Original Article

Monitoring a New England Cottontail Reintroduction with Noninvasive Genetic Sampling

MELISSA L. BAUER, *Department of Natural Resources and the Environment, University of New Hampshire, Durham, NH 03824, USA*
BRETT FERRY, *New Hampshire Fish and Game, Concord, NH 03301, USA*
HEIDI HOLMAN, *New Hampshire Fish and Game, Concord, NH 03301, USA*
ADRIENNE I. KOVACH [1] *Department of Natural Resources and the Environment, University of New Hampshire, Durham, NH 03824, USA*

ABSTRACT Careful monitoring of reintroduced threatened species is essential for informing conservation strategies and evaluating reintroduction efforts in an adaptive management context. We used noninvasive genetic sampling to monitor a reintroduction of a threatened shrubland specialist, the New England cottontail (*Sylvilagus transitionalis*), in southeastern New Hampshire, USA. We monitored the apparent survival and breeding success of founder individuals and tracked changes in population size and genetic diversity for 5 years following an initial reintroduction in 2013. We released 42 rabbits, documented 29 unique offspring in years following releases through noninvasive surveys, and identified 6 founder individuals and 9 recruited offspring that bred. Apparent survival of founders was variable and greatest in the first year of the reintroduction. Predation was the primary cause of mortality and greatest in the first month after release and after heavy snowfall. Population size remained small but relatively stable until a stochastic decline in the fourth year following reintroduction, followed by a slight rebound after population augmentation and offspring production by wild-born rabbits. Genetic diversity increased after the initial founders with diverse genetic backgrounds were released and then they and their subsequent offspring bred. We documented successful dispersal 700 m from the release site to a high-quality patch of habitat, which remained occupied throughout the study. For New England cottontail reintroductions to be successful in the long term, releases will be needed at multiple patches within dispersal distance, and habitat corridors need to be restored among patches to create a functioning metapopulation. For small or isolated reintroduced populations, continued intensive monitoring is needed to detect stochastic declines in population size or changes in sex ratios and guide subsequent management reactions via additional reintroductions or population augmentations. Noninvasive genetic sampling is a valuable tool to monitor reintroductions of the New England cottontail and other threatened species to provide managers with detailed information to inform decision-making in an adaptive management framework. © 2020 The Wildlife Society.

KEY WORDS monitoring, New England cottontail, noninvasive genetic sampling, reintroduction, *Sylvilagus transitionalis*.

Reintroduction—an attempt to reestablish a self-sustaining population through translocation or release of captive-bred individuals to an area from where it has been extirpated— is an important strategy to conserve endangered wildlife species (Fischer and Lindenmayer 2000, Fritts et al. 2001, Seddon et al. 2007, Jachowski and Lockhart 2009). Successful reintroductions must overcome obstacles such as unstable demographics (Murrow et al. 2009), skewed

sex ratios (Tella 2001, Clout et al. 2002), disease (Viggers et al. 1993), inbreeding depression (Brook et al. 2002, O'Grady et al. 2006), stochastic events related to weather or predation (Stacey and Taper 1992), and limited habitat or population connectivity in metapopulation systems (Chandler et al. 2015). Genetic monitoring is a valuable tool to evaluate the success of reintroductions and facilitate decision-making in an adaptive management context (Schwartz et al. 2007, De Barba et al. 2010, Cullingham and Moehrenschlager 2013, DeMay et al. 2017). Specifically, noninvasive genetic sampling of DNA extracted from hair, feathers, feces, or other shed tissues can be used to monitor populations. Accordingly, it is an effective

method to document the presence of elusive species, identify and count individuals, estimate population sizes, determine sex, and evaluate genetic diversity of a population without handling or observing animals (Waits and Paetkau 2005).

We used noninvasive genetic sampling to monitor a reintroduction of a threatened habitat specialist, the New England cottontail (*Sylvilagus transitionalis*), in a historically occupied landscape where a declining population was thought to be extirpated in the years prior to reintroduction. The New England cottontail is the only native rabbit east of the Hudson River and a species of high conservation concern throughout the northeastern United States. It is sympatric with the nonnative eastern cottontail (*S. floridanus*) in the southern portion of its range and snowshoe hare (*Lepus americanus*) in the northern portion of its range. This specialist species requires dense thicket habitat (shrubland, early successional forest, or dense understory underneath forest edge canopy) for forage, thermoregulation, and cover from predators, both within its home range (Barbour and Litvaitis 1993, Litvaitis 2003), and during dispersal (Fenderson et al. 2014, Amaral et al. 2016). Although patchy and ephemeral by nature, shrubland habitats have declined in area and experienced extensive fragmentation in the northeastern United States due to forest maturation, widespread development (e.g., suburban, industrial), and suppression of natural disturbance regimes that maintain early successional habitat (Litvaitis 1993, 2003; Schlossberg and King 2007). Today, New England cottontails are isolated into 5 geographically and genetically distinct regional populations (Litvaitis et al. 2006, Fenderson et al. 2011). Further subdivisions occur within each of these geographic areas, resulting in small, local metapopulations, in which extinctions and recolonizations occur independently from each other (Fenderson et al. 2011, 2014; Bauer 2018; Cheeseman et al. 2019).

Loss and fragmentation of shrubland habitat have limited dispersal within New England cottontail metapopulations (Fenderson et al. 2011, 2014; Bauer 2018; Cheeseman et al. 2019; B. Ferry, unpublished data). Historically, dispersal movements to occupied patches and patches of newly available shrubland habitat would have offset patch extinctions within metapopulations. Currently, cottontails persist within each metapopulation on remnant patches surrounded by an inhospitable landscape matrix, with roads, development, and mature forest posing dispersal barriers (Fenderson et al. 2014, Amaral et al. 2016). New England cottontails exhibit low dispersal capabilities in these landscapes. A telemetry study in New York, USA, documented a mean movement distance of approximately 75 m (Cheeseman 2017). In that study, dispersal movements (movements >250 m) were exceedingly rare; for New England cottontails that did disperse, the median dispersal distance was 512 m. Further, New England cottontails made nearly 10 times as many exploratory movements as dispersal movements, suggesting a natural propensity for dispersal impeded by an impermeable matrix in a fragmented landscape. Similarly, a telemetry study

in the Merrimack Valley region of New Hampshire, USA, documented one dispersal event out of 37 collared New England cottontails, and the dispersing cottontail moved 900 m before being predated (B. Ferry, personal observation).

In response to declining New England cottontail populations and their 9-year (2006–2015) candidate listing status under the Endangered Species Act, conservation efforts on behalf of the species have been underway since 2008 via a collaborative, range-wide New England Cottontail Conservation Initiative. Efforts to restore habitat and population connectivity have included widespread creation and restoration of shrubland habitat, with approximately 12,700 acres (~5,140 ha) restored or managed across the species' range as of 2018 (in addition to existing self-sustaining habitat), and the development of a captive breeding program (New England Cottontail Technical Committee 2019). These collaborative conservation efforts among federal, state, and private organizations and landowners were deemed sufficient to preclude federal listing of the species in 2015 (USFWS 2015). Captive breeding efforts have progressed from rearing individuals at the Roger Williams Park and Queens Zoos to the establishment of an island breeding colony in Rhode Island, USA, and outdoor breeding and holding pens in New Hampshire and Rhode Island. Releases of individuals reared in zoos, the island colony, and outdoor pens were initiated at Bellamy River Wildlife Management Area (WMA) in New Hampshire in 2013, at Great Swamp WMA in Rhode Island in 2016, and at Wells National Estuarine Research Reserve in Maine, USA, in 2017. Our goal was to use noninvasive genetic sampling to monitor the success of the first reintroduction at Bellamy River WMA from 2013 to 2018. Specifically, our objectives were to 1) track the survival and reproduction of founder cottontails across multiple releases at Bellamy River WMA, and 2) quantify changes in population size and genetic diversity following releases. We use our results to evaluate factors that contribute to successful reintroduction and monitoring and make suggestions to aid ongoing and future efforts at additional reintroduction sites. Our expectation was that successful reintroductions in the short term should produce high survival of released individuals, reproduction by both founders and wild-born individuals, and dispersal into additional patches of available habitat nearby in the landscape. In the long term, successful reintroductions should produce a self-sustaining metapopulation (i.e., multiple occupied patches within dispersal distance) that can persist without additional input from the captive breeding program.

STUDY AREA

This study was conducted at Bellamy River WMA (43.156030, −70.857880), a 162-ha property in Dover, New Hampshire, in a historically agricultural landscape that presently consists of a variety of land cover types including mature forest, wetlands, fallow fields, and shrublands (Fig. 1). Habitats in the portion of the WMA targeted for cottontail reintroduction included dense shrubland under sparse canopy
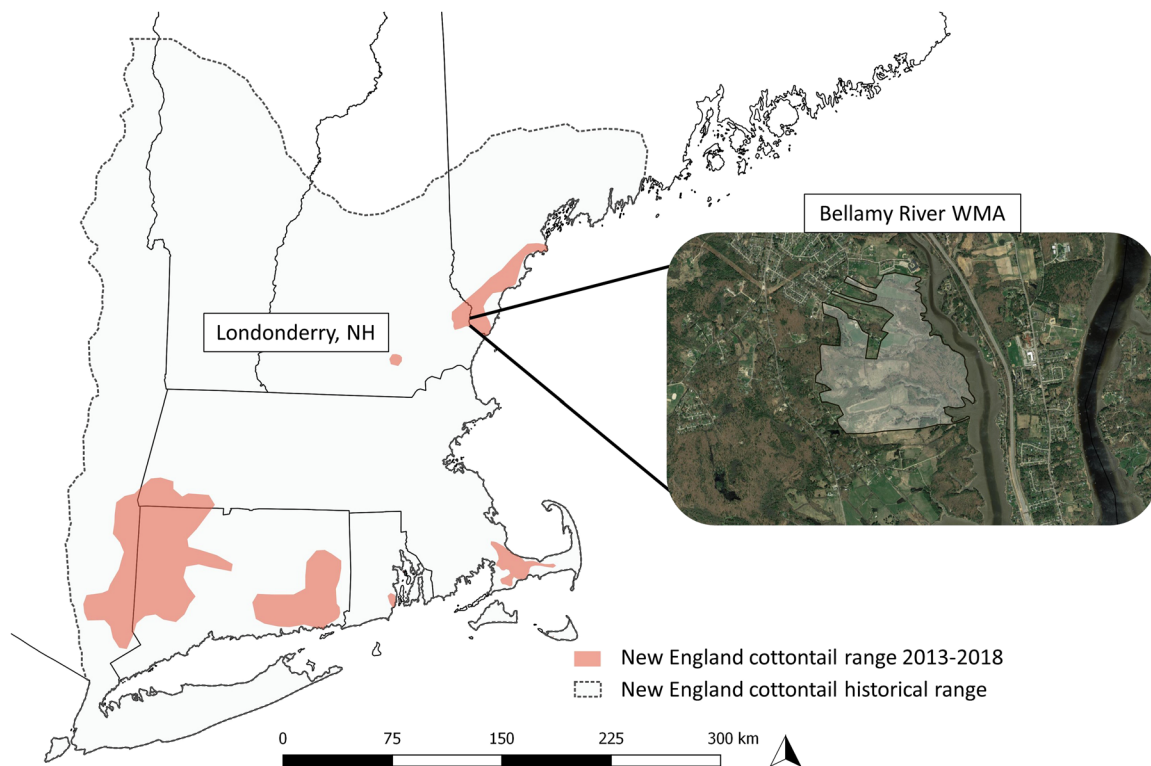
Figure 1. Location of the first New England cottontail reintroduction site at Bellamy River Wildlife Management Area (WMA), in New Hampshire, USA, shown in relation to the current range (2013–2018; red shading) and historical range (ca. 1960). The other region consistently occupied by New England cottontails in recent years in New Hampshire is shown in Londonderry.

with native understory species (including *Swida* spp., *Rhus* spp., *Rubus* spp., *Vaccinium* spp., *Spirea* spp., *Vitus* spp., and regenerating hardwoods and *Pinus strobus*) and invasive understory species (including *Rosa multiflora*, *Elaeagnus umbellata*, *Lonicera* spp., *Celastrus orbiculatus*, *Berberis* spp., and *Euonymus alatus*), moderate to dense native and invasive shrubs under moderate canopy (including canopy species such as *Acer* spp., *Prunus* spp., *Malus* spp., *Betula* spp., *Populus* spp., *Quercus* spp., *Pinus strobus*, *Juniperus virginiana*, *Robinia pseudoacacia*, *Salix* spp., and *Ulmus americana*), and wet, open areas dominated by grasses and forbs. The surrounding landscape was composed of mature forest, agricultural fields, suburban development, and additional managed shrubland and young forest patches.

Approximately 46 ha of habitat projects were completed to create shrubland habitat on this property; about half of the restored area has grown into the dense shrub habitat required by New England cottontails. Two key shrubland patches included a 10-ha area on the northern portion of the property, where cottontails were released during this reintroduction; and an additional 10-ha patch of dense shrub habitat 700 m southwest of the release site. Remnant New England cottontail individuals were present on the site until 2012, after which winter surveys for fecal pellets did not identify any remaining individuals and the patch and surrounding landscape were assumed to be vacant. Bellamy River WMA and the surrounding landscape are a focal area for New England cottontail conservation in the New Hampshire seacoast region, with the goal of restoring a

functional landscape for cottontail metapopulations. Bellamy River WMA was selected as a reintroduction site because of its large size and ongoing habitat restoration work at the site, including large-scale volunteer shrub planting projects since 2010. Additional habitat management projects totaling approximately 63 ha have been completed at nearby sites within a 3 km distance from Bellamy River WMA, and of that, 9 ha of dense shrubland habitat have been restored that could support cottontails. The potential for multiple shrubland patches in the surrounding landscape to support a cottontail metapopulation was another motivation for selecting this area as a release site. Mean temperature was 13.8° C (0.3° C higher than the long-term average) during the founder release period (Apr–Nov, 2013–2017) and –2.3° C (consistent with the long-term average) during the winter monitoring period (Dec–Mar, 2014–2018) (NOAA 2010). Total snowfall during the four-month winter monitoring period ranged from 94.5 to 259.3 cm (average 190.2 cm) (NOAA 2020).

METHODS

Founder Releases
We released 42 founder individuals (hereafter founders) in Bellamy River WMA (Fig. 1, Table 1) in 2013, 2014, 2015, and 2017 from Roger Williams Park Zoo (Providence, RI, USA), Queens Zoo (Queens, NY, USA), and outdoor breeding enclosures at Great Bay National Wildlife Refuge (Newington, NH, USA) and Ninigret National Wildlife

Table 1. Outcome of New England cottontail releases at Bellamy River Wildlife Management Area in New Hampshire, USA, from 2013 to 2017, including the number of founder New England cottontails released each year, number of mortalities within 1 month of the release date, number of founders surviving into the winter survey period or long enough to breed, percent apparent survival, and percent of founders known to be on the site through telemetry or parentage analyses that were detected in winter pellet surveys.

Year (release period)	No. released	No. mortalities within 1 month[a]	No. survived	Apparent survival (%)	Founder detection (%)
2013 (Jul–Oct)	8	0	6	75.0	80.0
2014 (Apr–Nov)	18	5	0	0.0	NA[b]
2015 (Jul–Oct)	7	3	1	14.3	100.0
2017 (Aug–Nov)	9	5	3	33.3	66.6

[a] In most years, mortality was highest during the first month postrelease. All released rabbits were monitored with telemetry until a mortality signal was detected or the signal was lost.

[b] All founders released in 2014 died prior to pellet surveys as a result of starvation or predation following heavy snowfall and prolonged deep snowpack.

Refuge (Charlestown, RI, USA). Founders were released based on availability from the breeding program, primarily in the autumn, with some released in spring and summer. We released founders in the center of the release patch. Prior to release, we collected a tissue biopsy from the ear of each founder and stored it in 100% ethanol until DNA extraction. We outfitted founder individuals with radio-collars (Advanced Telemetry Systems M1555, Isanti, MN, USA) with a mortality signal to track survival and monitored them 1–5 times weekly, as long as the collars were active and indicated that the rabbit was alive. For all mortalities, we confirmed date of mortality with telemetry and recorded cause of mortality when the carcass could be recovered. Methods of rearing and handling cottontails were consistent with the Association of Zoos and Aquariums code of ethics and standards maintained by the U.S. Fish and Wildlife Service and New Hampshire Fish and Game.

Winter Pellet Surveys

To monitor the reintroduced population, we collected cottontail fecal pellet samples during winter surveys conducted on 20 ha of available habitat surrounding the release site (the 10-ha release patch and the additional 10-ha managed shrubland patch). We collected pellets in a systematic, fine-scale sampling scheme, in which the patch was searched with transects spaced 30 m apart and pellets were collected every 30 m along a transect when present, following methods of Kristensen and Kovach (2018). We recorded spatial information for search transects and pellets (Garmin GPSMAP 64 s, Olathe, KS, USA). Pellet surveys consisted of 1 survey during December–March in winter 2013/2014 (hereafter winter 2014) and 2 independent surveys annually in 2014/2015 through 2017/2018 (hereafter winter 2015 through 2018), under optimal survey conditions to detect New England cottontails (2–5 days after snowfall, with snow depth <30.5 cm when possible and wind speed <40 km/hour; Brubaker et al. 2014). We collected fecal pellets with gloves to prevent contamination in the field and stored them in 15-mL conical tubes at −20° C until DNA extraction.

Molecular Methods and Data Analyses

We extracted DNA from pellets with the QIAamp® DNA Stool Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions with minor modifications (Kovach et al. 2003) and from tissue samples with the Qiagen DNeasy® Blood and Tissue Kit (Qiagen). We amplified DNA in 3 multiplex polymerase chain reactions (PCR; see Appendix A [in Supporting Information] for protocols) with a panel of 16 microsatellite markers, including 14 loci developed for the New England cottontail (King et al. 2017), 1 locus developed for the eastern cottontail (Berkman et al. 2009), and 1 Y-chromosome marker developed for sex identification in the European rabbit (*Oryctolagus cuniculus*; Vašíček et al. 2011). To increase amplification success rates, we used a high-fidelity hot-start technique in PCR reactions (AmpliTaq Gold® 360 DNA Polymerase; Applied Biosystems, Foster City, CA, USA) and a Solid Phase Reverse Immobilization paramagnetic bead purification on PCR products when needed. We sent PCR products to Yale DNA Analysis Facility (New Haven, CT, USA) for electrophoresis on a 3730*xl* 96-capillary DNA Analyzer. We manually scored alleles in PeakScanner (Applied Biosystems) and compiled multilocus genotypes for each sample. To prevent contamination, we performed fecal and tissue extractions in dedicated spatially segregated spaces and performed post-PCR work in a different laboratory. We performed extractions in small batches (8–16 samples) and included negative controls in each batch.

For quality control of low-copy DNA, we used a multiple-tubes amplification approach (Frantz et al. 2003, Waits and Paetkau 2005) and negative and positive PCR controls. We required 2 replicate allele observations for heterozygous loci and 3 replicate observations for homozygous loci to determine a consensus genotype (Frantz et al. 2003). We quantified the per locus genotyping error by comparing genotypes of all replicates with the consensus genotype (Pompanon et al. 2005). Samples missing data at ≥3 loci were excluded from analyses, thus final multilocus genotypes included ≥14 loci. To check for null alleles, we used MICRO-CHECKER (Van Oosterhout et al. 2004). To identify samples collected from the same or unique individuals, we used the multilocus matches option in GenAlEx 6.5 (Peakall and Smouse 2006, 2012). We reevaluated samples differing at only 1–2 loci and considered samples to be from the same individual when these mismatches appeared to be due to allelic dropout. We calculated the probability of identity of siblings ($P_{ID-SIBs}$), the probability that 2 siblings drawn at random from a population will have the same genotype (Waits et al. 2001), and

retained unique genotypes (i.e., individuals) for further analyses.

To identify founders and offspring that were present each year, we tracked individual genotypes detected through successive survey years. We used COLONY 2.0 (Jones and Wang 2010) to identify parent–offspring and sibling relationships on an annual basis and across years, when appropriate (considering individuals potentially alive in each year's sampling period, excluding known mortality events). We used the following settings in COLONY: male and female polygamy, inbreeding, very long run length, full-likelihood analysis, high likelihood precision, no allele frequency updates, and no sibship prior. Apparent survival was calculated on an annual basis as the percent of released individuals surviving through the winter, including founders detected during winter pellet surveys and those identified as parents of wild-born offspring.

To compare genetic diversity over time following the release of founder rabbits into the population, we calculated heterozygosity metrics and number of alleles for each yearly collection of samples in GenAlEx 6.5 (Peakall and Smouse 2006, 2012). We calculated allelic richness corrected for sample size in FSTAT 2.9.3.2 (Goudet 1995, 2001). We estimated average pairwise relatedness each year in ML-Relate (Kalinowski et al. 2006). For comparison, we also calculated genetic diversity metrics for a remnant New England cottontail population in the urbanized landscape of Londonderry, New Hampshire, and separately for each of 4 patches in the Londonderry population. We estimated census population size using a single-session mark–recapture method in the Program R package *capwire* 1.1.4 (Pennell and Miller 2015) for each year, separately, with sufficient recapture data.

RESULTS

Survey Detection and Founder Survival
We collected 191 pellet samples during the 5 winter survey seasons (2014–2018), successfully genotyped 175 samples, and identified 36 unique individuals, 5 of which were detected over multiple years (Table 2). Of the individuals detected, 7 were released founders and 29 were offspring recruited into the population as determined through parentage analyses. Genotyping success varied across years

from 87.2% to 100.0% and 1–21 samples were collected per individual (Table 2). Average false allele genotyping error rates across loci were 0.001/locus, and average allelic dropout rates were 0.025/locus. The probability of identity for siblings was 3.5×10^{-5} for 16 loci for this population, meaning that there was a one in 28,571 chance that 2 siblings share the same genotype at these genetic markers. $P_{ID\text{-}SIBs}$ for 14 loci, the minimum number required to retain a sample for analysis, was 6.3×10^{-4}. For retained samples, an average of 15.7 of 16 loci were genotyped. Molecular sex identification agreed with field sex for all founder individuals.

Of the 42 founders that were released, 6 bred, 9 survived into the winter following their release (detected through telemetry or in winter fecal pellet surveys), and 1 survived long enough to breed but did not survive into the first winter. Founders that survived into the first winter following release or long enough to breed included 6 of 8 released in 2013, 0 of 18 released in 2014, 1 of 7 released in 2015, and 3 of 9 released in 2017 (Table 1). Apparent survival of founders, the percentage of founders that survived through the winter survey period following release or survived long enough to breed, ranged from 75% in 2013 to 0% in 2014 (due to mortality following heavy snow and prolonged deep snowpack; Table 1).

Detection of surviving founders was high overall, but imperfect and varied by year (Table 1). Parentage analyses were useful in identifying individuals that were not detected in pellet surveys but were identified as breeders the summer following winter surveys or identified as undetected offspring based on parentage (1–3 undetected individuals/yr). Of the founders known to be present on the site during winter pellet surveys (i.e., known from telemetry observations to have survived; or detected via parentage analyses that identified individuals breeding the summer following winter surveys), 1 founder was not detected in 2014 winter surveys, 2 surviving founders from the 2013 release (as determined through parentage analyses) were not detected in the 2015 winter surveys, and 1 founder was not detected from the 2018 release. Parentage analyses also identified 2 wild-born individuals that were not detected in pellet surveys until their second winter. Parentage analyses identified known founders and their subsequent offspring as the parents of all detected offspring, except in the first year

Table 2. Number of New England cottontail fecal pellet samples collected during winter (Dec–Mar) surveys at Bellamy River Wildlife Management Area in New Hampshire, USA, during each year of the reintroduction (winter 2014 through winter 2018), number of samples successfully genotyped, percent genotyping success, number of unique individuals identified through pellet surveys, range of number of samples collected (i.e., captures) per individual, and population size estimates based on pellet surveys, telemetry, parentage analyses, and *capwire* estimates for years with sufficient capture history data.

Year (winter)	No. samples collected	No. samples genotyped	Genotyping success (%)	No. individuals detected	Range of captures	Population estimate (95% CI)
2014	20	18	90.0	10	1–5	11 (10–13)[a]
2015	23	21	91.3	8	1–8	11
2016	78	68	87.2	12	1–21	12 (12–14)[a]
2017	18	18	100.0	4	2–8	5
2018	52	50	96.2	7	1–15	8[a]
Total	191	175	x̄ = 92.9	8.2	4.1	9.4

[a] Indicates *capwire* population estimate for years with sufficient capture history data.

of the study when an unknown male (not a released founder) was determined to be the sire of 2 of the offspring produced in summer 2013. This revealed that one remnant male was present on the patch prior to the first releases of the reintroduction and that male bred with initial founder females.

Population Size and Genetic Diversity

The population remained relatively stable for the first 3 years after the initial reintroduction in 2013, experienced a decline in 2017, and increased slightly in 2018, following the last release and offspring production by wild-born rabbits (Figs. 2 and 3). Population estimates from telemetry data, estimates based on capture history in *capwire*, and reconstructed parentage ranged from 5 to 12 individuals/year, largely consistent with the number of unique individuals detected in pellet surveys (Table 2). Parentage analysis in COLONY identified an average of 4.6 breeding individuals/year (range = 2–7 breeding individuals/yr).

Allelic richness and heterozygosity of the population increased as founders from the initial release and their offspring bred over multiple years and their alleles were incorporated into the population. Allelic richness decreased with a population decline from 12 individuals in the winter of 2016 to 5 in the winter of 2017. Allelic richness and heterozygosity continued to decrease following the decline in 2017, at which point individuals detected on the patch were highly related. In years when the population increased at Bellamy River WMA, allelic richness and heterozygosity were higher than in the only other region consistently occupied by New England cottontails in New Hampshire, located in Londonderry. Genetic diversity metrics for the remnant Londonderry population are provided for comparison with the reintroduced Bellamy River WMA population (Table 3; data from Bauer 2018).

Founder Reproduction and Dispersal

The number of recruited offspring identified from winter pellet surveys and reconstructing breeding history with parentage analyses ranged from 3 to 9 per year. During each of the first 3 years of the reintroduction, the number of breeding individuals ranged from 4 to 7. There was only 1 breeding pair following the population decline in 2017 (Table 4). Two males and one female bred over 2 consecutive years and one female bred twice over a 3-year period. One male successfully sired offspring with 3 females in 2013 and 3 females in 2014. Females bore offspring with 2 males during a season 50% of the time, but not with >2 males. Females produced as many as 4 surviving offspring/season (i.e., the offspring were born in the summer and had to survive at least ~6 months until the winter to be detected in pellet surveys), with an average of 2.2 recruited offspring/ season. Individuals born in the wild were also documented breeding, producing second-generation wild-born individuals. Six founders bred – all from the initial release in 2013 – and 9 recruited offspring bred. Four individuals were detected surviving through 2 winter survey seasons, and one male was detected in pellet surveys for 3 consecutive years. In the second winter survey season, and in all subsequent years, individuals were detected in the 10-ha patch of shrubland 700 m southwest of the release site, indicating that dispersal occurred (Fig. 3). In 2018, offspring from a female detected on the southwestern patch were detected both on the southwestern patch, and on the northeastern patch, showing additional dispersal between the 2 patches.

DISCUSSION

Noninvasive genetic sampling provides critical insight into the viability and recovery of populations of rare or cryptic species (Waits and Paetkau 2005, Smith et al. 2009, Cullingham and Moehrenschlager 2013, Woodruff et al. 2016, DeMay et al. 2017). In this study, we showed the value of noninvasive genetic sampling to monitor a population of a threatened habitat specialist, the New England cottontail, through 5 years of reintroduction efforts. Tracking this reintroduction with genetic monitoring has produced insights to guide future reintroductions of New England cottontails.

Our findings show that reintroductions of New England cottontails can result in successful breeding by both
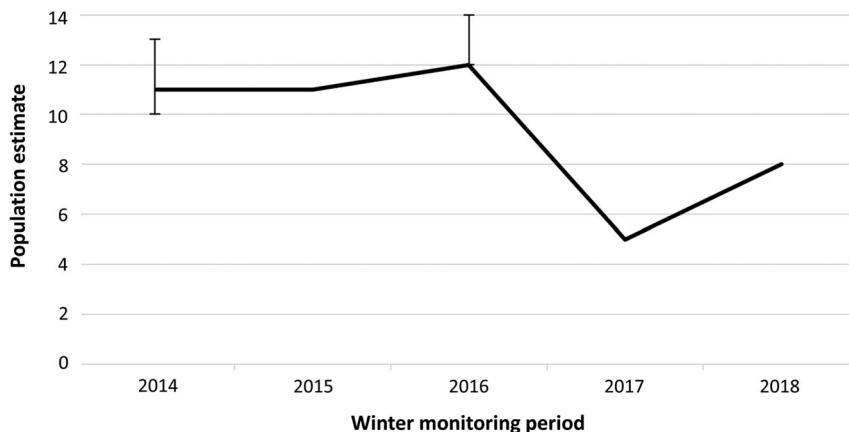
Figure 2. New England cottontail winter (Dec–Mar) population size estimates at Bellamy River Wildlife Management Area in New Hampshire, USA, from 2014–2018 based on pellet surveys, telemetry, parentage analyses, and *capwire* estimates. Displayed 95% confidence intervals are for *capwire* population estimates in years with sufficient capture history data from pellet surveys.
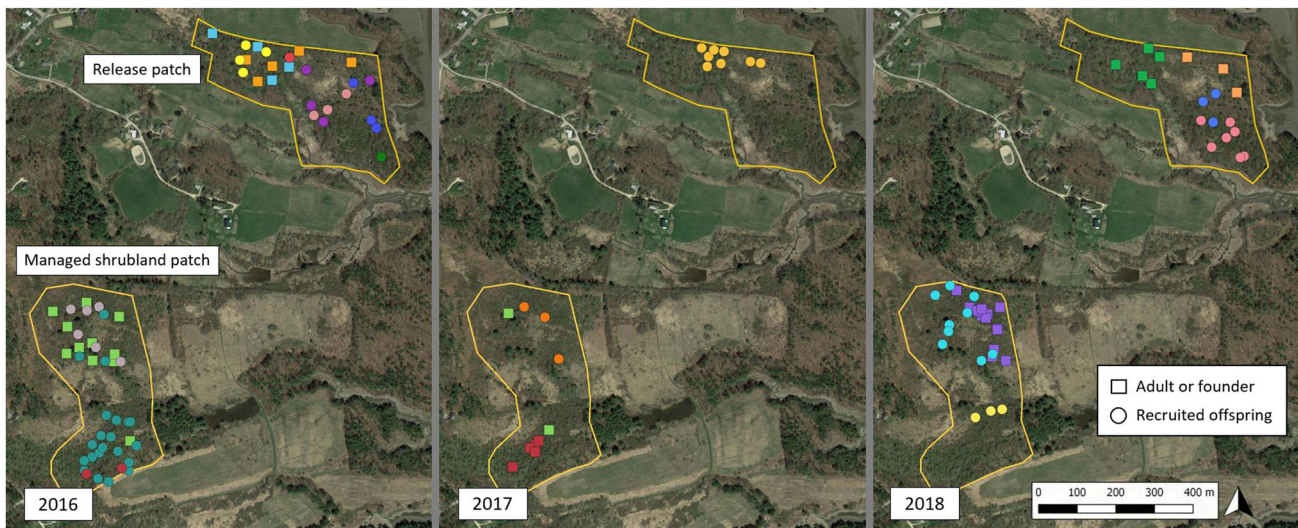
Figure 3. New England cottontail pellet samples collected in winter surveys from 2016–2018 at Bellamy River Wildlife Management Area in New Hampshire, USA, with a 10-ha release patch and additional 10-ha managed shrubland patch delineated in yellow. Each point color represents a unique individual. Squares indicate adults from previous years or founders and circles indicate offspring recruited into the population. The 2016 panel shows dispersal from the release patch to the managed shrubland patch to the southwest. The 2017 panel shows the population had declined and then increased in the 2018 panel following recruitment on the southwest patch, dispersal from the southwest patch to the release patch, and the release of additional founders in autumn of 2017.

Table 3. Genetic diversity metrics of the reintroduced New England cottontail population at Bellamy River Wildlife Management Area (WMA), in New Hampshire, USA, including individuals detected as breeders or alive on the patch during winter (Dec–Mar) pellet surveys from winter 2014 through winter 2018. Cumulative metrics from 4 patches in the Londonderry, New Hampshire New England cottontail population surveyed with winter pellet surveys and live-trapping from 2015–2017 are provided for comparison (data from Bauer 2018). H_O: observed heterozygosity; r: relatedness calculated in ML-Relate.

Year or population	Patch size (ha)	No. individuals[a]	No. of alleles	Allelic richness	H_O	r
Bellamy River WMA						
2014	20	10	2.7	2.8	0.514	0.134
2015	20	9	3.1	3.0	0.567	0.143
2016	20	12	3.5	3.4	0.569	0.170
2017	20	5	2.7	2.6	0.569	0.083
2018	20	8	2.0	2.0	0.400	0.147
Londonderry patches						
Stonyfield	8.5	21	3.3	3.2	0.549	0.080
Buckthorn St.	5.7	11	3.3	2.6	0.524	0.122
Cohas Brook	8.0	16	3.0	2.9	0.453	0.136
Charlotte St.	4.5	8	2.9	2.7	0.450	0.127
Londonderry total	26.7	57[b]	3.7	3.7	0.503	0.098

[a] Number of individuals on each patch in Londonderry is cumulative including all New England cottontails detected from 2015–2017.
[b] Londonderry total includes 1 additional isolated individual not grouped with any of the 4 patches.

Table 4. Number of offspring identified in the reintroduced New England cottontail population at Bellamy River Wildlife Management Area in New Hampshire, USA, during each winter (Dec–Mar) survey season from 2014 through 2018, number of males and females identified as parents, number of breeding founders, number of parents identified through parentage analysis that were not detected in pellet surveys, and number of offspring with full and half sib relationships.

Year	Offspring	Males breeding	Females breeding	Founders breeding[a]	Unsampled parents	Full sibs	Half sibs
2014	6	2	3	4	1[b]	2	4
2015	9	4	3	4	1	5	7
2016	7	1	3	2	0	6	10
2017	3	3	2	0	1	0	2
2018	4	1	1	0	0	4	0

[a] All breeding founders were from the original release in 2013. No founders survived into the winter from the 2014 release, 1 founder survived from the 2015 release into the 2016 winter survey period but was released in Oct 2014 after the breeding season, and 3 founders survived from the 2017 release into the 2018 winter survey period but were released in mid-Aug 2017 and did not breed prior to winter surveys.
[b] An unsampled parent detected by parentage analysis of samples collected in winter 2014 revealed that a remnant, wild male was on the patch at the time of the first releases of the reintroduction in 2013.

founders and wild-born individuals, with some individuals surviving and reproducing over multiple years. Reintroductions can stimulate population growth, increase genetic diversity, and create the opportunity for individuals to disperse and colonize nearby suitable patches. However, this reintroduction is not yet self-sustaining and has limited metapopulation function. We found that annual survival was highly variable, and a population decline and skewed sex ratio 4 years postreintroduction highlighted that stochastic events can have dramatic implications for both demography and genetic diversity. Annual monitoring revealed changes in the population status that allowed for adaptive management responses, such as releasing more females after genetic monitoring revealed a male-biased sex ratio.

The greatest limitation to the short- and long-term success of cottontail reintroduction in our study was survival—both postrelease and over winter. Recent studies of New England cottontail survival in the wild indicate extremely variable survival annually, with estimates ranging from approximately 10% to 75% (A. E. Cheeseman, State University of New York, College of Environmental Science and Forestry and B. Ferry, unpublished data). New England cottontail survival has been found to be lower on small patches (Barbour and Litvaitis 1993, Litvaitis and Villafuerte 1996) with estimates of 23% survival on sink patches and 45% survival on source patches (Litvaitis and Villafuerte 1996). Here we found that apparent survival of founder individuals varied from 0% to 75% of founders surviving through the winter survey period or long enough to breed. Of all individuals detected, including wild-born offspring, we detected only 5 of the 36 surviving through 2 winter survey periods, indicating low survival past age 1. However, we detected 1 male in winter pellet surveys for 3 years and 2 of the original founder females bred in their third summer, indicating occasional survival to age 3. New England cottontails are thought to have an average lifespan in the wild similar to that of the closely related eastern cottontail, averaging 15 months (Chapman et al. 1980).

Predation was the most common source of mortality for New England cottontails in this reintroduction based on recovered collared carcasses. Mortality from predation was generally high in the first month following release and was also high following severe winter snowfall events. Mortality during the first few weeks following release could be due to increased movement in a novel environment and concomitant increase in predation risk (Metzgar 1967, Ambrose 1972, Snyder et al. 1976, Sievert and Keith 1985, Ebenhard 1987). High mortality in the first weeks following release has also been noted as a major obstacle in restocking efforts for the European rabbit (Calvete et al. 1997, Letty et al. 2002) and was documented in translocations of swamp rabbits (*Sylvilagus aquaticus*; Watland et al. 2007). Letty et al. (2008) note mortality rates of European rabbits as high as 50% in the first 2 days following release, and 69% within the first month due to predation by mammalian predators. Mortality of New England cottontails from predation following heavy snowfall and declines in body condition were

observed on several occasions. In winter 2015, 100% mortality of founders was linked to extensive deep snowpack. Three snowstorms with >12-inch (>30.5-cm) accumulation occurred between mid-January and mid-February 2015, resulting in snowpack between 20 and 32 inches (50.8 and 81.3 cm). We determined mortality by radiotelemetry during this period of heavy snowfall and deep snowpack for all 11 founders still alive on the patch prior to winter pellet surveys. In 2016, we documented a mortality 5 days after a 6-inch (15.2-cm) snow event and, in 2018, we documented a mortality 2 days after an 11-inch (27.9-cm) snow event. This trend has been noted in other studies, with increased predation of both New England and eastern cottontails documented with an increase in the number of days of snow cover as well as snow depth and persistence (Brown and Litvaitis 1995, Boland and Litvaitis 2008).

Apparent survival was highest in the first year of the reintroduction, a trend which has also been noted in reintroductions of pygmy rabbits (*Brachylagus idahoensis*; DeMay et al. 2017) and riparian brush rabbits (*S. bachmani riparius*; Hamilton et al. 2010). The trend of decreased survival following the initial release could be due to an increased predator response (O'Donoghue et al. 1997, Sinclair et al. 1998, Stoddart et al. 2001), stochastic environmental and demographic processes (Crawford et al. 2010, Price et al. 2010), or competition with established rabbits (Hamilton et al. 2010). Competition could have been a factor in this reintroduction because territoriality and aggressive conspecific interactions among males have been documented in both New England cottontails (Tefft and Chapman 1987) and eastern cottontails (McKinney 1970, Brenner and Flemming 1979). Individuals released after the first year may have had to search farther for an open territory, increasing vulnerability to predation. For example, when founders were released in 2015, there were 11 individuals on the patch from the previous winter and apparent survival was 14.3%, and when founders were released in 2017 there were only 5 individuals on the patch from the previous winter and apparent survival was slightly larger at 33.3%.

Despite variable annual survival, several metrics of short-term success of this reintroduction were attained over the 5-year period. We documented successful breeding by founder individuals (albeit only those released in the first of four years of releases) and wild-born offspring. Results of parentage analyses indicate a promiscuous breeding strategy. Males produced offspring with up to 3 females/season, and females often produced offspring sired by 2 different males in a season. This study also showed that reintroductions have the potential to bolster genetic diversity, a valuable management objective for isolated and highly related remnant populations of New England cottontails. After only 2 breeding seasons, the observed heterozygosity in this reintroduced population surpassed that of the largest remnant population in New Hampshire (Londonderry [NH] population), and after 3 breeding seasons, allelic richness surpassed that of the Londonderry population as additional founder alleles were incorporated into the reintroduced population.

In another indication of the short-term success of this reintroduction, we documented dispersal from the release site to another high-quality shrubland patch 700 m away within the wildlife management area. This successful dispersal event and subsequent continued occupancy of the new patch exemplify the potential for this reintroduced population in the longer term to occupy a landscape in a metapopulation context through reproduction and dispersal. Bellamy River WMA is conducive to relatively long cottontail dispersal movements, with shrubby field–forest edges to act as corridors, and no major barriers (e.g., roads and development; Fenderson et al. 2014, Amaral et al. 2016). In addition, during the first year of the study, one female dispersed 2.4 km south to another property, but there were no rabbits to breed with present on patches surrounding the wildlife management area.

Intensive monitoring and parentage analyses allowed us to make observations about cottontail detection in winter pellet surveys and suggest considerations to improve detection in future monitoring efforts. Detection of New England cottontails varies with survey conditions such as number of days after a snowfall event, days with high wind before a survey, and snow depth (Brubaker et al. 2014). In this reintroduction, one collared founder that was known to be on the site was not detected in pellet surveys in 2 of the 4 years that individuals were released. We did not sample all contributing breeders in each year (i.e., in some years, unsampled individuals had the greatest parentage probability in COLONY analyses). This led to gaps in the pedigree in later years of the study; similar challenges occurred in fecal survey monitoring of a pygmy rabbit reintroduction (DeMay et al. 2017). Specifically, through parentage analyses, we identified 3 founders and 1 wild-born individual that were present at the time of pellet surveys, not detected, but identified breeding the summer after winter surveys. In addition, one wild-born individual that did not breed was not detected until its second winter, based on parents identified in the analysis. Similar detection results were documented for a New England cottontail reintroduction at Wells National Estuarine Research Reserve in Wells, Maine, where 1 of 7 radiocollared rabbits known to be on the site was not detected during 2 intensive winter pellet surveys (M. L. Bauer and A. I. Kovach, unpublished data). Decreased detection following heavy snow events could be due to subnivean behavior (Katzner and Parker 1997, Brubaker et al. 2014), decreased cottontail movement, or snow falling off branches and covering pellets following heavy storms (J. P. Tash, United States Fish and Wildlife Service, personal communication). This may explain some of the missed detections at Bellamy River WMA; however, surveys conducted at Wells Reserve did not follow heavy snow events, indicating that variation in individual cottontail movement ranges (smaller winter movement ranges for some females compared with males; M. L. Bauer, personal observation) in combination with factors such as deviation from 30-m transect spacing by surveyors may account for decreased detection. Conducting 2 independent surveys adhering to 30-m spacing between search transects

(Kristensen and Kovach 2018) and avoiding surveying after heavy snowfall events could increase detection and improve the ability to track founder survival and reproduction in cottontail reintroductions.

Reintroductions of other lagomorphs have focused on releasing a large number of individuals into a relatively large area. Due to high postrelease mortality, releasing large groups of individuals simultaneously may be necessary to ensure stable breeding populations following the acclimation period (Armstrong and Seddon 2008, Hamilton et al. 2010). For example, 100–800 pygmy rabbit individuals were released into a wildlife management area of 1,514 ha in each of 3 years (DeMay et al. 2017), in a much larger scale reintroduction effort than that for the New England cottontail. Yet, even at that scale, after 3 years, a self-sustaining population of pygmy rabbits had not yet been achieved, similar to our finding for this New England cottontail reintroduction. Continued supplemental releases were deemed necessary for the pygmy rabbit, but researchers also questioned whether continued releases at the same site might have negative consequences for reproduction and survival in the wild by contributing to reduced fecundity, habitat saturation, or increased predator responses (DeMay et al. 2017). Low annual survival, limited founder reproduction, and minimal population growth after the first year of release suggests a similar conundrum for New England cottontail reintroductions, leaving lingering questions about the optimal number of rabbits to release on the landscape. Exploring factors that can be manipulated to increase survival rates of reintroduced populations, such as release date, acclimation, supplemental feeding, and site quality, may be more cost-effective and potentially successful in the long term than a strategy of multiple releases.

Given the recurring challenges confronted across lagomorph reintroductions, more research is needed on the optimal number of New England cottontail individuals and releases necessary to combat postrelease mortality and establish a self-sustaining population, and decisions should take into consideration both mortality rates of released individuals and the density of cottontails in the reintroduction landscape. Presuming an average winter density of 2 cottontails/ha (Barbour and Litvaitis 1993, Kristensen and Kovach 2018), it is unlikely that the reintroduced population in the 20 ha of habitat in the Bellamy River WMA had reached carrying capacity because the winter population never rose above 12 individuals. Cottontail densities are variable in the wild (Kristensen and Kovach 2018), and the expected density of 2 cottontails/ha may not be typical of all habitat types or possibly not attainable for relatively small, isolated patches. Further research on cottontail densities in relation to habitat and patch characteristics is needed to establish realistic target densities for reintroductions. Further, it is unknown if success may have been greater with a single release or fewer larger releases, rather than the multiple releases – based on availability from the breeding program – of 42 individuals across 5 years; for example, it is unknown whether it would have minimized competition for occupied home ranges, stress to resident rabbits, or predator

responses that may occur over time with the multiple release strategy (Hamilton et al. 2010; DeMay et al. 2016, 2017). Given the small amount of available habitat in the landscape for New England cottontails, continued augmentation of reintroduced populations will likely be necessary, until sufficient habitat is established in the context of a larger landscape.

Winter population size remained small but relatively stable for the first 3 years of the reintroduction and was able to rebound after the decline in 2017. However, given the lack of expansion of this population across the years, without further monitoring and additional reintroductions, this population remains at high risk of decline as a result of stochastic events, skewed sex ratios, or inbreeding depression. Populations with such a low number of breeding individuals are extremely susceptible to stochastic decline and could be extirpated given a year with heavy storms, high predation, an absence of either males or females, or isolation of a male and female on different patches within a site preventing breeding. Following the 2017 decline, we observed effects of such stochasticity, resulting in a male-biased sex ratio, with 4 males and only 1 female in the population. This prompted the decision to release new individuals to augment the population; however, none of those founders produced offspring that season likely due to the timing of the release in August (one of the founders survived to reproduce in the following summer of 2018; M. L. Bauer and A. I. Kovach unpublished data). This led to elevated within-patch relatedness in winter 2018, as the one remaining wild-born female produced a litter of four offspring in summer 2017. For this isolated population, to buffer the potential impacts of stochastic population declines, continued monitoring and additional reintroductions are needed. Restoring habitat and releasing individuals into additional shrubland patches within dispersal distance could restore a functioning metapopulation to more sustainably buffer such stochasticity in the long term. Augmenting existing metapopulations with connectivity between patches to promote dispersal could also be a successful use of captive breeding resources and could lessen the challenges of reintroducing a population in isolation. For reintroductions to be effective and maintain increased population sizes and genetic diversity into the long term, a functioning metapopulation (i.e., multiple occupied patches within dispersal distance) is needed.

MANAGEMENT IMPLICATIONS

Trends identified by studying the first New England cottontail reintroduction at Bellamy River WMA can inform future management for successful cottontail reintroductions that produce high survival, breeding success, and dispersal in the short term, and a self-sustaining metapopulation in the long term. Key recommendations for a successful reintroduction of a small cottontail population include 1) repeated reintroductions of sufficient numbers of individuals to combat high postrelease mortality; 2) distributing released individuals spatially throughout the patch to minimize competition with established rabbits; and 3) annual monitoring to track population size, sex ratios, number of breeders, and genetic diversity. To aid these efforts, spatially explicit capture–recapture models in conjunction with intensive noninvasive genetic sampling (Kristensen and Kovach 2018) will increase detection and provide robust population estimates. We recommend use of these models in wild populations to provide a better understanding of cottontail carrying capacities on the landscape in relation to varying habitat; this will enable establishing realistic target densities for releases. Ultimately, reintroduction efforts should aim to restore a self-sustaining metapopulation that includes multiple occupied patches within dispersal distance (<2 km) in a landscape conducive to dispersal, conditions that historically supported New England cottontail populations and offset local patch extinctions. With a limited number of cottontails available for release from the captive breeding program and the importance of dispersal for maintaining cottontail populations, expanding existing metapopulations may be more successful than reestablishing populations in isolation. Focusing restoration efforts in existing metapopulations will have the important benefit of augmenting genetic diversity in addition to population size; evidence suggests this genetic rescue may be warranted in parts of the species' range (Fenderson et al. 2011, 2014; Cheeseman et al. 2019).

LITERATURE CITED

Amaral, K. E., M. Palace, K. M. O'Brien, L. E. Fenderson, and A. I. Kovach. 2016. Anthropogenic habitats facilitate dispersal of an early successional obligate: implications for restoration of an endangered ecosystem. PLoS ONE 11:e014884.

Ambrose, H. W. III. 1972. Effect of habitat familiarity and toe-clipping on rate of owl predation in *Microtus pennsylvanicus*. Journal of Mammalogy 53:909–912.

Armstrong, D. P., and P. J. Seddon. 2008. Directions in reintroduction biology. Trends in Ecology and Evolution 23:20–25.

Barbour, M. S., and J. A. Litvaitis. 1993. Niche dimensions of New England cottontails in relation to habitat patch size. Oecologia 95:321–327.

Bauer, M. L. 2018. Assessing the effects of habitat restoration on shrubland specialists: case study on the New England cottontail and shrubland birds. Thesis, University of New Hampshire, Durham, USA.

Berkman, L. K., M. J. Saltzgiver, E. J. Heist, C. K. Nielsen, C. L. Roy, and P. D. Scharine. 2009. Hybridization and polymorphic microsatellite markers for 2 lagomorph species (Genus *Sylvilagus*): implications for conservation. Conservation Genetics Resources 1:419–424.

Boland, K. M., and J. A. Litvaitis. 2008. The role of predation and hunting on eastern cottontail mortality at Cape Cod National Seashore, Massachusetts. Canadian Journal of Zoology 86:918–927.

Brenner, F. J., and D. P. Flemming. 1979. Behavioral determination of dominance in captive male cottontail rabbits. Proceedings of the Pennsylvania Academy of Science 53:139–142.

Brook, B. W., D. W. Tonkyn, J. J. O'Grady, and R. Frankham. 2002. Contribution of inbreeding to extinction risk in threatened species. Conservation Ecology 6:16.

Brown, A. L., and J. A. Litvaitis. 1995. Habitat features associated with predation of New England cottontails: what scale is appropriate? Canadian Journal of Zoology 73:1005–1011.

Brubaker, D. R., A. I. Kovach, M. J. Ducey, W. J. Jakubas, and K. M. O'Brien. 2014. Factors influencing detection in occupancy surveys of a threatened lagomorph. Wildlife Society Bulletin 38:513–523.

Calvete, C., R. Villafuerte, J. Lucientes, and J. J. Osacar. 1997. Effectiveness of traditional wild rabbit restocking in Spain. Journal of Zoology 241:271–277.

Chandler, R. B., E. Muths, B. H. Sigafus, C. R. Schwalbe, C. J. Jarchow, and B. R. Hossack. 2015. Spatial occupancy models for predicting metapopulation dynamics and viability following reintroduction. Journal of Applied Ecology 52:1325–1333.

Chapman, J. A., J. G. Hockman, and M. M. Ojeda C. 1980. *Sylvilagus floridanus*. Mammalian Species 136:1–8.

Cheeseman, A. E. 2017. Factors limiting recovery of the New England cottontail in New York. Dissertation, State University of New York, College of Environmental Science and Forestry, Syracuse, USA.

Cheeseman, A. E., J. B. Cohen, C. M. Whipps, A. I. Kovach, and S. J. Ryan. 2019. Hierarchical population structure of a rare lagomorph indicates recent fragmentation has disrupted metapopulation function. Conservation Genetics 20:1237–1249.

Clout, M. N., G. P. Elliott, and B. C. Robertson. 2002. Effects of supplementary feeding on the offspring sex ratio of kakapo: a dilemma for the conservation of a polygynous parrot. Biological Conservation 107:13–18.

Crawford, J. A., R. G. Anthony, J. T. Forbes, and G. A. Lorton. 2010. Survival and causes of mortality for pygmy rabbits (*Brachylagus idahoensis*) in Oregon and Nevada. Journal of Mammalogy 91:838–847.

Cullingham, C. I., and A. Moehrenschlager. 2013. Temporal analysis of genetic structure to assess population dynamics of reintroduced swift foxes. Conservation Biology 27:1389–1398.

De Barba, M., L. P. Waits, E. O. Garton, P. Genovesi, E. Randi, A. Mustoni, and C. Groff. 2010. The power of genetic monitoring for studying demography, ecology and genetics of a reintroduced brown bear population. Molecular Ecology 19:3938–3951.

DeMay, S. M., P. A. Becker, J. L. Rachlow, and L. P. Waits. 2017. Genetic monitoring of an endangered species recovery: demographic and genetic trends for reintroduced pygmy rabbits (*Brachylagus idahoensis*). Journal of Mammalogy 98:350–364.

DeMay, S. M., P. A. Becker, L. P. Waits, T. R. Johnson, and J. R. Rachlow. 2016. Consequences for conservation: population density and genetic effects on reproduction of an endangered lagomorph. Ecological Applications 26:784–795.

Ebenhard, T. 1987. High activity in bank voles in relation to colonization ability. Oikos 49:297–302.

Fenderson, L. E., A. I. Kovach, J. A. Litvaitis, and M. K. Litvaitis. 2011. Population genetic structure and history of fragmented remnant populations of the New England cottontail (*Sylvilagus transitionalis*). Conservation Genetics 12:943–958.

Fenderson, L. E., A. I. Kovach, J. A. Litvaitis, K. M. O'Brien, K. M. Boland, and W. J. Jakubas. 2014. A multiscale analysis of gene flow for the New England cottontail, an imperiled habitat specialist in a fragmented landscape. Ecology and Evolution 4:1853–1875.

Fischer, J., and D. B. Lindenmayer. 2000. An assessment of the published results of animal relocations. Biological Conservation 96:1–11.

Frantz, A. C., L. C. Pope, P. J. Carpenter, T. J. Roper, G. J. Wilson, R. J. Delahay, and T. Burke. 2003. Reliable microsatellite genotyping of the Eurasian badger (*Meles meles*) using faecal DNA. Molecular Ecology 12:1649–1661.

Fritts, S. H., C. M. Mack, D. W. Smith, K. M. Murphy, M. K. Phillips, M. D. Jimenez, E. E. Bangs, J. A. Fontaine, C. C. Niemeyer, W. G. Brewster, and T. J. Kaminski. 2001. Outcomes of hard and soft releases of reintroduced wolves to central Idaho and the greater Yellowstone area. Pages 125–147 in D. S. Maehr, R. F. Noss, and J. L. Larkin, editors.

Large mammal restoration: ecological and sociological challenges in the 21st century. Island Press, Washington, D.C., USA.

Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. Journal of Heredity 86:485–486.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). https://www2.unil.ch/popgen/softwares/fstat.htm. Accessed 13 Feb 2020.

Hamilton, L. P., P. A. Kelly, D. F. Williams, D. A. Kelt, and H. U. Wittmer. 2010. Factors associated with survival of reintroduced riparian brush rabbits in California. Biological Conservation 143:999–1007.

Jachowski, D. S., and J. M. Lockhart. 2009. Reintroducing the black-footed ferret *Mustela nigripes* to the Great Plains of North America. Small Carnivore Conservation 41:58–64.

Jones, O. R., and J. Wang. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10:551–555.

Kalinowski, S. T., A. P. Wagner, and M. L. Taper. 2006. ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. Molecular Ecology Notes 6:576–579.

Katzner, T. E., and K. L. Parker. 1997. Vegetative characteristics and size of home ranges used by pygmy rabbits (*Brachylagus idahoensis*) during winter. Journal of Mammalogy 78:1063–1072.

King, T. L., M. Eakles, A. Aunins, T. J. McGreevy, Jr., T. P. Husband, A. Tur, and A. I. Kovach. 2017. Microsatellite marker development from next generation sequencing in the New England cottontail (*Sylvilagus transitionalis*) and cross-amplification in the eastern cottontail (*S. floridanus*). BMC Research Notes 10:741.

Kovach, A. I., M. K. Litvaitis, and J. A. Litvaitis. 2003. Evaluation of fecal mtDNA analysis as a method to determine the geographic distribution of a rare lagomorph. Wildlife Society Bulletin 31:1061–1065.

Kristensen, T. V., and A. I. Kovach. 2018. Spatially explicit abundance estimation of a rare habitat specialist: implications for SECR study design. Ecosphere 9:e02217.

Letty, J., J. Aubineau, and S. Marchandeau. 2008. Improving rabbit restocking success: a review of field experiments in France. Pages 327–348 in P. C. Alves and K. Hackländer, editors. Lagomorph biology: evolution, ecology, and conservation. Springer-Verlag, Berlin, Germany.

Letty, J., S. Marchandeau, F. Reitz, J. Clobert, and F. Sarrazin. 2002. Survival and movements of translocated wild rabbits (*Oryctolagus cuniculus*). Game and Wildlife Science 19:1–23.

Litvaitis, J. A. 1993. Response of early successional vertebrates to historic changes in land use. Conservation Biology 7:866–873.

Litvaitis, J. A. 2003. Are pre-Columbian conditions relevant baselines for managed forest in the northeastern United States? Forest Ecology and Management 185:113–126.

Litvaitis, J. A., J. P. Tash, M. K. Litvaitis, M. N. Marchand, A. I. Kovach, and R. Innes. 2006. A range-wide survey to determine the current distribution of New England cottontails. Wildlife Society Bulletin 28:614–621.

Litvaitis, J. A., and R. Villafuerte. 1996. Factors affecting the persistence of New England cottontail metapopulations: the role of habitat management. Wildlife Society Bulletin 24:686–693.

McKinney, T. D. 1970. Behavioral interactions in grouped male cottontails. Journal of Mammalogy 51:402–403.

Metzgar, L. H. 1967. An experimental comparison of screech owl predation on resident and transient white-footed mice (*Peromyscus leucopus*). Journal of Mammalogy 48:387–391.

Murrow, J. M., J. D. Clark, and E. K. Delozier. 2009. Demographics of an experimentally released population of elk in Great Smoky Mountains National Park. Journal of Wildlife Management 73:1261–1268.

National Oceanic and Atmospheric Administration [NOAA]. 2010. U.S. Department of Commerce, Washington D.C., USA. https://www.ncdc.noaa.gov/data-access/land-based-station-data/land-based-datasets/quality-controlled-local-climatological-data-qclcd. Accessed 24 Jan 2020.

National Oceanic and Atmospheric Administration [NOAA]. 2020. U.S. Department of Commerce, Washington D.C., USA. https://www.ncdc.noaa.gov/cdo-web/search?datasetid=GHCND. Accessed 2 Feb 2020.

New England Cottontail Technical Committee. 2019. New England cottontail regional initiative 2018 performance report. New England Cottontail Technical Committee, Concord, New Hampshire, USA.

O'Donoghue, M., S. Boutin, C. J. Krebs, and E. J. Hofer. 1997. Numerical responses of coyotes and lynx to the snowshoe hare cycle. Oikos 80:150–162.

O'Grady, J. J., B. W. Brook, D. H. Reed, J. D. Ballou, D. W. Tonkyn, and R. Frankham. 2006. Realistic levels of inbreeding depression strongly affect extinction risk in wild populations. Biological Conservation 133:42–51.

Peakall, R., and P. E. Smouse. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539.

Pennell, M. W., and C. R. Miller. 2015. Package capwire. R package version 1.1.4. https://cran.r-project.org/web/packages/capwire/capwire.pdf. Accessed 17 Jan 2020.

Pompanon, F., A. Bonin, E. Bellemain, and P. Taberlet. 2005. Genotyping errors: causes, consequences, and solutions. Nature Reviews Genetics 6:847–856.

Price, A. J., W. Estes-Zumpf, and J. Rachlow. 2010. Survival of juvenile pygmy rabbits. Journal of Wildlife Management 74:43–47.

Schlossberg, S., and D. I. King. 2007. Ecology and management of scrub-shrub birds in New England: a comprehensive review. Report submitted to Natural Resources Conservation Service, Resource Inventory and Assessment Division, Beltsville, Maryland, USA.

Schwartz, M. K., G. Luikart, and R. S. Waples. 2007. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology and Evolution 22:25–33.

Seddon, P. J., D. P. Armstrong, and R. F. Maloney. 2007. Developing the science of reintroduction biology. Conservation Biology 21:303–312.

Sievert, P. R., and L. B. Keith. 1985. Survival of snowshoe hares at a geographic range boundary. Journal of Wildlife Management 49:854–866.

Sinclair, A. R. E., R. P. Pech, C. R. Dickman, D. Hik, P. Mahon, and A. E. Newsome. 1998. Predicting effects of predation on conservation of endangered prey. Conservation Biology 12:564–575.

Smith, S., P. McRae, and J. Hughes. 2009. Faecal DNA analysis enables genetic monitoring of the species recovery program for an arid-dwelling marsupial. Australian Journal of Zoology 57:139–148.

Snyder, R. L., W. Jenson, and C. D. Cheney. 1976. Environmental familiarity and activity: aspects of prey selection for a ferruginous hawk. Condor 78:138–139.

Stacey, P. B., and M. Taper. 1992. Environmental variation and the persistence of small populations. Ecological Applications 2:18–29.

Stoddart, L. C., R. E. Griffiths, and F. F. Knowlton. 2001. Coyote responses to changing jackrabbit abundance affect sheep predation. Journal of Range Management 54:15–20.

Tefft, B. C., and J. A. Chapman. 1987. Social behavior of the New England cottontail, *Sylvilagus transitionalis* (Bangs), with a review of social behavior in New World rabbits (Mammalia: Leporidae). Revue d'Écologie La Terre et La Vie 42:235–276.

Tella, J. L. 2001. Sex-ratio theory in conservation biology. Trends in Ecology and Evolution 16:76–77.

United States Fish and Wildlife Service [USFWS]. 2015. Endangered and threatened wildlife and plants; 12-month finding on a petition to list the New England cottontail as an endangered or threatened species. Federal Register 80:55286–55304.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538.

Vašíček, D., K. Vašíčková, V. Parkányi, and L.' Ondruška. 2011. Non-invasive PCR sexing of neonatal rabbits selected for islet cell culture. Slovak Journal of Animal Science 44:43–47.

Viggers, K. L., D. B. Lindenmayer, and D. M. Spratt. 1993. The importance of disease in reintroduction programmes. Wildlife Research 20:687–698.

Waits, L. P., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10:249–256.

Waits, L. P., and D. Paetkau. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. Journal of Wildlife Management 69:1419–1433.

Watland, A. M., E. M. Schauber, and A. Woolf. 2007. Translocation of swamp rabbits in southern Illinois. Southeastern Naturalist 6:259–270.

Woodruff, S. P., P. M. Lukacs, D. Christianson, and L. P. Waits. 2016. Estimating Sonoran pronghorn abundance and survival with fecal DNA and capture–recapture methods. Conservation Biology 30:1102–1111.

Associate Editor: Berkman.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Appendix A. Microsatellite primers and multiplex polymerase chain reaction conditions for the analysis of New England cottontail pellet and tissue samples.