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Managing hybridization of a recovering endangered species: The red wolf *Canis rufus* as a case study

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Abstract Hybridization presents a unique challenge for conservation biologists and managers. While hybridization is an important evolutionary process, hybridization is also a threat formany native species. The endangered species recovery effort for the red wolf *Canis rufus* is a classic system for understanding and addressing the challenges of hybridization. From 1987–1993, 63 red wolves were released from captivity in eastern North Carolina, USA, to establish a free-ranging, non-essential experimental population. By 1999, managers recognized hybridization with invasive coyotes *Canis latrans* was the single greatest threat to successful recovery, and an adaptive management plan was adopted with innovative approaches for managing the threat of hybridization. Here we review the application and results of the adaptive management efforts from 1993 to 2013 by comparing: (1) the numbers of wolves, coyotes, and hybrids captured, (2) the numbers of territorial social groups with presumed breeding capabilities, (3) the number of red wolf and hybrid litters documented each year and (4) the degree of coyote introgression into the wild red wolf gene pool. We documented substantial increases in the number of known red wolves and red wolf social groups from 1987–2004 followed by a plateau and slight decline by 2013.The number of red wolf litters exceeded hybrid litters each year and the proportion of hybrid litters per year averaged 21%. The genetic composition of the wild red wolf population is estimated to include < 4% coyote ancestry from recent introgression since reintroduction. We conclude that the adaptive management plan was effective at reducing the introgression of coyote genes into the red wolf population, but population recovery of red wolves will require continuation of the current management plan, or alternative approaches, for the foreseeable future. More broadly, we discuss the lessons learned from red wolf adaptive management that could assist other endangered species recovery efforts facing the challenge of minimizing hybridization [*Current Zoology* 61 (1): 191–205, 2015].

Keywords Canid, Conservation, Genetics, Hybrid, Management

Hybridization, the interbreeding among distinct taxa, presents a unique challenge for conservation biologists and managers. While hybridization is an important evolutionary process for speciation (Arnold, 1992; Allendorf et al., 2001), hybridization also poses a threat to the conservation of native species, particularly when it is facilitated by anthropogenic alteration of habitats, translocation of species, and excessive exploitation (Wayne et al., 2004). Such human activities have caused a global escalation in hybridization, resulting in multiple extinctions of plant and animal populations and species (Rhymer and Simberoff, 1996; Wolf et al., 2001). The need to develop strategies to minimize anthropogenic-driven hybridization is a key conservation challenge (Allendorf et al., 2001).

Hybridization followed by introgression is the most difficult type of hybridization to control and manage (Allendorf et al., 2001). Over time, breeding among hybrids and backcrossing of hybrids and parentals can lead to the formation of a hybrid swarm and the loss of

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the gene pool of one or both parental species (Rhymer and Simberloff, 1996). This process, known as genomic extinction (Allendorf and Luikart, 2007), has been documented as a major threat for a diverse group of plant and animal taxa (McCarley, 1962; Rogers et al., 1982; Dowling and Childs, 1992; Abernethy, 1994; Rhymer et al., 1994), including several species of wild canids (Wayne et al., 2004).

One intensive effort to address the threat of hybridization and introgression has been implemented for the endangered red wolf (*Canis rufus*; USFWS, 1989). This species, first described by Bartram (1791), was listed as endangered in 1967, and starting in 1973 the last known wild individuals were captured and placed in a captive breeding program to avoid genomic extinction due to hybridization with coyotes *C. latrans*. The red wolf recovery effort has been clouded by debate over the taxonomic status and evolutionary history of this species. It has been classified as a distinct species (Nowak, 1979, 2002), a species of hybrid origin due to breeding between gray wolves *C. lupus* and coyotes (Wayne and Jenks, 1991; Roy et al., 1994, 1996), and as member of a third group of independently evolving North American canids called the eastern wolf *Canis lycaon* that includes the Algonquin wolf and wolf-like canids in the Great Lakes region (Wilson et al., 2000, 2003; Kyle et al., 2006, 2007). The grouping of red wolves and eastern wolves as a distinct species was challenged by results from a large-scale genomic survey of grey wolves, coyotes, red wolves and eastern wolves (VonHoldt et al., 2011). Using over 48,000 single nucleotide polymorphism (SNP) loci, VonHoldt et al. (2011) rejected the hypothesis that red wolves were part of a third species group of North American canids and concluded there were only two main groups of canids in North America (coyotes and gray wolves), and red wolves and eastern wolves have a hybrid origin. In response, Rutledge et al. (2012b) argued the VonHoldt et al. (2011) study included insufficient sampling of Algonquin wolves (n = 2) and flawed analyses. After reanalysis of the VonHoldt et al. (2011) data, they concluded that the three species hypothesis grouping Algonquin wolves and red wolves cannot be rejected.

The goal of this study was not to address the red wolf taxonomic debate but instead to evaluate the efforts of the U.S. Fish and Wildlife Service (USFWS) to prevent introgression of coyote genes into the reintroduced wild population. Between 1987 and 1993, the USFWS reintroduced red wolves to the Alligator River National Wildlife Refuge (ARNWR) in northeastern North Carolina to re-establish a free-ranging experimental population (Phillips et al., 2003). The experimental population area (Fig. 1) primarily encompassed the Albemarle Peninsula, which was characterized by a diversity of habi-

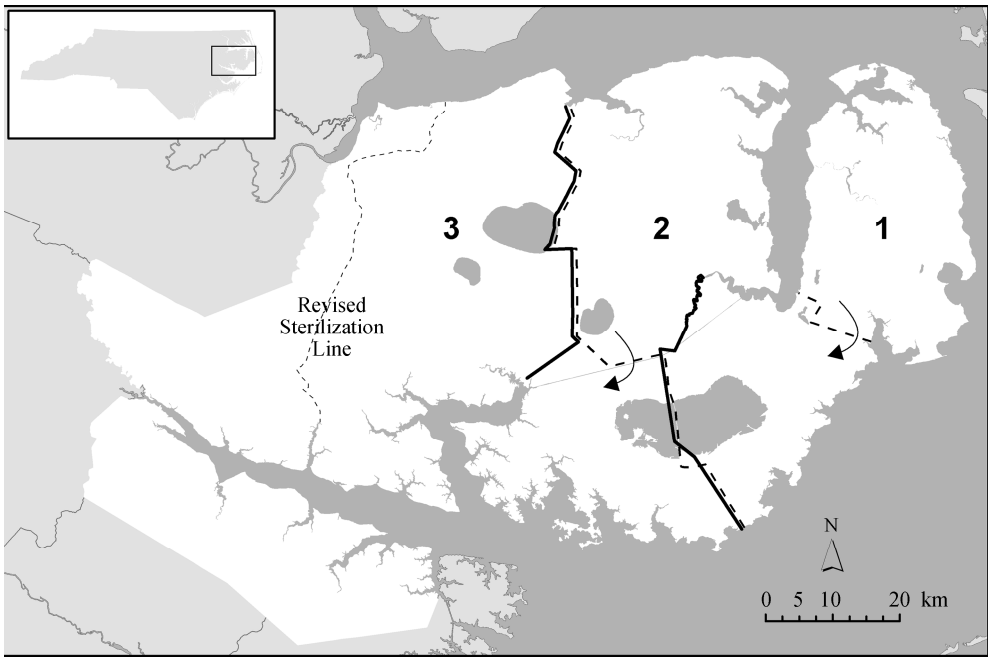

Fig. 1 Historic and current management zones within the red wolf experimental area on the Albemarle Peninsula, North Carolina

In 2002, based on an evaluation of the known spatial distribution of red wolves and non-red wolf canids, the boundaries of the management zones were realigned (dotted lines to solid lines).

tats (Hinton and Chamberlain, 2010; Dellinger et al., 2013). Initially, coyotes were not thought to occupy the experimental population area, but by the early 1990's their presence was documented and shortly thereafter hybridization between red wolves and coyotes occurred (Phillips et al., 1995, 2003; Adams et al., 2003; Adams, 2006). In 1999, a population and habitat viability assessment recognized several threats to establishing a free-ranging red wolf population (Kelly et al., 1999), and the group acknowledged hybridization with coyotes was the greatest risk to recovery of the species. Subsequently, the USFWS adopted a Red Wolf Adaptive Management Plan (RWAMP) to reduce or eliminate this threat (Kelly, 2000).

By its very nature, an adaptive management plan incorporates new or modified procedures as new information becomes available. Such changes in procedures, as well as the amount and geographic distribution of effects, precludes a rigorous quantitative approach, however, we have documented and evaluated the actions taken and their effectiveness. Here we review the results of management actions for the red wolf ARNWR experimental population area from 1993–2013 by evaluating: (1) the numbers of wolves, coyotes, and hybrids captured and monitored each year, (2) the numbers of territorial social groups with presumed breeding capabilities, (3) the number of red wolf and hybrid litters documented each year, and (4) the degree of coyote introgression into the wild red wolf gene pool. If the RWAMP was successful at controlling hybridization and facilitating recovery, we expected (1) an increase in the number of red wolves and the number of canid territories controlled by red wolves, (2) a decrease in the number of hybrid and coyote-like animals occupying the recovery area, (3) more red wolf litters than hybrid litters and a decline in the proportion of hybrid litters over time, and (4) < 10% introgression of coyote ancestry into the wild red wolf population. These results are examined for their implications concerning the future of red wolf recovery, and more broadly, other conservation efforts facing the challenge of hybridization.

1 Materials and Methods

1.1 Field methods

This study occurred within the Red Wolf Recovery Experimental Population Area on the Albemarle Peninsula in northeastern North Carolina (Phillips et al., 2003; Dellinger et al., 2013). During 1993 to 2013, USFWS personnel used padded foot-hold traps to capture all adult (> 9 months old) red wolves, coyotes, and hybrids. Prior to implementing the RWAMP, management efforts concentrated on capturing, radio-collaring, and radio-tracking as many red wolves as possible. In addition, biologists attempted to locate dens and mark pups with microchip "PIT" tags for future identification during subsequent capture operations. At the request of landowners, red wolves were removed from areas where they were not wanted and released at other locales. Coyotes were removed and euthanized when they were encountered.

Conceptually, the RWAMP partitioned the Peninsula into three management zones (Fig. 1), with the most intensive efforts initially deployed in the eastern-most zone and progressing successively westward (Stoskopfet al., 2005). The goals for the eastern-most zone (Zone I) were to radio-collar and release all red wolves, and to remove all coyotes and hybrids. In Zone II the goals were to radio-collar and release all red wolves, and either remove or sterilize (via tubal ligation or vasectomy) and release all coyotes and hybrids at their points of capture. Surgical procedures were performed by a licensed veterinarian following methods described in Seidler and Gese (2012). These sterile animals were left as "placeholders" to defend and maintain their territories (Bromley and Gese, 2001; Seidler and Gese, 2012) with minimal risk to the red wolf gene pool before being removed when there were dispersing red wolves seeking to establish territories, or a red wolf naturally displaced a placeholder. In the remainder of the area (Zone III), Zone II management activities were opportunistically extended westward as resources allowed. In theory, creating a functional red wolf population occupying the entire Albemarle Peninsulawould ultimately saturate the landscape and naturally exclude immigrating coyotes (Kelly, 2000).

Field personnel located radio-collared animals via ground and aerial telemetry every 3- to 7-days to define home ranges and territorial limits, and locate mortalities and identify causes of death. Personnel conducted field surveys to identify areas occupied by unknown canids, translocated red wolves from areas where landowners objected to their presence, located dens to collect samples for genotyping pups, and cross-fostered red wolf pups from captivity to wild parents to augment wild productivity particularly after removing a hybrid litter (cf. Kitchen and Knowlton, 2006). The radio-telemetry data was also used to estimate the proportion of the recovery area occupied by red wolf territories (see online supplemental). Scat sampling for DNA analyses, coupled with location data, was intermittently applied to

provide additional information concerning the genetic characteristics and distribution of canids without capturing and handling animals (Adams et al., 2003, 2007; Adams and Waits, 2007; Bohling, 2011).

1.2 Species identification methods

We defined a red wolf as an individual whose genealogy could be traced directly to the 14 captive red wolf population founders (see online supplemental), or an individual whose genotype contained no coyote-specific alleles and was classified as red wolf using a maximum likelihood assignment test (Miller et al., 2003; Adams, 2006). The genetic assignment test uses a maximum-likelihood approach to compare the genotype of an unknown individual to the allele frequencies of the red wolf founders (with modeled drift) and North Carolina coyotes using 18 nuclear DNA microsatellite loci (Miller et al., 2003). This test considers allele frequency differences, as well as the presence of coyote-specific alleles, which are absent in the red wolf founders but observed in the current coyote population in northeastern North Carolina. Results from the genetic analyses were integrated with data on morphology and parentage to determine whether to retain, sterilize, or euthanize an individual (Stoskopf et al., 2005; Adams, 2006). To be retained in the wild population, animals originally had to have at least 75% red wolf ancestry (Stoskopf et al., 2005). This threshold was raised to ≥ 87.5% red wolf ancestry in 2002. The percentage of red wolf ancestry for each individual was determined in two ways: directly based upon a genetically reconstructed pedigree (e.g., 75% red wolf female x 100% red wolf male = 87.5% red wolf offspring, Adams, 2006) and, in cases where parentage is unknown, from the maximum-likelihood assignment test (Miller et al., 2003). Pedigree analysis methods are described in more detail in online supplemental. For our 2014 sample of known red wolves, 100% can be placed into the pedigree, and the percentage of ancestry that can be traced to the red wolf founders and the proportion of coyote introgression are estimated from the pedigree.

1.3 Assessment of progress

Our assessment of population numbers relies on the number of radio-collared canids ≥ 5 months old known to be alive on 1 March and 1 September each year, 1993–2013. Individuals not identified as being alive on or after specific inventory dates were subsequently censored after that date. By design, the RWAMP was flexible and adaptive (Kelly, 2000). Consequently, we provide results from a management process in which data interpretations are confounded by changes in procedures as well as changes in the geographic distribution of efforts. An example is the more stringent criteria adopted for genetically discriminating between red wolves and hybrids in 2002 (Miller et al., 2003), forcing re-evaluation of all current and former animals in each management zone. Also in 2002, based on an evaluation of the known spatial distribution of red wolves and non-red wolf canids, the boundaries between zones were moved westward, enlarging Zone I and decreasing the size of Zone III (Fig. 1; Stoskopfet al., 2005). Results and interpretations that follow are presented in accord with the zone boundaries recognized in 2007 rather than those accepted at times during which specific management actions were taken. Similarly, the more conservative assignment of genetic ancestry, based on microsatellite genotyping adopted in 2002, is used for animals from all years.

2 Results

2.1 Summary of population management

In the 6 years preceding adoption of the RWAMP, the average number of canids captured for the 1st time ("1st captures") was about 28 per year, and most (75%) were retrospectively identified via genetic analysis as being red wolves (Table 1). During 1999–2013, the number of first captures averaged 63.5 per year, but during this time the proportion of red wolves declined and that of coyotes increased (Table 1).

Table 1 Numbers, by genetic assignment, of adult canids captured for the first time on the Albemarle Peninsula, North Carolina, during four periods, 1993 through 2013

Period	No. canids captured	Mean No. captures/yr.	Mean No. by genetic assignment (%)		
			Red wolf	Hybrid	Coyote
1993–1998[1]	167	27.8	20.8 (75)	2.8 (10)	4.2 (15)
1999–2000[2]	129	64.5	40.5 (63)	16.5 (26)	7.5 (11)
2001–2002[3]	87	43.5	26.5 (61)	10.0 (23)	7.0 (16)
2003–2013[4]	735	66.8	22.6 (34)	10.1 (15)	34.1 (51)

[1] Prior to adoption of RWAMP. [2] Post-adoption of RWAMP relying on physical characteristics. [3] Initiation of reliance on genetic testing. [4] Full implementation of genetic testing of all canids.

Prior to adoption of the RWAMP, the number of canids (> 5 months of age) removed from the Peninsula averaged 11.2 per year (6.5 red wolves, 1.0 hybrids, and 3.7 coyotes; Fig. 2A). Red wolves were primarily removed to accommodate landowners, to initiate breeding on island populations and to establish a second release site in the Great Smoky Mountains National Park. Following implementation of the RWAMP, 13–63 ($\bar{x}$ = 28.2) canids were removed per year. As the years progressed, the genetic classification of animals that were removed changed, with red wolf captures declining and numbers of hybrids and coyotes removed increasing dramatically (Fig. 2A). The high incidence of red wolves removed in 2000 and 2001 (12 and 11, respectively) occurred while management efforts increased substantially but prior to implementing use of genetic criteria for assessing ancestry. Between 2004 and 2013, the number of red wolves removed declined while the removal of animals with coyote ancestry increased (Fig. 2A).

No animals were sterilized prior to 1999, but after that 252 animals were sterilized and released, including 3 red wolves inaccurately classified as hybrids before genetic testing (Fig. 2B); 35 of these occurred in the

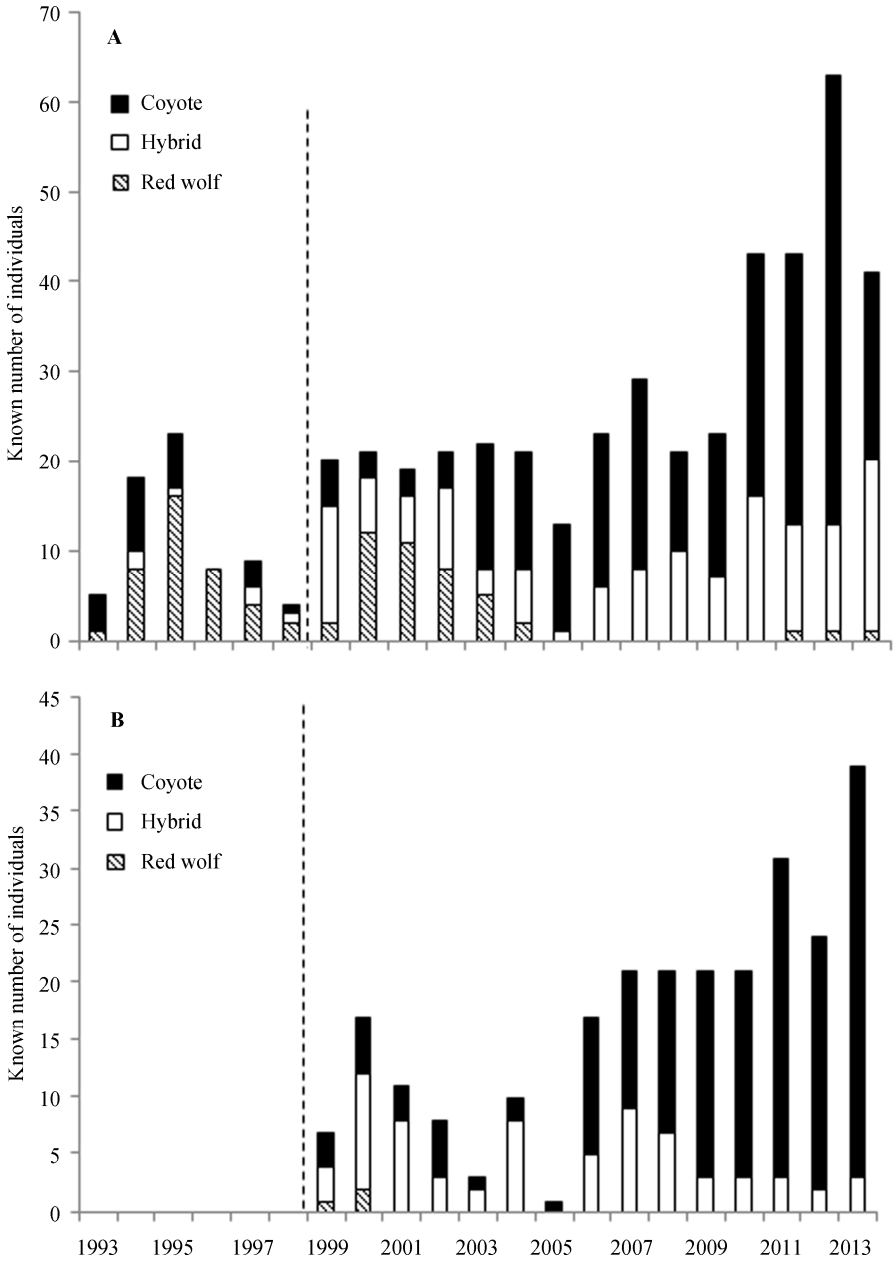

Fig. 2 Numbers of canids (A) removed,and(B) sterilized and released, by genetic classification and year, within the red wolf experimental area, Albemarle Peninsula, North Carolina, 1993–2013

Vertical lines represent initiation of the Red Wolf Adaptive Management Plan.

first 3 years of the RWAMP. The number sterilized was relatively small (1–10 per year) between 2003 and 2005. In 2006 as efforts increased toward the west, 17 animals were sterilized. During 2007–2013, an increasing number of coyotes were sterilized to serve as "placeholders" to hold space on the landscape and prevent genetic introgression (Fig. 2B). Many of these sterilized animals were eventually removed from the population (n = 19) when red wolves appeared to be seeking new territories in areas occupied by sterile animals. In addition, many of these sterile animals were naturally displaced (n = 50) by red wolves.

Other types of management actions were sporadically employed. An additional 41 wolves born in captivity or on island propagation sites were released within the experimental population area, 29 prior to 1999 and 12 afterwards. Between 1999 and 2013, 27 captive-born red wolf pups were cross-fostered into wild litters to augment wild recruitment and enhance genetic diversity after removing a hybrid litter. All cross-fostered pups were accepted by the wild, surrogate parents and at least seven became breeders responsible for 98 red wolf pups born from 2004 to 2013 (A. Beyer, USFWS, unpubl. data).

2.2 Canid population demography and social groups

Sixty-three red wolves (32 adults and 31 juveniles) were released on the Alligator River National Wildlife Refuge (within Zone I) between 1987 and 1994 (Phillips et al., 2003). Fourteen of the releases (11 adults and 3 juveniles) were considered successful and breeding was documented in the wild. Our initial census indicates 33 red wolves known to be present in March 1993 (22, 8, and 3 in Zones I, II, and III, respectively; Fig. 3A). Between 1993 and 1998, 125 additional red wolves > 5 months of age ($\bar{x}$ = 20.8 annually) were captured (Table 1), with the spring 1999 census indicating 52 red wolves within the experimental area (22, 18, and 12 in Zones I, II, and III, respectively; Fig. 3A). During the same 6-year period, 43 red wolf litters were located.

In the first 2 years after implementation of the RWAMP, 81 additional red wolves were captured, plus another 303 red wolves in the ensuing 13 years. Despite the large number of potential recruits to the population, in the next 3 years the census of known living red wolves only increased to 85–90 ($\bar{x}$ = 86.7) animals in the fall, with slightly lower numbers ($\bar{x}$ = 77.0) in spring (Fig. 3A). Thereafter, the known number of free-ranging red wolves across the recovery area has remained relatively stable at around 90–95 adult red wolves.

The relative distribution of red wolves on the landscape changed over time. Both the number of wolves (Fig. 3A) and the number of social units in Zone I declined to about half after implementation of the RWAMP (Fig. 3B), without evidence that hybrids and/or coyotes had appropriated those territories. In Zone II, known numbers of red wolves increased from around 30 to perhaps 50, while an increase from 15 to 25 occurred in Zone III (Fig. 3A).

Coyotes have increased in numbers of first captures (Table 1), numbers removed (Fig. 2A), and numbers sterilized (Fig. 2B) during the recovery effort. During inventories for all intact canids on the Albemarle Peninsula, most coyotes captured and identified were removed and were not alive at our inventory dates, or were sterilized and released. Coyotes were routinely removed in small numbers during the pre-RWAMP period (Fig. 4B) with an increasing number of coyotes being removed throughout the recovery area. Only sterile coyotes were documented in our inventories; intact coyotes were removed. Since 2009, extensive trapping efforts in Zones II and III have resulted in removal of 15–41 ($\bar{x}$ = 24.0) coyotes annually (Fig. 4B). The attempt to capture and genotype all *Canis* on the Peninsula, starting in 1999, resulted in a dramatic surge in the number of hybrids removed, principally in Zone II (Fig. 4A). Additional hybrid individuals were regularly removed, mostly in Zones II and III. Another surge in hybrid removal followed adoption of the more stringent genotype criteria in 2002, resulting in removal of 9 hybrid individuals, including 7 within Zone I (Fig. 4A). Subsequently, the number of hybrids removed declined erratically (Fig. 4A) with surviving individuals being removed from Zone I and increased removals from Zones II and III.

The number of recognized red wolf social groups increased from 5 in 1993 to 14 by 1999 (Fig. 5D). Subsequently, this increased to about 20 social units between 2003 and 2008 (Fig. 5D) and then declined to about 15 social units during 2009 to 2013 as breeding pairs have been disrupted by gunshot mortalities associated with coyote hunting in the recovery area during the past several years (USFWS, 2009–2013). In Zone I, the number of social units increased from 4 in 1993 to 10 by 2001, where it remained through 2003 but then dropped to 5 by 2005, and subsequently declined to 2 breeding units during 2011–2013 (Fig. 5A). The change in known numbers of desirable social units in Zone II from one in 1993 to 10 in 2004 was associated with an intermediate shift to "neutral" social units associated with the sterilization of one or both alpha animals (Fig. 5B). The

known number of wolf social units in Zone III was relatively stationary (12) until implementation of the RWAMP. As in Zone II, it appears the use of sterilization assisted in an increase to 5–6 social units with desirable red wolf ancestry (Fig. 5C).

2.3 Summary of genetic results

As the number of radio-collared animals increased, so did the location of natal dens (8.5/yr before RWAMP adoption to 12.6/yr afterward). Genetic assessment of litters indicated the number of hybrid litters fluctuated over time (0–5/yr) with an average of 1.5/year (Fig. 6). The number of red wolf litters per year was always higher than the number of hybrid litters and averaged 6.9/year (Fig. 6). The ratio of hybrid to red wolf litters

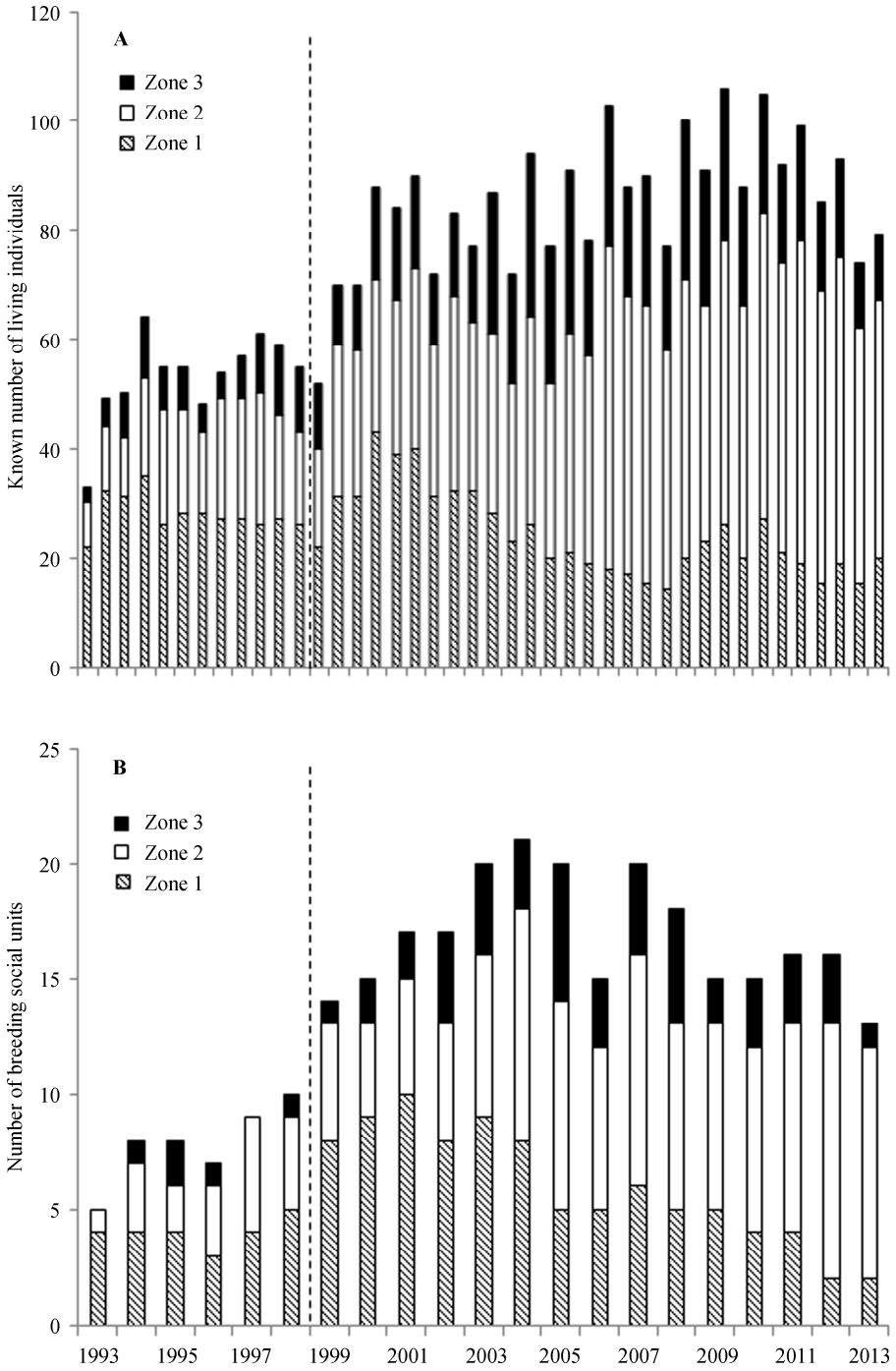

Fig. 3 Known numbers and distribution of (A) red wolves during spring (March 1st) and fall (September 1st) inventories, and (B) known red wolf social units in spring, among management zones within the red wolf experimental area on the Albemarle Peninsula, North Carolina, 1993–2013

Vertical lines represent initiation of the Red Wolf Adaptive Management Plan.

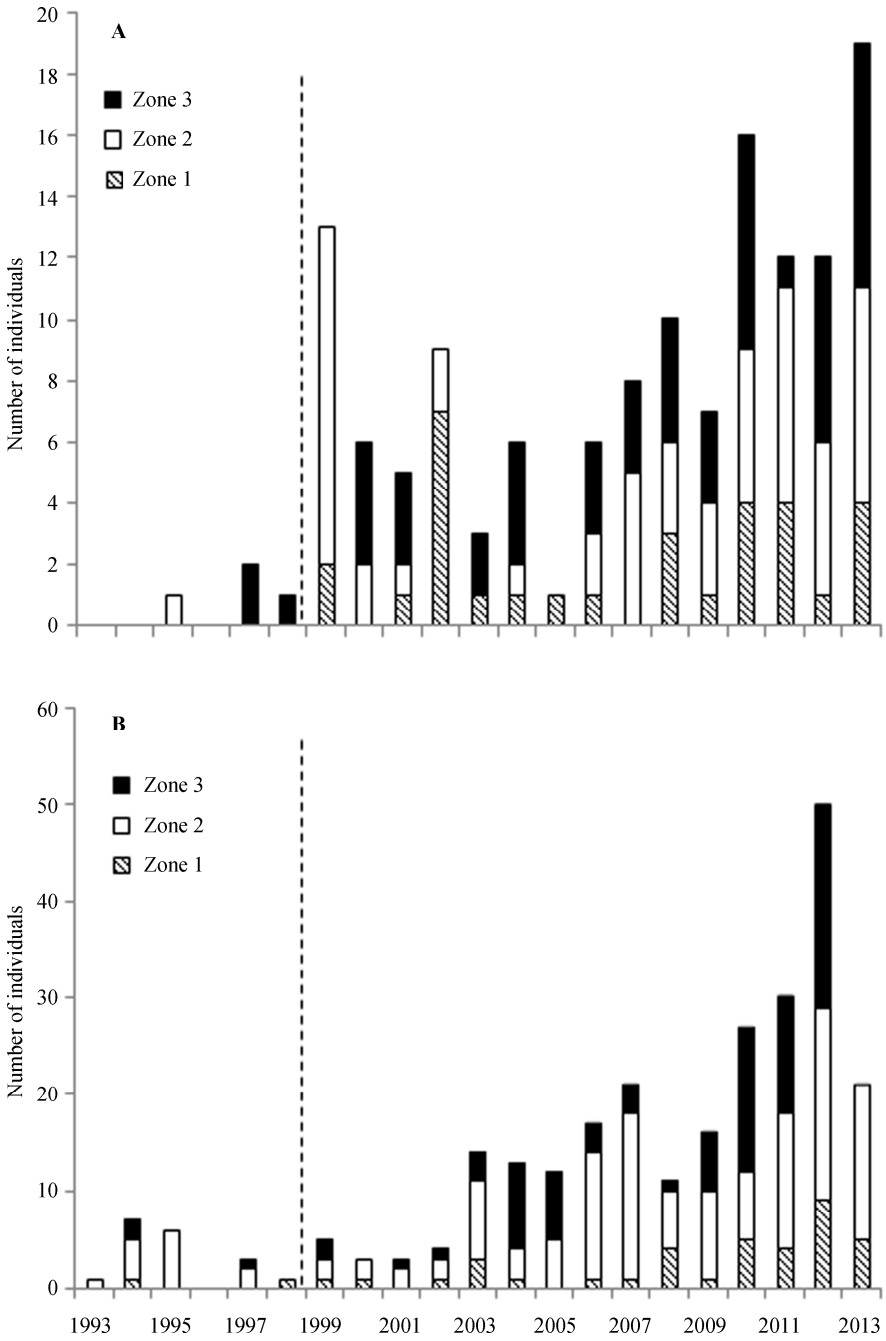

Fig. 4 Numbers of (A) hybrids removed, and (B) coyotes removed, by zone and year, from the red wolf experimental area on the Albemarle Peninsula, North Carolina, 1993–2013

Vertical lines represent initiation of the Red Wolf Adaptive Management Plan.

averaged 21% and peaked at 55% in 2006 (Fig. 6). Overall, 37 of the 40 (92.5%) litters with coyote ancestry were detected and removed, while 7 of 147 (4.8%) red wolf litters were mistakenly removed before genetic testing.

Retrospective molecular genotyping suggested the known number of free-ranging reproductively-intact hybrids alive at any inventory point in the pre-RWAMP period never exceeded two. No reproductively-intact hybrids were noted at any inventory date from 2004 through 2013 (i.e., all known hybrids were removed or sterilized). The average ancestry of all known, reproductively intact red wolves and introgressed individuals in the recovery zone in 2014 is 96.5% based on genetic testing and pedigree analysis.

3 Discussion

3.1 Success of current program

Minimizing the threat of hybridization for threatened and endangered species is particularly challenging when

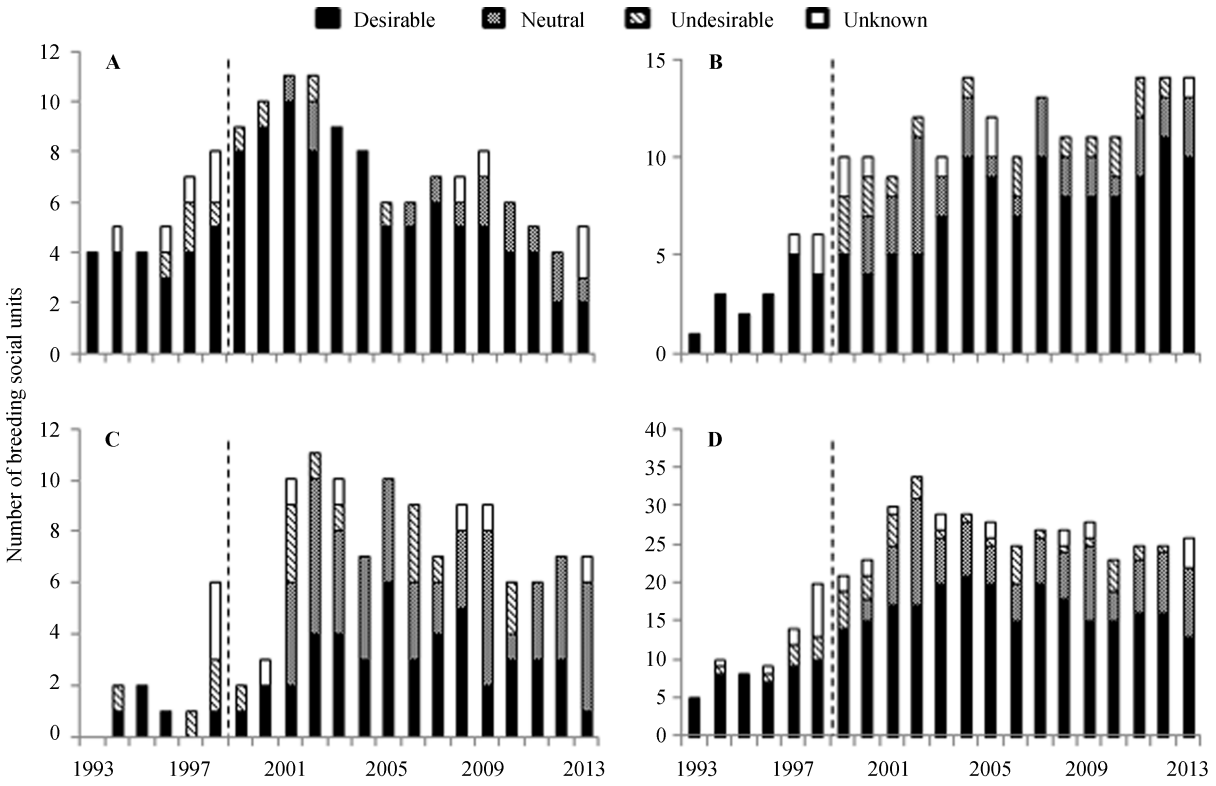

Fig. 5 Numbers and suitability of canid social units in Zones (A) I, (B) II, and (C) III, and (D) the entire red wolf experimental area, Albemarle Peninsula, North Carolina, 1993–2013

"Desirable" indicates the alpha male and female individuals are ≥75 % red wolf ancestry; "neutral" indicates one or both alpha individuals are sterile; "undesirable" indicates both breeding individuals are reproductively intact and one or both are genotypically identified as coyote or hybrid; and "unknown" indicates that the genotype of one individual of the breeding pair is unknown. Vertical lines represent initiation of the Red Wolf Adaptive Management Plan.

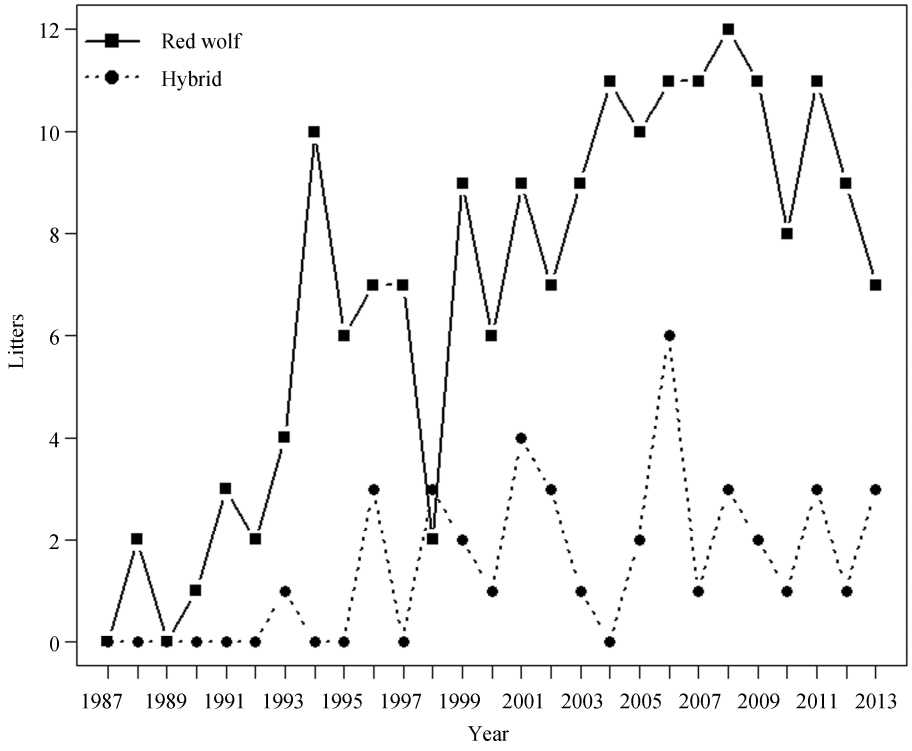

Fig. 6 Number of red wolf and hybrid litters detected each year since the reintroduction of red wolves into North Carolina

the hybridizing species greatly outnumbers the threatened population (Allendorf et al., 2001) as with red wolves and coyotes in North Carolina. The success of the RWAMP at controlling hybridization and facilitating red wolf recovery was mixed, based our criteria. The number of red wolves did increase over time but plateaued around 2009 and declined slightly thereafter. The number of coyotes and hybrids detected did not decrease over time as desired. Despite predictions of genetic swamping (Kelly et al., 1999; Fredrickson and Hedrick, 2006), our estimate of average ancestry of all known, reproductively intact red wolves and introgressed individuals in the recovery zone in 2014 is currently 96.5% indicating the success of the RWAMP at limiting introgression of coyote genes into the reintroduced population. We also documented more red wolf litters than hybrid litters, but the ratio of hybrid litters to red wolf litters did not decline over time indicating hybridization is an ongoing challenge.

The RWAMP is an intensive long-term management effort that includes removal of coyotes and hybrids, sterilization and release of others to control space (i.e., the "placeholder" concept), the release of red wolves from captive-breeding programs, genetic testing of litters, cross-fostering captive born pups to wild parents, and a public relations effort to promote the recovery program and reduce anthropogenic mortalities. It is difficult to speculate about the relative contribution of individual activities, but we consider the removal, as well as sterilization and release, of coyotes and hybrids as critical components. Another key management activity has been the genetic testing of wild born litters to provide the opportunity to remove hybrids before they reach breeding age. Although such activities were not a part of the original recovery effort, they now constitute a core component of the program, and in the absence of such efforts it seems unlikely that introgression of coyote genes into the red wolf population could be adequately controlled (Fredrickson and Hedrick, 2006). We recognize the potential biases of monitoring hybridization based on capture efforts alone and suggest complementary, non-invasive sampling of scats (Adams and Waits, 2007; Bohling and Waits, 2011) to assess the genetic composition and distribution of canids. In 2010, this type of analysis was conducted in the recovery area and revealed that 1) only 4% of samples had hybrid ancestry, and 2) red wolf ancestry was highest in zone 1 (> 80%) and decreased from East to West (Bohling, 2011) consistent with results from the trapping efforts presented here.

3.2 Implications for future management of red wolves

The U.S. Fish and Wildlife Service continues to actively promote recovery efforts of the red wolf in eastern North Carolina (USFWS, 2007; Hinton et al., 2013). These efforts are consistent with the conclusion that we should "protect the red wolf as a component of the evolutionary legacy of canids" (Allendorf et al., 2001), and recent analyses of North American canids indicating this species has a distinct genetic signature (VonHoldt et al., 2011; Rutledge et al, 2012b). We acknowledge that these efforts have required considerable financial and social investments each year (USFWS 2013), and the population is not self-sustaining. In theory, efforts to remove or sterilize coyotes might be relaxed with time as red wolves fully occupy available habitat within the recovery area. Under such conditions, wolves dispersing within the recovery area would be successful in finding conspecific mates and coyotes immigrating to the area would be naturally excluded by resident wolves (Murray and Waits, 2007; Roth et al., 2008; Wheeldon et al., 2010). However, we believe this scenario is unlikely because wolf habitat is discontinuous within the recovery area and anthropogenic habitat changes will continue to favor coyotes because of their ability to more effectively colonize landscapes in closer proximity to human activity (Benson et al., 2012; Gese et al., 2012; Benson and Patterson, 2013). Further, there is little evidence red wolves naturally control the coyote population through strife, which is a core prediction derived from the competitive exclusion hypothesis (Murray et al., 2015). However, it is notable that recent records also report gunshot mortality remains prevalent for coyotes, indicating that mistaken identity by coyote hunters could continue to disrupt red wolf breeding pairs. Yet, a recent legal ruling banning coyote hunting in the recovery area (Red Wolf Coalition et al., v. Cogdellet al., No. 2:13-cv-60-BO, 2014 WL 1922234 [E.D. N.C. May 13, 2014]) may help promote stability of red wolf social groups.

While the wolf population had a relatively high baseline mortality risk relative to other wolf populations (Fuller et al., 2003; Smith et al., 2010) and the majority of deaths were related to anthropogenic activities, it does not appear the additive nature of human-related mortality exceeds that observed in other wolf populations (Creel and Rotella, 2010; Murray et al., 2010; Sparkman et al., 2011). However, anthropogenic mortality can lead to increased hybridization in other canid systems (Rutledge et al., 2012a). In red wolves, over half of the detected hybridization events followed the

disruption of a stable breeding pair of red wolves due to mortality of one or both breeders (Bohling, 2011). Of these 69% were due to anthropogenic causes, primarily gunshot mortality during the local fall hunting season, which occurs just prior to the red wolf breeding season (Bohling and Waits, press).

The number of known wolves appeared to plateau at around 90 to 95 adult red wolves, indicating the population may have reached carrying capacity, as also suggested by Murray et al. (2015). In 2007, red wolf social units were using about 1,043 km^2 – about 48% of "usable" (cumulatively used) habitat and about 23% of the total recovery area (USFWS 2007, online supplemental), but the remainder of acceptable habitat is fragmented in small patches located across the recovery area and less likely to be colonized by wolves given recent habitat studies (Dellinger et al., 2013). In addition, we consider expansion of the red wolf population beyond the current recovery area unlikely given recent survey results showing few red wolves in adjacent areas (Bohling and Waits, 2011). The current USFWS recovery goals require establishing 3 independent populations (USFWS, 1989), and such efforts would require a rigorous assessment of red wolf habitat availability, combined with empirical and modeling analysis of coyote abundance and potential hybridization, in candidate recovery areas. If reintroduction efforts are initiated in new geographic areas, the management actions for controlling hybridization described here will likely be critical to success as most of the historical red wolf range is now occupied by coyotes. Given the extensive loss of habitat and the challenge of hybridization with invasive coyotes, the red wolf is a species fitting the definition of "conservation reliant" (Scott et al., 2005), and the ongoing program review should be considered an opportunity to chart a new direction that reflects the changing standards and expectations regarding endangered species recovery (Scott et al., 2010; Jackowski et al., 2014; Murray et al., 2015).

3.3 Implications for other species

Our assessment suggests that access to appropriate resources can curtail or reverse genetic introgression in some situations. Our data indicate the use of sterilization and the removal of hybrids to limit introgression of unwanted coyote genes has enhanced effectiveness of red wolf recovery efforts. Red wolves are relatively long-lived, territorial, form social hierarchies, and develop strong and persistent social bonds. This enables the use of sterile individuals of the introgressing species and hybrids to control space without compromising the status of the target species. In our case the introgressing species, the coyote, is abundant and adaptable to human-modified landscapes. While procedures similar to those used in the RWAMP might work in the case of European gray wolves or Ethiopian wolves *Canis simensis*, there could be additional social conflicts because domestic dogs represent the introgressing species. Perhaps more realistically, the population of eastern wolves in Algonquin Provincial Park (Patterson and Murray, 2008) ultimately may benefit from removal of hybrids occurring in the same region, especially given the unique genetic and taxonomic status of wolves inside the park (Rutledge et al., 2010). Similar considerations might apply for conserving the European wildcat *Felis silvestris*, with the added caveat that felids may not have as persistent social bonds and strong territorial constraints common among many canids, thereby precluding some of the measures enacted in North Carolina to protect wolves. Reduced social fidelities among cervids (e.g., red deer *Cervus elaphus*), or among aquatic species, may reduce the utility of such efforts.

An important contribution of the RWAMP has been to help elucidate mechanisms of hybridization affecting recovering populations, and to test methods of managing such hybridization to improve chances of recovery success (Murray and Waits, 2007). Another novel management method used for red wolves that might be beneficial in other systems is the genetic testing of litters to remove hybrid individuals and cross-fostering pure offspring from captivity to increase recruitment into the wild population. Aggressive management actions designed specifically to undermine the negative influence of invasive species can enhance population recovery efforts (Peterson et al., 2008; Finlayson et al., 2010), at least over the short-term. Such management, based on intensive and adaptive research, is a much- needed addition for other species threatened by hybridization and introgression (Laikre et al., 2010).

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Supplemental information

Red Wolf Founders

The red wolf founders are the 14 individuals removed from the wild along the Gulf coast of Texas and Louisiana who were chosen, based upon morphology, skull radiographs, sonographic analysis, breeding experiments, and electrophorectic and chromosomal analysis, to initiate the captive breeding program (Carley, 1975; Riley and McBride, 1975). These individuals also have a unique mitochondrial DNA haplotype that has not been observed in coyotes (Adams et al., 2003).

Pedigree Analysis Methods

Pedigree analysis methods are described in detail in Adams 2006, but are summarized here. Parentage was determined using a combination of field and genetic data. USFWS biologists typically identified potential parents of a newly captured red wolf or litter of puppies based upon observational knowledge of breeding pairs and the proximity of the various red wolf packs. Parents were unknown or uncertain for approximately 25% of captured individuals. Genotypic data at 18 microsatellite loci was used to determine parentage relationships using the program Cervus (Marshall et al., 1998; Kalinowski et al., 2007). We used Cervus to identify the most likely parents from the potential pool of reproductive individuals in the population. We allowed a maximum of one mismatch for a potential parent pair, but only if the mismatch was due to allelic dropout. We also checked all parentage assignments with a 1 allele mismatch to confirm that the pairing was realistic based on detailed field observations and/or telemetry of wolves during the breeding season. Fifteen percent of identified parent-offspring relationships had 1 genotypic mismatch; the remainder had zero mismatches.

Red Wolf Pack Territory Estimates

Using data from 1987–2007, wolf pack territory estimates were generated by including data for every known pack member in a 95% kernel density estimation with a root-n bandwidth estimator (Worton, 1989; Wu and Tsai, 2004; Steury et al., 2010). Locations from all wolves (> 75% ancestry) within a pack were combined for home range estimation, although exploratory and emigrant movements were excluded, and more than one location per pack per day was included only if individual wolves were > 500 m apart (Oakleaf et al., 2006). We considered any habitat that had ever been occupied by a red wolf pack between 1987 and 2007 as “usable habitat”.

The recovery area encompasses about 4,600 km^2 (not including large water features). From 1987 through 2007 wolf pack territories cumulatively covered a total of 2,172 km^2, or about 47% of the total experimental area. In 2007, red wolf social units were using about 1,043 km^2 – about 48% of “usable” (cumulatively used) habitat and about 23% of the total recovery area (Fig. 1).

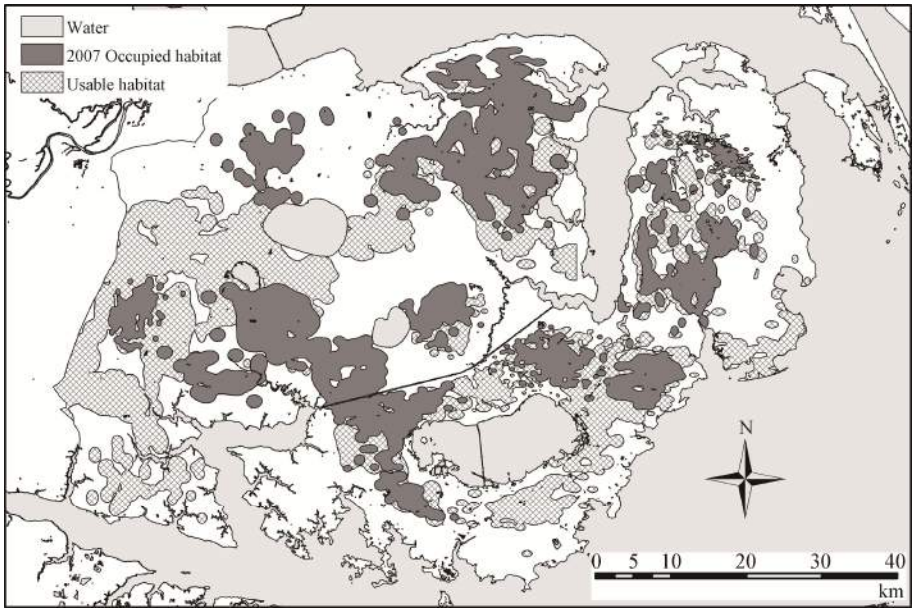

Fig. 1 Availability of red wolf habitat on the Albemarle Peninsula, North Carolina

Usable habitat includes any habitat known to be used by red wolves (1987–2007).

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