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# Using the “placeholder” concept to reduce genetic introgression of an endangered carnivore

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ABSTRACT

One of the most endangered species is the red wolf, *Canis rufus*. Reintroduction of the red wolf began in 1987, but in 1993 hybridization between coyotes (*Canis latrans*) and wolves was documented. To reduce genetic introgression, coyotes and coyote–wolf hybrids were captured, sterilized, and released as “placeholders”. Placeholders held territories until either displaced or killed by a wolf, or management personnel removed them before releasing a wolf. We evaluated the placeholder concept by examining the number of animals sterilized and released, likelihood of displacement by a wolf, factors influencing displacements, territory fidelity of placeholders, and survival rates and causes of mortality of placeholders and wolves. Of the 182 placeholders, 125 were coyotes and 57 were hybrids. From 1999 to 2013, 51 placeholders were displaced or killed by wolves, and 16 were removed by management personnel. Thus, 37% of the placeholders were displaced leading to occupancy by a wolf. Most displacements occurred in winter (43%) and were always by the same sex. Males were more likely to be displaced than females. Home range characteristics influencing the probability of displacement included home-range size (i.e., more placeholders displaced from larger home ranges) and road density (i.e., more placeholders displaced from home ranges with lower road density). Annual survival of placeholders was higher than wolves in 12 of 14 years, with cause-specific mortality similar among wolves and placeholders. Placeholders provided territories for wolves to colonize, yet reduced the production of hybrid litters, thereby limiting genetic introgression to <4% coyote ancestry in the wolf population.

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## 1. Introduction

There is increasing concern about the status and distribution of many carnivore populations throughout the world (Schaller, 1996; Gittleman et al., 2001; Woodroffe, 2001; Ripple et al., 2014). With increasing human populations, many populations of carnivores are exposed to changes in land-use practices, increased habitat loss and fragmentation, increased human persecution, declines in natural prey species, increased disease transmission from domestic and wildlife species, illegal poaching, and increased competition with other carnivores (Gese, 2001; Sillero-Zubiri et al., 2004; Loveridge et al., 2010). As a result of these varied and diverse influences, many populations of large, medium, and small-bodied carnivores have undergone a general decline with some species now occupying a fragment of their former range (IUCN, 1990; Cole and Wilson, 1996; Woodroffe, 2001).

One threat facing a few carnivore species is hybridization resulting in genetic introgression with sympatric species (Wayne et al., 2004). While hybridization is an important evolutionary process (Allendorf et al., 2001), it poses a threat to the persistence and conservation of several wild canid species. Hybridization with domestic dogs poses a threat to the Ethiopian wolf (*Canis simensis*; Gottelli et al., 1994) and the European gray wolf (*Canis lupus*). Hybridization among several related canids in Ontario, Canada, could threaten the genetic integrity of a population of eastern wolves (*Canis lycaon*) in Algonquin Provincial Park (Patterson and Murray, 2008). In the United States, hybridization between red wolves (*Canis rufus*) and coyotes (*Canis latrans*) was identified as one of the greatest threats to conservation efforts and recovery of red wolves in eastern North Carolina (Kelly et al., 1999; Stoskopf et al., 2005). Reducing genetic introgression of coyote genes into the red wolf population presents a unique challenge for the U.S. Fish and Wildlife Service (USFWS), the agency charged with reintroducing and managing the current red wolf population (U.S. Fish and Wildlife Service, 1989, 2007).

In 1987, four pairs of red wolves were released at the Alligator River National Wildlife Refuge (ARNWR) in eastern North Carolina (Phillips and Parker, 1988). By 1993, the wolves had successfully bred and reestablishment of a free-ranging experimental population was considered to be a success (Phillips et al., 2003). The experimental population area primarily encompassed the Albemarle Peninsula, which did not have coyotes present during the initial reintroduction. However, by the early 1990s the presence of coyotes was documented and shortly thereafter hybridization between red wolves and coyotes occurred

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(Adams et al., 2003; Phillips et al., 2003). In 1999, a population and habitat viability assessment recognized several threats to the free-ranging red wolf population (Kelly et al., 1999), with hybridization with coyotes being the greatest threat to recovery of the species. Subsequently, the USFWS adopted a Red Wolf Adaptive Management Plan (RWAMP) with one of the objectives to reduce hybridization between coyotes and red wolves (Kelly, 2000).

As part of the RWAMP (Kelly, 2000), sterilization of coyotes and hybrid animals was proposed to reduce genetic introgression into the red wolf population. While sterilization has been tested as a management tool to reduce predation on domestic livestock and wild neonatal ungulates (Bromley and Gese, 2001a; Seidler et al., 2014) and proposed as a method for population control (Mech et al., 1996; Haight and Mech, 1997), using sterilization to reduce genetic introgression was a novel application. In essence, sterilized coyotes and hybrids would be allowed to remain on the landscape, maintaining social bonds and territories (Bromley and Gese, 2001b; Seidler and Gese, 2012), and serve as "placeholders" that would maintain territories, thereby reducing residency of home ranges in the recovery area by reproductive coyotes or hybrids, and thus reducing the threat of hybridization with a red wolf (i.e., producing hybrid offspring if pairing with a red wolf occurred; Stoskopf, 2012) and facilitating expansion of the red wolf population. The sterile placeholders could be displaced from their territories by a red wolf, or the USFWS could remove these sterile animals and release red wolves at that site when either a captive or wild-born red wolf was available for release. Sterilization was not used to control or manage the coyote population in the recovery area, but to create non-reproductive territories with sterile animals that were incapable of successfully reproducing with intact red wolves.

In late 1999, a plan to sterilize coyotes and hybrids to serve as placeholders in the Red Wolf Recovery Experimental Population Area (RWREPA) in eastern North Carolina was initiated. In this paper, we introduce and evaluate the placeholder concept as a management tool, covering its use in the red wolf recovery area from 1999 to 2013. As part of this evaluation, we examined (1) the number of animals (coyotes and hybrids) that were sterilized and released as placeholders, (2) the likelihood of a placeholder being displaced by a red wolf and the biotic and abiotic factors influencing these displacements, (3) the degree of territory fidelity of placeholders (i.e., the likelihood of dispersing after being sterilized), (4) survival rates and causes of mortality of both placeholders and red wolves, and (5) the number of hybrid litters born per year in the recovery area. Ultimately, the management goal is the reduction and eventual elimination of genetic introgression from coyotes into the red wolf population, thus allowing for continued persistence of a free-ranging population of red wolves in the wild.

## 2. Materials and methods

### 2.1. Study area

The Red Wolf Recovery Experimental Population Area (RWREPA) study area was located in northeastern North Carolina on the Albemarle Peninsula and encompassed approximately 4900 km$^2$ (Fig. 1). The peninsula is part of the South Atlantic Coastal Plain and is a combination of tidal (estuarine) and non-tidal (palustrine) wetlands, and mixed upland forests. The western region is dominated by mixed pine-hardwood forests of loblolly pine (*Pinus taeda*), white oak (*Quercus alba*), hickory (*Carya tomentosa*), beech (*Fagus grandifolia*), tulip tree (*Liriodendron tulipifera*), sweetgum (*Liquidambar styraciflua*), and red maple (*Acer rubrum*) (Hartshorn, 1972). Pocosins are palustrine wetlands endemic to the Atlantic coast and are found throughout the study area. The acidic and nutrient poor soils of pocosins facilitate dominance by pond pine (*P. serotina*) although loblolly and longleaf pine (*P. lalustris*) are common. The vegetation of the central region exhibits a gradual west-to-east change from upland species to palustrine wetlands dominated by tupelo (*Nyssa sylvatica*), Atlantic white cedar (*Chamaecyparis thyoides*), loblolly pine, and bald cypress (*Taxodium distichum*) (Lynch and Peacock, 1982; Moorhead and Brinson, 1995). Estuarine wetlands have their highest incidence in the eastern region of the study area (mainly Dare and Hyde counties), primarily along the coastline and are dominated by black rush (*Juncus roemerianus*) with areas of wetland grasses (*Spartina alterniflora*, *S. patens*, *Cladium jamaicense*), marsh elder (*Iva frutescens*), and false willow (*Baccharis angustifolia*) (Moorhead, 1992).

Within the RWREPA the principal landowners were private timber and agricultural corporations with federal and state governments having the next highest proportions of land ownership. There were numerous wildlife refuges contained within the study area with the two largest being the ARNWR and Pocosin Lakes National Wildlife Refuge

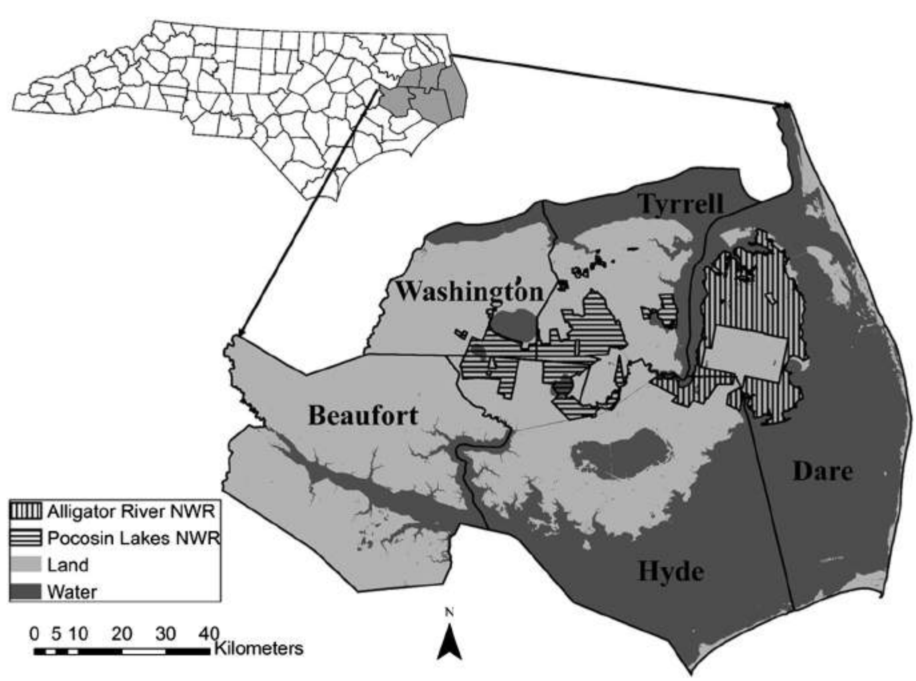


**Fig. 1.** The five county Red Wolf Recovery Experimental Population Area in northeastern North Carolina including the location of the two largest National Wildlife Refuges.

(PLNWR; Fig. 1). The ARNWR was located in the extreme northeastern section of the study area and was designated as the initial red wolf reintroduction site in 1987 due to a lack of coyotes and human presence, but with abundant prey (Phillips and Parker, 1988). Contained within the ARNWR was a 19,020-ha U.S. Air Force bombing range. The average annual rainfall for ARNWR was 145 cm without seasonal fluctuations, although 4.8 cm of snow falls annually during the winter (U.S. Department of the Interior, 2008). The 44,560-ha PLNWR was located in the central portion of the study area (Fig. 1). The total human population for the study area in 2010 was 105,124 people (U.S. Census Bureau, 2010).

### 2.2. Capture, sterilization, and monitoring of study animals

All capture, handling, aerial telemetry, and monitoring of red wolves, coyotes, and hybrids was conducted by USFWS personnel. Genetic analysis of blood samples collected from captured animals was used for species identification (Miller et al., 2003; Adams, 2006; Bohling et al., 2013). Beginning with the reintroduction in 1987, all red wolves released from captivity were equipped with a very high frequency (VHF) radio-collar (Telonics, Mesa, Arizona, USA; Phillips and Parker, 1988). Adults (>9 months old) born in the wild were trapped with a padded, foot-hold trap, immobilized, and fitted with a VHF radio-collar, body measurements and weight recorded, and a blood sample drawn. Pups born in the wild were implanted with an integrated transponder (PIT) tag (Trovan®; Beck et al., 2009). Radio-collared adult red wolves were located 2–3 times/week from an airplane or ground based vehicle. Starting in 2007, many red wolves were fitted with a GPS radio-collar (Lotek Wireless, Newmarket, Ontario, Canada) which obtained a location every 5 h (Dellinger et al., 2013).

Starting in 1999 and continuing through to 2013, adult (>9 months old) coyotes and hybrids within the RWREPA were sterilized to examine the feasibility of the placeholder concept. Captured coyotes and hybrids were either sterilized or removed (euthanatized) from the recovery area (Kelly, 2000; Gese et al., 2015), and thus there were no intact coyotes and hybrids monitored during this study. Upon capture in a padded, foot-hold trap, coyotes and hybrids were transported to a surgical facility, sterilized, then fitted with a VHF radio-collar (Telonics, Mesa, Arizona, USA), body measurements and weight recorded, and blood drawn. Females were sterilized by tubal ligation or spay, while males were vasectomized or neutered (Bromley and Gese, 2001b; Seidler and Gese, 2012). Animals spayed or neutered were classed as "hormones not intact", while animals undergoing tubal ligation or vasectomy were classed as "hormones intact" (Asa, 2005). All surgical procedures were conducted by a licensed veterinarian after the animals were anesthetized. Animals were monitored overnight for post-operative complications and released at their capture site the following day. Research techniques and animal care procedures were conducted under permits and standard operating protocols approved by the U.S. Fish and Wildlife Service.

Sterilized coyotes and hybrids wearing VHF radio-collars were located on a regular basis (2–3 times/week) during the same aerial telemetry flights as the red wolves. Locations of the placeholders provided spatial information including home range location and boundaries (USFWS, unpublished data) for the 182 placeholders (Fig. 2). Data were also recorded for the date of displacement, the species which displaced or killed the coyote or hybrid, and if available, the specific individual that displaced the placeholder. Because aerial telemetry was conducted during the day, we were concerned if the home ranges determined from daytime locations may underestimate space use (Gese et al., 1990). However, the average home range size of the 182 VHF radio-collared resident placeholders in the study area was 23.5 ± 12.0 (range 5.5–64.5 $km^2$), similar to the mean home range of 27.2 $km^2$ for coyotes later equipped with GPS-collars (Hinton, 2014).

### 2.3. Biotic and abiotic factors influencing displacement

For each placeholder's home range, we determined the percent composition of 10 land cover types within their home range using ArcGIS 10.2.2 (ESRI, Redlands, California, USA). Land cover types were obtained from LANDFIRE 1.3.0 (LANDFIRE 1.3.0., 2012) and included agriculture, sparse, developed, herbaceous, marsh, riparian, shrubland, swamp, forest, and water. Land ownership was compiled from state GIS databases and included federal, state, private, and non-governmental organizations (NGO). A digital representation of primary and secondary roads was obtained from the North Carolina Department of Transportation (https://connect.ncdot.gov/resources/gis/; accessed July 2014). The length of primary and secondary roads in each home range was

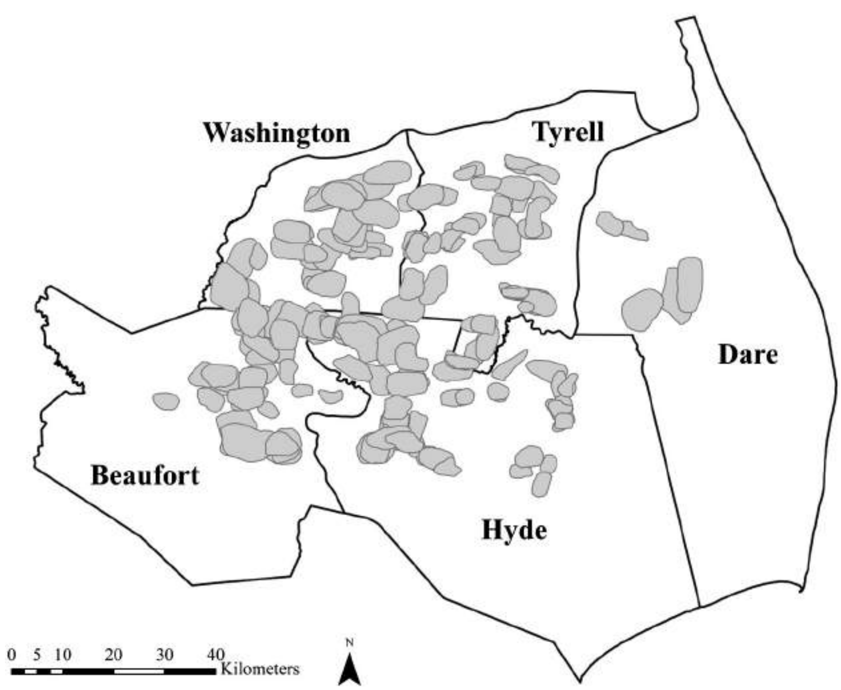


**Fig. 2.** Home ranges of placeholders (i.e., sterilized coyotes and hybrids) in the Red Wolf Recovery Experimental Population Area, North Carolina, 1999–2013.

converted to road density (km/km$^2$). We used generalized linear models (GLM) with a binomial distribution and logit link function to examine the influence of abiotic (home range characteristics) and biotic (placeholder characteristics) factors on the probability of being displaced ($y = 1$) or not displaced ($y = 0$) by a red wolf. Home range characteristics were assessed for each placeholder's home range, including home range size (km$^2$), road density (km/km$^2$), percent occurrence of each land cover type, dominant land cover type, percent occurrence of each land owner type, and dominant land owner type. Placeholder characteristics included sex of the placeholder, body length, and sterilization procedure (hormones intact or not intact). We developed separate GLMs to examine the effects of the home range and placeholder characteristics. Correlated variables ($r > 0.25$) were not allowed to enter the same model as additive or interactive effects.

We ranked all home range and placeholder characteristic GLMs and the null model using the Bayesian Information Criteria (BIC; Schwarz, 1978). Variables from the highest ranked model of home range characteristics were combined with variables from the highest ranked model of placeholder characteristics to generate a set of models containing both combinations of predictor variables, and we again used BIC (Schwarz, 1978) to compare models (Scheiner, 2004). All model development and analysis was conducted in the R statistical software (R Core Team, 2014).

*2.4. Cause-specific mortality and survival rates*

Radio-collared adult red wolves, coyotes, and hybrids were monitored 2–3 times/week allowing for the early detection of a mortality signal and facilitating recovery of the carcass to determine the cause of death. If applicable, a field necropsy was conducted, or if the cause of death was not apparent, the carcass was examined by a veterinary pathologist. We classified mortalities into one of three classes: anthropogenic, natural, or unknown. Anthropogenic mortality included any human-caused death not due to removal of coyotes or hybrids by agency personnel to make that home range available to a red wolf. Thus, anthropogenic mortality included causes of death from gunshot, vehicle collision, foul play, trapping, and poisoning. Foul play included suspected gunshot or suspected illegal take. Natural mortalities included health-related incidences such as disease or parasite load, and interspecific and intraspecific aggression resulting in death of the animal. A total of 182 placeholders and 410 red wolves were monitored from 1 January 1999 to 31 December 2013. We calculated annual survival rates for red wolves, sterile coyotes, and sterile hybrids using the program MICROMORT (Heisey and Fuller, 1985), but limited our survival analysis to the time period of 2000 to 2013 as there was only one sterile coyote and four sterile hybrids available for monitoring in 1999.

*2.5. Composition of litters*

During spring, personnel from the USFWS monitored radio-collared red wolves and located breeding females at active dens to determine the composition of the litter (Bohling and Waits, 2015; Gese et al., 2015). Pups born in the wild were implanted with an integrated transponder (PIT) tag (Trovan®; Beck et al., 2009) for future identification during subsequent capture operations in the fall when pups were large enough to be radio-collared. If the genetic origin of the litter was questionable, blood samples were obtained and examined using 18 nuclear DNA microsatellite loci to determine their ancestry and red wolf pedigree (Miller et al., 2003; Adams, 2006; Bohling et al., 2013).

## 3. Results

*3.1. Displacement events*

From 1999 to 2013, the USFWS captured, sterilized, and released 218 animals to serve as placeholders within the RWREPA. Of these, 15 were classed as transients (cf Gese et al., 1988), 13 were killed <3 months after release, and 8 disappeared (i.e., lost contact with the radio-collar) <3 months after release, thereby leaving 182 individuals for analysis. These 182 placeholders included 66 female and 59 male coyotes, and 26 female and 31 male hybrids. Of the 182 placeholders monitored, 51 were displaced by wolves (37 were spatially displaced by wolves from their territories and 14 were killed by a red wolf). In addition, 16 placeholders were removed by USFWS personnel and a red wolf released into the territory. Thus, 67 (37%) of the 182 placeholders were naturally displaced or artificially removed, leading to occupancy of the territory by a red wolf. During the same time period, 146 (35%) displacements out of 410 red wolves monitored were also documented. No coyote or hybrid displaced a red wolf; red wolves were displaced only by another red wolf. All displacements (100%) of placeholders were by a red wolf of the same sex. Similarly, for red wolves 98% of red wolf displacements were by a red wolf of the same sex.

Of the 51 naturally occurring displacements of placeholders, the frequency of displacements varied seasonally ($\chi^2 = 9.37$, df $= 3$, $P = 0.025$) with the most displacements occurring in winter (43%; 1 December–28 February), followed by spring (25%; 1 March–31 May), fall (18%; 1 September–30 November), and summer (14%; 1 June–31 August). Similarly, the 146 displacements of red wolves by red wolves varied seasonally ($\chi^2 = 31.64$, df $= 3$, $P < 0.001$) with most displacements occurring in winter (41%), followed by spring (26%), fall (25%), and summer (8%).

Although there were similar numbers of female ($n = 92$) and male ($n = 90$) placeholders, sterilized males were more likely to be displaced than sterilized females (males: 34.4% displaced, females: 21.7% displaced; $\chi^2 = 3.64$, df $= 1$, $P = 0.056$), regardless if the male was a sterile coyote (32.2%) or a sterile hybrid (38.7%; Fig. 3). Female placeholders that underwent tubal ligation and were hormonally intact were no more likely to be displaced than females that underwent a spay and were not hormonally intact (tubal ligation: 19.4% displaced; spay: 30.0% displaced; $\chi^2 = 1.025$, df $= 1$, $P = 0.31$; Fig. 4). Similarly, males that underwent vasectomy and were hormonally intact were also no more likely to be displaced than males that underwent a neuter surgery and were not hormonally intact (vasectomy: 32.9% displaced, neuter: 42.3% displaced; $\chi^2 = 0.519$, df $= 1$, $P = 0.47$; Fig. 4). The weight at capture of displaced female placeholders ($13.21 \pm 2.57$ kg, standard deviation [SD]) was no different than female placeholders that were not displaced ($13.50 \pm 2.58$ kg; $t = 0.450$, df $= 30.499$, $P = 0.65$). Similarly, the weight at capture of male placeholders that were displaced ($15.84 \pm 3.48$ kg) was not different than the male placeholders that were not displaced ($14.94 \pm 2.58$ kg; $t = -1.265$, df $= 47.725$, $P = 0.2119$).

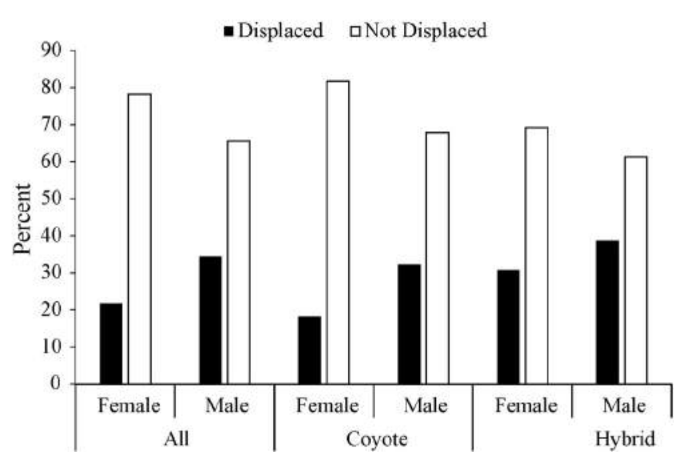


**Fig. 3.** The percent of male and female coyotes and hybrids serving as placeholders that were displaced and not displaced by red wolves in the Red Wolf Recovery Experimental Population Area, North Carolina, 1999–2013.

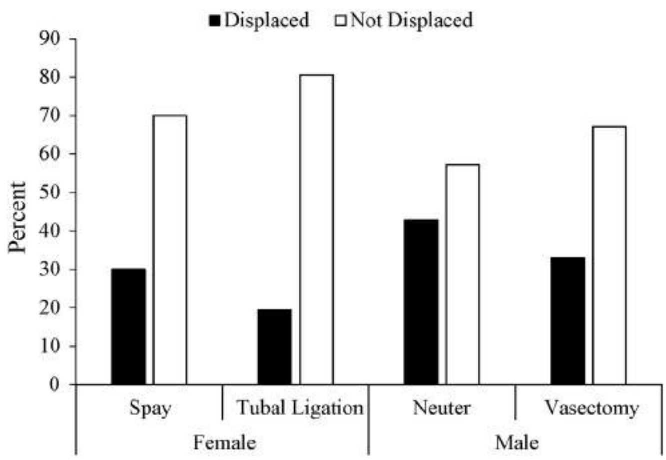


**Fig. 4.** The percent of 182 placeholders, sterilized by four methods, which were displaced and not displaced by red wolves in the Red Wolf Recovery Experimental Population Area, North Carolina, 1999–2013.

### 3.2. Biotic and abiotic factors influencing displacement

We examined the abiotic (home range characteristics) and biotic (placeholder characteristics) factors influencing the likelihood of a placeholder being displaced. Of the 63 models of home range characteristics examined plus the null model, the highest ranked was the null model followed by models containing home-range size or road density (Table 1). We found that the percent of placeholders displaced by a red wolf increased as home-range size increased (Fig. 5A). At home ranges $<20$ km$^2$, 17 of 85 (20%) placeholders were displaced by red wolves, while in contrast, 10 of 26 (38%) of the placeholders with home ranges $>35$ km$^2$ in size were displaced. In contrast, we found that the percent of placeholders displaced by a red wolf decreased with increasing road density, with displacements being highest at low road densities (Fig. 5B). All other models of home range characteristics had ΔBIC values $>10$ and model weights $<0.01$, thus home-range size and road density were carried forward to the combined models (Burnham and Anderson, 2002). Interestingly, neither the composition of land ownership or the dominant land ownership, nor the composition of land cover type or dominant land cover type influenced whether a placeholder was displaced by a red wolf.

Of the seven models of placeholder characteristics and the null model, the highest ranked was the null model followed by the univariate model of placeholder sex (Table 2). As described previously, we found male placeholders were more likely to be displaced than female placeholders (males: 34.4%, females: 21.7%). All other models of placeholder characteristics had ΔBIC values $>4$ and model weights $<0.08$, thus placeholder sex was the single variable carried over to generate the combined models. Of the eight combined models examined and the null model, the highest ranked model was the null model followed by the univariate model containing placeholder sex, then the univariate models containing home-range size and road density (Table 3).

**Table 1**
The ΔBIC and model weights for the generalized linear models and the null model examining the influence of home range characteristics within a placeholder's home range and the likelihood of being displaced by red wolves in the Red Wolf Recovery Experimental Population Area, North Carolina, 1999–2013.

| Model | ΔBIC | df | Weight |
|---|---|---|---|
| Null | 0.0 | 1 | 0.68 |
| Home-range size (km$^2$) | 2.6 | 2 | 0.19 |
| Road density (km/km$^2$) | 3.8 | 2 | 0.10 |
| Home-range size (km$^2$) + Road density (km/km$^2$) | 7.0 | 3 | 0.02 |

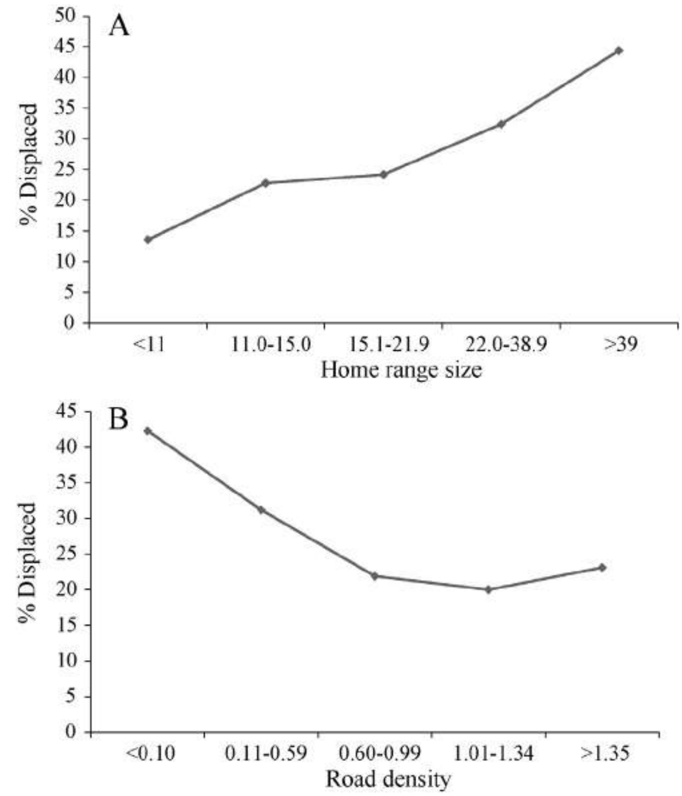


**Fig. 5.** The percent of placeholders displaced by a red wolf across (A) five classes of home-range size (km$^2$) of the placeholder, and (B) five classes of road density (km/km2) within a placeholder's home range, Red Wolf Recovery Experimental Population Area, North Carolina, 1999–2013.

### 3.3. Territory fidelity

Dispersal of juvenile animals from their natal home range is a common occurrence among most canid species. However, we emphasize that because only adult coyotes and hybrids $>9$ months of age were sterilized and used as placeholders, we only examined territory fidelity for adult canids in the study area (i.e., we did not include juvenile dispersal from their natal home ranges). Territory fidelity of adult canids was high during the study. During the 14 years of monitoring (2000–2013), of the 125 adult coyotes serving as placeholders, only 2 (1.6%) adult sterile coyotes dispersed from their resident territory. Of the 57 adult hybrid animals serving as placeholders, 4 (7.0%) adult hybrids dispersed from their territory. Similarly, of the 410 adult red wolves monitored during the same time period, 11 (2.7%) adult red wolves dispersed from their resident territory. In contrast to and for

**Table 2**
The ΔBIC and model weights for the generalized linear models and the null model examining the influence of placeholder characteristics on the likelihood of being displaced by red wolves in the Red Wolf Recovery Experimental Population Area, North Carolina, 1999–2013.

| Model | ΔBIC | df | Weight |
|---|---|---|---|
| Null | 0.0 | 1 | 0.56 |
| Sex | 1.5 | 2 | 0.26 |
| Hormones intact | 4.1 | 2 | 0.07 |
| Body length (cm) | 5.2 | 2 | 0.04 |
| Sex + Hormones intact | 5.3 | 3 | 0.04 |
| Sex + Body length (cm) | 6.6 | 3 | 0.02 |
| Hormones intact + Body length (cm) | 9.3 | 3 | 0.01 |
| Sex + Hormones intact + Body length (cm) | 10.4 | 4 | 0.00 |

**Table 3**
The ΔBIC and model weights for eight generalized linear models and the null model combining biologically meaningful characteristics of the placeholder and the placeholder's home range on the likelihood of being displaced by red wolves in the Red Wolf Recovery Experimental Population Area, North Carolina, 1999–2013.

| Model | ΔBIC | df | Weight |
|---|---|---|---|
| Null | 0.0 | 1 | 0.48 |
| Sex | 1.5 | 2 | 0.22 |
| Home-range size ($km^2$) | 2.6 | 2 | 0.13 |
| Road density ($km/km^2$) | 3.8 | 2 | 0.07 |
| Home-range size ($km^2$) + Sex | 5.0 | 3 | 0.04 |
| Road density ($km/km^2$) + Sex | 5.0 | 3 | 0.04 |
| Home-range size ($km^2$) + Road density ($km/km^2$) | 7.0 | 3 | 0.01 |
| Home-range size ($km^2$) + Road density ($km/km^2$) + Sex | 9.1 | 4 | 0.00 |
| Home-range size ($km^2$) * Road density ($km/km^2$) + Sex | 10.4 | 5 | 0.00 |

comparative purposes, we found that 103 (25.1%) of the juvenile red wolves dispersed at some time from their natal home range.

### 3.4. Survival rates and cause-specific mortality

We estimated annual survival rates for the 182 adult placeholders that were monitored for 137,784 radio-days (sterile coyotes: 84,093 radio-days; sterile hybrids: 53,691 radio-days) during 1999 to 2013. For comparison, we examined survival rates of 410 adult red wolves monitored for 388,587 radio-days during the same time period. In general, the sterilized adult placeholders (coyotes and hybrids combined) had higher survival rates than adult red wolves (Fig. 6). Mean annual survival was highest for sterilized hybrids (0.876 ± 0.11, standard deviation, SD), lowest for red wolves (0.80 ± 0.04) and intermediate for coyotes (0.843 ± 0.12). Red wolves exhibited higher annual survival than the placeholders in only two (14%) of the 14 years of the study, while placeholders had the highest survival in 12 (86%) of the 14 years monitored. Interestingly, sterilized coyotes had the highest survival in 6 (43%) of the 14 years and hybrids also had the highest survival in 6 (43%) of the 14 years.

Some sources of mortality among adult red wolves and adult placeholders were similar, while some specific causes were more species related (Table 4). Anthropogenic causes of mortality was similarly high for both adult red wolves and adult placeholders (red wolves vs. placeholders: $\chi^2 = 0.47$, 1 df, $P = 0.49$), and the number of deaths due to natural and unknown causes was similar (Table 4). A similar high percentage of red wolves and placeholders were killed by gunshot and foul play (red wolves vs. placeholders: $\chi^2 = 0.07$, 1 df, $P = 0.788$). Six red wolves were killed by poisoning and no placeholders were killed by poisoning (red wolves vs. placeholders: $\chi^2 = 1.65$, 1 df, $P = 0.199$). No red wolves were killed by placeholders (sterile coyotes or sterile hybrids), but 19% of the sterile coyote mortalities and 21% of the sterile hybrids mortalities were caused by interspecific aggression from red wolves (red wolves vs. placeholders: $\chi^2 = 50.36$, 1 df, $P = 0.0001$). Red wolves were rarely killed (~6% of mortality) by conspecifics (i.e., intraspecific aggression) and no placeholders were recorded as killed by conspecifics (red wolves vs. placeholders: $\chi^2 = 3.95$, 1 df, $P = 0.0469$).

**Table 4**
Anthropogenic, natural, and unknown causes of mortality for adult red wolves and sterile placeholders (coyotes, hybrids) in the Red Wolf Recovery Experimental Population Area, northeastern North Carolina, 1999–2013.

| | Red Wolves % (n) | Sterile Coyotes % (n) | Sterile Hybrids % (n) |
|---|---|---|---|
| *Anthropogenic* | | | |
| Gunshot | 37.1 (91) | 23.8 (10) | 33.3 (8) |
| Vehicle | 17.6 (43) | 19.0 (8) | 8.3 (2) |
| Foul Play | 4.1 (10) | 14.3 (6) | 8.3 (2) |
| Trapping | 2.4 (6) | 4.8 (2) | 4.2 (1) |
| Poisoning | 2.4 (6) | 0 (0) | 0 (0) |
| Total | 63.7 (156) | 61.9 (26) | 54.2 (13) |
| *Natural* | | | |
| Health-related | 11.8 (29) | 0 (0) | 4.3 (1) |
| Interspecific | 0 (0) | 19.0 (8) | 20.8 (5) |
| Intraspecific | 5.7 (14) | 0 (0) | 0 (0) |
| Total | 17.6 (43) | 19.0 (8) | 25.0 (6) |
| Unknown | 18.8 (46) | 19.0 (8) | 20.8 (5) |
| Total deaths | 245 | 42 | 24 |

### 3.5. Composition of litters

In general there was little variation in the number of hybrid litters from 2000 to 2013 with a mean of 2 hybrid litters/year (±1, standard deviation) with a maximum of 5 litters in 2006 and no hybrid litters in 2004 (Fig. 7). During the same time period, the number of red wolf litters has varied with a mean of 9 litters (±2) and ranged from 6 to 12 litters each year.

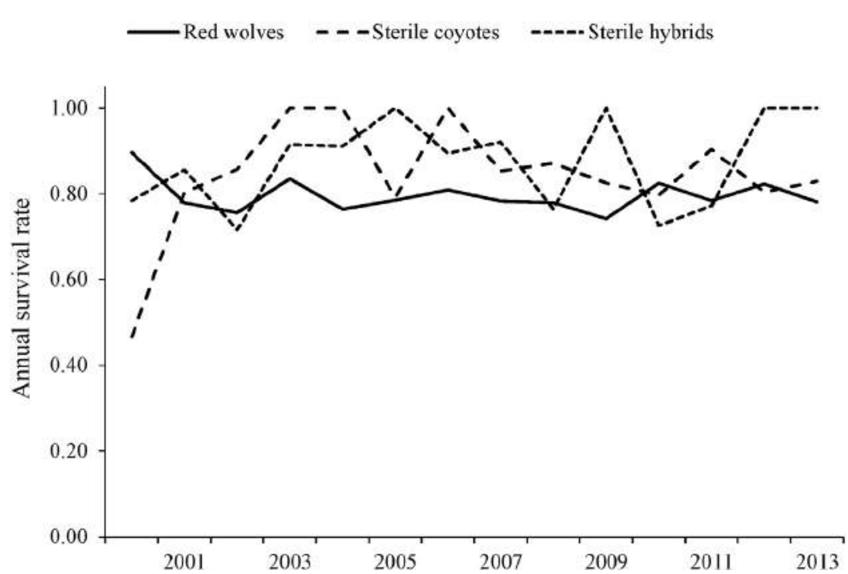


**Fig. 6.** Annual survival rates of adult red wolves (n = 410), sterilized adult coyotes (n = 125), and sterilized adult hybrids (n = 57), in the Red Wolf Recovery Experimental Population Area, North Carolina, 2000–2013.

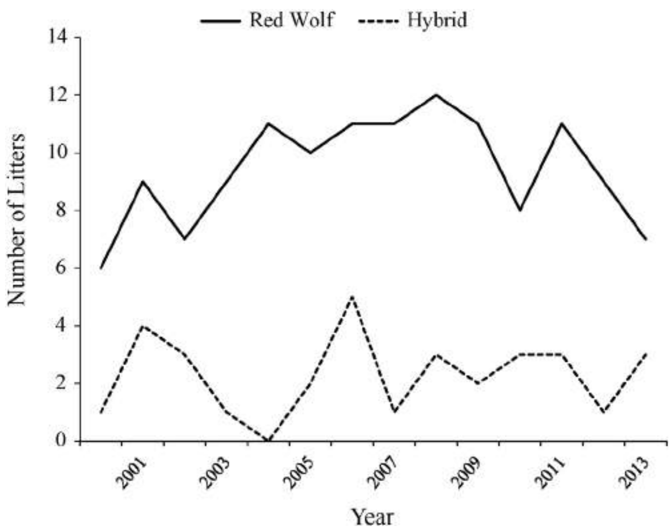


**Fig. 7.** The number of red wolf and hybrid litters in the Red Wolf Recovery Experimental Population Area, North Carolina, 2000–2013.

## 4. Discussion

Many factors threaten the persistence of canid populations throughout the world (Gittleman et al., 2001; Woodroffe, 2001; Ripple et al., 2014). Hybridization with coyotes followed by genetic introgression was identified as one of the greatest threats to recovery of red wolves in North Carolina (Kelly et al., 1999). Sterilization of coyotes and hybrid individuals was proposed to serve as placeholders to reduce hybridization and genetic introgression of the red wolf population (Kelly, 2000). This is the first documented case of using sterilization and the placeholder concept to mediate hybridization and genetic introgression between similar taxonomic canids. The primary objective of the placeholder concept was to limit opportunities for intact red wolves to produce viable offspring during mating events with coyotes or hybrid animals, as well as keeping space available for red wolves without the threat of producing hybrid offspring if pairing with a red wolf occurred (Stoskopf, 2012). These sterile placeholders could then be displaced from these territories by a red wolf, or these sterile animals could be removed and red wolves released into the now empty territory. Sterilization was not used to control or manage the coyote population in the recovery area, but to create non-reproductive territories with sterile animals that were incapable of successful reproduction (i.e., hybridization).

Natural displacements and strategic management removals of placeholders resulted in 37% of those sterile placeholders being replaced by red wolves in that territory. Displacements were unidirectional with red wolves displacing and replacing placeholders, but no placeholder displaced red wolves during the 14 years of monitoring. Interestingly, animals not having hormonal systems intact (i.e., animals spayed or neutered) were not displaced at a higher frequency than sterile animals with intact hormones (i.e., animals tubal ligated or vasectomized). Intact hormonal systems are generally believed to be necessary for pair bonding and territorial maintenance (Asa, 1995). The higher frequency of displacements in winter is not surprising given that the breeding season would compel animals to seek mating opportunities. The finding that male red wolves displaced male placeholders, and female red wolves displaced female placeholders reinforces the mating opportunity hypothesis.

We found that home range size and road density influenced the percentage of placeholders displaced by red wolves. At home ranges <20 km$^2$, 20% of the placeholders were displaced by red wolves, while 38% of the placeholders with home ranges >35 km$^2$ were displaced. Red wolves have larger home ranges (Chadwick et al., 2010) than coyotes, and may thus prefer to acquire larger areas in which to establish residency. Similarly, home ranges of placeholders that contained low road densities were preferred by red wolves, leading to higher displacement rates. Dellinger et al. (2013) reported red wolves avoided areas with high human density, and suggested red wolves will use human-associated landscapes, but modify their habitat selection patterns with increased human presence. Thus large home ranges with low road density appear to be preferred by red wolves and placeholders occupying said home ranges have a higher likelihood of being displaced. Interestingly, of the 26 placeholders with home ranges >35 km$^2$, the 10 placeholders displaced had a median home range size of 47 km$^2$ and a median road density of 0.48 km/km$^2$, while the 16 placeholders not displaced had a median home range size of 41 km$^2$ and a median road density of 0.63 km/km$^2$. Past studies on gray wolves have suggested wolves tended to survive where human density was low and road density was <0.58 km/km$^2$ (Thiel, 1985; Mech et al., 1988). Red wolves and coyotes used similar habitats and space (Hinton, 2014), thus the lack of habitat variables influencing displacements was likely due to similar habitat selection and requirements.

Annual survival rates of placeholders were higher than red wolves in 12 of the 14 years of monitoring. Coyotes and hybrids each had the highest survival rates in 6 of the 14 years. Even first generation hybrids had survival values more similar to coyotes than red wolves, indicating that hybridization conferred some level of increased survival abilities more reminiscent of coyotes. Perhaps the smaller body size, dietary breadth (Hinton, 2014), and behavioral plasticity of hybrids, which are more similar to coyotes than red wolves, also allowed for increased survival rates. Coyotes are adaptable to human-modified environments (Bekoff and Gese, 2003; Gehrt, 2004; Gese et al., 2012), and hybridization appeared to confer similar “coyote-like” survival traits to hybrid individuals.

While causes of mortality were similar among red wolves, coyotes, and hybrid animals, red wolves did experience a higher frequency of gunshot and health-related mortality. The high red wolf mortality due to gunshot is cause for concern as many of these mortalities occurred in the breeding season during the past 2–3 years (Hinton et al., in review) and not only limited potential litter production of red wolf pairs in the last 2 years (Fig. 7), but also opened opportunities for hybridization between red wolves and coyotes by reducing mating opportunities with red wolves (Bohling and Waits, 2015; Gese et al., 2015). While sterilization of placeholders does limit successful reproduction between red wolves and coyotes, it is impractical to capture and sterilize all coyotes in the recovery area.

While only 37% of the placeholders were naturally or artificially displaced leading to red wolf occupancy of the territory, the remaining 63% did protect space in which no hybrid litters could be produced. Ultimately, limiting genetic introgression into the red wolf population is the overall goal of the use of the placeholder concept. In 2014, the genetic composition of the wild red wolf population was estimated to include <4% coyote ancestry from recent introgression since reintroduction (Gese et al., 2015). Use of placeholders, combined with removal of coyotes and hybrids, release of captive adult red wolves, and cross-fostering of captive pups into wild red wolf litters, appeared to be effectively limiting genetic introgression into the red wolf population (Gese et al., 2015). Continued intensive management will likely be necessary in the future to limit hybridization and genetic introgression. Using the placeholder concept to limit hybridization in other canid species has potential. Hybridization with domestic dogs poses a threat to the Ethiopian wolf (Gottelli et al., 1994) and the European gray wolf, but sterilization to generate placeholders may not be an effective strategy in these situations because domestic dogs are the introgressing species and sterilizing all free-ranging domestic dogs would be impossible. Using the placeholder concept to reduce or limit hybridization among several related canids in Ontario and reduce the threat of genetic introgression into a population of eastern wolves in Algonquin Provincial Park (Patterson and Murray, 2008) may be more practical.

## 5. Conclusions

Sterilization has been used in the recent past to reduce predation rates by coyotes on domestic and native ungulates (Bromley and Gese, 2001a; Seidler et al., 2014), but using sterilization to limit genetic introgression into the red wolf population is the first use of sterile animals within the context of the "placeholder" concept. We emphasize that sterilization was not used to limit the distribution or size of the coyote population, but to reduce the incidence of hybridization between coyotes and red wolves and genetic introgression into the red wolf population. Results from this experiment demonstrate the utility of the placeholder concept to limit genetic introgression of coyotes into the recovering red wolf population in northeastern North Carolina. Territories were held by sterilized placeholders and then being successfully displaced by red wolves resulting in red wolf occupancy. Equally important was production of hybrid litters was limited to a few each year in the recovery area, and the genetic composition of the red wolf population in 2014 contained <4% coyote introgression. The utility and application of the placeholder concept may be practical for limiting genetic introgression in similar situations where an introgressing species threatens the genetic integrity of a sympatric carnivore.

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