Population Viability Analysis of the Red Wolf (*Canis rufus*)

Integrated Management of In Situ and Ex Situ Populations in Support of Species Recovery in a Mixed Canid Landscape


B. Bartel – USFWS

Final Report

22 August 2023

Analysis conducted by

Philip S. Miller, Ph.D.
Director of Science, Single-Species Planning
IUCN SSC Conservation Planning Specialist Group

In consultation with

US Fish and Wildlife Service Red Wolf Recovery Team

Supporting population data analyses prepared by

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Cover photo by B. Bartel, USFWS.

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Executive Summary

In 2020, the US Fish and Wildlife Service (Service) initiated a process for revising the Recovery Plan for the red wolf (*Canis rufus*). As part of this revision process, a population viability analysis (PVA) was to be developed in order to assist with the creation of science-based recovery criteria and site-specific actions for achieving long-term demographic and genetic recovery of the species in the wild. When the PVA was initiated, approximately 15 wild red wolves were known to survive in the five-county area of the Albemarle Peninsula, northeastern North Carolina (hereafter referred to as the Eastern North Carolina Red Wolf Population, or ENCRWP). This remnant population is the outcome of a significant rate of population decline that began in the mid-2010s. Major threats to the species include unsustainable mortality resulting from anthropogenic activities (primarily gunshot and vehicle collisions) and hybridization with a much larger population of coyotes that inhabit the ENCRWP habitat. This hybridization threat may lead to the dilution, degradation and ultimate disappearance of the red wolf as a distinct taxonomic entity on the landscape.

This analysis was to build upon and extend a 2015-2016 PVA effort that examined a suite of scenarios for promoting viability of both the in situ (wild) and ex situ (captive, now SAFE) red wolf populations through careful demographic management and releases of captive-bred animals. The current PVA effort brought forward two important additions to previous effort: the explicit addition of coyotes and their demographic interactions with red wolves, and the intention to explore the conditions necessary to establish new red wolf populations through release of additional animals from the SAFE population to habitats thought suitable for red wolf reintroduction. The present analysis does not include canid populations found in Texas and Louisiana that may have red wolf ancestry, as research addressing this issue is ongoing.

The PVA model was built in the software package Vortex, an individual-based model used around the world to evaluate the impact of threats to the future growth and stability of small populations of endangered species and the potential for future improvements to species status through implementing alternative management actions. The Vortex software package used to create this demographic simulation model has a number of features that fit well with the needs of this recovery planning effort, including a highly flexible structure for adapting the basic model structure to the characteristics of the species of interest. Additionally, an extensive capacity exists within the software to simulate the intensive management of captive (ex situ) populations serving as a source for release to the wild. However, the software does not always have the capacity to simulate complex social breeding systems in species like wolves that have an explicit pack structure. Consequently, some simplifying assumptions must be made when customizing the tool to the characteristics of the species being assessed.

The red wolf – coyote system with its prominent hybridization process presented particular challenges to traditional methods of PVA model development that focus on a single species in a simpler demographic environment. Technical subgroups of the newly-formed Red Wolf Recovery Team were formed, and engaged in extensive discussions around the available data and information about red wolf biology, demography, and population genetic structure in order to create input datasets for the modeling process. Based on the valuable outcomes of these discussions, a protocol was devised whereby each

individual was assigned an "ancestry" tag that defined its taxonomy – red wolf, coyote, or hybrid – on the basis of the taxonomy of its parents. In this way, the PVA was capable of tracking the abundance of each taxonomic unit within the same interbreeding population. Complex rules were developed that calculated likelihood of pair formation and litter production as a function of taxonomic identities of the mates; moreover, these rules were sufficiently flexible to allow for modifications to function parameters that attempted to replicate the outcomes of management strategies designed to improve opportunities for red wolf pair formation, reproduction, and population growth. Extensive analysis of data on breeding and survival rates of red wolves in the ENCRWP since 2000, and in the SAFE population since 1990, formed the basis for detailed model input datasets. Vortex then projected forward in time the current red wolf and coyote population, beginning 1 January 2022 and continuing for 100 years. Key model output includes the probability of red wolf population persistence or extinction (disappearance of the distinct taxonomic entity), the average size of persisting red wolf populations, and the extent of gene diversity retained as a proportion of the original diversity brought in from the wild to begin the captive population in the 1970s.

A baseline analysis of the ENCRWP as of 1 January 2022 included an initial abundance of just 12 red wolves and a preliminary estimate of 500 coyotes occupying the red wolf recovery area in northeastern North Carolina. This model assumed no future additions of animals from the SAFE population, designed as a control scenario to then more clearly evaluate the potential benefits to be gained by releases from the SAFE population. If there were no changes in the average rates of red wolf breeding or survival from rates estimated at that time, this baseline analysis indicated that the very small remnant population of red wolves is not capable of surviving for more than 10 to 20 years. This is an even more dire prediction of future population persistence than the previous PVA which started with 74 red wolves as of 1 January 2015 and predicted population extinction within approximately 35 years from that starting point. Overall, demographic conditions are now even less favorable for red wolf stability than they were less than a decade ago, which is reflected in the updated model results.

A set of scenarios was then evaluated that portrayed a suite of potential management actions designed to directly improve red wolf breeding and/or survival. Across nearly all scenarios, captive-born red wolves (four to six adults and six to eight pups) from the SAFE population were chosen for release annually to the ENCRWP population over a period of 20 years and were, in some scenarios, followed by periodic reinforcement of pups through year 50. These releases increased population abundance during the release period, but population declines returned immediately after cessation of the release efforts. Specific actions designed to improve rates of wolf-wolf pairing and the likelihood of litter production among those pairs in the presence of release efforts did not lead to measurable improvements to the prospects for red wolf population growth without additional management actions. Mitigation of anthropogenic mortality was more effective in promoting population growth, but was not also successful as a means of facilitating long-term viability – with continued hybridization of red wolves with coyotes identified as the primary cause of the renewed decline.

Direct management of coyote reproductive output – primarily in the form of sterilization of intact coyotes over 25 years in a manner that allowed them to retain the ability to form pairs and occupy territories but fail to produce offspring – was shown overwhelmingly to foster reduction in the coyote breeding population, to open up the possibility of more frequent wolf-wolf pair formation and, consequently, to improve the chances of red wolf population growth and long-term viability. Increasing the duration of pup releases from the SAFE population further improved conditions for red wolves in the ENCRWP population. The model was very sensitive to the level of coyote sterilization, with a major improvement in red wolf viability resulting from an increase in coyote sterilization rate from 5% to just 10%. The success of coyote management, of course, is strongly dependent on the overall abundance of coyotes on the landscape. A set of models was constructed in which the initial abundance of coyotes in northeastern North Carolina was increased to 1000 or 2000 animals. These estimates are informed by reported numbers of coyotes removed by hunters and trappers across northeastern North Carolina. The prospects for ENCRWP recovery are reduced in models featuring these larger coyote abundances, even with targeted management of reproductively intact animals over a 25-year period.

Long-term viability of the red wolf population, and of the source SAFE population, is also defined in terms of the extent of gene diversity retention over time, with the goal of maximizing that rate of retention over a period of 100 years. The SAFE population can best contribute to the continued demographic and genetic viability of red wolves in the wild by removing current space restrictions and increasing the carrying capacity across participating breeding facilities from the current value of about 300 to 400 individuals. This will involve creating more enclosures among institutions currently participating in the SAFE program, and/or bringing additional facilities into the program. Even more importantly, the SAFE program would greatly benefit from an effort to improve the reproductive output of the population, producing more litters among paired females so that opportunities for releasing a larger number of animals to the wild are expanded and the retention of population-wide gene diversity is increased. A proportional decrease in the litter production failure rate among paired females of just 15% annually can contribute greatly to achieving this goal.

The current draft Recovery Plan identifies the goal of maintaining three distinct red wolf populations within the species' historic range to ensure both redundancy as protection against catastrophic population loss and broad representation of habitat types across diverse geographies. This analysis also explored a range of conditions under which new populations might be established beyond eastern North Carolina. If the same types of red wolf demographic management interventions recommended for the ENCRWP were implemented in candidate release sites, sustained release strategies such as those constructed here can help foster sustained growth of fledgling red wolf populations. However, this optimistic outcome can be achieved only through thorough and vigilant management of resident coyote populations, should they exist in these sites. Even modest reductions in the intensity of coyote sterilization and removal, particularly in sites that have at least 150 coyotes in an area that can support sustained growth of that population if left to its own devices, can significantly jeopardize the chances for successful red wolf population establishment. The source SAFE population can tolerate the relatively larger release effort needed to establish multiple populations in a relatively shorter period of time if, as with the ENCRWP modeling results, both expansions to available space to accommodate up to 400 animals and improvements to reproductive output (reductions in the proportion of adult females failing to produce a litter) can be achieved.

All of the scenarios explored in this PVA assume that the habitat areas of interest – the northeastern North Carolina habitat area or any hypothetical candidate site chosen for future reintroduction efforts – are effectively isolated with respect to demographic exchange with other canids outside those areas. This assumption might be at least partially justified by the known dynamic whereby established red wolf pairs can effectively displace coyotes from areas now dominated by red wolves and create a barrier of sorts to larger-scale movement of coyotes into a habitat filled with functional red wolf pairs and associated individuals. Nevertheless, assuming a closed demographic system almost certainly overestimates the efficacy of a given level of coyote management effort in the models described here. In an open demographic system, some number of intact coyotes from outside the management area would be expected to move into the area and replace those that have been removed as the coyote population declines.

Conclusions regarding the strength of this immigration effect are explicitly dependent on the assumptions built into our simulation of the process. When revisions to this PVA are undertaken, authorities may consider developing an updated set of scenarios to explore this dynamic and its impacts on red wolf recovery. In the absence of this type of analysis, reasoned logic can be used to recognize that when there is evidence that significant immigration is occurring of reproductively capable coyotes into a habitat that is being managed to reduce coyote breeding success, additional management effort must be employed to offset the presence of those immigrants. This adaptive approach to management is critically dependent on obtaining reliable estimates of standing coyote abundance in a specific defined area and the extent of immigration of coyotes into that area.

Overall, where scenario characteristics overlap across analyses, the results coming from this updated PVA effort are very much in line with those of its immediate predecessor (Faust et al. 2016). Both analyses generate high probability of red wolf population extinction in northeastern North Carolina, with recent observed declines in the wild outpacing those predicted in the earlier modeling effort. Importantly, the earlier analysis also highlighted two critical needs for the SAFE population: (1) to increase SAFE population carrying capacity by adding breeding and holding spaces across a greater number of participating institutions, and (2) to improve breeding success across those institutions to both better maintain genetic diversity through time but to also satisfy the need for larger number of individuals for release. With the additional analyses in this PVA focusing on efforts to establish additional populations beyond northeastern North Carolina, the benefits of expanding the ex situ population and its central role in supporting in situ conservation become that much more evident.

The PVA described here is a valuable platform for highlighting gaps in our knowledge of red wolf and coyote demography, and the complex ways in which these two species interact. Continued collection and analysis of key data – including refinements in red wolf demographic parameters, the intrinsic and extrinsic factors that influence reproduction and survival, demographic responses to threat management, and core characteristics of coyote populations that interact with red wolves – will improve the inputs to future implementations of population viability analysis to guide red wolf recovery.

The analytical results presented here suggest that recovery of red wolves in the wild can be achieved – and can perhaps be realized in 40 to 50 years if conditions are right. However, success will likely require substantial management efforts beyond many of those already implemented by the range of dedicated state and federal management authorities and field biologists already engaged in red wolf conservation. This report will hopefully serve as a valuable blueprint in the conservation toolbox to help the Red Wolf Recovery Team identify key targets for population recovery and detail important actions to make that ultimate goal of recovery a reality.

Introduction

The only species of wolf endemic to the United States, the red wolf (*Canis rufus*) was first officially listed as "threatened with extinction" in 1967 under theEndangered Species Preservation Act of 1966 (32 FR 4001) and remains listed as an endangered species under the Endangered Species Act (ESA) of 1973 (16 U.S.C, 1531 et seq.). The red wolf was declared extinct in the wild in 1980 (Hinton et al. 2013), but an ex situ (captive management) program initiated in the 1970s prevented the disappearance of the species altogether. Reintroduction of the wolves to eastern North Carolina began in 1987; while the program enjoyed early success after documenting breeding among released animals (Phillips and Parker 1988; Phillips et al. 2003), the presence of coyotes first observed in the area in the early 1980s (NCWRC 2018) led to subsequent hybridization with red wolves and a new threat to long-term species recovery. In response to this new threat, the Red Wolf Adaptive Management Plan (Kelly 2000) was drafted to lay out a strategy for mitigating genetic erosion of the red wolf population resulting from this introgression. After releases from the ex situ population (in the form of pup fostering or releases of adults) were significantly curtailed during the period 2010-2020, a sustained effort was renewed in 2021 and continues to today. Despite previous reintroduction efforts and other forms of ongoing management, the red wolf population in North Carolina began to decline rapidly in the absence of releases beginning in 2014 (Figure 1) as mortality from gunshots and vehicle collisions continued to increase and negative interactions with coyotes were becoming more severe (USFWS 2018).

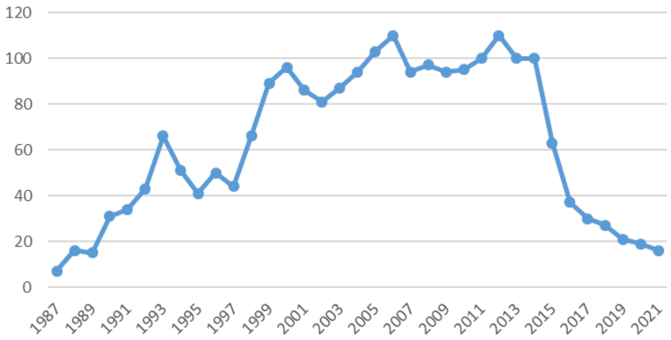


Figure 1. Estimated abundance of red wolves in the Eastern North Carolina Red Wolf Population (ENCRWP), 1987 – 2021. Graphic courtesy of U.S. Fish and Wildlife Service.

The United States Fish and Wildlife Service (hereafter, Service) approved a Recovery Plan (USFWS 1989) for the species in 1990 which remains in effect to this day. Despite the age of this core recovery document, significant efforts have been directed over the past few decades towards improving the status of the species in its historic range. A Population and Habitat Viability Assessment (PHVA) workshop convened by the Service identified key threats to the species and developed a relatively simple population viability analysis (PVA) to evaluate future resilience of red wolves in the remnant population in northeastern North Carolina, hereafter referred to as the eastern North Carolina red wolf population or ENCRWP (Kelly et al. 1999). In particular, this workshop clarified the acute threat of introgression with coyotes to long-term persistence of red wolves as a distinct taxonomic entity, ultimately prompting the development of an adaptive management strategy to reduce hybridization and retain red wolves on the landscape (Kelly et al. 2000). More recently, a detailed PVA (Faust et al. 2016) explored alternative scenarios for effectively integrating the ex situ (captive) and in situ (wild) populations to improve both demographic and genetic viability in the wild ENCRWP without compromising ex situ population stability. The Service used the results of this analysis in creating their Species Status Assessment (SSA) that reviewed current knowledge of red wolf biology and conservation and the conditions necessary for improved viability in the wild (USFWS 2018). The publication of this SSA was soon followed by a National Academy of Sciences document that, after thorough review of the available scientific information, confirmed the taxonomic identity of the red wolf as a valid species (NAS 2019).

In 2021, the Service embarked on a process to update the red wolf Recovery Plan, which is now in draft form (USFWS 2022). This updated Plan is to include specific recovery criteria that help to define conditions for recovery of the species in its historic range. In order to derive these criteria, the Service requested an updated and expanded PVA that would address some key aspects of wild red wolf population dynamics not explicitly included in the 2016 analysis – namely, interactions with coyotes and the implications of hybridization on red wolf taxonomic integrity, and the characteristics of reintroduction efforts to establish red wolf populations in new sites beyond northeastern North Carolina. The Service then contracted with the Conservation Planning Specialist Group, of the IUCN's Species Survival Commission, to conduct the PVA in consultation with the Red Wolf Recovery Team.

The analysis was designed to address the following key questions:

- What are the demographic targets for each population (e.g., adult abundance, number of packs, population growth rate) that would constitute viability?
- Can we identify threshold rates of anthropogenic mortality (e.g., shooting deaths) that should not be exceeded if wild populations are to grow in size and become viable?
- Can we identify coyote management parameter targets (abundance, sterilization efforts, etc.) that must be achieved to secure demographic and/or genetic viability of red wolf populations?
- How should releases be conducted in order to maximize the chances of successful population establishment at a given site?
    - Number of release events
    - Interval between release events
    - Number of individuals in each release
    - Age/sex of released individuals
- Can the target abundance of the captive (SAFE) population adequately support recommended releases to the wild, while also remaining demographically and genetically viable on its own?

This report describes that expanded analysis and how the results can assist with development of effective criteria to guide red wolf recovery.

General Approach to PVA Model Development

Collaborative work on the analysis began in February 2022 with a series of online (virtual) sessions with the contractor and the Recovery Team. These sessions helped to shape the overall purpose of the analysis, develop the key questions for consideration in the analysis, and to gather the appropriate information to serve as input to the evolving PVA model. At the conclusion of these sessions, four expert subgroups were formed to continue more in-depth discussions on the following topics: Red Wolf Taxonomy/Genetics; Wild Canid Demography; Disease Pathogen Identification and Prioritization; and Ex Situ (Captive) Population Dynamics. The lead contractor also solicited the assistance of experts in population data analysis from the Alexander Center for Applied Population Biology (Lincoln Park Zoo, Chicago IL) and the Association of Zoos & Aquariums (AZA) Reproductive Management Center (Saint Louis Zoo) to coordinate development of input parameters for both wild and captive components of the model. Once these work streams were in place, occasional online meetings were held between May 2022 and April 2023 to update the Recovery Team on data analysis progress and the current status of PVA model development and implementation.

The software package chosen to conduct the PVA was Vortex (version 10.6.0), a stochastic individual-based simulation of endangered species population dynamics (Lacy and Pollak 2022). Vortex models population dynamics as discrete, sequential events that occur according to probabilities that are random variables following user-specified distributions. *Vortex* simulates a population by stepping through a series of events that describe an annual cycle of a typical sexually reproducing, diploid organism: mate selection, reproduction, mortality, increment of age by one year, dispersal among populations, removals, supplementation, and then truncation (if necessary) to the carrying capacity. An earlier version of Vortex was used by Faust et al. (2016) in their red wolf PVA, so an element of continuity was maintained across modeling efforts. In addition, the choice of Vortex was based on a number of advantages inherent to its design, including:

- Individual-based model structure allows for explicit simulation of random (stochastic) variation in annual birth and death rates among age-sex classes, and the impact of this variation on the growth dynamics of small populations.
- Ability to define individual, population, or global state variables that can be used to derive complex expressions for age/sex-specific demographic rates, which can change over time in response to evolving environmental conditions, management regimes, etc.
- Capacity for incorporating metapopulation structure, with multiple populations that are linked by occasional dispersal and distributed across a landscape.
- Incorporation of detailed genetic processes, such as mating between known relatives (as tracked through kinship calculations between selected parents) leading to inbreeding depression, or erosion of genetic diversity over time through random genetic drift.
- Explicit linkage of an existing captive (initially Species Survival Plan®, or SSP; now Saving Animals from Extinction, or SAFE) population with the wild population through designated releases of specific individuals determined by genetic or demographic criteria.

For more information on the features of Vortex and its application to wildlife population management, see Lacy (2000; 2019) and Lacy et al. (2021). Despite the software's capacity for simulating a variety of life histories and breeding systems across many vertebrate species, creating models in the defined Vortex environment for species featuring breeding dynamics with distinct social structure or complex spatial patterns of habitat use can be particularly challenging. In those cases, it may be advantageous to develop fully customized simulation or analytical models from scratch using advanced programming tools. Extensive testing of models created in Vortex across a broad range of taxonomic diversity helps to instill greater confidence in the mathematical core of the software and, by extension, the broad insights to be gained through its use.

Specification of canid taxonomy

In addition to the aforementioned linkage between wild and captive populations, creating a type of managed metapopulation, a core feature of the current red wolf PVA is the explicit identification of three distinct taxonomic entities within a single wild canid population: "pure" red wolves, "pure" coyotes, and fertile hybrids resulting from matings between the two. This delineation is made possible by the use of an Individual State Variable (ISVar) that defines the genetic ancestry of offspring ($\mathrm{Anc}_{\mathrm{Off}}$) as the simple linear combination of its parents' ancestry:

$$\mathrm{Anc}_{\mathrm{Off}} = 0.5*(\mathrm{Anc}_{\mathrm{Dam}} + \mathrm{Anc}_{\mathrm{Sire}})$$

If we define a "pure" red wolf with an ancestry of 1.0 and a "pure" coyote with an ancestry of 0.0, a litter of hybrids resulting from their pairing would therefore have an ancestry of 0.5. By giving each individual in the canid population an ISVar "tag" that remains constant throughout its life, we can tally the number of red wolves, coyotes and hybrids that make up the total canid population in a given area. More specifically, experts in the Recovery Team's Taxonomy/Genetics subgroup proposed to classify canids in this system as follows:

Red wolves	Ancestry ≥ 0.75
Coyotes	Ancestry ≤ 0.25
Hybrids	0.25 > Ancestry < 0.75

Extensive statistical analysis by this subgroup using both individuals of known ancestry and a highly diagnostic morphometric – in this case, hind foot length – resulted in correctly assigning ancestry with a high degree of confidence (vonHoldt et al., 2022). Based on this analysis, the threshold was adopted for this PVA. (Note that this choice does not equate to an official decision by USFWS on red wolf ancestry thresholds.) Further discussions within this working group led to the logical conclusion of adopting a symmetrical threshold for coyote ancestry, thereby leading to hybrids occupying a relatively wide range of intermediate ancestry values.

Spatial scope of analysis

All models constructed as part of this PVA feature a metapopulation structure composed of at least two distinct populations. However, where typical metapopulations feature some level of natural exchange of individuals through dispersal or migration, this analysis defines connectivity on the basis of translocation of captive-born animals from the SAFE population to the wild by managed releases. Initially, the existing population in North Carolina (ENCRWP) occupying habitat within the five-county area (Beaufort, Dare, Hyde, Tyrrell, and Washington Counties) is the only wild population within the simulated metapopulation. Additional models include two more “hypothetical” wild populations in order to determine the feasibility of establishing new populations in the species’ historic range through releases of animals from the SAFE population. In this analysis, a “hypothetical” population is one that is not associated with a particular habitat area chosen by the Service or other partners based on systematic ecological and/or socioeconomic assessment of candidate habitat areas. In these models, it is assumed that the wild populations are physically separated from each other so that they do not exchange individuals through dispersal.

Universal features of analysis

All final models described in this analysis were designed and implemented using Vortex Version 10.6.0.0 (May 2023). Specific elements of the software were enhanced to make this complex analysis possible.

The start date for each prospective simulation scenario was effectively 1 January 2022 or, technically speaking, just before the process of pair formation takes place in the early stages of the calendar year. This date was chosen in accordance with the timing of creating the studbook for both the ENCRWP and SAFE populations, and having an end date of 31 December 2021.

Each scenario was projected forward in time from 1 January 2022 over a period of 100 years, i.e., until 31 December 2121. This period of time allows us to examine long-term impacts of management actions (e.g., retention of population gene diversity over a 100-year timeframe) implemented across shorter timeframes. Note that this is a slightly shorter projection window than the most recent PVA of Faust et al. (2016), but is consistent with the 100-year window of recovery defined in the current Recovery Plan. Because of the stochastic nature of the simulation, where random variation in reproduction and survival rates lead to unpredictable outcomes over time, each scenario is repeated 1000 times in order to better assess both likely outcomes as well as the frequency of specific population outcomes such as risk of extinction and retention of genetic diversity.

Summary of Demographic and Genetic Input Data for PVA Simulations

This section provides a general summary of the input used for the PVA. For a more detailed description of these input parameters and the statistical analyses used to derive them, see Appendices II and III.

Initial population abundance

ENCRWP: As of 31 December 2021, there were a minimum of 12 red wolves on the landscape, known through monitoring of radio-collared animals on the ground. Consultation with Service personnel responsible for tracking and management of the population resulted in the addition of three animals to this original group, for a total of 15 known or suspected animals. These 15 individuals consisted of nine females (seven adults) and six males (four adults). A small number of additional animals may have been alive as of that date, but there was no information to confirm their existence. We therefore used a conservative approach to include only those animals known or suspected to be alive as of the start date. Only one pair of adults was known at that time – the Milltail pair composed of female 2225 and male 2323. These individuals were assigned as a functioning pair in the model studbook, thereby enhancing the likelihood of producing a litter of red wolves early in the simulation.

As the second canid component of this model, we assumed a range of coyote abundance values across selected PVA scenarios. The first set of models features an estimated total of 500 coyotes occupying the approximately 3,500km^2 of suitable red wolf habitat in the five-county area of northeastern North Carolina. This number is based on an estimated mean territory size of 27km^2 for coyotes fitted with GPS radio collars on the Albemarle Peninsula (Hinton et al. 2015). If we assume an uneven and inefficient distribution of territories within that habitat, the Canid Demography working group estimated a total of about 100 functional coyote-sized territories on that landscape. This serves as a low end to the abundance estimate, and can be viewed as consistent with similar estimates based on general knowledge of coyote population dynamics (e.g., Gese and Patterson 2021).

Data provided by the North Carolina Wildlife Resources Commission (NCWRC) are consistent with a smaller mean coyote territory size approaching 21km^2, in line with the value of 23.5km^2 reported by Gese and Terletzky (2015). If we assume a more efficient distribution of territories across the available habitat space, we can identify an alternative estimate for current coyote abundance of 1000 individuals across approximately 170 territories. Finally, as an upper bound on initial coyote abundance for this analysis, additional data provided by NCWRC on hunting and trapping rates suggests a coyote population in the five-county area approaching 2,000 to 3,000 individuals. Selected model scenarios include an initial abundance of 2,000 coyotes, which is an attempt to balance the value of exploring the potential impact of higher coyote numbers on red wolf population viability with the computational challenges inherent to running complex individual-based models featuring large population sizes.

Finally, we included a total of five known hybrids that were listed in the studbook as of the start date of the simulation. It is possible that more hybrids existed on the landscape but, as with our treatment of initial red wolf abundance, we adopted a conservative approach in the absence of information and therefore did not include unidentified individuals in the analysis.

SAFE: The captive population was comprised of 235 living individuals as of 31 December 2021. The breeding status (paired or unpaired) and parentage of all living animals and their ancestors was captured through the studbook provided by AZA red wolf studbook keeper N. Davis (Point Defiance Zoo and Aquarium, Tacoma WA). All red wolves, in both the SAFE population and the ENCRWP, are descended from 14 wild founder animals, and their full pedigree is tracked in the AZA studbook. With these data, the genetic structure of each population – levels of inbreeding, gene diversity retained, etc. – was available for incorporation into the model.

Dynamics of reproduction – pairing, litter production

In all simulations, all canids in both wild and captive populations become reproductively active (i.e., adults) after they become two years of age. If reproducing in a given year, an adult female can produce only one litter of pups, and has the capacity to produce a litter each year through her adult lifespan.

ENCRWP: Explicitly including coyotes and hybrids in this PVA significantly increases the overall complexity of the analysis. Mechanisms for processes like mate choice and the consequences of alternative canid management practices need to be considered and, to the extent possible, coded into the typical demographic description of populations included in this analysis.

We employ a breeding system featuring long-term monogamy, where paired individuals remain together until one individual dies. Vortex does not explicitly model wolf pack structure, with social status and breeding priority assigned to specific individuals, pack fission/fusion dynamics, etc. This more simplified approach has been used effectively in other wolf PVAs without compromising the validity of the analysis and outcomes (Carroll et al. 2014; Miller 2017). At the beginning of each year, unpaired females are given the opportunity to choose their mate. The number of adult unpaired females that are given the opportunity to pair is a dynamic function of (a) the number of existing pairs that already occupy a territory, and (b) the total number of female canids on the landscape. In a given year, if there are unoccupied territories and unpaired female canids, individual females are then chosen at random across all adult age classes, regardless of taxonomy, to determine if they are candidates to pair with a suitable male and establish a territory. We are able to specify a reasonable form of mate choice in the simulation through the specification of criteria defining an acceptable mate for each female that has been chosen for pairing. These criteria can be based on natural mate choice mechanisms (e.g., some form of assortative mating: Fredrickson and Hedrick 2006) or the outcome of human interventions that are designed to improve opportunities for wolf-wolf pairings. The model allows each unpaired female up to 25 "encounters" with individual unpaired males, with an encounter being defined as an unpaired female located in the same spot as an unpaired male for a brief amount of time. For each male encountered, a pair can be formed with a specific probability that is based on the taxonomic identity of the two potential mates. In this way, wolf-wolf or coyote-coyote pairs are more likely as a function of general suitability characteristics between prospective mates. Despite the higher suitability of same-canid pairs, there is a chance of hybrid pair formation when a male of a different taxonomy is encountered. When an unpaired female successful pairs with a male, that female is no longer available to encounter other unpaired males. If a female has not selected a suitable mate after the maximum number of encounters (a very rare event probabilistically), she has a final opportunity to pair with the next selected male, irrespective of identity.

Based on the average territory size, and if we consider the low estimate for coyote population abundance as discussed previously, we assume that a maximum of approximately 100 adult coyote females can establish breeding territories in the five-county area of northeastern North Carolina. Furthermore, we assume that red wolf territories are approximately 2.5 times larger than the typical coyote territory, or about 70km^2 (Hinton et al. 2016). Therefore, and again assuming some inefficiency in overall habitat use, we assume a maximum of about 40 red wolf territories to be available in that habitat. The Vortex model is set up to account for the dynamic shift in overall breeding rates as a function of the relative number of adult females of each type of ancestry in the canid population and some expression of their preference for choosing suitable mates.

Analysis of the ENCRWP field data indicate that a red wolf female paired with a red wolf male has, on average, a 60% chance of producing a litter. When that female is paired with a non-wolf (coyote or hybrid) male, that success rate drops to about 37%. Our description of coyote demography is informed primarily by the detailed literature review of Gese and Patterson (2021); here, we assume that a coyote or hybrid paired with another non-wolf has an 80% chance of successfully producing a litter.

Litter size across all canids ranges from 1-10 pups with a mean of about five individuals. The actual size of each litter produced is the result of a value drawn at random from a statistical distribution described by a mean and a standard deviation. For red wolves, we further specify a mathematical function that describes a negative impact of the kinship of the pair on the size of the resulting litter; inbred litters are smaller than non-inbred litters. This inbreeding effect is not specified for non-wolf pairs. All litters assume a mean 50:50 sex ratio, with the sex of each pup drawn at random.

SAFE: In addition to the advanced pairing mechanics used in the wild population, we use the detailed genetic management capabilities built into Vortex to define breeding in the SAFE population. Specifically, the model determines how close the population is to the maximum number of spaces available (defined by carrying capacity, K: see below) and then estimates the number of pairs that are required to bring the population up to that value, given a pair's expected reproductive success and the number of deaths across all age classes expected to occur that year (see below). Therefore, this "breed to K" function regulates the SAFE population breeding rate to avoid excessive pup production from one year to the next in response to space limitations. In addition, pairs are selected on the basis of mean kinship (Ballou and Lacy 1995), a metric that describes the mean relatedness of an individual to the rest of the population in which it resides. Selecting mates with low mean kinship reduces inbreeding and improves the retention of population genetic diversity overall. Finally, prospective pairings that exceed a given threshold of relatedness (1 – [0.9*GD], where GD is population-wide gene diversity retained) are not created; in that case, another prospective male mate is selected (maximum of 10 opportunities) until a genetically suitable pair can be formed. If such a male cannot be identified, that female does not breed in that year.

We used a slightly modified estimate of the percentage of adult females in this population that are expected to be paired each year (subject to modification according to the "breed to K" protocol above). The recent past featured a pairing rate that was relatively low during a time when captive-born individuals were not being released to the wild. The 60% pairing rate used in the PVA represents a more accurate portrayal of current breeding efforts across the institutions housing animals for ex situ breeding as reproduction rates are increased to support a more active release program.

Litter production rates were based on a detailed reproductive viability analysis (RVA: Bauman et al. 2019) in which various biological factors were evaluated for their influence on reproductive success (see Appendix III for more details on this analysis). Ultimately, the analysis revealed that litter production was a function of (a) the age of both sire and dam, and (b) whether each parent had successfully bred in the past. When younger and previously experienced individuals were paired, the probability of that pair producing a litter approached 60%; in contrast, the chance of success for a pair made up of older animals that have not previously produced pups was as low as 5%.

The RVA was also used to specify the mean litter size in the SAFE population. Both the age and the past reproductive experience of the dam were identified as key factors influencing litter size, with younger females with previous breeding experience producing larger litters. The sex ratio of SAFE litters showed a slight bias towards more males as inbreeding coefficient of the pups increased.

Mortality

ENCRWP, SAFE: Age-specific annual mortality rates are presented in Table 1. There is no statistical difference in mortality between sexes, so only one value is shown for each age class and for each taxonomic entity. In the absence of wide-ranging data on the coyote population in northeastern North Carolina, annual mortality rate estimates are based on general studies of coyote populations in the southeastern US (e.g., Stevenson et al. 2016) and on a specific study in red wolf habitat of impacts of sterilizing coyotes to limit genetic introgression into the red wolf population (Gese and Terletzky 2015). The intent with this model was to generate a coyote population that was capable of modest sustained growth in the absence of specific management interventions.

Note the inclusion of a high mortality rate for hybrid litters, which is an attempt to capture the current practice among Service biologists to reduce the reproductive output of hybrid pairs. The value included in this model may be a high estimate relative to that realized on the ground, but is intended to assess the impact of aggressive management of hybrids to reduce introgression with red wolves and, by extension, increase long-term red wolf population viability. In addition, the consistently lower mortality seen in the SAFE population results from the comparatively more benign ex situ environment where intensive management is designed to optimize survival when possible.

Table 1. Annual mortality rates (mean (standard deviation)) of canid taxa in northeastern North Carolina and the ex situ SAFE population. Standard deviations for red wolves in the northeastern North Carolina and SAFE populations are directly calculated from demographic data, while those for coyotes and hybrids are theoretical values in the absence of specific field data.

	Northeastern North Carolina			SAFE
Age Class	Red Wolves	Coyotes	Hybrids	
Pups (0-1)	55.2 (18.6)	56.0 (10.0)	90.0 (5.0)	36.3 (13.6)
Subadults (1-2)	27.8 (10.0)	25.0 (5.0)	25.0 (5.0)	5.6 (3.3)
Adults (2-6)	22.7 (7.6)	21.0 (5.0)	21.0 (5.0)	3.4 (1.5)
Adults (7+)	36.5 (11.5)	21.0 (5.0)	21.0 (5.0)	18.6 (7.6)

Infectious disease as a mortality modifier

Disease was included here as a “catastrophe” in all wild populations; we assume that such events are carefully monitored and managed in the ex situ environment. A catastrophe is defined in the PVA context as a rare event, but with the potential for significant negative impacts to rates of reproduction and/or survival. In each year of the simulation, the PVA software determines if a catastrophe occurs according to a defined annual probability; if so, a specified multiplicative modifier is applied in that year to normal rates of reproduction and/or survival. The Disease Pathogen Identification and Prioritization working group reviewed relevant literature on disease agents of interest in this system and concluded that an outbreak of a highly infectious disease such as canine distemper would be the most likely catastrophic event impacting canids in northeastern North Carolina. Following multiple iterations of trial simulations and resulting discussion, the group agreed that a canine distemper outbreak would in fact be likely to have relatively minor impacts on canid populations in the region, especially in the red wolf component of the population where vaccination against this type of pathogen is a routine element of capturing and handling animals. Specifically, a significant event would be expected to occur approximately once in 20 years, with a 15% reduction in canid pup survival and a 5% reduction in adult canid survival. In simulations where multiple wild populations were considered, it was assumed that they were separated spatially so that outbreaks would occur independently across populations.

Population carrying capacity

In the typical Vortex modeling framework, a population is allowed to increase in abundance under favorable demographic conditions (and without explicit specification of density dependence) until the carrying capacity *K* is reached. When this occurs, individuals are randomly removed (simulating additional mortality under these limiting conditions) according to the age and sex structure of the population in order to bring the population back down to the value of *K*. In this manner, we therefore simulate a ceiling-type density dependence.

ENCRWP: Extensive discussion in the Wild Canid Demography working group led to a consensus estimate for red wolf carrying capacity in the five-county area of northeastern North Carolina of 200 individuals across all subadult and adult age classes (Age-1+). This was consistent with the specified maximum number of approximately 40 red wolf territories that could be distributed across the northeastern North Carolina landscape, with each territory accounting for a pair of adults and approximately three surviving pups. If that landscape were dominated by coyotes, the corresponding carrying capacity would equate to approximately 750 individuals (100 territories) assuming an initial coyote abundance of 500. In a mixed canid system, habitat carrying capacity is a dynamic function of the relative number of red wolves and coyotes occupying the habitat. In scenarios featuring larger initial coyote abundance values of 1000 or 2000, this estimate of coyote carrying capacity increases proportionally to 1500 or 3000 individuals, respectively.

SAFE: In ex situ populations such as this, the carrying capacity is estimated in part through knowledge of the availability of spaces among the various institutions participating in the ex situ program. However, as pointed out by Faust et al. (2016), carrying capacity is not equivalent to the number of spaces or enclosures distributed across participating institutions since different facilities may practice different methods for housing animals in comparatively larger or smaller enclosures. Currently, ex situ population managers estimate K for red wolves to be about 300 animals among approximately 125 enclosures. An explicit question to be addressed in this PVA is the value of expanding this carrying capacity through the addition of a larger number of enclosures in existing institutions or the addition of new institutions to the current collection of zoos and breeding centers participating in the program.

Simulating red wolf management strategies: Establishing new populations beyond northeastern North Carolina

In addition to managing the existing red wolf population in northeastern North Carolina, a key element of the current draft Recovery Plan is to establish three viable red wolf populations within the historic range of the species. Therefore, this PVA explores how those populations could be established and the conditions in which they can best contribute to species recovery. It is important to make clear that these models are not explicitly linked to any specific location or site that has been chosen for this purpose; they are instead abstract scenarios that make multiple assumptions about habitat size, quality, threats, etc. As a result, all establishment scenarios refer to "wild population 2" or "wild population 3" (alternatively, "Wild-2" or "Wild-3" given that the ENCRWP can be thought of as "Wild-1") instead of specific locations.

Throughout this analysis, new populations are assumed to be established through releases from the SAFE population (see next section on release scenario details). It is certainly possible that establishment will be assisted by translocation of individuals from an abundant and viable ENCRWP, but the details of this protocol have not been addressed. All establishment scenarios assume that red wolf reproduction and mortality can be managed effectively as described below, and that specific coyote management strategies can be effectively implemented as also described below. Furthermore, new populations Wild-2 and Wild-3 are assumed to have the same pairing and breeding dynamics and the same demographic characteristics for all canids occupying the northeastern North Carolina five-county area as defined by successful in situ management discussed above (increased red wolf pairing opportunities, reductions in anthropogenic mortality, etc.).

In each reintroduction scenario, the Wild-2 and Wild-3 populations are assigned the same characteristics. Each of the hypothetical release sites is assigned a red wolf carrying capacity of either 200 or 300, with a correspondingly larger carrying capacity for coyotes/hybrids in accordance with the discussion above. In addition, each of the sites is assigned an initial population of coyotes ranging from 0 to 200 individuals. This relatively small coyote population is acknowledged to be considerably smaller than the existing population in northeastern North Carolina. While also maintaining computational feasibility, this component of the analysis is perhaps optimistic in assuming the existence of candidate release sites (as yet unidentified) with relatively low coyote densities.

Simulating red wolf management strategies: Releases from the SAFE population

A critical question in this PVA is focused on the capacity of the ex situ SAFE population to provide sufficient numbers of captive-bred red wolves for release to support improved viability of the existing ENCRWP population and to establish new populations of red wolves in suitable habitat within the species' historic range. To address this question, most scenarios presented here include some type of release strategy.

Protocols for releasing captive-born red wolves from the SAFE population to the wild are defined on the basis of three main variables: over how many years the releases are conducted, the interval between release events, and the demographic characteristics of the individuals released (number, age, and sex). General characteristics of the various release strategies tested here include:

- The model does not explicitly consider releases from St. Vincent National Wildlife Refuge, an island propagation site off the Gulf Coast of Florida. However, a wild breeding pair of red wolves will continue to be maintained on the island to propagate pups in the wild in a somewhat controlled (e.g., no paved roads, no residents, limited hunting), but natural environment, that will provide them with experience in the wild as juveniles for the purpose of strategically translocated them into the ENCRWP when they reach the age of natural dispersal. Consequently, St. Vincent National Wildlife Refuge will continue to serve in adding to the benefits gained by selecting SAFE population animals for release.
- Releases are implemented through the Translocation module in the Vortex PVA software. In this mechanic, individuals are "harvested" from the source SAFE population according to specified criteria, transferred to a temporary "holding facility", and then immediately "supplemented" to the recipient wild population. In this way, the specific identity of each individual and, most importantly, their genetic profile (relatedness to others in the population, etc.) remain intact through the transfer process.
- Under current space availability in the SAFE population, the model does not select animals for release if the population is less than 80% of its carrying capacity so as to avoid further reductions in abundance that could lead to undesirable loss of gene diversity. In scenarios where the number of spaces in the SAFE population begins to grow over time (see below), this threshold is relaxed to 50% in recognition of the growth potential offered by the increase in available breeding space.
- Adults less than six years of age are selected from the SAFE population for release in order to add younger individuals with a higher reproductive potential to better support wild population growth.
- Individuals of a given age-sex class are selected at random for release. Existing pairs and/or family groups are not maintained for selection and release. This is acknowledged as a simplification of the actual practice of retaining family groups for release and attempting to identify individuals for release based on desirable behavioral profiles (e.g., observed extent of habituation to humans).
- Analysis of field data indicate no distinct post-release mortality. Consequently, released animals are subject to the standard wild red wolf mortality rates appropriate for their age-sex class from the time they are released to the next census just before pair formation and breeding. Additionally, the model does not account for the occasional event in which a released red wolf is returned to the SAFE population because of behavioral incompatibilities, i.e., high tolerance of human presence.

The release strategies examined in this PVA are defined below. These schedules are loosely based on past release efforts, but are designed primarily to help determine the intensity of future release efforts required to achieve broad recovery objectives while hopefully retaining sufficient practicality to be implementable. Note that in the early years of the release program, the model does not differentiate between the release of pups through pup fostering and their release with family groups. The specific method of release would be at the discretion of the authorities conducting the releases, based on their assessment of a successful outcome using one or another technique. In addition, note that the model adds random variation around the mean values listed below so that, in any given year, slightly more or fewer individuals could be selected for release.

Releases to ENCRWP only

- Release-Low
  - Adults: Four (two female, two male) each year for model years 1 through 5
  - Pups: Six (three female, three male) each year for model years 1 through 20

- Release-High
  - Adults: Six (three female, three male) each year for model years 1 through 5
  - Pups: Eight (four female, four male) each year for model years 1 through 20

- Release-High50
  - Adults: Six (three female, three male) each year for model years 1 through 5
  - Pups: Eight (four female, four male) each year for model years 1 through 20, then eight every five years thereafter for model years 25 through 50

Establishing new populations

- Large Release
  - ENCRWP
    - Adults: Eight (four female, four male) each year for model years 1 through 5
    - Pups: Eight (four female, four male) each year for model years 1 through 20

  - Wild-2
    - Adults: Eight (four female, four male) each year for model years 6 through 10
    - Subadults: Eight (four female, four male) each year for model years 6 through 10
    - Pups: Eight (four female, four male) each year for model years 11 through 20, then eight every five years thereafter for model years 22 through 52

  - Wild-3
    - Adults: Eight (four female, four male) each year for model years 11 through 15
    - Subadults: Eight (four female, four male) each year for model years 11 through 15
    - Pups: Eight (four female, four male) each year for model years 16 through 25, then eight every five years thereafter for model years 29 through 59

- Small Release
  - All populations: 50% of the Large Release values

These schedules clearly show a transition to greater reliance on pup fostering in later years of the release protocol, in keeping with USFWS intentions. In addition, the multi-population release schedule features a staggered approach to population establishment that facilitates identification and preparation of the selected release site while reducing the demands on the SAFE source population in the early years of the protocol. Nevertheless, the releases as simulated here put fairly heavy demands on the ex situ program to provide individuals for release while also maintaining demographic and genetic integrity of that source population. Towards that end, selected release scenarios include a gradual increase over 10 or 20 years in SAFE population space availability (carrying capacity) from the baseline value of 300 individuals to either 350 or 400.

Simulating red wolf management strategies: Improving red wolf reproduction and survival in northeastern North Carolina

Three different forms of direct management of red wolf demography were simulated in this PVA:

- Shift in mortality timing – Hinton et al. (2017) documented a marked increase in mortality among red wolves in the later months of the calendar year during pair formation, even though the total mortality rate across the time period of their analysis (1987 – 2013) remained rather constant. A higher mortality rate during the latter months of the year likely coincides with the onset of hunting season and the harvest of agricultural crops. This disruption in pair integrity will likely be manifest in a reduced rate of litter production among paired females. Scenarios featuring some form of management with respect to the timing of mortality were included in this PVA. In these scenarios, the baseline value for the probability of litter production among wolf-wolf pairs was increased from 60% to 70%.

- Reduced anthropogenic mortality – As frequently noted in the red wolf conservation literature, anthropogenic activities make up a large proportion of red wolf mortalities. Using data from 1987 to 2012, Hinton et al. (2017) showed a steady increase in the proportion of overall red wolf mortality attributable to anthropogenic sources, reaching approximately 85% of red wolf mortalities in 2012. Moreover, approximately 80-85% of those mortalities were attributable to gunshot and vehicle collisions. Analysis of more recent data (2017 – 2021) by USFWS indicates that the proportion of overall mortality attributable to known anthropogenic sources declined slightly to 78%, so the analysis from the earlier time period may represent a maximum estimate of anthropogenic impact. This mortality has been shown to increase the rate of hybridization with coyotes as wolf-wolf pairs are broken up after the death of one of the members (Bohling and Waits 2015). It is assumed that these anthropogenic sources impact subadults and younger adults (up to Age-6) as these age classes seem to be the most vulnerable. Scenarios were constructed for this PVA that simulated proportional reductions of 25%, 37.5%, or 50% in this human source of mortality among the most vulnerable age classes. It is implicitly assumed here that anthropogenic mortality is additive to overall mortality so that a 25% reduction in this source of mortality will result in an equal drop in overall mortality. This is likely an optimistic assumption as some mortality is probably compensatory (Hinton et al. 2016), but the issue has remained unresolved in the literature (Sparkman et al. 2011). Finally, an assumption is made that this mitigation of mortality applies only to the red wolf component of the population, i.e., mortality rates of coyotes and hybrids remain unchanged. Mortality rates for the affected age-classes across these mitigation scenarios are shown in Table 2.

Table 2. Annual mortality rates (mean (standard deviation)) for red wolves in northeastern North Carolina under different levels of anthropogenic (gunshot and vehicle collision) mortality mitigation. Cells in gray indicate the age-classes subject to the mitigation. See text for additional details and assumptions concerning scenario construction.

Age Class	Base Value	25% Reduction	37.5% Reduction	50% Reduction
Pups (0-1)	55.2 (18.6)	55.2 (18.6)	55.2 (18.6)	55.2 (18.6)
Subadults (1-2)	27.8 (10.0)	22.5 (8.1)	19.9 (7.2)	17.2 (6.0)
Adults (2-6)	22.7 (7.6)	18.4 (6.2)	16.2 (5.4)	14.1 (4.5)
Adults (7+)	36.5 (11.5)	36.5 (11.5)	36.5 (11.5)	36.5 (11.5)

- Improved wolf – wolf pairing – The Wild Canid Demography working group discussed many tactics devoted to reducing the rate of wolf – non-wolf pairing and targeting the rate of wolf – wolf pairing through active management of pair composition. These tactics are simulated here by (a) including a rule whereby identified wolf – non-wolf pairs are physically broken up without the death of the non-wolf mate; and (b) increasing the mean probability of wolf – wolf pairing. It is assumed that managers are 90% effective at targeting mixed pairs for break-up (recognized as perhaps slightly optimistic), and that the annual probability of wolf-wolf pairing is increased from the baseline value of 63.5% (derived from analysis of field data) to 80%. However, because of current limitations in the Vortex model structure, pairs can be broken up only after their first breeding season in which they could have produced a hybrid litter. This may be compensated, however, by the high mortality rate of hybrid litters discussed previously. The numerical process of pair formation in the PVA model remains probabilistic, but the increased likelihood of wolf – wolf pairing, in combination with the annual break-up of wolf – non-wolf pairs, is intended to capture much of the targeted management of canid pairs on the northeastern North Carolina landscape.

Simulating canid management strategies: Coyote population management

In addition to improved red wolf demographic rates through simulated management, this PVA features two explicit interventions in the coyote/hybrid component of the model:

- Sterilization: As described in detail by Gese and Terletzky (2015), adult coyotes and hybrids that are sterilized (using tubal ligation or vasectomy to keep hormones intact: Asa 2005) can serve as "placeholders" that hold territories but do not reproduce. This reduces the rate of production of both coyote as well as hybrid litters, thereby slowing the rate of coyote population growth and the rate of coyote introgression into the red wolf population. The numbers of coyotes and hybrids that have been sterilized over the course of the recent recovery effort have been relatively low, i.e., on the order of about a 10-15 animals per year (Madison, pers. comm.). However, there is a strong interest in exploring the potential impacts of more systematic sterilization efforts on red wolf population viability.

  To explore this management option in the PVA, scenarios were constructed that used a new Individual State Variable in Vortex to "flag" an animal that has transitioned from reproductively intact (fertile) to sterile. In order to evaluate long-term impacts of a determined coyote management effort over a defined time period, the simulated process of sterilization was conducted for the first 25 years of each coyote management scenario. A group of reproductively intact subadult and adult coyotes and hybrids comprising 5% or 10% of the total non-wolf population was selected randomly and sterilized each year through model year 25. It is assumed that intact and sterilized individuals can be identified in the field through some type of external marking such as an ear tag or tattoo. Sterilized females were allowed to form pairs at the same rate as intact females, but would fail to produce a litter. Similarly, intact females who paired with sterile males would also fail to produce a litter.

  In scenarios exploring new population establishment, it is assumed that 25% of the initial coyote population has been sterilized before red wolves are released. This attempts to simulate a modest effort at reducing coyote reproductive capacity in the area without the ability to fully prepare the site for optimal red wolf population growth.

- Removal: A simpler form of non-wolf management involved the removal of 5% of the total adult non-wolf population each year. The actual fate of these animals is not specified, but the process is akin to trapping followed by euthanasia. In order to simulate maximum efficiency of this management method in conjunction with sterilization, only intact non-wolves were targeted for removal.

An important assumption across this PVA, for both northeastern North Carolina and new population reintroduction scenarios, is that red wolf habitats are considered to be demographically isolated from adjacent landscapes. This means that no coyotes are entering red wolf habitats from outside the geographic areas of interest, i.e., they are demographically closed populations. This assumption is employed to more explicitly evaluate the impact of specific coyote population management alternatives, while also recognizing the potential impacts that result if the assumption is violated.

Scenario Structure Used in PVA Simulations

In total, more than 80 scenarios are included in this PVA, with each scenario defined by a distinct set of input parameter values that represent a specific set of assumptions and alternative management interventions. Thirty-two scenarios comprise a set of analyses focused only on the ENCRWP wild population and its linkage to the SAFE population, while thirty scenarios were developed to assess the outcomes of a range of new population establishment efforts, also featuring linkage to the SAFE population as a source for release animals. Additional information on scenario characteristics can be found in Appendix I.

The “Current – No Releases” scenario that forms the basis of all subsequent scenario definitions includes demographic input parameters described in the previous section, using field data analyzed through 2021, but with releases of animals from the SAFE population excluded going forward in time. This is not meant to imply that current management also does not include releases. Instead, the baseline scenario provides a “control” of sorts to which scenarios featuring releases of different intensity can be compared. In this way, the efficacy of releases can be directly evaluated for their contribution to establishing conditions for red wolf population viability in the wild.

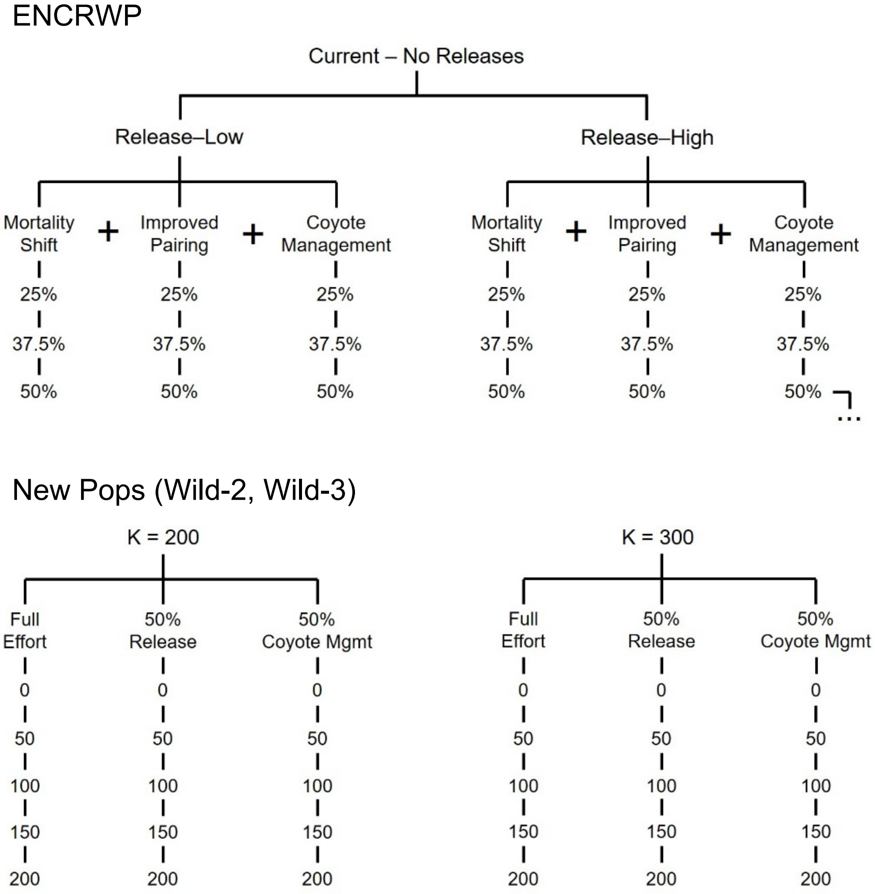


Figure 2. Simple schematics of PVA work flow for primary scenarios focused on the ENCRWP only (top section) and on the establishment of new populations in addition to the existing site (bottom section). ENCRWP scenarios are defined on the basis of the release protocol, up to three additive canid management efforts (mortality shift, improved pairing, and coyote management) and the percentage reduction in anthropogenic red wolf mortality (25% to 50%). The “...” symbol denotes a small set of additional scenarios based on the 50% anthropogenic mortality mitigation scenario. All scenarios here are compared to a “control” scenario defined by current conditions and with no releases.
New population establishment scenarios are defined by red wolf habitat carrying capacity (K), full or partial effort targeting numbers of released wolves and extent of coyote management, and the number of coyotes in the release site.
See text and Appendix I for more information on scenario details.

Results of PVA Simulations

Output metrics

Key output variables for each scenario in this analysis are given below. These variables are used for the red wolf component of the ENCRWP and hypothetical newly-established wild populations, as well as the SAFE population.

P(E)	Probability of population extinction over the 100-year simulation timeframe, i.e., the proportion of iterations in which red wolf population size declines to zero or is composed of only sex.
N	Mean population size after 100 years, calculated across only those iterations that do not decline to extinction (extant populations). Removing from the calculation those iterations in which red wolves become extinct provides a more clear interpretation of expected number of individuals IF the population survives across the full duration of the simulation.
GD	Mean population gene diversity retained, calculated across only those iterations that do not decline to extinction (extant populations). In this version of the report, GD is reported for each population and not the aggregate metapopulation that includes all wild populations and the SAFE population collectively.

Because of our ancestry-based definition of red wolves and other canid entities in this analysis, “extinction” of red wolves results not only from the death of animals over time but is also facilitated by insufficient rates of breeding within this taxonomic component of the population.

The complex taxonomic identification of individuals within a single canid population presents some challenges when interpreting standard Vortex model output. For example, the red wolf portion of the ENCRWP may decline to extinction, but the population of hybrids may survive for a longer period of time and the coyote population could persist throughout the time period of the simulation. In this case, the standard model would predict no risk of overall population extinction. In response to this challenge, because red wolves are a distinct taxonomic component of the larger canid population, it was necessary to create custom output variables to describe the demographic and genetic structure of the wild red wolf populations of interest. Where helpful, the abundance of coyotes and/or hybrids for a given scenario is also reported.

ENCRWP: Current conditions, no releases

Based on the best demographic information available for this analysis, and with no significant changes to management activities and with no future releases from the SAFE population, the very small population of red wolves currently residing in the northeastern North Carolina is projected to continue to decline rapidly and become extinct within the next 20 years (Figure 3). The coyote population is expected to grow towards the assigned carrying capacity and maintains a steady size throughout the 100-year simulation. The hybrid population shows a predicted trajectory very similar to that of red wolves, disappearing within 30 years as continued breeding with coyotes reduces the red wolf genetic signature in this population to negligible levels. It is important to note that we are not including in this scenario releases from the SAFE population as a management intervention; this scenario is designed to examine the inherent instability of this small red wolf population without a functional demographic connection to the ex situ source population.

As a form of model validation, it is useful to evaluate more detailed elements of model performance against our expectations in this scenario. The model restricts the maximum mean number of pairs produced each year to our expectation of 100 when only coyotes occupy northeastern North Carolina (Figure 4). Red wolf females are able to pair with other canids (Figure 4), but are almost surely likely to pair with coyotes despite their greater tendency to choose red wolves. [Unfortunately, the complex structure of this model prevents us at this time from recording the taxonomic identity of a red wolf female's mate.] It is also important to note that the number of paired red wolf females is recorded after the mortality event, so the actual number of paired females earlier in the annual model sequence would be approximately 25% higher than the value recorded here. Nevertheless, the detailed results here (and those presented elsewhere) help to confirm proper model function and, by extension, improve confidence in the general reliability of subsequent scenario output.

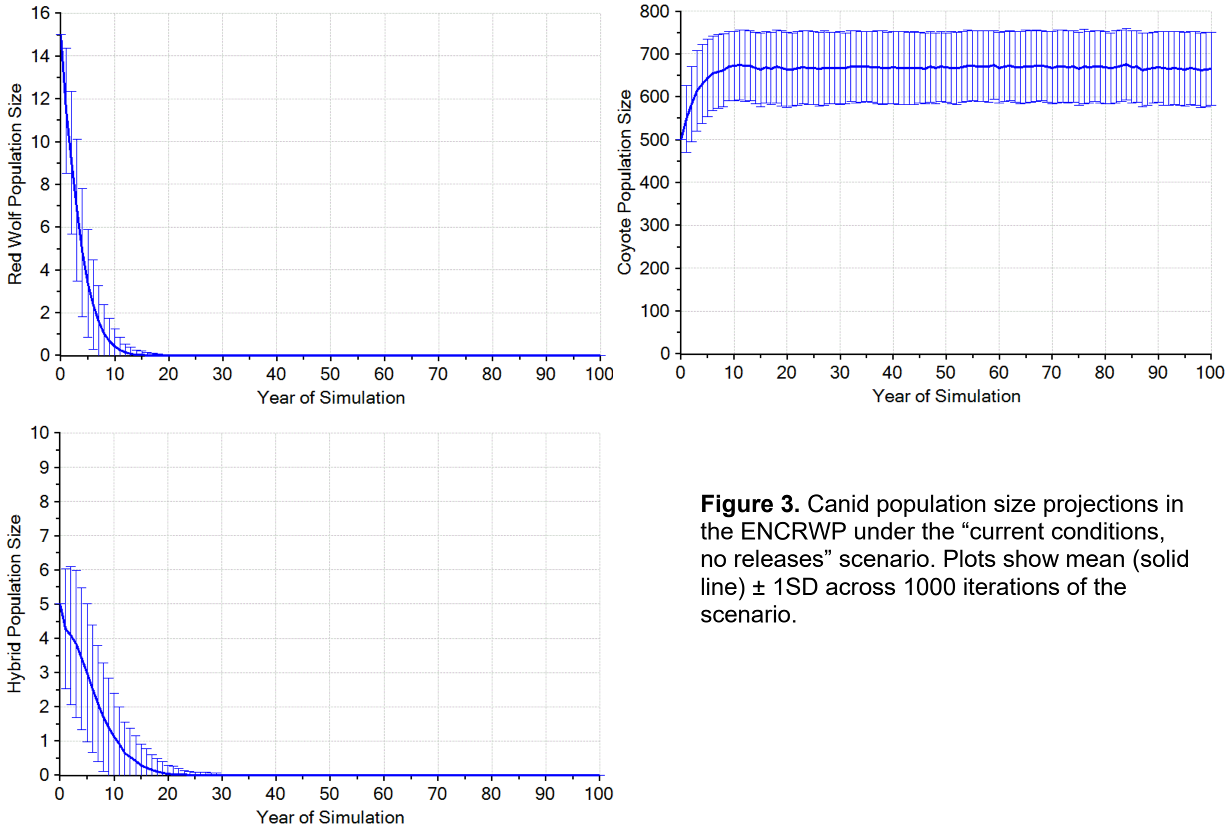


Figure 3. Canid population size projections in the ENCRWP under the "current conditions, no releases" scenario. Plots show mean (solid line) ± 1SD across 1000 iterations of the scenario.

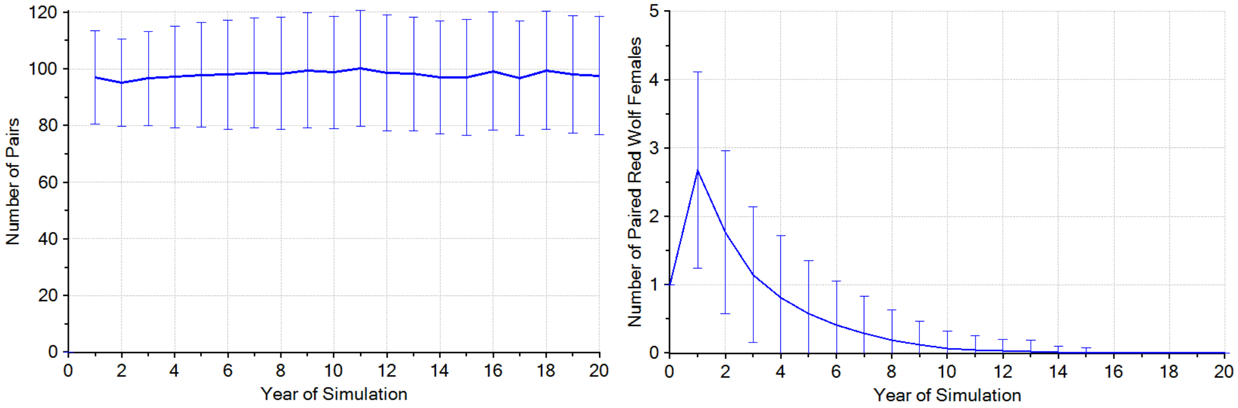


Figure 4. Total number of canid pairs (left panel) and number of mated red wolf females (right panel) in the ENCRWP under the "current conditions, no releases" scenario. Plots show mean (solid line) ± 1SD across 1000 iterations of the scenario.

Under the “current, no releases” scenario, The SAFE population shows a slight but persistent annual rate decline in size over 100 years (Figure 5). Over the course of the simulation, this population declines on average to 192 individuals from an initial 235 animals, with an annual growth rate of λ = 0.998. This decline is accompanied by an erosion of gene diversity retained from the initial value of 0.886 to 0.762, a 14% proportional decline.

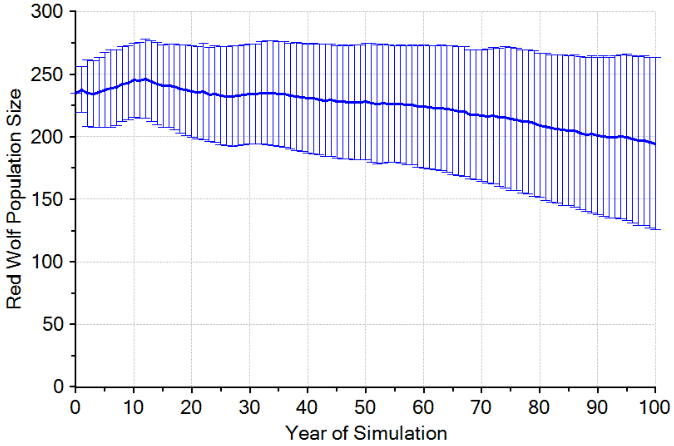


Figure 5. Projection of the number of red wolves in the SAFE population under the “current conditions, no releases” scenario. Plots show mean (solid line) ± 1SD across 1000 iterations of the scenario.

The simulated SAFE population increases for the first 10-15 years of the projection, perhaps suggesting that space limitations are restricting continued population growth. Additionally, the potential for low rates of litter production could also help explain these results. To test these hypotheses, a separate simulation was constructed in which the carrying capacity was gradually increased over the first 10 years from 300 to 350 and, in another scenario, litter production among paired adults females was improved (this was implemented technically within the Vortex software as a 15% proportional reduction in the probability of an individual female failing to produce a litter in a given year).

Increasing the carrying capacity led to a significant increase in population size in the first 15 years of the simulation, but thereafter the same general rate of decline occurred (Figure 6A). However, when combining this increased space with a higher rate of litter (pup) production, the SAFE population was able to grow to an equilibrium size of 300 individuals and maintain that abundance throughout the duration of the simulation. Moreover, the rate of gene diversity loss was slowed considerably at just over a 6% proportional loss relative to the initial value (Figure 6B), resulting in a final value of 0.832.

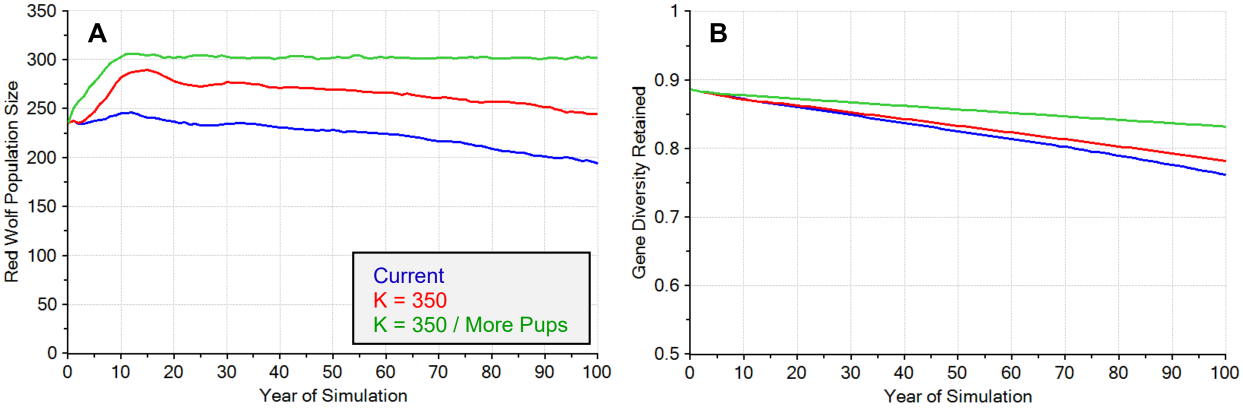


Figure 6. Projection of the number of red wolves (A) and gene diversity retained (B) in the SAFE population under different assumptions governing carrying capacity and litter production relative to the “current, no releases” scenario.

ENCRWP: Improving red wolf reproduction and survival with releases

Releases combined with various forms of red wolf demographic management (and, in all cases, removal of a large proportion of hybrid litters) can increase ENCRWP population size in the short term, especially in the first five years of the release program when adults are part of the release cohort (Figure 7). Enacting a seasonal shift in mortality to facilitate higher rates of litter production appears to have a negligible impact under the conditions modeled here. Reducing anthropogenic mortality leads to modest improvements in population size and a corresponding increase in the abundance of mated females (results not shown here) with, as expected, the largest increase seen with the greatest extent of mortality management. However, as soon as the releases are terminated in model year 20, the population demonstrates a similar rapid rate of decline to extinction.

The small "humps" seen in model years 35 – 40 represent mean population size across the very small number of iterations (0.2% to 1.5% of the 1000 total) in which the red wolf population has not become extinct. Because these plots show the mean red wolf population size across extant population iterations only (as per our definition of mean population size), the number of iterations that constitute that average declines dramatically in those final years and, consequently, can produce unusual results.

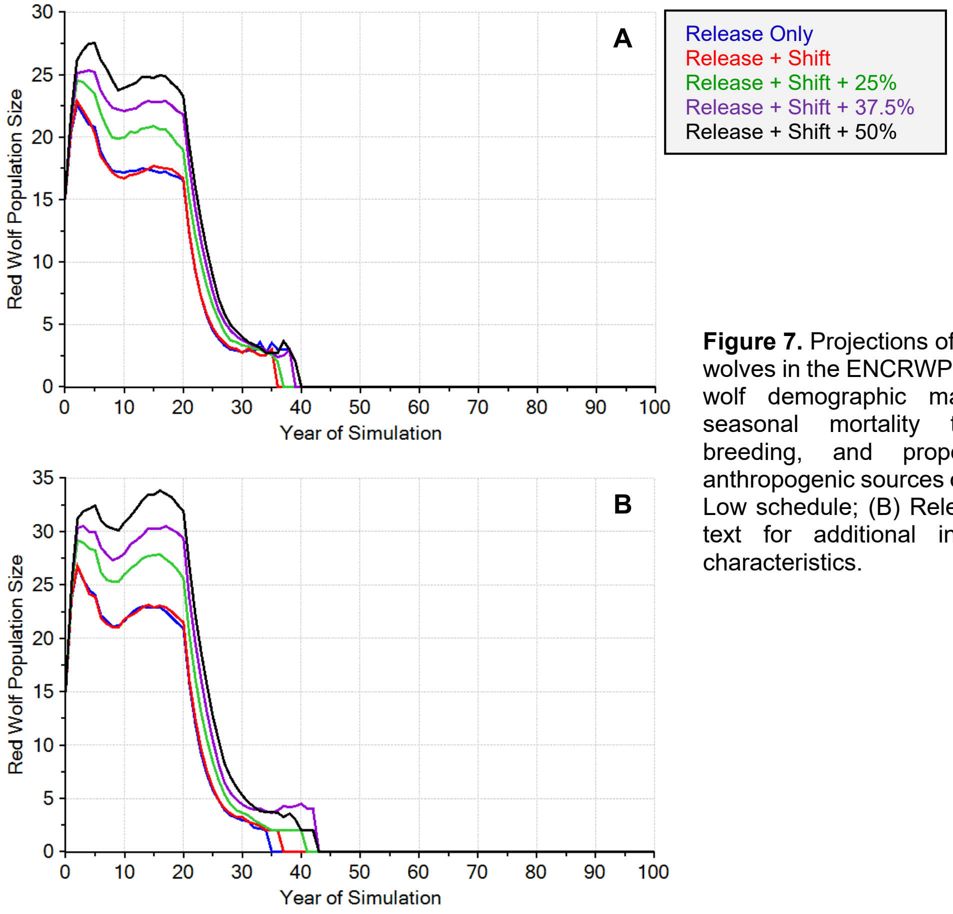


Figure 7. Projections of the mean number of red wolves in the ENCRWP with varying levels of red wolf demographic management: a shift in seasonal mortality to facilitate improved breeding, and proportional reductions in anthropogenic sources of mortality. (A), Release-Low schedule; (B) Release-High schedule. See text for additional information on scenario characteristics.

Adding improved wolf-wolf pairing to the suite of red wolf demographic strategies, in the absence of other forms of canid management in northeastern North Carolina, does not improve the outlook for red wolves (Figure 8). Note again the small collection of anomalous iterations that persist at relatively large population size around model year 40 in the Release-Low scenario with 37.5% mortality reduction.

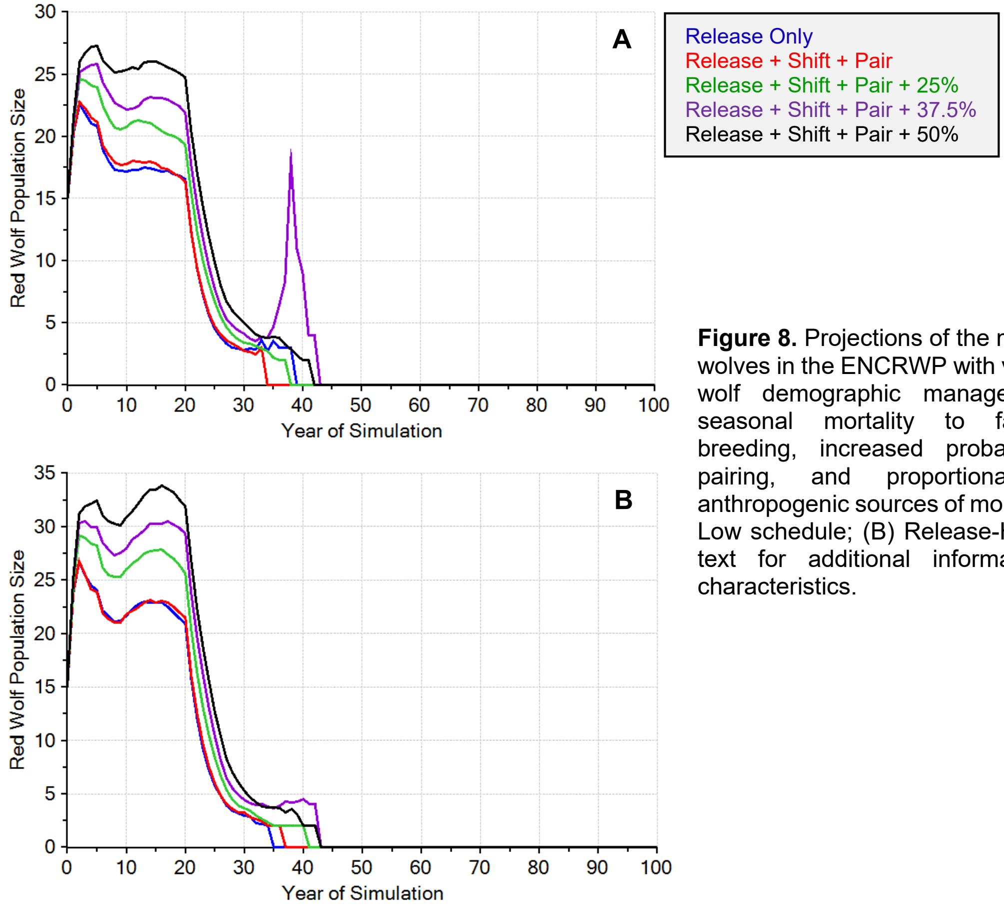


Figure 8. Projections of the mean number of red wolves in the ENCRWP with varying levels of red wolf demographic management: a shift in seasonal mortality to facilitate improved breeding, increased probability of wolf-wolf pairing, and proportional reductions in anthropogenic sources of mortality. (A), Release-Low schedule; (B) Release-High schedule. See text for additional information on scenario characteristics.

ENCRWP: Combining red wolf demographic improvements and releases with coyote management

In stark contrast to the scenarios discussed above that only target red wolf demographic management, scenarios that also include sterilization (10% of intact animals) and removal (5% of intact animals) of coyotes for 25 years, under the conditions and assumptions simulated in this PVA, can lead to high probabilities of red wolf population persistence and substantial population growth. Under both release schedules, the red wolf population can persist for 100 years in northeastern North Carolina, ranging in likelihood from 29% with the Release-Low strategy and improved red wolf breeding rates (Release + Shift + Pair: Figure 9A) to more than 96% with the Release-High strategy and improved red wolf breeding rates coupled with a 50% reduction in anthropogenic mortality (Release + Shift + Pair + 50%: Figure 9C). If the population persists in accord with these probabilities, red wolf numbers in these two scenarios increase to an average minimum of 130 animals (Figure 9B) to an average maximum of 183 animals (Figure 9D). In all scenarios featuring some form of canid management, the red wolf population size increases consistently and begins to stabilize approximately 30 to 60 years into the simulation – even with the cessation of release activities at year 20. This indicates that some regulation of population size by carrying capacity is taking place, especially at higher levels of anthropogenic mortality mitigation.

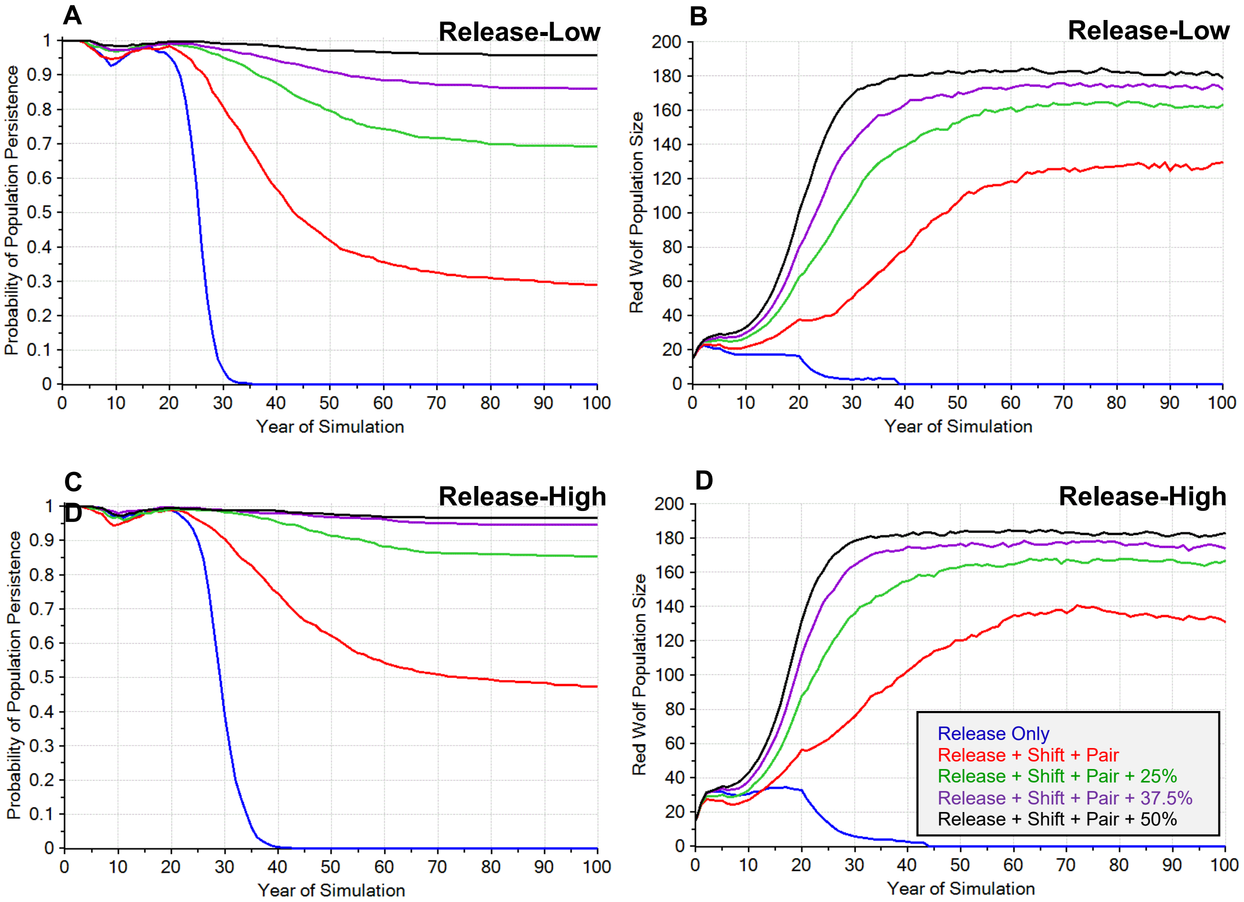


Figure 9. Projections of (panels A and C) the probability of red wolf population persistence, and (panels B and D) the mean number of red wolves if the population is to persist, in the ENCRWP in scenarios featuring Release-Low or Release-High strategies as described in the text. Red wolf demographic management in these scenarios is identical to that featured in Figures 7 and 8, but with the addition of coyote population management in the form of 10% annual sterilization and the 5% annual removal of intact animals. See text for additional information on scenario characteristics.

Sustained sterilization and removal of coyotes over the first 25 years of the simulation leads to a significant decline in the coyote population within the first five years, ultimately dropping by at least 90% by the end of the management phase (Figure 10A). At that point, anthropogenic mortality of red wolves that is largely unchecked can result in a modest rebound in the coyote population (e.g., blue and red plots in Figure 10A), but that increase is quickly followed by a renewed decline in that population through the remainder of the simulation as red wolves are now the dominant canid in northeastern North Carolina. As expected given the desired model mechanics and with a 10% annual sterilization rate of intact animals, approximately 50 coyotes are sterilized in the first year of the simulation, with the cumulative number reaching 125 animals within about five years (Figure 10B).

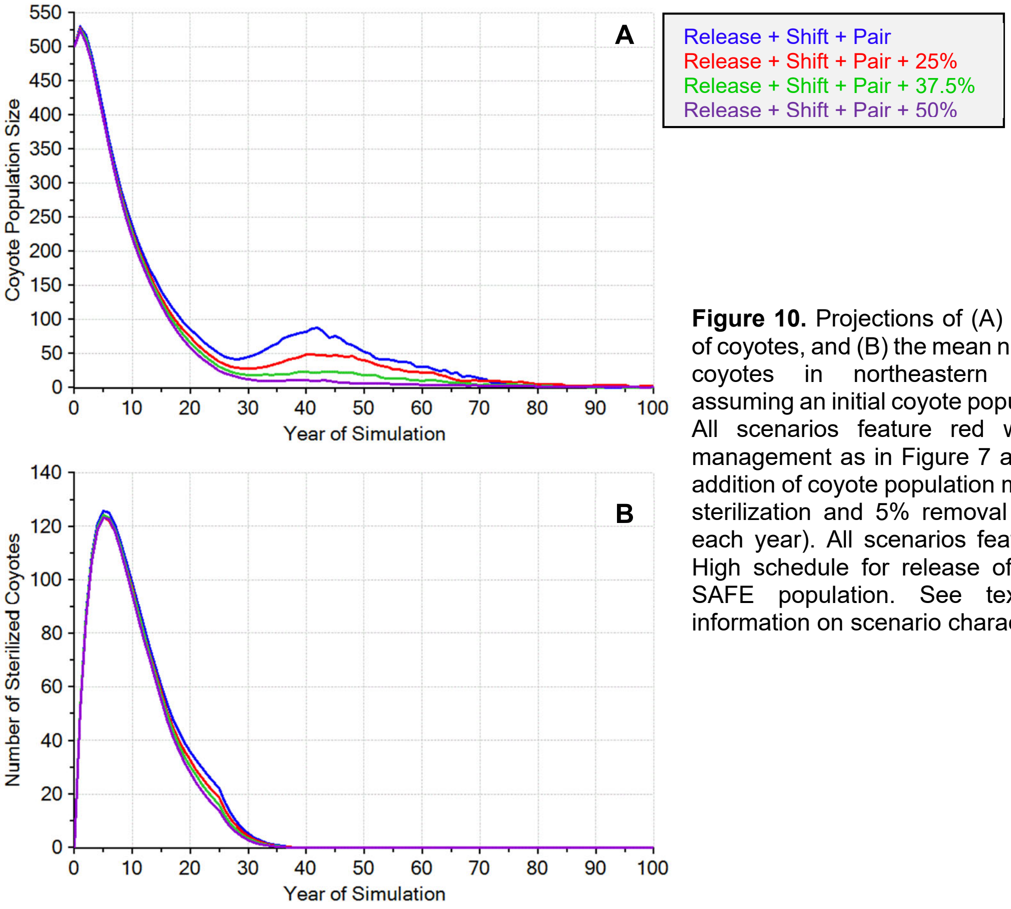


Figure 10. Projections of (A) the mean number of coyotes, and (B) the mean number of sterilized coyotes in northeastern North Carolina, assuming an initial coyote population size of 500. All scenarios feature red wolf demographic management as in Figure 7 and 8, but with the addition of coyote population management (10% sterilization and 5% removal of intact animals each year). All scenarios feature the Release-High schedule for release of wolves from the SAFE population. See text for additional information on scenario characteristics.

As further confirmation of model performance across a broad range of management intensity, Figure 112 shows the total number of pairs formed among all canids transitioning from nearly 100 pairs (maximum number in a coyote-only landscape) in the early years of the simulation when large numbers of intact coyotes rapidly fill the available territories to approximately 40 pairs (maximum number in a red wolf-only landscape) when red wolves are the dominant canid. The higher variance in the Release-Low scenario reflects greater variability in the competition between the remaining intact coyotes and the relatively less robust red wolf population for available territories. This observation is further emphasized by the lower probability of red wolf population persistence and final extant population size in the relatively less intensive "Release-Low + Shift + Pair" coyote management scenario.

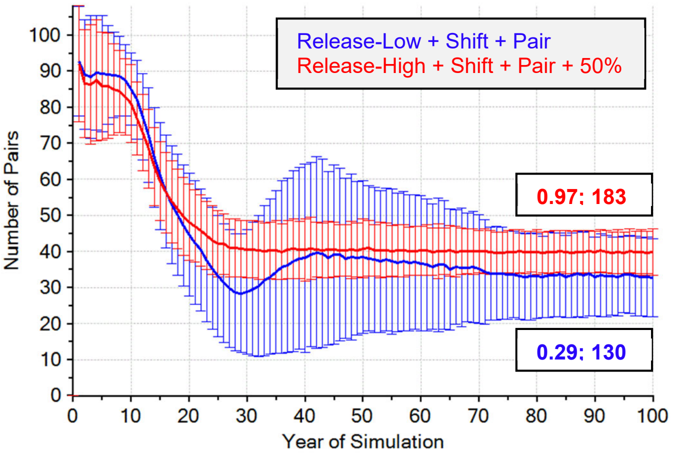


Figure 11. Projections of the number of pairs (mean±SD) in the northeastern North Carolina canid population under alternative release strategies and red wolf demographic regimes, and with the addition of coyote population management in the form of sterilization and the removal of intact animals. Each box in the lower right portion of the plot gives the probability of red wolf population persistence and final extant red wolf population size. See text for additional information on scenario characteristics.

An additional set of scenarios was constructed to evaluate the relative efficacy of coyote sterilization vs. removal in promoting red wolf population growth. Each management method was implemented separately, i.e., either sterilization or removal, and at two different levels of intensity (5% or 10% annual rate). Sterilization was clearly more effective at promoting red wolf population persistence and facilitating robust population growth (Figure 12). The difference in outcomes between the 5% and 10% sterilization scenarios is particularly striking, with the probability of red wolf population persistence increasing from 0.03 to 0.88 after 100 years under the higher sterilization rate. The 10% sterilization scenario also resulted in significantly improved population growth within the first 20 years of the simulation, as the intact coyote population declines rapidly (data not shown here).

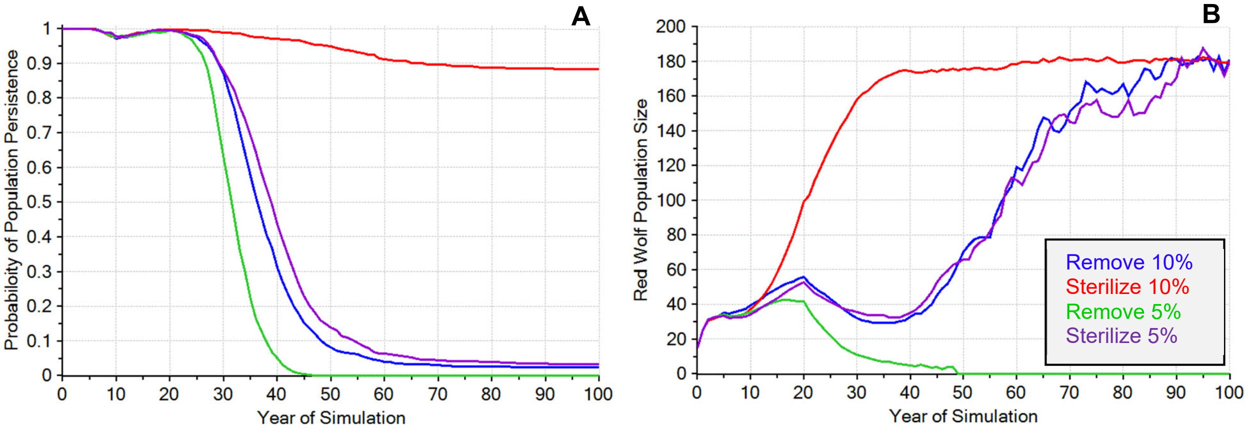


Figure 12. Projections of (A) the probability of red wolf population persistence, and (B) the mean number of red wolves if the population is to persist, in the ENCRWP in scenarios featuring different types and intensities of coyote management for the first 25 years of the simulation. All scenarios feature the Release-High schedule for release of red wolves from the SAFE population. See text for additional information on scenario characteristics.

SAFE: Impact of demographic management with alternate release strategies to benefit ENCRWP

Recovery of red wolf populations in the wild should be assessed both on the basis of demographic viability (probability of population persistence and long-term population size) and genetic viability (long-term retention of gene diversity among those founders that initiated the ex situ SAFE population). In order to examine a range of ex situ management strategies that could improve viability of both the ENCRWP and SAFE populations, a set of scenarios was constructed with the following modifications to the base scenario featuring the full range of red wolf demographic management activities (shift in mortality timing, improved red wolf pairing, 50% reduction in anthropogenic mortality, and coyote sterilization/removal):

- A new release strategy (Release-High50; see page 10), with periodic release of pups every five years in years 25 – 50 after the standard release protocol in model years 1-20;
- SAFE population carrying capacity increased gradually from 300 to 350 (over 10 years) or 400 (over 20 years), representing an increase in the number of facilities and/or spaces available to house individuals; and
- the probability of failure to produce a litter among established pairs was reduced proportionally by 15%, thereby improving the rate of litter production in the population. This component of pup production was identified previously as a key variable influencing population growth and long-term viability.

[A subset of these scenarios have already been discussed in the text accompanying Figure 6.]

All release strategies tested here facilitate sustained population growth in the ENCRWP (e.g., Figure 13; Release-Low and Release-High50 scenarios omitted here for clarity but give very similar results). Increasing SAFE carrying capacity allows for expansion of the ex situ population, thereby increasing the opportunities for larger release cohorts and, therefore, improved growth of the ENCRWP. Additional improvements in ex situ population management in the form of improved litter production provides even greater benefits to the recipient wild population. It is worth noting here that the scenarios featuring improved ex situ management also allow releases to occur more easily by reducing the population size threshold required from N/K = 0.8 to N/K = 0.5 (see page 10 for more information). This explains the increased ENCRWP growth rate in the first 5 – 10 years of the simulation over the original scenario featuring the smaller carrying capacity of 300 individuals. All scenarios tested here give ENCRWP persistence probabilities >0.95 over 100 years.

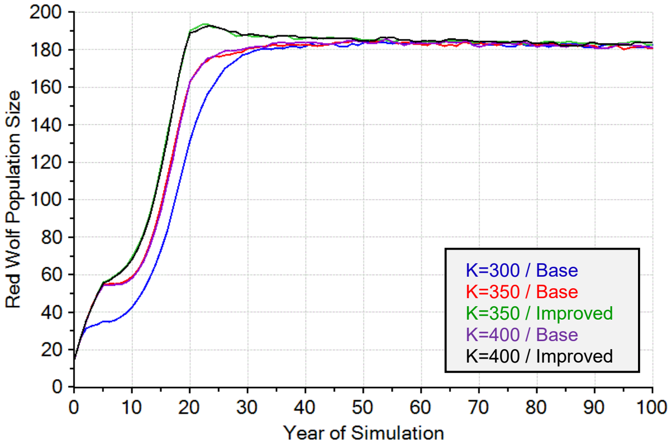


Figure 13. Projections of the number of red wolves in the ENCRWP under the Release-High strategy and assuming different demographic conditions in the SAFE population. Information in the legend gives the carrying capacity K of the SAFE population, followed by the rate of litter production among paired females. See accompanying text for more information on scenario characteristics.

Similarly, increasing SAFE carrying capacity and improving mean rates of litter production improve growth opportunities for the ex situ population (Figure 14). All scenarios tested here give SAFE persistence probabilities >0.95 over 100 years. Nevertheless, growth is impeded in the first five years of the simulation as adults are selected during this time period for release. This outcome is especially pronounced when litter production rates are not improved (compare “Base” and “Improved” trajectories in Figure 14). The continued selection of pups for release after model year 20 in the Release-High50 scenarios does not significantly alter population growth dynamics in the years following those releases (lower-left panel of Figure 14; compare this panel with the upper-right panel showing Release-High scenarios). Scenarios using the base rates of litter production (estimated using the reproductive viability analysis: Appendix III) show sustained and gradual decline of population size beginning around model year 30. If those litter production rates are improved by proportionally reducing the fraction of pairs that fail to produce a litter by 15%, this decline disappears and populations maintain a constant abundance across the duration of the simulation. It is interesting to note that the long-term population size in these scenarios is just under 90% of the scenario-specific carrying capacity. This is likely due to restrictions in litter production imposed by the Vortex “Breed to K” protocol (see page 6 for more information), coupled with the relatively strict regulation of abundance when the population reaches the reflective boundary defined by K.

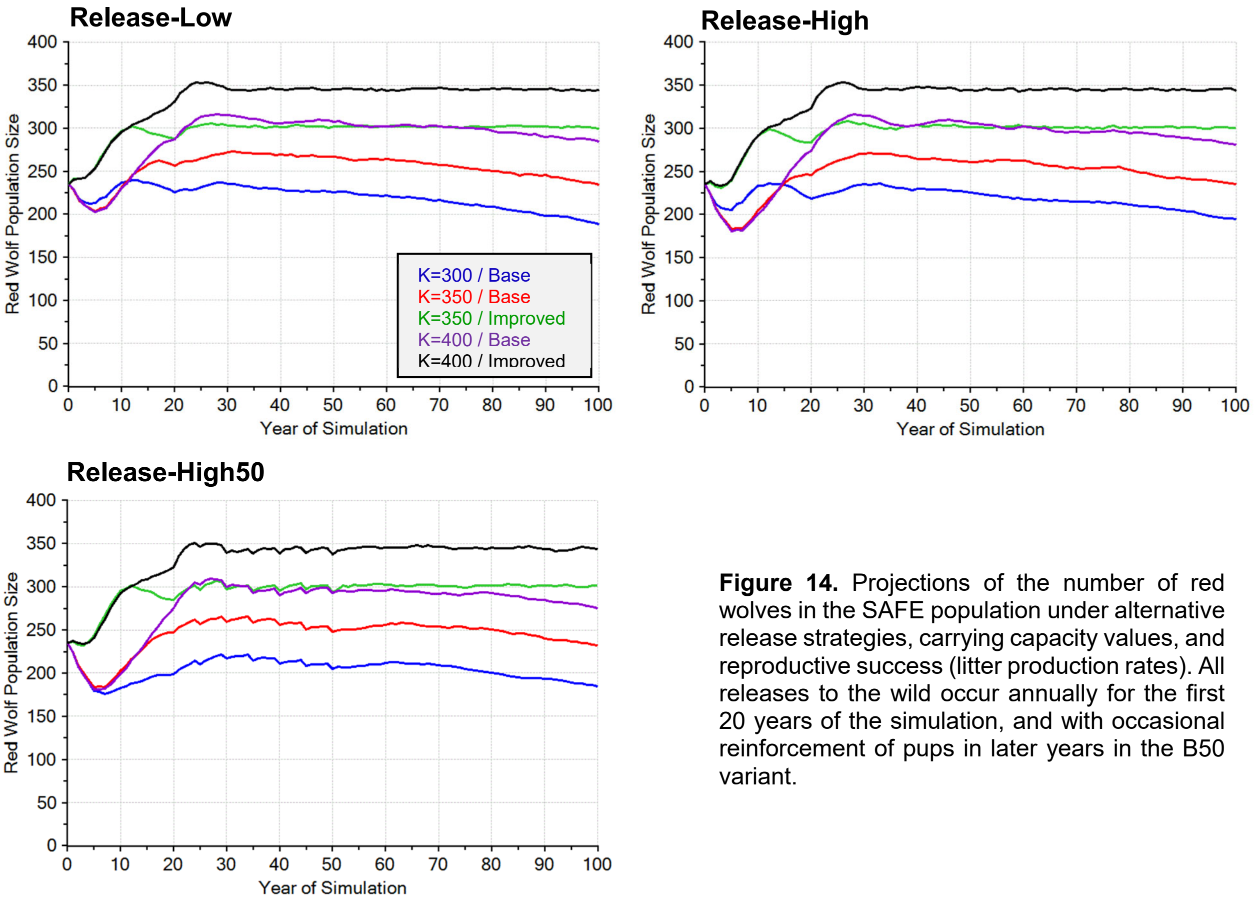


Figure 14. Projections of the number of red wolves in the SAFE population under alternative release strategies, carrying capacity values, and reproductive success (litter production rates). All releases to the wild occur annually for the first 20 years of the simulation, and with occasional reinforcement of pups in later years in the B50 variant.

The ENCRWP retains a larger proportion of SAFE population founder gene diversity (GD) over the duration of the simulations as the level of release effort increases from the Release-Low strategy through Release-High and ultimately to Release-High50 (Table 3). Across the scenarios within a given release strategy, increasing the SAFE population carrying capacity facilitates a greater level of GD retention in the ENCRWP, with an even greater level of retention observed when that increase in SAFE K is coupled with increased litter production (proportional 15% reduction in failure to produce a litter among paired females) in the ex situ population. In the most favorable scenarios featuring expanded carrying capacity and improved litter production rates, gene diversity retention exceeds 0.85 for 29-36 years depending on the release strategy employed. In those same scenarios, gene diversity retention exceeds 0.80 for 76-90 years.

Improving demographic characteristics in the SAFE population leads to even greater gains in gene diversity retention in the source population. When carrying capacity is expanded to at least 350 and litter production is improved, final GD retained in the SAFE population nearly meets or exceeds 0.830 after 100 years and exceeds 0.85 for 59-67 years. However, increasing release effort from Release-Low to Release-High50 reduces the extent of GD retention in scenarios where K is increased but litter production is not improved. For example, increasing K to 400 yields a final GD retained of 0.788 under Release-Low, 0.784 under Release-High, and 0.780 under Release-High50. This type of gradual reduction is not pronounced when litter production is improved.

Table 3. Proportional founder gene diversity (GD) retained after 100 years among red wolves in the ENCRWP and SAFE populations for alternative PVA scenarios. Numerical values in parentheses at the top of the center and right column groups give the starting GD values for the ENCRWP and SAFE red wolf populations. Columns labeled "Y < x" give the model year in which the mean value for GD across all iterations with extant red wolf populations dropped below 0.85 or 0.80. All scenarios feature the full range of red wolf demographic management (a shift in seasonal mortality to facilitate improved breeding, increased probability of wolf-wolf pairing, and a 50% reduction in anthropogenic sources of mortality) as well as coyote management (10% sterilization and 5% removal of intact animals annually for 25 years).

			ENCRWP (0.819)			SAFE (0.886)		
Release Strategy	K_{SAFE}	Litter Production	GD_{100}	Y < 0.85	Y < 0.80	GD_{100}	Y < 0.85	Y < 0.80
Low	300	Base	0.758	14	57	0.756	27	70
	350	Base	0.765	18	65	0.774	26	77
	400	Base	0.765	18	65	0.788	28	87
	350	Improved	0.775	29	76	0.830	61	–
	400	Improved	0.775	29	76	0.836	68	–
High	300	Base	0.764	20	65	0.759	26	68
	350	Base	0.766	20	66	0.769	22	73
	400	Base	0.766	18	66	0.784	22	82
	350	Improved	0.779	33	80	0.829	60	–
	400	Improved	0.779	33	80	0.835	67	–
High50	300	Base	0.773	20	74	0.760	27	69
	350	Base	0.777	21	77	0.768	22	70
	400	Base	0.776	18	76	0.780	22	80
	350	Improved	0.789	36	89	0.829	59	–
	400	Improved	0.789	36	90	0.835	66	–

ENCRWP: Impact of coyote abundance

Under the lowest estimate of initial coyote abundance used in this analysis, and when using SAFE population release strategy Release-High and implementing the various in situ demographic management activities described in previous scenarios, the model predicts that the ENCRWP would increase in abundance relatively rapidly over 30 years and approach the habitat carrying capacity defined for the five-county area of northeastern North Carolina (Figure 15). The probability of persistence for this population over 100 years is greater than 0.95. [The trajectories labeled "500 / 10%" in this figure correspond to the trajectories labeled "Release + Shift + Pair + 50%" in Figures 9C and 9D.] Employing a lower level of coyote management (sterilization / removal), even under this lowest estimate of initial coyote abundance, would fail to create favorable conditions for a viable red wolf population as was graphically demonstrated in Figure 12 (page 22).

When estimates of initial coyote abundance are increased to 1000 or 2000 individuals in northeastern North Carolina, lower levels of coyote management are also not favorable for red wolf population persistence (Figure 15). The red wolf population is projected to grow slowly through releases from the SAFE population for the first 20 years of the simulation, but once those releases are terminated the red wolf populations decline rapidly in the presence of the much larger coyote population, as has been seen in previous model scenarios. Extinction of the red wolf population occurs in approximately 50 to 60 years, depending on the abundance of coyotes in those years.

In contrast, if the intensity of coyote management is increased from 5% to 10% sterilization (2.5% to 5% removal) of intact animals annually, the probability of red wolf population persistence under conditions of larger initial coyote abundance can increase substantially (Figure 15A). The model featuring an initial abundance of 1000 coyotes resulted in a probability of red wolf population persistence over 100 years approaching 0.9, with a mean size of successful (persisting) populations of 193 individuals (Figure 15B). If the initial coyote population is, however, assumed to be 2000 individuals, the probability of red wolf population persistence drops significantly to 51% over 100 years. If management of that large coyote population is successful, the red wolf population can grow to about 195 individuals and becomes regulated by territory size restrictions at the habitat carrying capacity. The expansion of the red wolf population beyond the established carrying capacity between model years 30 and 50 is most likely a consequence of the way in which average territory availability is calculated in a highly mixed red wolf – coyote system, as is likely the case in this model where the two taxa are competing more intensely for available breeding habitat.

As expected, the cumulative number of sterilized coyotes in the habitat increases considerably with a larger number of coyotes occupying the landscape (Figure 16). In the scenario with 2000 coyotes and the higher level of coyote management, the cumulative number of sterile animals in the population peaks in model year 5 at 423, which is about 32% of the total coyote population at that time (total coyote abundance: 1,310). At the end of the management program in model year 20, the 110 sterile coyotes comprise nearly 40% of the 283 animals make up the total population (detailed results not shown here).

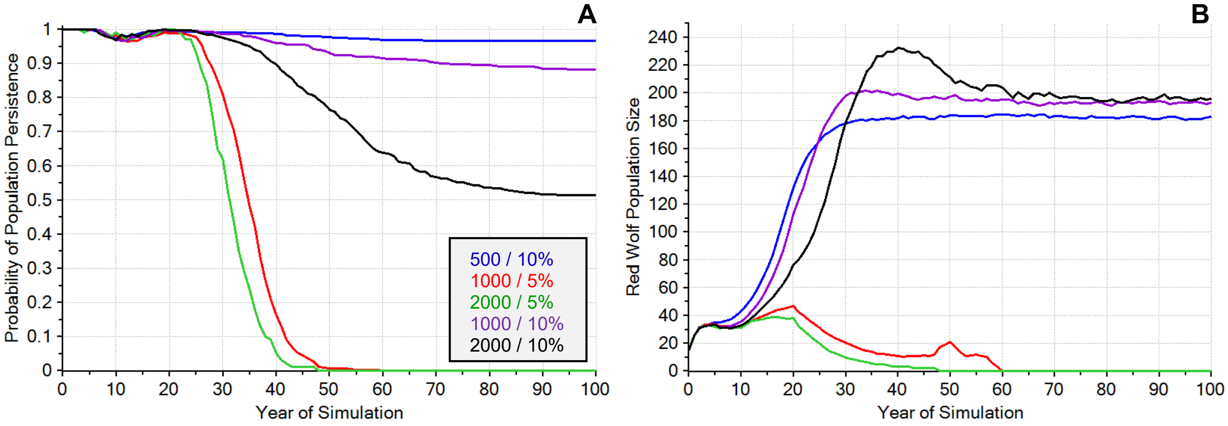
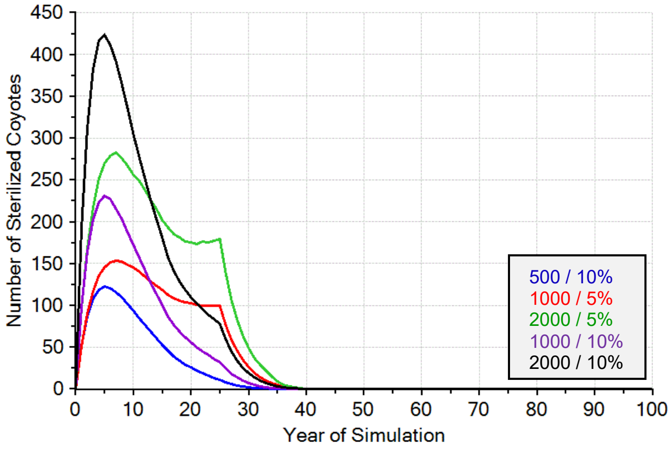


Figure 15. Projections of (A) the probability of red wolf population persistence, and (B) the mean number of red wolves if the population is to persist, in the ENCRWP with different values of initial coyote abundance and coyote management intensity (% sterilized annually for 20 years; removal rates are half of sterilization rates). All trajectories feature the Release-High strategy of releases from the SAFE population. See text for additional information on scenario characteristics.

Figure 16. Projections of the cumulative number of sterile coyotes in the northeastern North Carolina red wolf habitat area under different assumptions of initial coyote abundance and level of coyote management effort. See legend for Figure 15 for more detailed information on scenario characteristics.



Establishing new wild populations: Impact of coyote abundance, management activity, and carrying capacity

Under the baseline Large release schedule discussed previously for the establishment of new populations beyond northeastern North Carolina (page 11), and when implementing the kinds of demographic management regimes demonstrated earlier in this PVA to foster long-term population viability, red wolf populations established through releases from the SAFE population can increase to an equilibrium abundance near to the identified habitat carrying capacity (e.g., Figure 17). As briefly described in the section on model input data (page 9), the model assumes that demographic management in the new habitat can be implemented effectively so that wolf-wolf pairing opportunities and litter production among paired females are improved, anthropogenic sources of subadult and adult red wolf mortality are effectively mitigated (i.e., reduced to 50% of the level documented in northeastern North Carolina: see Table 2), and the baseline rate of 10% annual sterilization and 5% annual removal of intact coyotes occurs for the first 25 years of the simulation. In addition, this set of models assumes that the red wolf population carrying capacity in each of the three habitats is 200 – equivalent to the value for the existing northeastern North Carolina habitat area. Finally, the scenarios assume no immigration of coyotes from outside the new sites, identical to the assumption made for the ENCRWP habitat area.

Logically, the red wolf population growth rate is impacted by the initial abundance of coyotes in the new habitat area, but the probability of successful population establishment in a candidate site (i.e., acceptably low probability of failure) does not appear to be appreciably affected by coyotes as long as sterilization and removal are implemented fully as per the results of relevant ENCRWP simulations. Populations that become established show very consistent results for final abundance and population gene diversity: red wolf population size stabilizes at a mean of 180 – 190 individuals, with proportional gene diversity retained after 100 years ranging from 0.783 – 0.788. However, the likelihood of successful establishment of these hypothetical new populations in suitable reintroduction sites is influenced by the intensity of releases from the SAFE population and, in keeping with the ENCRWP management simulation results, much more strongly on the extent of local coyote management in the presence of higher initial coyote abundances (Figure 18).

If the release effort is reduced by 50% over the Large Release strategy, leading to only half the original number of red wolves to be released across the three populations from the SAFE source, only the Wild-3 population displays any significant detrimental effect. When the initial number of coyotes in the habitat area is less than 150, the likelihood of successful population establishment remains 95% or higher. When the coyote abundance equals or exceeds 150, this likelihood drops to 90%. The likelihood of establishment for the Wild-2 population does not drop below 95% under any level of coyote abundance tested here. This reduced probability of successful establishment for the Wild-3 population under decreased release effort most likely results from the fact that releases to Wild-3 begin later in the simulation (see page 11 for detailed information on the Large Release strategy), thereby making it more likely that the resident coyote population has increased to an abundance that compromises the likelihood of red wolf population growth and establishment (long-term persistence).

In contrast to the relatively benign impact of reducing release efforts as simulated here, Figure 18 shows that if coyote management is implemented with only half the intensity of the "full management" scenario – specifically, 5% annual sterilization and 2.5% annual removal rates for 25 years – both the Wild-2 and Wild-3 populations demonstrate reduced likelihoods of establishment when more coyotes are present in the release area. This likelihood for the Wild-2 population drops from 100% in the absence of coyotes and with implementation of the "full management" scenario, to just 57.2% when initial coyote abundance is 200 animals and when employing the "50% coyotes" management scenario. For the Wild-3 population, in which release efforts are delayed for five years after the beginning of Wild-2 population releases, the chances for successful establishment are reduced even further. A 100% chance of successful establishment in the absence of coyotes drops to just 12.0% with an initial abundance of 200 coyotes and when employing the "50% coyotes" management scenario.

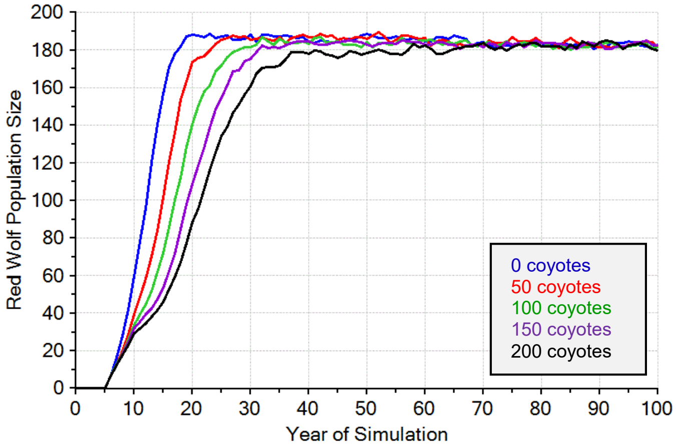


Figure 17. Projections of the number of red wolves in the new Wild-2 population under alternative values of initial coyote abundance and using the "Large Release" schedule (see page 10 for a description). Demographic management of red wolves and coyotes follows effective levels suitable for promoting red wolf population growth (i.e., the "Release + Shift + Pair + 50%" + coyote management scenario presented in Figure 9). Probabilities of successfully establishing the Wild-2 population under these conditions exceed 99%. See text for additional information on scenario characteristics.

When the red wolf population carrying capacity is increased from 200 to 300, the chances for successful red wolf population establishment across the range of initial coyote abundances and management regimes is reduced, particularly when initial coyote abundance is 100 or greater and coyote management is only 50% of the standard effort level. This seemingly paradoxical result is likely due to the assumption that an increased red wolf carrying capacity, which would equate to a generally larger habitat area, would also mean a larger coyote carrying capacity. In the case of the Wild-3 population which begins receiving animals from the SAFE population in simulation year 11, the standing coyote population has had a chance to grow and become a greater barrier to successful wolf-wolf pair formation. This would serve to inhibit red wolf population growth in the earlier stages of the simulation and reduce the opportunity for sustained population growth. However, if populations are successfully established, the larger carrying capacity facilitates population growth to a larger final abundance of 270 – 280 individuals and, perhaps more importantly, a higher retained proportion of population gene diversity at 0.805 – 0.809 in Wild-2 and Wild-3. This retention value is slightly reduced to approximately 0.795 under the "50% Releases" scenarios where a smaller number of SAFE animals are released over time.

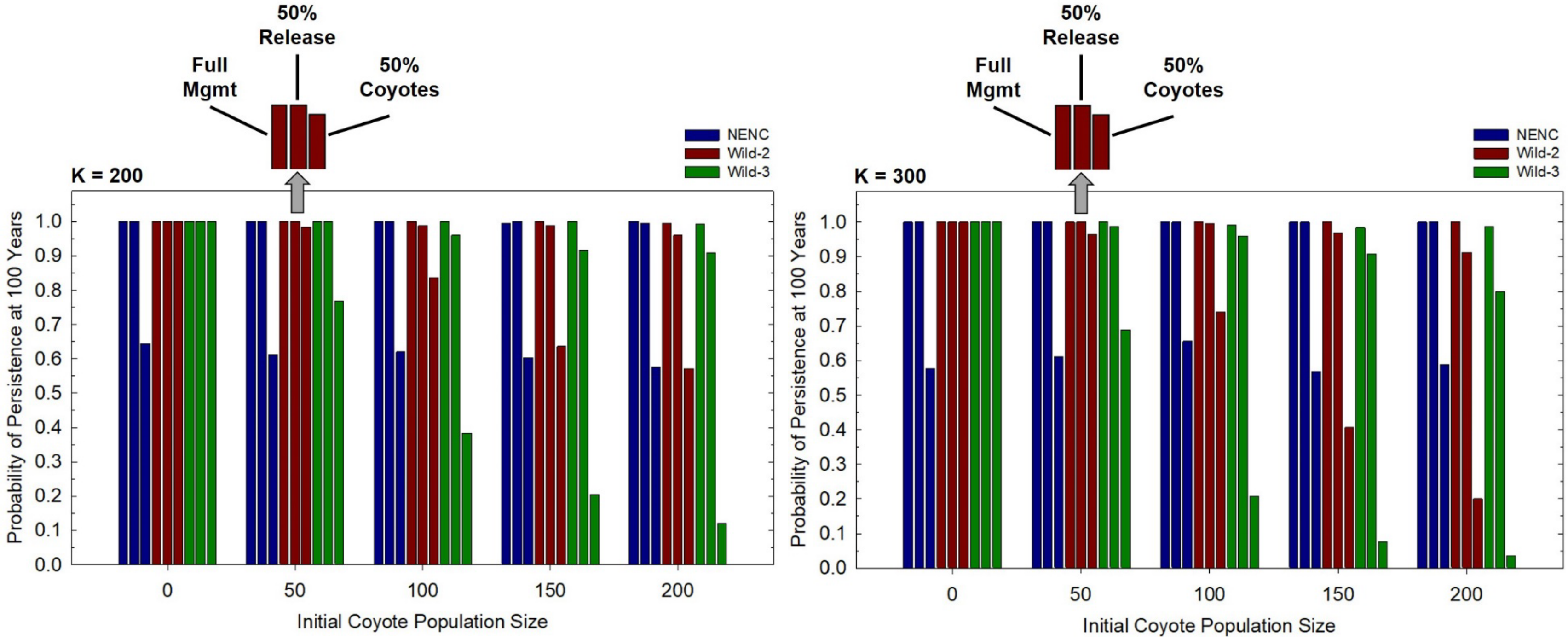


Figure 18. Summary of likelihood of population establishment for the existing ENCRWP and the two hypothetical new populations initiated through releases from the SAFE population. Habitat carrying capacity is set at either K=200 (left panel) or K=300 (right panel). For each color-coded population and for each value of initial coyote population abundance, the three bars denote three alternative management regimes (gray vertical arrow: example given above the sets of results for red wolf K = 200 and an initial coyote abundance of 50): “Full management” where the standard Large Release schedule is used (see page 11 for a description); “50% Release” in which the release schedule is reduced by half (the “Small Release” schedule listed on page 10) while maintaining the standard coyote management effort; and “50% Coyotes” in which the standard rate of coyote sterilization and removal is reduced by 50% while retaining the Large Release strategy. Demographic management of red wolves and coyotes follows effective levels suitable for promoting red wolf population growth (i.e., the “Release + Shift + Pair + 50%” + coyote management scenario presented in Figure 9).

Across the range of scenarios tested here, carrying capacity of the SAFE source population is increased from 300 to 400 in an attempt to offset the removal of a relatively large number of individuals over an extended period of time. Additionally, the probability of litter production among paired females is improved as in previous scenarios. Representative demographic and genetic results for the SAFE population are shown in Figure 19 for scenarios featuring a habitat carrying capacity of 200 for each wild release site and an initial coyote abundance at each site of 200 individuals. [Other scenarios featuring changes to these wild population parameters do not impact results for the SAFE population.]

When the full complement of releases from the source population are employed, the SAFE population abundance remains rather constant at about 235 animals for the first 15 years of the full release effort. At this point, the selection of subadults and adults for release from this source population is terminated, which helps to promote growth of the population to a long-term mean abundance of approximately 350 animals (with no risk of extinction across the duration of the simulation). If the release effort is reduced by 50%, the population grows towards carrying capacity earlier in the time course of releases to the wild habitats and achieves the same long-term stable abundance around more year 25. Gene diversity retained in the SAFE population declines at a slightly higher rate over the first 10-15 years of the simulation as adults and subadults are removed from the population for release to the wild. Following this initial period, gene diversity erodes at a steadily declines more slowly from a value of approximately 0.87 to 0.83 after 100 years. In the two scenarios depicted in Figure 19, gene diversity retained in the SAFE population after 100 years is 0.829 and 0.833 for the Full Releases and 50% Releases scenarios, respectively.

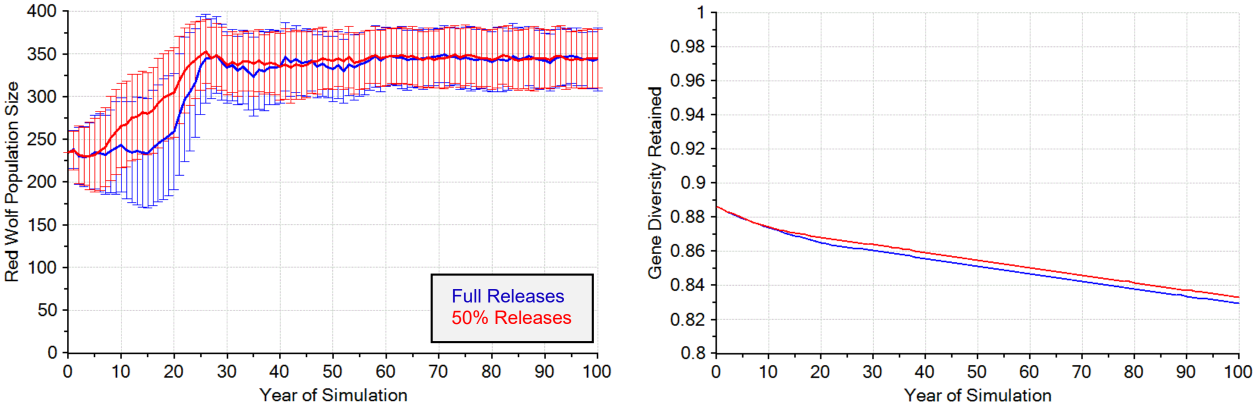


Figure 19. Projections of mean red wolf population size (mean±SD) (left panel) and proportional gene diversity retained (right panel) in the SAFE population under two different levels of release effort designed to establish red wolf populations in new candidate sites in addition to the existing population in northeastern North Carolina. Both scenarios include a red wolf carrying capacity of 200 individuals for all wild populations and an initial abundance of 200 coyotes for each of the new habitat sites designated “Wild-2” and “Wild-3”. See text for additional information on scenario characteristics.

Discussion

The population viability analysis described in this report is a complex application of individual-based simulation modeling to examine population dynamics of an endangered species. A substantial body of detailed information is available to develop robust reproduction and survival estimates for the northeastern North Carolina (ENCRWP) and SAFE red wolf populations, and to begin detailing the complex interactions between red wolves and coyotes in a mixed canid habitat. Despite the availability of this quality information, significant uncertainty remains in key areas of our understanding of this system. It is important to recognize that PVA methodologies are not intended to give absolute and accurate "answers" for what the future will bring for a given wildlife species or population. Many practitioners caution against the exclusive use of absolute results from a PVA in order to promote specific management actions for threatened populations (e.g., Ludwig 1999; Beissinger and McCullough 2002; Reed et al. 2002; Ellner et al. 2002; Lotts et al. 2004; Lacy 2019). Instead, we can use PVA results to make comparisons of the relative performance of a simulated population under alternative management activities or different assumptions of environmental conditions. Even in this comparative framework, results from PVA efforts can provide a critical base of evidence when deriving meaningful and justifiable endangered species recovery criteria (Himes Boor 2014; Doak et al. 2015).

Viability of the ENCRWP

This analysis is intended to update and expand the previous analysis of red wolf population viability (Faust et al. 2016) in two important ways: by explicitly incorporating coyotes into the population simulation, and by exploring opportunities for establishing new populations beyond the existing ENCRWP. Like the previous analysis, the present model predicts the continued rapid decline and disappearance of the ENCRWP if the population is not supported by releases from the captive SAFE population. The small population of red wolves does not have sufficient reproductive capacity to overcome unsustainable mortality when available territories are in short supply given the existing coyote density in the area. Releases from the SAFE population no doubt improve the situation, and incremental reductions in anthropogenic sources of mortality lead to further improvements. However, those benefits are quickly lost after release efforts are halted; the red wolf population once again quickly declines and disappears when left on its own, even if additional improvements to the likelihood of successful wolf-wolf pairing and litter production are implemented along with mortality mitigation.

The model suggests that substantial improvements to ENCRWP viability through management are realized only in the presence of aggressive and, particularly in the early phases of red wolf population growth, sustained coyote management – namely sterilization and removal of intact subadults and adults. This result reinforces the hypothesis that the breeding population of coyotes in the northeastern North Carolina habitat is effectively "swamping" the ability of red wolves to effectively establish sufficient numbers of productive wolf-wolf breeding pairs as a first step to building a viable red wolf population in the area. Detailed inspection of model results indicates that the likelihood of red wolf population growth may be relatively modest but, if it happens, that growth can be quite robust. In other words, red wolf population viability as simulated here seems to be critically dependent on the relative proportion of wolf-wolf vs. mixed pairs early in the simulation. If more wolf-wolf pairs can become established early on, this outcome has a reinforcing impact on their ability to produce more pairs in the presence of sustained coyote breeding management. Besides direct coyote management, reducing anthropogenic mortality appears to be the most effective management activity to support this early phase of red wolf population growth. Reductions in this source of mortality of at least 35% to 40% seem to be most effective in supporting red wolf population growth when breeding opportunities are improved.

Based on the mechanics of this model and the underlying data and assumptions built into it, sterilization of coyotes leads to significantly greater benefits for the red wolf population compared to a comparable level of direct removal of coyotes. This is because sterile coyotes – both males and females –

continue to form pairs and occupy territories but fail to produce offspring on that territory as long as both members of the pair survive. Even if the sterile pair breaks up due to death or other circumstances, a new pair that forms on that territory may also be sterile as the proportion of sterilized animals in the population continues to increase with sustained reproductive management. This is the explicit intention of the "placeholder" concept as practiced in the area (Gese and Terletzky 2015). In contrast, removal of individuals without sterilization leaves a full complement of reproductively intact coyotes in the population; reproduction among those remaining individuals quickly replaces those individuals that were removed. This process is akin to the "vacuum effect" often cited in the feral animal and wildlife management literature (e.g., Killian et al. 2007). It is encouraging to note that the model predicts the efficacy of such coyote sterilization programs even if the population of coyotes in the area is substantially larger than what is used in the base input dataset. The efficacy of sterilization in this scenario, however, requires a correspondingly larger effort in the treatment of a constant proportion of animals based on the initial size of the population to be treated.

Specifically, the coyote management scenarios tested here suggest that sterilizing about 10% of the intact coyote population each year is desirable to achieve sustained red wolf population growth, with the removal of some intact coyotes also providing a modest added benefit. Developing credible demographic parameters for the coyote component of this analysis highlighted the difficulty in generating accurate estimates of coyote population abundance in northeastern North Carolina. As a result of this uncertainty, reliably estimating a target proportion of reproductively intact animals to capture and sterilize at any point in time can be reasonably considered a daunting task at best. Simple calculations using some customized model output data can provide a guidepost for the level of sterilization effort recommended here. If we assume a mid-range estimate of 1000 coyotes currently occupying the red wolf recovery area in northeastern North Carolina, and we impose a 10% intact animal sterilization rate (and assuming all animals begin the simulation intact) across the entire coyote population occupying the entire five-county area, we will expect to target approximately 100 animals – males and females, random across age classes – for sterilization in the first year, with the precise number a function of when the sterilization is occurring relative to the timing of litter production among paired females. After five years, as the coyote population begins to decline through reduced reproductive output among an increasing number of sterile adults, approximately 50 to 55 intact animals would be targeted for treatment. After 10 and 20 years, the number of targeted intact animals would be approximately 25 and six, respectively. Summing these estimates across the full 20 years of sterilization effort, on the order of 700 coyotes would be targeted for sterilization according to this protocol.

The level of sterilization effort estimated in the calculations just summarized is considerably greater than what is currently implemented by state and federal managers. There are two important points to consider in the practical application of these results. Firstly, the scenarios tested here featured coyote management for only the first 25 years of the simulation. When coyote population density has been reduced through this management effort, and the red wolf population has grown to a size where wolf-wolf pairing is much more common, the coyote population would likely continue to decline without the need for sustained levels of sterilization over longer periods of time. Secondly, the non-random or clumped spatial distribution of red wolves across northeastern North Carolina may support targeted management of a portion of the local coyote population in order to promote wolf-wolf pairing in a specific area. Once success has been achieved there, the process can be repeated in another portion of the area where red wolves are present. In this way, the prospect of managing the entire coyote population across the entire five-county area can be approached in a more feasible step-wise manner. This PVA makes no comment on the specific coyote management protocols that could be employed to promote red wolf population viability; discussions on the desire and ability of state and federal agencies to increase these types of efforts should occur outside the bounds of this analysis.

The challenging question of the existence of red wolf – coyote hybrids and their fate in northeastern North Carolina and future reintroduction sites has not been explicitly addressed in this analysis. The broader issue of treatment of hybrids in the context of the Endangered Species Act is discussed elsewhere (e.g., Doremus 2010; Ellstrand et al. 2010), with some studies advocating a flexible approach to addressing the existence and management of hybrids on a case-by-case basis at the discretion of appropriate management authorities (e.g., Lind-Riehl et al. 2016). Intentionally maintaining some proportion of hybrids in a red wolf population, with the goal of retaining red wolf genes that may otherwise disappear as the red wolf population declines, also facilitates the retention of coyote genes on that same landscape. The management strategies included in this analysis – targeted removal of hybrid litters and intensive effort around sterilizing coyotes to create "placeholders" designed to limit both coyote population growth and the production of hybrid litters – are consistent with an implicit intent to minimize the number of red wolf – coyote hybrids on a landscape that is being managed for red wolf recovery. At a basic level, it can be argued that unique red wolf genetic material is best maintained by working to improve the growth of the pure red wolf population.

This PVA does not address the threat of sea-level rise and potential habitat inundation in the five counties that define red wolf habitat in northeastern North Carolina. This threat was discussed in some detail in the red wolf Species Status Assessment (USFWS 2018), where habitat models demonstrated the gradual loss of available habitat under different scenarios of sea-level rise and subsequent inundation. This loss of habitat across the five-county area could be simulated in the PVA model as a gradual reduction in canid carrying capacity, further reducing breeding territory availability and, by extension, population growth potential. Red wolf populations could potentially exist at a lower abundance in these sea-level rise scenarios than what is currently possible, although the likelihood of this outcome would be reduced and the genetic viability of those populations would be compromised beyond what is described in this PVA. Expansion of the ENCRWP outside of the current five-county area could be considered as a countermeasure to this threat of habitat loss through inundation. Future revisions to this PVA could include some treatment of this threat and its potential mitigation.

SAFE population dynamics when supporting ENCRWP viability through releases

While demographic viability may be possible in the ENCRWP with releases from the ex situ SAFE source population and with aggressive coyote management, genetic viability requires an even tighter linkage to the SAFE population to maximize introduction and retention of founder gene diversity in the wild. In order to improve the likelihood of ENCRWP viability, a long-term release effort like the Release-High50 strategy tested here is the preferred option. Releases of approximately six adults and eight pups for an initial period of five years, followed by pup-only releases annually for another 15 years with occasional reinforcement every five years for an additional 30 years (total release timeframe: 50 years) can improve red wolf wild population genetic viability over the less intensive Release-Low or Release-High strategies. Despite the benefits to the wild population that result from this strategy, total gene diversity retention in the ENCRWP still drops below 80% after 65 to 75 years, even under more favorable management conditions. The model used here selects individuals of a given age and sex at random in the SAFE population for release to the wild. In practical application of these results, population managers could go a step further and select individuals not only on the basis of age, behavior, or other social considerations but also on an individual animal's genetic characteristics, such as its mean kinship value in the ex situ population. Although this type of targeted selection of animals for release can have many advantages, it is often impractical to apply because of difficulties in properly classifying individual animal temperament, proper matching of age among pups for fostering efforts, etc.

Improvements to wild population genetic diversity through releases from the SAFE population can come at a cost to gene diversity retention in the source population as more individuals are selected from this source population, and over a longer period of time, for release to the wild. The genetic effects of this release program can be best offset by a combination of: (1) expanding the carrying capacity of the SAFE population by increasing the available space across participating institutions, and (2) improving the reproductive output of the SAFE population – specifically, the rate of litter production among paired females. Reducing the proportion of mated females that do not produce a litter by just 15% over current levels can lead to major improvements in gene diversity retained in the SAFE population and, therefore, in the ENCRWP as well.

Discussing improvements to SAFE population reproductive success in terms of reducing the probability of failure of a specific female to produce a litter is recognized as confusing. The choice was made to discuss probability of failure in this report because this is the explicit data input field available in the Vortex software to describe probability of litter production for each adult female. Once the probability of not producing a litter is specified, the software automatically calculates the probability of success, without direct input by the user. Moreover, this probability is a complex function of both the age and past reproductive success (as a categorical variable) of each member of the pair. As a result, the specific probability of success or failure for each female and her mate is calculated individually during the annual breeding event. For example, a pair of four-year-old experienced animals will have a 31.9% chance of failure (68.1% chance of success). Proportionally reducing the failure probability by 15% to 27.1% translates into a 72.9% chance of success, which is a 7% proportional increase. By contrast, a pair of eight-year-old experienced animals will have a 66.4% chance of failure (33.6% chance of success). Reducing this pair's chance of failure by 15% yields a proportional increase in the chance of success by nearly 30% to 43.6%. Ex situ breeding facilities can analyze historical data on both success and failure of litter production and use these data to target improvements in litter production by reducing risks of failure. If we look generally across all recommended pairings in the time window 2001 – 2021, we find that 26.7% of all recommended pairs (independent of age and previous breeding success) were successful in producing a litter (Appendix II, Table 16). This equates to a 73.3% risk of failure among recommended pairs. Therefore, a 15% proportional decrease in the probability of litter production failure – from 73.3% to 62.3% – translates into a 41% increase in success from 26.7% to 37.7%. This could be used as a basic guidepost among ex situ population managers in their efforts to strengthen the ex situ population's capacity as a robust source of animals for release to the wild.

The estimation and simulation of litter production in the SAFE population is a complex process involving many influential variables that emerged from the reproductive viability analysis (RVA) used for this PVA (Appendix III; Bauman et al 2019). The RVA revealed that the age of both dam and sire of any litter that is produced, as well as their reproductive history (whether either of them had produced a litter in the past), were primary factors in determining current success. In fact, the analysis also revealed that the sire's inbreeding coefficient was also a determining factor. Early runs of the PVA model, however, showed that including this sire inbreeding factor led to a sustained projected decline in the SAFE population, rendering it unsustainable on its own and certainly as a source of animals for release to the wild. Further exploration of the RVA revealed some statistical anomalies in the relationship between sire inbreeding coefficient and the likelihood of litter production across a range of inbreeding values that extends far beyond the actual range of values among males in the red wolf studbook. Based on the sensitivity of the RVA model to this factor, particularly outside the range of values currently seen in the studbook, the decision was made to remove this factor from the analysis as releases from the SAFE population would not be an option to promote long-term viability of red wolves in the wild. Nevertheless, this analysis may provide motivation to more fully investigate the effect of male inbreeding on reproductive success in the captive red wolf population.

Establishing new populations through releases from the SAFE population

By necessity, a number of important assumptions must be enumerated when developing scenarios exploring the possibility of establishing viable red wolf populations in hypothetical habitat sites. First is the assumption that there are in fact candidate sites that could be used for red wolf reintroduction. There appears to be no systematic evaluation of candidate sites currently underway; however, a body of literature exists that can provide important guidance in the search for candidate habitats (e.g., O'Neal 2018; Toivonen et al. 2022; Drobes 2022). Site review, analysis and selection will be a detailed and collaborative process that can occur only after the red wolf Recovery Plan has been completed. Second is the assumption that the favorable red wolf demographic rates identified in the ENCRWP modeling as critical for future recovery can be achieved in other sites. Thirdly, these models assume that sites can be found that have both sufficient habitat to support potentially viable red wolf populations and sufficiently low coyote densities to facilitate red wolf population growth. Modeling of the ENCRWP in this analysis demonstrates that an initial population of 15 red wolves can potentially expand to favorable levels with as many as 500 – 800 coyotes on the landscape, given proper management of the many threats to red wolf population growth and viability.

The observation that increasing the red wolf habitat carrying capacity of candidate sites leads to lower chances of population establishment in the presence of coyotes is a direct outcome of the assumption that a larger carrying capacity for red wolves is also a larger carrying capacity for coyotes – if coyotes are present on the landscape when red wolves are to be established. This may not be a logical assumption based on the diversity of canid densities across the eastern United States, but it is a reasonable place to start in this analysis. Identifying coyote densities among a suite of candidate sites should, of course, be an important criterion for site establishment. Whether or not sites can be identified with coyote densities lower than those found in northeastern North Carolina's Albemarle Peninsula is an open topic for discussion.

All of the scenarios explored in this PVA assume that the habitat areas of interest – the northeastern North Carolina habitat area or any hypothetical candidate site chosen for future reintroduction efforts – are effectively isolated with respect to demographic exchange with other canids outside those areas. In other words, reducing the coyote population through sterilization and removal in any given area does not lead to immigration of coyotes from nearby populations into the managed habitat. This assumption might be at least partially justified by the known dynamic whereby established red wolf pairs can effectively displace coyotes from areas now dominated by red wolves and create a barrier of sorts to larger-scale movement of coyotes into a habitat filled with functional red wolf pairs and associated individuals. Nevertheless, assuming a closed demographic system almost certainly overestimates the efficacy of a given level of coyote management effort in the models described here. In an open demographic system, some number of intact coyotes from outside the management area would be expected to move into the area and replace those that have been removed as the coyote population declines. This immigration will reduce the population-wide impact of sterilization efforts – a dynamic that has been explored in similar model-based studies of feral animal control in urban environments (e.g., Miller et al. 2014).

Models that explore the impact on red wolf viability of coyote immigration in managed habitats are themselves composed of many additional assumptions about the nature of demographic connectivity with neighboring habitats: the nature of ecological triggers that prompt immigrant coyotes to move into the managed habitat; the number of coyotes expected to immigrate into the managed population each year; the age and sex of immigrating individuals; the processes by which immigrating animals are able to participate in successful pairing and breeding with residents; etc. By extension, conclusions regarding the strength of this immigration effect are explicitly dependent on the assumptions built into our simulation of the process. While this general principle is also true for the current analysis in total, it can also be argued that the extensive datasets on both in situ and ex situ red wolf populations that underlie the simulation models presented here provide a strong foundation for realistic model construction and informed interpretation of their output. Therefore, after considering these sources of additional uncertainty, the decision was made to exclude these types of coyote immigration scenarios from the current PVA. State and federal authorities that may be interested in revising/updating the present analysis may consider developing realistic scenarios that explore this dynamic and its impacts on red wolf recovery.

In the absence of this type of analysis, reasoned logic can be used to recognize that when there is evidence that significant immigration is occurring of reproductively capable coyotes into a habitat that is being managed to reduce coyote breeding success, additional management effort must be employed to offset the presence of those immigrants. This adaptive approach to management is critically dependent on obtaining reliable estimates of standing coyote abundance in a specific defined area and the extent of immigration of coyotes into that area. Demographic studies of sterilization impacts on free-roaming cats in urban environments highlight the importance of "front-loading" sterilization efforts – maximizing treatment rates in the early years of a population management campaign – in achieving desired longer-term population outcomes (Miller et al. 2014; Benka et al. 2021).

Overall, where scenario characteristics overlap across analyses, the results coming from this updated PVA effort are very much in line with those of its immediate predecessor (Faust et al. 2016). Both analyses generate high probability of red wolf population extinction in northeastern North Carolina, with recent observed declines in the wild outpacing those predicted in the earlier modeling effort. Importantly, the earlier analysis also highlighted two critical needs for the SAFE population: (1) to increase SAFE population carrying capacity by adding breeding and holding spaces across a greater number of participating institutions, and (2) to improve breeding success across those institutions to both better maintain genetic diversity through time but to also satisfy the need for larger number of individuals for release. With the additional analyses in this PVA focusing on efforts to establish additional populations beyond northeastern North Carolina, the benefits of expanding the ex situ population and its central role in supporting in situ conservation become that much more evident. The close cooperation between ex situ and in situ conservation activities is central to the One Plan approach to effective integrated conservation planning (Byers et al. 2013; McGowan et al. 2016).

Thoughts on future data collection priorities

As should be the case in any structured and deliberate analysis of this type, the PVA process has identified a number of areas of uncertainty in our knowledge of red wolf demography and the ways in which they interact with coyotes. These data gaps include but are not restricted to:

- Assessment of the effects of inbreeding on red wolf fitness: Analysis of available data identified a significant impact of inbreeding (kinship between parents) on litter size in the ENCRWP. Similar analyses suggested a somewhat paradoxical increase in survival with higher levels of inbreeding, and a potentially significant impact of inbreeding on the probability of litter production in the SAFE population. Given the very small number of red wolves currently in the wild, and despite active management to reduce the occurrence of inbreeding, it is important to monitor the wild population for evidence that inbreeding depression may be reducing aspects of reproductive success and/or survival of specific age classes.
- Ongoing evaluation of factors influencing pairing success among red wolves in the ex situ environment: The current PVA and other analyses of red wolf population dynamics in the ex situ environment indicate that pairing success should be improved for long-term sustainability of the valuable source population. It is important to continue assembling and analyzing data from ex situ breeding facilities on annual rates of pairing success and the potential factors that help determine

success or failure among the various pairing recommendations made annual in AZA's Breeding and Transfer Plan process.

- Assessment of post-release survival among individuals added to the wild from the SAFE population: Analysis of existing data suggests that released individuals are immediately subject to the same mortality risk as wild-born individuals to the next census period and beyond. There are recent years, however, in which there has been significantly higher mortality of individuals released from the SAFE population. There will always be variability in the immediate success of those releases, but continuing to monitor the fate of released individuals will become increasingly important as releases likely to increase in magnitude.
- Ongoing monitoring of red wolf population responses to threat management: This PVA highlights quantitative targets for reducing anthropogenic mortality, primarily from gunshot and vehicle collisions. If expanded mitigation efforts are undertaken to further reduce these sources of mortality, it is vital to monitor the extent to which the red wolf population displays increased growth in response to alleviating these and other threats.
- Ongoing analysis of variables influencing mate choice among red wolves and coyotes in a mixed canid system: The present PVA model requires, for better or worse, numerical specification of the probability of successful pairing between canids after an encounter. While an extensive literature exists around mate choice in this system (e.g., Fredrickson and Hedrick 2006; Bohling and Waits 2015; Gese et al. 2015; Bohling et al. 2016; Hinton et al. 2018), gaps in our knowledge remain. Additional data can help determine if red wolves and coyotes are in fact choosing mates based on specific attributes, or if pairing is effectively random between individuals, independent of taxonomic identity. Other important questions include the influence of canid densities on mate choice, inbreeding levels, etc.
- Ongoing monitoring of local coyote abundance: Determining the actual number of coyotes in northeastern North Carolina or other candidate red wolf release sites remains challenging. Multiple methods have been used to generate abundance estimates, often giving quite different results. If possible, developing a consistent method for robust estimation of coyote abundance can provide a critical piece of information to assist in red wolf recovery.
- Application of information on coyote movement ecology and immigration rates to managing current and future red wolf habitats: Predicting the extent of immigration of coyotes into a managed red wolf recovery site from neighboring landscapes -- numbers of individuals, the age-sex composition of the immigrant cohort, their ability to successfully integrate into the breeding pool, etc. – is also critical to our understanding of how coyote populations respond to management in open demographic environments.

Taking everything together in this report – recognizing both the extensive body of information collected by dedicated experts in wild and captive environments as well as the broad areas of uncertainty in our understanding of the complex dynamics of mixed canid populations in wild habitats – it is appropriate to consider the present PVA as a "living document" subject to continued scrutiny, review and revision. As management actions are implemented across the in situ – ex situ spectrum, and as continued research provides the red wolf management community with new data on population demography and genetics, effects of those management actions and implications of those research findings can be evaluated in light of this analysis. The modeling tool can then be adjusted or expanded accordingly to provide new insights and guidance on how to proceed more effectively with red wolf recovery.

Conclusions

- The existing Eastern North Carolina Red Wolf Population (ENCRWP) is not currently viable without significant additional management intervention. Despite active current management of this very small population, declines in abundance will likely continue in the face of persistent threats including high anthropogenic mortality and continued hybridization with coyotes. If releases from the ex situ source SAFE population were to be discontinued, ENCRWP extinction is likely in the next 2-3 decades.
- Continued releases to the ENCRWP of red wolves from the SAFE population are required to build red wolf numbers as an early step in creating a viable wild population. Model results suggest that release efforts should strive to release six adults and eight pups (equal sex ratio) each year over the first five years of the release protocol, with pup fostering also included for 20 years followed by periodic reinforcement of six pups every five years for a total program duration of 50 years (the Release-High50 strategy). Compared to other strategies tested in this analysis, employing this longer-term strategy maximizes the introduction and retention of genetic diversity from the SAFE source population in wild red wolves while also greatly improving that wild population's growth and demographic stability.
- As a target for future management, anthropogenic sources of mortality should be reduced, if possible, by 50% over current levels. Management should target deaths of red wolves by gunshot and vehicle collisions. These efforts to reduce mortality must be sustained over the coming decades to facilitate increased red wolf abundance and improved breeding opportunities.
- Direct intervention in coyote population breeding success in northeastern North Carolina appears to be the most effective management action to promote red wolf population growth and viability. Sterilization of intact male and female coyotes is considerably more effective as a population management strategy compared to an equivalent rate of removal of individuals from the population.
- An annual sterilization rate of 10% of the intact coyote population each year for up to 25 years is effective in reducing the coyote population to a level that allows sufficient wolf-wolf pair formation for promoting red wolf population growth. Defining a proportional management target instead of an absolute number of coyotes to target for sterilization or removal provides greater flexibility for applying this action to coyote populations of different size. However, because of the difficult nature of reliably estimating coyote population size across red wolf habitats where coyotes coexist, developing these types of targets for annual sterilization activities is recognized as challenging. If possible, continued methodological improvements in coyote population abundance estimation techniques in both northeastern North Carolina and other potential red wolf sites will be an important contribution to improved evidence-based red wolf management.
- In order to satisfy its role as a source of individuals for release to the wild while maintaining its own genetic and demographic viability, the red wolf SAFE program should explore ways in which reproductive output can be increased among paired females. First, the present analysis indicates the value of increasing available space from the current capacity of 300 individuals to 400 individuals. Second, the increased space can be used effectively through a 15% reduction in the proportion of mated pairs that do not produce a litter – conversely, after assessing all recommended pairings in this population since 2001, this equates to an increase in annual successful litter production across all adult ages and previous reproductive experience on the order of 40%.
- In keeping with the ENCRWP modeling results, managing coyote densities in candidate release sites appears to be a critical factor influencing the potential success of efforts to establish viable red wolf populations in new areas. Establishment models suggest that aggressive coyote management efforts should accompany red wolf release efforts from the beginning; if efforts are not at sufficient levels, coyote populations of just 100 to 150 individuals could significantly compromise long-term release efforts.

- If new population establishment activities are to begin in the relatively short term (10 to 15 years), the added strain of releasing larger numbers of animals from the SAFE program could put that population at risk of reduced genetic viability. If space to accommodate wolves among the many participating SAFE program institutions can be increased from 300 to 400 animals, and reproductive success can be improved (15% reduction in paired females failing to produce a litter), gene diversity loss in this valuable source population can be reduced.
- The PVA described here is a valuable platform for highlighting gaps in our knowledge of red wolf and coyote demography, and the complex ways in which these two species interact. Continued collection and analysis of key data – including refinements in red wolf demographic parameters, the intrinsic and extrinsic factors that influence reproduction and survival, demographic responses to threat management, and core characteristics of coyote populations that interact with red wolves – will improve the inputs to future implementations of population viability analysis to guide red wolf recovery.
- The analytical results presented here suggest that recovery of red wolves in the wild can be achieved – and can perhaps be realized in 40 to 50 years if conditions are right. However, success will likely require substantial management efforts beyond many of those currently implemented by the range of dedicated state and Federal management authorities and field biologists already engaged in red wolf conservation. This report will hopefully serve as a valuable blueprint in the conservation toolbox to help the Red Wolf Recovery Team identify key targets for population recovery and identify important actions to make recovery a reality.

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Appendix I

Scenario Designations and Characteristics

Scenario #	Scenario Label	Scenario Description
ENCRWP		
1	Current, no releases (Figs. 3-5)	Current demographic management (status quo), but with an assumption of no future releases. Initial coyote population assumed to be 500 individuals.
2	Current, K=350 (Fig. 6)	As in scenario #1, with SAFE carrying capacity set to 350
3	Current, K=350, More Pups (Fig. 6)	As in scenario #2, with improved litter production in the SAFE population (15% proportional reduction in the litter production failure rate)
4	Release Only	As in scenario #1, with the addition of releases from SAFE population: Release-Low: Four adults each year for model years 1 through 5; six pups each year for model years 1 through 20 Release-High: Six adults each year for model years 1 through 5; eight pups each year for model years 1 through 20 Release-High50: Six each year for model years 1 through 5; Eight each year for model years 1 through 20, then eight every five years thereafter for model years 25 through 50
5	Release + Shift	As in scenario #4, with the addition a shift in seasonal timing of mortality leading to increased annual chance of litter production among wild red wolves from baseline of 60% to 70%
6	Release + Shift + Pair	As in scenario #5, with the addition of an increase in the probability of wild wolf-wolf pairing from baseline of 63.5% to 80%
7	Release + Shift + Pair + [X%]	As in scenario #6, with the addition of a proportion reduction in total mortality resulting from a reduction in anthropogenic mortality of X = 25%, 37.5%, or 50%. Scenarios #6 and #7 run in the absence or presence of coyote management (10% sterilization / 5% removal of intact animals each year for first 20 years of simulation)
8	Remove 10%	As in scenario #7, with a 50% reduction in anthropogenic mortality and the Release-High release strategy. Coyote management restricted to 10% sterilization of intact coyotes for first 20 years of simulation.
9	Sterilize 5%	As in scenario #8, with coyote management restricted to 50% sterilization of intact coyotes for first 20 years of simulation.
10	Remove 10%	As in scenario #8, with coyote management restricted to 10% removal of intact coyotes for first 20 years of simulation.
11	Remove 5%	As in scenario #8, with coyote management restricted to 50% sterilization of intact coyotes for first 20 years of simulation.

Scenario #	Scenario Label	Scenario Description
12	K=300 / Base	As in scenario #7, with a 50% reduction in anthropogenic mortality, the Release-High50 release strategy, and coyote management (10% sterilization / 5% removal). SAFE population carrying capacity set to 300. Baseline rate of SAFE population litter production.
13	K=350 / Base	As in scenario #12, with SAFE population carrying capacity set to 350. Baseline rate of SAFE population litter production.
14	K=350 / Improved	As in scenario #12, with SAFE population carrying capacity set to 350. Improved rate of SAFE population litter production (15% proportional reduction in the litter production failure rate).
15	K=400 / Base	As in scenario #12, with SAFE population carrying capacity set to 400. Baseline rate of SAFE population litter production.
16	K=400 / Improved	As in scenario #12, SAFE population carrying capacity set to 400. Improved rate of SAFE population litter production (15% proportional reduction in the litter production failure rate).
17	[Low / High] – [300 / 350 / 400] – [Base / Improved]	As in scenario #12, with different combinations of release strategy, SAFE population carrying capacity, and SAFE population litter production success.
18	500 / 10%	As in scenario #7, with a 50% reduction in anthropogenic mortality and the Release-High release strategy. Standard rate of coyote management (10% sterilization / 5% removal of intact animals each year for first 20 years of simulation).
19	1000 / 5%	As in scenario #18, with initial coyote population of 1000 individuals and a lower rate of coyote management (5% sterilization / 2.5% removal of intact animals each year for first 20 years of simulation).
20	2000 / 5%	As in scenario #18, with initial coyote population of 2000 individuals and a lower rate of coyote management (5% sterilization / 2.5% removal of intact animals each year for first 20 years of simulation).
21	1000 / 10%	As in scenario #18, with initial coyote population of 1000 individuals and the standard rate of coyote management (10% sterilization / 5% removal of intact animals each year for first 20 years of simulation).
22	2000 / 10%	As in scenario #18, with initial coyote population of 2000 individuals and the standard rate of coyote management (10% sterilization / 5% removal of intact animals each year for first 20 years of simulation).

Scenario #	Scenario Label	Scenario Description
NEW SITES		
23	[0 / 50 / 100 / 150 / 200] + [K=200] + [Full]	As in scenario #7, with a 50% reduction in anthropogenic mortality. SAFE population carrying capacity set to 400, with Improved litter production. Range of initial coyote abundance from a minimum of 0 to a maximum of 200. K=200: Carrying capacity for each of new populations Wild-2 and Wild-3. Full: Standard coyote management (10% sterilization / 5% removal of intact animals each year for first 20 years of simulation). Also includes Large Release (See page 11 for details of Large Release).
24	[0 / 50 / 100 / 150 / 200] + [K=200] + [50% Releases]	As in scenario #23, with the number of individuals released according to the Large Release strategy reduced by 50%, i.e., the Small Release strategy.
25	[0 / 50 / 100 / 150 / 200] + [K=200] + [50% Coyotes]	As in scenario #23, with a 50% reduction in the coyote management rate (5% sterilization / 2.5% removal of intact animals each year for first 20 years of simulation).
26	[0 / 50 / 100 / 150 / 200] + [K=300] + [Full]	As in scenario #23, with carrying capacity for each wild population set to 300.
27	[0 / 50 / 100 / 150 / 200] + [K=300] + [50% Releases]	As in scenario #24, with carrying capacity for each wild population set to 300.
28	[0 / 50 / 100 / 150 / 200] + [K=300] + [50% Coyotes]	As in scenario #25, with carrying capacity for each wild population set to 300.

Appendix II

Red Wolf *(Canis rufus)* Data Analysis Report for Population Viability Analysis

2 February 2023
REVISED 24 May 2023

Developed by:
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Vortex Model Supporting Analyses

This Appendix details the supporting analyses run for set-up of the metapopulation model of the red wolf (*Canis rufus*) for use in subsequent PVA modeling in Vortex. Supporting analyses were developed by Dr. Arielle Parsons of Lincoln Park Zoo as a member of and in collaboration with the Red Wolf Population Viability Analysis Team. All code used for supporting analyses may be made available on request and/or will be archived with later publication of this research.

Sections

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1. Data sources

Data for the red wolf population and its management were collected from the Species Survival Plan® (SSP) Studbook (Davis 2022), the US Fish and Wildlife Red Wolf Population Database (USFWS, unpublished data), and PMCTrack (www.PMCTrack.org; (Faust et al. 2021). For the SAFE (ex-situ) population the demographic window, a period with "modern management" practices that are likely to be similar to future practices, was considered 1 January 1990 – 31 December 2021. There have been two major demographic windows identified for the Northeastern North Carolina (ENCRWP; in-situ) red wolf population. The first window is 1 January 2000 – 31 December 2014 when regular releases from the SAFE to ENCRWP were happening and active control of coyote hybridization was taking place. During this window, coyote hunting was allowed year-round from 2000-2012, prohibited at night in 2013 in the eastern North Carolina counties adjacent to and including the red wolf in-situ release site (Dare, Hyde, Tyrrell, Washington, and Beaufort counties) and prohibited completely in these counties in 2014. During this window, regular intensive monitoring of pairs took place, contributing to a higher level of certainty in reproductive data.

The second window is 1 January 2015-31 December 2021 when releases and active management of hybridization were majority halted and coyote hunting allowed only during daylight hours and by permit in Dare, Hyde, Tyrrell, Washington, and Beaufort counties. During this window, regular monitoring of pairs was hampered by reduced personnel and lack of access to private land, contributing to low certainty in reproductive data. Mortality data were not as affected by these changes in monitoring.

In the following analysis, we test differences in mortality rates between these windows, summarizing them both within each window separately and together over both windows where warranted. We only analyze reproductive data, specifically pairing and breeding success rates, from the 2000-2014 window, when certainty in data quality are higher.

2. A note on discrepancies with results from (Faust et al. 2016)

Some results in this report, including mortality tables and breeding tables, exhibit discrepancies when compared to the Faust et al. 2016 PVA report. Most of the analyses include an additional 5 years of data so we do not expect identical results to the 2016 analysis. Nevertheless, even when we replicated the same date range as the 2016 report, some discrepancies exist. The main reasons for these discrepancies are data improvements, including: 1) birthdates missing from the 2016 dataset but present in the 2022 dataset following studbook updates which enable inclusion of additional animals in age-specific analyses; 2) more consistent censoring of lost-to-follow-up (LTF) animals so that they are excluded in the 2022 analysis after they have gone LTF; 3) more consistent use of staggered entry in mortality and breeding analyses in the 2022 analysis; 4) inclusion of released animals in the SAFE population prior to release instead of censoring them out completely; and 5) more accurate genetic information available on which to base whether animals were full red wolves, hybrids, or coyotes.

Additionally, sample size discrepancies can be seen for the number of pups considered in the analysis of first year mortality and pup sex ratio where sample sizes are smaller than the sample considered in (Faust et al. 2016). These smaller sample sizes are due to a decrease in pups from the ENCRWP for two main reasons: 1) only litters and pups confirmed to be red wolf litters were considered, based on better genetic information available in the present analysis 2) some birthdates for pups were missing from the dataset available in 2016 but are available in this analysis (n=15 additional litters included in present analysis). Ultimately, we feel confident that these analyses use the highest quality and most up-to-date data available, with appropriate analytical assumptions.

3. Supporting analyses

For all following analyses, unless otherwise indicated, we restricted the animals included to only full red wolves, defined as those with >=75% red wolf ancestry. Ancestry values were predominantly assessed through genetic testing (J. Adams, pers. comm), but some were inferred from pedigree associations within the studbook (Davis 2022). Some of the following analyses specifically compared red wolves (>=75% red wolf ancestry) to hybrids (<75% and >=0.25% red wolf ancestry) and coyotes (<25% red wolf ancestry) and are noted as such. The cutoff values (>= 75%, <25%) for delineating different types of canids were based on recommendations and consensus discussions within the larger PVA Team.

The census date was set as 31 December each year and classes included individuals of that age as of 1 January of that year. Released animals were included in the analysis in the SAFE population up until their release (and if they return later to the SAFE) with appropriate non-death exit age. After release, they were included in the ENCRWP population with appropriate entry age EXCEPT pup releases which were not included in the 0-1 age class. Pup releases (pups fostered in the first few weeks of life or releases of any animals younger than 1) into ENCRWP are not included in that population's data until they are 1 year old so they don't bias the 0-1 age class after spending at least some of their first year in the SAFE population; this the ENCRWP 0-1 mortality reflects that of wild-born red wolves. All analyses assume LTF animals are dead unless a true death was recorded after an animal went LTF in which case we know the animal was alive during the LTF period and the LTF is ignored. We also assumed any animal that went LTF less than 1 year before the window end-date was alive.

3a. Mortality analysis

We analyzed overall mortality patterns of red wolves using a Cox proportional hazard model performed in program R (R Core Team 2021) using the survival package (Therneau 2022) and functions survfit and coxph. We included both SAFE (n=1054) and ENCRWP (n=790) individuals in the analysis and assessed whether mortality rates differed between the two populations or sexes and (in the case of the ENCRWP) hybrid status, as well as between time periods.

Difference in mortality between ENCRWP time windows

We started by testing differences between the two ENCRWP time periods (1 January 2000-31 December 2014 and 1 January 2015-31 December 2021). **We found no significant difference in cumulative survival rates (i.e., 1-mortality; p=0.98), supporting the pooling of mortality rates for the two ENCRWP time periods (Figure II-1).** Mortality rates for the 2015-2021 window were particularly variable and are likely not representative, due to low sample sizes (2000-2014 mean sample size = 131, sd = 171; 2015-2021 mean sample size = 23.8, sd = 11.4). Full data on which this analysis is based is shown in Table II-1.

Difference in mortality between ENCRWP and SAFE populations

We tested for differences in mortality rates between each population (SAFE and ENCRWP with both ENCRWP windows combined: 1 January 2000 – 31 December 2021)). **We found that the SAFE cumulative survival rate (i.e., 1-mortality rate) was significantly higher than the ENCRWP cumulative survival rate such that ENCRWP red wolves have a 3.52 times higher risk of mortality than SAFE red wolves (p < 0.0001; Figure II-2).** Full data on which this analysis is based is shown in Table II-2.

Difference in mortality between sexes

We tested for differences in mortality rates between sexes for each population separately (SAFE (1990-2000) and ENCRWP with both windows combined (2000-2021)). **We found no significant difference in cumulative survival rates (1-mortality) between the sexes for either the SAFE population (Cox p value = 0.71) or the ENCRWP population (Cox p-value = 0.43), supporting the pooling of sexes in each population for further analysis.**

Difference in mortality between red wolves and hybrids/coyotes in the ENCRWP

We tested for differences in mortality rates between red wolves and “not red wolves” (i.e., pooled hybrids and coyotes, red wolf ancestry <75%). **We found no significant difference in cumulative survival (i.e., 1-mortality rate) between red wolves and pooled hybrids/coyotes (Cox p-value = 0. 09).** However, we note that sample size for pooled hybrids and coyotes for which we had complete information on which to base analysis is small (n=44) because most lacked birthdates and/or clear ancestry in the database to call them “not red wolves”. Therefore, we recommend treating this result with caution.

ENCRWP mortality timing

We assessed how the timing of mortality in comparison to the pre-breeding and breeding seasons changed over time by treating each mortality as a binary variable: 1 = occurred inside the breeding season, 0 = occurred outside the pre-breeding and breeding seasons where the breeding season is defined as 10/1 to 3/31. This analysis only considered animals for which a death could be corroborated (i.e., no LTFs were considered). We used a logistic regression to determine if the fraction of mortalities occurring during the pre-breeding and breeding seasons has changed over time. **Indeed, our analysis indicates there has been a significant (p <0.0001) increase in the fraction of mortalities that occur during the pre-breeding and breeding seasons (Figure II-3). The mean probability of death during the pre-breeding and breeding seasons was 0.39 (SE=0.02) in 2000-2014 and 0.70 (SE=0.05) in 2015-2021**.

While overall mortality may not necessarily be increasing over time, the timing of mortality during the breeding season means that deaths may be disruptive to population dynamics, possibly impacting reproductive rates.

Annual stage-based mortality to estimate mean mortality rates and environmental variation
The amount of environmental variation caused by typical fluctuations in the local environment (food, predation, disease, weather) is important to understanding annual variation in demographic rates. Vortex simulates mortality using a mean rate with environmental variation (EV), annually drawing an estimate from within this distribution for each age/stage class. Rather than using age-based cumulative rates across the demographic window and using expert opinion for the EV rates as was done in Faust et al. 2016 (i.e. Table II-2), we chose to calculate annual, stage-based mortality rates and directly calculate EV. We partitioned annual variation into contributions from demographic stochasticity and contributions from the environment following Lacy et al. (2021). To calculate EV, we obtained means of annual rates by first calculating a rate for each year of data (described below), then averaging those annual rates which was unweighted by annual sample size as recommended by Lacy et al. (2021). We used the resulting average mortality rates and estimates of annual environmental variation (EV) as inputs into Vortex.

First, we calculated annual morality rates for four stage classes: 0-1 years old (<1), 1-2 years old (>=1, <2) when red wolves are pre-reproductive, 2-7 years old (>=2, <7), the years of peak reproduction, and 7+ years old (>=7), the years when age-based mortality rates begin to increase (Table II-2). We calculated these for each population (SAFE 1990-2021 and ENCRWP 2000-2021, with the sexes pooled together; Table II-3, II-4). For the 0-1 age class, the number at risk is the number of animals born in a given year. We calculated the number of deaths for the 0-1 class based on following those individuals for 365 days (even if their death occurred in the next calendar year), and removed released animals from this age class for both the SAFE and ENCRWP populations. We note that the mean estimate for mortality in the 0-1 age class (s3) is higher than for the 0-1 age class in the age-based tables (Table II-2) since individuals in the 0-1 age class in Table II-2 were able to enter into the class at any point (e.g., 6 months old) and because a cumulative (across years) estimate can dampen some of the inter-annual variability observed. In the 1-2, 2-7 and 7+ age classes, we considered at-risk animals to be those whose age fell within the age class on 1 January of a given year, thus there is a discrepancy between those surviving the 0-1 age class and those entering the 1-2 age class in a given year. Further discrepancies with Table II-2, especially in the 1-2 class, are due to individuals that were born in the preceding year (thus not included in the 0-1 age class) and died before the census date in the next year, thus were not included in the 1-2 age class. Pup releases were not included in the 0-1 age class but were included in the older age classes after they survived 1-year post-release. We considered non-pup (>= 1 year old) releases only after they had survived to the next census date (12/31) following their release. We only considered full red wolves in this analysis (>=75% red wolf ancestry). The resulting annual stage-based tables are found in Tables II-3 (SAFE) and II-4 (ENCRWP).

We summarized EV as the annual variation that exceeds the variation expected as sampling error for a binomial distribution (Akçakaya 2002) following the methodology shown in the Vortex manual (Lacy et al. 2020). A summary of the resulting mean mortality rates and levels of environmental variation (standard deviation of EV) to be used in Vortex modeling are shown in Table II-5. We calculated these with all years, and with only years with $\geq$ 10 individuals, which limits the data to years where estimates are more robust based on larger sample sizes.

3b. Analysis of post-release mortality

For the ENCRWP 1986-2021, we analyzed red wolf mortality post-release using a Cox proportional hazards model. This time window differed from the 2000-2021 time window due to non-pup releases only occurring with frequency prior to 2000. We analyzed mortality from release to 12/31 of the release year (i.e., census date). We performed this analysis separately for those animals released as non-pups (>=1 year of age at release) and those fostered as pups (<1 year of age at release) and tested any differences between the type of release and between the sexes. We split non-pup releases into those released directly from the SAFE population and those released from other wild sites (St. Vincent Island and Great Smoky Mountains National Park). Sample size was not high enough to assess post-release survival for different non-pup age classes (i.e., 1-2 years old subadults and 2+ years old adults) thus all non-pup ages were pooled. All released animals were full red wolves (>=75% red wolf ancestry).

There was no significant difference in survival to the next census for non-pups released from the SAFE and non-pups translocated from other wild sites (p=0.23) and these release types were pooled for the remaining analyses presented here. There was no significant difference in mortality between the sexes for non-pup releases (all release types; p=0.30) or pup fosters (p=0.41). There was no significant difference between pup fosters and non-pup release survival to the next census (all release types; p=0.80). A results summary of this analysis is found in Table II-6.

Released animals were censored out of the baseline mortality rates calculated in Table II-5 until they survived to the next census (12/31) post-release, so that the Vortex model can include post-release mortality explicitly in the Translocation (Harvest/Release) process. This would allow modeling of the assumption that the first months post-release are riskier, after which red wolves settle into ENCRWP and take on wild mortality rates. To test this assumption, we assessed differences in survival to the next census between releases (including non-pup and pup fosters) and wild-born red wolves of the same age ranges to determine if releases have similar mortality rates to ENCRWP mortality rates. Since releases, especially for non-pups, occurred throughout the year, we included ENCRWP individuals (wild-born) in the analysis who were alive in the ENCRWP at the start of each month/year where releases occurred and tracked their survival to the following census date (12/31 of that year). We used animals aged 1-7 years for comparison with non-pup release survival rates and individuals aged 0-1 for comparison with pup fosters. We censored out any pups that were older than 1-year-old by the census date.

There was no significant difference in survival to the next census of non-pup releases (all non-pup releases included) and wild-born animals of the same age range during the same months (p=0.69). There was also no significant difference in survival to the next census for pup fosters and wild-born pups of the same approximate age (p=0.18).

3c. First year mortality and inbreeding

We analyzed the impact of inbreeding on first-year mortality using only individuals who spent their entire first year in a population (i.e., no pup releases). Inbreeding was measured as kinship between the parents (i.e., the F-value of the pups). We used a logistic regression (generalized linear model with logit link) to predict mortalities as a function of pup inbreeding value (F) separately for the SAFE population (1990-2021) and ENCRWP population (2000-2021) using the general logistic relationship:

$$\text{logit}(p_m) = \beta_0 + \beta_1 * F$$

where the probability of a pup dying within the first year of life (p_m) is given as the inverse logit of a linear function of pup inbreeding (F) where β_0 is the intercept term and β_1 the slope coefficient associated with F. Since we analyzed each population separately, there is an intercept and slope term associated with each population:

	SAFE 1990-2021			ENCRWP 2000-2021		
Parameter	Estimate	SE	p-value	Estimate	SE	p-value
β_0: intercept	-0.71	0.21	<0.001	0.06	0.15	0.71
β_1: slope	-1.54	2.83	0.59	1.00	1.00	0.32

This analysis only considers animals of known (numeric) ancestry and all tests include only full wolves (>=75% red wolf ancestry). Since mortality did not differ significantly between the sexes or ENCRWP time periods based on the previous analyses, sexes were pooled. In total, we analyzed mortality for 1609 pups, 620 in the ENCRWP and 989 in the SAFE. Inbreeding values of the pups ranged from 0 to 0.341 in the ENCRWP (median = 0.11, mean = 0.13, standard deviation = 0.09) and 0 to 0.29 in the SAFE population (median = 0.07, mean = 0.07, standard deviation = 0.04).

For the SAFE population, inbreeding was associated with a decreased probability of dying in the first year, but results were not statistically significant (p=0.59). For red wolves in the ENCRWP population, inbreeding was associated with an increased probability of dying in the first year, but results were not statistically significant (p=0.31; Figure II-4).

3d. Analysis of the impact of inbreeding on litter size and birth sex ratio

Impact of inbreeding on litter size

We used all ENCRWP full red wolf litters (>=75% red wolf ancestry) from 2000-2021 and all SAFE litters 1990-2021 for which we had full birthdates and parent identification. This analysis does not include any ENCRWP litters that were euthanized via management as those are not considered full red wolves. In total, our dataset included 430 litters, 134 of which were ENCRWP-born and 296 of which were SAFE-born. Kinship between parents (i.e., the inbreeding value of the pups; F) ranged from 0 to 0.341 in the ENCRWP population (median = 0.12, mean = 0.11, standard deviation = 0.08) and 0 to 0.25 in the SAFE population (median = 0.07, mean = 0.06, standard deviation = 0.04). We used a Poisson regression (generalized linear model with log link), to evaluated the impact of kinship between parents and population on litter size. The data are well-approximated by a Poisson distribution, except from the lack of 0s. However, mean litter size over both populations is large enough (mean=4.51) that the probability mass of 0 is only 0.015 (relatively infrequent). The data are slightly over-dispersed, but not excessively so as the variance (var=4.18) is only 1.08 times the mean.

We tested for differences in the relationship between litter size and pup inbreeding by coding populations as 1 (ENCRWP) or 0 (SAFE) and adding terms for pup inbreeding (F), population (Pop) and an interaction term of ($F * Pop$) to determine if each population had a different relationship slope. Specifically, we set up the linear relationship:

$$\log(s) = \beta_0 + \beta_1 * F + \beta_2 * Pop + \beta_3 * (F * Pop)$$

Where s is the litter size which is modeled on the log scale as a function of a linear combination of predictors which include pup inbreeding value (F), population (SAFE or ENCRWP coded as 0 and 1, respectively; Pop) and the interaction between these two predictors ($F * Pop$). β_0 is the intercept term, β_1 is the slope coefficient associated with F, β_2 is the slope coefficient associated with Pop and β_3

is the slope coefficient associated with the interaction $F * Pop$, allowing inference on whether the two populations differ in their relationship between s and F.

There was no significant difference in the relationship between kinship and litter size between the populations (p-value associated with slope of interaction term (F*Pop) =0.10; Figure II-6).
We tested for a difference in litter size between the two populations and tested the relationship between litter size and pup inbreeding F using the relationship:

$$\log(s) = \beta_0 + \beta_1 * F + \beta_2 * Pop$$

Where s is the litter size which is modeled on the log scale as a function of a linear combination of predictors which include pup inbreeding value (F) and population (SAFE or ENCRWP coded as 0 and 1, respectively; Pop). β_0 is the intercept term, β_1 is the slope coefficient associated with F, β_2 is the slope coefficient associated with Pop.

Parameter	Estimate	SE	p-value
β_0: intercept	1.51	0.39	<0.0001
β_1: slope (Pop)	0.18	0.06	0.001
β_2: slope (F)	-1.62	0.42	0.0001

ENCRWP mean litter size (mean=4.41, sd=2.16) was significantly higher than SAFE mean litter size (mean=4.07, sd=2.10) across all values of F (p = 0.001; Figure II-5, Table II-7). There was a significant negative relationship between litter size and kinship between parents (p=0.0001; Figure II-6).

Based on this model, the expressions for litter size in each population become:

$$\log(s_{SSP}) = 1.51 - 1.62 * F$$
$$\log(s_{NENC}) = (1.51 + 0.18) - 1.62 * F$$

Impact of inbreeding on offspring sex ratio
We analyzed the impact of inbreeding on the birth sex ratio of SAFE (1990-2021) and ENCRWP (2000-2021) red wolves (>=75% red wolf ancestry). We included 1865 total pups: 956 females (51.3%) and 909 males (48.7%) with 639 pups being from the ENCRWP (34.3%) and 1226 from the SAFE (65.7%). We removed unknown sex individuals and individuals with unknown parents but retained individuals whose birthdates were unknown. Kinship between parents (i.e., the inbreeding value of the pups; F) ranged from 0 to 0.341 in the ENCRWP population (median = 0.12, mean = 0.11, standard deviation = 0.08) and 0 to 0.25 in the SAFE population (median = 0.07, mean = 0.06, standard deviation = 0.04). Note that the number of pups considered in the ENCRWP in this analysis is lower than considered in Faust et al. 2016 because the present analysis included only animals verified to be >=75% red wolf ancestry. We used a logistic regression (generalized linear model with logit link), using the pup's inbreeding value and population to predict the sex and test differences between the populations. First, we tested random effects for dam and litter, but neither were supported as assessed by AIC over models without random effects. Second, we tested for differences in birth sex ratio between the populations by only including F-value and a population term in our model.

$$\text{logit}(r) = \beta_0 + \beta_1 * F + \beta_2 * Pop$$

where r is pup sex coded as Male=1 and Female=0 which is modeled on the logit scale as a function of a linear combination of predictors which include an intercept, pup inbreeding value (F), population (SAFE or ENCRWP coded as 0 and 1, respectively; Pop). β_0 is the intercept term, β_1 is the slope coefficient associated with F and β_2 is the slope coefficient associated with Pop.

Parameter	Estimate	SE	p-value
β_0: intercept	-0.20	0.08	0.010
β_1: slope (F)	1.52	0.85	0.072
β_2: slope (Pop)	0.07	0.11	0.549

There was no significant difference in the birth sex ratio between the SAFE and ENCRWP populations (p = 0.549).

Then, we tested for differences in the relationship between birth sex ratio and pup inbreeding by coding populations as 1 (ENCRWP) or 0 (SAFE) and adding terms for pup inbreeding (F), population (Pop) and an interaction term of $(F * Pop)$ to determine if each population had a different relationship (i.e., slope) between birth sex ratio and pup inbreeding value. Specifically, we set up the linear relationship:

$$\text{logit}(r) = \beta_0 + \beta_1 * F + \beta_2 * Pop + \beta_3 * (F * Pop)$$

Where r is pup sex coded as Male=1 and Female=0 which is modeled on the logit scale as a function of a linear combination of predictors which include an intercept, pup inbreeding value (F), population (SAFE or ENCRWP coded as 0 and 1, respectively; Pop) and the interaction between pup inbreeding and population (F*Pop). β_0 is the intercept term, β_1 is the slope coefficient associated with F, β_2 is the slope coefficient associated with Pop and β_3 is the slope coefficient associated with the interaction F*Pop, allowing inference on whether the two populations differ in their relationship between r and F.

There was a significant difference in the relationship between inbreeding and birth sex ratio between the populations (slope of the interaction term $(F * Pop)$ p-value=0.046) such that the birth sex ratio is more likely to be skewed male at larger levels of inbreeding in the SAFE that in the ENCRWP.

Parameter	Estimate	SE	p-value
β_0: intercept	-0.36	0.11	0.001
β_1: slope (F)	4.15	1.57	0.008
β_2: slope (Pop)	0.36	0.18	0.050
β_3: slope $(F * Pop)$	-3.72	1.86	0.046

Modeling the two populations separately, we see that **there is a significant positive relationship between pup inbreeding and the probability a pup will be born male in the SAFE (p=0.008) but the relationship is not significant in the ENCRWP (p=0.668; Figure II-7).**

	SAFE 1990-2021			ENCRWP 2000-2021		
Parameter	Estimate	SE	p-value	Estimate	SE	p-value
β_0: intercept	-0.36	0.11	0.001	0.00	0.15	0.996
β_1: slope (F)	4.15	1.57	0.008	0.43	1.00	0.668

3e. Post-reproductive ages

We assessed post-reproductive ages by calculating the percent of females available for breeding in each age class for each population (ENCRWP 2000-2014, SAFE 1990-2021) that successfully whelped a litter. This analysis included only females with known birthdates and those that were full red wolves (>=75% red wolf ancestry). We did not include pup releases in the 0-1 age class. We did not censor any living individuals, assuming any living individual capable of breeding. Results of this analysis are shown in Table II-8. **Based on this table, we consider breeding ages to be between 2 and 10 years old with senescence after 10 years of age.**

3f. Pairing of adult ENCRWP red wolves with other red wolves and non-wolves

Using the results in Table II-8, we considered breeding ages to be between 2-10 years old for the ENCRWP (2000-2014). For each year of the window (2000-2014), we assessed the percent of available male and female red wolves (>=75% red wolf ancestry) that paired with either another red wolf (WW pair) or a non-wolf (i.e., hybrid or coyote <75% red wolf ancestry; WnonW pair). Annual rates and the average from 2000-2014 are summarized in Table II-9 with averages pooled across years shown in Table II-10. We note that in some cases the numbers in Table II-9 differ from findings in Faust et al. 2016 due to updates to the studbook in the intervening years and more accurate genetic data on which to base percent red wolf ancestry.

We used the annual ENCRWP pairing rates in Table II-9 to estimate the amount of environmental variation in pairing rates caused by typical fluctuations in the local environment. Analogous to mortality rates, we partitioned annual variation into contributions from demographic stochasticity and contributions from the environment following Lacy et al. (2021). To calculate EV, we obtained means of annual rates by first calculating a rate for each year of data (described below), then averaging those annual rates which was unweighted by annual sample size as recommended by Lacy et al. (2021). We used the resulting average pairing rates and estimates of annual environmental variation (EV) as inputs into Vortex.

We calculated the number of adult males and females aged 2-10 years on the ENCRWP from 2000-2014 available for pairing and the annual pairing rates for each sex. We summarized EV as the annual variation that exceeded the variation expected as sampling error for a binomial distribution (Akçakaya 2002) following the methodology shown in the Vortex manual (Lacy et al. 2020). A summary of the resulting mean pairing rates and levels of environmental variation (standard deviation of EV) to be used in Vortex modeling are shown in Table II-11. We calculated these with all years included since both sexes had $\geq$ 10 individuals available for pairing in each year considered.

3g. Breeding success of adult ENCRWP red wolves

Building on the results from Tables II-9 and II-10, we assessed breeding success for paired adult female breeding-aged (2-10 years old) ENCRWP red wolves (>=75% red wolf ancestry) with other red wolves and non-wolves (i.e., hybrids and coyotes, <75% red wolf ancestry). For each year of the window (2000-2014), we assessed the percent of available female red wolves that paired with either another red wolf (WW pair) or a non-wolf (WnonW pair) and were successful at producing a litter (i.e., female breeding success). Annual rates are shown in Table II-12 with pooled rates for 2000-2014 shown in Table II-13.

Similar to the analysis of pairing rates, we used the annual ENCRWP female breeding success rates in Table II-12 to estimate the amount of environmental variation in female breeding success rates caused by typical fluctuations in the local environment. We calculated the number of adult females aged 2-10 years on the ENCRWP from 2000-2014 that were paired with either a male red wolf or a male nonwolf and the percent of those that successfully whelped a litter. We summarized EV as the annual variation that exceeded the variation expected as sampling error for a binomial distribution (Akçakaya 2002) following the methodology shown in the Vortex manual (Lacy et al. 2020). A summary of the resulting mean female breeding success rates and levels of environmental variation (standard deviation of EV) to be used in Vortex modeling are shown in Table II-14. We calculated these with all years since sample sizes for females paired with non-wolves were consistently low (< 10 pairs), thus removing those would result in no information gained.

3h. Reasons for ENCRWP new pairings

We assessed reasons for re-pairings (i.e., new mates) of ENCRWP (2000-2014) red wolves (>=75% red wolf ancestry). For each red wolf sex, we considered four types of mate changes: from a red wolf mate to another red wolf mate (Wolf-Wolf), from a red wolf mate to a non-wolf mate (i.e., hybrid or coyote; <75% red wolf ancestry; Wolf-nonWolf), from a non-wolf mate to a red wolf mate (nonWolf-Wolf) and from a non-wolf mate to another non-wolf mate (nonWolf-nonWolf). We then tallied the total number of each type of pair transitions and the number of those that coincided with a mate death event (i.e., in the same year or previous year). While Vortex automatically puts females back into the breeding pool after a mate's simulated death, these data can give some context for how frequently re-pairing occurs without a mate dying/going LTF, as well as the frequency of doing so with different canids. Results are shown in Table II-15.

3i. SAFE population pairing and breeding success

We used data from the Reproductive Viability Analysis (Franklin and Bauman 2022) which is based partially on the Recommendation Outcomes Detailed report in PMCTrack to determine how many females aged 2-10 were recommended for breeding in on SSP® Breeding and Transfer Plans. The RVA process eliminates some pairs that were definitely not attempted (i.e. one mate was never transferred to the other's facility), but we cannot know for certain whether pairs were actually put together to breed. Thus, some "unsuccessful" pairs included in the analyses could represent those that were never given a breeding opportunity.

We assessed pairing and breeding success in each year from 2001 – 2021 representing years with available data in PMCTrack. We defined the number of at-risk females as those alive and of breeding age at the beginning of the year (1 January) just prior to the breeding season. We defined the number of recommended pairings as those recommended and assumed attempted in the breeding season of a given year. The number of attempted pairings that were successful are those that resulted in a litter born in the following year. We determined the percent paired as the percent of at-risk females that received a recommendation to breed and the percent successful as the percent of recommended pairings that produced a litter (Table II-16).

3j: Age distributions for the SAFE and the ENCRWP initial populations (31 December 2021)

We assessed the age distributions for the SAFE and ENCRWP populations as of 31 December 2021 for Vortex model initialization. Only full red wolves are considered (>=75% red wolf ancestry). Age pyramids are shown in Figure II-8.

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5. Figures

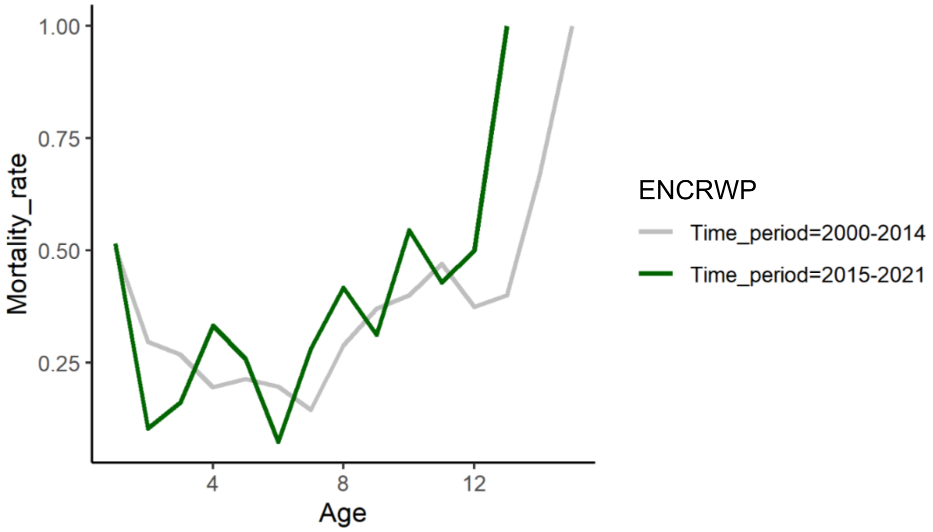

Figure II-1: Mortality rates of red wolves (>= 75% red wolf ancestry) for each ENCRWP window (1 January 2000-31 December 2014 and 1 January 2015-31 December 2021) were not statistically different (Cox proportional hazards p-value = 0.84) indicating mortality rates can be pooled.

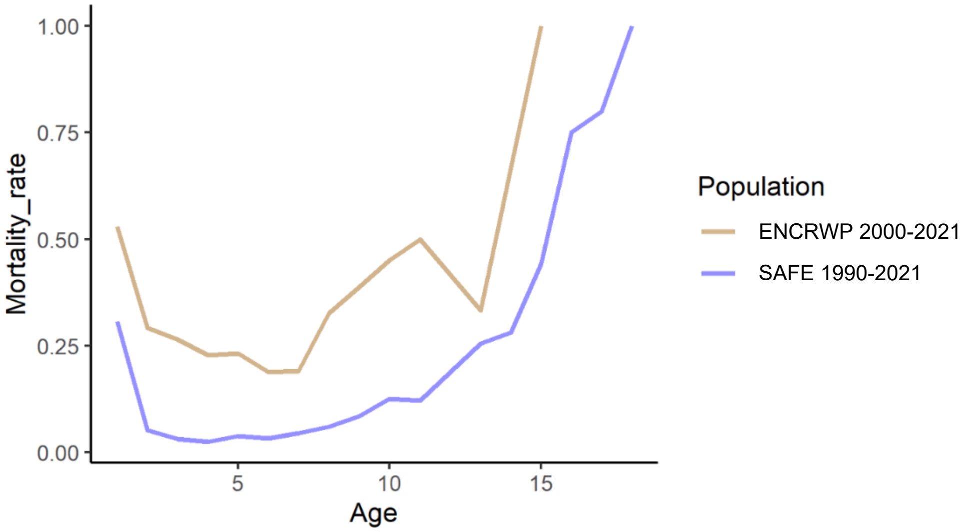

Figure II-2: Mortality rates of red wolves (>= 75% red wolf ancestry) in the ENCRWP (2000- 2021) and SAFE (1990-2021) populations. Mortality rates were statistically different between populations with NNEC rates being 3.49 times higher (Cox proportional hazards p-value = <0.0001).

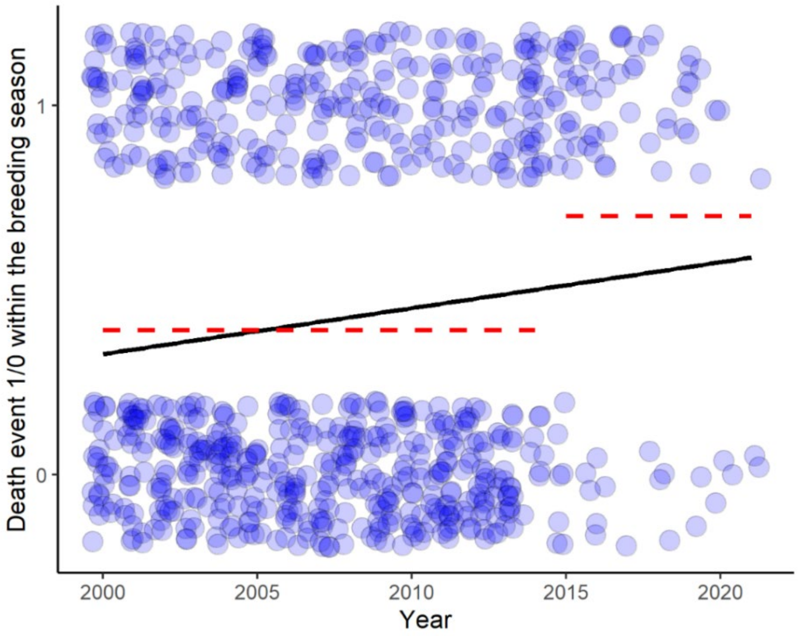

Figure II-3: Mortalities within the pre-breeding and breeding seasons (defined as 10/1 to 3/31) each year from 2000-2021 in the ENCRWP population. Only mortalities of red wolves (>=75% red wolf ancestry) were considered. The solid black line represents predictions of a logistic regression showing the proportion of deaths taking place within the breeding season has increased over time. The dashed red lines show mean mortality rates within the breeding seasons for 2000-2014 and 2015-2021, respectively.

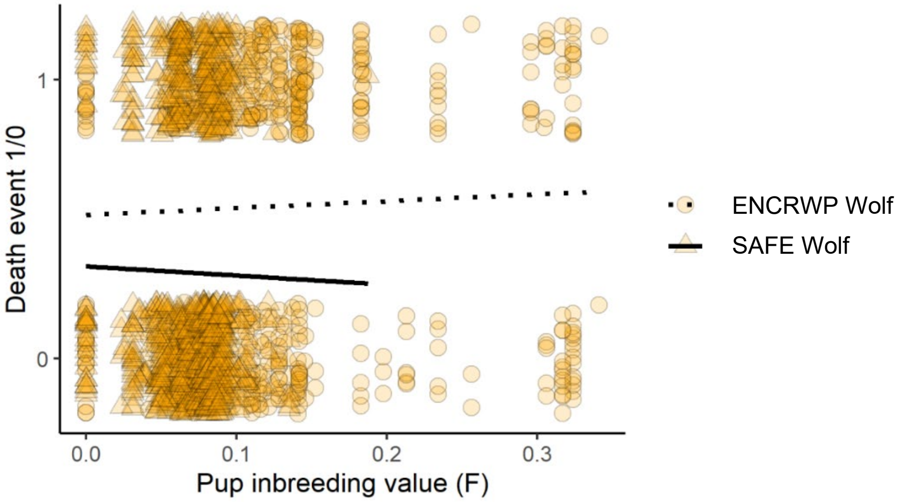

Figure II-4: Impact of inbreeding on mortalities within the first year of life in the ENCRWP (2000-2021) and SAFE populations (1990-2021). Only mortalities of red wolves (>=75% red wolf ancestry) were considered. The dots represent each pup being considered and the lines represent predictions of a logistic regression showing the proportion of deaths taking place within the first year of life. In the ENCRWP, pups with higher inbreeding were more likely to die in their first year and in the SAFE pups with higher inbreeding were less likely to die in their first year but both relationships were not statistically significant.

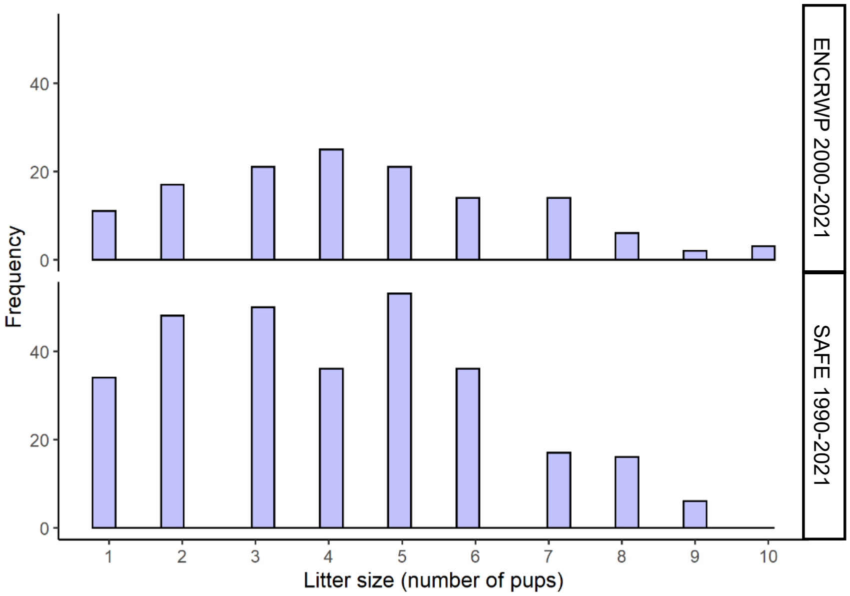

Figure II-5: Litter size distributions for ENCRWP (2000-2021) and SAFE populations (1990-2021). Only litters of full red wolves (>=75% red wolf ancestry) were considered.

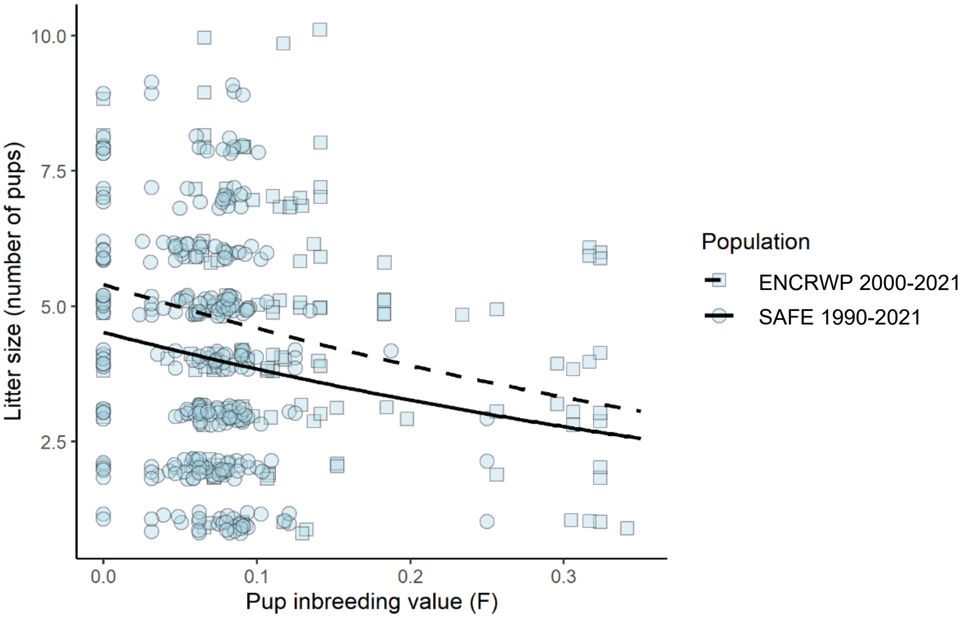

Figure II-6: The relationship between litter size and pup inbreeding (i.e., kinship between the parents; F) for ENCRWP (2000-2021) and SAFE populations (1990-2021). Only litters of full red wolves (>=75% red wolf ancestry) were considered. The dots represent each pup considered and the lines represent model predictions under a Poisson regression (generalized linear model with a log link). Both populations exhibit a statistically significant negative relationship between litter size and kinship (i.e., inbreeding) but the relationships were not statistically different between populations.

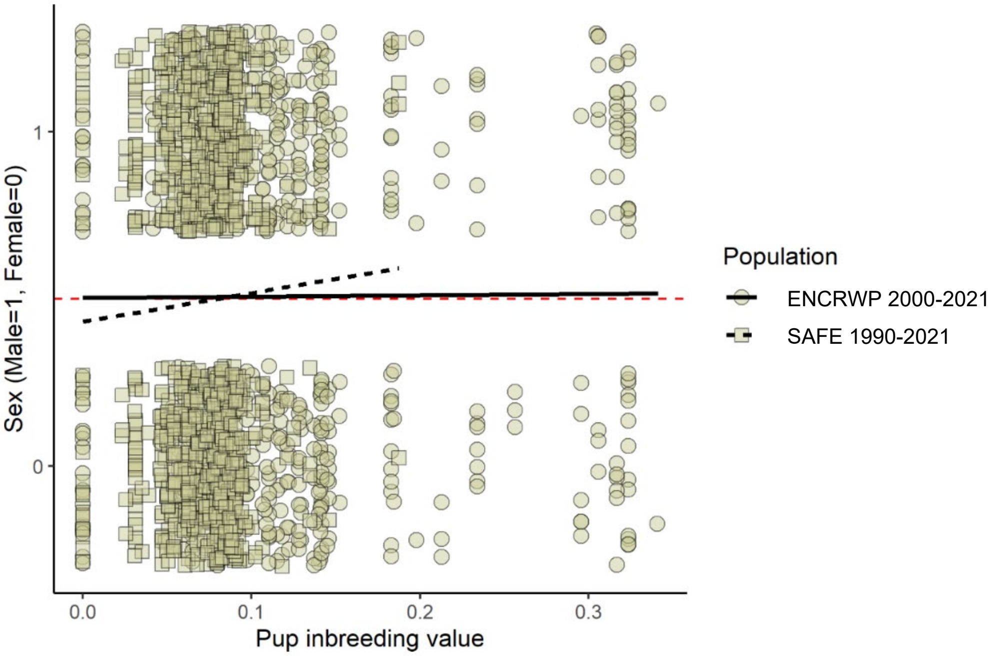

Figure II-7: The relationship between birth sex ratio and pup inbreeding value (F) for ENCRWP (2000-2021) and SAFE populations (1990-2021). Only litters of full red wolves (>=75% red wolf ancestry) were considered. The y-axis shows male pups (1) and female pups (0) with the red dotted line representing a 50/50 sex ratio. The yellow dots represent each pup considered and the black lines represent model predictions under a logistic regression (generalized linear model with a logit link). The ENCRWP exhibited a neutral relationship while the SAFE population exhibited a significant positive relationship between birth sex ratio and pup inbreeding value, with the two populations having significantly different relationships between birth sex ratio and pup inbreeding value.

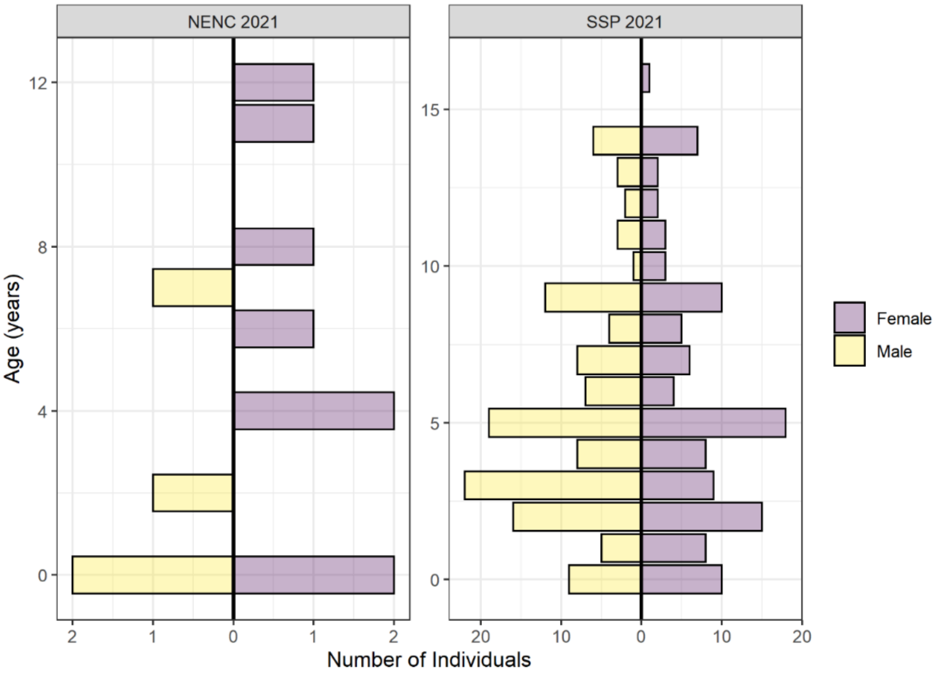

Figure II-8: Age pyramids for initial ENCRWP and SAFE populations on 31 December 2021. Only full red wolves are considered (>=75% red wolf ancestry).

6. Tables

Table II-1: Age-based mortality tables for ENCRWP red wolves (>=0.75 known ancestry) in two time windows: 2000-2014 and 2015-2021. Since there was no significant difference in survival by sex, these have been pooled. The number at risk accounts for censors, releases and animals returned back to the SAFE. Animals that were alive at the end of the study are right-censored, as are animals that went LTF within 1 year of the end of the study (i.e., assumed alive at the end of the study). Pups released from the SAFE to the ENCRWP are not included in the 0-1 age class in either population window. This analysis considered non-pup (>= 1 year old) releases only after they had survived to the next census date (12/31) following their release. Note that there are 15 fewer mortalities than in Table II-2 (with the cumulative time window) due to 15 individuals in the 0-1 age class that went LTF on 2014-12-31 and were not counted as deaths since the LTF was within 1 year of the end date for the window.

	ENCRWP 2000-2014				ENCRWP 2015-2021			
Age	#At_risk	#Mortalities	#Censored	%Mortality	#At_risk	#Mortalities	#Censored	%Mortality
0-1	630	318	16	50.5%	31	16	0	51.6%
1-2	340	101	13	29.7%	29	3	0	10.3%
2-3	250	67	12	26.8%	37	6	1	16.2%
3-4	179	35	5	19.6%	36	12	0	33.3%
4-5	154	33	8	21.4%	31	8	2	25.8%
5-6	122	24	7	19.7%	27	2	1	7.4%
6-7	96	14	8	14.6%	32	9	1	28.1%
7-8	76	22	2	29.0%	24	10	1	41.7%
8-9	54	20	4	37.0%	16	5	1	31.3%
9-10	30	12	1	40.0%	11	6	0	54.6%
10-11	17	8	2	47.1%	7	3	0	42.9%
11-12	8	3	0	37.5%	4	2	1	50.0%
12-13	5	2	0	40.0%				
13-14	3	2	0	66.7%				
14-15	1	1	0	100.0%				

Table II-2: Age-based mortality tables for red wolves (>=0.75 known ancestry) in two populations: SAFE 1990-2021 and ENCRWP 2000-2021. The number at risk accounts for censors, releases and animals returned back to the SAFE. Animals are censored from each population while they are in the other population (i.e., censored from the SAFE after release). Animals that were alive at the end of the study are right-censored, as are animals that went LTF within 1 year of the end of the study (i.e., assumed alive at the end of the study). Pups released to the ENCRWP are not included in the 0-1 age class in either population. This analysis considered non-pup (>= 1 year old) releases only after they had survived to the next census date (12/31) following their release. Since there was no significant difference in mortality by sex, these have been pooled. Since there was no significant difference between ENCRWP time windows, these have also been pooled.

	SAFE 1990-2021				ENCRWP 2000-2021			
Age	#At risk	#Mortalities	#Censored	%Mortality	#At risk	#Mortalities	#Censored	%Mortality
0-1	989	303	19	30.6%	659	349	0	53.0%
1-2	679	35	44	5.2%	356	104	0	29.2%
2-3	609	19	37	3.1%	276	73	2	26.5%
3-4	567	14	39	2.5%	210	48	0	22.9%
4-5	523	20	19	3.8%	177	41	2	23.2%
5-6	490	16	37	3.3%	143	27	1	18.9%
6-7	444	20	11	4.5%	120	23	1	19.2%
7-8	415	25	14	6.0%	98	32	1	32.7%
8-9	379	32	9	8.4%	67	26	1	38.8%
9-10	343	43	22	12.5%	40	18	0	45.0%
10-11	281	34	4	12.1%	22	11	0	50.0%
11-12	244	46	6	18.9%	12	5	1	41.7%
12-13	192	49	4	25.5%	6	2	1	33.3%
13-14	139	39	5	28.1%	3	2	0	66.7%
14-15	95	42	13	44.2%	1	1	0	100.0%
15-16	40	30	0	75.0%				
16-17	10	8	1	80.0%				
17-18	1	1	0	100.0%				

Table II-3: SAFE population 1990-2021 annual mortality rates for four stage classes: 0-1 years old (<1), 1-2 years old (>=1, <2), 2-7 years old (>=2, <7) and 7+ years old (>=7).

Year	At-risk 0-1	Dead 0-1	% Mortality 0-1	At-risk 1-2	Dead 1-2	% Mortality 1-2	At-risk 2-7	Dead 2-7	% Mortality 2-7	At-risk 7+	Dead 7+	% Mortality 7+
1990	37	21	56.8%	11	1	9.1%	40	2	5.0%	13	2	15.4%
1991	41	8	19.5%	21	3	14.3%	41	2	4.9%	18	4	22.2%
1992	53	16	30.2%	16	0	0.0%	53	2	3.8%	20	9	45.0%
1993	54	19	35.2%	32	3	9.4%	56	5	8.9%	18	2	11.1%
1994	45	17	37.8%	35	1	2.9%	69	2	2.9%	26	4	15.4%
1995	13	5	38.5%	36	2	5.6%	98	9	9.2%	30	3	10.0%
1996	16	10	62.5%	29	2	6.9%	116	4	3.4%	34	7	20.6%
1997	17	6	35.3%	10	2	20.0%	127	2	1.6%	37	9	24.3%
1998	17	11	64.7%	7	1	14.3%	119	9	7.6%	41	6	14.6%
1999	13	8	61.5%	13	1	7.7%	95	8	8.4%	55	12	21.8%
2000	11	5	45.5%	8	0	0.0%	72	3	4.2%	70	11	15.7%
2001	15	2	13.3%	5	1	20.0%	50	1	2.0%	82	6	7.3%
2002	26	11	42.3%	6	0	0.0%	33	0	0.0%	97	14	14.4%
2003	21	5	23.8%	13	2	15.4%	33	0	0.0%	88	13	14.8%
2004	20	10	50.0%	15	1	6.7%	39	2	5.1%	80	19	23.8%
2005	45	6	13.3%	15	1	6.7%	40	1	2.5%	73	14	19.2%
2006	29	9	31.0%	9	0	0.0%	46	0	0.0%	66	15	22.7%
2007	59	10	16.9%	37	0	0.0%	51	2	3.9%	55	6	10.9%
2008	14	2	14.3%	19	0	0.0%	81	1	1.2%	53	18	34.0%
2009	8	2	25.0%	49	2	4.1%	91	2	2.2%	42	19	45.2%
2010	24	13	54.2%	12	0	0.0%	121	2	1.7%	39	7	17.9%
2011	13	7	53.8%	6	0	0.0%	117	4	3.4%	45	8	17.8%
2012	38	5	13.2%	10	0	0.0%	110	6	5.5%	46	9	19.6%
2013	22	10	45.5%	6	0	0.0%	87	2	2.3%	64	7	10.9%
2014	36	11	30.6%	33	5	15.2%	78	3	3.8%	70	11	15.7%
2015	21	9	42.9%	12	1	8.3%	62	2	3.2%	100	13	13.0%
2016	53	13	24.5%	23	0	0.0%	60	0	0.0%	99	15	15.2%
2017	23	4	17.4%	11	0	0.0%	76	1	1.3%	91	13	14.3%

2018	46	14	30.4%	40	0	0.0%	76	3	3.9%	88	22	25.0%
2019	36	4	11.1%	20	2	10.0%	108	2	1.9%	70	14	20.0%
2020	25	10	40.0%	33	1	3.0%	99	3	3.0%	81	12	14.8%
2021	37	18	48.6%	33	2	6.1%	116	7	6.0%	80	16	20.0%

Table II-4: ENCRWP population 2000-2021 annual mortality rates for four stage classes: 0-1 years old (<1), 1-2 years old (>=1, <2), 2-7 years old (>=2, <7) and 7+ years old (>=7). This analysis included only red wolves >=75% red wolf ancestry and did not consider pup fosters in the 0-1 age class, only animals born on the ENCRWP. All other age classes did include pup fosters after they survived their first year. This analysis considered non-pup (>= 1 year old) releases only after they had survived to the next census date (12/31) following their release.

Year	At-risk 0-1	Dead 0-1	% Mortality 0-1	At-risk 1-2	Dead 1-2	% Mortality 1-2	At-risk 2-7	Dead 2-7	% Mortality 2-7	At-risk 7+	Dead 7+	% Mortality 7+
2000	42	19	45.2%	14	6	42.9%	64	10	15.6%	5	1	20.0%
2001	39	21	53.8%	23	4	17.4%	58	15	25.9%	12	3	25.0%
2002	33	15	45.5%	17	9	52.9%	51	10	19.6%	16	4	25.0%
2003	39	25	64.1%	13	2	15.4%	40	10	25.0%	19	4	21.1%
2004	53	28	52.8%	17	6	35.3%	38	5	13.2%	15	11	73.3%
2005	42	15	35.7%	9	3	33.3%	41	11	26.8%	4	0	0.0%
2006	51	31	60.8%	18	3	16.7%	32	7	21.9%	7	2	28.6%
2007	30	18	60.0%	27	14	51.9%	37	6	16.2%	8	3	37.5%
2008	52	25	48.1%	20	8	40.0%	40	7	17.5%	9	3	33.3%
2009	40	18	45.0%	11	4	36.4%	38	8	21.1%	13	8	61.5%
2010	43	20	46.5%	22	4	18.2%	36	7	19.4%	8	4	50.0%
2011	38	24	63.2%	20	6	30.0%	45	7	15.6%	5	4	80.0%
2012	38	27	71.1%	16	4	25.0%	47	10	21.3%	6	0	0.0%
2013	36	21	58.3%	10	3	30.0%	44	10	22.7%	11	5	45.5%
2014	19	18	94.7%	11	0	0.0%	37	9	24.3%	10	4	40.0%
2015	10	5	50.0%	12	2	16.7%	37	14	37.8%	8	4	50.0%
2016	11	6	54.5%	1	0	0.0%	30	9	30.0%	6	3	50.0%
2017	4	0	0.0%	4	2	50.0%	19	4	21.1%	6	3	50.0%
2018	4	4	100.0%	5	0	0.0%	14	2	14.3%	6	2	33.3%
2019	0	0		3	0	0.0%	16	9	56.3%	5	2	40.0%
2020	0	0		1	1	100.0%	9	2	22.2%	4	2	50.0%
2021	0	0		1	0	0.0%	6	2	33.3%	4	1	25.0%

Table II-5: Estimated mean mortality rates and environmental variation for each population (SAFE 1990-2021 and ENCRWP 2000-2021) and stage class comparing mean rates and levels of environmental variation when all years are included versus when only years with sample sizes of >=10 individuals are included (i.e., when only estimates based on robust sample sizes are included).

	SAFE 1990-2021				ENCRWP 2000-2021			
	All Years				**All Years**			
Stage	Class 0-1	Class 1-2	Class 2-7	Class 7+	Class 0-1	Class 1-2	Class 2-7	Class 7+
Mean # at risk	28.12	19.33	78.36	59.06	27.13	11.96	34.04	8.30
Mean annual mortality rate	35.30%	5.60%	3.40%	18.60%	55.2%	27.8%	22.7%	36.5%
Mean sd due to environment	13.2%	3.3%	1.5%	7.6%	18.6%	19.6%	7.6%	11.5%
	Years >=10 individuals				**Years >=10 individuals**			
Mean # at risk	29.68	22.73	78.36	59.06	36.24	16.73	38.20	13.71
Mean annual mortality rate	35.60%	5.80%	3.40%	18.60%	55.8%	28.6%	23.3%	41.6%
Mean sd due to environment	13.6%	2.8%	1.5%	7.6%	10.4%	9.6%	6.8%	14.4%

Table II-6: Post-release mortality of pup (<1 year old) and non-pup (>=1 year old) releases on the ENCRWP 1986-2021. All releases are full red wolves (>=75% red wolf ancestry). Post-release is assessed as survival from release up to the next census (12/31). For pups, only those pups still under 1-year-old by the census were included. Mortality rates for releases are compared with mortality rates of *in-situ* red wolves (i.e., born on the ENCRWP) of the same age ranges, starting in the same months and calculated until the next census.

Release/Wild Type	#At risk	#Mortalities	%Mortality
Non-pups releases (Wild translocation)	20	5	25.0%
Non-pups releases (SAFE)	34	11	32.4%
All non-pup releases	54	16	29.6%
Pup fosters	69	23	33.3%
ENCRWP wild-born non-pup	935	306	33.1%
ENCRWP wild-born pup	122	39	31.9%

Table II-7: Litter size distributions for ENCRWP (2000-2021) and SAFE (1990-2021) populations.

Population	Litter size	Frequency
ENCRWP 2000-2021	1	11
ENCRWP 2000-2021	2	17
ENCRWP 2000-2021	3	21
ENCRWP 2000-2021	4	25
ENCRWP 2000-2021	5	21
ENCRWP 2000-2021	6	14
ENCRWP 2000-2021	7	14
ENCRWP 2000-2021	8	6
ENCRWP 2000-2021	9	2
ENCRWP 2000-2021	10	3
SAFE 1990-2021	1	34
SAFE 1990-2021	2	48
SAFE 1990-2021	3	50
SAFE 1990-2021	4	36
SAFE 1990-2021	5	53
SAFE 1990-2021	6	36
SAFE 1990-2021	7	17
SAFE 1990-2021	8	16
SAFE 1990-2021	9	6

Table II-8: Ages of breeding success for the ENCRWP (2000-2014) and SAFE (1990-2021) populations for female red wolves (>=75% red wolf ancestry). The number of potential breeders represents the number of living females in each age class. We did not include pup releases in the 0-1 age class. The number of successful breeders represents the numbers of available females that produced litters.

	SAFE 1990-2021			ENCRWP 2000-2014		
Age	# Potential Breeders	# Successful Breeders	%Reproductive	# Potential Breeders	# Successful Breeders	%Reproductive
0-1	501	1	0.2%	328	0	0.0%
1-2	346	12	3.5%	173	23	13.3%
2-3	314	17	5.4%	133	65	48.9%
3-4	293	36	12.3%	104	56	53.9%
4-5	277	38	13.7%	89	60	67.4%
5-6	260	38	14.6%	70	45	64.3%
6-7	239	39	16.3%	56	24	42.9%
7-8	224	26	11.6%	43	21	48.8%
8-9	204	20	9.8%	30	12	40.0%
9-10	186	13	7.0%	16	4	25.0%
10-11	153	5	3.3%	11	0	0.0%
11-12	131	7	5.3%	8	0	0.0%
12-13	101	0	0.0%	5	0	0.0%
13-14	74	0	0.0%	2	0	0.0%
14-15	51	0	0.0%	1	0	0.0%
15-16	23	0	0.0%			
16-17	6	0	0.0%			
17-18	1	0	0.0%			

Table II-9: Pairing of adult breeding-aged (2-10 years old) ENCRWP red wolves (>=75% red wolf ancestry) with other red wolves and non-wolves (i.e., hybrids and coyotes, <75% red wolf ancestry). For each year of the window (2000-2014), we assessed the percent of available male and female red wolves that paired with either another red wolf (WW pair) or a non-wolf (WnonW pair).

	Males					Females					
Year	#Breeding-aged	#WW pairs	% Paired WW	#WnonW Pairs	% Paired WnonW	#Breeding-aged	#WW pairs	% Paired WW	#nonWW Pairs	% Paired WnonW	%Paired any canid
2000	32	6	18.8%	3	9.4%	29	6	20.7%	7	24.1%	44.8%
2001	31	13	41.9%	4	12.9%	28	13	46.4%	6	21.4%	67.9%
2002	32	10	31.3%	8	25.0%	28	10	35.7%	4	14.3%	50.0%
2003	30	19	63.3%	1	3.3%	24	19	79.2%	4	16.7%	95.8%
2004	27	18	66.7%	1	3.7%	22	18	81.8%	2	9.1%	90.9%
2005	23	15	65.2%	0	0.0%	22	15	68.2%	0	0.0%	68.2%
2006	17	15	88.2%	1	5.9%	20	15	75.0%	5	25.0%	100.0%
2007	20	19	95.0%	0	0.0%	23	19	82.6%	4	17.4%	100.0%
2008	23	18	78.3%	1	4.3%	22	18	81.8%	4	18.2%	100.0%
2009	24	15	62.5%	3	12.5%	24	15	62.5%	3	12.5%	75.0%
2010	25	15	60.0%	1	4.0%	18	15	83.3%	1	5.6%	88.9%
2011	28	15	53.6%	1	3.6%	20	15	75.0%	4	20.0%	95.0%
2012	31	16	51.6%	2	6.5%	22	16	72.7%	3	13.6%	86.4%
2013	30	13	43.3%	3	10.0%	25	13	52.0%	5	20.0%	72.0%
2014	27	7	25.9%	1	3.7%	20	7	35.0%	5	25.0%	60.0%

Table II-10: Average pairing rates of adult breeding-aged (2-10 years old) ENCRWP red wolves (>=75% red wolf ancestry) with other red wolves and non-red wolves (i.e., hybrids and coyotes, <75% red wolf ancestry) between 2000-2014. Values were generated by taking the average of percentages over the years from Table II-9.

Pair type	Average pairing rate
Female Wolf-Wolf	63.5%
Female Wolf-nonWolf	16.2%
Female Wolf-any canid	79.7%
Male Wolf-Wolf	56.4%
Male Wolf-nonWolf	7.0%
Male Wolf-any canid	63.4%

Table II-11: Estimated mean pairing rates for adult breeding-aged (2-10 years old) ENCRWP red wolves (>=75% red wolf ancestry) with other red wolves and non-wolves (i.e., hybrids and coyotes, <75% red wolf ancestry) from 2000-2014. A comparison of male and female mean rates is shown with estimated levels of environmental variation when all years are included versus when only years with sample sizes of >=10 individuals are included (i.e., when only estimates based on robust sample sizes are included). All years have more than 10 individuals available for pairing of each sex (Table II-9).

	Males			Females		
Pair type	Wolf-Wolf	Wolf-NonWolf	Wolf-Any Canid	Wolf-Wolf	Wolf-NonWolf	Wolf-Any Canid
Mean number adults available	26.7	26.7	26.7	23.1	23.1	23.1
Mean annual pairing rate	56.4%	7.0%	63.4%	63.5%	16.2%	79.7%
Mean sd due to environment	19.5%	3.9%	16.7%	17.8%	0.0%	16.5%

Table II-12: Breeding success for paired adult female breeding-aged (2-10 years old) ENCRWP red wolves (>=75% red wolf ancestry) with other red wolves and non-wolves (i.e., hybrids and coyotes, <75% red wolf ancestry). For each year of the window 2000-2014, we assessed the percent of available female red wolves that paired with either another red wolf (WW pair) or a non-wolf (WnonW pair) and were successful at producing a litter (i.e., female breeding success).

Year	#Females aged 2-10	#WW Pairs	%Females paired WW	#WW successful	%WW successful	#WnonW Pairs	%Females paired WnonW	#WnonW successful	%WnonW successful
2000	29	6	20.7%	4	66.7%	7	24.1%	5	71.4%
2001	28	13	46.4%	7	53.8%	6	21.4%	1	16.7%
2002	28	10	35.7%	7	70.0%	4	14.3%	3	75.0%
2003	24	19	79.2%	9	47.4%	4	16.7%	1	25.0%
2004	22	18	81.8%	11	61.1%	2	9.1%	0	0.0%
2005	22	15	68.2%	10	66.7%	0	0.0%	0	
2006	20	15	75.0%	11	73.3%	5	25.0%	5	100.0%
2007	23	19	82.6%	11	57.9%	4	17.4%	0	0.0%
2008	22	18	81.8%	12	66.7%	4	18.2%	1	25.0%
2009	24	15	62.5%	11	73.3%	3	12.5%	0	0.0%
2010	18	15	83.3%	9	60.0%	1	5.6%	0	0.0%
2011	20	15	75.0%	10	66.7%	4	20.0%	2	50.0%
2012	22	16	72.7%	8	50.0%	3	13.6%	2	66.7%
2013	25	13	52.0%	5	38.5%	5	20.0%	4	80.0%
2014	20	7	35.0%	4	57.1%	5	25.0%	0	0.0%

Table II-13: Summary of successful breeding (i.e., litter produced) of paired female ENCRWP red wolves (>=75% red wolf ancestry) aged (2-10 years old). Success rates are shown for female red wolves paired with other red wolves and non-wolves (i.e., hybrids and coyotes, <75% red wolf ancestry) from 2000-2014. Values were generated by taking the average of percentages over the relevant years from Table II-12.

Pair type	Pair breeding success rate
Wolf-Wolf	60.6%
Wolf-nonWolf	36.4%
Wolf-Any canid	57.6%

Table II-14: Estimated mean breeding success rates for adult breeding-aged (2-10 years old) female ENCRWP red wolves (>=75% red wolf ancestry) with other red wolves and non-wolves (i.e., hybrids and coyotes, <75% red wolf ancestry) from 2000-2014. Due to low sample sizes, especially with the number of Wolf-NonWolf pairs available, comparisons are shown including years with <10 pairs.

Pair type	Wolf-Wolf	Wolf-NonWolf	Wolf-Any Canid
Mean number females paired	14.3	3.8	18.1
Mean annual breeding success rate	60.6%	36.4%	57.6%
Mean sd due to environment	0.0%	22.0%	6.5%

Table II-15: Reasons for re-pairings (i.e., new mates) of ENCRWP (2000-2014) red wolves (>=75% red wolf ancestry). For each red wolf sex, we consider four types of mate changes: from a red wolf mate to another red wolf mate (From Wolf-To Wolf), from a red wolf mate to a non-wolf mate (i.e., hybrid or coyote; <75% red wolf ancestry; From Wolf-To nonWolf), from a non-wolf mate to a red wolf mate (From nonWolf-To Wolf) and from a non-wolf mate to another non-wolf mate (From nonWolf-To nonWolf). We then tally the total number of each type of pair transition, and the number of those that coincided with a mate death event (i.e., in the same year or previous year).

	Male Red wolves >=75% red wolf			
	Mate Death/LTF	Other reason	Total	% Re-pairing coinciding with death
From Wolf-To Wolf	17	13	30	56.7%
From Wolf-To nonWolf	4	2	6	66.7%
From nonWolf-To Wolf	0	11	11	0.0%
From nonWolf-To nonWolf	0	5	5	0.0%
Total that changed mates	21	13	37	56.8%
	Female Red wolves >=75% red wolf			
From Wolf-To Wolf	25	12	37	67.6%
From Wolf-To nonWolf	8	3	11	72.7%
From nonWolf-To Wolf	0	16	16	0.0%
From nonWolf-To nonWolf	0	9	9	0.0%
Total that changed mates	33	40	73	45.2%

Table II-16: The percent of at-risk SAFE females aged 2-10 years-old that were paired in each year based on SSP® Breeding and Transfer Plans. The number of at-risk females are those alive and of breeding age at the beginning of the year (1 January) just prior to the breeding season. The number of recommended pairings are those recommended and assumed attempted in the breeding season of a given year. The number of attempted pairings that were successful are those that resulted in a litter born in the following year. The percent paired is the percent of at-risk females that received a recommendation to breed and the percent successful is the percent of recommended pairings that produces a litter.

Year	# Females aged 2-10 at risk as of 1 January	Recommended pairings attempted by breeding season of that year	Attempted pairings successful that breeding season	% Paired	% Pairs successful
2001	59	22	6	37.3%	27.3%
2002	56	29	8	51.8%	27.6%
2003	49	30	5	61.2%	16.7%
2004	41	28	7	68.3%	25.0%
2005	40	37	14	92.5%	37.8%
2006	36	32	9	88.9%	28.1%
2007	40	23	14	57.5%	60.9%
2008	57	13	3	22.8%	23.1%
2009	63	12	2	19.0%	16.7%
2010	87	29	8	33.3%	27.6%
2011	92	28	5	30.4%	17.9%
2012	89	27	7	30.3%	25.9%
2013	85	27	3	31.8%	11.1%
2014	79	28	8	35.4%	28.6%
2015	82	32	5	39.0%	15.6%
2016	80	40	13	50.0%	32.5%
2017	76	34	5	44.7%	14.7%
2018	74	29	11	39.2%	37.9%
2019	68	25	8	36.8%	32.0%
2020	72	27	6	37.5%	22.2%
2021	76	28	9	36.8%	32.1%
Averages all years:				45.0%	26.7%

Appendix III

Red Wolf (*Canis rufus*)
AZA Animal Program
Reproductive Viability Analysis (RVA) Report

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Karen Bauman
AZA Canid and Hyaenid TAG Chair

10 August 2022 (DRAFT DATE)

AZA Reproductive Management Center
at the Saint Louis Zoo

ASSOCIATION
OF ZOOS &
AQUARIUMS

Introduction to Reproductive Viability Analysis (RVA)

Accredited zoos and aquariums strive to maintain genetically and demographically stable animal populations so that those populations can support conservation, education, research, and recreational goals for many years to come (Traylor-Holzer, Leus, and Byers 2018; Powell, Dorsey, and Faust 2019). However, analyses have revealed that many populations of animals managed in human care are not sustainable (Lees and Wilcken, 2009) and struggle with unexplained low or inconsistent rates of reproduction (Long, Dorsey, and Boyle 2011; Faust et al. 2019). The Association of Zoos and Aquariums (AZA) Reproductive Management Center (RMC) has developed Reproductive Viability Analysis (RVA) as a tool to identify the inherent biological and reproductive characteristics of animals in breeding pairs and the pairs themselves that correlate with successful reproduction in order to inform population managers on best practices for population management and to improve genetic and demographic predictions. The RVA process identifies predictors of reproductive success based on the past performance of breeding pairs in the population, using modeling techniques that consider multiple individual and breeding pair attributes simultaneously.

The data used for RVA come from multiple sources, beginning with the Breeding and Transfer Plans (BTPs) and the associated outcomes, as well as the studbook. Then, additional information is added such as contraception history and/or inbreeding coefficients, as well as any interim recommendations that did not appear in the published BTPs. RVA relies on a core set of variables (e.g., age), but is designed to be flexible, so variables may be added depending on the species being evaluated and its unique biological characteristics. Once all of this information is compiled into a single data set, the data are ready for analysis. Any pairs that were recommended to breed in the published BTP, but did not get the opportunity to do so, are excluded from analyses.

Multiple statistical approaches can be utilized for RVA, some of which are described in Bauman et al. (2019). Results from the RVA elucidate factors that are driving reproductive success in the population of interest. In addition, the resulting statistical models can be used to predict the probability of success of pairs for which we do not yet know the outcome.

Red Wolf Program Challenges

The role of the ex situ red wolf population is to support the reintroduction and conservation of the species in its historical range (Lasher et al. 2021). Historically this population has experienced periodic bursts in population growth due to changes in management strategies, as well as unpredictable and variable litter sizes (Lasher et al. 2021). Unfortunately, due to limited spaces available for holding red wolves, these boosts in population size lead to other negative effects on population demographics, as they can prevent the dispersal of offspring from the natal pack for the establishment of new breeding pairs, which then also hinders re-breeding of parents in subsequent years (Lasher et al. 2021). One potential long-term effect of this is the creation of surplus post-reproductive animals occupying breeding spaces 12 years into the future (Lasher et al. 2021). Therefore, maintaining a more predictable and stable birth rate is important for the long-term management of the population.

Litter size in the SAFE population ranges between one and nine, with four pups born per litter on average (Lasher et al. 2021). Increasing female age and increasing inbreeding in males have both been shown to lead to reduced litter sizes and a decrease in breeding success in previous studies (Franklin et al. 2020, Lockyear et al. 2009; Rabon and Waddell 2010). Franklin, Waddell, and Goodrowe (2018) also found increasing inbreeding in males to be associated with a decrease in sperm concentration. Unfortunately, the SAFE population is descended from just 12 founders, with no potential founders remaining, therefore, although inbreeding should be avoided and/or minimized to reduce the impacts of inbreeding on reproductive success in the population, it is increasingly difficult to avoid, since no additional founders are known to exist (Lasher et al. 2021).

Red Wolf RVA

Data

The red wolf RVA utilized data from 21 BTP periods (2000 – 2020), across which there were 580 breeding attempts made. Of the 580 breeding attempts, 156 attempts (26.9%) successfully resulted in offspring (live or stillborn). It should be noted that some of these attempts may have been the result of interim recommendations that were not captured in the BTPs. See "Limitations, Caveats, and Cautions" for more information.

Variables

The response variable ("Success") for the RVA models is reproductive success (or failure) for each breeding pair, based on whether offspring were produced (including stillborn offspring) during the breeding season after which the breeding and transfer plan was finalized. All explanatory variables included in the red wolf RVA and their descriptions, as well as their sources, are listed in Table III-1. Descriptive statistics for each variable are presented in Appendix A.

Methods

Chi-square Tests of Association and T-Tests

Chi-square tests of association were performed on the descriptive statistics for all categorical variables, specifically to investigate the association between the different levels of each factor and reproductive success. It should be noted that these tests do not control for any confounding variables and should therefore be interpreted with extreme caution. T-Tests were used to investigate for possible differences in the mean age of individuals in successful vs. unsuccessful pairs, the age difference between the male and female in successful vs. unsuccessful pairs, as well as the mean inbreeding coefficient of individuals in successful vs. unsuccessful pairs. For the complete list of tests performed and their associated p-values, see Appendix A.

Conditional Random Forest and LASSO Regression Analyses

For the red wolf RVA, conditional random forest (CRF) analyses and LASSO logistic regression analyses were used to identify what factors are the most important for predicting reproductive success. The conditional random forest (CRF) models construct a "forest" of conditional inference trees, which are

Table III-1. Description of variables used in the RVA with definition and data source information.

Variable Name	Level Definitions	Data Source
Attributes of Pairs		
Success	"Yes" if offspring were produced (live or stillborn) during the breeding season following publication of the B&T plan. "No" if zero offspring were produced.	Studbook
PairType	"New" if pair has the opportunity to breed together for the first time; "Experienced" if the pair had a previous opportunity together and they successfully produced offspring; "Carryover" if the pair had a previous opportunity together but has never produced offspring; “Unknown” if pair type was otherwise unknown.	Breeding & Transfer Plans; Studbook; SAFE coordinator
AtLocation	"Yes" if both the male and female in the pair were at the same institution already prior to the date the B&T plan was published (or interim recommendation made). "No" if one or both individuals had to be transferred to a new institution to fulfill the breeding recommendation.	Breeding & Transfer Plan; Studbook
IntSuccess5	Has the institution successfully bred this species in the five years prior to the published date of the B&T plan? "Yes" if the institution has had success, "No" if the institution has attempted to breed in the last 5 years without success, if the institution has not had a recommendation or attempted to breed in the last 5 years, or if prior institutional attempts to breed this species was otherwise unknown.	Breeding & Transfer Plan; Studbook
IntSuccess10	Has the institution successfully bred this species in the ten years prior to the published date of the B&T plan? "Yes" if the institution has had success, "No" if the institution has attempted to breed in the last 5 years without success, if the institution has not had a recommendation or attempted to breed in the last 5 years, or if prior institutional attempts to breed this species was otherwise unknown.	Breeding & Transfer Plan; Studbook
PairParity	"YY" if both individuals are parous; "YN" if male is parous and female is nulliparous; “NY” if male is nulliparous and female is parous; “NN” if both individuals are nulliparous.	Studbook
AgeDifference	Absolute value of the difference in age (in years) between the male and female.	Calculated field; requires individual age variables
Attributes of Individuals		
MAge	Male's age at the time the B&T plan was published.	Breeding & Transfer Plan; Studbook
FAge	Female's age at the time the B&T plan was published.	Breeding & Transfer Plan; Studbook
MInbreed	Male’s individual inbreeding coefficient (i.e., F)	PMx
FInbreed	Female’s individual inbreeding coefficient (i.e., F)	PMx
MBirthType	“W” if male was born in the wild, “C” if male was born in captivity	Studbook
FBirthType	“W” if female was born in the wild, “C” if female was born in captivity	Studbook
MContraceptWPrior Reversal	"Yes" if the male was contracepted in the past but reversed prior to the current breeding recommendation; “No” if the male was contracepted in the past, but has never successfully produced offspring since treatment; “NC” if the male has never been contracepted	RMC's contraception database
FContraceptWPrior Reversal	"Yes" if the female was contracepted in the past but reversed prior to the current breeding recommendation; “No” if the female was contracepted in the past, but has never successfully produced offspring since treatment; “NC” if the female has never been contracepted	RMC's contraception database

grown based on bootstrapping samples with only a subset of variables available for splitting each node. For generating predictions, weighted means of all the observed responses (or decisions) from all the inference trees created are used. While conditional inference trees utilize significance tests for determining the splitting variables and split points for the creation of nodes within the decision trees, there are no classical significances for the explanatory variables. Rather, this analysis tells you which variables were most important in constructing the classification trees, and variable importance scores are assigned (Strobl et al. 2008; Strobl et al. 2009). Unfortunately, this method does not tell you about the directionality of the effects. Least absolute shrinkage and selection operator (LASSO) logistic regression analyses optimize the fit to the data while constraining its complexity; therefore, the regression coefficients for some of the explanatory variables are forced to become zero, resulting in simpler models compared to traditional regression techniques, decreasing the likelihood of overfitting and improving the accuracy of predictions made on new data (James et al. 2014). All variables listed in Table III-1 were included for both the CRF and LASSO regression models. One thousand iterations of each of the analyses were run. See Bauman et al. 2019 for the full detailed description of these methods. The analyses were executed within R® Studio software (R version 4.2.1, R Core Team). Since CRF analyses and the LASSO regression technique are sensitive to the scale of the input variables, female age, male age, the age difference between the male and female, and individual inbreeding coefficients were standardized prior to these analyses (by subtracting the mean and dividing by 2 standard deviations) in order to be compatible with the other binary inputs (Gelman 2007).

Performance of the RVA models is measured using the area under the receiver operating characteristic (ROC) curve (AUC). The AUC assesses the accuracy of models for classifying a binary outcome, in this case reproductive success or failure (Jimenez-Valverde, 2012). AUC values range from 0 to 1, with an AUC of 0.5 expected as being the same as random and values close to 1 representing high accuracy in predicting realized outcomes.

Results

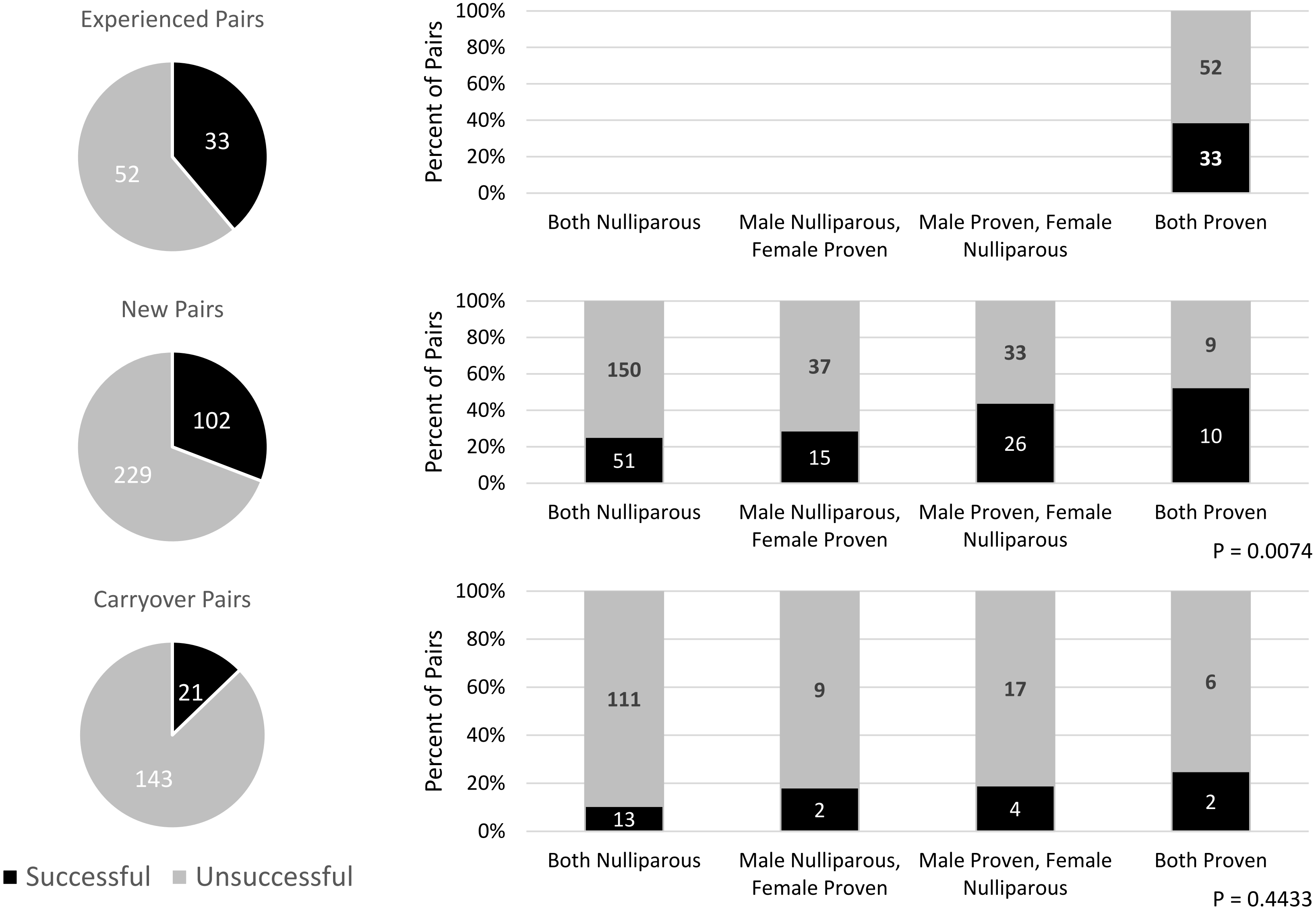
Chi-square Tests of Association

There is a significant association between Pair Type (i.e., new, carryover, or experienced) and reproductive success (Figure III-1; P <0.0001). Experienced pairs show the greatest rate of reproductive success (38.8%), followed by new pairs (30.8%). Carryover pairs were only successful 12.8% of the time.

Similarly, pair parity is associated with reproductive success (Figure III-1; P <0.0001). 40.2% of breeding pairs in which both the male and female are proven breeders are successful. When only the male or the female has previously produced offspring, the rate of success decreases to 37.5% and 27.0%, respectively. Only 19.7% of pairs are successful when both the male and female are nulliparous.

Breeding pairs at an institution with prior success breeding red wolves within the last 5 or 10 years also have a higher rate of reproductive success (P = 0.0037 and P = 0.0295, respectively). This result indicates that there could be some influence of environment or institutional knowledge on success rates; however, prior institutional success is likely confounded with pair type, as experienced pairs are likely to remain at the same institution for subsequent breeding seasons, and may similarly be confounded with pair parity.

Figure III-1. Proportion of red wolf breeding pairs that were reproductively successful or unsuccessful by pair type and pair parity. The p-values are based on a chi-square test of association between pair parity and success within each pair type.



There was no association between male contraception history and reproductive success. In contrast, there was a significant association between female contraception history and reproductive success (P = 0.0005): 29.6% of pairs in which the female has no prior history of contraceptive use are successful (N = 150/506). This is compared to only 7.9% of pairs in which the female was previously contracepted and had not yet reversed (N = 5/63) and 9.1% of pairs in which the female had already reversed from prior contraception use (N = 1/11).

There were no associations between male or female birth types (i.e., wild or captive) and reproductive success. There was also no difference in the rate of reproductive success between pairs that were already at the institution where the breeding recommendation was to be attempted versus those pairs in which the male, the female, or both individuals needed to be translocated for breeding.

T-Tests

Among red wolf breeding pairs, the average age of females was significantly younger in successful pairs compared to unsuccessful pairs, 5.0 ± 2.4 years old vs. 6.4 ± 2.8 years old, respectively ($P < 0.0001$). There was a similar trend for male age, 6.0 ± 2.6 years old vs. 6.5 ± 3.0 years old, for successful and unsuccessful pairs, respectively (P = 0.0539). The age difference between the male and female also appears to be important: Successful pairs have a significantly smaller age difference on average (2.7 ± 2.1 years) than unsuccessful pairs (3.2 ± 2.5 years; P = 0.0189).

Males in unsuccessful pairs also had higher individual levels of inbreeding compared to males in successful pairs, 0.068 ± 0.020 vs. 0.064 ± 0.020, respectively (P = 0.0203). There were no differences observed in individual levels of inbreeding among females between those in successful vs. unsuccessful pairs.

NOTE: The results from the chi-square and t-tests above do not take into account any possible interactions with other variables and these results alone should not directly lead to changes in management or husbandry.

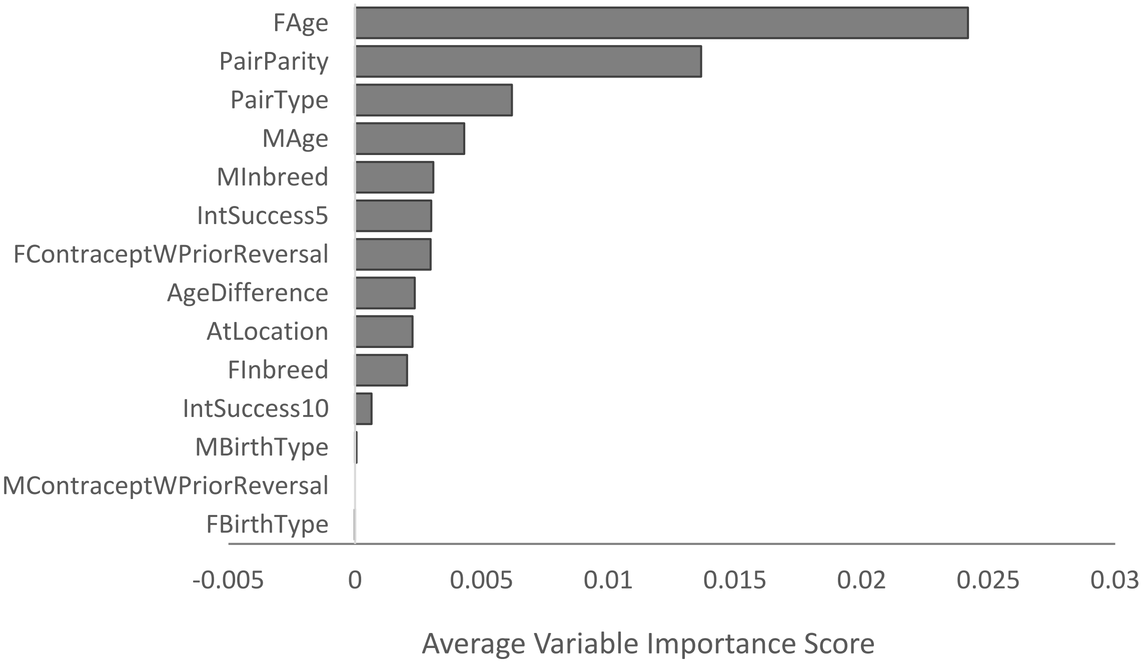
Conditional Random Forest (CRF)

On average, the CRF models were considered “acceptable”, as the average AUC was 0.75 (Hosmer, Lemeshow, and Sturdivant 2013). Only variable importance scores for iterations with an AUC > 0.7 (considered “acceptable”) were used to identify which factors may be most important in predicting the success of red wolf breeding pairs. On average, the CRF models (N = 840) indicated that the primary predictors of reproductive success are female age and pair parity (Figure III-2). Pair type and male age were the 3rd and 4th most important variables, respectively (Figure III-2).

LASSO Regression

Overall, the LASSO regression models are underperforming compared to the CRF models as 42.4% of the models (N = 424) would be considered “poor”, with AUCs < 0.7 (Hosmer, Lemeshow, and Sturdivant 2013). Only results from iterations (N = 576) with an AUC > 0.7 (considered “acceptable”) are being presented. The proportion of “acceptable” iterations in which each variable included in the red wolf RVA

Figure III-2. Average variable importance scores across 840 iterations for each factor used in the creation of nodes within the conditional inference trees for the red wolf conditional random forest models. Factors with higher importance scores are the variables that are more predictive of reproductive success (or failure).



had a non-zero regression coefficient is presented in Table III-2. Variables that were found to be significantly associated with reproductive success in at least 20% of iterations (in any combination) included pair type, institutional success in the last 5 years, pair parity, female age, male age, the age difference between the male and female, male's individual level of inbreeding, and the female's contraception history (Table III-2). Carryover pairs have a significantly lower probability or reproductive success compared to new pairs. There was no difference in reproductive success observed between new and experienced pairs. Breeding pairs at an institution that has had success breeding red wolves within the last 5 years also have a higher probability of success. Male age, female age, and the age difference between the male and female are all significantly associated with success, such that increasing male and female age, and a larger age difference between the male and female are associated with lower reproductive success. When it comes to reproductive history, pairs in which both the male and female are proven have the highest chance of reproductive success. Additionally, pairs in which only the male is proven also have a higher probability of success compared to pairs in which both individuals are nulliparous or only the female is proven. Females who have never been contracepted have a significantly higher rate of reproductive success than females who have a history of contraception, regardless of whether or not those females have already produced offspring post-treatment. Lastly, only inbreeding among males appears to be associated with reproductive success: as individual levels of inbreeding increase, the probability of reproductive success decreases.

Table III-2. Average LASSO regression coefficients resulting from iterations with an AUC > 0.7 of the ex situ red wolf population Reproductive Viability Analysis. Levels of variables highlighted in green increase reproductive success relative to the reference level (i.e., vs. level). Levels of variables (or quantitative variables) highlighted in red decrease, or are negatively associated with, reproductive success, relative to the reference level, or as the value increases. Levels of variables (or quantitative variables) highlighted in yellow do not have a consistent positive or negative association with reproductive success relative to the reference level across iterations, or had non-zero coefficients in fewer than 20% of iterations.

Variable: Level	Percentage of Non-Zero Coefficients	Average Effect/ Regression Coefficient	SD of Non-Zero Coefficients	Min	Median	Max	Lower 95% CL	Upper 95% CL
PairType: Carryover (vs. New)	100%	-0.53	0.12	-0.93	-0.53	-0.14	-0.54	-0.52
PairType: Experienced (vs. New)	0.9%	-0.24	0.14	-0.45	-0.15	-0.13	-0.26	-0.12
AtLocation: Yes (vs. No)	10.8%	-0.09	0.07	-0.29	-0.06	0.00	-0.10	-0.07
IntSuccess5: Yes (vs. No)	93.6%	0.19	0.09	0.01	0.19	0.52	0.19	0.20
IntSuccess10: Yes (vs. No)	8.3%	0.10	0.08	0.00	0.09	0.33	0.08	0.12
PairParity: YY (vs. NN)	95.5%	0.35	0.17	0.00	0.33	1.06	0.34	0.37
PairParity: YN (vs. NN)	59.5%	0.25	0.16	0.00	0.23	0.74	0.23	0.27
PairParity: NY (vs. NN)	15.6%	0.23	0.14	0.00	0.22	0.63	0.20	0.26
MAge (Male Age)	81.1%	-0.18	0.11	-0.58	-0.17	0.00	-0.19	-0.17
FAge (Female Age)	100.0%	-0.70	0.12	-1.07	-0.71	-0.14	-0.71	-0.69
DiffAge (|FAge - MAge|)	94.1%	-0.19	0.09	-0.53	-0.19	0.00	-0.20	-0.26
MBirthType: Wild (vs. Captive)	3.0%	0.23	0.21	0.01	0.14	0.61	0.13	0.33
FBirthType: Wild (vs. Captive)	0.0%	NA	NA	NA	NA	NA	NA	NA
MInbreed	93.6%	-0.26	0.12	-0.65	-0.25	-0.01	-0.27	-0.24
FInbreed	4.0%	0.07	0.09	-0.07	0.05	0.26	0.00	0.15
MContraceptWPriorReversal: NC (vs. No)	0%	NA	NA	NA	NA	NA	NA	NA
MContraceptWPriorReversal: Yes (vs. No)	0%	NA	NA	NA	NA	NA	NA	NA
FContraceptWPriorReversal: NC (vs. No)	92.4%	0.43	0.23	0.01	0.39	1.24	0.41	0.45
FContraceptWPriorReversal: Yes (vs. No)	0%	NA	NA	NA	NA	NA	NA	NA
AUC of ROC		0.74	0.03	0.70	0.73	0.86		

Consensus Findings

Both statistical approaches used for the red wolf RVA (CRF and LASSO regression) identified female age as likely the most significant predictor of reproductive success (Table III-3). On average, successful females were 1.4 years younger than unsuccessful females (5.0 ± 2.4 years vs. 6.4 ± 2.8 years). Though females were paired up to 13 years of age, the oldest successful female was 11 years old. When success is broken down by female age categorically, it becomes more apparent that the reproductive success of pairs significantly declines when the female is > 6 years old, regardless of prior reproductive success (Figure III-3).

To a slightly lesser extent, male age was also found by both methods to be associated with breeding success. Successful males were 0.5 years younger, on average, compared to unsuccessful males (6.0 ± 2.6 years vs. 6.5 ± 3.0 years). Similar to females, the oldest male paired was 13 years old, and the oldest male to successfully breed was 12 years old. When success is broken down by male age categorically, it becomes more apparent that the reproductive success of pairs significantly declines when the male is > 8 years old, particularly among proven males, as nulliparous males have consistently lower reproductive success (Figure III-4).

The association between breeding success and the age difference between the male and female detected using the LASSO regression appears to be heavily driven by the lack of success between pairs with an age difference greater than 7 (female is older) or 8 years (male is older). This again suggests the lack of success among older males and females.

Pair parity is arguably the next most important factor in predicting the reproductive success of red wolf pairs based on both RVA approaches. 40.2% of breeding pairs in which both the male and female are proven are successful. When only one individual (either the male or the female) has previously produced offspring, the rate of success decreases to 37.5% and 27.0%, respectively, and only 19.7% of pairs are successful when both the male and female are nulliparous. When it comes to only one individual in the pair being proven, a proven male appears to be a better driver of reproductive success compared to a proven female, based on the proportion of LASSO iterations in which the effect was found to be significant and the magnitude of the regression coefficient.

The last factor that is likely most important to consider when making future breeding recommendation is pair type. Experienced breeding pairs (pairs who had a previous opportunity together and successfully produced offspring) had the highest rate of reproductive success (38.8%) among red wolf pairs, compared to new pairs (30.8% successful) and carryover pairs (pairs who have had an opportunity to breed together in a previous BTP period but were unsuccessful; only 12.8% successful).

Table III-3. Factors significantly associated with breeding success based on different analyses. Statistical significance was declared where $P < 0.05$. Factors that are consistent across all methods are in bold.

	Factors associated with breeding success
Chi-square Test of Association or T-Test	**Pair Type** Institutional Success in Last 5 Years Institutional Success in Last 10 Years **Pair Parity** Female Contraception History **Female Age** **Male Age (Trend)** Age Difference Male Inbreeding
Conditional Random Forest Models (Top 4 based on Variable Importance Scores)	**Pair Type** **Pair Parity** **Female Age** **Male Age**
LASSO Regression Models	**Pair Type** Institutional Success in Last 5 Years **Pair Parity** Female Contraception History **Female Age** **Male Age** Age Difference Male Inbreeding

Figure III-3. Proportion of red wolf breeding pairs that were reproductively successful or unsuccessful by female age whether the female was proven (A) or nulliparous (B). The p-value is based on a chi-square test of association between age and success.

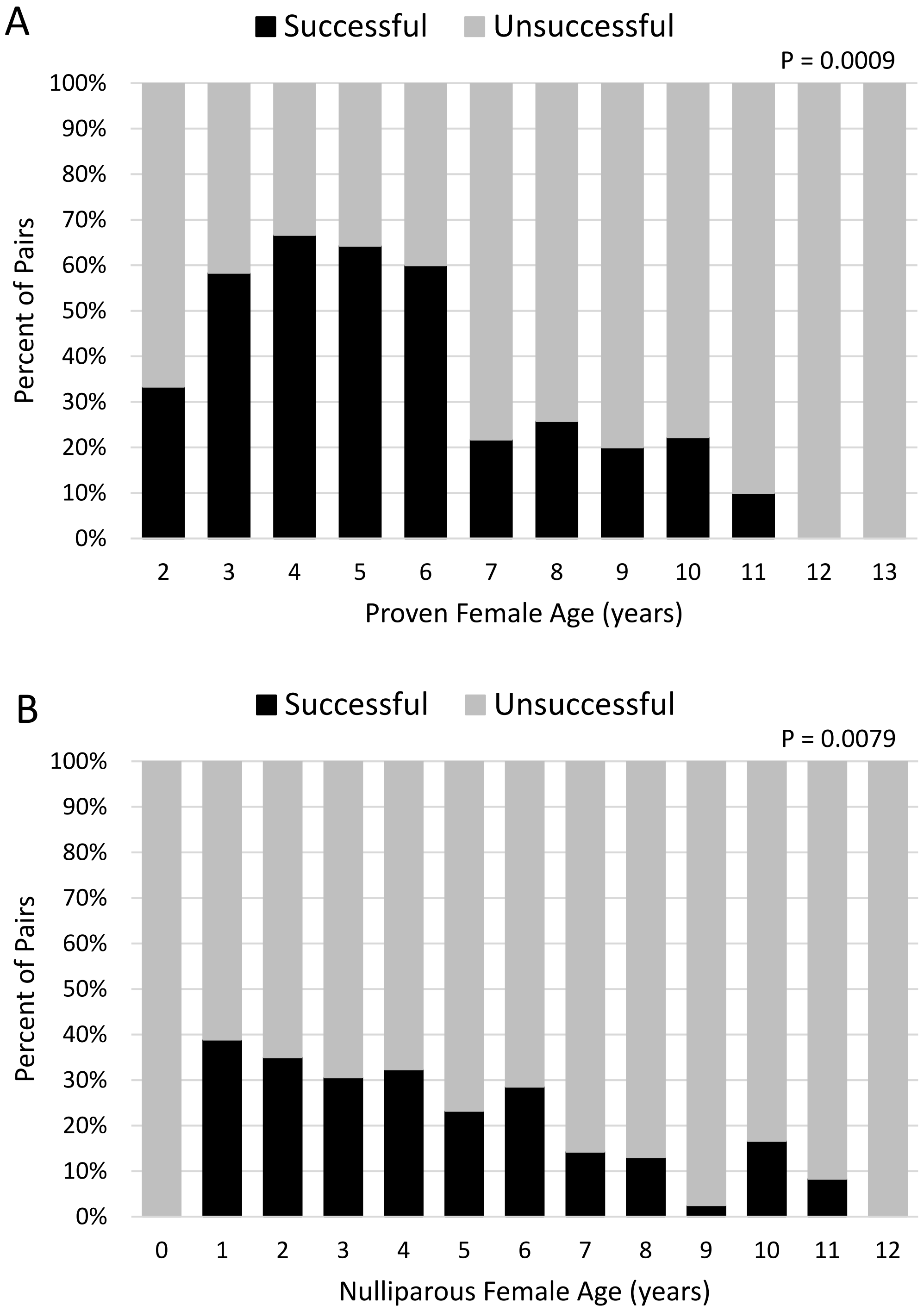
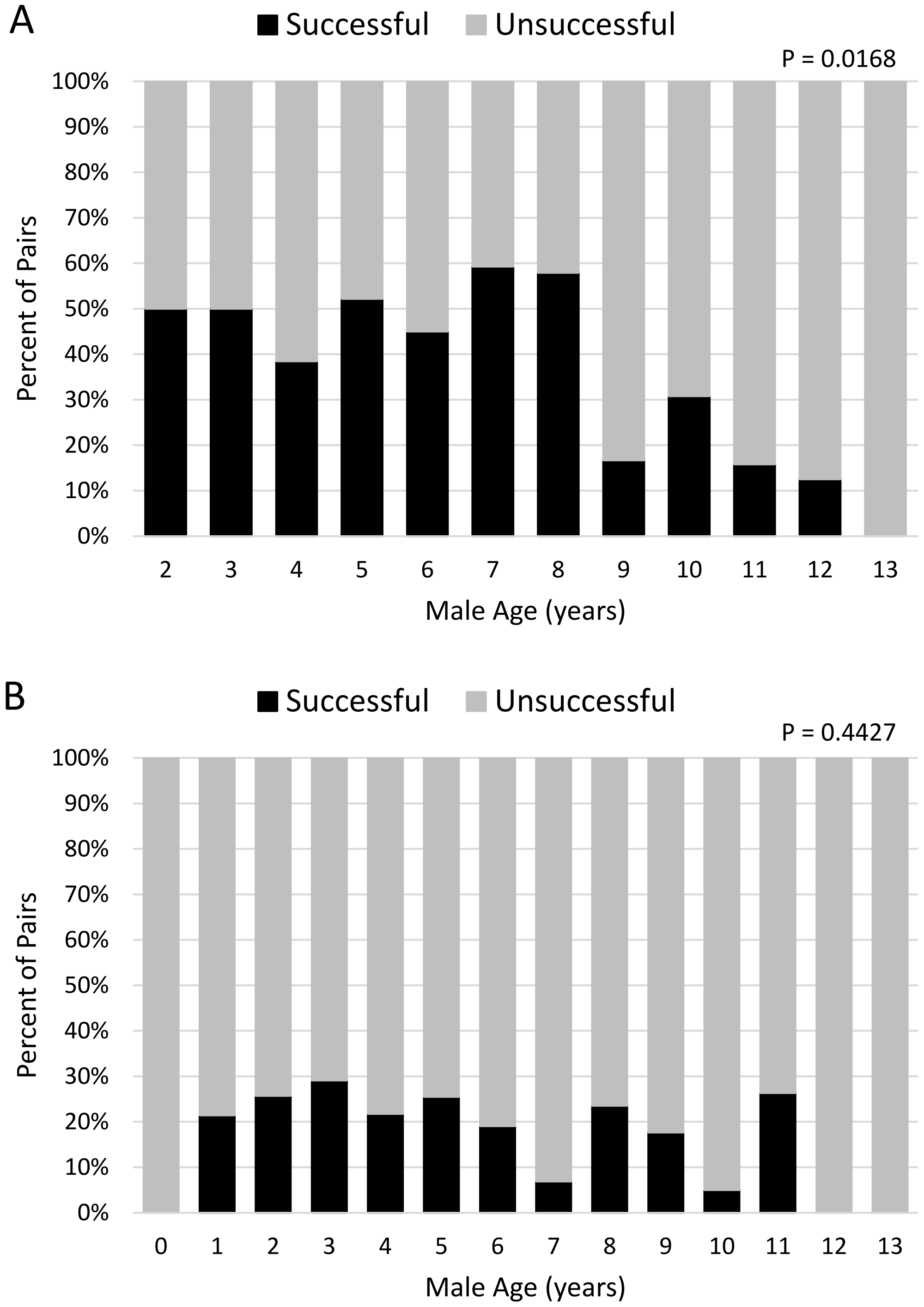


Figure III-4. Proportion of red wolf breeding pairs that were reproductively successful or unsuccessful by male age whether the male was proven (A) or nulliparous (B). The p-value is based on a chi-square test of association between age and success.



Limitations, Caveats, and Cautions

Based on our current data sources, we can see if animals are located at the same institution during the BTP period, but we cannot know based on this information alone if those animals were actually put together for breeding, therefore we don't always know if the breeding recommendation was actually attempted. Thus, there may be some "unsuccessful" pairs included in the analyses that were actually never given a breeding opportunity. The AZA Population Management Center has recently made improvements to their PMCTrack software in order to collect this type of information which will allow us to better refine our data sets in the future. Currently, we rely on SAFE program leaders to provide this information. When we know a breeding recommendation was never attempted, we do remove that pair from our dataset.

Similarly, if good records have not been kept on interim recommendations, we can only capture those pairs if they are successful, because their offspring will show up in the studbook. Therefore, any interim recommendations that were not formally recorded and did not result in offspring production will not appear in the dataset. PMCTrack has recently been updated in order to formally capture information on interim recommendations as well, so going forward this should become less of an issue.

Lastly, the statistical modeling techniques used for the RVA require that there is no missing information for any of the variables in the model. Therefore, there may be some additional breeding pairs listed in the BTPs that were not included in the analysis if any information was missing (e.g., inbreeding coefficient).

CONCLUSIONS & PROPOSED MANAGEMENT ACTIONS

Within this report we have presented the results of a Reproductive Viability Analysis for red wolves managed within AZA. Based on the results of our analyses, the RMC has the following recommendations for the AZA Red Wolf Animal Program:

- Consider splitting up pairs that have previously been unsuccessful, as the likelihood of success among carryover pairs is low (12.8%) compared to new pairs (30.8%), particularly if the male is nulliparous. Given the short prime reproductive lifespan, splitting up pairs after one or two years of unsuccessful attempts is recommended.
- Females should be bred before age 7. Even proven females over age 7 have a low probability of reproductive success.
- Males should be bred before age 9. Even proven males over age 9 have a low probability of reproductive success.
- Genetically valuable individuals (priority breeders) should be paired with younger, proven partners, as advancing age decreases likelihood of success, yet the age difference between the male and female is of minimal concern.
- Animal managers should feel comfortable moving animals between institutions when necessary to fulfill the breeding recommendations set forth by the SAFE, as breeding success generally appears unaffected when red wolves have been moved (see also Franklin et al. 2020).

- When space allows, more breeding pairs need to be created overall to meet demographic targets when making pairs with a lower probability of success (e.g., carryover pairs, nulliparous individuals, and older animals).
- More research is needed into the effect of contraception on future fertility among females. The use of contraception in females is significantly associated with female age ($P < 0.0001$) making its effect more difficult to detect; however, there is generally a consistent trend of lower success among females previously treated with contraceptives across ages. Unfortunately, sample sizes are too small to declare these differences significant. Regardless, contraception use in females appears to hinder future reproductive potential, and use should therefore be considered carefully in genetically valuable individuals and potential breeders. Contact the AZA RMC for current guidelines and recommendations prior to using contraceptives.

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APPENDIX A: Descriptive Statistics for Variables included in the RVA

NOTE: T-Test, F-Test, and χ^2 test p-values indicate a significant difference or association between levels of the variable and reproductive success; however, these tests do not control for any confounding variables, therefore interpret with extreme caution.

Pair Condition (B&T plans 2000 – 2020)	**N Pairs**	**% Successful (N Pairs)**	**χ^2 p-value**
Breeding pairs	580	26.90% (156)	
<u>Pair Type:</u>			
Experienced pairs	85	38.82% (33)	<0.0001
Carryover pairs	164	12.80% (21)	
New pairs	331	30.82% (102)	
<u>Pair at an Institution with success within previous 5 years:</u>			
Yes	322	31.68% (102)	0.0037
No	258	20.93% (54)	
<u>Pair at an Institution with success within previous 10 years:</u>			
Yes	379	29.82% (113)	0.0295
No	201	21.39% (43)	
<u>Male and Female were both at breeding location prior to B&T plan:</u>			
Yes	336	24.11% (81)	0.0754
No	244	30.74% (75)	
<u>Pair Parity:</u>			
Parous (Both Male and Female)	112	40.18% (45)	<0.0001
Nulliparous (Neither Male or Female)	325	19.69% (64)	
Male Parous, Female Nulliparous	80	37.50% (30)	
Female Parous, Male Nulliparous	63	26.98% (17)	
<u>Birth Type:</u>			
Female born in the Wild	18	27.78% (5)	0.9317
Female born in Captivity	562	26.87% (151)	
Male born in the Wild	21	38.10% (8)	0.2384
Male born in Captivity	559	26.48% (148)	
<u>Contraception:</u>			
Female with Prior Contraception Without Reversal	63	7.94% (5)	0.0005
Female with Prior Contraception Post-Reversal	11	9.09% (1)	
Female without Prior Contraception History	506	29.64% (150)	
Male with Prior Contraception Without Reversal	3	33.33% (1)	0.7371
Male with Prior Contraception Post-Reversal	2	50.0% (1)	
Male without Prior Contraception History	575	26.78% (154)	

Age/Inbreeding Related Variables (B&T plans 2000 – 2020)	**N**	**Mean ± SD**	**Median**	**Min**	**Max**	**T-Test**
Male Age	580	6.3 ± 2.9	6	0	13	
Female Age	580	6.0 ± 2.7	6	0	13	
<u>Male Age by Success:</u>						
Yes	156	6.0 ± 2.6	6	1	12	0.0539
No	424	6.5 ± 3.0	6	0	13	
<u>Female Age by Success:</u>						
Yes	156	5.0 ± 2.4	5	1	11	<0.0001
No	424	6.4 ± 2.8	7	0	13	
<u>Age Difference by Success:</u>						
Yes	156	2.7 ± 2.1	2	0	8	0.0189
No	424	3.2 ± 2.5	3	0	11	
<u>Male Inbreeding Coefficient by Success:</u>						
Yes	156	0.064 ± 0.020	0.068	0	0.091	0.0203
No	424	0.068 ± 0.020	0.074	0	0.135	
<u>Female Inbreeding Coefficient by Success:</u>						
Yes	156	0.068 ± 0.022	0.072	0	0.135	0.6853
No	424	0.067 ± 0.021	0.070	0	0.135	