (2001) reported that deer, raccoons, and rabbits were consumed more frequently by red wolves occupying non-agricultural habitats than by wolves occupying agricultural habitat; conversely, rodents were consumed more frequently by wolves occupying agricultural habitat than by wolves occupying non-agricultural habitats.
Social Structure and Reproduction

The red wolf is a social canid that forms permanent or semi-permanent pair bonds. Riley and McBride (1972) postulated that it was less social than the gray wolf, but more so than the coyote. It appears that their premise was based on the number of red wolves (naturally occurring in Louisiana and Texas) observed traveling in a group and occupying a range, and a diet consisting of prey undeserving of pack hunting (i.e., small prey size). As previously noted, availability of prey may have affected the behavior of the red wolf in the last naturally occurring population.

Red wolves live in extended family units called packs. A pack consists of a breeding adult male and a breeding adult female, occasionally with juveniles from previous whelping seasons and young of the year. Pack sizes can range from a mated pair to 12 individuals (Kelly et al., 2004); although larger packs have formed in RWRA. In the reintroduced population a pack of at least 20 wolves (two adults [Studbook (SB) #1199 and SB#1162], eight juveniles [SB#1424–1431] plus one unconfirmed, and 10 pups [SB#1497–1506]) formed for approximately five months before some of the juveniles dispersed in the fall of 2006 (USFWS, unpublished data).

Courtship and mating in the red wolf occurs from late-December or early January to March, and pups are whelped in the spring (April and May) after a gestation period of 60-63 days (Paradiso and Nowak, 1972). Riley and McBride (1972) reported that pups were born in March and April; however, they provided no data to support their assertion, and it may be a simple error in calculation. In 30 years of the captive breeding of the red wolf
only two litters were born in March: a litter of six (4:2) born 25 March 2002 at Alligator River National Wildlife Refuge (captive facility) to sire SB# 980 and dam SB# 896; two females born 26 March 2007 at Lowry Park Zoo, Tampa, FL to sire SB# 779 and dam SB# 1274. No litters have been born in June.

Typically, only one litter is born to a pack in a given year. A pack producing two litters in a single breeding season (2001) has been documented in the RWRA (J. Adams, Department of Fish and Wildlife Resources, University of Idaho, pers. comm.; USFWS, unpublished data). The two females [mother (SB#756) and daughter (SB#1051)] shared a den to whelp their 11 pups sired by an unrelated male (SB#1052) (USFWS, unpublished data). Multiple litter production has been reported in the gray wolf, but separate dens are typically used by the pregnant females (Ballard et al., 1987; Mech and Nelson, 1989; Mech et al., 1998). Multiple paternity (i.e., polyandry) in a red wolf pack also has been documented in the RWRA and confirmed through genetic analysis (J. Adams, Department of Fish and Wildlife Resources, University of Idaho, pers. comm.; USFWS, unpublished data). Two males (SB#1427 and SB#1418) sired four pups (SB#1638–1641) with a single female (SB#978) in 2007 [male SB#1427 and female SB#978 were paired, but the female bred with male SB#1418 sometime during the breeding season. Male SB#1427 disappeared shortly after the breeding season, and SB#1418 moved into the pack.].

Red wolves rear their young in dens of shallow depressions with dense vegetation for cover or in deep burrows along the slopes of brushy windrows or canal banks or at the base of large trees (Phillips et al., 2003; USFWS, unpublished data). Pregnant females may dig several dens during the breeding season. The same den may be used in consecutive
years, although use in consecutive years seems to be limited to deep burrows rather than above-ground depressions (Phillips et al., 2003). Dens are used from April through July, corresponding to the whelping and pup-rearing periods. The den may be frequented by all members of a pack, but attendance at dens is most common by the breeding pair; females attended pups more often than did males and adults tended pups more often than did juveniles (Hinton, 2006; USFWS, unpublished data).

Reported litter sizes (captive and wild; n = 396 litters) range from 1 to 11 pups, with an average of four pups (mean = 4.0) per litter (Waddell, 2009). Both parents participate in rearing of the young (Wagener, 1998). Yearlings are often found within the vicinity of the dens, and may help in rearing young (Hinton, 2006; USFWS, unpublished data). Juvenile dispersal occurs when the wolves are between 16 and 22 months of age (Phillips et al., 2003). Full size is attained in one year, and sexual maturity is reached by three years; although younger animals have been known to successfully produce offspring (see Chapter 3).

The median life expectancy of the red wolf (including animals only surviving to at least 30 days of age) is about three years in the wild (Waddell, 2009), but wolves 13 years of age currently (2009) exist in the RWRA. Red wolves have lived to 15 years of age in captivity and in the wild (USFWS, unpublished data; see also Chapter 3).
THE DECLINE OF THE SPECIES

The plight of the red wolf was first brought to the attention of scientists in the early 1960s (McCarley, 1962). While the red wolf was extirpated throughout most of its range by the early part of the twentieth century as a result of hunting, aggressive predator control programs, and habitat destruction (Nowak, 1970, 1972), small populations were known to exist in portions of Arkansas (Bond and Crawley, 1968; McAnally and Crawley, 1968; 1969; Sealander, 1956; Wilson and Crawley, 1967), Louisiana (Lowery, 1936, 1943), Missouri (Elder and Hayden, 1977; Leopold and Hall, 1945; Paradiso and Nowak, 1971), Oklahoma (Blair, 1939; Halloran, 1958; Halloran and Glass, 1959; McCarley, 1952), and Texas (Bailey, 1905; Halloran, 1959, 1961; but see McCarley, 1959). At the same time, many of these reports (Bailey, 1905; Blair, 1939; Lowery, 1936, 1943; Sealander, 1956) and others (e.g., Paradiso, 1966) noted the scarcity or absence of coyotes in areas where red wolves occurred. The continued conversion of the landscape from wooded areas to prairie and agricultural space, however, reduced the red wolf’s habitat and allowed the coyote to expand its range further eastward (Paradiso, 1966; Paradiso and Nowak, 1971). The red wolf and the coyote, once primarily allopatric species (except along a line of the mid-west where the drier grasslands met the eastern forested zone), had become sympatric.

The remaining red wolf populations had rapidly declined by the middle of the twentieth century, and were replaced by the coyote and coyote-like animals (e.g., coyote-wolf hybrids, coyote-dog hybrids, wolf-dog hybrids). By the early 1960s, McCarley (1962) concluded that the red wolf was extant only in eastern and southern Louisiana. Paradiso
(1965), based on specimens collected in 1961 and 1963 or early 1964, concluded that the red wolf also was present in some Gulf-Coast counties of Texas. However, populations of pure red wolves, present just a decade earlier, could not be confirmed in Arkansas (Gipson et al., 1974), Missouri (Elder and Hayden, 1977) or Oklahoma (Freeman and Shaw, 1979). In fact, these studies and others (e.g., McCarley, 1962; Paradiso and Nowak, 1971) reported the coyote to be the predominant wild canid in most areas, and that red wolf influence was diminishing. The coyote appeared to have replaced the red wolf in Oklahoma and Missouri (Paradiso and Nowak, 1971). However, in central Texas, southern Louisiana, and portions of Arkansas, the substantial morphological diversity of the canid population indicated extensive hybridization between the red wolf and the coyote (Paradiso and Nowak, 1971; but see Russell and Shaw, 1971). This generated a concern that hybridization between coyotes or coyote-like animals and red wolves would genetically swamp the remaining red wolves and threaten its continued existence (Carley, 1975).

**Protected Status**

On 11 March 1967, the red wolf was listed (as *C. niger*) as endangered under the provisions of the Endangered Species Preservation Act of 1966 (USFWS, 1967). Under this act, the Departments of Interior, Agriculture, and Defense were directed to protect listed species and, if consistent with their respective primary purposes, preserve the species’ habitats. Furthermore, the taking of listed species was prohibited only within the National
Wildlife Refuge system. Although acquisition of land for protection of species also was authorized, the legislation did little more than identify species threatened with extinction.

Two years later, the Endangered Species Conservation Act of 1969 was passed. This act provided additional protections by listing species threatened with global extinction that might be endangered in some parts of the United States but not in danger elsewhere (e.g., Canada), and prohibited the import and subsequent sale of such species within the United States. Of specific importance, though, is that this legislation called for an international ministerial meeting to adopt a convention on the conservation of endangered species. That meeting was held as a conference in 1973 in Washington, D.C., and led to the signing of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which restricted international commerce in animal and plant species believed to be harmed by trade. Later that year and following the conference, the United States passed the Endangered Species Act (as amended; ESA; 16 United States Code 1531 et seq.), combining and strengthening the provisions of the Endangered Species Preservation Act and the Endangered Species Conservation Act, and the United States implementation of CITES.

The purposes of the ESA are “to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved, to provide a program for the conservation of such endangered species and threatened species, and to take such steps as may be appropriate to achieve the purposes of the treaties and conventions” (USFWS, 1992a). Simply stated, the goal of the ESA is to use all means
necessary to recover endangered and threatened species such that they no longer need protection under the act.

The red wolf is currently listed as endangered under the provisions of the ESA. The species also is listed as critically endangered by the International Union for Conservation of Nature and Natural Resources (IUCN) World Conservation Union (Hilton-Taylor, 2000). Listing the red wolf as an endangered species in 1967 attracted immediate attention in regards to its recovery, but it wasn’t until the passage of the ESA that measures to preserve the species were spurred into action.

RESTORING A SPECIES

By the early 1970s, the range of the red wolf was reduced to the coastal counties and parishes of southeastern Texas and southwestern Louisiana (Riley and McBride, 1972; Russell and Shaw, 1972); fewer than 300 pure red wolves were believed to exist (Mech and Rausch, 1975). In anticipation of the passage of the ESA, the Service established a recovery program for the red wolf in the fall of 1973. The objectives of the program were to: (1) determine the location and abundance of each surviving red wolf subspecies; (2) restore surviving red wolf subspecies in their present ranges to desirable population levels; (3) maintain an adequate gene pool; and, (4) reestablish surviving red wolf subspecies in additional locations within their historic range (McCarley and Carley, 1979). However, field studies soon determined that a “hybrid swarm” had spread eastward from central
Texas and threatened to genetically swamp the remaining red wolves (Carley, 1975, 1977; Nowak, 1970).

Shortly after the ESA was signed into law, the USFWS realized the red wolf could no longer be saved in the wild (Carley, 1975). At that time, a decision was made to shift the program from an “objective of local preservation to one of planned extirpation of the species in the wild” (USFWS, 1989: 10). This decision was defensible only with the development of a long-range plan to eventually restore the species to the wild. Accordingly, the recovery program’s objectives were adapted to “(1) locate and capture as many red wolves as possible in an attempt to preserve the species in captivity and (2) explore the feasibility of reestablishing red wolf populations in areas of the species’ historic range” (USFWS, 1984: 9). Because of the urgency to save the species, early efforts in recovery of the red wolf had to be multifaceted, including development and dissemination of public information about the species and responding to complaints of damage caused by animals on private lands within the species’ remaining range. Responding to complaints had the benefit of giving program biologists access to canids on private lands, reducing human persecution of the wolf, and building a rapport with landowners.

Efforts at recovery also required the development of methods and procedures for distinguishing wolves from wolf-like hybrids, and evaluating suspected red wolves already housed in zoos and other captive facilities (USFWS, 1989). However, extensive hybridization in the red wolf’s remaining range made it difficult to identify distinguishable characteristics of wolves. As a result, no one characteristic could be used to identify pure red wolves. Morphometric measurements with minimal taxonomic standards, skull x-ray,
and electrophoretic and vocalization analyses were used in distinguishing red wolves from wolf-like hybrids (Carley, 1975, 1979b; McCarley, 1978; McCarley and Carley, 1979). These techniques were used in conjunction with a captive breeding/certification program administered through a cooperative agreement between the USFWS and Metropolitan Park District’s (Tacoma, Washington) Point Defiance Zoological Park (Point Defiance Zoo & Aquarium; PDZA).

Between the years 1973 and 1980, the USFWS captured more than 400 canids in coastal areas of Texas and Louisiana. Canids determined to be probable wolves were placed in the certification program. Only 43 animals met the criteria established to define the red wolf and were admitted to the program for verification. Because of the complexities of identification, final confirmation of red wolves was made through the captive breeding process and production of offspring. Ultimately, only 14 red wolves could be confirmed through the captive breeding process. By 1980, the red wolf was considered extirpated and biologically extinct in the wild (McCarley and Carley, 1979; Parker, 1988; USFWS, 1984).

The Founders

The founding wolves of the captive breeding program included nine males and five females (Table 1) trapped from two coastal counties in Texas and two coastal parishes in Louisiana (Figure 4). In 1977, a pair of wolves captured together in Louisiana was the first to produce a litter (6 pups) in captivity (USFWS, unpublished data). Over the next four years (1978 to 1981) the species demonstrated its reproductive vitality in captivity by
producing 79 pups in 15 litters (USFWS, unpublished data). [In subsequent years, two of
the founding lines (SB#17, Pines Inholding Male and SB#30, Crain Female) would be lost
– no living descendants are represented in the current population (USFWS, unpublished
data). Sperm from SB#17’s last living descendant (SB#255) was collected and preserved so
that this founding line might be resurrected at a later date through artificial insemination.]
With the species securely reproducing in captivity, the recovery program immediately
turned its attention to reintroducing the red wolf into the wild.

Return to the Wild

In 1976 and 1978 the recovery program conducted experimental releases of wild-
caught, mated pairs on Bulls Island of the Cape Romain National Wildlife Refuge (South
Carolina) (Carley, 1979a, 1981a,b). The experimental releases were temporary because
Bulls Island was not large enough to support a viable population of red wolves. However,
the one-year experiments demonstrated that reestablishment of wild-caught, and potentially
captive-born wolves, was feasible. To begin the reestablishment process, the recovery
program drafted a proposal to experimentally reintroduce a viable population of red wolves
on the Tennessee Valley Authority’s Land Between the Lakes (Carley and Mechl, 1981,
1983), a 689 km² (68,894 ha) peninsula of public-owned lands between Kentucky Lake and
Lake Barkley in western Kentucky and Tennessee. The five-year experimental plan called
for the release of three to five adult mated pairs over a two-year period. However, the
proposal was abandoned a few years later before any wolves were ever released, in part, because of public misunderstanding, opposition, and a general lack of support.

In 1984, another potential reintroduction site was presented when the Prudential Insurance Company donated nearly 485 km$^2$ (48,562 ha) on the mainland of Dare and Tyrrell counties (North Carolina) to the Federal government. The lands would be administered by the USFWS as the newly formed Alligator River National Wildlife Refuge (ARNWR). A plan to reestablish a population of wolves on the new refuge, based primarily on the proposal for Land Between the Lakes, was soon developed (Parker, 1987a). Early surveys of the area proved the refuge contained suitable prey for the red wolf and, equally important, that coyotes, feral dogs, and other wild *Canis* were absent. After considerable evaluation and public engagement, the USFWS began the first reintroduction by releasing eight, captive-born wolves (four adult male-female pairs) on the refuge in the fall of 1987 (Rabon, 2007). Additional releases followed in subsequent years, but by 1993 the release of captive-born adult wolves had ceased (A. Beyer, Red Wolf Recovery Program, U.S. Fish and Wildlife Service, pers. comm.). The release of island-born wolves continues, and more recently, captive-born pups have been fostered into wild litters within the RWRA to be raised as wild wolves (USFWS, unpublished data; Waddell et al., 2002, in litt.).

A second reintroduction was initiated by the USFWS in 1991 in the Great Smoky Mountains National Park (Parker, 1990). However, the USFWS terminated this effort in 1998 based on the lack of survivability of pups and the inability of the wolves to establish home ranges within the park (Henry and Lucash, 2000; USFWS, 1998).
Three insular propagation sites were established in South Carolina (Bulls Island, Cape Romain National Wildlife Refuge), Florida (St. Vincent National Wildlife Refuge) and Mississippi (Horn Island, Gulf Island’s National Seashore) as a component of the recovery program. These were designed to breed captive wolves in a natural environment but with limited dispersal ability, and to release the resulting offspring in the RWRA or other mainland reintroduction sites (Parker, 1987b). Funding limitations have resulted in a termination or suspension of the use of two insular propagation sites; Horn Island propagation site was terminated in the late 1990s, and the Bulls Island site was temporarily suspended in 2005. Cape Romain National Wildlife Refuge retains a small number of wolves for public education and captive propagation at the Sewee Visitor and Environmental Education Center; these wolves are not free-roaming on the island.

The reintroduction of the red wolf in northeastern North Carolina and eastern Tennessee was made possible, in part, by using a special designation under section 10(j) of the Endangered Species Act.

**Experimental Status**

The USFWS designated reintroduced populations of the red wolf “Experimental Population, Non-Essential” in North Carolina on 19 November 1986 (USFWS, 1986) and in North Carolina and Tennessee on 4 November 1991 (USFWS, 1991). This designation provides for limited protected status on private lands, with full protection as a threatened species on National Wildlife Refuge lands (North Carolina) and National Park Service
lands (Tennessee). The non-essential experimental designation “regulates taking, and other actions,” but affords flexibility in management of the species that “is less restrictive than the mandatory prohibitions covering endangered species” (USFWS, 1986: 41792). For example, the non-essential experimental designation allows for the “take” of red wolves, provided such taking “is not intentional or willful” and “is incidental to lawful activities” such as hunting and trapping of other species (USFWS, 1995: 18947).

Current Population Status

The red wolf captive breeding program is jointly managed by the USFWS and the Association of Zoos and Aquariums (AZA) under the Red Wolf Recovery/Species Survival Plan® (Red Wolf Recovery/SSP). A recovery plan is the USFWS’s document that delineates specific actions required to protect and recover listed species, and the SSP is the “zoological community’s plan for addressing biological and organizational questions regarding long-term propagation of an endangered species” (USFWS, 1989: 1). The red wolf captive population (data current as of 3 February 2009) consists of about 189 animals held in 42 participating institutions around the country (Waddell and Long, 2009). The

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1 Take is defined as “harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or attempt to engage in any such conduct.” The term “harm” has been further defined in the ESA implementation regulations as “an act which actually kills or injures wildlife. Such act may include significant habitat modification or degradation where it actually kills or injures wildlife by significantly impairing essential behavioral patterns including breeding, feeding or sheltering” (50 CFR Part 17.3). The regulations define the term “harass” as “an intentional or negligent act or omission which creates the likelihood of injury to wildlife by annoying it to such an extent as to significantly disrupt normal behavioral patterns which include, but are not limited to breeding, feeding or sheltering” (50 CFR Part 17.3). Endangered Species Act of 1973, as amended (USFWS, 1992a).
target captive population set by the Red Wolf Recovery/SSP is 330 red wolves (USFWS, 1989).

The reintroduced population in northeastern North Carolina and the island propagation sites are managed by the U.S. Fish and Wildlife Service’s Red Wolf Recovery Program and the respective National Wildlife Refuges. The restored population has grown to between 100 and 130 wolves, in about 20 packs, in the RWRA (data current as of 28 September 2007; USFWS, 2007). The target wild population set by the Red Wolf Recovery/SSP is 220 red wolves maintained in three reintroduction populations within the historic range of the species (USFWS, 1989).

NEW (AND OLD) PROBLEMS AND SOLUTIONS

The red wolf was the first carnivore declared biologically extinct in the wild to be successfully reintroduced into a portion of its former range, and has served as a model for reintroducing other imperiled carnivores. New and old problems, however, are affecting the management of the red wolf in the wild and in captivity that could seriously impair recovery and the continued survival of the species.

When the red wolf was reintroduced in 1987, the eastern coyote (C. latrans var.) was not known to occur in ARNWR (Parker, 1987a), and its occurrence in eastern North Carolina was sparse (DeBow et al., 1998). By the early 1990s, however, the coyote had expanded its range to nearly all counties around the RWRA. Although the reintroduced red wolf population was being intensely managed, coyotes were entering the RWRA and
mating with red wolves (Henry and Lucash, 2000; Phillips et al., 2003; USFWS, unpublished data). The renewed hybrid threat in the RWRA and the recent unsuccessful attempt to restore wolves in the Great Smoky Mountains National Park caused the recovery program to evaluate its efforts at recovery.

The USFWS organized a Population and Habitat Viability Assessment (PHVA) workshop, facilitated by the IUCN/World Conservation Union’s Conservation Breeding Specialist Group, to develop solutions on the issues facing the recovery of the red wolf. Principal among the issues challenging the expansion of the recovery program were (1) the selection of new reintroduction sites, (2) assessing and managing the threat of hybridization, (3) the need for an effective and feasible monitoring program, and (4) an assessment of the captive breeding program’s role in facilitating recovery in the wild (Kelly et al., 1999). However, participants of the workshop quickly concluded that hybridization of the red wolf with the eastern coyote threatened the viability of the wild red wolf population, superseding all other issues (Kelly et al., 1999). As a result, the other issues were refocused with greater emphasis on wolf-coyote hybridization and its threat to the genetic integrity of the red wolf.

The workshop resulted in the development of an adaptive management plan to assess and manage the threat of hybridization (Fazio et al., 2005; Kelly, 2000). The multi-year plan specified annual goals and an implementation strategy to address red wolf-coyote hybridization in the RWRA. Two important components of the plan included (1) eliminating areas where the presence of a canid is unknown, or where a red wolf is present, but its breeding status or transience is unknown (so called “zones of ignorance”), and (2)
ensuring all known breeding units within the RWRA were red wolves. To facilitate the plan, the RWRA was divided into three regions (Figure 5a), and the goals implemented in order of priority according to management region (i.e., if the goals in Region 1 have not been achieved, then pursuit of those goals should not be undertaken in Region 2).

A Red Wolf Recovery Implementation Team (RWRIT) was formed following the PHVA workshop to assist the USFWS in data analysis and offer advice and recommendations on its recovery efforts implemented under the adaptive management plan (Stoskopf et al., 2005). Through the cooperative efforts of the USFWS and RWRIT, the introgression of coyote genes into the red wolf population has been reduced and red wolf numbers and range have increased. Their collective efforts also have decreased the total area where the status of canids is unknown, thereby modifying the prioritized goals and area of each region (Figure 5b) (Stoskopf et al., 2005). Region 1 is presently managed as a “coyote free” area; generally non-wolf *Canis* are removed. In regions 2 and 3, hybrids are typically removed, but coyotes are sterilized and used as place-holders until wolves are available through management actions such as translocation (McLellan and Rabon, 2006) or naturally move into areas currently held by non-wolves. Region 3 is managed as the western boundary area to limit non-wolf movements into northeastern North Carolina and the RWRA.

Although the introgression of coyote genes in the red wolf population can and is being managed with success, the potential for hybridization continues to present challenges to the success of the red wolf recovery program and to future reintroductions (Kelly et al., 1999). With the threat of hybridization, participants of the PHVA workshop identified the
role of the captive population as being more important than ever as the repository of original genetic composition of the species. As such, increased breeding in the captive population was identified as a critical need. Evaluating the reproductive success of individuals, as well as investigating factors affecting reproduction and mate preference/selection, were identified as important measures for maximizing reproduction (Kelly et al., 1999). However, without knowledge of how a red wolf chooses a mate, we cannot begin to understand how to manage wolf-coyote matings. Currently, information on mate choice does not exist for the red wolf.

Furthermore, the small founding population (n = 14) requires intensive management to maintain genetic diversity and demographic stability in the red wolf population (Kelly et al., 1999). Nonetheless, in the mid-1990s the proportion of litters produced in the captive population each year began to decline (Waddell, 2002). In addition, the captive population’s age structure showed a large proportion of animals in the older age classes and unequal representation of founders (Long et al., 2001). In response, a database of physiological reproductive characteristics and, subsequently, assisted reproductive technologies were developed for the red wolf (Goodrowe et al., 1998, 2000, 2001). These technologies, however, did not consider behavioral information in evaluating the reproductive potential of an individual red wolf or of the managed population (e.g., Wedekind, 2002). For example, the effects of inbreeding in animal and plant species have long been observed to affect traits related to reproductive fitness. In addition, many studies suggest that reproductive performance improves with age during the first few years of reproductive life, but reaches a plateau or even declines in older, senescent individuals.
Solutions

More than 30 years have passed since the inception of the red wolf captive breeding program and 20 years since the first wolves were reintroduced in the wild. However, several consecutive years of reduced fecundity in the captive population (Waddell, 2002) and the continued threat of hybridization (Fazio et al., 2005) provide rationale that additional information about the red wolf’s reproductive behavior and factors that may be affecting the species’ reproductive potential are required if we are to continue with a successful recovery program. Assessing the available reproductive histories is important for understanding factors affecting reproduction and for developing management tools to improve reproduction. Furthermore, information on how this species selects a mate among conspecifics and congeners is important for expanding the existing wild population and to ensure the success of future red wolf reintroductions. Such knowledge will minimize wolf-coyote matings and will improve management decisions that maximize the reproductive potential of the species.

Studies have shown that multi-institutional research is valuable in revealing factors affecting an individual’s behavior and reproductive performance (see reviews Mellen, 1994; Shepherdson and Carlstead, 2001). In this study I conducted a systematic, retrospective, behavioral assessment to identify characteristics that have influenced reproduction in the captive population. The central dogma of the Red Wolf Recovery/SSP is to: (1) breed individuals with lowest mean kinship; and (2) breed individuals whose
alleles may soon be lost. This often results in breeding the oldest animals in the population. Based on this premise, I developed two general hypotheses:

(1) reproductive output declines as sire/dam inbreeding coefficient increases; and

(2) reproductive output declines as sire/dam age increases.

I also conducted a comprehensive, behavioral preference study using olfactory presentations of excreta (i.e., sex-specific and species-specific) to determine those factors that are important in the selection of mates. Studies have shown that canids use feces and urine as a form of scent-marking, and excreta might be important to canids for recognizing individuals and for identifying and selecting mates (Asa and Mech, 1995; Asa et al., 1985, 1986; Bekoff, 2001; Mech, 1970; Peters and Mech, 1975; Raymer et al., 1984, 1986; Sillero-Zubiri and Macdonald, 1998; Vilà et al., 1994). Based on the results of these studies and the premise that observed preferences will provide information about the olfactory mechanisms that are important in mate selection, I developed three general hypotheses:

(1) male and female wolves show a preference for excreta over a control;

(2) male and female wolves show a stronger preference for excreta during the breeding season than during the non-breeding season; and

(3) male and female wolves show a preference for excreta from a conspecific over that from a congener.

The following chapters represent an assessment of the validity of these hypotheses.


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Table 1. The founding red wolves of the captive breeding program and their gender and provenances.

<table>
<thead>
<tr>
<th>SB#</th>
<th>Name</th>
<th>Sex</th>
<th>Capture Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Mr. Sabine</td>
<td>Male</td>
<td>Jefferson County, TX</td>
</tr>
<tr>
<td>8</td>
<td>Happy Face</td>
<td>Male</td>
<td>Chambers County, TX</td>
</tr>
<tr>
<td>11</td>
<td>Herbilding Male</td>
<td>Male</td>
<td>Cameron Parish, LA</td>
</tr>
<tr>
<td>12</td>
<td>Mrs. Sabine</td>
<td>Female</td>
<td>Jefferson County, TX</td>
</tr>
<tr>
<td>13</td>
<td>Ms. Wessie</td>
<td>Female</td>
<td>Jefferson County, TX</td>
</tr>
<tr>
<td>14</td>
<td>Mrs. McBride</td>
<td>Female</td>
<td>Jefferson County, TX</td>
</tr>
<tr>
<td>16</td>
<td>Fortenberry Female</td>
<td>Female</td>
<td>Jefferson County, TX</td>
</tr>
<tr>
<td>17</td>
<td>Pines Inholding Male</td>
<td>Male</td>
<td>Cameron Parish, LA</td>
</tr>
<tr>
<td>24</td>
<td>Louisiana Radio Male</td>
<td>Male</td>
<td>Cameron Parish, LA</td>
</tr>
<tr>
<td>26</td>
<td>John</td>
<td>Male</td>
<td>Calcasieu Parish, LA</td>
</tr>
<tr>
<td>30</td>
<td>Crain Female</td>
<td>Female</td>
<td>Cameron Parish, LA</td>
</tr>
<tr>
<td>33</td>
<td>Gulf Oil Split Lip</td>
<td>Male</td>
<td>Jefferson County, TX</td>
</tr>
<tr>
<td>34</td>
<td>Sabine Ranch Male</td>
<td>Male</td>
<td>Jefferson County, TX</td>
</tr>
<tr>
<td>42</td>
<td>‘78 Pines Inholding Male</td>
<td>Male</td>
<td>Cameron Parish, LA</td>
</tr>
</tbody>
</table>
Figure 1. Distribution of the red wolf, with subspecific ranges (dashed lines): *Canis rufus floridanus* (east); *Canis rufus gregoryi* (central); *Canis rufus rufus* (southwest).

Modified with permission from Nowak, 2002.
Figure 2. Map of the five counties in North Carolina comprising the red wolf recovery area.
Figure 3. Photographs of variations in the coloration of pelage in the red wolf – (a) typical tawny pelage (photograph taken by the author); (b) black phase pelage (photograph taken by Gregory, 1935); (c) rare white-phase pelage (photograph taken by the author).
Figure 4. Map of coastal counties and parishes of Texas and Louisiana, respectively, where the remaining wild red wolves were trapped before being placed in the captive breeding program.
Figure 5. Maps of red wolf recovery area depicting (a) the original zones and (b) the modified zones of the adaptive management plan to assess and manage the threat to the restored population of wolves posed by hybridization with coyotes. Zone 1 is managed as a “coyote free” area; Zones 2 and 3 manage all non-wolf canids as placeholders after sterilization; Region 3 also is managed to limit non-wolf movements into the red wolf recovery area.
CHAPTER 2

The following chapter has been prepared in the format of, and accepted for publication (i.e., in press) in, the journal Zoo Biology. The article is reproduced here as accepted for publication.
EFFECTS OF INBREEDING ON REPRODUCTIVE SUCCESS, PERFORMANCE, LITTER SIZE, AND SURVIVAL IN CAPTIVE RED WOLVES (CANIS RUFUS)

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Running head: Inbreeding in captive red wolves
ABSTRACT

Captive-breeding programs have been widely used in the conservation of imperiled species, but the effects of inbreeding, frequently expressed in traits related to fitness, are nearly unavoidable in small populations with few founders. Following its planned extirpation in the wild the endangered red wolf (*Canis rufus*) was preserved in captivity with just 14 founders. In this study, we evaluated the captive red wolf population for relationships between inbreeding and reproductive performance and fitness. Over 30 years of managed breeding, the level of inbreeding in the captive population has increased, and litter size has declined. Inbreeding levels were lower in sire and dam wolves that reproduced than in those that did not reproduce. However, there was no difference in the inbreeding level of actual litters and predicted litters. Litter size was negatively affected by offspring and paternal levels of inbreeding, but the effect of inbreeding on offspring survival was restricted to a positive influence. There was no apparent relationship between inbreeding and method of rearing offspring. The observable effects of inbreeding in the captive red wolf population currently do not appear to be a limiting factor in the conservation of the red wolf population. Additional studies exploring the extent of the effects of inbreeding will be required as inbreeding levels increase in the captive population.

Keywords: *Canis rufus*, inbreeding coefficient, reproductive performance, fitness
The red wolf (*Canis rufus*) was once distributed across the eastern and south-central United States. As a result of hunting, aggressive predator-control programs, and habitat destruction it was extirpated throughout most of its range by the early part of the twentieth century (Nowak, 1970, 1972, 2002). The coyote (*C. latrans*) and coyote-like animals (e.g., coyote-wolf hybrids, coyote-dog hybrids) expanded their range into former red wolf habitats (McCarley, 1962; Paradiso, 1965; Paradiso and Nowak, 1971) and threatened to genetically swamp the remaining red wolf populations (Carley, 1975; Nowak, 1970). An official program to recover the red wolf in the wild began when the species was listed as endangered in 1967 (USFWS, 1967). However, the recovery program shifted its focus from local preservation to one of species conservation through captive breeding when recovery in the wild appeared unlikely (Carley, 1975).

Over a seven-year period (1973-1980), the USFWS captured more than 400 canids in the red wolf’s remaining range (i.e., coastal areas of Texas and Louisiana), but only 43 animals met the species-defining standards established for admittance to a captive-breeding program for further evaluation (McCarley and Carley, 1979; Phillips et al., 2003). Because of the complexities of identification, final confirmation of red wolves was made through the captive-breeding process and production of offspring. Only 14 red wolves (9.5; ♂, ♀) would eventually be confirmed through the captive-breeding process, and become the founding stock of the recovery program. By 1980, the red wolf was considered extirpated.
and biologically extinct in the wild (McCarley and Carley, 1979; Parker, 1988; USFWS, 1984).

Captive-breeding programs have been widely used in the conservation of imperiled carnivores (e.g., Association of Zoos & Aquariums lists 28 Species Survival Plans® for carnivores), but few of these species’ populations have been evaluated for inbreeding depression (e.g., Kalinowski et al., 1999; Ralls et al., 1988). The deleterious effects of inbreeding in animal and plant species have long been observed (e.g., Darwin, 1868, 1876), and are expressed most severely in traits related to fitness (DeRose and Roff, 1999; Falconer, 1981). Although, the degree to which populations suffer from inbreeding depression varies (e.g., Brewer et al., 1990; Ralls et al., 1988), reduction in survivorship and fertility, impairment of seminal traits, loss of vitality, and decline in competitive ability have been documented in inbred populations (Eklund, 1996; Lacy et al., 1993; Ralls et al., 1979, 1980, 1988; Wildt et al., 1983, 1987).

The primary objective of a captive-breeding program is to maintain the demographic security of the population by preserving genetic diversity over an extended period of time (Ballou and Foose, 1996; Soulé et al., 1986). The red wolf recovery program established a goal to preserve 80-90% of the genetic diversity of the founders for 150 years (USFWS, 1989). Following 30 years of managed breeding, the current gene diversity for the captive red wolf population is 89.52%, and is equivalent to a population descended from approximately five founders (Waddell and Long, 2008). These calculations are inferred from estimates of relatedness (e.g., mean kinship, inbreeding coefficients).
The small number of founders, as well as potential introgression and inbreeding depression, are challenges inherent in the conservation of the red wolf. The present study is the first known to determine the effect inbreeding has on the production of offspring. We also conducted a retrospective analysis of reproductive events of captive red wolves in order to examine relationships between the level of inbreeding and various measures of reproductive fitness (litter size, rearing, composition, and viability).

METHODS

Longitudinal reproductive events of captive red wolves were examined to test the relationships between the level of inbreeding of sires, dams, and offspring and the following measures of reproductive performance: litter size; litter-rearing type; sex ratio of the litter; and, viability of offspring. Demographic data for the red wolf population (e.g., pedigrees, date and place of birth, date of death, reproductive experience, litter size) were obtained from the red wolf studbook (Waddell, 2007) and from documentation, notes and general correspondence maintained in the files of the red wolf captive-breeding program (USFWS, unpublished data). Several contradictions between the studbook and file reports on wolf demography, breeding events, and provenance were discovered. Inconsistencies in the data were evaluated, reconciled, and the red wolf studbook modified accordingly before data were analyzed.

A breeding pair was defined as a male-female wolf pair having had access and an opportunity to breed (see exceptions for artificial insemination events below). A breeding
pair that remained together for multiple years was identified annually as a potential 
breeding pair. For several years in the captive-breeding program, pairs of wolves that failed 
to produce offspring were not recorded in any database; these pairings could not be 
included in the analyses. Breeding success was classified in a binary manner as either (1) 
production of offspring or (2) reproductive failure. The first record of a captive-born red 
wolf litter was 1977. Therefore, evaluations of breeding success were based on 554 
recorded pairing events from 1977 through 2006.

Artificial insemination events (n = 41) were excluded from all analyses, with two 
exceptions (one in 1995 and one in 1999). These two events were included as breeding 
pairs in the analyses because the females were artificially inseminated with the paired 
(housed) male’s semen. Artificial insemination was necessary in these two cases because 
normal reproduction was considered to have been difficult as a result of medical anomalies 
(the 1995 male had frenulum breve of the penis; the 1999 male had a torn anterior ligament 
preventing him from mounting the female). However, because these two pairs failed to 
reproduce in their respective breeding years they were excluded from any analyses of 
reproducing pairs.

An inbreeding coefficient \( f \) (Wright, 1922) was calculated for each wolf in the 
captive breeding population using the Single Population Analysis and Record Keeping 
System (SPARKS) v1.54 (International Species Information System, Eagan, Minnesota, 
USA 55121-1170). An \( f \) also was calculated for predicted offspring that might result from a 
pairing event. Methods for calculating \( f \) in a pedigree are provided by Ballou (1983). In 
determining the \( f \) of founders, SPARKS software designates wild-caught individuals as
unrelated, although there is a possibility that some of the wild-caught wolves could have been inbred or related through recent common ancestry in the wild, given their capture locations. Therefore, an individual’s \( f \) can only be verified to represent minimum estimates because the extent of inbreeding is measured as a loss of heterozygosity relative to the founders (Lacy et al., 1993).

Sex ratio of the litter (i.e., the proportion of males, females, and pups of unknown sex produced in a litter) was calculated at birth and at two subsequent benchmark ages (i.e., pups aged 3-months and 12-months). Litter sizes at birth may be underestimated because litters were not always observed on the date of birth; pups may have been stillborn and consumed by the parent or otherwise lost to infanticide before observer detection.

Viability of pups also was calculated when the pups were 3-months and 12-months of age. In studying the relationship between inbreeding and juvenile mortality, the costs of inbreeding may be underestimated if an age less than reproductive maturity is selected because “inbred mortality increases more rapidly than [does] non-inbred mortality with increasing age in some species” (Ralls et al., 1988: 187). Therefore, the 3-month age category was chosen to represent survival of pups to juvenilehood (Scott and Fuller, 1965), and the 12-month age category to represent survival to adult size (Mech, 1970), but likely before attaining reproductive maturity (Medjo and Mech, 1976; Rausch, 1967; USFWS, unpublished data). In calculating survival, those pups that were listed as “lost to follow-up” before 12-months of age in the studbook (\( n = 3 \)) were resolved as alive or dead based on additional information (e.g., field reports) before analyses. “Lost to follow-up” is a data entry term used in the studbook when the final disposition of a specimen is unknown and is
applied following the “Guidelines for Data Entry and Maintenance of North American Regional Studbooks” (Thompson and Earnhardt, 1996). However, the disposition of animals involved in a release program may not always conform to established zoo-based data entry standards for studbooks. Such was the case for the three wolves designated as “lost to follow-up” in the studbook and used in our data analyses. Of the three individuals, one pup disappeared at about two weeks of age while being held in an acclimation release pen with his parents and siblings. Therefore, we considered this animal dead before 3-months of age. The other two wolves were released as juveniles (older than 3-months of age) with their parents and littermates into the wild as part of the species’ restoration effort (e.g., Parker, 1987), but were presumed dead before 12-months of age based on continued radio telemetry monitoring of the respective packs.

Rearing type of offspring was categorized into three groups: litters in which all pups were reared by their parents were categorized as the “Parent” group (n = 169); litters in which at least one pup was reared by hand was categorized as the “Hand” group (n = 12); and, litters in which at least one pup was reared by foster parents was classified in the “Foster” group (n = 11). Fostered litters were excluded from most analyses of rearing type because pups chosen for fostering are based on factors other than the pups’ level of inbreeding (e.g., number of pups in the donor litter, the availability of a foster litter, and the size of potential foster litters).

Statistical analyses were conducted using JMP® 7.0 (SAS Institute Inc., Cary, North Carolina, USA 27513). To determine if inbreeding was a factor in the production of offspring, reproduction was evaluated as a function of $f$ on actual and predicted litters using
nominal logistic regression. Changes in $f$ and litter size over time were evaluated using least squares regression, as were the effects of $f$ on the sex ratio of litters. Procedures for modeling relationships between inbreeding and survivorship have been extensively evaluated (e.g., Armstrong and Cassey, 2007; Kalinowski and Hedrick, 1998; Morton et al., 1956; Templeton and Read, 1983, 1984, 1998; Willis and Wiese, 1997). As noted by Armstrong and Cassey (2007), generalized linear modeling offers advantages to traditional linear and non-linear (e.g., Kalinowski and Hedrick, 1998; Morton et al. 1956; Templeton and Read, 1983, 1984, 1998) methods, for example, by incorporating statistics to indicate if the model is a reasonable fit to the data and for ease in changing the form of the relationship using different link functions. Therefore, relationships between viability and $f$ were evaluated using generalized linear modeling techniques with a binomial distribution and logit-link model (see Armstrong and Cassey, 2007). Generalized linear modeling techniques with a Poisson distribution and an overdispersion parameter ($\hat{c}$) were used to test for a relationship between litter size and $f$. To determine if inbreeding was a factor in the rearing of offspring, rearing type was evaluated as a function of $f$ using nominal logistic regression. Means of groups were compared using Student’s t-tests or Wilcoxon-Mann-Whitney tests, as appropriate. All data are depicted as mean ± standard error of the mean (SEM), unless otherwise noted; statistical significance was set at $p \leq 0.05$. 
RESULTS

Of the 554 pairings between 1977 and 2006, a total of 192 litters consisting of 797 offspring (359.406.32; ♂.♀.undetermined) were produced. During this period, the reproducing population consisted of 92 sires (f range = 0.0-0.25, mean = 0.027 ± 0.003) and 101 dams (f range = 0.0-0.125, mean = 0.030 ± 0.003) with a population mean f of 0.029 ± 0.002 (Figure 1). The overall population mean f was 0.036 ± 0.001 (n = 1108). Litter size ranged from 1 to 9 pups (mean = 4.15 ± 0.14), and has significantly decreased over time (F = 18.029, df = 1, p < 0.0001). In the early years of the captive breeding program (1977-1979) the mean litter size was 5.143 ± 0.459 pups (n = 7 litters, range 1-6 pups/litter), whereas more recently (2005-2006) the mean litter size was 3.391 ± 0.354 (n = 23 litters, range 1-8 pups/litter). Approximately 26% of the births had an f of 0; the rest were distributed across 60 levels of inbreeding (Table 1). The level of inbreeding in offspring in the captive population has increased over time (F = 23.639, df = 1, p < 0.0001). The mean f of litters was 0.0 between 1977 and 1979 (n = 7), but increased to a mean of 0.072 ± 0.001 in 2005 and 2006 (n = 23).

To determine whether inbreeding was a factor in the production of offspring, reproduction as a function of f on actual (n = 192) and predicted (n = 362) litters (all pairings) was evaluated. The mean f of sires was lower ($\chi^2 = 18.832, df = 1, p < 0.0001$) in actual litters (0.027 ± 0.003; range 0-0.250) than in predicted litters (0.041 ± 0.002; range 0-0.135). The mean f of dams also was lower ($\chi^2 = 6.506, df = 1, p = 0.011$) in actual litters (0.030 ± 0.003; range 0-0.125) than in predicted litters (0.039 ± 0.002; range 0-0.500).
However, there was no difference ($\chi^2 = 0.128$, df = 1, $p = 0.720$) in the mean $f$ of offspring from actual litters ($0.054 \pm 0.003$; range 0-0.250) and predicted litters ($0.053 \pm 0.002$; range 0-0.188).

The following results are calculated from all pairing events that resulted in reproduction ($n = 192$), unless otherwise noted.

**Offspring Effects**

A negative relationship was found between offspring $f$ and litter size ($\chi^2 = 5.522$, df $= 1$, $\hat{\epsilon} = 0.938$, $p = 0.019$). When the sex ratio of litters was measured as a function of the offspring’s $f$, the proportion of males increased ($F = 6.076$, df $= 1$, $p = 0.015$; Figure 2) and the proportion of females decreased ($F = 3.898$, df $= 1$, $p = 0.049$) with increasing $f$. The relationship between offspring’s $f$ and the proportion of pups of undetermined sex was not statistically significant ($F = 0.464$, df $= 1$, $p = 0.497$). There was a positive relationship between offspring $f$ and pup survival at 3-months of age ($\chi^2 = 7.163$, df $= 1$, $p = 0.007$) (Figure 3), but the relationship between inbreeding and the survival of pups at 12-months of age ($\chi^2 = 1.599$, df $= 1$, $p = 0.206$) was not statistically significant.

There was no difference ($n = 181$, $\chi^2 = 2.14$, df $= 1$, $p = 0.144$) between the $f$ of parent-reared litters (mean $= 0.054 \pm 0.003$, $n = 169$) and that of hand-reared litters (mean $= 0.037 \pm 0.009$, $n = 12$). Foster-reared litters were excluded from this analysis because pups chosen for fostering are based on factors other than the pups’ level of inbreeding (see above). However, for all captive-born red wolves the mean $f$ of foster-reared litters (mean
= 0.073 ± 0.015, n = 11) was significantly higher than that of hand-reared litters (t = 1.973, p < 0.05), but did not differ significantly from that of parent-reared litters.

**Parental effects**

A negative relationship was found between the sire’s f and litter size (\(\chi^2 = 5.482, \text{df} = 1, \hat{c} = 0.939, p = 0.019\)); the relationship between the dam’s f and litter size was not statistically significant (\(\chi^2 = 2.356, \text{df} = 1, \hat{c} = 0.951, p = 0.125\)). When the sex ratio of litters was measured as a function of the parental f, there were no significant effects of the sire’s f on the proportion of males (F = 0.279, df = 1, p = 0.598), females (F = 0.977, df = 1, p = 0.324), or pups of undetermined sex (F = 0.499, df = 1, p = 0.480) in a litter, nor were there significant effects of the dam’s f on the proportion of males (F = 0.846, df = 1, p = 0.359), females (F = 0.061, df = 1, p = 0.806), or pups of undetermined sex (F = 1.022, df = 1, p = 0.313) in a litter. When offspring survival was measured as a function of parental f, the sire had a positive effect on the number of pups surviving at 3-months of age (\(\chi^2 = 20.880, \text{df} = 1, p < 0.0001\)) and 12-months of age (\(\chi^2 = 21.739, \text{df} = 1, p < 0.0001\); Figure 4). Similar positive relationships were found between the dam’s f and pup survival at 3-months of age (\(\chi^2 = 3.829, \text{df} = 1, p = 0.050\)) and 12-months of age (\(\chi^2 = 4.224, \text{df} = 1, p = 0.039\)).

There was no apparent relationship between parental f and offspring rearing type (sire, n = 181, \(\chi^2 = 1.291, \text{df} = 1, p = 0.256\); dam, n = 181, \(\chi^2 = 0.063, \text{df} = 1, p = 0.802\)). For the reasons stated above, foster-reared litters were excluded from this analysis.
DISCUSSION

The captive-breeding program’s primary objective to preserve genetic diversity in the population is achieved by selecting sires and dams based on their mean kinship coefficient (i.e., a measure of the relatedness of an individual to all individuals in the population) and the $f$ of the resulting offspring. Using breeding animals with the lowest mean kinship coefficient increases the opportunity to reduce the loss of gene diversity and minimizes the harmful effects of mating closely related relatives (Lacy, 1995; Lacy et al., 1995). Therefore, it is not surprising to find that a relationship did not exist between the level of inbreeding in the offspring and the production of offspring in the captive red wolf population.

However, the effects of inbreeding depression observed in the captive red wolf population are supported by studies showing a relationship between a decline in fitness of individuals and higher inbreeding coefficients (e.g., Lacy, 1993; Lacy et al., 1993; Ralls et al., 1979, 1980, 1988). In the present study, litter size was negatively affected by offspring and paternal levels of inbreeding, but pup survival was positively affected. Inbreeding appeared to distort the sex ratio, but there was no apparent relationship between inbreeding and offspring rearing type.

The results presented here are in contrast to the findings of Kalinowski et al. (1999) who reported no observable effect of inbreeding on litter size and juvenile viability (to 180 days) in the red wolf. There are several potential explanations for these differences in results. First, captive breeding techniques used to avoid inbreeding may have resulted in
statistical power insufficient to detect inbreeding depression. Kalinowski and Hedrick (1999) reported that varying the total number of births and the average $f$ of the population affected the probability of detecting inbreeding depression. However, the red wolf population analyzed by Kalinowski et al. (1999) had a higher mean $f$ and nearly twice the number of inbreeding levels than did the red wolf model population of Kalinowski and Hedrick (1999), suggesting that the probability of detecting inbreeding depression in the red wolf population is expected to be equal to or higher than the estimated probability of their model population.

Second, the dataset used by Kalinowski et al. (1999) may have included too few generations (i.e., litters from 1977 to 1996) to detect inbreeding effects in the captive population. This supposition is supported by the findings of Lockyear (2006) who, by including additional reproductive events (i.e., litters from 1977 to 2005), reported a significant decline in litter size with paternal inbreeding in red wolves. However, the period of time used in the Kalinowski et al. (1999) study included second and later generations. Documentable inbreeding effects are expected to be seen in the second captive generation (Lacy et al., 1993). Furthermore, Kalinowski et al. (1999) did not report on paternal inbreeding effects. Therefore, it is difficult to determine if the increase in the number of reproductive events used by Lockyear (2006) is the sole justification for observable inbreeding depression.

A third possible explanation for the difference in results is that the dataset used by Kalinowski et al. (1999) may have inadvertently included hybrid canids and/or free-ranging wolves which may have misestimated the results of inbreeding depression. An evaluation
of our dataset representative of the period used by Kalinowski et al. (1999) (i.e., 1977 to 1996) yielded only 584 births in 131 litters opposed to their reported 688 births in 157 litters. The majority of the litters representing the difference (21 of 26) had an $f$ of 0. The inclusion of 53% more non-inbred individuals in their analyses could have underestimated inbreeding depression in wolves in the captive population. A complete comparison of the datasets representative of the period used by Lockyear (2006) (i.e., 1977 to 2005) could not be accomplished. However, Lockyear (2006) only reported 165 litters born compared with 183 litters identified during the same period in our dataset, indicating that some captive red wolf reproductive events were excluded from the analyses. We are confident that our dataset represented the most comprehensive record of known red wolf pairs and captive reproductive events, and adequately excluded hybrids and free-ranging wolves, because the dataset was constructed from multiple file sources rather than the studbook criteria for defining a dataset for analysis. As noted earlier, inconsistencies between the studbook and file reports were discovered and reconciled before data were analyzed.

To determine whether or not differences in the datasets affected the detection of inbreeding, we analyzed our dataset using reproducing pairs from 1977 to 1996 (as conducted in Kalinowski et al., 1999). Our finding of a non-significant trend toward decreased litter size with maternal inbreeding ($n = 131, \chi^2 = 0.225, df = 1, \hat{c} = 0.928, p = 0.635$) was consistent with the findings of Kalinowski et al. (1999; $p = 0.94$). A decrease in the average litter size with time also was consistent with the findings of Kalinowski et al. (1999); however, the present dataset provided a statistically significant result ($n = 131, F = 5.723, df = 1, p = 0.018$). Viability of offspring could not be directly compared because of
differences in the benchmark ages used. However, an analysis of survivorship using our
dataset revealed a significant positive relationship between viability and the $f$ of offspring at
3-months of age ($n = 131, \chi^2 = 5.213, df = 1, p = 0.022$), but not at 12-months of age ($n =
131, \chi^2 = 0.247, df = 1, p = 0.619$). Kalinowski et al. (1999) found no association between
inbreeding and offspring viability at 6-months of age.

The biological significance of the association between inbreeding and offspring
survival in the current study is not immediately evident. The increase in offspring survival
may be influenced by factors unrelated to inbreeding. For example, captive red wolves may
be exhibiting some level of adaptability to the captive-breeding environment that is
positively manifested in pup survival. Improvements in husbandry, veterinary care, and
nutrition also may positively contribute to pup survival. Nevertheless, the positive effect of
inbreeding on survival does not support altering population management strategies (i.e.,
increasing the level of inbreeding to improve offspring survival) at the expense of reduced
litter size or other potential negative effects. It also is important to consider that the level of
inbreeding in the red wolf population is relatively low compared with other inbred
carnivore populations that show increases in juvenile mortality (e.g., \textit{Canis lupus}, Laikre
and Ryman, 1991; \textit{Panthera tigris amoyensis}, Xu et al., 2007). The level of inbreeding
affecting mortality in the red wolf may be higher than that required for evidence of
inbreeding depression in other aspects of fitness. Inbreeding depression has been shown to
reduce weight, body size, longevity, and productivity in other captive populations of wolves
with varying degrees of inbreeding (e.g., Fredrickson and Hedrick, 2002; Laikre and
Ryman, 1991), but a threshold relationship appears to exist between inbreeding and
extinction (Frankham, 1995; Wayne et al., 1991). Kalinowski et al. (1999) acknowledged the limited examination of fitness in their analyses. It is possible that the analyses of additional components of fitness could have resulted in observable inbreeding effects in the captive red wolf population.

In the current study, the effects of parental and offspring inbreeding were evaluated on multiple aspects of fitness in the captive red wolf population. While there is observable inbreeding depression in the population, it currently does not appear to be a limiting factor in the conservation of the red wolf population. For example, although sex ratio in the present study is affected by inbreeding, the intensity of sex ratio depression does not appear to be sufficient to affect the probability of population survival (e.g., Senner, 1980). Similarly, although semen parameters and sperm characteristics for the red wolf are reported to be less robust and more highly variable compared to other canids, the effects inbreeding depression have on red wolf seminal traits and fertility appear to be, at this time, only minor (Goodrowe et al., 1998; Koehler et al., 1994, 1998; but see also Lockyear, 2006). This is perhaps encouraging news for red wolf captive-breeding efforts. However, caution should be taken in interpreting the results of the present study in relation to past findings and for future decisions about management of the population. For instance, while the observed effect of inbreeding on sex ratio (toward a male bias) supports the premise that inbreeding distorts sex ratios by reducing the proportion of the homogametic sex (e.g., Hook and Schull, 1973; Senner, 1980), others have reported a female bias in the sex ratio of the red wolf (Frankham and Wilcken, 2006). Unfortunately, the red wolf dataset used by Frankham and Wilcken (2006) may have been influenced by the inclusion of non-wolf
canids or free-ranging wolves. Nonetheless, Frankham and Wilcken (2006), using data from 25 vertebrate taxa, also reported that the distortion of sex ratio was not a consistent indicator of inbreeding depression. This suggests that further analyses are required to elucidate the influence inbreeding has, if any, on the sex ratio of the red wolf.

Caution also is warranted because small, incremental increases in inbreeding may result in an unobservable loss of genetic variation, but over a few generations the effect could lead to a cumulative loss of fitness (Lacy, 1995). Furthermore, a failure to consider the effects of inbreeding on adult traits (e.g., adult survival, competitive ability, ability to provide parental care) may underestimate the total cost of inbreeding (Ryan et al., 2003). As inbreeding levels increase in the captive red wolf population, more studies will be required to understand the impact of genetic diversity on red wolf reproduction and to determine ways to abate and reverse the effects of inbreeding. We recommend managers of captive and endangered populations frequently evaluate the effects of incremental increases in inbreeding. We also recommend including a broader spectrum of parameters potentially affected by inbreeding (e.g., offspring and parental components of fitness, and adult traits related to fecundity, physiological response to stress, survival, social dominance, and parental capabilities) in the analyses.

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Table 1. The number of litters ($N_{litters}$), the number of individual pups ($N_{ind}$), the mean litter size at birth ($\text{Mean}_{\text{litter size birth}}$) and when pups are 3-months ($\text{Mean}_{\text{litter size 3}}$) and 12-months of age ($\text{Mean}_{\text{litter size 12}}$), the number of individual pups surviving 3-months ($N_{survive\ 3}$) and 12-months of age ($N_{survive\ 12}$) and the inbreeding coefficients ($f$) of offspring for the red wolf captive-breeding population.

<table>
<thead>
<tr>
<th>Offspring $f$</th>
<th>$N_{litters}$</th>
<th>$N_{ind}$</th>
<th>$\text{Mean}_{\text{litter size birth}}$</th>
<th>$N_{survive\ 3}$</th>
<th>$\text{Mean}_{\text{litter size 3}}$</th>
<th>$N_{survive\ 12}$</th>
<th>$\text{Mean}_{\text{litter size 12}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0000</td>
<td>40</td>
<td>205</td>
<td>5.13 ± 0.30</td>
<td>123</td>
<td>3.08 ± 0.27</td>
<td>106</td>
<td>2.65 ± 0.26</td>
</tr>
<tr>
<td>0.0001 – 0.0499</td>
<td>34</td>
<td>145</td>
<td>4.27 ± 0.39</td>
<td>102</td>
<td>3.00 ± 0.38</td>
<td>96</td>
<td>2.82 ± 0.36</td>
</tr>
<tr>
<td>0.0500 – 0.0999</td>
<td>105</td>
<td>390</td>
<td>3.71 ± 0.18</td>
<td>280</td>
<td>2.67 ± 0.19</td>
<td>236</td>
<td>2.25 ± 0.18</td>
</tr>
<tr>
<td>0.1000 – 0.2500</td>
<td>13</td>
<td>57</td>
<td>4.39 ± 0.58</td>
<td>44</td>
<td>3.39 ± 0.68</td>
<td>35</td>
<td>2.69 ± 0.70</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>192</strong></td>
<td><strong>797</strong></td>
<td></td>
<td><strong>549</strong></td>
<td></td>
<td><strong>473</strong></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Inbreeding coefficients ($f$) of sires (open circles) and dams (solid squares) plotted against year bred.
Figure 2. Proportion of males in a litter at birth plotted against offspring inbreeding coefficients ($f$) for the red wolf captive-breeding population. Fitted line is based on least-squares regression.
Figure 3. Observed viability of pups at 3-months of age plotted against offspring inbreeding coefficients ($f$) for the red wolf captive-breeding population. Fitted line is based on least-squares regression.
Figure 4. Observed viability of pups at (a) 3-months of age and (b) 12-months of age plotted against inbreeding coefficients ($f$) of sires for the red wolf captive-breeding population. Fitted line is based on least-squares regression.
CHAPTER 3

The following chapter has been prepared in the format of the journal Animal Conservation.
EFFECTS OF AGE AND BREEDING EXPERIENCE ON REPRODUCTIVE PERFORMANCE OF THE RED WOLF

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Running head: Age-specific effects on red wolf reproduction
ABSTRACT

Propagation programs contribute to the conservation of a species by preserving genetic and demographic stock that can be used to reinforce or re-establish populations in the wild. Identifying traits that affect the reproductive success of individuals is essential to achieve this goal. Longitudinal reproductive events of a captive population of endangered red wolves (*Canis rufus*) were investigated to determine whether parental age, breeding experience, and rearing-type were factors in the production of offspring, litter size and sex ratio, litter-rearing type, and viability of offspring. Younger males and females were more likely to reproduce, and were more likely to produce larger litters, than were older individuals. The age of the dam, but not the sire, had a significant negative effect on pup survival. Sires and dams that had prior experience in the production of offspring were more likely to reproduce again than were individuals without prior reproductive success, but prior sexual experience alone was not a factor in the production of offspring. Parental breeding experience had a significant negative effect on pup survival, but no apparent relationships with size or sex ratio of the litter. Rearing-type of sires and dams was not a factor in the production of offspring. Declines in reproduction, fitness and survival with advancing age suggest the effect is due to senescence, the onset of which occurs as early as eight years of age in females. Of those hypotheses put forth to explain positive correlations of age and breeding experience on reproduction, the present results are most consistent with the breeding-experience hypothesis.
Key-words: age hypothesis; breeding-experience hypothesis; reproductive experience; reproductive strategies; senescence
INTRODUCTION

A principal objective of propagation programs is to contribute to the conservation of a species by preserving genetic and demographic stock that can be used to reinforce or re-establish populations in the wild (Ballou & Foose 1996). Propagation programs for imperilled species can achieve this objective by using both short-term and long-term management strategies (Frankham et al. 1986). Short-term strategies generally aim to rapidly multiply the population to insure the species’ survival in captivity and/or for immediate release into the wild. Long-term strategies include retaining as much of the founders’ genetic diversity as possible and maintaining demographic stability within a captive population. Theoretical analyses suggest that 20 to 30 founders can enable a propagation program to retain 90% of the original (i.e., founders’) genetic diversity for 200 years (Soulé et al. 1986).

The critically endangered red wolf (*Canis rufus* Audubon & Bachman) has been propagated in captivity for more than 30 years, following its planned extirpation in the wild (Carley 1979; U.S. Fish and Wildlife Service 1989). This propagation program is jointly managed by the U.S. Fish and Wildlife Service and the Association of Zoos and Aquariums under the auspices of the Red Wolf Recovery/Species Survival Plan® (Red Wolf Recovery/SSP). With only 14 red wolves as the founding stock, the Red Wolf Recovery/SSP established a goal to preserve 80-90% of the genetic diversity of the founders for 150 years, with a target captive population of 330 individuals (U.S. Fish and Wildlife Service 1989). To insure that the captive population maintains genetic diversity
and demographic stability, a Population Analysis and Breeding/Transfer Plan is produced annually (e.g., Waddell & Long 2008). This plan constructs breeding recommendations for individuals within the population and sets an objective for the number of offspring to produce. Each year’s plan is determined using age, reproductive status, and mean kinship of individuals, potential offspring inbreeding coefficients, and genetic and demographic projections that take into consideration the population’s past and potential growth rates ($\lambda$), mortality rates, and the amount of breeding and holding space available at cooperating institutions.

The captive red wolf population steadily increased from the late 1970s to the mid-1990s when a sharp decrease in births, due primarily to spatial limitations, resulted in a decline (Waddell & Long 2008) that was further exacerbated by fewer annual breeding recommendations, the pairing of individuals with marginal reproductive potential, and the use of reproductive inhibitors that may have compromised future reproductive potential when the inhibitors were removed. The distribution of ages within the population also became skewed toward a greater number of older cohorts. Since the mid-1990s this population has stabilized, and in recent years it has experienced a marked increase, in part, because of a focused effort to breed a greater number of younger animals and of older individuals that are genetically desirable (i.e., under-represented founder alleles). As of July 2007, the captive red wolf population consisted of 208 individuals, with an effective population ($N_e$) size of 172 (Waddell & Long 2008). The current gene diversity for the captive population is 89.52%, and is equivalent to a population descended from about five founders (Waddell & Long 2008). Under current conditions, gene diversity is expected to
be maintained at or above 85% for at least three more decades. However, inbreeding within
the captive population has resulted in lower reproductive performance and smaller litter
sizes (Rabon & Waddell, in press). Demographic projections to achieve the target
population size require a 2% annual growth rate ($\lambda = 1.02$), but current spatial limitations
necessitate that the population maintain a 0% growth rate ($\lambda = 1.00$) and approximately 30
births annually (Waddell & Long 2008).

Preserving genetic and demographic security while increasing the captive
population can be challenging because genetic diversity is lost during the expansion of the
population (Nei, Maruyama & Chakraborty 1975). As the captive population grows, a shift
from a short-term strategy to a long-term strategy may result in the selection of individuals
for breeding with under-represented alleles or older animals that retain greater
representation of the founders' genetic makeup. If reproductive output increases with age,
then breeding older individuals can serve to maintain the genetic composition desired by
the propagation program. In contrast, if reproductive success declines with age, then
breeding older individuals may diminish the program’s reproductive output. Similarly, if
breeding experience is correlated with breeding performance, then pairing experienced
individuals may increase reproductive output, whereas breeding inexperienced individuals
may require an increase in the reproductive effort (i.e., more pairs for breeding) to achieve
output goals.

Although there are exceptions (e.g., Sæther 1990; Weimerskirch 1990), most studies
suggest that reproductive performance improves with age (e.g., Nol & Smith 1987; Festa-
Bianchet 1988; Lunn, Boyd & Croxall 1994; Côté & Festa-Bianchet 2001; Broussard et al.)
During the first few years of reproductive life before reaching a plateau, or even declining in older, senescent individuals (e.g., Williams 1957; Clutton-Brock 1988). Several hypotheses have been proposed to explain age-specific variation in reproductive performance (e.g., Nol & Smith 1987; Broussard et al. 2003), including (1) the breeding-experience hypothesis, which predicts previous experience in reproduction improves later breeding performance; and, (2) the age hypothesis, which suggests that breeding performance improves with age, and that older, first-time breeders are more likely to be successful in reproduction than are younger, first-time breeders. However, few studies have separated the effects of age from those of reproductive experience, in part because longitudinal data on the reproductive histories of known individuals are sparse. In studies evaluating both reproductive experience and age, reproductive experience contributed to the improvement of reproductive performance (e.g., Raveling 1981; Harvey, Stenning & Campbell 1985; Wooller et al. 1990; Pyle et al. 1991; Sydeman et al. 1991; Forslund & Larsson 1992; Hepp & Kennamer 1993; Lunn et al. 1994; but see also Pärt 1995).

Maintaining demographic stability is a challenge inherent in the conservation of the red wolf. Identifying those traits that affect the reproductive success of individuals is essential for achieving the annual target for population growth rate, understanding life history and population dynamics, and managing the species both in captivity and the wild. In this paper I conducted a retrospective analysis of reproductive events of captive red wolves in order to examine relationships of parental age and experience with reproductive performance and fitness.
MATERIALS AND METHODS

As part of a larger study of traits that affect reproduction (e.g., Rabon & Waddell, in press), longitudinal reproductive events of captive red wolves were examined to determine whether age, reproductive experience (i.e., prior sexual experience and prior reproductive success), and parental rearing-type influenced the production of offspring. I also examined longitudinal reproductive events to test the relationships between the age and reproductive experience of sires and dams and the following measures of reproductive fitness: litter size, litter-rearing type, sex ratio of the litter, and viability of offspring. A detailed description of the data collected and the manner in which the data were categorized for analyses can be found in Rabon & Waddell (in press).

Evaluations of breeding success were based on 554 recorded pairing events from 1977 through 2006 (i.e., all pairings). Data on breeding success also were evaluated with repeated pairings by the same sire and dam excluded. For analyses of the 554 pairings, all subsequent pairings after the initial pairing of a sire and dam were excluded regardless of the reproductive result of the initial pairing (leaving 351 pairing events). In addition, for those pairings that resulted in the production of offspring, all subsequent pairings after the first reproductive event were excluded (leaving 152 pairing events).

The age of wild-caught wolves introduced into the captive-breeding program were estimated at time of capture, but these estimates were not likely accurate. For example, one wild-caught male (Studbook (SB) #11) survived to an age of 17.6 years based on an estimated age at time of capture. The oldest captive-born male red wolf (SB#543) survived
to 15.9 years of age; the oldest captive-born female (SB#409) survived to 16.1 years of age. Using the estimated ages of wild-caught wolves could overestimate the effects of age on reproduction. Therefore, for the purposes of analyses I considered wild-caught wolves (n = 14) to be at least one-year of age if captured before 1 June and young of the year (i.e., less than one-year of age) if captured after 1 June. Wolves that were younger than a year-old when paired for breeding were considered age 0 for the purposes of analyses. The ages of wolves were recorded at time of pairing in whole-year increments (e.g., 5-years-old, not 5.8-years-old); pairs maintained for subsequent years are accordingly age-adjusted.

Reproductive experiences of individuals were categorized according to their previous participation in, and the results of, pairing events. If a wolf had not previously been paired with a mate, I recorded the individual as having no prior sexual experience or reproductive success. Wolves that had been previously paired but with no offspring produced were categorized as having had prior sexual experience, but not reproductive success. Wild-caught wolves were classified as “unknown” and excluded from analyses of reproductive experience, unless otherwise noted, because their sexual and reproductive history was uncertain.

Rearing-type of sires and dams were categorized into two groups: the “Parent” group if reared by a parent (sires, n = 330; dams, n = 319); the “Hand” group if reared by a human (sires, n = 20; dams, n = 29). Foster-reared sires and dams were excluded from the analyses of parental rearing-type because there were too few individuals to conduct the analyses (sires, n = 1; dams, n = 3). Wild-caught wolves were included in the “Parent”
group for analyses because wild-caught wolves were most likely reared primarily by their parents.

Rearing-type of offspring was categorized into three groups: “Parent” group (n = 169) contained litters in which all pups were reared by their parents; “Hand” group (n = 12) litters in which at least one pup was reared by hand; and, “Foster” group (n = 11) litters in which at least one pup was reared by foster parents. Fostered litters were excluded from most analyses of rearing-type because pups chosen for fostering are based on factors other than parental age (e.g., number of pups in the donor litter, the availability of a foster litter, the size of potential foster litters).

Statistical analyses were conducted using JMP® 7.0 (SAS Institute Inc., Cary, North Carolina, USA 27513). To determine if parental age influenced the production of offspring, reproduction was evaluated as a function of sires’ and dams’ ages on actual and predicted litters using nominal logistic regression. To determine if parental experience was a factor in the production of offspring, reproduction was evaluated as a function of (1) prior sexual experience and reproductive success of the sire and dam and (2) the rearing type of the sire and dam on actual and predicted litters using Pearson's chi-square ($\chi^2$) test. To determine whether parental age and reproductive experience were factors in breeding performance, performance was evaluated as a function of the parents’ age and experience on litter size, litter-rearing type, sex ratio of the litter, and viability of offspring. Relationships between offspring viability and parental age and experience were evaluated using generalized linear modeling techniques with a binomial distribution and logit-link model. Generalized linear modeling techniques with a Poisson distribution, identity-link model, and an overdispersion
parameter (c̃) were used to tests for a relationship between litter size and parental age and experience. To determine if parental age or reproductive experience was a factor in the rearing of offspring, rearing type was evaluated as a function of parental age and experience using nominal logistic regression. Changes in age over time were evaluated using least squares regression, as were the effects of parental age and experience on the sex ratio of litters. Means of groups were compared using Student’s t-tests or Wilcoxon-Mann-Whitney tests, as appropriate. All data are depicted as mean ± standard error of the mean (SEM), unless otherwise noted; statistical significance was set at p ≤ 0.05.

RESULTS

A total of 192 litters (range = 1 to 9 pups per litter; mean litter size = 4.15 ± 0.14) consisting of 797 offspring (359:406:32, ♂♂:♀♀:unknown) was produced between 1977 and 2006. Of the 192 reproductive events, males as young as 1.8 years of age (e.g., SB#327) have sired offspring, and females have whelped at 11 months of age (e.g., SB#132). The oldest captive-born male (SB#294) known to have sired offspring was 12.8 years of age. A wild-caught adult male (SB#11), estimated at four years of age at time of capture, continued to sire pups 11 years after capture, possibly until 15 years of age. Several captive-born female red wolves (e.g., SB#195, 205, 315, 576, 725, 819) whelped litters at 11 years of age. However, the reported upper-age limits of reproducing individuals may be underestimated as the Red Wolf Recovery/SSP has established a
maximum breeding age of 13 years for males and 11 years for females in breeding recommendations designed to maximize reproductive output (Waddell & Long 2008).

To determine whether age was a factor in the production of offspring, I evaluated reproductive performance as a function of parental age on actual (n = 192) and predicted (n = 362) litters (all pairings). The mean ages of males and females paired for breeding were 5.49 ± 0.14 (range = <1 to 15 years) and 5.62 ± 0.14 (range = <1 to 14 years), respectively. Younger males and females were more likely to reproduce than were older animals, and this pattern continued when repeated pairing events were excluded from the analysis (Table 1). A similar pattern was observed when pairing events were restricted to the Red Wolf Recovery SSP recommended age-range for breeding sires (i.e., 1 to 12 years of age) and dams (i.e., 1 to 10 years of age) for all pairing events and when repeated pairing events were excluded (Table 1). The mean ages of sires and dams have significantly increased over time (Figure 1).

Prior sexual experience of the sire ($\chi^2 = 0.419, \text{df} = 1, p = 0.517$) or dam ($\chi^2 = 0.588, \text{df} = 1, p = 0.443$) was not a factor in the production of offspring (n = 542, no unknowns). However, prior reproductive success was important. Both sires ($\chi^2 = 25.799, \text{df} = 1, p < 0.0001$) and dams ($\chi^2 = 7.415, \text{df} = 1, p = 0.007$) were more likely to produce offspring when they had previously produced a litter (n = 542). When repeated pairings were removed from the analyses, this pattern continued for sires (prior sexual experience, $\chi^2 = 1.557, \text{df} = 1, p = 0.212$; prior reproductive success, $\chi^2 = 11.367, \text{df} = 1, p = 0.0007$; n = 343), but not for dams (prior sexual experience, $\chi^2 = 0.355, \text{df} = 1, p = 0.551$; prior reproductive success, $\chi^2 = 0.537, \text{df} = 1, p = 0.464$; n = 343).
After statistically accounting for age using nominal logistic regression, the likelihood of producing offspring significantly increased with prior reproductive success, but not with prior sexual experience, both in sires ($\chi^2 = 22.814, \text{df} = 1, p < 0.0001$) and dams ($\chi^2 = 11.184, \text{df} = 1, p = 0.0008$). Likewise, after accounting for prior reproductive success, younger sires ($\chi^2 = 20.892, \text{df} = 1, p < 0.0001$) and dams ($\chi^2 = 29.555, \text{df} = 1, p < 0.0001$) had a significantly greater production of offspring than did older sires and dams.

Rearing-type of sires ($\chi^2 = 0.666, \text{df} = 1, p = 0.414$) and dams ($\chi^2 = 1.473, \text{df} = 1, p = 0.225$) was not a factor in the production of offspring (n = 351; no repeat pairings), although there was a slight trend for Parent-reared sires and dams to be more likely to produce offspring than did Hand-reared sires and dams.

The following results are calculated from reproducing pairs, unless otherwise noted.

**Effects of Age**

Older sires ($\chi^2 = 3.904, \text{df} = 1, \hat{c} = 0.943, p = 0.048$) and dams ($\chi^2 = 4.500, \text{df} = 1, \hat{c} = 0.946, p = 0.034$) produced smaller litters (all pairings). When the sex ratio of litters was measured as a function of parental age, there were no significant effects of the sire’s age on the proportion of males ($F = 0.004, \text{df} = 1, p = 0.947$), females ($F = 1.037, \text{df} = 1, p = 0.309$), or pups of undetermined sex ($F = 2.751, \text{df} = 1, p = 0.099$) in a litter at birth, nor were there significant effects of the dam’s age on the proportion of males ($F = 0.034, \text{df} = 1, p = 0.854$), females ($F = 0.079, \text{df} = 1, p = 0.306$), or pups of undetermined sex ($F = 1.652, \text{df} = 1, p = 0.200$) in a litter at birth (all pairings). When offspring survival was
measured as a function of parental age (all pairings), there was a significant negative relationship between the dam’s age and pup survival at 3-months of age ($\chi^2 = 7.776$, df = 1, $p = 0.005$), but not when pups were 12-months old ($\chi^2 = 0.321$, df = 1, $p = 0.571$; Figure 2). There was no relationship between the sire’s age and pup survival at 3-months ($\chi^2 = 0.468$, df = 1, $p = 0.494$) or 12-months of age ($\chi^2 = 0.111$, df = 1, $p = 0.739$).

Similar trends were found when repeated pairings were excluded from the analyses (n = 152 pairing events). There was a non-significant trend toward younger sires ($\chi^2 = 1.774$, df = 1, $\hat{c} = 0.990$, $p = 0.183$) and dams ($\chi^2 = 3.758$, df = 1, $\hat{c} = 0.981$, $p = 0.053$) producing larger litters. There were no effects of parental age on the proportion of males (sires, $F = 0.428$, df = 1, $p = 0.514$; dams, $F = 1.559$, df = 1, $p = 0.214$), females (sires, $F = 0.011$, df = 1, $p = 0.917$; dams, $F = 0.002$, df = 1, $p = 0.963$), or pups of undetermined sex (sires, $F = 1.411$, df = 1, $p = 0.237$; dams, $F = 3.569$, df = 1, $p = 0.061$) in a litter at birth. There was no significant relationship between parental age and pup survival at 3-months (sire, $\chi^2 = 0.014$, df = 1, $p = 0.906$; dam, $\chi^2 = 2.337$, df = 1, $p = 0.126$) or 12-months of age (sire, $\chi^2 = 0.559$, df = 1, $p = 0.455$; dam, $\chi^2 = 0.380$, df = 1, $p = 0.538$).

There was no relationship between parental age and offspring rearing-type (Table 2). A similar result was found when repeat pairings of the sire and dam were excluded (Table 2). Foster-reared litters were excluded from this analysis because pups chosen for fostering are based on factors other than parental age (see above).
Effects of Reproductive Experience

When offspring survival was measured as a function of parental experience (no unknowns), there was a negative relationship between the sire’s (n = 186) prior breeding experience and pup survival at 3-months (sexual experience, $\chi^2 = 10.392$, df = 1, $p = 0.001$; reproductive success $\chi^2 = 3.881$, df = 1, $p = 0.049$) and 12-months of age (sexual experience, $\chi^2 = 7.711$, df = 1, $p = 0.006$; reproductive success, $\chi^2 = 7.650$, df = 1, $p = 0.006$). A similar relationship was found between the dam’s (n = 189) experience and pup survival at 3-months (sexual experience, $\chi^2 = 10.655$, df = 1, $p = 0.001$; reproductive success $\chi^2 = 9.578$, df = 1, $p = 0.002$) and 12-months of age (sexual experience, $\chi^2 = 1.475$, df = 1, $p = 0.225$; reproductive success, $\chi^2 = 6.963$, df = 1, $p = 0.008$).

When litter size was measured as a function of parental experience (no unknowns), there were no relationships between litter size and prior sexual experience of sires ($\chi^2 = 0.118$, df = 1, $\hat{c} = 0.974$, $p = 0.731$, n = 186) or dams ($\chi^2 = 0.052$, df = 1, $\hat{c} = 0.975$, $p = 0.819$, n = 189). Furthermore, there was no relationship between litter size and prior reproductive success of sires ($\chi^2 = 0.038$, df = 1, $\hat{c} = 0.977$, $p = 0.847$, n = 186) or dams ($\chi^2 = 0.349$, df = 1, $\hat{c} = 0.973$, $p = 0.555$, n = 189).

There were no relationships between prior sexual experience of the sire (n = 186) or the dam (n = 189) on the proportion of males (sires, $F = 1.897$, df = 1, $p = 0.170$; dams, $F = 0.396$, df = 1, $p = 0.529$), females (sires, $F = 1.097$, df = 1, $p = 0.296$; dams, $F = 0.587$, df = 1, $p = 0.445$), or pups of undetermined sex (sires, $F = 0.254$, df = 1, $p = 0.615$; dams, $F = 0.059$, df = 1, $p = 0.809$) in a litter at birth. Furthermore, there were no relationships.
between prior reproductive success of the sire (n = 186) or the dam (n = 189) on the proportion of males (sires, F = 1.717, df = 1, p = 0.192; dams, F = 0.058, df = 1, p = 0.810), females (sires, F = 0.772, df = 1, p = 0.381; dams, F = 0.008, df = 1, p = 0.929), or pups of undetermined sex (sires, F = 0.445, df = 1, p = 0.506; dams, F = 0.306, df = 1, p = 0.581) in a litter at birth.

When litter rearing-type was measured as a function of parental experience (no unknowns), there were no relationships between the sire’s prior sexual experience ($\chi^2 = 0.094, df = 1, p = 0.759, n = 175$) or prior reproductive success ($\chi^2 = 0.010, df = 1, p = 0.920, n = 175$) and litter rearing-type, nor were there relationships between the dam’s prior sexual experience ($\chi^2 = 0.004, df = 1, p = 0.950, n = 178$) or prior reproductive success ($\chi^2 = 0.560, df = 1, p = 0.454, n = 178$) and litter rearing-type.

**DISCUSSION**

The effects of age and experience observed in the captive red wolf population are supported by studies showing a relationship between age and experience and reproductive performance (e.g., Raveling 1981; Harvey *et al.* 1985; Wooller *et al.* 1990; Pyle *et al.* 1991; Sydeman *et al.* 1991; Forslund & Larsson 1992). In the present study, age and reproductive experience of sires and dams increased production of offspring, and the effects were independent of one another in successful reproduction. Younger sires and dams were more likely to reproduce than were older individuals, and litter size tended to decrease with increasing age of sires and dams. There also was a trend for survival of offspring to be
negatively affected by increasing parental age, but the effect was sex specific. Other relationships between breeding experience or parental-age and reproductive performance and fitness were not apparent.

Of the hypotheses put forward to explain positive correlations of age with reproductive experience, the present results are most consistent with the breeding-experience hypothesis. However, the cumulative benefits of breeding experience on reproductive success in the red wolf are unknown. That is, the advantages of multiple years of experience may offer no advantage over inexperienced individuals. For example, Sydeman et al. (1991) reported that breeding experience in younger female northern elephant seals (*Mirounga angustirostris*) was beneficial for weaning success, but detrimental later in life. Moreover, the advantages of multiple years of breeding experience on breeding success may be sex specific. Ollason and Dunnet (1978) reported that male fulmars (*Fulmarus glacialis*) show a general improvement in breeding success with breeding experience, whereas in early and later years female fulmars had lower breeding success than did males with similar breeding experience.

The effects of age on reproductive performance are more difficult to explain, as the results presented here do not support the age hypothesis. Differential effects of age and experience on breeding success have been reported in studies investigating both. In the Antarctic fulmer (*Fulmarus glacialisoides*), experience positively contributed to breeding success after the first reproduction, but age had no significant effect (Weimerskirch 1990). Similarly, in a study of three species of European ducks, reproductive performance varied with age and experience, but the effects of age were explained more by female body mass
and nesting date (Blums, Hepp & Mednis 1997). Experimental manipulation of breeding experience or the analysis of other age-related improvements in reproduction may be required to understand age-specific variation in breeding success in the red wolf (e.g., Pärt 1995). Several additional hypotheses (e.g., residual reproductive-value hypothesis, selection hypothesis, evolutionary-restraint hypothesis) also have been proposed that may explain age-specific variation in reproductive performance (e.g., Nol & Smith 1987; Broussard et al. 2003). In the present study, data (e.g., parental survival rates, somatic investment) were not collected or recorded in a manner permitting assessment of these hypotheses.

The present study is the first to investigate the relationships between parental age, breeding experience, and reproduction in the red wolf. A previous study investigated changes in age structure in the captive red wolf population and how those changes affected the population’s reproductive potential (Lockyear 2006). However, the accuracy of the dataset used in that study has been questioned (e.g., Rabon & Waddell, in press), and relatively few results of the effects of age on reproduction are similar between the two studies. For example, Lockyear (2006) reported that breeding success was negatively correlated with female age, but found no effect of sires’ or dams’ ages on litter size. In the present study, breeding success was negatively affected by the age of both males and females, and litter size significantly declined with increasing age of the dam. In fact, dams older than seven years of age had significantly smaller litter sizes (mean litter size = 3.14 ± 0.33, n = 28) compared to those of younger dams (mean litter size = 4.32 ± 0.16, n = 164) (F = 8.704, df = 1, p = 0.004; Figure 3). Lockyear (2006) also reported a reduction in pup
survival (at 12-months of age) with increasing age of the dam. While I found that older dams (i.e., ≥ 7 years of age) had significantly fewer offspring than did younger dams, the effect was significant for pups of 3-months of age, but not of 12-months of age (F = 4.815, df = 1, p = 0.029; Figure 2).

Differences in the results between the two studies may be explained by differences in the datasets. Lockyear (2006) reported only 165 litters born in captivity compared with 183 litters identified during the same period (i.e., 1977 to 2005) in my dataset. Furthermore, Lockyear (2006) conducted some of her analyses using data restricted to breeding events from 1992 through 2005. Failure to include data from all litters born in the propagation program may have affected the results. Unfortunately, a complete comparison of the datasets representative of the period(s) used by Lockyear (2006) could not be accomplished. Therefore, it is difficult to determine whether the differences between these two studies can be solely attributed to the number of reproducing events considered in the analyses. Nevertheless, the simultaneous declines in reproduction, and in fitness and survival of offspring with advancing maternal age seen in the present study suggest that the effect is due to senescence (Bonduriansky & Brassil 2002; Jones et al. 2008; for review see also Nussey et al. 2008). Furthermore, females showed signs of reproductive senescence at an earlier age (about 8 years old) than previously reported (e.g., Lockyear 2006).

The recorded range in age for successful reproduction in captivity both in sires and dams is similar to that of the wild population. However, reproduction in individuals less than two years of age is uncommon in either population. In males, reproduction at less than two years of age has only been recorded in the wild (Phillips, Henry & Kelly 2003),
whereas successful reproduction by females at less than one year of age has only been recorded in captivity (this paper). The upper age limit for successful reproduction in captivity extends a few years beyond the ages recorded for wild-born wolves, at least for males (i.e., the oldest wild-born males have sired pups at 8.8 years of age (e.g., SB#795, 771)). Both captive-born and wild-born (e.g., SB#978) female wolves have whelped litters when they were 11 years of age, although wild-born females are unlikely to reproduce beyond 10 years of age (U.S. Fish and Wildlife Service, unpublished data). The differences in the maximum age for successful reproduction in the red wolf may be attributed to differential rates of survival between wild and captive animals (Mech 1988; Phillips et al. 2003), thus reducing the opportunity for older wild wolves to reproduce. Captive animals are generally expected to live longer because they are protected, better fed, and receive consistent care.

The age range for reproduction in red wolves appears similar to that of grey wolves (C. lupus) and coyotes (C. latrans). In captivity, male and female grey wolves have bred as young as 10 months (Medjo & Mech 1976) and as old as 14-15 years of age (Kreeger 2003). Both male and female coyotes are capable of breeding in their first year, but the likelihood of reproduction appears to be related to the availability and abundance of food (Gier 1968; Knowlton 1972). Data on the reproductive activity of very old coyotes are sparse. Gese (1990) reported that the behaviour of an estimated-age male coyote associating with an oestrus female that was later observed with pups, suggested that the male was reproductively active at 11 and 12 years of age. In addition, a few studies have reported a decline in fecundity in female coyotes older than nine years of age (e.g.,
Windberg 1995; Green, Knowlton & Pitt 2002). The maximum breeding age of wild grey wolves also is not well known, but Mech (1988) reported reproduction in a female of at least 10 years of age.

Understanding the factors influencing reproductive performance is important for managing and conserving the red wolf both in captivity and in the wild. Nol and Smith (1987) cautioned that the detection of effects of age and experience could be affected by the relative quality of environment conditions. While there are recognizable geographic and spatial differences among Red Wolf Recovery/SSP propagation facilities, the general protective environment, food resources, and animal care are relatively consistent. In addition, differences in geography and enclosure do not appear to affect the likelihood of successful reproduction (David Rabon, unpublished data), although a more complete analysis of breeding location and reproductive success is required.

The results presented here may prove useful in the preparation of the annual Population Analysis and Breeding/Transfer Plan for the red wolf. Additional data on the traits affecting reproductive performance may allow for an improvement in breeding success while minimizing reproductive effort. For example, an analysis of the effects of the presence of juveniles from the previous year’s litter during the breeding season may prove important for successful reproduction. If spatial limitation of propagation facilities persist, improving the ratio between reproductive success and effort will be necessary to achieve the estimated growth rates required to maintain demographic stability and to grow the red wolf population toward stated recovery goals.
ACKNOWLEDGEMENTS

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REFERENCES


Table 1. Unrestricted and age-restricted mean ages (in years ± SEM) of sires and dams that produced offspring and failed to reproduce for all pairing events and when repeat pairing events of the sire and dam were excluded.

Unrestricted age range of sires (0 to 15 years) and dams (0 to 14 years)

<table>
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<th>All Pairings (n = 554)</th>
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<tbody>
<tr>
<td></td>
<td>Reproduction</td>
<td>No Reproduction</td>
<td>$\chi^2$</td>
<td>df</td>
<td>P</td>
<td>Reproduction</td>
<td>No Reproduction</td>
<td>$\chi^2$</td>
<td>df</td>
<td>P</td>
<td>Reproduction</td>
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<td>$\chi^2$</td>
<td>df</td>
<td>P</td>
<td></td>
<td></td>
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<tr>
<td>Sires</td>
<td>4.68 ± 0.23</td>
<td>5.92 ± 0.17</td>
<td>18.83</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>4.58 ± 0.28</td>
<td>5.34 ± 0.22</td>
<td>4.62</td>
<td>1</td>
<td>0.0316</td>
<td>4.17 ± 0.28</td>
<td>5.72 ± 0.21</td>
<td>19.23</td>
<td>1</td>
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<tr>
<td>Dams</td>
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<td>&lt;0.0001</td>
<td>4.17 ± 0.28</td>
<td>5.72 ± 0.21</td>
<td>19.23</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>4.50 ± 0.23</td>
<td>6.22 ± 0.17</td>
<td>36.56</td>
<td>1</td>
<td>&lt;0.0001</td>
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<tr>
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Red Wolf Recovery/SSP age-restricted range of sires (1 to 12 years) and dams (1 to 10 years)

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<th></th>
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<tbody>
<tr>
<td></td>
<td>Reproduction</td>
<td>No Reproduction</td>
<td>$\chi^2$</td>
<td>df</td>
<td>P</td>
<td>Reproduction</td>
<td>No Reproduction</td>
<td>$\chi^2$</td>
<td>df</td>
<td>P</td>
<td>Reproduction</td>
<td>No Reproduction</td>
<td>$\chi^2$</td>
<td>df</td>
<td>P</td>
<td></td>
<td></td>
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<tr>
<td>Sires</td>
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<td>5.82 ± 0.18</td>
<td>16.05</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>4.55 ± 0.27</td>
<td>5.34 ± 0.22</td>
<td>5.09</td>
<td>1</td>
<td>0.0241</td>
<td>4.25 ± 0.26</td>
<td>5.40 ± 0.21</td>
<td>12.17</td>
<td>1</td>
<td>0.0005</td>
<td></td>
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<tr>
<td>Dams</td>
<td>4.55 ± 0.20</td>
<td>5.65 ± 0.16</td>
<td>18.10</td>
<td>1</td>
<td>&lt;0.0001</td>
<td>4.55 ± 0.27</td>
<td>5.34 ± 0.22</td>
<td>5.09</td>
<td>1</td>
<td>0.0241</td>
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<td>5.40 ± 0.21</td>
<td>12.17</td>
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Table 2. Mean ages (in years ± SEM) of sires and dams by offspring rearing-type for all pairing events and when repeat pairing events of a sire and dam were excluded.

<table>
<thead>
<tr>
<th></th>
<th>All Pairings</th>
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<th>No Repeat Pairings</th>
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<tbody>
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<td>Sires</td>
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<td>Dams</td>
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</tr>
<tr>
<td>n</td>
<td>169</td>
<td>12</td>
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</table>
Figure 1. Mean ages (in years) of (a) sires and (b) dams at time of pairing each year for the captive population (n = 192). Fitted line is based on least-squares linear regression.
Figure 2. Age of dam (mean in years ± SEM) plotted against offspring viability at (a) 3-months of age and (b) 12-months of age. Fitted (red) and quadratic lines are based on least-squares regression.
Figure 3. Age of dam (mean in years ± SEM) plotted against litter sizes at birth for (a) all pairings (n = 192) and (b) when repeated pairings were excluded (n = 152) and the dams’ ages plotted against litter sizes when offspring were three months of age for (c) all pairings and (d) when repeated pairings were excluded. Fitted (red) and quadratic lines are based on least-squares regression.
CHAPTER 4

BEHAVIORAL RESPONSE OF RED WOLVES (*Canis rufus*) TO CONSPECIFIC AND CONGENERIC SOCIAL ODORS

“Apart from man, most other mammals think through their noses. [If] we too were olfactory animals there would be no bird watchers, but in their place we would have mammal smelling societies.”

Alec Nisbett (1977: 30)

ABSTRACT

Studies have shown that canids use urine and feces to scent mark, and that these social odors might be important in recognizing individuals and in selecting mates. The purposes of this study were to determine whether male and female red wolves (*Canis rufus*) exhibited seasonal, sex-specific, and species-specific preferences for conspecific and congeneric (i.e., *Canis latrans*) social odors. In general, females responded to the presentation of male social odors more quickly than did males, and males responded more quickly to female social odors than did females. There were no differences in the proportion of time males and females spent proximal to social odors. Males and females typically responded more quickly to and spent more time investigating social odors during the non-breeding season than during the breeding season. Species-specific responses to
social odors were indeterminate. The results provide a foundation for understanding the complex nature of olfactory communication and social organization in the red wolf.

INTRODUCTION

Olfaction is an important sensory function in most mammals, and especially in carnivores (Ewer, 1973; Macdonald, 1985). Many carnivores lead complex social lives in which information between conspecifics is conveyed through the transmission of odors. Carnivores also transmit and receive chemical signals to and from sympatric congeners and prey. Among the carnivores, the Canidae have been extensively investigated for the role odors play in relation to social behaviors (e.g., Asa and Mech, 1995; Harrington and Asa, 2003; Kleiman, 1966; Macdonald, 1985).

Canids (and most mammals) produce odors from a variety of scent-producing organs, such as sebaceous and apocrine glands of the skin and salivary glands, as well as from secretions originating in the vagina and anal sacs, and in urine and feces (Adams, 1980; Asa and Mech, 1995; Gorman and Trowbridge, 1989). Mammalian odors typically are more complex than the chemical signals (i.e., pheromones) used by lower animals to elicit a natural, stereotypic behavioral response. Furthermore, the behavioral responses to mammalian odors, unlike pheromones, are often complex and are largely dependent on context, experience, and individual discrimination or recognition (Beauchamp et al., 1976). Thus, Brown (1979) recommended using the term “social odors” to differentiate the odors mammals use to communicate information from behavior-eliciting pheromones.
Social odors are typically transmitted via scent marking, which is characterized by the deposition of scented compounds on the substrate or objects in the environment, or applied to the individual’s own body or that of a conspecific (Eisenberg and Kleiman, 1972; Gorman and Trowbridge, 1989; Ralls, 1971). Although presumably rare in canids, scent marking also may take the form of releasing odiferous compounds into the air (Eisenberg and Kleiman, 1972; Gorman and Trowbridge, 1989). Urine, feces, and anal-sac secretions are commonly used by canids to scent mark (Asa et al., 1985a; Asa et al., 1985b; Kleiman, 1966; Peters and Mech, 1975; Vilà et al., 1994). The use of anal-sac secretions in scent marking is somewhat difficult to determine because secretions can be voluntarily expelled independently of feces or they may be deposited on feces during defecation (Asa et al., 1985a; Asa et al., 1985b). In gray wolves (Canis lupus), the rate at which anal-sac secretions are deposited on feces is relatively low, but appears to be associated with social dominance (Asa et al., 1985a; Asa et al., 1985b). Discerning the use of urine and feces as social odors also is somewhat complicated by the quantities in which they can be deposited. The deposition of small “token” quantities of both urine and feces are typically regarded to be communicative in nature, whereas urination or defecation in large amounts is considered primarily an eliminative function (Asa et al., 1985a; Kleiman, 1966; Macdonald, 1985; Peters and Mech, 1975). However, eliminative amounts of urine and feces also may serve a communicative function. Studies have shown that eliminative amounts of urine and feces are deposited by dominant members of a pack in a non-random manner (Asa et al., 1985a; Barja, 2003; Barja et al., 2004; Peters and Mech, 1975; Vilà et al., 1994).
Canids scent mark throughout the year, but there are temporal variations in the frequency of marking. Several studies have reported that the frequency of scent marking with urine by male gray wolves increased during the winter months (Asa et al., 1985a; Asa et al., 1990; Barja Núñez and de Miguel, 2004; Peters and Mech, 1975; but also see Rothman, 1977; Rothman and Mech, 1979), which coincides with the breeding season (Mech, 1970). Scent marking with urine by female gray wolves also appeared to increase in frequency during the winter (Kleiman, 1966; but also see Barja Núñez and de Miguel, 2004). The frequency of urine scent marks by male gray wolves increased during the summer or post-partum period as well, but not at the same rate as during the winter season (Barja Núñez and de Miguel, 2004). Coyotes (*Canis latrans*) exhibited similar temporal trends in urine scent-marking behavior (Bekoff and Wells, 1986; Bowen and McTaggart Cowan, 1980; Gese and Ruff, 1997; Wells and Bekoff, 1981; but also see Barrette and Messier, 1980). In contrast, scent marking with feces by both male and female gray wolves and coyotes appeared to occur at a low and relatively stable rate throughout the year (Gese and Ruff, 1997; Peters and Mech, 1975; Vilà et al., 1994; Wells and Bekoff, 1981). However, male gray wolves marked with feces more frequently than did females (Asa et al., 1985a).

Scent marking with social odors may serve a number of functions. As previously noted, scent marking in canids appears to be closely associated with high social status or dominance (Ralls, 1971). In gray wolves, the dominant male and female, and rarely subordinate males, scent marked with urine (Asa et al., 1985a; Asa et al., 1990; Peters and Mech, 1975; Ryon and Brown, 1990). Similarly, dominant and subordinate males, but not
females, scent marked with feces (Asa et al., 1985a). Dominant coyotes also scent marked at a higher rate than did subordinates (Gese and Ruff, 1997; but also see Barrette and Messier, 1980). Moreover, scent marking appears to be related to establishment and maintenance of territories. Peters and Mech (1975) reported that gray wolves scent marked in higher proportions at junctions and along the edges of their territory (but also see Paquet and Fuller, 1990). Likewise, adult coyotes scent marked with urine with greater frequency along the boundary of their territory than in the interior (Allen et al., 1999; Bowen and McTaggart Cowan, 1980; Gese and Ruff, 1997; but also see Barrette and Messier, 1980). Gese and Ruff (1997) also reported that transient coyotes scent marked less frequently than did resident coyotes. In a study of Ethiopian wolves (Canis simensis), dominant individuals scent marked along narrow, concentrated “contact zones” where territorial boundaries were shared with another wolf pack (Sillero-Zubiri and Macdonald, 1998). When the boundaries of inter-pack territories shifted, scent marking in the contact zones shifted as well.

The use of social odors in scent marking also implies that the odors may function in identification. In their seminal book on wolves, Young and Goldman (1944) noted that urine “carries a strong and, to wolves apparently, an indentifying odor for each individual.” In fact, we now know that each individual animal (except identical twins) has a distinct, genetically determined odor, allowing for identification using odor cues (Conover, 2007). Several studies have demonstrated that canids are capable of discriminating between their social odors and those made by conspecifics. For example, Brown and Johnston (1983) demonstrated that gray wolves can distinguish between urine odors of conspecifics. Moreover, Tegt (2004) demonstrated that coyotes can distinguish among odors created by
themselves, individuals of varying degrees of relatedness, and genetically unrelated animals.

Distinguishing between related and unrelated individuals also is an important aspect of reproduction and of avoidance of inbreeding. Several studies have shown that animals prefer to mate with a conspecific whose genetically distinctive odor differs from their own (e.g., Beauchamp et al., 1985; Gilder and Slater, 1978; Moore, 1965; Yamazuki et al., 1976). This suggests that the recognition of individual odors may be a factor not only in mate discrimination but in the preferential choice of mates. However, the ability to discriminate individual odors is not limited to conspecifics.

Social odors and scent marking may also play a role in interspecific communication. Indeed, many predator-prey interactions involving olfactory predators are influenced by the use of odors of prey species (for review see Conover, 2007). But what is the function of scent marking between closely related, sympatric species? Scent marking of territories does not appear to present an “impregnable barrier” to movement of conspecifics, but rather delimits territorial boundaries to limit intrusion (i.e., territory avoidance) and identification of the territory (i.e., resource) holder (e.g., Allen et al., 1999; Peters and Mech, 1975; Rothman, 1977; Rothman and Mech, 1979; but also see Wells and Bekoff, 1981). Scent marking may play a similar role with congeners (e.g., Paquet, 1991a, 1991b). Furthermore, although canids are reported to overmark novel scents with greater frequency when they are encountered at their territorial periphery (Allen et al., 1999; Gese and Ruff, 1997; Harris and Knowlton, 2001; Peters and Mech, 1975), the behavioral responses to scent marking are not equal across species. For example, both gray wolves and coyotes are reported to
overmark urine marks of the other species, but coyotes appeared to increase their marking behavior in the presence of gray wolf urine, whereas gray wolves did not exhibit such behavior in the presence of coyote urine (Paquet, 1991a). Between conspecifics, scent marking is purported to function as a signal of tenure, status, pack structure (Sillero-Zubiri and Macdonald, 1998), and relative competitive ability (Gosling, 1982, 1990; Gosling and Roberts, 2001). Scent marking may serve a similar function with sympatric congeners. That is, scent marking may function as an interference mechanism of competition (i.e., the displacement of competitively subordinate individuals) if sympatric congeners represent potential competitors (Gosling, 1982; Peterson, 1995).

As previously noted, social odors appear to be associated with sexual interactions and reproduction, but what is being signaled is inconclusive. Scent marking is thought to convey important information about the sexual status or receptiveness of individuals (Ryon and Brown, 1990), and the method of marking may play a role in signaling reproductive status. Gosling and Roberts (2001) argued that there are distinct costs associated with scent marking that make such signals honest indicators of the general health and status of the signaler. Several studies seem to support this premise. For example, the frequency of scent marking with urine increases just before and during the breeding season as females approach estrus (Asa et al., 1985a; Bekoff and Wells, 1986; Gese and Ruff, 1997; Ryon and Brown, 1990; Wells and Bekoff, 1981; but also see Escobar-Ibarra et al., 2006). However, Asa and colleagues (1990) reported that the increase in scent marking (with urine) in female gray wolves was well before the onset of proestrus or estrus. This suggests that increases in scent marking are not reliable indicators of immediate sexual or reproductive status,
although they could portend approaching estrus. Nevertheless, increases in scent marking during the reproductive season may provide other information important for mate choice. For example, increased scent marking may be involved in signaling competitive ability or be an indication of the dominance status of potential mates (Gosling, 1990). Furthermore, tandem scent marking (i.e., double urine marking) by dominant males and females during the reproductive season suggests that such behavior may have a multifunctional role in courtship, mating and reproductive synchrony, and the creation and maintenance of pair bonds (Escobar-Ibarra et al., 2006; Gese and Ruff, 1997; Harrington et al., 2000; Rothman, 1977; Rothman and Mech, 1979).

Social odors can provide important biological and ecological information about a species or individuals (e.g., presence, territory or home-range, diet, and general health, or parasitic infection) to wildlife managers who, in turn, can use the information in managing the species. For example, understanding the social odors that facilitate or contribute to the selection of mates can increase our understanding of mating behavior and assist in conservation of the species. Furthermore, such knowledge can assist in discerning any interspecific behavioral significance among closely related or sympatric species. As noted above, social and reproductive behaviors of the gray wolf (e.g., Mech and Boitani, 2003) and the coyote (e.g., Bekoff, 1978; Bekoff and Wells, 1986) have been studied extensively, but similar behavioral data for the critically endangered red wolf (*Canis rufus*) generally are lacking (e.g., Riley and McBride, 1972; Shaw, 1975). The paucity of data on the red wolf can be attributed, in part, to the species’ near demise before extensive examination of its behavior and ecology.
The red wolf was extirpated throughout most of its range by the early part of the twentieth century (e.g., Nowak, 2002). The species was eventually considered biologically extinct in the wild after remaining individuals were systematically removed for captive propagation and reintroduction (U.S. Fish and Wildlife Service 1989). [A complete review of the status of the red wolf can be found in Chapter 1 of this volume.] Fortunately, captive breeding efforts were successful, and in the fall of 1987 captive-reared adult red wolves were released onto Alligator River National Wildlife Refuge in eastern North Carolina (USA) as part of an experimental restoration effort (Parker, 1987). Eastern North Carolina appeared appropriate for the reintroduction of red wolves, in part, because the human population density was low, prey species were abundant, and coyotes rarely occurred in North Carolina (Parker, 1987). Within a few years of the red wolf’s reintroduction, however, coyotes had become denizens of the red wolf recovery area (eastern North Carolina), introducing a conservation challenge for wildlife managers charged with conserving the red wolf.

Prior to the arrival of the coyote, the red wolf was likely the only species of *Canis* occupying the southeastern United States (Nowak, 2002). Coexistence between potentially competing canids is not uncommon (e.g., Paquet, 1991b), but competitive effects between species are more evident when the species are similar in size (Peterson, 1995). The red wolf and coyote are morphologically similar, as noted by Young and Goldman (1944: 488): “there appear to be no… dependable characters distinguishing in all cases small specimens of *rufus* [subspecies] from large ones of the coyote.” However, these two species have
undergone changes during the past century that made the issues of size and coexistence of greater concern.

Once trapped and killed to near extinction, the remaining red wolves represented the smallest of the subspecies (e.g., Nowak, 2002), and may have already suffered from introgression with coyotes (Paradiso and Nowak, 1971). Moreover, coyotes had expanded their range across former red wolf habitats, crossbreeding with wolves, coyote-wolf types, and dogs in the process. By the time of the red wolf’s reintroduction into eastern North Carolina, the coyote had transformed into a larger, more aggressive form in the eastern United States (e.g., *C. latrans* var; Gipson, 1978; Hilton, 1978). The concomitance of red wolves and coyotes in eastern North Carolina, now even more morphologically and behaviorally similar, amplified the potential for competition between the species.

Interspecific competition is purported to have contributed to morphological patterns, and possibly taxonomic displacement, among carnivores (e.g., Van Valkenburgh, 1991). There are a number of factors that influence the outcome of competition between closely related species, such as territory, availability and abundance of food, population characteristics, and the prevailing influence of humans. However, a lack of reproductive isolation and/or the availability of mates may be the most critical issue between restored red wolves and coyotes in eastern North Carolina. Indeed, the first red wolf-coyote hybridization in the red wolf restoration area was recorded in 1993 (Phillips et al., 2003). By early 1999, hybridization with coyotes was identified by participants of a Population and Habitat Viability Assessment (PHVA) workshop for the red wolf as the primary threat to the conservation of the red wolf in the wild (Kelly et al., 1999).
In order to abate the threat of hybridization, it is important to determine how red wolves select mates among conspecifics and congeners. Understanding the factors affecting mate choice also was identified as important for maximizing reproduction in both the wild and captive breeding populations. Like many canids, the red wolf is monoestrous, and maximizing reproductive success in each breeding season is essential to the viability and long-term survival of the species. Understanding how red wolves respond to olfactory stimuli and select mates among conspecifics and congeners will assist managers in reducing the threat of hybridization in the wild. For example, specific social odors may be used to target individual wolves or coyotes when trapping for the purposes of relocating animals to minimize wolf-coyote matings. Furthermore, knowing the olfactory parameters important in mate choice may assist managers in pairing red wolves for mating or in releasing potentially suitable mates in the territory of a single wolf to improve the chances for successful reproduction.

**Research Objectives**

The objectives of this study were to determine if male and female red wolves exhibited seasonal, sex-specific, and species-specific preferences for conspecific or congeneric social odors. In Experiment 1, I examined whether male-female pairs of red wolves exhibited a preference for urine from unrelated, reproductively sound, male red wolves over a control, and whether such preferences were seasonal. In Experiment 2, I examined whether male-female pairs of red wolves exhibited a preference for feces from
unrelated, reproductively sound, female red wolves over a control. Seasonal preferences were again examined. Finally, in Experiment 3, I examined whether male-female pairs of red wolves exhibited a preference for urine from unrelated conspecifics (reproductively viable male red wolf) over urine from congenerics (reproductively viable male coyote). Although this study is not an examination of red wolf scent-marking behavior, preferences exhibited for certain odors may indicate that there are behavioral or physiological mechanisms associated with olfaction, and possibly scent marking, that are important in mate selection.

Based on the known use of social odors by gray wolves and coyotes, I hypothesized that: (1) male and female red wolves would show a preference for excreta over a control; (2) male and female red wolves would show a stronger preference for excreta during the breeding season than during the non-breeding season; and, (3) male and female red wolves would show a preference for excreta from a conspecific over that from a congener.

MATERIALS AND METHODS

Two-choice preference tests using excreta (urine and feces) from donor red wolves and coyotes were used to assess those factors important for the red wolf in choosing mates. Subjects were adult red wolves (range in age of males 1.8 to 13 years, mean = 6.48 ± 0.52 years; range in age of females 1.8 to 13 years, mean = 5.89 ± 0.54 years) reared and maintained in captive breeding facilities participating in the Red Wolf Recovery/Species Survival Plan® (Red Wolf Recovery/SSP). All animals were maintained in outdoor pens at
least 225 m$^2$ in size, some on public exhibit and others in off-exhibit propagation areas.

Red wolves were housed as male-female pairs; although some pairs were unrelated companion animals or otherwise not allowed to breed (e.g., use of reproductive inhibitor). Red wolves were fed dog chow every other day or, during warmer months, fasted for a day every six days. Water was available *ad libitum*. Veterinary attention was provided *sī opus sit*.

**Sample Collection**

In a parallel study evaluating red wolf endocrinology, feces were collected 3-7 times per week (7 January through 25 April) from nine female red wolves housed in Red Wolf Recovery/SSP institutions. Urine was collected during the breeding season (19 February through 8 March) from 13 male red wolves housed at Point Defiance Zoo & Aquarium (Tacoma, Washington USA) via expression of the bladder following catheterization prior to electro-ejaculation in a study for semen evaluation and cryopreservation. Feces and urine collected in conjunction with those studies were used in this olfactory preference study. Coyote urine was retrieved (5-15 January) from collection pans below pens individually housing coyotes reared and maintained at the National Wildlife Research Center’s Predator Research Facility (United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services) in Millville, Utah (USA). Urine and feces were not evaluated or used as an indicator of reproductive status of the donor. However, male and female donor red wolves and male donor coyotes were of reproductive age and active...
participants of their respective captive breeding programs. As such, these animals were considered to be reproductively viable.

Given the size of the red wolf population and the limited number of generations in the captive breeding program (i.e., all animals descended from 14 founders), it is implausible to preclude a level of relatedness between donors and subjects. However, in all trials the donor animal was selected so that they shared no more than one common grandparent with the subjects. The distribution of red wolves in the captive breeding program reduced the likelihood for subjects and donors having had prior experience or interaction with one another, but lack of familiarity was not a prerequisite when pairing a donor to subjects.

**Olfactory Presentations**

Urine and feces from donor animals were presented to a male-female red wolf pair (subjects) during the breeding season (15 January through 15 March) and non-breeding season (15 July through 15 September). Approximately 3-5 ml of frozen-thawed urine was placed on a sterile 5 x 5 cm gauze pad before being inserted into a vented, plastic (PVC) container. Frozen-thawed feces, about 35 to 55 g, were placed directly inside the PVC container. The containers were positioned at least 1.5 m apart along the perimeter fence line of the subjects’ enclosure, near the ground in a manner mimicking natural deposition of the excreta. Subjects did not have direct access to the donor urine or feces.
Testing began by placing a stimulus container and a control (i.e., empty) container (when only red wolf excreta was examined) or two stimuli containers (one red wolf and one coyote when examining congeneric differences) along the perimeter of the subject animals’ pen. All experiments began as soon as the stimuli were introduced to the subjects. The subjects’ behavioral response was recorded for 30-50 minutes for conspecific trials or 10-20 minutes for congeneric trials. Latencies for red wolves to approach (< 1 m away) and contact (< 10 cm away) the stimulus and control were recorded. Because the time of each test varied, the tests were standardized by dividing the total time a subject spent in approach to, or contact with, the stimulus or control by the total time of the test. Observations were conducted between 0900 and 1600 hours.

The subject’s behavioral response (latency to respond to excreta, time spent in proximity to excreta, scent-marking) was recorded via remote video. The video recording was reviewed for any preferences exhibited and the data coded and analyzed using observational multi-media equipment and software. The experimenter wore disposable vinyl surgical gloves to reduce contamination by undesired odors. The PVC containers were cleaned using warm water and unscented antibacterial soap, and the experimenter’s hands were cleaned using unscented antibacterial gel and the gloves replaced between tests and stimulus presentations.

Statistical analyses were conducted using JMP® 7.0 (SAS Institute Inc., Cary, North Carolina, USA 27513). Relationships between proportions or age of subjects and response to stimuli were evaluated using nominal logistic regression. Responses of male and female wolves to a stimulus and a control were compared using two-tailed, matched-pairs t-tests.
Means of seasonal responses within each sex were compared using two-tailed Welch’s t-tests (allowing for unequal variances). All data are depicted as mean ± standard error of the mean (SEM), unless otherwise noted, and statistical significance was set at p ≤ 0.05.

RESULTS

Three experimental designs were employed for the investigation of olfactory preferences in red wolves: (1) subjects’ response to urine from sexually mature and viable male red wolves (n = 24 tests); (2) subjects’ response to feces from reproductively viable female red wolves (n = 22 tests); and, (3) subjects’ response to urine from sexually mature and viable male red wolves and from male coyotes (n = 10 tests). Results are presented by study design.

Experiment 1 – Urine from a Conspecific Male

Nearly half of the individuals of each sex (12 of 24 males; 10 of 24 females) responded to the presentation of urine from a conspecific male. When comparing the latency to respond within the sexes, there were no differences for males or females in their response to the stimulus or the control (Table 1). When comparing the latency to respond between the sexes, there was a trend for females to respond to the control, but not the stimulus, more quickly than did the males (Table 1). There were no seasonal differences in the latency to respond, but there was a slight trend for females to approach (F = 3.82, p =
and contact \( (F = 3.80, p = 0.92) \) the stimulus more quickly during the breeding season than during the non-breeding season (Table 2).

There were no differences within or between the sexes in the total time spent in proximity of the stimulus or the control (Table 3). Female wolves spent more time in approach \( (F = 7.45, p = 0.029) \) and contact \( (F = 7.84, p = 0.027) \) with the stimulus during the non-breeding season than during the breeding season (Table 4). There were no seasonal differences in the total time males spent in proximity of the stimulus or the control, but there was a slight trend for males to spend more time in approach of the control during the non-breeding season than during the breeding season \( (F = 3.80, p = 0.080; \text{Table 4}) \).

Age did not appear to be a factor in the response of male red wolves to conspecifics’ urine or to the control \( (\chi^2 = 0.44, n = 24, \text{df} = 1, p = 0.507) \). However, older female red wolves \( (n = 10) \) were more likely to respond both to urine from males \( (\text{mean age} = 11.6 \text{ years}, \chi^2 = 10.97, \text{df} = 1, p < 0.001) \) and to the control \( (\text{mean age} = 11.5 \text{ years}, \chi^2 = 9.42, \text{df} = 1, p = 0.002) \) than did younger female red wolves \( (\text{mean ages} = 7.7 \text{ and } 7.8 \text{ years, respectively, } n = 14) \).

Experiment 2 – Feces from a Conspecific Female

Fewer than half of the individuals of each sex \( (8 \text{ of } 22 \text{ males; } 10 \text{ of } 22 \text{ females}) \) responded to the presentation of feces from conspecific females. There were no differences within the sexes or between the sexes in the latency to respond to the stimulus or the control (Table 5). When considering seasonal differences in the responses, however, males
approached (F = 64.82, p < 0.001) and females approached (F = 9.60, p = 0.021) and contacted (F = 8.96, p = 0.024) the stimulus more quickly during the non-breeding season than during the breeding season (Table 6).

When comparing the total time spent in proximity within the sexes, there was a slight trend for females to spend more time in approach of the control rather than the stimulus (Table 7). There were no differences in the total time spent in proximity between the sexes (Table 7). When considering seasonal differences in the responses, female wolves spent more time in contact (F = 12.96, p = 0.011) with the stimulus during the non-breeding season than during the breeding season (Table 8).

Age did not appear to be a factor in the response of male red wolves to feces from conspecific females or to the control ($\chi^2 = 0.04, df = 1, p = 0.839$), but younger female red wolves (n = 10) were more likely to respond both to feces from females (mean age = 2.0 years, $\chi^2 = 11.62, df = 1, p < 0.001$) and to the control (mean age = 2.1 years, $\chi^2 = 7.56, df = 1, p = 0.006$) than did older female red wolves (n = 12, mean ages = 3.83 and 3.75 years, stimulus and control, respectively).

**Experiment 3 – Urine from Congeneric Males**

All tests (n = 10) were conducted during the non-breeding season, thus seasonal differences in the subjects’ response could not be evaluated. Only one male red wolf responded, and in just two of the tests with red wolf urine and one of the tests with coyote urine. Similarly, only one female red wolf responded to tests involving urine from the red
wolves and from coyotes (three times each). There were no differences by either sex in the latency of the responses to red wolf urine or to coyote urine (Table 9). There were no differences in the total time male and female wolves spent in proximity to urine from conspecific or congeneric males (Table 10). When combining responses from both sexes, there were no differences in the total time spent in proximity to urine whether from red wolves or from coyotes (approach: $t = 0.14$, df = 1, $p = 0.894$; contact: $t = 0.34$, df = 1, $p = 0.745$). Although the data are inconclusive, the responding female red wolf was younger (age = 3.0 years) than were the female wolves that failed to respond (mean age = 4.4 years).

Scent Marking

Scent marking behaviors (i.e., urination, defecation, flank-rubbing, digging, scratching) in response to the presentation of social odors did not appear to be an important component of the red wolves’ interaction with social odors. Of the red wolves that responded to the presentation of male red wolf social odor, few individuals scent marked (2 of 12 males; 3 of 10 females), and these values did not differ from the numbers of red wolves that scent marked in response to the control (2 of 12 males; 2 of 10 females). Similar incidences of scent marking behavior were noted in response to female red wolf social odor (2 of 8 males; 1 of 10 females) and to a control (2 of 8 males; 1 of 10 females). When comparing all scent marking behavior of red wolves by season, male red wolves scent marked more frequently during the non-breeding season ($n = 7$) compared to the breeding season ($n = 1$). Females scent marked equally during the breeding ($n = 3$) and
non-breeding seasons (n = 4). Comparable rates of scent marking were noted when both red wolf and coyote social odors were presented, but female red wolves (n = 4) scent marked more frequently than did males (n = 0), and equally in response both to red wolf and coyote social odors (2 of 3 responses, each species).

DISCUSSION

The results suggest that red wolves exhibit some degree of seasonal and sex-specific responses to social odors, but they show no preferences. Species-specific responses to social odors represent too small a dataset to extend observation to inference. Nevertheless, a few trends emerged from the data that may prove useful in deciphering the complex processes of species identification and mate selection, and assist in future studies of the use of social odors in mate selection by the red wolf.

Tegt (2004) acknowledged two caveats for the investigatory response to olfactory presentations and the discrimination of social odors in her study of olfactory cues and kin recognition in coyotes. The first, related to recognition of kinship, posits that if olfactory sensations are processed through a filter of “self,” then odors of “non-self” may be investigated with greater interest because odors of “self” are ubiquitous and of little interest or possibly even undetectable by the individual. Similarly, olfactory sensations may be processed through a filter of “familiarity.” In such a construct, individuals may be inclined to exhibit preferences for the odors of animals with which they have some familiarity (e.g., related) or have been previously exposed (i.e., prior olfactory experience). For example,
Ferkin and Rutka (1990) reported that meadow voles (*Microtus pennsylvanicus*) preferred the odors of familiar individuals, and behaved agonistically toward unfamiliar individuals, independently of genetic relatedness. The recognition of, and response to, odors may be species-specific, however, as Tegt (2004) reported that genetic relatedness, and not familiarity, was the greatest predictor in the time coyotes spent investigating conspecific odors. In the present study, olfactory cues were evaluated against a non-odor control (except in the red wolf-coyote experiments), and social odors were from donors of similar degrees of relatedness to the subject (i.e., sharing no more than one common grandparent). Thus, the discrimination of odors processed through the filter of “self” were not likely relevant to the subjects’ responses. The familiarity of subjects and donors could not be completely controlled; therefore, my study could not resolve this issue.

The second caveat Tegt (2004) noted was related to the issue of investigatory time, as the strength of the odor may affect the time spent investigating the odor, thus affecting the level of “interest” in the odor. For example, a weak odor may require more time of investigation to obtain the important information. Similarly, a weak odor may take longer to detect than a strong odor. In the present study, I accepted investigatory time as a suitable proxy for relative interest in an odor, but I also evaluated the latency to respond as an additional metric to substantiate investigatory time being affected by odor strength. A comparison between the latency to respond and the time spent in investigation might provide additional insight into the behavioral response to odors that a single metric could not. My results indicate that red wolves are able to discriminate novel social odors from a control, but whether investigatory time is a suitable proxy for relative interest in an odor
remains largely unresolved. Canids have an acute sense of smell. Although I attempted to remove confounding odors from the presentations, odors undetectable by the experimenter could have easily been detected by, and of interest to, the red wolves, and may factor into their responses.

The presentation of a novel object introduces another caveat in discerning olfactory “interests” in social odors. That is, a novel object itself may elicit an investigatory response irrespective of odor quality or magnitude. For example, Harris and Knowlton (2001) reported that captive coyotes spent more time investigating novel objects in a familiar environment than in an unfamiliar environment. Glickman and Sroges (1966) attributed the relatively uninhibited exploratory behavior of novel objects by carnivores to the evolutionary demands of their prey-seeking habits (i.e., an immediate and unafraid response to finding food). Red wolves generally responded more quickly to social odors than to a control, but the amount of time spent investigating the odors did not differ from the amount of time spent investigating the control. It is possible that the time the red wolves spent investigating the control could be that they simply were exhibiting an interest in a novel object rather than any interest in its odor.

Several studies have discussed a relation between the quantity of social odors used in scent marking and their communicative function. For example, Peters and Mech (1975) considered squat urination and defecation as basically an eliminative function that may serve in intra-pack communication, whereas raised-leg urination was primarily associated as a scent-marking behavior. The limited response of subjects in the present study offers little in resolving the issue of quantity of social odor relative to its communicative abilities.
However, small amounts of urine and feces, similar to the amounts deposited as scent marks (e.g., Peters and Mech, 1975), as well as their placement mimicking deposition by scent marking (e.g., Barja et al., 2004), were used in the present study. Unfortunately, urine used in the present study was collected by means that differ from natural deposition during scent marking. Although feces were collected after being naturally deposited, their original placement and/or possible use as scent marking was not recorded.

The differential responses by male and female red wolves to social odors may be associated with the temporal variation in the use of social odors in scent marking. For example, female red wolves responded more quickly to urine from males during the breeding season than they did during the non-breeding season. Furthermore, female red wolves tended to respond more quickly than did male wolves to urine from male wolves. These behaviors correspond to studies that report increases in the frequency of scent marking with urine during the breeding season by male gray wolves (Asa et al., 1985a; Asa et al., 1990; Barja Núñez and de Miguel, 2004; Peters and Mech, 1975) and coyotes (Bekoff and Wells, 1986; Bowen and McTaggart Cowan, 1980; Gese and Ruff, 1997; Wells and Bekoff, 1981). Increases in investigatory behavior during the breeding season also correspond with increases in volatile compounds in the male gray wolf’s urine (Raymer et al., 1984). Moreover, both male and female red wolves investigated females’ feces with greater frequency during the non-breeding season than during the breeding season, which corresponds to the period of time when scent marking with urine is likely to be low, and scent marking with feces is relatively (albeit at a low rate) constant (Gese and Ruff, 1997; Peters and Mech, 1975; Vilà et al., 1994; Wells and Bekoff, 1981).
This study explored social odors used by red wolves that might be important in mate selection between conspecifics and congeners. The findings indicate that urine and feces from conspecific animals might provide important information about conspecifics, and that the information may be processed differently by the sexes and differently at different times of the year. However, additional study is warranted to elucidate the differential investigatory responses of male and female red wolves to the social odors of conspecific and congeneric animals. The experiments in the present study provide a foundation for understanding the complex nature of olfactory communication and social organization in the red wolf, but additional research is needed to understand how red wolves recognize social odors of conspecifics and congeners, and how they use the information in their social interactions and organization.

LITERATURE CITED


Table 1. Latency (in seconds; mean ± SEM) of male and female wolves to approach and contact urine of male wolves and to approach and contact a control (combined seasons), and the proportion (in parentheses) of males and females responding.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Approach</th>
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<th>Contact</th>
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<tbody>
<tr>
<td></td>
<td>Males’ Urine</td>
<td>Control</td>
<td>t</td>
<td>P</td>
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<tr>
<td>Male</td>
<td>361.58 ± 103.02</td>
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<td>Female</td>
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<tr>
<td>P</td>
<td>0.533</td>
<td>0.052</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Latency (in seconds; mean ± SEM) of male and female wolves to approach and contact urine of males and to approach and contact a control during the breeding and non-breeding seasons.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Approach</th>
<th>Males’ Urine</th>
<th>Control</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Breeding</td>
<td>Non-breeding</td>
<td>t</td>
<td>P</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Approach</td>
<td>Males’ Urine</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>Breeding</td>
<td>Non-breeding</td>
<td>t</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Proportion of total time (unitless mean \( \times 10^{-3} \pm \text{SEM} \)) that male and female wolves spent proximal to males' urine and to a control (combined seasons), and the proportion (in parentheses) of males and females responding.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Approach</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males' Urine</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>( t )</td>
<td>( P )</td>
</tr>
<tr>
<td>Male</td>
<td>( 19.90 \pm 6.68 ) ( (25) )</td>
<td>( 26.48 \pm 12.91 ) ( (25) )</td>
</tr>
<tr>
<td>Female</td>
<td>( 17.43 \pm 3.57 ) ( (21) )</td>
<td>( 9.38 \pm 2.35 ) ( (21) )</td>
</tr>
<tr>
<td></td>
<td>( t )</td>
<td>( P )</td>
</tr>
<tr>
<td>Male</td>
<td>1.28</td>
<td>0.99</td>
</tr>
<tr>
<td>Female</td>
<td>1.36</td>
<td>0.211</td>
</tr>
</tbody>
</table>
Table 4. Proportion of total time (unitless mean ± SEM) male and female wolves spent proximal to urine of males and to a control during the breeding and non-breeding seasons.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Approach</th>
<th>Males’ Urine</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Breeding</td>
<td>Non-breeding</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Approach</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Males’ Urine</td>
<td>9.16 ± 2.03</td>
<td>30.65 ± 12.09</td>
</tr>
<tr>
<td></td>
<td>Contact</td>
<td>4.64 ± 1.01</td>
<td>16.15 ± 6.41</td>
</tr>
<tr>
<td>Female</td>
<td>Approach</td>
<td>12.75 ± 2.80</td>
<td>20.54 ± 5.50</td>
</tr>
<tr>
<td></td>
<td>Contact</td>
<td>5.18 ± 1.18</td>
<td>10.16 ± 3.29</td>
</tr>
</tbody>
</table>
Table 5. Latency (in seconds; mean ± SEM) of male and female wolves to approach and contact females’ feces compared to a control (combined seasons), and the proportion (in parentheses) of males and females responding.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Approach</th>
<th></th>
<th></th>
<th></th>
<th>Contact</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Females’ Feces</td>
<td>Control</td>
<td>t</td>
<td>P</td>
<td>Females’ Feces</td>
<td>Control</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>35.25 ± 13.93 (18)</td>
<td>36.38 ± 10.48 (18)</td>
<td>0.89</td>
<td>0.410</td>
<td>58.25 ± 21.24 (18)</td>
<td>52.00 ± 15.05 (9)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>233.90 ± 105.62 (23)</td>
<td>66.10 ± 26.89 (23)</td>
<td>0.07</td>
<td>0.945</td>
<td>133.89 ± 59.86 (21)</td>
<td>69.50 ± 26.56 (23)</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>1.20</td>
<td>0.74</td>
<td></td>
<td>0.48</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.352</td>
<td>0.515</td>
<td></td>
<td>0.681</td>
<td>0.427</td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Latency (in seconds; mean ± SEM) of male and female wolves to approach and contact females’ feces compared to a control during the breeding and non-breeding seasons.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Approach</th>
<th>Females’ Feces</th>
<th>Control</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Breeding</td>
<td>Non-breeding</td>
<td>t</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>Females’ Feces</td>
<td>82.33 ± 1.45</td>
<td>7.00 ± 3.29</td>
<td>20.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>63.67 ± 11.87</td>
<td>15.00 ± 0.00</td>
<td>2.89</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>319.29 ± 137.30</td>
<td>1.33 ± 0.33</td>
<td>2.32</td>
<td>0.059</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>121.00 ± 41.57</td>
<td>11.20 ± 4.49</td>
<td>2.63</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t</td>
<td>P</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Contact</th>
<th>Females’ Feces</th>
<th>Breeding</th>
<th>Non-breeding</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>84.67 ± 2.73</td>
<td>42.40 ± 33.07</td>
<td>1.27</td>
<td>0.271</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>199.83 ± 77.39</td>
<td>2.00 ± 0.58</td>
<td>2.56</td>
<td>0.051</td>
<td></td>
</tr>
<tr>
<td></td>
<td>122.40 ± 41.91</td>
<td>16.60 ± 4.40</td>
<td>2.51</td>
<td>0.065</td>
<td></td>
</tr>
</tbody>
</table>

“--” indicates too few samples for statistical analysis.
Table 7. Proportion of total time (unitless mean x 10^{-3} ± SEM) male and female wolves spent proximal to females’ feces and to a control (combined seasons), and the proportion (in parentheses) of males and females responding.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Approach</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females’ Feces</td>
<td>Females’ Feces</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15.41 ± 6.69 (18)</td>
<td>4.63 ± 1.37 (18)</td>
</tr>
<tr>
<td></td>
<td>13.89 ± 7.30 (18)</td>
<td>6.39 ± 3.42 (18)</td>
</tr>
<tr>
<td>Female</td>
<td>10.38 ± 1.11 (23)</td>
<td>5.04 ± 0.87 (21)</td>
</tr>
<tr>
<td></td>
<td>22.58 ± 6.70 (23)</td>
<td>8.03 ± 2.68 (23)</td>
</tr>
<tr>
<td>t</td>
<td>0.07 1.74</td>
<td>0.29 0.15</td>
</tr>
<tr>
<td>P</td>
<td>0.949 0.180</td>
<td>0.801 0.893</td>
</tr>
</tbody>
</table>
Table 8. Proportion of total time (unitless mean $\times 10^{-3} \pm$ SEM) male and female wolves spent proximal to females’ feces and to a control during the breeding and non-breeding seasons.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Approaches</th>
<th>Females’ Feces</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Breeding</td>
<td>Non-breeding</td>
</tr>
<tr>
<td>Male</td>
<td>Approach</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.81 ± 3.10</td>
<td>19.37 ± 10.58</td>
</tr>
<tr>
<td>Female</td>
<td>Contact</td>
<td>9.17 ± 1.20</td>
<td>13.21 ± 1.62</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>Contact</th>
<th>Females’ Feces</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Breeding</td>
<td>Non-breeding</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>4.47 ± 0.95</td>
<td>4.72 ± 2.23</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>4.09 ± 0.82</td>
<td>6.93 ± 1.76</td>
</tr>
</tbody>
</table>
Table 9. Latency (in seconds; mean ± SEM) of male and female wolves to approach and contact wolf urine and coyote urine, and the proportion (in parentheses) of males and females responding.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Approach</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wolf Urine</td>
<td>Coyote Urine</td>
</tr>
<tr>
<td>Male</td>
<td>9.00 ± 8.00 (20)</td>
<td>62.00 ± 21.00 (20)</td>
</tr>
<tr>
<td>Female</td>
<td>14.00 ± 11.06 (30)</td>
<td>23.33 ± 3.38 (30)</td>
</tr>
<tr>
<td>t</td>
<td>0.60</td>
<td>1.91</td>
</tr>
<tr>
<td>P</td>
<td>0.656</td>
<td>0.307</td>
</tr>
</tbody>
</table>

“--” indicates too few samples for statistical analysis.
Table 10. Proportion of total time (unitless mean x 10^{-3} ± SEM) male and female wolves spent proximal to wolf urine and to coyote urine, and the proportion (in parentheses) of males and females responding.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Approach</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wolf Urine</td>
<td>Coyote Urine</td>
</tr>
<tr>
<td>Male</td>
<td>10.24 ± 5.36 (20)</td>
<td>7.37 ± 2.33 (20)</td>
</tr>
<tr>
<td></td>
<td>42.55 ± 6.22 (30)</td>
<td>48.27 ± 17.55 (30)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>3.11</td>
<td>1.88</td>
</tr>
<tr>
<td>P</td>
<td>0.198</td>
<td>0.311</td>
</tr>
</tbody>
</table>

“--” indicates too few samples for statistical analysis.
CHAPTER 5

GENERAL DISCUSSION

The red wolf recovery program has celebrated many successes and achieved notable milestones over the past four decades. For example, restoring the red wolf to the wild has the distinction of representing a series of successful “firsts” in the reintroduction of a species, including of a species declared extinct in the wild, of a large predator, of a mammal from captive stock, and of a wolf. The red wolf recovery program also has had its share of challenges and setbacks. An ageing captive population with a greater proportion of individuals approaching reproductive senescence, unequal representation of founders, and spacing limitations resulting in age-related prioritizations (i.e., older animals) and fewer recommendations for breeding (Waddell and Long, 2008), as well as habitat fragmentation, gunshot and vehicle related mortality, and the continued threat of wolf-coyote hybridization in the wild (U.S. Fish and Wildlife Service, 2007), are constant reminders that recovery of the red wolf is far from realized.

In this dissertation I took a two-pronged approach to investigate factors affecting reproduction in the red wolf. In the first, I conducted a systematic, retrospective assessment of longitudinal reproductive events of captive red wolves to identify characteristics that have influenced reproductive success, performance, and fitness. I evaluated the information relevant to two general hypotheses:

(1) reproductive output declines as sire/dam inbreeding coefficient increases; and
(2) reproductive output declines as sire/dam age increases.

The results of the experiments, presented in chapters 2 and 3, provide support for these hypotheses. I show that younger, less inbred male and female wolves were more likely to reproduce, and were more likely to have larger litters, than older, inbred individuals. Measures of fitness, such as litter size and offspring survival, were generally negatively affected by inbreeding and age. However, there were no relationships between inbreeding or age and the sex ratio or rearing method of the offspring. Additional analyses also revealed that the mean level of inbreeding and age of sires and dams in the captive red wolf population have increased and that the average litter size has decreased since the inception of the captive breeding program. Moreover, prior reproductive success increased the likelihood of reproduction, but prior sexual experience alone was not a factor in the production of offspring. Prior reproductive success also negatively affected survival of offspring, but had no effect on other measures of fitness (i.e., litter size, sex ratio, or rearing method).

For the second approach, I conducted a behavioral preference study using olfactory presentations of conspecific and congeneric social odors to determine the olfactory cues that are important for the red wolf in the selection of mates. Three general hypotheses were evaluated:

(1) male and female wolves show a preference for excreta over a control;

(2) male and female wolves show a stronger preference for excreta during the breeding season than during the non-breeding season; and
(3) male and female wolves show a preference for excreta from a conspecific over that from a congener.

The results of the experiments presented in chapter 4 are inconclusive in their support for these hypotheses, but the data suggest that there are sex-specific and seasonal differences in red wolves’ responses to social odors. Males responded more quickly than did females to the social odors of females, whereas females responded more quickly than did males to the social odors of males. The time spent in proximity to the social odors, however, did not differ between the sexes. Generally males and females responded more quickly and spent more time in proximity to social odors during the non-breeding season than during the breeding season. When considering red wolf responses to the social odors of conspecifics and congeners, red wolves did not show a preference for species in the latency to respond to or the time spent in proximity of social odors.

In this dissertation I have shown that assessing the available reproductive histories of red wolves can assist in our understanding of the factors that are affecting the species’ reproduction, and can guide the development of management techniques and tools to improve reproduction. For example, the result presented here may be used when preparing annual breeding recommendations to increase breeding success while minimizing reproductive effort by pairing wolves with traits that are more likely to reproduce and maintain the demographic stability of the population. In addition, I have provided insight into the behavioral responses of red wolves to social odors of conspecifics and congeners, and how these responses differ by gender and season. This information adds to our understanding of red wolf social behaviors, and can serve as a foundation for future studies.
on the use of olfactory cues in mate choice, territorial establishment and defense, and in other wolf-wolf and wolf-coyote interactions. The information also may prove useful in improving techniques for pairing wolves for breeding and/or reintroduction in order to expand the existing wolf populations or to build future ones. For example, social odors may be used to introduce unfamiliar wolves to each other’s scent and to encourage pair bonding between a specific male and female to improve the chances for successful reproduction.

As a species, the red wolf is unique, from its taxonomy to the chronicle of its restoration. Recently in the red wolf’s story the species has progressed along the continuum of recovery from dependent (i.e., occurring only in captivity) toward an independent existence. However, we are far from meeting the objectives of the species’ recovery plan (U.S. Fish and Wildlife Service, 1989), and the threats currently faced by the red wolf are pervasive and recurrent. That is not to say the threats are unmanageable, or the species unrecoverable. Rather, recovery of the red wolf will most likely rely on continuous implementation of management actions against persistent threats to maintain a recovered status (Fazio, 2008; Scott et al., 2005). If so, the information in the present study may prove useful in identifying and implementing the conservation measures necessary for protecting, conserving, and enhancing the red wolf so that the protections of the Endangered Species Act are no longer necessary.


Recovery of imperiled species under the Endangered Species Act: the need for a

Service, Atlanta, Georgia. 110 pp.

U.S. Fish and Wildlife Service. 2007. Red wolf (Canis rufus) 5-year status review:
summary and evaluation. Red Wolf Recovery Program, Alligator River National
Wildlife Refuge, Manteo, NC. 58 p.


*Canis rufus gregoryi* Species Survival Plan®. Point Defiance Zoo & Aquarium,
Tacoma, WA. 41 p.