

Research Article

Ex Situ Breeding Program With Wild-Caught Founders Provides the Source For Collaborative Effort To Augment Threatened New England Cottontail Populations.

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Abstract

In collaboration with the NEC Population Working Group, the Providence, Rhode Island-based Roger Williams Park Zoo (RWPZ) runs a NEC ex situ breeding program as part of a science-based conservation strategy for the New England cottontail (NEC). The mission of RWPZ is to support research on a species that is challenging to observe in the wild, in addition to breeding fragile cottontail rabbits for future release to support wild populations. Being the first to breed this species in a zoological setting, RWPZ was crucial to the survival of the only native rabbit in New England.

Keywords : *New England cottontail; ex situ breeding; conservation.*

INTRODUCTION

Previously found throughout much of New England, the New England cottontail (NEC) (*Sylvilagus transitionalis*) is a vulnerable rabbit species that is rated as Vulnerable on the IUCN Red List. Only small subpopulations in Connecticut, Massachusetts, Maine, New York, New Hampshire, and Rhode Island still have it now. According to estimates, the populations of the latter two states were less than 180 and 100 people, respectively, in 2015 [1]. Due to land development, succession, and the suppression of natural disturbances (such as flooding and wildfires), this species' early successional habitat is disappearing. For decades, NEC populations have been declining, and if nothing is done, the genetic diversity of the surviving isolated groups would also disappear. The non-native eastern cottontail (*Sylvilagus floridanus*), which was brought from Missouri in the 1930s to help hunters, may also be displacing them in the fight for resources [2]. The creation of a breeding management program for NEC was the responsibility of the NEC Population Management Group, which was made up of funding organizations, state universities, RWPZ, and wildlife agencies at the federal and state levels. In order to restore sustainable population numbers, this effort aims to breed NECs for

release into the wild. As part of this effort, RWPZ collaborates with academics and students from several universities to supply data for numerous studies examining the biology and ecology of NEC rabbits. Field biologists can learn more about NEC behavior and RWPZ can refine best practices for NEC breeding management with the help of this research. The whole recovery project involves numerous partners who are attempting to reunite and grow isolated NEC populations by establishing or restoring early successional habitat on both public and private lands [Appendix A]. A supply of genetically varied NECs to (re)populate these areas was the missing component. State scientists contacted Roger Williams Park Zoo (RWPZ) zookeepers and Lou Perrotti, Director of Conservation Programs, in 2010 because they possessed the resources and know-how to support a breeding effort at the zoo.

EX SITU BREEDING PROGRAM

State wildlife biologists collect wild rabbits in Connecticut, Massachusetts, Maine, New Hampshire, and New York, then transport them to zoos to breed. These rabbits, known as "founders," are thought to be unrelated and will serve as the population's foundation. To verify that they are NECs

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and not the non-native eastern cottontail, which has a similar appearance, fecal samples are taken upon arrival and forwarded to The University of Rhode Island (URI) for genetic analysis. After that, the rabbits are quarantined for 30 days. They receive treatment for both internal and external parasites during this time, and they are watched for symptoms of illness. They are also examined for tularemia as of 2022. The rabbits are given ear tags and microchips for identification if they are determined to be healthy NECs. Before entering the breeding program, they get a thorough medical examination and receive a vaccination against the Rabbit Hemorrhagic Disease Virus (RHDV2). The founders are kept separately indoors in lab-style suspended caging when they are not engaged in breeding [Figure 1]. Timothy hay is used as bedding for the cages, which are also equipped with woody browse and a hide box. Grain and water bottles are fixed to the cage door. Every day, husbandry tasks include sifting waste onto the liner, replacing soiled hay with fresh, refilling feed hoppers, changing water bottles, and giving out fresh greens. Every other day, cage liners are changed. The area features windows and climate control to promote a more organic photoperiod. Figure 1: Racks for caging in a laboratory. The rabbits live in a more hygienic environment with this type of caging, where waste drips through into the liner below, and cleaning is much less intrusive. Breeding occurs in one of four covered outdoor breeding pens [Figure 2], each measuring around 100 x 150 and featuring fully meshed walls and a dig-proof concrete slab floor. To reduce male hostility, visual barriers are erected between pens. Each pen has a section of timothy hay in the middle for food and bedding. The animals can take refuge in a number of hides of different sizes, either together or separately. They can get water, food, and woody browse from a variety of sources. Every day, pens are cleaned by removing and replacing dirty hay, sweeping the floors, restocking grain sources, changing out the water bottles, and providing fresh greens. Two cameras with overlapping areas of view are mounted on opposite walls in each pen. Students at URI use camera video for their study. Figure 2: A breeding pen outside. Rabbits are transported in their hide boxes, which stay in the pen with them to offer extra cover, when they come here to breed. In order to investigate stress levels in NECs under managed care, fecal samples are either taken from cages or pens upon request from URI for research studies or submitted to the State University of New York, College of Environmental Science and Forestry. The success of the wildlife scientists' trapping efforts determines the precise number of males and females in the program each year. Having 4–5 males and 13–14 females per season is the goal. The goal of rabbit pairings is to maximize genetic diversity; each male is given a comparable number of females, and females are paired with a different male throughout each breeding cycle. Unless there are indications of aggressiveness, NEC pairs are

kept together in the breeding enclosures for about 48 hours. Males may stay outside to mate once more following this breeding opportunity, while females are taken back inside to their separate cages. Throughout their window for giving birth, the females are constantly observed. 40 days after the last day in the breeding pen, this window closes. It starts 27 days after the first day. In an attempt to lessen stress on the dam, once kits are delivered, there is very little noise and the cage is not cleaned for three days, with the exception of removing any dead kits or delivering fresh browse [Figure 3]. It is crucial that the dam raises these children since they will be released into the wild. While taking a detached approach, the staff aims to deliver the finest service possible. Figure 3: Kits for newborns. A litter of one-day-old kits with their ears back and their eyes closed. Roger Williams Park Zoo is the source. Until they are weaned at 23 days, the kits stay with the dam [Figure 4]. The female can now breed again after the dam or kits are transferred to a different cage. Each season, which normally lasts from February to August, accommodates as many mating rounds as feasible. Some of the adults will be released at the conclusion of the season, while those that did well in the program and turned out to be good breeders might remain at RWPZ for an additional season. Rotating founders is crucial for population genetics to ensure that no rabbit's genes are overrepresented. RWPZ veterinarians start the process of vaccinating the kits against the Rabbit Hemorrhagic Disease Virus (RHDV2), determining their sex, and providing them with microchips and ear tags after they have been weaned. To get a DNA sample from each animal, they also cut off a small piece of one ear. To assist them get used to live outside, kits are taken outside to one of the vacant breeding cages after they have been tagged and identified. Until the NEC Population Management Working Group determines their release location based on their genetic profile, the kits stay at RWPZ. After that, the rabbits will be picked up by state wildlife biologists and taken to a hardening pen for additional acclimatization. There are currently two hardening pens: one at Great Bay National Wildlife Refuge (Newington, New Hampshire) and one in Ninigret National Wildlife Refuge (Charlestown, RI). In order to prepare the young kits for life in the wild, the pens are utilized for a soft-release procedure. They are predator-proof and occupy around one acre of prime NEC habitat. They are covered with nylon netting and have a perimeter fence made of hardware cloth that extends in an apron below ground level. Before being finally released into the wild, the kits are taught to survive in the outdoors and search for food here. Post-release survival is increased by time spent in pens like these, according to studies [1]. NECs in the hardening pens may be returned to RWPZ's veterinary division if it is determined that they require medical care. A dam with her 15-day-old kits is shown in Figure 4. Before weaning, the kits will spend a further eight days with the

dam.3. FindingsOf the 139 founders that have been brought to RWPZ since 2010, 38 have passed away, 84 have been released, and 17 are presently part of the breeding program. Despite the high newborn rabbit mortality rate, more than 250 of the 600 NEC kits born at RWPZ have been reintroduced into the wild [Table 1]. The great majority of losses are caused by pre-weaning mortality. The 2022 season is now underway. However, with 94 kits born so far, this year has already set a record. Table 1. Pre-weaning mortality rate, total number of NEC kits born at Roger Williams Park Zoo (RWPZ) since 2012, and number of kits released. In New Hampshire, Rhode Island, Maine, and Massachusetts, RWPZ kits have been deployed on areas owned by public and private partners. In 2016, Queens Zoo (Queens, NY) launched a second breeding program to build on the success at RWPZ. over 1000 NEC births and over 400 individuals released into the wild are the combined totals from the two zoos. mainland. At least 200 NEC were thought to be there in 2017 after 73 rabbits hatched at RWPZ were released onto the island over a five-year period. Because the pilot experiment was effective, there are now enough members of the Patience Island colony to serve as a starting point for new populations. At RWPZ and Queens Zoo, NECs were used to produce four self-sustaining populations.

CONSERVATION IMPACT

When it comes to the conservation of this species, the long-running NEC breeding management program—which was started at RWPZ with additional assistance from Queens Zoo—is a crucial component. It becomes more difficult for the juvenile bunnies to spread the 1–3 kilometers they usually travel to reproduce because of the severely fragmented habitats and populations [2]. The population's capacity to preserve genetic diversity and adjust to predators, illnesses, and habitat changes is diminished in the absence of this choice. These populations will be able to spontaneously diversify if they become more connected. RWPZ offers a supply of NEC rabbits to colonize these areas as conservation partners address this issue by establishing and maintaining new habitat throughout New England and New York. NECs may also be introduced into already colonized regions where low population size poses a risk of genetic bottlenecks in order to enhance genetic variety. As a preferred non-invasive monitoring method, DNA analysis of collected feces is utilized to identify released individuals using the genetic profile completed at RWPZ prior to release. The number of RWPZ-bred NEC that survive, whether they procreate, and how far they spread from the release sites may all be ascertained in this approach. In order to ascertain whether additional management measures are required, this technique can also be used to track population trends like size and sex ratio. According to data from a five-year research conducted in

New Hampshire, after the release of Genetic diversity was increased as a result of the breeding program's NEC surviving and reproducing with wild-born NEC [3]. The possibility of repopulation through the introduction of individuals from the ex situ breeding program was demonstrated by the fact that some rabbits were even able to effectively disperse to an area of suitable habitat 700 meters from the release location. Insights from past and present research conducted by University of Rhode Island (URI) graduate students continue to assist us understand the species and adjust the breeding program. RWPZ has directly used data from camera footage to ascertain the optimal breeding pen design to lessen antagonism and increase mating success, as well as the amount of time breeding pairs spend together. A mate choice study is currently underway as a result of earlier breeding behavior research suggesting pre-copulatory mate choosing is taking place [4]. In the future, the findings of this study might indicate that a new strategy is necessary to enable females to select more suitable males for mating, hence reducing breeding aggression and boosting the number of births. It might also affect the way biologists perceive natural populations. Knowing whether pre-copulatory mate choice is taking place in wild populations that are already fragmented might be helpful in order to further reduce the number of potential mate options. The invasive animal illness Rabbit Hemorrhagic illness Virus (RHDV2) has begun to spread across the United States from the west coast towards the east since efforts to save this species began. All individuals brought in and children leaving are now vaccinated against RHDV2 in order to combat this. It was a difficult process, and in order to have the vaccine imported for usage, RWPZ veterinary professionals had to collaborate with the state veterinarian of Rhode Island to submit a federal application. As a result, before being released, all Queens Zoo rabbits are vaccinated at RWPZ. It is more crucial than ever to ensure that NEC are not eradicated by this new threat, especially since the first instance of RHDV2 was documented in Connecticut in September 2022 (in domestic rabbits) [5].

CONCLUSIONS

This once-considered species for Endangered Species Act protection no longer satisfies the listing requirements, thanks to the efforts of RWPZ and all of the other partners. While this is no small accomplishment, RWPZ will continue to support population growth and genetic diversification through the breeding program until the habitat and population objectives of the conservation strategy are fulfilled. Without the assistance of the NEC Population Management Group partners, the program itself would not have been able to succeed. In order to supply founders for breeding, RWPZ depends on field biologists from neighboring states where native populations

still exist. Furthermore, there is dependence on the university researchers who analyze hundreds of hours of breeding pens' worth of video footage to obtain fresh comprehension of NEC behavior. This species cannot be saved by a single endeavor; even the NEC Population Management Group is only one component of the greater picture, a division of a larger partner group that is striving to support the recovery of the NEC populations.

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Data Availability Statement

Not relevant.

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Conflicts of Interest

No conflicts of interest are disclosed by the writers. The study's design, data collection, analysis, and interpretation, manuscript preparation, and the choice to publish the findings were all done independently of the sponsors.

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