

# Effects of age and experience on reproductive performance of captive red wolves (*Canis rufus*)

David R. Rabon, Jr.

**Abstract:** Propagation programs contribute to the conservation of a species by preserving genetic and demographic stock that may be used to reinforce or re-establish wild populations. Identifying traits that affect reproductive success is essential to achieve this goal. Longitudinal reproductive events of the captive population of endangered red wolves (*Canis rufus* Audubon and Bachman, 1851) were investigated to determine whether parental age, breeding experience, and rearing type were factors in reproduction, litter size, and sex ratio, as well as viability of offspring. Younger wolves were more likely to reproduce and produce larger litters than were older individuals. The age of the female, but not the male, had a negative effect on pup survival. Wolves that had prior experience in offspring production were more likely to reproduce again than were individuals that had no prior reproductive success, but prior sexual experience alone was not a factor in offspring production. Parental breeding experience had a negative effect on pup survival, but no apparent relationships with litter size or sex ratio. Declines in reproduction, fitness, and survival with advancing age suggest the effect is due to senescence, the onset of which occurs at 8 years of age in females. The results are consistent with the breeding-experience hypothesis.

**Key words:** reproductive experience, breeding-experience hypothesis, senescence, age hypothesis, reproduction, red wolf, *Canis rufus*.

**Résumé :** Les programmes de propagation contribuent à la conservation des espèces en préservant des stocks génétiques et démographiques pouvant être utilisés pour renforcer ou rétablir les populations sauvages. L'identification des caractères qui ont une incidence sur le succès de reproduction est essentielle à l'atteinte de cet objectif. Les événements de reproduction longitudinaux de la population captive de loups roux (*Canis rufus* Audubon et Bachman, 1851) ont été étudiés dans le but de déterminer si l'âge des parents, leur expérience en matière de reproduction et le type d'élevage avaient une incidence sur le succès de reproduction, la taille et le rapport de masculinité de la portée, ainsi que la viabilité des louveteaux. Les jeunes loups étaient plus susceptibles de se reproduire et de produire de grandes portées que les loups plus âgés. Il y avait une corrélation négative entre l'âge de la femelle, mais pas celui du mâle, et la survie des louveteaux. Les loups ayant déjà produit une progéniture étaient plus susceptibles de se reproduire à l'avenir que les individus qui ne s'étaient pas encore reproduits avec succès, mais l'expérience sexuelle seule n'avait pas d'incidence sur la production de petits. Il y avait une corrélation négative entre l'expérience parentale de reproduction et la survie des louveteaux, mais aucun lien apparent avec la taille ou le rapport de masculinité de la portée. Des baisses de la reproduction, de l'aptitude et de la survie avec l'âge donnent à penser que cet effet est dû à la sénescence, qui commence à l'âge de huit ans chez les femelles. Ces résultats sont compatibles avec l'hypothèse de l'expérience de reproduction. [Traduit par la Rédaction]

**Mots-clés :** expérience de reproduction, hypothèse de l'expérience de reproduction, sénescence, hypothèse de l'âge, reproduction, loup roux, *Canis rufus*.

## Introduction

The red wolf (*Canis rufus* Audubon and Bachman, 1851) is a critically endangered canid (IUCN 2013) that was once widely distributed across the eastern and south-central United States (for review see Hinton et al. 2013). Hunting, aggressive predator-control programs, and habitat destruction led to the species' extirpation throughout most of its range by the early part of the twentieth century. By the early 1970s, fewer than 300 pure red wolves were believed to exist (Mech and Rausch 1975). The remaining red wolves were threatened by hybridization with coyotes (*Canis latrans* Say, 1823) and coyote-like animals (e.g., coyote-wolf hybrids, coyote-dog hybrids) expanding their range into red wolf habitats (McCarley 1962; Paradiso 1965; Paradiso and Nowak 1971). A plan to propagate the red wolf in captivity was considered the only practical means for the species' preservation when its extinction

appeared inevitable. Because coyotes and coyote-like animals had replaced many red wolf populations and threatened remaining populations with hybridization, a comprehensive species verification program was implemented; only 14 red wolves were confirmed through the program. By 1980, the red wolf was considered extirpated and biologically extinct in the wild.

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 and Foose 1996). Propagation programs for imperiled 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 ensure the species' survival in captivity and (or) for immediate release back into the wild. Long-term strategies include retaining as much of the founders' genetic diversity as

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possible and maintaining demographic stability within a captive population. Theoretical analyses suggest that 20–30 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 red wolf 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; U.S. Fish and Wildlife Service 1990). 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 species for 150 years, with a target captive population of 330 individuals. To ensure that the captive population maintains genetic diversity and demographic stability, a Population Analysis and Breeding/Transfer Plan is produced annually. 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 number of facilities housing red wolves withdrew from participating in the Red Wolf Recovery/SSP causing a decline in the number of breeding pairs formed and a sharp decrease in births (Waddell and Long 2013). The population decline 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. However, since the mid-1990s the captive population has stabilized, and in recent years the population has experienced a marked increase, in part, from 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 December 2013, the captive red wolf population consisted of 193 individuals. Current gene diversity is 89.29%, and is equivalent to a population descended from about five founders (Waddell and Long 2013). Under current conditions, gene diversity is expected to be maintained at or above 85% for at least 16 years (Waddell and Long 2013). However, inbreeding within the captive population has resulted in lower reproductive performance, smaller litter sizes, and reduced survivability of offspring (Rabon and Waddell 2010). Demographic projections to achieve the 5-year target population size (recently reduced to 200 individuals) require a 3% annual growth rate ( $\lambda = 1.03$ ), but current spatial limitations necessitate that the population maintain a 2% growth rate ( $\lambda = 1.02$ ) and approximately 26–35 births annually to offset the expected annual mortality rate (Waddell and Long 2013).

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 et al. 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 in mammals and birds (e.g., Nol and Smith 1987; Festa-Bianchet 1988; Lunn et al. 1994; Côté and Festa-Bianchet 2001; Broussard et al. 2003) 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 and Smith 1987; Broussard et al. 2003), including (i) the breeding-experience hypothesis, which predicts that experience in reproduction improves later breeding performance, and (ii) the age hypothesis, which suggests that breeding performance improves with age, and that older first-time breeders are more likely to be successful at reproduction than 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 that have evaluated both reproductive experience and age, reproductive experience contributed to the improvement of reproductive performance (e.g., Raveling 1981; Harvey et al. 1985; Wooller et al. 1990; Pyle et al. 1991; Sydesman et al. 1991; Forslund and Larsson 1992; Hepp and Kenamer 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 red wolf life history and population dynamics, and managing the species toward recovery.

## Materials and methods

As part of a larger study of traits that affect reproduction (e.g., Rabon and Waddell 2010), I examined longitudinal reproductive events of captive red wolves to determine whether parental age, reproductive experience (i.e., prior sexual experience and prior reproductive success), and parental rearing type were factors in the production of offspring. I also examined longitudinal reproductive events to test the relationships between the parental age and reproductive experience of adult males and females and the following measures of reproductive fitness: litter size; litter rearing type; sex ratio of the litter; viability of offspring. I obtained demographic data for the red wolf population (e.g., pedigrees, date and place of birth, date of death, reproductive experience, rearing method, litter size) 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 (U.S. Fish and Wildlife Service, unpublished data). Several contradictions between the studbook and the file reports on wolf demography, breeding events, and provenance were discovered. Therefore, I evaluated and reconciled inconsistencies in the data, and modified the red wolf studbook accordingly before analyzing the data.

I evaluated reproductive success based on 554 recorded pairing events from 1977 through 2006 (i.e., all pairings). I also evaluated data on reproductive success with repeated pairing events by the same male and female excluded. That is, all subsequent pairings after the initial pairing of a male and female 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. Therefore, for the purposes of

**Table 1.** (a) Unrestricted mean ( $\pm$ SE) ages (years) and (b) Red Wolf Recovery/Species Survival Plan (SSP) age-restricted mean ( $\pm$ SE) ages (years) of adult breeding male and female red wolves (*Canis rufus*) that produced offspring and failed to reproduce for all pairing events and when repeat pairing events of the adult breeding male and female were excluded.

(a) Unrestricted age range of males (0–15 years) and females (0–14 years).						
	All pairings ( $n = 554$ )			No repeat pairings ( $n = 355$ )		
	Reproduction	No reproduction	$p$	Reproduction	No reproduction	$p$
Males	4.682 $\pm$ 0.193	5.923 $\pm$ 0.181	0.0001	4.592 $\pm$ 0.234	5.436 $\pm$ 0.232	0.0175
Females	4.500 $\pm$ 0.178	6.218 $\pm$ 0.181	<0.0001	4.208 $\pm$ 0.226	5.667 $\pm$ 0.229	<0.0001
$n$	192	362		130	225	

(b) Red Wolf Recovery/SSP age-restricted range of males (1–12 years) and females (1–10 years).						
	All pairings ( $n = 498$ )			No repeat pairings ( $n = 324$ )		
	Reproduction	No reproduction	$p$	Reproduction	No reproduction	$p$
Males	4.679 $\pm$ 0.195	5.821 $\pm$ 0.188	<0.0001	4.500 $\pm$ 0.236	5.309 $\pm$ 0.237	0.0206
Females	4.547 $\pm$ 0.177	5.653 $\pm$ 0.171	<0.0001	4.277 $\pm$ 0.221	5.335 $\pm$ 0.223	0.0014
$n$	190	308		130	194	

analyses I considered wild-caught wolves ( $n = 14$ ) to be at least 1 year of age if captured before 1 June and young of the year (i.e., less than 1 year of age) if captured after 1 June. I considered wolves that were younger than 1 year old when paired for breeding age 0 for the purposes of analyses. I recorded the age of wolves at their time of pairing in whole-year increments (e.g., 5 years old, not 5.8 years old); wolf pairs maintained for subsequent years are accordingly age-adjusted.

I categorized the reproductive experiences of individual wolves according to their 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. I categorized wolves that had been previously paired but with no offspring produced as having had prior sexual experience, but not reproductive success. I classified wild-caught wolves as unknown and excluded them from analysis of reproductive experience, unless otherwise noted, because their sexual and reproductive history was uncertain.

I categorized rearing type of breeding adult males and females into two groups: the “parent” group if reared by a parent (males,  $n = 331$ ; females,  $n = 321$ ) or the “hand” group if reared by a human (males,  $n = 20$ ; females,  $n = 30$ ). I excluded foster-reared males and females from the analyses of parental rearing type because there were too few individuals (males,  $n = 1$ ; females,  $n = 3$ ). I included wild-caught wolves in the “parent” group for analysis because wild-caught wolves were most likely reared primarily by their parents.

I calculated the sex ratio of the litter at birth (i.e., number of males and females produced in a litter and the proportion of males and females produced in a litter) and at two subsequent benchmark ages (i.e., pups aged 3 and 12 months). I also calculated viability of pups when the pups were 3 and 12 months of age. In calculating survival, I considered pups that were listed as “lost to follow-up” before 12 months of age in the studbook ( $n = 10$ ) to have died.

I categorized the rearing type of offspring into three groups: “parent” group ( $n = 169$ ) contained litters in which all pups were reared by their parents; “hand” group ( $n = 12$ ) contained litters in which at least one pup was reared by hand; “foster” group ( $n = 11$ ) contained litters in which at least one pup was reared by foster parents. I excluded fostered litters 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).

I conducted statistical analyses using JMP® version 7.0 (SAS Institute Inc., Cary, North Carolina, USA). To determine if parental age influenced the production of offspring, I evaluated reproduc-

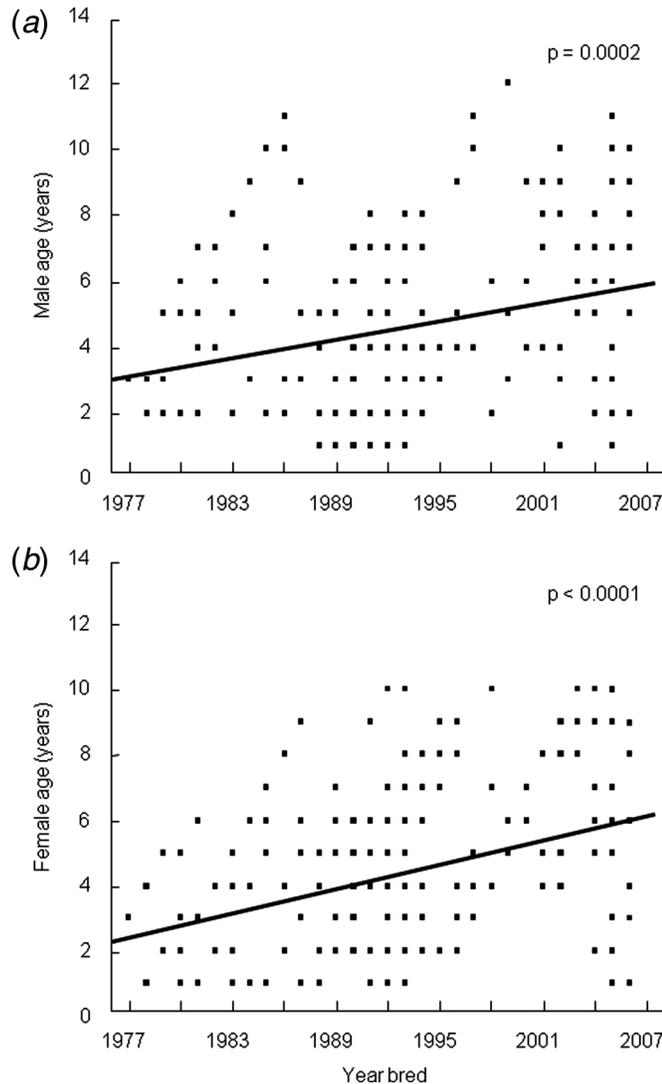
tion as a function of the ages of the adult breeding males and females on actual and predicted litters using nominal logistic regression. To determine if parental experience was a factor in the production of offspring, I evaluated reproduction as a function of (i) prior sexual experience and reproductive success of the adult breeding male and female and (ii) the rearing type of the adult breeding male and female on actual and predicted litters using Pearson’s  $\chi^2$  test. To determine whether parental age and reproductive experience were factors in breeding performance, I evaluated performance as a function of the parents’ age and experience on litter size, litter rearing type, sex ratio of the litter, and viability of offspring. I evaluated relationships between offspring viability and parental age and experience using generalized linear modeling techniques with a binomial distribution and logit-link model. I used generalized linear modeling techniques with a Poisson distribution, identity-link model, and an overdispersion parameter ( $\hat{c}$ ) to test for a relationship between litter size and parental age and reproductive experience. I used least-squares regression to evaluate changes in age over time and the effects of parental age and experience on the sex ratio of litters. I compared the means of groups using Student’s  $t$  test or Wilcoxon–Mann–Whitney test, as appropriate. Statistical significance was set at  $p \leq 0.05$ .

## Results

A total of 192 litters (range = 1–9 pups per litter; litter size = 4.15  $\pm$  0.14, mean  $\pm$  SE) consisting of 797 offspring (359 males, 406 females, 32 unknowns) were produced between 1977 and 2006. Males as young as 1.8 years of age sired offspring, and females first whelped at 11 months of age. The oldest captive-born male known to have sired offspring was 12.8 years of age. Several captive-born female red wolves 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 and Long 2013).

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 ( $\pm$ SE) ages of males and females paired for breeding were 5.49  $\pm$  0.14 years (range = <1 to 15 years) and 5.62  $\pm$  0.14 years (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 1a). A similar pattern was observed when pairing events were restricted to the

**Fig. 1.** Mean ages of adult (a) males and (b) females at time of pairing each year for the captive red wolf (*Canis rufus*) population ( $n = 192$ ). Fitted line is based on least-squares linear regression.



Red Wolf Recovery/SSP recommended age range for breeding males (i.e., 1–12 years of age) and females (i.e., 1–10 years of age) for all pairing events and when repeated pairing events were excluded (Table 1b). The mean ages of breeding males and females have significantly increased over time (Figs. 1a, 1b).

Prior sexual experience of the breeding male ( $\chi^2_{[1]} = 0.419$ ,  $p = 0.517$ ) or female ( $\chi^2_{[1]} = 0.588$ ,  $p = 0.443$ ) was not a factor in the production of offspring ( $n = 542$ , no unknowns). However, prior reproductive success was important. Both adult breeding males ( $\chi^2_{[1]} = 25.799$ ,  $p < 0.0001$ ) and females ( $\chi^2_{[1]} = 7.415$ ,  $p = 0.007$ ) 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 males (prior sexual experience,  $\chi^2_{[1]} = 1.557$ ,  $p = 0.212$ ; prior reproductive success,  $\chi^2_{[1]} = 11.367$ ,  $p = 0.0007$ ;  $n = 343$ ), but not for females (prior sexual experience,  $\chi^2_{[1]} = 0.355$ ,  $p = 0.551$ ; prior reproductive success,  $\chi^2_{[1]} = 0.537$ ,  $p = 0.464$ ;  $n = 343$ ).

After statistically accounting for age using nominal logistic regression, the likelihood of producing offspring significantly increased with prior reproductive success, but not prior sexual experience, both in adult breeding males ( $\chi^2_{[1]} = 22.814$ ,  $p < 0.0001$ ) and females ( $\chi^2_{[1]} = 11.184$ ,  $p = 0.0008$ ). Likewise, after accounting for

prior reproductive success, younger breeding males ( $\chi^2_{[1]} = 20.892$ ,  $p < 0.0001$ ) and females ( $\chi^2_{[1]} = 29.555$ ,  $p < 0.0001$ ) had a significantly greater likelihood of producing offspring than did older breeding males and females.

Rearing type (i.e., parent, hand) of adult breeding males ( $\chi^2_{[1]} = 0.666$ ,  $p = 0.414$ ) and females ( $\chi^2_{[1]} = 1.473$ ,  $p = 0.225$ ) was not a factor in the production of offspring ( $n = 351$ ; no repeat pairings), although there was a slight trend that adult males and females that were parent-reared were more likely to produce offspring than were hand-reared adult males and females.

The following results are calculated using only pairs that successfully reproduced offspring, unless otherwise noted.

Older breeding males ( $\chi^2_{[1]} = 3.904$ ,  $\hat{c} = 0.943$ ,  $p = 0.048$ ) and females ( $\chi^2_{[1]} = 4.500$ ,  $\hat{c} = 0.946$ ,  $p = 0.034$ ) produced smaller litters, but the trend was not significant (males,  $\chi^2_{[1]} = 1.774$ ,  $\hat{c} = 0.990$ ,  $p = 0.183$ ; females,  $\chi^2_{[1]} = 3.758$ ,  $\hat{c} = 0.981$ ,  $p = 0.053$ ) when repeated pairings were excluded from the analyses ( $n = 152$  pairing events). When litter size was measured as a function of parental reproductive experience (no unknowns), there were no relationships between litter size and prior sexual experience of breeding males ( $\chi^2_{[1]} = 0.118$ ,  $\hat{c} = 0.974$ ,  $p = 0.731$ ,  $n = 186$ ) or females ( $\chi^2_{[1]} = 0.052$ ,  $\hat{c} = 0.975$ ,  $p = 0.819$ ,  $n = 189$ ). Furthermore, there was no relationship between litter size and prior reproductive success of breeding males ( $\chi^2_{[1]} = 0.038$ ,  $\hat{c} = 0.977$ ,  $p = 0.847$ ,  $n = 186$ ) or females ( $\chi^2_{[1]} = 0.349$ ,  $\hat{c} = 0.973$ ,  $p = 0.555$ ,  $n = 189$ ).

Parental age (Table 2a) and prior reproductive experience (Table 3a) had no apparent effect on the proportion of male pups, female pups, or pups of an undetermined sex in a litter at birth. Similar trends occurred when repeated pairings were excluded from the parental age analyses (Table 2a).

There was a significant negative relationship between the breeding female's age and pup survival at 3 months of age, but no other relationships between parental age and pup survival were apparent (Figs. 2a, 2b; Table 2b). When offspring survival was measured as a function of parental experience (no unknowns), there was a negative relationship between the male's prior reproductive experience and the pup survival at 3 and 12 months of age (Table 3b). A similar relationship was found between the female's prior reproductive experience and the pup survival at 3 and 12 months of age (Table 3b). There was no relationship between parental age and rearing type of their offspring (Table 4).

## Discussion

The Red Wolf Recovery/SSP has contributed to the genetic and demographic conservation of the red wolf. The restoration of the wolf to the wild less than 10 years after the species was declared extinct in the wild is a testament to the effectiveness of the short-term strategy to rapidly multiply the population in captivity for the purposes of re-establishing a wild population. Accomplishing the long-term strategy of retaining the founders' genetic diversity and maintaining demographic stability within the captive population is realized over time. Current population demographics suggest that the captive population can persist and accomplish its genetic-retention goal (Waddell and Long 2013). However, a fundamental component to achieving this goal is the ability of the Red Wolf Recovery/SSP to adjust for the traits, such as parental age or reproductive experience, that affect the reproductive success of individuals in the managed population.

The recorded range in age for successful reproduction in captivity in both male and female red wolves is similar to that of the wild population. However, reproduction in individuals less than 2 years of age is uncommon in both populations. In males, reproduction at less than 2 years of age has only been recorded in the wild (Phillips et al. 2003; U.S. Fish and Wildlife Service, unpublished data), whereas successful reproduction in females at less than 1 year of age has only been recorded in captivity (this paper). The upper age limit for successful reproduction in captivity ex-

**Table 2.** Relationships between parental age of red wolves (*Canis rufus*) and (a) proportion of pups in a litter at birth and (b) offspring survival of pups at 3 and 12 months of age for all pairings resulting in reproduction and when repeat pairing events of a breeding male and female were excluded.

(a) Proportion of pups in a litter at birth.												
	All pairings						No repeat pairings					
	Sires			Dams			Sires			Dams		
	F	df	p	F	df	p	F	df	p	F	df	p
Males	0.004	1	0.947	0.034	1	0.854	0.428	1	0.514	1.559	1	0.214
Females	1.037	1	0.309	0.079	1	0.306	0.011	1	0.917	0.002	1	0.963
Unknown	2.751	1	0.099	1.652	1	0.200	1.411	1	0.237	3.569	1	0.061
n	186			189			152			152		

(b) Offspring survival of pups at 3 and 12 months of age.												
	All pairings						No repeat pairings					
	Sires			Dams			Sires			Dams		
	$\chi^2$	df	p	$\chi^2$	df	p	$\chi^2$	df	p	$\chi^2$	df	p
3 months	0.468	1	0.494	7.776	1	0.005	0.014	1	0.906	2.337	1	0.126
12 months	0.111	1	0.739	0.321	1	0.571	0.559	1	0.455	0.380	1	0.538
n	186			189			152			152		

**Table 3.** Relationships between parental reproductive experience (i.e., prior sexual experience only or prior reproductive success) of red wolves (*Canis rufus*) and (a) proportion of pups in a litter at birth and (b) offspring survival of pups at 3 and 12 months of age.

(a) Proportion of pups in a litter at birth.												
	Sexual experience only						Reproductive success					
	Sires			Dams			Sires			Dams		
	F	df	p	F	df	p	F	df	p	F	df	p
Males	1.897	1	0.170	0.396	1	0.529	1.717	1	0.192	0.058	1	0.810
Females	1.097	1	0.296	0.587	1	0.445	0.772	1	0.381	0.008	1	0.929
Unknown	0.254	1	0.615	0.059	1	0.809	0.445	1	0.506	0.306	1	0.581
n	186			189			186			189		

(b) Offspring survival of pups at 3 and 12 months of age.												
	Sexual experience only						Reproductive success					
	Sires			Dams			Sires			Dams		
	$\chi^2$	df	p	$\chi^2$	df	p	$\chi^2$	df	p	$\chi^2$	df	p
3 months	10.392	1	0.001	10.655	1	0.001	3.881	1	0.049	9.578	1	0.002
12 months	7.711	1	0.006	1.475	1	0.225	7.650	1	0.006	6.963	1	0.008
n	186			189			186			189		

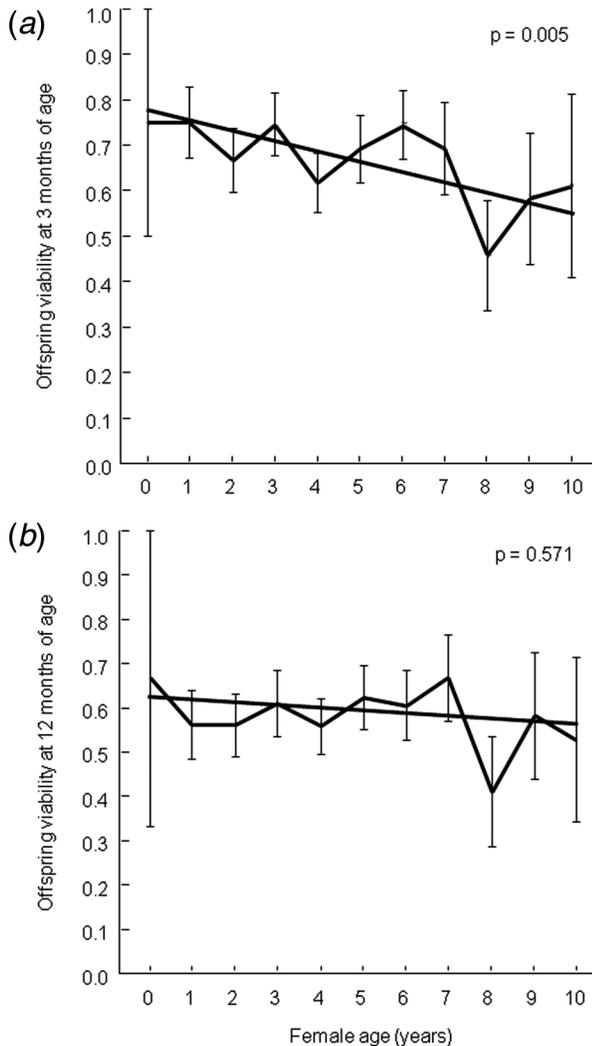
tends a few years beyond those ages recorded for wild-born wolves, at least for males. The oldest captive-born, male red wolf known to have successfully bred was 12.8 years of age when the pups were sired, while the oldest known wild-born males have sired pups at 9.8 years of age (U.S. Fish and Wildlife Service, unpublished data). Both captive-born and wild-born female wolves have whelped litters when they were 11 years of age, though 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; U.S. Fish and Wildlife Service, unpublished data), 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 gray wolves (*Canis lupus* L., 1758) and coyotes. In captivity, male and female gray wolves have bred as young as 10 months of age (Medjo and 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 is sparse. Gese (1990) reported that the behavior of an estimated-age male coyote associating with an estrus female, and 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 9 years of age (e.g., Windberg 1995; Green et al. 2002). The maximum breeding age of wild gray wolves also is not well known, but Mech (1988) reported reproduction in a female of at least 10 years of age.

The effects of parental age and reproductive 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 and Larsson 1992). In the present study, parental age and reproductive experience of male and female red wolves were positive

**Fig. 2.** Mean ( $\pm$ SE) age of adult breeding female red wolf (*Canis rufus*) plotted against offspring viability at (a) 3 months of age and (b) 12 months of age. Fitted line is based on least-squares regression.



factors in the production of offspring, and the effects were independent of one another in successful reproduction. Younger males and females were more likely to reproduce than were older individuals, and litter size tended to decrease with increasing age of the breeding males and females. There also was a trend for survival of offspring to be affected by increasing maternal age, but the effect was limited, in part, by multiple reproductive events. Conversely, the reproductive experience of the parents appeared to have a greater negative affect on the survival of offspring. Other relationships between reproductive experience or parental age and reproductive performance and fitness were not apparent.

Of the hypotheses put forward to explain positive correlations of parental age and reproductive experience on reproduction, the results in the present study are most consistent with the breeding-experience hypothesis. However, the cumulative benefits of reproductive experience on reproductive success in the red wolf are unknown. That is, multiple years of reproductive experience may offer no advantage over inexperienced individuals. For example, Sydeman et al. (1991) reported that reproductive experience in younger female northern elephant seals (*Mirounga angustirostris* (Gill, 1866)) was beneficial for weaning success, but that experience was detrimental to weaning success later in life. Moreover, the advantages of multiple years of reproductive experience on reproductive success may be sex-specific. Ollason and Dunnet

(1978) reported that male Northern Fulmars (*Fulmarus glacialis* (L., 1761)) show a general improvement in reproductive success with reproductive experience, whereas female Northern Fulmars had lower reproductive success than did males with similar reproductive experience in early and later years.

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 parental age and reproductive experience on reproductive success have been reported in studies investigating both. In the Antarctic Fulmar (*Fulmarus glacialis* (A. Smith, 1840)), reproductive experience positively contributed to reproductive success after the first reproduction, but parental age had no significant effect (Weimerskirch 1990). Similarly, in a study of three species of European ducks, reproductive performance varied with parental age and reproductive experience, but the effects of age were explained more by the covariates female body mass and nesting date (Blums et al. 1997). Experimental manipulation of reproductive experience or the analysis of other age-related improvements in reproduction may be required to understand age-specific variation in reproductive success in the red wolf (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 and 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 results presented here are the first investigating the relationships between parental age, reproductive 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 et al. 2009). However, the accuracy of the data set used in the previous study has been questioned (Rabon and Waddell 2010), and relatively few results of the effects of age on reproduction are similar between the two studies. For example, Lockyear et al. (2009) reported that reproductive success was negatively correlated only with female age. They also reported no effect of the age of either the male or the female on litter size, but found pup survival was reduced with increasing age of the females. In the present study, breeding success was negatively affected by age of both males and females, and litter size significantly declined with increasing age of the female. Indeed, females older than 7 years of age had significantly smaller litter sizes when compared with the litter sizes of younger females ( $p = 0.004$ ; Figs. 3a, 3b). This result suggests that female red wolves show signs of reproductive senescence at a younger age than previously reported. Although the results of parental age on pup viability are inconclusive, there appear to be similar trends in their effects between the two studies.

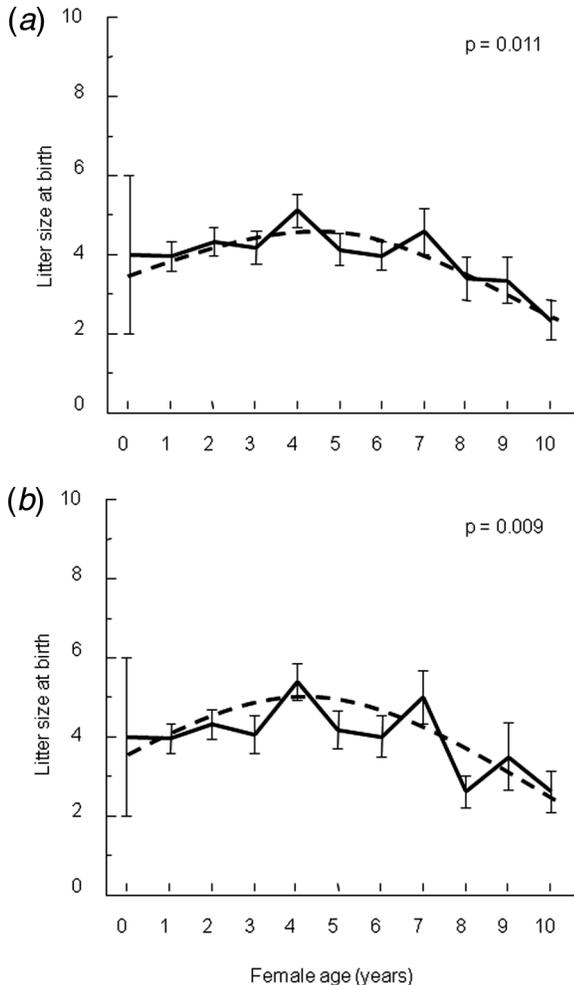
Differences in the results between the two studies may be explained by differences in the data sets. Lockyear et al. (2009) reported only 165 litters born in captivity compared with 183 litters identified during the same period (i.e., 1977–2005) in my data set. Furthermore, Lockyear et al. (2009) conducted some of the analyses using data restricted to breeding events from 1992 through 2005. The failure to include data from all litters born in the propagation program in the analyses may have affected the results. Unfortunately, a complete comparison of the data sets representative of the period(s) used by Lockyear et al. (2009) 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.

In the current study, the effects of parental age and reproductive experience were evaluated on multiple aspects of fitness in the captive red wolf population. Understanding the factors influencing reproductive performance is important for managing and conserving the species both in captivity and in the wild. Nol

**Table 4.** Mean ( $\pm$ SE) ages (years) of adult breeding male and female red wolves (*Canis rufus*) by the rearing type of their offspring for all pairing events and when repeat pairing events of a breeding male and female were excluded.

	All pairings			No repeat pairings		
	Parent-reared litters	Hand-reared litters	<i>p</i>	Parent-reared litters	Hand-reared litters	<i>p</i>
Males	4.550 $\pm$ 0.200	5.500 $\pm$ 0.933	0.320	4.410 $\pm$ 0.223	5.222 $\pm$ 1.199	0.583
Females	4.497 $\pm$ 0.189	5.250 $\pm$ 0.789	0.349	4.254 $\pm$ 0.216	4.778 $\pm$ 0.954	0.609
<i>n</i>	169	12		134	9	

**Fig. 3.** Mean ( $\pm$ SE) age of adult breeding female red wolf (*Canis rufus*) plotted against litter sizes at birth for (a) all pairings ( $n = 192$ ) and (b) when repeated pairings were excluded ( $n = 152$ ). Quadratic (broken lines) and *p* values are based on least-squares regression.



and Smith (1987) cautioned that the detection of effects of age and reproductive experience could be affected by the relative quality of environment conditions, including population density and the availability of resources. Although 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 factors for the captive wolves. In addition, differences in geography and enclosure do not appear to affect the likelihood of successful reproduction (D. 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 reproductive suc-

cess 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 or the proximity of other breeding pairs may prove important to 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.

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