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Retrospective Investigation of Captive Red Wolf Reproductive Success in Relation to Age and Inbreeding

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The critically endangered red wolf (Canis rufus) has been subject to a strictly managed captive breeding program for three decades. A retrospective demographic analysis of the captive population was performed based on data from the red wolf studbook. Data analyses revealed a decrease in the effective population size relative to the total population size, and changes in age structure and inbreeding coefficients over time. To varying degrees, the probability of successful breeding and litter sizes declined in association with increasing dam age and sire inbreeding coefficients. Neonate survival also declined with increasing dam age. Recent changes in strategies regarding breed-pair recommendations have resulted in moderate increases in reproductive success. Zoo Biol 28:214–229, 2009.

Keywords: canid; captive breeding; genetic diversity; population size

INTRODUCTION

The maintenance of genetic diversity and demographic security are usually primary population management goals of captive breeding programs and long-term conservation plans [Ballou and Foose, 1996]. Loss of genetic diversity is a function...
of population size and time: the smaller the population, the faster the loss of genetic diversity over time. However, the population size of relevance is not the absolute census but the genetically effective population (\(N_e\)), that is, actively breeding animals. Because the \(N_e\) is smaller than the total population, captive populations should be as large as possible to ensure long-term sustainability [Ballou and Foose, 1996; Hedrick, 1983; Lande and Barrowclough, 1987]. However, finite availability of captive resources strictly limits practical population sizes. In most captive conservation programs, optimal projected growth rates are designed to combine the maintenance of genetic diversity with the achievement of suitable population sizes within a reasonable amount of time [Ballou and Foose, 1996; Soulé, 1980].

Having suffered nearly complete extirpation from habitat loss, government-sponsored control programs, and the invasion of coyotes (\textit{Canis latrans}) leading to increased competition as well as extensive hybridization [Burdick et al., 1989; Nowak, 1972; Paradiso and Nowak, 1972; Pimlott and Joslin, 1968], the red wolf (\textit{C. rufus}) has been listed as endangered since the inception of the U.S. Endangered Species Act in 1973 [Hilton-Taylor, 2000; USFWS, 1989]. A captive-breeding population was established at Point Defiance Zoo & Aquarium (PDZA) in Tacoma, Washington in 1973. The Red Wolf Recovery Plan, administered by the United States Fish and Wildlife Service (USFWS), was combined with a zoo-based Species Survival Plan (SSP\textsuperscript{®}) in 1989 under the auspices of the Association of Zoos & Aquariums (AZA).

The captive red wolf population has been managed according to the goals of reintroducing individuals into selected locations within their historical range and long-term conservation through the maintenance of a captive population. Although some population interaction has occurred between the captive and reintroduced populations via island propagation sites and cross-fostering, they are physically independent units. The management strategy for the captive red wolf is to maximize genetic diversity while maintaining a demographically stable population within the limits imposed by the carrying capacity of available captive environments. To this end, the red wolf SSP and the AZA Canid Taxon Advisory Group has identified the goal of maintaining a captive population of 250 animals retaining 90% of the founders’ genetic diversity over a period of 20 years [Long and Waddell, 2005].

The data contained within studbooks are essential to monitor a population’s status, allowing informed decisions for breeding to maximize genetic diversity and promote demographic stability. Information contained within the red wolf studbook at the time of this study includes statistics for all animals from the first individual captured in 1969 through August 2005. An updated studbook is published annually, although pertinent events are recorded throughout the year. In addition to the information described above, the red wolf studbook contains a designation for each animal as “hybrid” (red wolf × coyote), “not hybrid”, or “unknown”, and “captive” or “free-ranging”.

The active management plan for the free-ranging red wolf population includes monitoring, identification of wolves via genetic, pedigree and morphometric assessment, and exclusion of coyotes and hybrids from red wolf home ranges within the experimental population area, but does not involve control of breeding except in cases of interspecific pairs [Adams et al., 2003; Miller et al., 2003; Stoskopf et al., 2005]. Whereas most of the free-ranging wolves are wild-born, captive-born pups are
occasionally cross-fostered into the population in the interests of supporting genetic diversity and demographic recruitment [Waddell et al., 2002].

Breeding recommendations for the captive red wolf population are made on an annual basis and are currently designed to achieve a 3% annual growth rate. The number of pairing recommendations made per year for the captive red wolf population is determined based on an approximate probability of 25% of pairs producing litters, an average litter size of four, and the amount of available housing spaces for new individuals [Long and Waddell, 2005]. Annual breeding-pair recommendations are based primarily on genetic considerations such as relative mean kinships, avoidance of inbreeding, and avoidance of linking rare with common lineages [Long and Waddell, 2005] with secondary consideration given to other demographic factors such as individual age and/or reproductive status [W. Waddell, K. Goodrowe, and R. Fulk, personal communication]. They are made with two measures in mind: mean kinship values and inbreeding coefficients ($f$).

Although studies involving long-term data for captive wildlife are limited, it is known that both age [Chen et al., 1994; James and Heywood, 1979; Lowseth et al., 1990; Peters et al., 2000a,b] and inbreeding [Charlesworth and Charlesworth, 1987; Day et al., 2003; Kosowska and Zdrojewicz, 1996a,b; Wildt et al., 1982] can affect reproduction. Among canids, reproductive senescence has been demonstrated in captive coyotes ($C. latrans$) [Green et al., 2002; Windberg, 1995], and inbreeding depression has been reported in the Mexican gray wolf ($C. lupus baileyi$) [Fredrickson and Hedrick, 2002], domestic dog [Wildt et al., 1982], and in animals with inbreeding coefficients above 0.125. Recently, in response to falling population growth rates, the minimal acceptable genetic diversity of 90% for the captive red wolf population was amended to 80–85%, allowing for an increase in the number of pairings recommended [Long and Waddell, 2005]. The red wolf SSP has identified 13 years as the maximal age for breeding males and 11 years as the maximal age for breeding female red wolves. As of March 24, 2008, the captive population numbered 193 individuals ($\delta$, $\varphi$, unknown = 85.108.0) spread across 41 institutions [Waddell, 2008].

This study is a retrospective demographic analysis of reproduction within the captive red wolf population. The purpose of this study was to investigate changes in inbreeding coefficients and age structure of the captive red wolf population over time and how these changes may be affecting the reproductive potential of the population. It is anticipated that information generated by this study can be applied to breeding decisions by the red wolf SSP to improve the management of the captive red wolf population.

**METHODS**

**Generation of Data**

Data pertaining to both the captive and current free-ranging red wolf populations (parent ages, population age structure, litter size, and family size) were derived from the red wolf studbook [Waddell, 2005]. The first record of a captive-born F1 generation was 1977, thus data for all analyses using studbook information are from 1977 through August 2005. The studbook contains data on 1257 (592.620.45) red wolves during this period, excluding animals identified as hybrids...
or of unknown taxonomy. Breeding success was classified in a binary manner according to the production or absence of a litter. Evaluations of breeding success within the captive population were based on records of pairing attempts made from 1992 through 2005, pertaining to 756 red wolves (378.378.0) [K. Goodrowe, unpublished data]. Inbreeding coefficients ($f$) were calculated for each animal using Single Population Analysis and Record Keeping System (SPARKS) v1.52.

### Comparison of Effective and Actual Population Sizes

The effective population size ($N_e$) of the captive red wolf population was considered as the total number of animals of breeding age, regardless of selection for or exclusion from breeding in any given year. To gauge changes in the effective population size over time, $N_e$ were calculated for the captive red wolf population at evenly spaced, discrete intervals over the history of the red wolf breeding program using a method developed by Lande and Barrowclough [1987] and described by Ballou and Foose [1996]:

$$N_e = \frac{4N_e(m)N_e(f)}{N_e(m) + N_e(f)}$$

where $N_e(m)$ and $N_e(f)$ respectively are the effective numbers of males and females in the population for each year in question, respectively. $N_e(m)$ and $N_e(f)$ incorporate the mean family size or lifetime reproductive fitness ($k$) and the variance in family size ($V_k$) and are calculated as follows:

$$N_e(m) = \frac{N_m * k_m - 1}{[k_m + (V_{km}/k_m) - 1]}$$

$$N_e(f) = \frac{N_f * k_f - 1}{[k_f + (V_{kf}/k_f) - 1]}$$

$N_m$ and $N_f$ represent the total number of adult males and females ($\geq 1$ year of age) in the population, respectively. The mean family size represents the mean total lifetime number of offspring surviving to adulthood per individual, including values of zero from animals that failed to breed. Mean family size and variance in mean family size were calculated separately for each year in question. Variance was calculated as the sum of squares of the difference between each individual family size and mean family size divided by the total number of males or females in the population, respectively. 1990 was chosen as the first year for evaluation of effective population sizes to allow for the first generation of captive pups (born in 1977) to complete their reproductive lifespan. Mean family size and variance calculations excluded family size data for males that were $<13$ and females that were $<11$ years old by the year 2005, as these animals were still in the active breeding population.

### Data Analyses

Data are reported as means $\pm$ standard error of the mean (SEM), unless otherwise specified. Data are based on the total population information (captive + free-ranging), or subsets representing: (1) the captive population, (2) the captive population at different times reflecting differing management strategies, or (3) the free-ranging population, as specified. Means of groups were compared using Student’s $t$-tests or Mann–Whitney Rank Sum tests as appropriate. Associative relationships with age, inbreeding coefficient and/or time were evaluated using Pearson Product Moment correlation or simple and/or multiple linear regression.
techniques. Because breeding success was a binary measure, regressions were evaluated using a binary logistic technique.

RESULTS

Changes in Population Demographics

The captive population of red wolves grew steadily until the early to mid-1990s, when space limitations necessitated a partial and temporary moratorium on breeding. Differences in demographic characteristics spanning that period are presented in Table 1. Since 1990, although the population has grown in absolute terms, the effective population size has been declining, showing significant decline from 1990 to 2000 \((P<0.05)\) and holding steady from 2000 to 2005 (Fig. 1a). Family size has also declined over time for both males and females \((\bar{c}: P=0.057, \bar{f}: P<0.05)\) (Fig. 1b).

One result of the reduced breeding during the 1990s was the disruption of the age distribution within the population. For the first 15 years of the breeding program, the captive red wolf population maintained a pyramid age distribution with older cohorts appearing over time (Fig. 2a). After the breeding restrictions, the pyramid distribution gradually became skewed towards older cohorts (Fig. 2b). In contrast, the free-ranging population, which was established during the late 1980s and early 1990s, has continued to maintain a pyramidal age structure with the majority of individuals occupying younger cohorts and progressively fewer individuals in each older cohort (Fig. 2c).

Mean inbreeding coefficients of captive red wolves have increased over the course of the captive-breeding program \((P<0.001)\) (Fig. 3a). Mean inbreeding coefficients within the free-ranging population from 1990 to 2005 have actually decreased \((P<0.001)\), although the means and ranges are greater than those found within the captive population (Fig. 3b).

Breeding Success

Since 1992, there have been 365 red wolf breeding pairs in zoological facilities. Within that group, the proportion of pairings resulting in litters per year has declined over time \((P<0.05)\). However, breeding success since 1992 has not followed a strictly

<table>
<thead>
<tr>
<th>Year</th>
<th>Sex</th>
<th>N</th>
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<th>Vk</th>
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<td>1990</td>
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<td>4.5±0.7 (0–15)</td>
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<td>26.4</td>
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<td>M</td>
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<td>39.9</td>
<td>0.35</td>
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<td></td>
<td>F</td>
<td>106/100</td>
<td>2.6±0.4 (0–15)</td>
<td>18.1</td>
<td>32.0</td>
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<tr>
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<td>M</td>
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<td>39.4</td>
<td>0.31</td>
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<td></td>
<td>F</td>
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<td></td>
</tr>
<tr>
<td>2005</td>
<td>M</td>
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<td>2.1±0.9 (0–7)</td>
<td>8.5</td>
<td>30.7</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
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<td>1.2±0.4 (0–11)</td>
<td>5.5</td>
<td>21.4</td>
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</tr>
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linear pattern. Although overall mean breeding success was $25 \pm 2.4\%$, the years 1992 and 1993 yielded exceptional success ($43 \pm 0.2\%$). When 1992 and 1993 were excluded from the analysis, there was no significant decline in the probability of breeding success over time ($P = 0.84$). Thus, breeding success has not declined appreciably since 1994. It is notable that the mean proportion of successful breedings from 1994 to 2004 was $21.6 \pm 1.4\%$. However, in 2005, when a larger proportion of younger animals were paired, $27.7\%$ of recommended pairings were successful.

As the age structure of the population has changed, so have the yearly mean (Fig. 4a) and modal (Fig. 4b) ages of paired males and females increased (means; $P < 0.01$, modes; $P < 0.001$), with a decrease in 2005 due to the changes in management described above. The yearly mean ages of paired individuals increased steadily from 1992 to 1996 in the case of females ($P < 0.001$) and from 1992 to 1997 in the case of males ($P < 0.001$), remaining stable thereafter (Fig. 4b). A significant jump in yearly mean ages occurred in both males and females between 1995 and 1996 ($P < 0.05$ respectively).

Breeding success was negatively correlated with the modal age of females ($r = -0.60$, $P < 0.05$), though not with males. However, regression analysis revealed a slight decline in breeding success over the entire period from 1992 to 2005 with increasing male age ($P < 0.05$) and to a somewhat greater extent with increasing female age ($P < 0.001$). Prior to 1996, neither female age nor male age correlated with

![Fig. 1. (a) Number of animals in the actual captive red wolf population compared with the effective population over time. (b) Changes in the mean family size ($k$) for males and females from 1990 to 2005. Regression lines indicate decline ($P = 0.057$) for males and significant decline ($P < 0.05$) for females, respectively.](image)
breeding success. However, from 1996 to 2005, breeding success declined considerably with increasing ages of males and females, with female age exerting the greatest effect ($P < 0.001$). Recall that recent policy restrictions exclude males 13 years old and beyond and females 11 years old and beyond from pairing recommendations. Within males aged 1–12 years, there was a nonsignificant trend.

Fig. 2. (a) Male and female age structures for the 1980, 1985, and 1990 captive (C) red wolf, prior to the partial breeding moratorium imposed during the 1990s; (b) Male and female age structures for the 1995, 2000, and 2005 captive red wolf, after undergoing a partial breeding moratorium; and (c) Male and female age structures for the 1995, 2000, and 2005 free-ranging (FR) red wolf population.
for breeding success to decrease as age increases ($n = 349, P = 0.08$). Within females aged 2–10 years ($n = 294$), age showed no particular association with breeding success.

Because breeding pairs are artificially selected in the captive population but not for free-ranging red wolves, it may be useful to compare the two populations. Although data are not available for free-ranging paired wolves that failed to reproduce, the overall mean ages of captive sires and dams ($6.1 \pm 0.2$ and $5.8 \pm 0.2$, respectively) were greater than those of their free-ranging counterparts ($4.5 \pm 0.2$ and $4.6 \pm 0.2; P < 0.05$ and $0.001$, respectively). Between the two populations, litter production was distributed across age groups in similar patterns for sires and a somewhat less similar pattern for dams ($r = 0.86$ and $0.64, P < 0.001$ and $0.01$, respectively), with some notable differences. Younger age groups accounted for a greater proportion of successful breedings in the free-ranging red wolf population than the captive population, and the numbers of litter-producing breedings in older age groups declined more steeply in the free-ranging population (Fig. 5).

The mean coefficients of inbreeding for captive animals were $0.041 \pm 0.002$ for males and $0.037 \pm 0.002$ for females (ranges $0–0.135$ respectively). Within the captive population, breeding success declined significantly with increasing $f$ in males ($P < 0.05$), but was not associated with female $f$ either independently or in combination with male $f$. Furthermore, in males, the effect of higher $f$ on the probability of breeding success was intensified as male age increased ($P < 0.01$, Fig. 6).
Litter Size

The mean litter size of all (captive and wild) red wolf litters born between 1977 and 2005 \((n = 291)\) was \(3.9 \pm 0.1\) pups (range 1–11). Although litter size has decreased slightly over time \((P < 0.01, \text{Fig. 7})\), no significant effect of either sire or dam age was evident for these data. However, there were differences between the captive and free-ranging populations \((n = 165\) and 126 respectively). Mean captive litter size tended to be lower than free-ranging litter size \((3.6 \pm 0.2\) and \(4.1 \pm 0.2\) respectively, \(P = 0.054)\). Neither sire nor dam age significantly affected litter size within the captive population, however within the free-ranging population, litter size decreased as dam age increased \((P < 0.001)\).

The specific relationship of dam age to litter size was more complicated than the overall trends implied, however. There was a nonsignificant trend for litter size to increase as dam age increased up to 5 years \((P = 0.07)\). Between 5 and 10 years of...

**Fig. 4.** (a) Mean \((\pm \text{SEM})\) and (b) Modal ages of male and female captive red wolves paired for breeding each year from 1992 to 2005. Regression line indicates the proportion of successful pairings each year within the same period.
age, there was no significant effect of dam age on litter size, although litter size appeared to decline slightly. Mean litter size for dams of age 11 was substantially less than that for dams of age 10 ($P < 0.05$).

With respect to inbreeding, litter size decreased as sire $f$ increased ($P < 0.01$). Litter size also tended to decrease with dam $f$. However, the relationship was not statistically significant ($P = 0.09$). The effect of sire $f$ was compounded slightly as dam $f$ increased ($P < 0.05$, Fig. 8), and also tended to be compounded by increasing dam age ($P = 0.08$). Sire age showed no interaction with litter size in combination with either sire or dam $f$.

**Pup Survival**

In general, the proportion of captive puppies within a litter that survived to one year of age was reduced as dam age increased ($P < 0.05$). The proportion of surviving pups was not associated with dam $f$, sire age, or $f$, and no combination effects were
Fig. 6. Regression of inbreeding coefficient \((f)\) and age in relation to breeding success for captive male red wolves \((P<0.01)\).

Fig. 7. Mean \((\pm\text{SEM})\) litter size for all red wolf litters born between 1977 and 2005 \((n = 291)\). Regression line indicates significant decline in litter size over time \((P<0.01)\).

Fig. 8. Regression of red wolf litter size with coefficients of inbreeding for sires and dams \((P<0.01)\).
noted. Pup survival is significantly greater in the free-ranging red wolf population (75.7±5.8%) than the captive population (59.0±3.3%) (P<0.05). However, censuses are not taken of free-ranging litters until > six months following the whelping season, therefore, pup survival may be overestimated in this population. Recall that mean dam age within the captive red wolf population increased significantly between 1995 and 1996. So, pup survival for the period from 1996 to 2005 was compared with pup survival for the period from 1977 to 1995. Post-1995 pup survival was significantly lower than pre-1995 pup survival within the captive red wolf population (61.6±3.2 and 69.5±5.7 respectively; P<0.05).

**DISCUSSION**

Rather than census data, it is the number of actively breeding individuals that determine the rate of loss of genetic diversity within a population; the effective population (\(N_e\)) [Ballou and Foose, 1996]. Therefore, the ratio of effective population size to census population size is important information in evaluating management strategy. In captive red wolves, the proportion of effective population to whole population declined from 1990 through 2004 and was maintained from 2004 through 2005.

The purpose of evaluating \(N_e\) in this study was to retrospectively compare changes in \(N_e\) over time. The values for red wolf captive \(N_e\) reported here were calculated based on discrete years and included population characteristics such as sex ratio, mean family size, and variation in family size. One aspect not taken into consideration was the fact that red wolves have overlapping generations. Thus, breeding animals for any one season are comprised of multiple members from single family lines. Because breeding does not generally occur between close family members, overlapping generations typically have the effect of reducing the effective population size [Lande and Barrowclough, 1987]. Furthermore, the \(N_e\) calculated herein included all potential breeders within the population; however, actual breeding pairs represent only a subset of this group. Therefore, the actual \(N_e\) for captive red wolves is likely to be lower than those reported here. However, because the calculations were consistent across all years measured, the comparison of change in \(N_e\) over time is useful. The aspect that contributed most to the reductions in \(N_e\) in this case was total lifetime family size, \(k\). From Table 1, it is apparent that \(k\) has also declined over time, particularly in females.

The emphasis of the red wolf SSP on maintaining genetic diversity has resulted in the preferential selection of older animals for mating. This is due to the fact that when red wolves are young, they typically have several surviving litter-mates. Within small populations such as the red wolf, multiple siblings have a large impact on relative mean kinships of individuals. As wolves age, the number of surviving siblings declines and unrelated litters are born, affecting relative kinships within the population. Thus, at least in terms of mean kinship, the most genetically rare individuals also tend to be the oldest individuals.

To varying degrees, the probability of successful breeding, litter size, and pup survival have all declined in association with increasing dam age. Although breeding success within the captive population has remained relatively stable since 1994, there was a significant negative correlation with modal female age, meaning that breeding success was reduced in years during which older females were preferentially paired.
for mating. Also, increases in mean age of both males and females, though primarily in females, were associated with reduced probability of successful breeding. A shift in the population age structure in the 1990s resulted in higher mean ages of both females and males from 1996 and later compared with the population prior to 1996. Interestingly, the observed associations of female age and breeding success were only apparent after that shift. Thus, female age may be of minor consequence to the probability of breeding success until animals have reached a certain age. No association of age and breeding success could be found in females aged 2–10 years, but a dramatic decline was apparent between 10 and 11 year-old animals. These data suggest that female red wolves reach reproductive senescence at approximately age 10. These findings are similar to reports of age-related loss of fecundity in coyotes (*C. latrans*), a closely related canid. Windberg [1995] found that pregnancy rates in female coyotes between 10 and 12 years of age were half those of females aged four to nine. In a recent study based on a 12-year data set of 24 captive wild-caught coyotes and comparing placental scars (indicative of fetal attachment sites), Green et al. [2002] found an 80–90% probability of breeding success in females aged 2 to 9 years but found that the probability declined thereafter to less than 40% in 12-year olds.

Litter size also was affected by dam age. Although there was no direct association of litter size and parent age in the captive population, litter size has declined slightly over time, whereas the age of mated individuals has increased. Furthermore, there was a direct association of litter size and dam age in the free-ranging red wolf population in that litter size declined at the upper limits of dam age. Captive litters were smaller than those produced by free-ranging red wolves, and captive dams were older than free-ranging dams. It is possible that an effect of dam age on captive litter sizes is masked by the unbalanced proportion of older animals paired for mating in captivity.

It should be noted that estimates of litter size for the free-ranging red wolf population may be artificially low because litters are not observed until some time after whelping season, and so the trend reported here for captive litters to be smaller than free-ranging litters is also likely to be underestimated. The finding that older females have smaller litters was also consistent with the findings of Green et al. [2002]. In that study, the mean number of placental scars rose in female coyotes aged 2 to 3 years, remained stable in animals aged 3 to 8, and progressively declined to a very low level by the age of 12. Finally, the proportion of red wolf pups surviving to the end of their first year was slightly reduced for older dams.

Breeding in the captive red wolf population is strictly controlled, with only selected wolves allowed the opportunity to mate, whereas free-ranging red wolves are free to select mates of their own volition, with the exception that interspecific mating pairs (red wolf × coyote) are actively disrupted. Although the captive red wolf population has been selected for the retention of genetic diversity, the mean coefficient of inbreeding within the captive population has risen slightly over time, whereas it has actually declined within the free-ranging population. However, mean inbreeding coefficients within the free-ranging population were generally higher and with a greater range than those within the captive population in concurrent years. Mean inbreeding coefficients within the captive red wolf population are still below levels at which inbreeding effects have been reported in other canid species [Fredrickson and Hedrick, 2002; Wildt et al., 1982]. The implications of inbreeding
within the free-ranging red wolf population are beyond the scope of this report; however, it is notable that the free-ranging population has shown a positive growth rate [B. Fazio, personal communication] and stable demographic structure.

In this study, both the likelihood of successful reproduction and the size of the litters produced declined with increasing inbreeding coefficients in captive males. Furthermore, the likelihood of successful reproduction was further reduced in males with higher inbreeding coefficients as they aged. Reduced male fecundity attributed to inbreeding has been recorded in various carnivore populations with small numbers of animals, including the cheetah (*Acinonyx jubatus*), [Menotti-Raymond and O’Brien, 1993; O’Brien et al., 1985, 1983; Wildt et al., 1983, 1995], Florida panther (*Felis concolor*) [Wildt et al., 1995], lion (*Panthera leo*) [Wildt et al., 1987], domestic dog [Wildt et al., 1982], and various populations of gray wolves (*C. lupus*) [Laikre and Ryman, 1991]. Notably, a direct link has been demonstrated between high levels of inbreeding, sperm quality, and reproductive success in the Mexican gray wolf [Asa et al., 2007]. With respect to red wolves, only litter size and neonate survival had been examined with respect to inbreeding coefficients prior to this study, with no inbreeding depression indicated; however, a nonsignificant trend towards reduced litter sizes was apparent from the data presented [Kalinowski et al., 1999]. Kalinowski et al. reported a mean inbreeding coefficient of 0.0403 (range 0–0.375) for captive red wolves, a value very similar to that reported here.

To include younger animals in the effective population, parameters for pairing recommendations were adjusted for recent breeding seasons. Minimum acceptable genetic diversity was revised from 90% to 80–85%, and females over 11 years of age and males over 13 years of age are now excluded from the breeding pool due to a low probability of litter-production [Fulk and Waddell, 2004]. This likely explains the moderate increase in the proportion of successful pairs seen from 2004 (20.0%) to 2005 (27.7%). Furthermore, the age structure of the population appears to be reverting to a stable distribution, with approximately 50% of individuals 4 years of age or younger, 20% between 5 and 10 years of age, and 30% over 10 as of August, 2005 [Waddell, 2005]. Although maintenance of genetic diversity is and should be a priority for the captive red wolf, evaluations of genetic diversity should be based on effective population size rather than simple census data. The results from this study indicate that population age structure has had a greater impact on reproduction in captive red wolves than inbreeding levels.

**CONCLUSIONS**

1. Management strategy for the captive red wolf should (and does) strive to preserve viable population demography, as well as maximal genetic diversity. Captive-population growth may be increased by further adjusting the maximal age of breeding females from 11 to 10 years of age, and, when possible, avoiding the recruitment of older males for breeding.

2. Although inbreeding does not yet appear to be a major limiting factor, it is advisable to continue to strive to maintain maximal diversity. As the level of inbreeding increases in the population, more studies aimed at discerning and overcoming possible inbreeding depression should be conducted. Furthermore, some
selective cross-breeding between the captive population and free-ranging individuals with low inbreeding coefficients could be considered to bolster genetic diversity within the captive population.

ACKNOWLEDGMENTS

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