Lack of Impact of Den Interference on Neonatal Red Wolves

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Abstract - Biologists handled *Canis lupus rufus* (Red Wolf) pups from 12 wild litters over 3 years to determine if den interference and handling negatively impacted neonatal survival. Litters were handled for blood collection and transponder placement on one of 2 den visits approximately 13 days apart when pups were approximately 5 days and 19 days old, respectively. No biologically important difference in the proportion of pups surviving was observed between subsequent visits, nor in comparison to historical data from dens where pups were not handled but rather documented based on autumn trapping surveys.

Introduction

Handling neonatal wild mammals provides researchers and managers the opportunity to collect data necessary for effective population monitoring and modeling. Benefits of handling neonates, however, should be weighed against potential risks. Assessments of the impact of handling neonates have been published for some mammals (Byers 1997, Dorney and Rusch 1953, Franklin and Johnson 1994, Henderson and Johanos 1988, White et al. 1972), but up until recently only anecdotal information guided management decisions for many canid species. In particular, the impact of human interference on wolf maternal behavior is controversial, with earlier work suggesting higher risk (Chapman 1979, Meck 1970, Smith 1998, Thiel et al. 1998) relative to recent work reporting relatively low risk of disturbance of dens of *Canis lupus* L. (Grey Wolf; Frame et al. 2007, Habib and Kumar 2007) and *Canis lupus lycaon* Schreber (Eastern Wolf; Argue et al. 2008). The potential benefits of early assessment and marking of *Canis lupus rufus* Audubon and Bachman (Red Wolf) pups are high considering the need to manage introgression of Coyote genes, a major threat to Red Wolf recovery (Kelly 2000, Kelly et al. 1999, US Fish and Wildlife Service [USFWS] 1989). This opportunistic study evaluates whether handling wild neonatal Red Wolves results in den abandonment, or negatively affects pup survival.

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Study Site

The Red Wolf recovery area consists of more than 6650 km^2 in five counties (Beaufort, Dare, Hyde, Tyrrell, and Washington) in northeastern North Carolina. The area contains three national wildlife refuges, a Navy and an Air Force bombing range, and considerable privately held property. Major land-cover types in the recovery area are agricultural (29%), pine (mostly *Pinus taeda.* L. [Loblolly Pine]) plantation (15%), pocosin (13%, consisting of *P. serotina* Michx. [Marsh Pine] and evergreen shrubs), and non-riverine swamp forests (10%, consisting of Loblolly Pine, *Nyssa* sp., and *Chamaecyparis thyoides* L. [Atlantic White Cedar]). Open water covers about 5% of the area, with minor land-cover types comprising the remaining 28% of the area. Mean temperatures range from 5.3 ºC in January to 26 ºC in July. Annual rainfall averages 126 cm, and elevation ranges from sea level to 50 m.

Methods

During Red Wolf denning season at the recovery site in North Carolina (approximately 15 March to 1 June), biologists located suspected den sites based on movement patterns of radio-collared female wolves. Because of the highly sensitive nature of the recovery effort, dens were included in this study opportunistically in the years 1999–2004 at the discretion of the USFWS biologists, based on pedigree of suspected parents, den accessibility, and management area in which the den was located. This eliminated any opportunity to randomly select dens for specific treatments, or to have equal groups of dens in each treatment in any given year. Dens in the study were assigned to 1 of 2 groups (early interference or delayed interference). Some dens known to biologists working in the recovery area were not included in the study because of clear biases. For example, dens where one or both of the parents were of unknown lineage, or suspected to be hybrid rather than pure wolf, were most likely to be assigned to the study and more likely to be assigned to the early interference group because the litters in those dens were not considered as "valuable" by project biologists. Also, dens in remote locations or with particularly difficult terrain were not included in the study.

Some degree of interference at a den is required to count the number of pups present. Fiber-optic video examination of dens could potentially allow enumeration of litter sizes with minimal interference. However, in our experience, accurate counting of pups with this technique was difficult. Pilot efforts with the fiber-optic video technique also proved logistically impractical in the difficult terrain of the recovery area. Direct entry of the dens and observation of the pups was more reliable and less likely to miss pups in the count. Without the option of a control group with no den interference, we chose to assess the potential effects of varying the timing of den interference. Specifically, we tested the hypothesis that earlier interference is more detrimental to pup survival than interference when the pups were older and the female more invested in the litter.

Each den was visited twice. Dens assigned to the early interference group ($n = 4$), were entered shortly after they were first discovered and the pups were at most approximately 1 week old. Pups were removed by a biologist wearing latex gloves and examined for any signs of illness, injury, or congenital defects. A 0.2-mL blood sample was drawn for DNA testing from the cephalic vein using a sterile 25-gauge needle. A 6-cm^2 area on the dorsal midline between the shoulder blades was lightly moistened with 70% isopropyl alcohol on a cotton ball, and a sterile passive integrated transponder (PIT) tag (Trovan®) was injected subcutaneously through a sterile 12-gauge needle. The transponder injection site was sealed with a drop of sterile cyanoacrylate (Nexaband®). All pups were then replaced in their den. Approximately 2 weeks later, observers returned to the dens in this group to count the pups present and note any reactions to the transponder injection. When dens had been moved between visits, the distance between the old and new den was recorded.

Pups in dens assigned to the delayed interference group ($n = 8$ dens) were not handled on the first visit. Observers only counted the number of pups by looking in the den without removing the pups. In some dens in the delayed interference group, a biologist had to crawl into the den with a flashlight to view the pups on the first visit. Approximately 2 weeks after the first den visit, observers returned to the delayed interference den sites and followed the same procedures used for the first visit to dens in the early interference group. Efforts were made to standardize the interval to the second den visit, but the impact of management schedules on den access did not allow the inter-visit interval to be tightly controlled. Consequently, some second visits made less than or more than 14 days after the first visit are included in the data set. No third visits were made to any dens, therefore no follow-up on implant and injection sites was performed for the pups in the delayed interference dens until autumn capture. Although a third check of dens in the delayed handling group would have allowed evaluation of the pups for reactions to transponder placement, and a better assessment of pup loss, this was not routinely feasible because of the mobility of the older pups. No problems attributable to transponder placement or early injections were identified for any wolves captured in the autumn.

As pups become mobile, verifying the number of pups in a litter becomes more difficult. Observations at rendezvous sites, a common technique in Gray Wolf studies, is not feasible in the Red Wolf restoration area in part because of the thick pocosin and forest vegetation, and the lack of elevated observation sites. The technique is also challenging because of lack of access to privately owned lands in the restoration area. For these reasons, we had to rely on counting the number of pups caught during the autumn trapping season (late August through September) to evaluate the impact of pup handling on den success. Autumn trapping is an imprecise method because of the challenge of successfully trapping all young wolves in an area. However, it is also a very conservative approach to evaluating pup survival because it would tend to overestimate mortality when living pups were not successfully trapped. Assessment by autumn trapping also does not differentiate mortality directly related to pup handling from other causes of early pup mortality. Assigning all unaccounted for pups after termination of autumn trapping to losses due to early pup handling would overestimate the impact of den interference.

Though it was not possible to accurately enumerate the number of pups from dens that were not entered, we did attempt to indirectly compare pup survival from the dens we entered to pup survival from dens never interfered with by comparing the autumn trapping data for the interfered with dens to the overall autumn trapping dataset accumulated since the initiation of the Red Wolf restoration effort. Any unmarked wolf caught and presumed to be a pup of the year based on morphology and tooth wear was designated as offspring of the pair occupying the area where the pup was caught. This assumption was later confirmed by genetic analysis. These numbers were totaled for all dens recorded over the history of the recovery effort, and the median value was compared to the trapping results from our interference dens.

Results

Table 1 lists for each den, the number of pups present at each visit and the number of pups caught in that territory during the autumn trapping season. Mean time between visits was approximately 13 days, with a range of 5 to 19 days. Seven of the 12 dens in the study, including 3 of 4 early interference and 4 of 8 delayed interference dens, had the same number of pups present

Table 1. Number of Red Wolf pups at successive den visits approximately 2 weeks apart, the proportion of pups caught during the following autumn, and the distance dens were moved between den visits.

	# Pups			Proportion	Visit	Distance
Group	Visit 1	Visit 2	Autumn capture	surviving to autumn	interval (days)	moved (m)
Early	5	5	1	0.2	7	0
Early	8	8	8	1.0	5	No data[A]
Early	3	3	2	0.7	14	124
Early	10	9	2	0.2	14	185
Mean				0.50		
Delay	4	6	6	1.0	14	No data[A]
Delay	6	5	2	0.4	17	211
Delay	2	2	2	1.0	17	1143
Delay	2	2	2	1.0	7	683
Delay	8	8	0	0.0	15	94
Delay	2	1	2[B]	1.0	14	240
Delay	4	3	2	0.7	16	1022
Delay	7	7	5	0.7	14	582
Mean				0.54		

[A]Den was moved but distance was not recorded.
[B]Biologists trapped during autumn what was thought to have been the second pup from this litter seen only at the first visit.

during the 2 successive den visits. There was no difference in the proportion of pups surviving to autumn for early versus delayed interference dens (Table 1).

Table 2 contains summary data for autumn litter sizes estimated as the number of pups ≥150 days old captured in each territory since the beginning of the restoration effort. Thirty-four of the 59 pups (56%) observed at the second visit for all interference dens were caught during autumn. Sixteen pups (6 males, 10 females) from these litters were subsequently documented to be members of territorial pairs, and 6 (2 males, 4 females) of them have produced litters.

Only 2 sibling pups of the total of 71 pups tagged with transponders showed reactions at the site of transponder injection at second visit. An attempt was made to drain the putative abscesses by puncturing a small hole in the dependent portion of each abscess with a scalpel blade. These 2 pups were not subsequently captured, and thus their fates remain unknown, though 2 of their siblings were captured during autumn trapping.

Discussion

Pups were caught during autumn from 11 of 12 litters in this study, suggesting the risk of a female abandoning a litter as a result of handling of pups using our protocol was low. The fate of the remaining litter is unknown.

Table 2. Median litter sizes for dens estimated from autumn trapping for years where pups were not handled in the den and years where pups were visited and handled in the den.

Year	Number of dens not visited*	Median autumn litter size of dens not visited	Number of dens visited	Median autumn litter size of visited dens
1988	2	1.0	0	
1990	1	3.0	0	
1991	3	3.0	0	
1992	2	2.0	0	
1993	5	3.0	0	
1994	8	3.0	0	
1995	5	2.0	0	
1996	7	2.0	0	
1997	4	2.0	0	
1998	1	1.0	0	
1999	4	2.5	4	2
2000	1	4.0	3	4
2001	1	1.0	5	3
2002	1	4.0	5	3
2003	3	2.0	4	3
2004	1	2.0	8	3
Total	49		30	

*Between 1988 and 1998, autumn capture data recorded 91 pups from the 38 dens. Between 1999 and 2004, the recorded number of pups from the dens listed for those years as not visited based on autumn capture data was 26 from 11 dens. Dens of the same parents were included in multiple years.

The mother of this litter had raised 2 of 5 pups successfully in the past in a different location. However, in prior years, when a different pair of wolves occupied the territory where the litter in our study was lost, there was no evidence of pups, suggesting the possibility that the particular territory may not be suitable for raising pups regardless of den interference.

Mother wolves in our study moved dens after human interference, a behavior documented for other canids including Gray Wolves and *Canis latrans* Say (Coyote) (Andelt et al. 1979, Chapman 1979, Frame et al. 2007, Habib and Kumar 2007, and Harrison and Gilbert 1985). Of the 12 litters included in the study, 11 were moved by the dam to new den sites between visits (see Table 1). The one litter not moved belonged to the early interference group. Movement of dens may be a normal behavior for Red Wolves. Female Coyotes prepare multiple dens prior to whelping (Harrison and Gilbert 1985) and move their pups quickly to alternate dens when needed. While searching for the dens included in our study, we found many recently excavated dens, some recently used. Red Wolves may prepare in advance for den movement, regardless of human disturbance.

One den in the early interference and 2 in the delayed interference groups in our study had 1 fewer pup present at the second visit. The missing pup in 1 of the delayed interference dens was apparently caught during autumn trapping, suggesting that perhaps the female was moving her pups during an interference visit. One delayed interference den had more pups present at the second visit, perhaps as a result of difficulty seeing pups in the den on the first visit.

The 2-week interval between visits was considered sufficiently long to observe negative impacts directly attributable to pup-handling protocols, and this assumption is supported by recent work by Argue et al (2008). Of the possible impacts, only infections from transponder placement were observed in our study and in only one den, involving 2 of 4 pups. These infections could have been the result of a break in sterile protocol during transponder placement. Handling neonatal pups did not appear to affect neonatal survival, and there was no apparent difference in pup survival between dens interfered with early after whelping and dens interfered with later (Table 1).

The number of pups trapped during autumn in our study was consistent with previous years where den interference was not a factor (Table 2; USFWS Red Wolf Program Database, unpubl. data). Causes of mortality through the age of 150 days for pups included in this study were largely undetermined because of the difficulty in detecting and recovering carcasses of pups without telemetry transmitters. At least 2 pups lost from one litter in the study were known to have died in a wildfire. Suspected causes of mortality for pups based on mortality patterns for all wolves in the recovery effort include starvation, inter- and intraspecific aggression, trauma from vehicles or farm equipment, other human-associated activities, and parasitic and viral diseases.

Juvenile survival (6 month to 1 year) rates as low as 0.34 are observed in stable or increasing populations of small carnivores (Fuller et al. 2003, Gese et al. 1989). Owing to low statistical power and the biased assignment to groups, we would only have been able to detect large differences in survival between our early and late interference groups. Nonetheless, we conclude that given the relatively high overall 6- to 8-month survival rates observed for animals in our study, any differences in survival between treatment groups were not large enough to be considered biologically significant. As such, the ability to safely interfere with dens without negative impact on pup survival opens many opportunities for the management of endangered wolves. These include early individual identification and genetic screening for efficient population management, better ability to assess early disease-related mortality, and the potential for use of cross fostering from captive populations.

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