

## RESEARCH ARTICLE

# Effects of Inbreeding on Reproductive Success, Performance, Litter Size, and Survival in Captive Red Wolves (*Canis rufus*)

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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. Zoo Biol 29:36–49, 2010. © 2009 Wiley-Liss, Inc.

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## INTRODUCTION

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 [U.S. Fish and Wildlife Service, 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 U.S. Fish and Wildlife Service 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; U.S. Fish and Wildlife Service, 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<sup>®</sup> 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 [U.S. Fish and Wildlife Service, 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 and 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. This 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 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, and 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 [U.S. Fish and Wildlife Service, 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 was also 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] noninbred mortality with increasing age in some species" [Ralls et al., 1988: p 187]. Therefore, the 3-month age category was chosen to represent survival of pups to juvenility [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; U.S. Fish and Wildlife Service, 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<sup>®</sup> 7.0 [SAS Institute Inc., Cary, North Carolina, USA 27513]. To determine whether 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 nonlinear [e.g., Kalinowski and Hedrick, 1998; Morton et al., 1956; Templeton and Read, 1983, 1984, 1998] methods, for example, by incorporating statistics to indicate whether 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 tests for a relationship between litter size and  $f$ . To determine whether 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  $\pm$  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 =  $0.027 \pm 0.003$ ) and 101 dams ( $f$  range = 0.0–0.125, mean =  $0.030 \pm 0.003$ ) with a population mean  $f$  of  $0.029 \pm 0.002$  (Fig. 1). The overall population mean  $f$  was  $0.036 \pm 0.001$  ( $n = 1108$ ). Litter size ranged from 1 to 9 pups (mean =  $4.15 \pm 0.14$ ), and

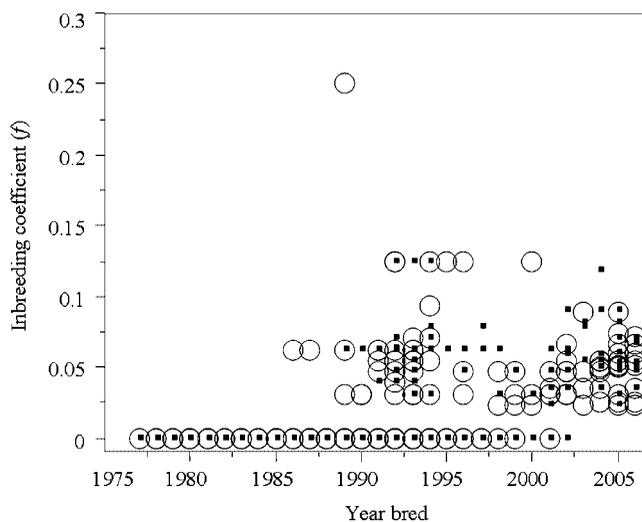


Fig. 1. Inbreeding coefficients ( $f$ ) of sires (open circles) and dams (solid squares) plotted against year bred.

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  $5.143 \pm 0.459$  pups ( $n = 7$  litters, range 1–6 pups/litter), whereas more recently (2005–2006), the mean litter size was  $3.391 \pm 0.354$  ( $n = 23$  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  $0.072 \pm 0.001$  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 ( $0.027 \pm 0.003$ ; range 0–0.250) than in predicted litters ( $0.041 \pm 0.002$ ; range 0–0.135). The mean  $f$  of dams also was lower ( $\chi^2 = 6.506$ ,  $df = 1$ ,  $P = 0.011$ ) in actual litters ( $0.030 \pm 0.003$ ; range 0–0.125) than in predicted litters ( $0.039 \pm 0.002$ ; 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 ( $\chi^2 = 5.522$ ,  $df = 1$ ,  $\hat{c} = 0.938$ ,  $P = 0.019$ ). When the sex ratio of litters was measured as a function of the offspring's  $f$ , the proportion of males increased ( $F = 6.076$ ,  $df = 1$ ,  $P = 0.015$ ; Fig. 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 ( $\chi^2 = 7.163$ ,  $df = 1$ ,  $P = 0.007$ ) (Fig. 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 =  $0.073 \pm 0.015$ ,  $n = 11$ ) 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 ( $\chi^2 = 5.482$ ,  $df = 1$ ,  $\hat{c} = 0.939$ ,  $P = 0.019$ ); the relationship between the dam's  $f$  and litter size was not statistically significant ( $\chi^2 = 2.356$ ,  $df = 1$ ,  $\hat{c} = 0.951$ ,  $P = 0.125$ ). 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

**TABLE 1.** The number of litters ( $N_{\text{litters}}$ ), the number of individual pups ( $N_{\text{ind}}$ ), 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 ( $N_{\text{survive 3}}$ ) and 12-months of age ( $N_{\text{survive 12}}$ ), and the inbreeding coefficients ( $f$ ) of offspring for the red wolf captive-breeding population

Offspring $f$	$N_{\text{litters}}$	$N_{\text{ind}}$	$\text{Mean}_{\text{litter size birth}}$	$N_{\text{survive 3}}$	$\text{Mean}_{\text{litter size 3}}$	$N_{\text{survive 12}}$	$\text{Mean}_{\text{litter size 12}}$
0.0000	40	205	$5.13 \pm 0.30$	123	$3.08 \pm 0.27$	106	$2.65 \pm 0.26$
0.0001–0.0499	34	145	$4.27 \pm 0.39$	102	$3.00 \pm 0.38$	96	$2.82 \pm 0.36$
0.0500–0.0999	105	390	$3.71 \pm 0.18$	280	$2.67 \pm 0.19$	236	$2.25 \pm 0.18$
0.1000–0.2500	13	57	$4.39 \pm 0.58$	44	$3.39 \pm 0.68$	35	$2.69 \pm 0.70$
Total	192	797		549		473	

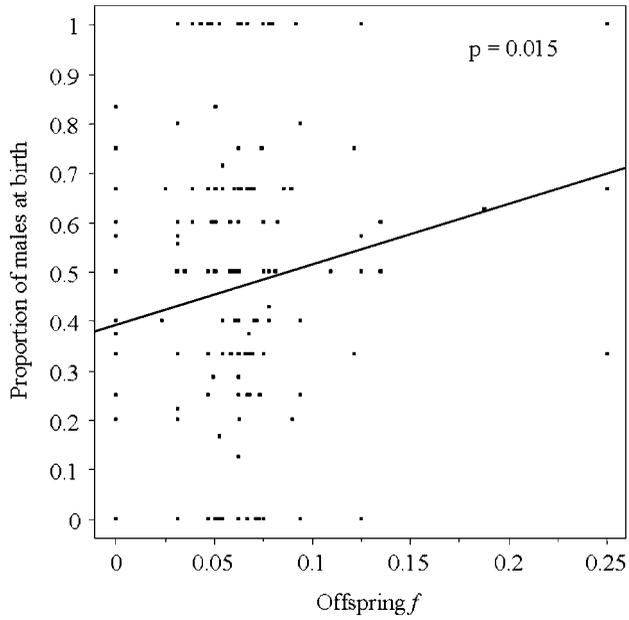


Fig. 2. Proportion of males in a litter at birth plotted against offspring inbreeding coefficients ( $f$ ) for the red wolf captive-breeding population. Fitted line is based on least-squares regression.

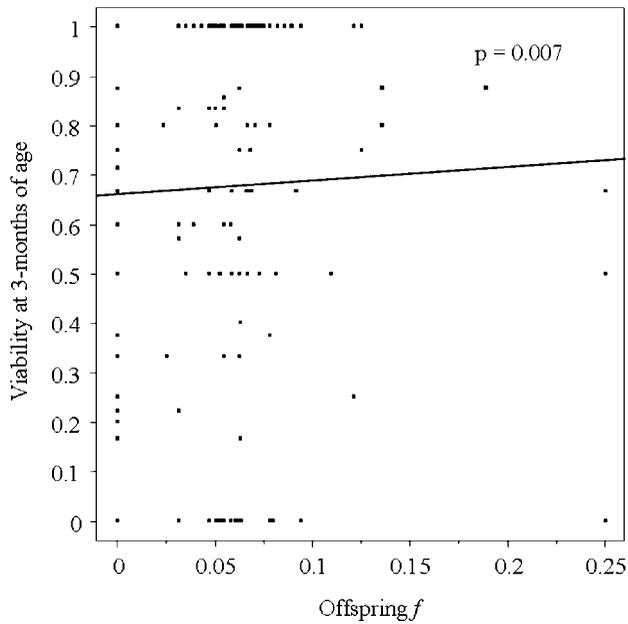


Fig. 3. Observed viability of pups at 3-months of age plotted against offspring inbreeding coefficients ( $f$ ) for the red wolf captive-breeding population. Fitted line is based on least-squares regression.

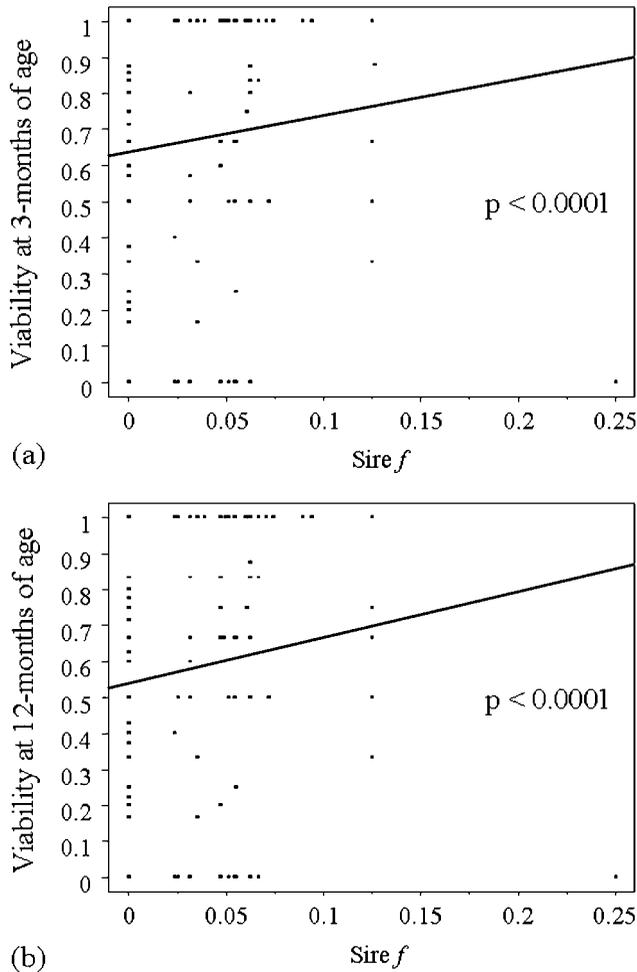


Fig. 4. Observed viability of pups at (a) 3-months of age and (b) 12-months of age plotted against inbreeding coefficients ( $f$ ) of sires for the red wolf captive-breeding population. Fitted line is based on least-squares regression.

( $F = 0.977$ ,  $df = 1$ ,  $P = 0.324$ ), 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 ( $\chi^2 = 20.880$ ,  $df = 1$ ,  $P < 0.0001$ ) and 12-months of age ( $\chi^2 = 21.739$ ,  $df = 1$ ,  $P < 0.0001$ ; Fig. 4). Similar positive relationships were found between the dam's  $f$  and pup survival at 3-months of age ( $\chi^2 = 3.829$ ,  $df = 1$ ,  $P = 0.050$ ) and 12-months of age ( $\chi^2 = 4.224$ ,  $df = 1$ ,  $P = 0.039$ ).

There was no apparent relationship between parental  $f$  and offspring rearing type (sire,  $n = 181$ ,  $\chi^2 = 1.291$ ,  $df = 1$ ,  $P = 0.256$ ; dam,  $n = 181$ ,  $\chi^2 = 0.063$ ,  $df = 1$ ,  $P = 0.802$ ). 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 this 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 whether 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., 1977–1996) 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

noninbred 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., 1977–2005) 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 nonsignificant 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 was also 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 this 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 is also 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., *Canis lupus*, Laikre and Ryman, 1991; *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 this 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 this 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 with 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 this 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 nonwolf 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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