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ASSESSING THE EFFECTS OF HABITAT RESTORATION ON SHRUBLAND SPECIALISTS: CASE STUDY ON THE NEW ENGLAND COTTONTAIL AND SHRUBLAND BIRDS

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ASSESSING THE EFFECTS OF HABITAT RESTORATION ON SHRUBLAND SPECIALISTS: CASE STUDY ON THE NEW ENGLAND COTTONTAIL AND SHRUBLAND BIRDS

BY

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THESIS

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ABSTRACT

ASSESSING THE EFFECTS OF HABITAT RESTORATION ON SHRUBLAND SPECIALISTS: CASE STUDY ON THE NEW ENGLAND COTTONTAIL AND SHRUBLAND BIRDS

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Loss and fragmentation of shrubland habitat in the northeastern United States due to succession, suppression of natural disturbance regimes, and development (Cronon 1983, Litvaitis 1993) have resulted in declines of populations of shrubland specialist species, including the New England cottontail (*Sylvilagus transitionalis*) and shrubland birds (Litvaitis et al. 2006, Schlossberg & King 2007). The New England cottontail's range has declined by over 86% (Litvaitis et al. 2006, Fenderson et al. 2014, Brubaker et al. 2014) and remaining populations are small and exhibit fine-scale structure, limited dispersal, and loss of metapopulation function (Fenderson 2011, 2014, Cheeseman 2017, Chapter 1). In the Northeast, declining species of shrubland birds outnumber increasing species three to one (Schlossberg & King 2007). In response to these losses, active management is ongoing to maintain and create shrubland habitat on the landscape to restore populations of specialists dependent on this habitat type. Given the extensive investments and collaboration focused on restoring shrubland habitats in New England, research is needed to monitor the effects of habitat creation on populations of shrubland specialists. Understanding how shrubland specialists are responding to ongoing habitat creation will inform additional restoration strategies in an adaptive management context, an iterative

process of incorporating new information into management practices and learning from previous management outcomes.

Shrubland habitat creation and management in New England is largely focused on restoring habitat for the New England cottontail, with the idea that other species will also benefit. The New England cottontail is a highly threatened shrubland obligate requiring multiple patches of shrubland habitat within a short dispersal distance to support viable metapopulations long-term. Much of this management has been initiated by the New England Cottontail Conservation Initiative in response to the cottontail's nine-year candidate listing status under the Endangered Species Act, and the National Fish and Wildlife Foundation Keystone Initiative. Conservation efforts implemented for the New England cottontail have included the development of a range-wide inter-agency conservation strategy, designation of focal habitat restoration areas and habitat acreage and cottontail population recovery goals, engagement with private organizations and landowners to create shrubland habitat, implementation of a range-wide cottontail occupancy monitoring protocol, and public outreach and education (Fuller & Tur 2012). Habitat management underway incorporates techniques to set back forest succession, including harvesting trees, cutting and mowing to promote shrub regrowth, planting to improve old field habitat, and controlled burning on fire-maintained habitats like pitch pine-scrub oak. Landscape-level conservation design has been initiated to identify best parcels in terms of vegetation type and patch size that can be targeted for restoration (Tash & Litvaitis 2007, Fuller et al. 2011).

Knowing how other species respond to widespread habitat management for cottontails is important to understand the full impacts and benefits of currently implemented management and to inform future management strategies. Given the resources invested in restoring shrubland habitat for cottontail populations, and the potential for other shrubland specialist species to benefit from this restoration, designating the New England cottontail a representative species could benefit strategic management for multiple species by the United States Fish and Wildlife Service. Representative species designations are a tool for strategic management to provide the greatest benefit for the most species with available resources. Research is needed to identify which additional species would benefit most from restoration to create habitat for cottontails. I investigated shrubland specialist birds as a suite of species that could benefit from habitat management for cottontails, given declining population trends for shrubland birds and their high conservation need in the Northeast.

In this thesis, I investigated the effects of habitat restoration on shrubland specialists in three case studies focused on New England cottontails and shrubland birds.

In Chapter 1, I used genetic tools to assess the population structure, genetic diversity, effective population size, and census population size of an isolated New England cottontail population in an urban landscape in Londonderry, New Hampshire. I documented attributes of small populations that pose conservation challenges, including limited dispersal and loss of metapopulation function (Chandler et al. 2015), low genetic diversity, high relatedness (Brook et al. 2002, O'Grady et al. 2006), skewed sex ratios (Tella 2001, Clout et al. 2002), and stochastic decline on isolated patches (Stacey & Taper 1992). I used a resistance surface approach to highlight areas of potential connectivity in the landscape, including powerlines, a shrub wetland, and rail corridor. I parameterized a spatially explicit individual-based simulation model that serves as a proof-of-concept for future work to compare the outcomes of alternate restoration scenarios on cottontail population size, genetic diversity, connectivity, and ability to persist in fragmented landscapes given best-case restoration scenarios.

In Chapter 2, I tracked the success of the first reintroduction effort for New England cottontails which has been ongoing at Bellamy River Wildlife Management Area since 2013. Using genetic analysis of fecal pellets collected in intensive winter surveys, I monitored survival and reproduction of founder cottontails and quantified changes in population size and genetic diversity following releases. Results indicate that reintroductions of New England cottontails can be successful. I documented successful breeding by both founders and wild-born cottontails, with some individuals reproducing and surviving over multiple years. Genetic diversity increased with the addition of breeding founders. However, I also found high post-release mortality following the first year of the reintroduction and variable survival that may be related to stochastic events, heavy snowfall, predator response, or competition for territory with established individuals. A population decline and skewed sex ratio, four years post-reintroduction, highlights the vulnerability of small reintroduced populations to stochastic decline. Key recommendations for a successful reintroduction of a small cottontail population include: **1)** importantly, restoring a functional metapopulation that includes multiple occupied patches within dispersal distance and shrubland corridors connecting patches; **2)** annual monitoring to track population size, sex ratios, number of breeders, and genetic diversity; and **3)** repeated reintroductions over time and reintroducing large numbers of individuals, distributed spatially to avoid exceeding carrying capacity and to combat high post-release mortality (Armstrong & Seddon 2008, Hamilton et al. 2010). Additional research is needed to improve our understanding of the factors influencing founder survival and how best to supplement reintroductions after the initial release to prevent collapse of a small population.

In Chapter 3, I conducted point counts and vegetation surveys and modeled shrubland bird occupancy to determine habitat and patch-level influences on shrubland bird presence at

sites occupied by or managed for New England cottontails. Of the five species of shrubland birds for which I modeled occupancy in relation to habitat covariates, Yellow Warbler and Prairie Warbler showed higher occupancy in microhabitat conditions suitable for New England cottontails. Yellow Warblers occupied wet sites with high stem densities and Prairie Warblers occupied sites with dense vegetation between 2-3 m. I also conducted indicator species analyses to identify shrubland bird specialists detected with high frequency at sites occupied by New England cottontails within the past five years and in microhabitat conditions associated with New England cottontail occupancy. Indicator species analyses identified 11 shrubland birds detected with high frequency in microhabitat conditions suitable for New England cottontail occupancy. In addition to Yellow Warbler and Prairie Warbler, which were associated with habitat covariates pertinent to New England cottontails in occupancy models, indicator species analyses identified Brown Thrasher, Field Sparrow, Blue-winged Warbler, Alder Flycatcher, Gray Catbird, Song Sparrow, Indigo Bunting, American Goldfinch, and Black-and-white Warbler as species sharing certain specific habitat requirements of New England cottontails or frequently detected on sites occupied by cottontails. Designating the New England cottontail a representative species would be an effective conservation strategy. Multiple shrubland specialist birds listed as Species of Greatest Conservation Need are associated with microhabitat conditions suitable for New England cottontail occupancy, and a suite of shrubland birds that use more open shrub habitats would benefit from habitat management before sites become suitable for cottontails.

INTRODUCTION

History of shrubland habitats in the Northeast

Changes in land use patterns have dramatic impacts on the amount and distribution of habitat types on the landscape. In the Northeast, landscape changes over the past several centuries have been complex, with concomitant consequences for wildlife species. Prior to colonial settlement, the landscape was a complex patchwork of multiple-aged forests, thickets, and wetlands (Cronon 1983). Native American agriculture and natural disturbances from fire, wind, flooding, and beavers maintained early successional habitat (DeGraff & Yamasaki 2003). The arrival of colonists imposed a more regular pattern on this diverse patchwork of ecological communities. Colonial deforestation began in the early seventeenth century and peaked by the mid-nineteenth century, by which time over 75% of the arable land had been cleared (Whitney 1994, Foster et al. 2002, Hall et al. 2002). Additionally, beaver populations had severely declined with the fur trade, reducing ephemeral wetlands and shrublands as a result (Cronon 1983).

In the mid-nineteenth century widespread farm abandonment in the Northeast, associated with competition with more productive agriculture in the Midwest (Black 1950, Irland 1982), resulted in an increase in early successional habitat. Shrubland habitats and abundances of species dependent on these habitats peaked in the early twentieth century (Litvaitis 1993, Foster 2002). However, due to succession, development, and suppression of natural disturbance regimes (Cronon 1983, Litvaitis 1993) shrubland habitats began declining in the mid and late 1900s, with marked declines in populations of shrubland species such as the New England cottontail noted during that period (Jackson 1973, Brooks & Birch 1988, Litvaitis et al. 1999). By the early 2000s over 80% of the combined land area of New Hampshire, Vermont, and Maine was dominated by second growth forests (Brooks 2003).

Today, natural scrub-shrub habitats persist on the landscape in poor or hydric soils, including pitch pine-scrub oak (Little & Garrett 1990), shrub wetlands (Cowardin et al. 1979), and coastal scrub-shrub, and due to natural disturbances including those from beavers and wind and ice storms (Askins 2000, Lorimer & White 2003, DeGraaf & Yamasaki 2003, Schlossberg & King 2007). Today, anthropogenic scrub-shrub habitats comprise a large portion of the early successional habitat in the Northeast, and include habitats resulting from silviculture, utility rights-of-way, succession of abandoned fields, and forest edges (Thompson & DeGraaf 2001, DeGraaf & Yamasaki 2003, Schlossberg & King 2007). Shrublands and young forests have a large variety of fruiting shrubs and herbaceous vegetation in comparison to mature forests (MacArthur & MacArthur 1961, Conner et al. 1983, Rice et al. 1984, Schulte & Niemi 1998, Keller et al. 2003), have high structural diversity, and provide protective cover (Gilbart 2012). Due to the ephemeral nature of these habitats, active management is essential for the persistence of shrublands and young forests on the landscape to maintain populations of specialist species (Litvaitis 1993, DeGraaf & Yamasaki 2003, Schlossberg & King 2007), as well as generalists and forest species that utilize these productive habitats during part or all of their life cycle (Anders et al. 1998, Vega Rivera et al. 1998, Askins 2001, Litvaitis 2001, Vitz & Rodewald 2006, 2007). There are extensive conservation efforts ongoing in the Northeast to create and maintain habitat for shrubland species.

Shrubland specialist species

Populations of a variety of taxa that rely on shrublands have been in decline in recent decades including birds (Dettmers 2003, Schlossberg & King 2007), mammals (Litvaitis 2001, Fuller 2003), pollinators (Wagner & Nelson 2003), reptiles (Kjoss & Litvaitis 2000), and plants (Latham 2003). There are 52s species of birds, mammals, and reptiles listed as Species of Greatest Conservation Need in the Northeast and additional species of rare invertebrates and pollinators that that rely on shrubland or young forest habitats for cover, nesting, and forage during part or all of their life cycle (Gilbart 2012). Some such species include the Blue-winged Warbler (*Vermivora cyanoptera*), American Woodcock (*Scolopax minor*), New England cottontail (*Sylvilagus transitionalis*), woodland jumping mouse (*Napaeozapus insignis*), frosted elfin butterfly (*Callophrys irus*), wood turtle (*Glyptemys insculpta*), spotted turtle (*Clemmys guttata*), northern black racer (*Coluber constrictor constrictor*), and timber rattlesnake (*Crotalus horridus*) (Gilbart 2012).

A focal species for shrubland restoration in the Northeast is the New England cottontail. The New England cottontail is a shrubland obligate that requires dense thicket habitat for forage, thermoregulation, and cover from predators, both within its home range (Barbour & Litvaitis 1993, Litvaitis 2003) and during dispersal (Fenderson et al. 2014, Amaral et al. 2016). New England cottontails have experienced extensive range contraction of >86% due to loss and fragmentation of shrubland habitat (Litvaitis et al. 2006, Fenderson et al. 2014, Brubaker et al. 2014, Fig. 2.1). Today, New England cottontails are isolated into five geographically (Litvaitis et al. 2006) and genetically (Fenderson et al. 2011) distinct regional populations. Further subdivisions occur within each of these geographic areas, resulting in small, local metapopulations, in which extinctions and recolonizations occur independently from each other due to extremely limited dispersal in fragmented landscapes (Fenderson 2011, 2014, Cheeseman 2017; B. Ferry, H. Holman, A. Kovach unpublished data). Research is needed on the response of cottontail populations to ongoing management to effectively implement habitat restoration and other management actions such as reintroductions. Understanding whether cottontail populations are growing or declining, identifying dispersal distances and patterns in managed landscapes, and tracking stochastic changes in cottontail abundance, relatedness, and sex ratios on isolated patches provides instrumental knowledge on where habitat connectivity can be improved or augmenting populations would be valuable.

Though a majority of shrubland habitat restoration projects in the Northeast are focused on the New England cottontail, the goal is that other species will also benefit. In addition to the New England cottontail, species of particular conservation concern that could benefit from this restoration are shrubland specialist birds. There are 41 species of birds that regularly breed in shrubland habitats in New England (Schlossberg & King 2007). Of those species, 21 have shown short or long-term declines (Askins 1993, Brawn et al. 2001, Dettmers 2003, Schlossberg & King 2007) and 12 additional species are of conservation concern locally or nationally, leaving only eight shrubland specialist bird species that are not declining or of conservation concern (Schlossberg & King 2007). Habitat use by shrubland specialist birds varies by species (DeGraaf & Yamasaki 2001, Schlossberg & King 2007). There are multiple management strategies for maintaining and creating shrubland habitat for birds (Askins 2001, Lorimer 2001, Thompson & DeGraaf 2001, DeGraaf & Yamasaki 2003), and no one strategy can accommodate all the region's specialist birds (Schlossberg & King 2007). Given the efforts in place to create the specific shrubland habitat conditions required by New England cottontails, research is needed on how other shrubland specialists will respond to this management. Certain species of shrubland birds are likely to benefit more than others from the dense, tall shrublands required by New England cottontails. Other species will benefit from more open shrublands, and species with particular conservation needs such as game birds or shrubland birds wintering in habitat in the Neotropics may require additional management outside the umbrella of cottontail habitat restoration. One goal of this study was to determine if habitat management focused on the specific requirements of New England cottontails will also benefit shrubland specialist birds, and which bird species will benefit most from that specific habitat restoration. Management that benefits multiple species is an efficient use of limited conservation resources.

Shrubland habitat restoration in New England

Extensive resources and collaboration between federal, state, and private organizations and landowners have been invested in creating and restoring shrubland and young forest habitats in the Northeast (Fuller & Tur 2012, NFWF Early Successional Forest Keystone Initiative Report 2015, Fuller et al. 2016). These shrubland restoration projects are focused primarily on restoring habitat for the New England cottontail (Fuller et al. 2016, NFWF Early Successional Forest Keystone Initiative Report 2015) and also for shrubland birds with a focus on the American Woodcock and Golden-winged Warbler (NFWF Early Successional Forest Keystone Initiative Report 2015). The New England Cottontail Conservation Initiative is pursuing a habitat restoration goal of 27,000 acres of shrubland and young forest. Nearly 18,700 acres were maintained or restored as of 2017, including 10,000 acres of self-sustaining habitat (New England Cottontail Executive Committee 2018). The focus of this thesis is to understand how both New England cottontails and shrubland specialist birds are responding to management to inform restoration that will generate New England cottontail responses at the population level and benefit shrubland specialist birds.

Research objectives

The objectives of this research were to investigate the effects of habitat restoration on shrubland specialist species in three case studies, with a focus on the New England cottontail and shrubland specialist birds.

The research objectives of this thesis, by chapter, were:

1. Quantify the genetic structure and diversity of an isolated New England cottontail population in an urban landscape where management was implemented to restore young forest. Parameterize a spatially-explicit model to compare the effects of alternate restoration scenarios on cottontail population size, persistence, genetic diversity, and connectivity; demonstrate this model as a proof-of-concept for applications to range-wide cottontail conservation scenarios.
2. Track the survival and breeding contributions of founder rabbits and the population growth of the first reintroduced New England cottontail population to identify trends and inform future reintroductions.
3. Evaluate the benefit of habitat management for the New England cottontail for multiple shrubland specialist species in a representative species context. Identify habitat influences on shrubland bird occupancy at sites occupied by or managed for New England cottontails. Identify shrubland bird specialists that are indicative of the specific habitats required by New England cottontails.

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CHAPTER 1

GENETIC STRUCTURE, CONNECTIVITY, AND PREDICTED RESPONSE TO RESTORATION OF AN ISOLATED NEW ENGLAND COTTONTAIL POPULATION IN AN URBAN LANDSCAPE

Abstract

Habitat loss and fragmentation from land use change reduce connectivity and dispersal of wildlife populations. Isolated populations are at greater risk of extinction due to small population sizes and decreased genetic diversity. These issues are germane to the conservation of the New England cottontail (*Sylvilagus transitionalis*), which has experienced extensive loss of population connectivity due to loss and fragmentation of shrubland habitat. New England cottontails in the highly developed landscape of Londonderry, New Hampshire were sampled for genetic monitoring with intensive winter fecal pellet surveys from 2016-2018 and live-trapping from 2015-2017. I used STRUCTURE to identify the number of distinct genetic groups in this population and tracked genetic diversity, relatedness, and population size on occupied patches across years. In this small population, I identified fine-scale population structure, low genetic diversity, high relatedness, and limited dispersal. I identified three distinct genetic groups in this population over a small extent of only 4 km of occupied habitat. Cottontails on a central patch that was restored as young forest 10 years ago showed the highest genetic diversity, lowest relatedness, and had the highest abundance until 2018 when abundance declined on that patch because the habitat had undergone succession to the point that it no longer provided ideal cover. Cottontails on isolated patches exhibited low genetic diversity and high relatedness. Hybridization between New England cottontails and an eastern cottontail was detected on an

isolated patch that was declining in abundance as eastern cottontails were expanding their range into Londonderry. I used a resistance surface approach in CIRCUITSCAPE to map and highlight areas of connectivity and barriers between groups of cottontails in the Londonderry landscape. Connectivity maps corroborated genetic data, indicating isolated patches on the landscape. Utility rights-of-ways, a shrub wetland, and rail corridor were highlighted as areas of potential connectivity. With an understanding of connectivity in this landscape, I developed a model to simulate the effects of restoration scenarios on cottontail population connectivity and persistence. I parameterized a spatially explicit individual-based model in CDPOP that incorporated the resistance surface landscape to simulate changes in cottontail population size, persistence, genetic diversity, and connectivity through 70 generations. This predictive framework will be used to evaluate the outcomes of planned restoration activities on cottontail populations to identify scenarios that will best restore metapopulation connectivity. The parameterized simulation model effectively projected cottontail population size, persistence, and genetic diversity given inputs based on the best available knowledge of New England cottontail vital rates. Sensitivity analyses identified dispersal as a key factor for New England cottontail population persistence in fragmented landscapes. High recruitment increased population stability, and high adult mortality within the range of mortality observed in empirical populations caused populations to crash. High adult mortality in consecutive years, for example due to a harsh winter, could be a concern for population persistence on isolated patches. Simulated restoration of multiple habitat patches in the Londonderry landscape increased population stability. This predictive model serves as a proof-of-concept for comparing restoration scenarios in managed cottontail populations range-wide. Predictive models are a potentially valuable tool to evaluate the effectiveness of alternate management strategies and determine whether small

cottontail populations in fragmented landscapes can be self-sustaining given best-case restoration scenarios.

Key Words:

New England cottontail, habitat fragmentation, landscape genetics, CDPOP, simulations

Introduction

Habitat loss and fragmentation influence biodiversity through altering multiple processes, including reproduction, mortality, dispersal, species interactions, and ecosystem functions (Addicott et al. 1987, Haddad et al. 2015). Fragmented populations persisting on small, isolated patches are at risk of extinction due to demographic processes including small effective population sizes and decreased mate choice (Bohonak 1999, Keyghobadi 2007); genetic processes such as reduced influx of new genetic variation (Bohonak 1999, Keyghobadi 2007), genetic drift (Hanski & Gilpin 1997, Keyghobadi 2007, Allendorf et al. 2012), inbreeding depression (Brook et al. 2002, O'Grady et al. 2006), and decreased adaptive potential (Lacy 1997); and stochastic processes including weather events, fire, and flooding (Stacey & Taper 1992).

Habitat loss and fragmentation have particularly detrimental impacts on species that are structured as metapopulations (Hanski & Gilpin 1997), such as the New England cottontail (*Sylvilagus transitionalis*). The New England cottontail is a shrubland obligate that has experienced extensive contraction over greater than 86% of its historical range (ca. 1960) (Litvaitis et al. 2006, Fenderson et al. 2014, Brubaker et al. 2014) due to habitat loss from widespread development, succession, and suppression of natural disturbance regimes that maintain early successional habitat (Litvaitis 1993, Litvaitis 2003, Schlossberg & King 2007). Today, New England cottontails persist in small, local metapopulations, in which extinctions and recolonizations occur independently from each other (Fenderson 2011, 2014, Cheeseman 2017), and dispersal is extremely limited (Cheeseman 2017, B. Ferry, unpublished data).

Classical metapopulations, disjunct but interacting populations prone to local extinction and recolonization, persist regionally when individuals dispersing from their natal patches create an extinction-recolonization balance (Levins 1969, Hanski & Gilpin 1997). By disrupting landscape connectivity, habitat loss and fragmentation create nonequilibrium metapopulations in which recolonizations are infrequent or absent. Disruption of landscape connectivity also creates source-sink metapopulation dynamics, whereby low dispersal rates between patches result in negative population growth in sink populations and may ultimately affect the persistence of these spatially structured populations (Hanski & Gilpin 1997). Dispersal is essential for rescuing sink populations, establishing new populations in unoccupied patches, and sustaining genetic diversity (Lande 1988, Gulve 1994, Stevens et al. 2006, Allendorf et al. 2012).

Despite its importance in maintaining metapopulations, dispersal is a difficult and understudied component of connectivity research and is a priority research area for conservation management (Lindenmayer et al. 2008, Jaquiéry et al. 2011). Genetic data provide a means to understand dispersal to enact effective restoration. The interdisciplinary field of landscape genetics provides tools to quantify successful dispersal as a measure of gene flow and uses spatial statistics to relate gene flow to specific features in complex, heterogeneous landscapes (Manel et al. 2003). Habitat of varying quality and the landscape matrix between habitat patches influence the ability of individuals to move and disperse through the landscape. Landscape genetics aims to understand the functional connectivity of populations, considering the effects of a variably permeable matrix on the dispersal or movement abilities of organisms (Taylor et al. 1993, With et al. 1997). One method to evaluate functional connectivity is to relate gene flow to specific landscape features in resistance surfaces built in a raster Geographic Information System (GIS) environment. Resistance surfaces are spatial layers that assign a cost value (representing landscape resistance to dispersal) to each raster cell based on the extent to which the underlying landscape feature is hypothesized to facilitate or impede gene flow in a species of interest (Spear et al. 2010). The effective distance between sampling sites or individuals can then be calculated as a cost distance through the resistance surface as opposed to Euclidean distance.

Landscape genetic studies have been conducted to identify landscape influences on dispersal in New England cottontail populations in southern Maine, seacoast New Hampshire, and Massachusetts on Cape Cod (Fenderson et al. 2014, Papanastassiou 2015, Amaral et al. 2016). Barrier features such as highways and large waterbodies limit dispersal and separate populations in southern Maine and seacoast New Hampshire (Fenderson et al. 2014). Linear shrubland habitat along roadsides, railroad beds, and utility corridors facilitates gene flow among patches in those populations (Fenderson et al. 2014, Amaral et al. 2016). Roads act as both barriers and facilitators (Fenderson et al. 2014, Amaral et al. 2016), due to the shrubby nature of roadsides and the risk that roads themselves pose in terms of vehicle collisions and lack of cover. Corridor analyses have emphasized the importance of linear anthropogenic features such as roadsides and utility rights-of-ways for restoring connectivity between focal sites (Amaral et al. 2016). The relative influence of barrier and facilitating features on gene flow varies among populations in relation to landscape composition (Fenderson et al. 2014, Amaral et al. 2016) and along a fragmentation gradient (Amaral et al. 2016). Previous research emphasizes the need to consider not only the amount of habitat created or restored, but the configuration of habitat patches to promote gene flow in restoration landscapes (Fenderson et al. 2014).

Simulation modeling is an additional advancement in the field of landscape genetics that enhances the power to investigate patterns of landscape influence on gene flow under a controlled, replicated design (Epperson et al. 2010, Manel & Holderegger 2013, Balkenhol et al. 2016). Simulations are a valuable tool for predicting population responses to restoration and landscape change (Wasserman et al. 2012, Van Strein et al. 2014, Hoban 2014, Cushman et al. 2015, Balkenhol et al. 2016). Landscape genetic simulations offer a means to predict how New England cottontail populations will respond to habitat creation given the extensive restoration efforts in place, and can be used to compare the effects of alternate restored landscapes on cottontail population connectivity, persistence, and genetic diversity.

The objectives of this study were to **1)** quantify the population genetic structure, genetic diversity, effective population size, and estimated census population size of an isolated New England cottontail population in an urban landscape, **2)** use a resistance surface approach to map areas of potential connectivity to planned habitat management projects surrounding a fragmented cottontail population, and **3)** parameterize a spatially explicit individual-based model for future use in comparing the effects of alternate restoration scenarios on cottontail population size, persistence, genetic diversity, and connectivity.

Methods

Study area

The Londonderry, New Hampshire population of New England cottontails is the largest remnant metapopulation of New England cottontails in New Hampshire. This population occupies shrubland habitat in a highly industrial landscape just south of the Manchester-Boston Regional Airport and east of the Merrimack River. Over 50% of the landscape is forested, and over 30% of the landscape is developed, including the airport, urban areas extending south of Manchester, industrial parks, and quarries. A 20-acre restoration site managed for New England cottontails on Stonyfield Yogurt property represents a stronghold for the species in this part of New Hampshire (New England Cottontail Conservation Initiative 2013). New England cottontails occupy additional shrubland patches within this complex industrial landscape matrix, and all occupied patches are within a 4 km geographic extent (Fig 1.1).

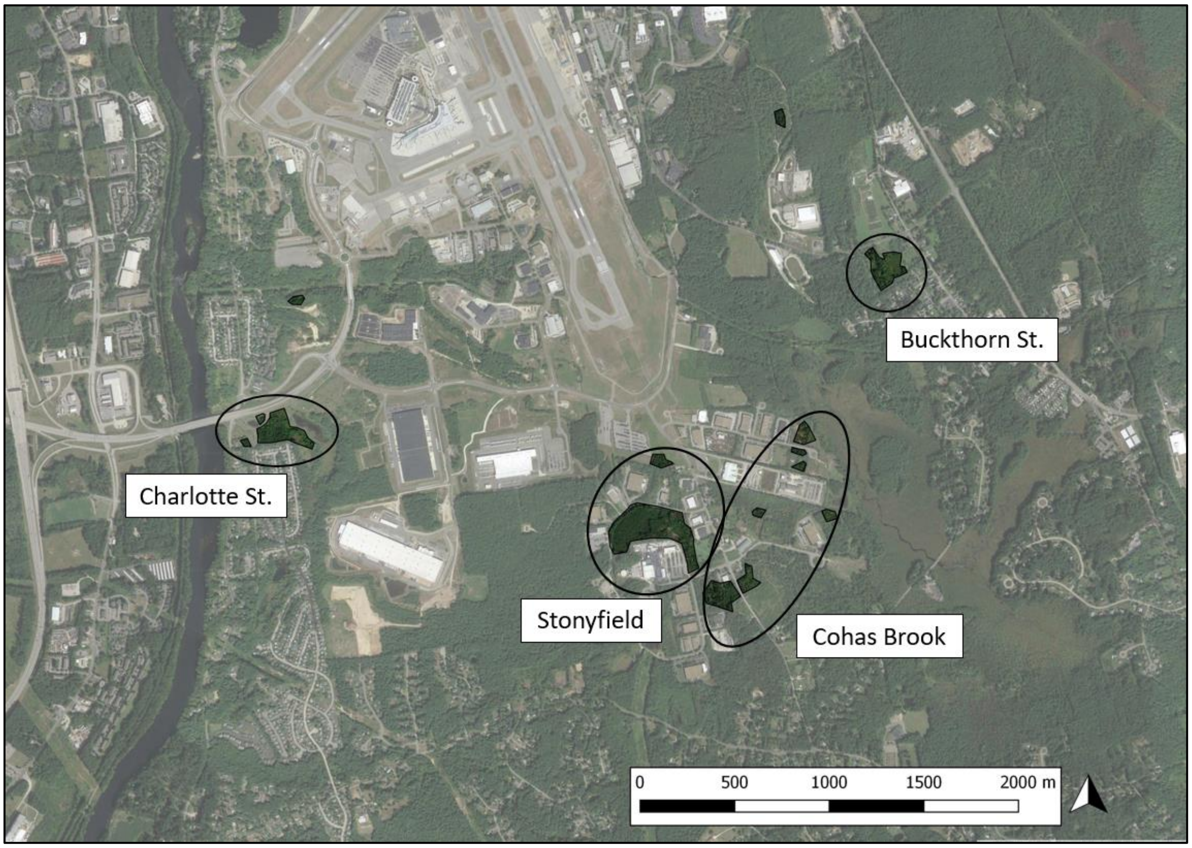


Fig. 1.1 The Londonderry, New Hampshire landscape consisting of a high proportion of forested and developed land, including industrial facilities just south of the Manchester-Boston Regional Airport. Highlighted patches indicate shrubland habitat occupied by New England cottontails from 2015-2017.

Sampling and genetic data

Spatially referenced (Garmin GPSMAP 64s, Olathe, KS) fecal samples were collected during winter pellet surveys conducted from 2016 through 2018 as part of an effort to monitor New England cottontail occupancy and abundance. Surveys were focused on known occupied cottontail patches, and additional shrubland habitat in the surrounding area. Pellet samples were collected under optimal survey conditions to detect New England cottontails (Brubaker et al. 2014) in a fine-scale sampling scheme, following the methods of Kristensen & Kovach (2018). Spatially referenced tissue samples were collected from cottontails captured in single-door box traps (Barbour & Litvaitis 1993) from 2015 through 2017 as part of a study to track individual dispersal and survival to assess population viability (B. Ferry, unpublished data). Methods of handling cottontails were approved by the Institutional Animal Care and Use Committee, IACUC #160609 (Appendix B), and consistent with standards maintained by New Hampshire Fish and Game. Fecal pellets were stored in 15-mL conical tubes at -20 ºC and tissue samples were stored in 100% ethanol until DNA extraction.

I 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, Valencia, CA, USA). DNA was amplified in three multiplex polymerase chain reactions (PCR) (see Appendix A 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 locus developed for sex identification in the European rabbit (Vašíček et al. 2011). PCR products were electrophoresed on a 3730*xl* 96-

capillary DNA Analyzer at the Yale DNA Analysis Facility (New Haven, CT, USA). Alleles were manually scored in PeakScanner (Applied Biosystems, Foster City, CA, USA).

To increase amplification success rates, I 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. For quality control of low copy DNA, I used a multiple tubes approach (Frantz et al. 2003, Waits & Paetkau 2005). I required two replicate allele observations for heterozygous loci, and three replicate observations for homozygous loci to determine a consensus genotype (Frantz et al. 2003). I quantified the per allele and per locus genotyping error by comparing the genotypes of all replicates to the consensus genotype (Pompanon et al. 2005). Samples missing data at three or more loci were excluded from analyses. To check for null alleles, I used MICRO-CHECKER (Van Oosterhout et al. 2004). To identify samples collected from the same or unique individuals, I used the multi-locus matches option in GenAlEx 6.5 (Peakall & Smouse 2006, 2012). I re-evaluated samples differing at only one to two loci and considered samples with mismatches that appeared to be due to allelic dropout the same individual. I calculated the probability of identity of siblings ($P_{ID\text{-}SIBs}$), 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.

Population genetic analyses and spatial autocorrelation

To identify the number of genetically distinct groups of cottontail individuals in Londonderry, I used a Bayesian approach in the program STRUCTURE 2.3.4 (Pritchard et al. 2000). I ran STRUCTURE 10 times at each K (the number of putative genetic groups), from

K=1-5, with a burn-in of 100,000 iterations and run-length of 500,000 iterations. I used the admixture model with correlated allele frequencies, which is appropriate for populations that exchange migrants (Pritchard et al. 2000). The best supported number of groups (K) was identified by the plateau of the average LnPD (Pritchard et al. 2000), the ΔK method (Evanno et al. 2005), and the evaluation of bar plots. Results were compiled with Structure Harvester 0.6.94 (Earl & vonHoldt 2012), averaged in CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007), and visualized in DISTRUCT 1.1 (Rosenberg 2004).

To compare genetic diversity among geographically segregated cottontail groups, I calculated heterozygosity metrics and number of alleles in GenAlEx 6.5 (Peakall & Smouse 2006, 2012). I calculated allelic richness corrected for sample size in FSTAT 2.9.3.2 (Goudet 1999, 2002). I estimated average pairwise relatedness by patch and for the overall population in ML-Relate (Kalinowski et al. 2006). I estimated census population size from 2016-2018 (years with sufficient recapture data) using a single session mark-recapture method in the R package *capwire* 1.1.4 (Pennell & Miller 2015) for the Londonderry population as a whole and for each geographically distinct patch. I estimated effective population size, the number of breeding individuals in a population, in NeEstimator 2.01 (Do et al. 2014) using the linkage disequilibrium method, with data from 2015-2017 combined.

To assess dispersal patterns and fine-scale genetic structure, I calculated spatial autocorrelation in GenAlEx. This technique compares genetic similarity of individuals as a function of geographic distance to identify the spatial extent over which individuals are highly related (Legendre 1993, Epperson 1993, Sokal et al. 1997). I assessed spatial autocorrelation for the population as a whole and for males and females separately. I used variable distance classes to maintain a sufficient number of pairwise comparisons in each distance class. Analyses were run with 9,999 permutations and 9,999 bootstraps to test for significance.

Landscape variables and univariate resistance surface modeling

I used a resistance surface approach to optimize cost values that different landscape features pose to cottontail movement in the Londonderry, New Hampshire landscape. This was a first step in developing connectivity maps for the Londonderry cottontail population. I mapped landscape variables identified as important to New England cottontail dispersal in previous landscape genetic studies (Fenderson et al. 2014, Amaral et al. 2016; Table 1.1). Landscape variables were mapped in ArcGIS 10.5 (Esri, Redlands, CA). To build a more comprehensive shrub and shrub wetland layer, data was combined from a 2011 National Land Cover Database (NLCD) layer (Homer et al. 2015) and 2012 LANDFIRE layer (LANDFIRE 2012) at 30 m resolution (EPSG 102003). Additional land cover variables (Table 1.1) were derived from LANDFIRE data layers, roads were derived from a New Hampshire Department of Transportation shapefile (NHDOT Bureau of Planning and Community Assistance 2018), rails from a U.S. rails TIGER/Line shapefile (U.S. Census Bureau 2015), and powerline rights-of-ways from a New England electrical transmission shapefile (NOAA 2013). In cases where land cover was misclassified in the immediate vicinity of patches occupied by cottontails (i.e. part of a shrubland patch occupied by a cottontail was misclassified as development), I digitized the misclassified area to the correct land cover type. The 2011 and 2012 land cover layers also did not account for a large industrial facility that had been constructed between two cottontail patches. To account for this, I digitized and reclassified that area as development based on 2015 aerial photography (NH GRANIT 2015).

Table 1.1 Facilitator and barrier landscape variables tested in univariate resistance surface optimization for New England cottontail population in Londonderry, New Hampshire.

Variable	Hypothesized effect on gene flow
Shrub	Facilitator
Shrub wetland	Facilitator
Herbaceous wetland	Facilitator
Linear facilitators (powerlines and buffered railroads)	Facilitator
Development	Barrier
Forested wetland	Barrier
Forest	Barrier
Fields/agriculture	Barrier
Water	Barrier
High-volume roads (multi-lane highways and statewide corridors)	Barrier
Moderate-volume roads (secondary and improved roads)	Barrier
Low-volume roads (trails and private roads)	Barrier

I completed univariate modeling to identify optimal cost values for landscape variables using two resistance surfaces, one based on the 2011/2012 landscape, and a second based on the 2015 landscape with additional digitized development. To identify the optimal cost value for a landscape variable in a resistance surface, each cell in a raster GIS surface is assigned a cost value based on the underlying land cover type or landscape feature, and pairwise genetic distances between sampled individuals on the landscape are compared to pairwise cost distance values. Cost values are then varied and the test repeated for each alternative cost. Model selection criteria such as AIC_C are used to identify the resistance surface parameterization for which cost values are best supported by genetic data. To test multiple cost values for each landscape variable, I systematically varied the cost value for each variable in separate resistance surfaces (e.g. Hohnen et al. 2016). Hypothesized facilitating features were assigned a reduced cost value of 1 relative to a background value of 100. Hypothesized barrier features were assigned elevated cost values of 2, 5, 10, 25, 50, 100, 250, 500, 750, and 1000 relative to a background value of 1. I also tested a null model of Euclidean distance in which every raster cell was assigned a value of 1.

I then calculated cost distances between all pairs of individuals in each surface using CIRCUITSCAPE 4.0 (McRae et al. 2013) and averaging resistances from an eight-neighbor connection scheme (e.g. Peterman et al. 2014). There are two common approaches to calculating cost distance from resistance surfaces: using the resistance surface to identify a single least-cost path through the landscape (Adriaensen et al. 2003) or representing the resistance surface as an electrical circuit to identify multiple paths of least resistance (McRae 2006). The latter approach is employed in CIRCUITSCAPE, a method that incorporates circuit and random walk theories to measure connectivity between sampling locations and identifies all possible pathways between individuals on the landscape. Identifying multiple paths is ecologically relevant because it does not assume that organisms have a complete knowledge of the landscape and would use an identified single path of least resistance. This approach is also advantageous if there are several potential paths of similar total cost (Balkenhol et al. 2016) and may better represent gene flow that occurs over multiple generations (McRae 2006). For these reasons, I used CIRCUITSCAPE analyses, rather than least cost path approaches. CIRCUITSCAPE provides valuable visualizations of habitat connectivity to aid in management planning (e.g. Emel & Storfer 2015, Warren et al. 2016, Alego et al. 2017).

To determine which parameterization for each variable was best supported by the genetic data, I built linear mixed effects models with a pairwise genetic distance matrix as the dependent variable, and a pairwise cost distance matrix as the independent variable. Genetic distance was calculated as the proportion of shared alleles between individual cottontails (Dps, Bowcock et al. 1994) in the R package *adegenet* (Jombart 2008). Cost distance was calculated in CIRCUITSCAPE. I used a maximum-likelihood population effects (MLPE) approach to account for the non-independence of pairwise comparisons (Clarke et al. 2002, Van Strein et al. 2012). In these models, explanatory variables (i.e. cost distances) are the fixed effect terms and sampling locations are included as a random effect to account for the proportion of the total variance related to non-independent sample locations that are incorporated in many sample pairs (Clarke et al. 2002). MLPE models were estimated with the lmer function in the *lme4* package (Bates et al. 2016) in R using a residual maximum likelihood approach (REML). REML is desirable for unbiased estimates of the variance components of mixed models (Clarke et al. 2002, Gurka 2006). To compare models with different fixed effects (i.e. cost distance) and to select the best fitting model, I used AIC_C. AIC has been demonstrated to be the best model selection index for linear mixed models in landscape genetics through simulations (Gurka 2006, Row et al. 2017). After identifying the best supported cost value for each landscape variable in the Londonderry landscape with mixed models and AIC_C, optimized cost values from Londonderry were compared to cost values optimized in previous research for landscapes in southern Maine and Cape Cod (Papanastassiou 2015, Amaral et al. 2016), and integrated with expert opinion. By comparing cost values from the small geographic extent of the Londonderry landscape to larger previously studied landscapes and expert opinion, I arrived at one cost value for each landscape variable and combined all landscape variables into a multivariate resistance surface for connectivity mapping.

Connectivity mapping

Next, I used a multivariate resistance surface and CIRCUITSCAPE to map connectivity in the Londonderry landscape to highlight corridors (i.e. regions of low resistance) for cottontail dispersal between both currently occupied patches and completed New Hampshire Fish and Game restoration sites in the larger landscape. CIRCUITSCAPE identifies all possible pathways between individuals or sites based on the resistance values of the underlying landscape. Corridors were identified as regions where the most pathways were concentrated. Analyses were run in the “all-to-one” mode, which is ideal for identifying areas of connectivity while minimizing run-time and memory usage (McRae & Shaw 2009). Identifying corridors of high connectivity between occupied and managed patches can provide insight for planning future landscape restoration that will support connected metapopulations.

Parameterization of a spatially explicit, individual-based framework to simulate the effects of restoration landscapes on population viability

I parameterized a spatially explicit, individual-based model, CDPOP, to simulate the effects of alternate restoration scenarios on cottontail population size, connectivity, and genetic diversity. For this project, I developed a proof-of-concept that can be pursued further for modeling the effects of restoration scenarios on the Londonderry, New Hampshire population, and in other parts of the New England cottontail’s range. CDPOP is an individual-based simulator of gene flow in complex landscapes (Landguth & Cushman 2010). CDPOP tracks alleles across individuals over time, with dispersal and mating governed by pairwise landscape distances between individuals on a continuous cost surface. Input parameters include vital rates (age-specific reproduction and mortality), demographic information (sex and age distributions), initial allele frequencies based on the empirical population, and a resistance surface and movement functions. Coordinates are input to indicate locations for individuals in the initial population and open coordinate locations in suitable habitat where individuals can move to, with the total number of coordinate locations totaling the carrying capacity of the landscape.

In the model, individuals move to mate based on the user-specified function and cost of the underlying landscape. Offspring are initialized in their mother's location, then mortality occurs for all age groups based on the user-specified probabilities. After mortality, offspring disperse to vacant locations according to the input dispersal function and cost distance threshold. If all locations are occupied (i.e. the population is at carrying capacity), remaining offspring are removed from the model. For each generation, the model outputs the number of individuals in the population, number of births and deaths, number of dispersers, number of breeding age individuals, heterozygosity and inbreeding coefficients, and average mating and dispersal distances. At specified time units, the model outputs the genotype and location of each individual.

Using the optimized multivariate resistance surface for the Londonderry landscape, I generated a pairwise cost distance matrix for input into CDPOP. Baseline parameters were selected using the best available knowledge of cottontail vital rates, demography, and movement from a literature review (Table 1.2). The baseline initial population size was 38 individuals, the maximum abundance estimate from the Londonderry population using recapture data from 2017. Simulated individuals were placed in starting locations corresponding to sampled individuals and additional individuals were randomly added to the surrounding landscape in suitable patches >2 ha in size to equal the initial population size. The first generation was randomly assigned 15 loci with alleles following the input allele frequency file based on sampled Londonderry cottontails. Loci followed a k-allele mutation model with a mutation rate of 0.0005 per locus per generation.

Table 1.2 CDPOP input parameters for the modeled New England cottontail population in Londonderry, New Hampshire, including vital rates, demographic information, and movement functions.

Parameter	Model value	Source
Carrying capacity	65 individuals	2 rabbits/ha ~32.55 ha on patches > 2 ha (Barbour & Litvaitis 1993, Litvaitis & Villafuerte 1996)
*Initial population size	**38 individuals**	Maximum abundance estimate based on recapture data in Londonderry, NH in 2017
	50 individuals	
	65 individuals	Estimated carrying capacity in the Londonderry restoration landscape based on a density of 2 rabbits/ha and ~32.55 ha of shrubland habitat
Female fecundity	5/litter, 3 litters/yr	USDOI 2009
Offspring sex ratio	Equal	Chapman & Morgan 1973
Multiple paternity option	Yes	M. Bauer & A. Kovach, unpublished data
Philopatry (strict)	No	
Population growth model	Exponential	
*Mortality, age 0	**0.85 (recruitment 2.25/female)**	*Mortality*: Litvaitis & Villafuerte 1996 *Recruitment*: Chapman & Litvaitis 2003, L. Perotti (unpublished report), Barbour & Litvaitis 1993, Warren 2017
	0.80 (recruitment 3/female)	
	0.73 (recruitment 4.05/female)	*Recruitment*: Chapman and Litvaitis (2003), L. Perrotti (unpublished report), Warren 2017
*Mortality, adult	0.70	Brown & Litvaitis 1995, Litvaitis & Villafuerte 1996, Warren 2017
	0.40	0.60 approximate maximum observed survival over a 3-year period in NY (A. Cheeseman, personal communication)
	0.23	0.77 maximum observed survival over a 4-year period in Londonderry, NH (B. Ferry, unpublished data)
Maximum age	3 years	Maximum observed age (i.e. individual was sampled over 3 winter seasons) in Londonderry, NH (Ch. 1) and Bellamy River WMA, NH (Ch. 2)
Age distribution	Even distribution	
Mate movement probability function	Gaussian	
Mean distance	50 m	Mean movement distance for New England and eastern cottontails from a telemetry study in NY (Cheeseman 2017)
Natal dispersal probability function	Negative exponential	
Mean distance	250 m	Minimum distance exceeding within-home-range movements from a telemetry study in NY (Cheeseman 2017)

*Alternate values for these parameters were evaluated in a sensitivity analysis. Bold values were used in the baseline scenario.

I simulated 70 overlapping generations on the cost surface landscape, with 10 Monte Carlo iterations per simulation. I evaluated model performance and the outcome of the baseline scenario on cottontail population size, persistence, observed heterozygosity, and allelic richness. I also conducted sensitivity analyses to evaluate the influence of three model parameters on model performance: mortality of age 0 individuals (i.e. recruitment, when combined with the female fecundity parameter), mortality of adults, and the initial population size (see Table 1.2 for values evaluated). These three parameters were evaluated with sensitivity analyses because they are likely to have a strong influence on cottontail population size and persistence, there is limited empirical data on recruitment and mortality, and mortality is highly variable annually.

Tracking predicted population response to a restoration scenario

After conducting sensitivity analyses, I used baseline model input and a resistance surface reflecting restored habitat patches to track predicted changes in cottontail population size, persistence, and genetic diversity under a restoration scenario. Shrubland restoration projects completed by New Hampshire Fish and Game that could undergo succession to become dense enough habitat to support New England cottontails were reflected as suitable habitat in a new resistance surface. For example, if a habitat project parcel was previously unsuitable habitat such as forest or field, the cost value of the raster cells underlying that parcel were reduced to a value of 1 (low cost to cottontail movement, i.e. suitable habitat) to reflect a restoration scenario in which that parcel had become suitable shrubland habitat. Managed habitat patches reflected as suitable habitat in the restoration scenario are outlined in Figure 1.2. CDPOP simulations were conducted with this new restoration resistance surface, and cottontail population size, persistence, number of alleles, and observed heterozygosity were tracked over 70 generations. Locations for every cottontail in the population were output at generations 10, 25, 50, and 70.

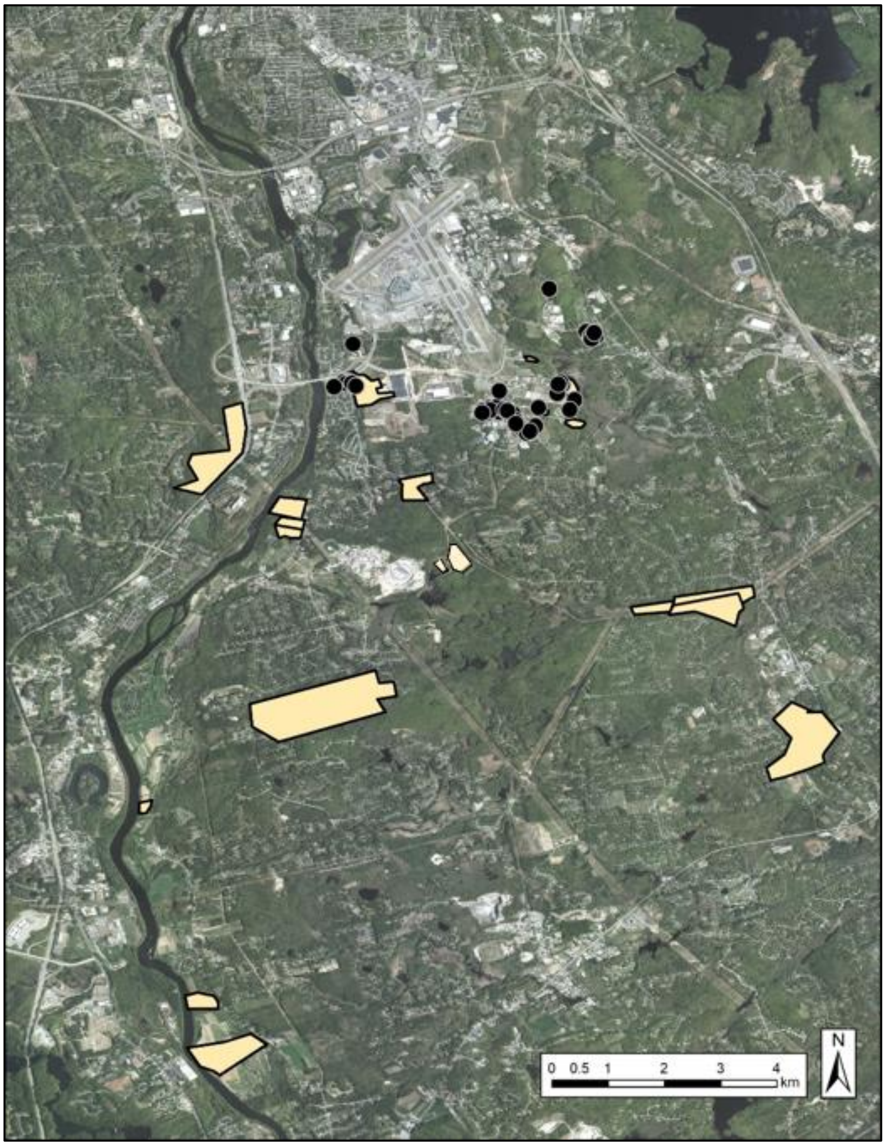


Fig. 1.2 Managed patches reflected as restored habitat suitable for New England cottontail occupancy in a CDPOP restoration scenario simulation are outlined and indicated in tan in the Londonderry, New Hampshire landscape. The current distribution of New England cottontails is indicated on the map as black points where individual cottontails were sampled through pellet surveys or live-trapping from 2015-2017.

Results

Survey results and genotyping success

Wildlife biologists at New Hampshire Fish and Game collected a total of 182 pellet samples over three winter survey seasons (2015-2017) and 33 tissue samples over three trapping seasons. I successfully genotyped 202 samples and identified 57 unique individuals over the three-year period (Table 1.3). Three individuals were detected in both 2016 and 2017, and one female was detected in both 2015 and 2017, surviving for three years. Pellet genotyping success varied across years from 86.1 to 100.0 percent, and a range of 1-16 samples were collected per individual (Table 1.3). The probability of identity for siblings was 1.1×10^{-4} for this population, meaning that there was a 1 in 9,090 probability that two siblings share the same genotype with these genetic markers. Molecular sex identification agreed with field sex for all trapped rabbits.

Table 1.3 Number of tissue samples collected from trapped rabbits each year in Londonderry, New Hampshire, number of fecal pellet samples collected during winter surveys, percent genotyping success for pellets, number of unique individuals identified, and range of number of samples collected (i.e. captures) per individual. NEC = New England cottontail. A portion of samples collected in 2018 were snowshoe hare and an eastern cottontail samples.

Year	No. tissue samples collected	No. pellet samples collected	Pellet genotyping success (%)*	No. unique individuals	Range of captures
2015	9	3	100.0	12	1
2016	12	51	100.0	16	1-16
2017	12	125	86.1	34	1-11
2018	NA	107 (76 NEC)	100.0	21	1-14
TOTAL:	33	255	**AVG:** 96.5	20.8	3.3

*Tissue genotyping success was 100% each year.

Population genetic analyses and spatial autocorrelation

Analyses in STRUCTURE indicated the presence of three distinct genetic groups (K=3; Fig. 1.4). Geographically, these groups correspond to the Charlotte Street, Cohas Brook, and Buckthorn Street patches, with admixture in the Stonyfield patch (Fig. 1.5). Allelic richness and observed heterozygosity calculated by patch and averaged over 2015-2017 were highest for the central Stonyfield patch and lower in the other three patches. Relatedness was high in each patch, ranging from 0.080 in Stonyfield to 0.136 in Cohas Brook, and 0.098 for the population overall (Table 1.4). *Capwire* abundance estimates varied by patch and by year, with Charlotte Street having the lowest abundance estimates, Stonyfield having the highest estimate in 2016 and 2017, and Buckthorn Street having the highest estimate in 2018 (Table 1.5). Abundance estimates for the entire study area (across all sampled patches) ranged from 18 (95%CI 16-24) in 2016 to 36 (95% CI 35-38) in 2017, though no samples were collected from Charlotte Street in 2016 contributing to the lower estimate that year. There was a marked decline in abundance on three of the four patches in 2018 (Table 1.5). The effective population size of the Londonderry population, the number of breeding individuals, was estimated to be 10.5 (95% CI 8.1-13.2).

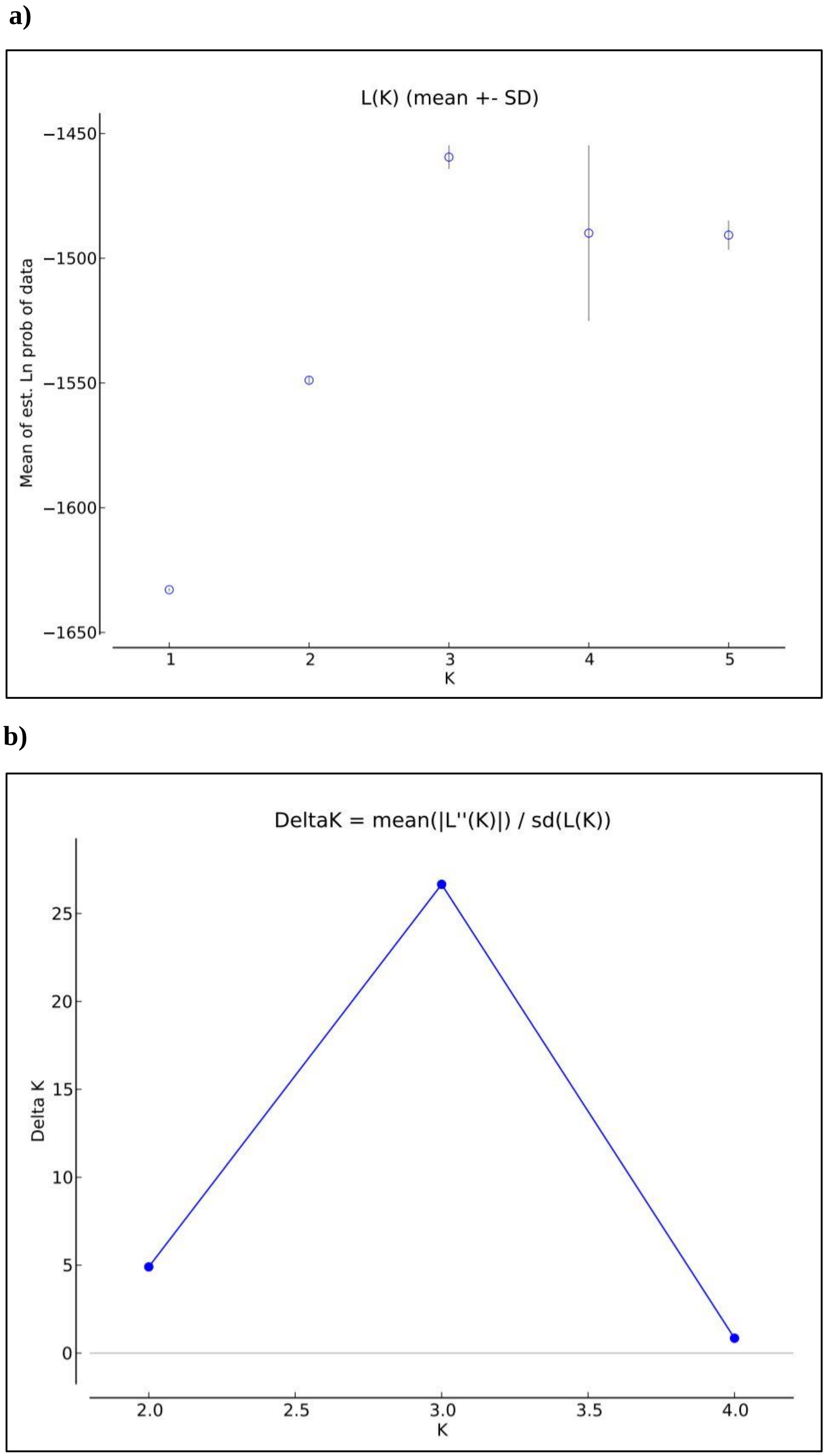


Fig. 1.3 a) Plot of the plateau of the average LnPD and **b)** peak ΔK at K=3 indicating the best supported number of genetic clusters of New England cottontails in Londonderry, New Hampshire from STRUCTURE analyses.

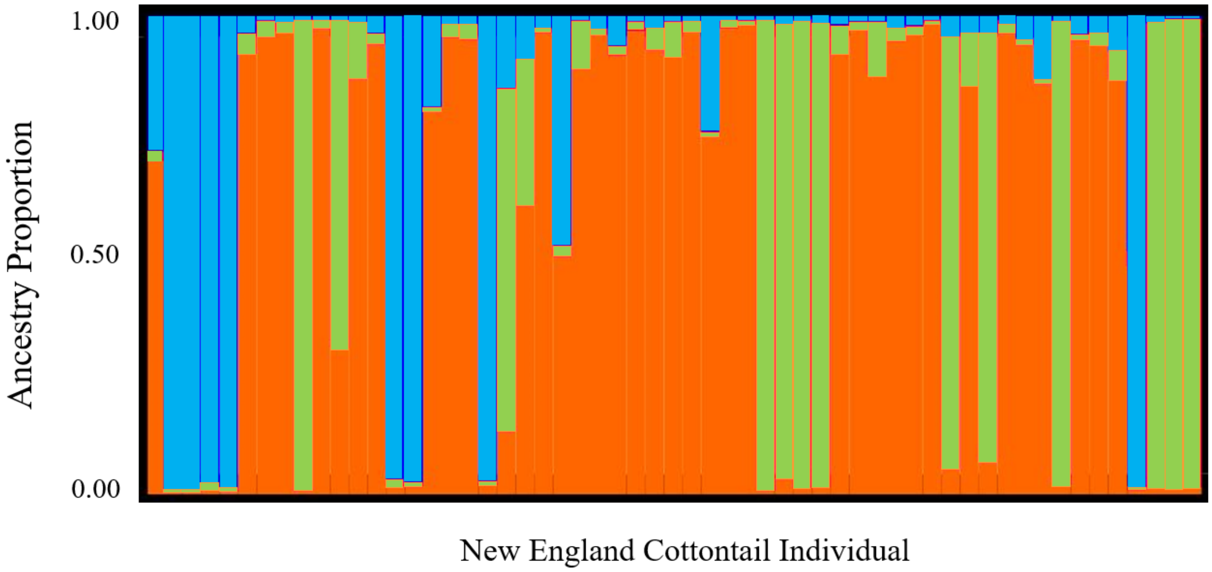


Fig. 1.4 Visualization of the results of STRUCTURE analyses for New England cottontails in Londonderry, New Hampshire showing three distinct genetic clusters. Each vertical bar is a cottontail individual and represents the proportion of the individual’s ancestry corresponding to the three genetically distinct clusters, represented by the blue, orange, and green colors.

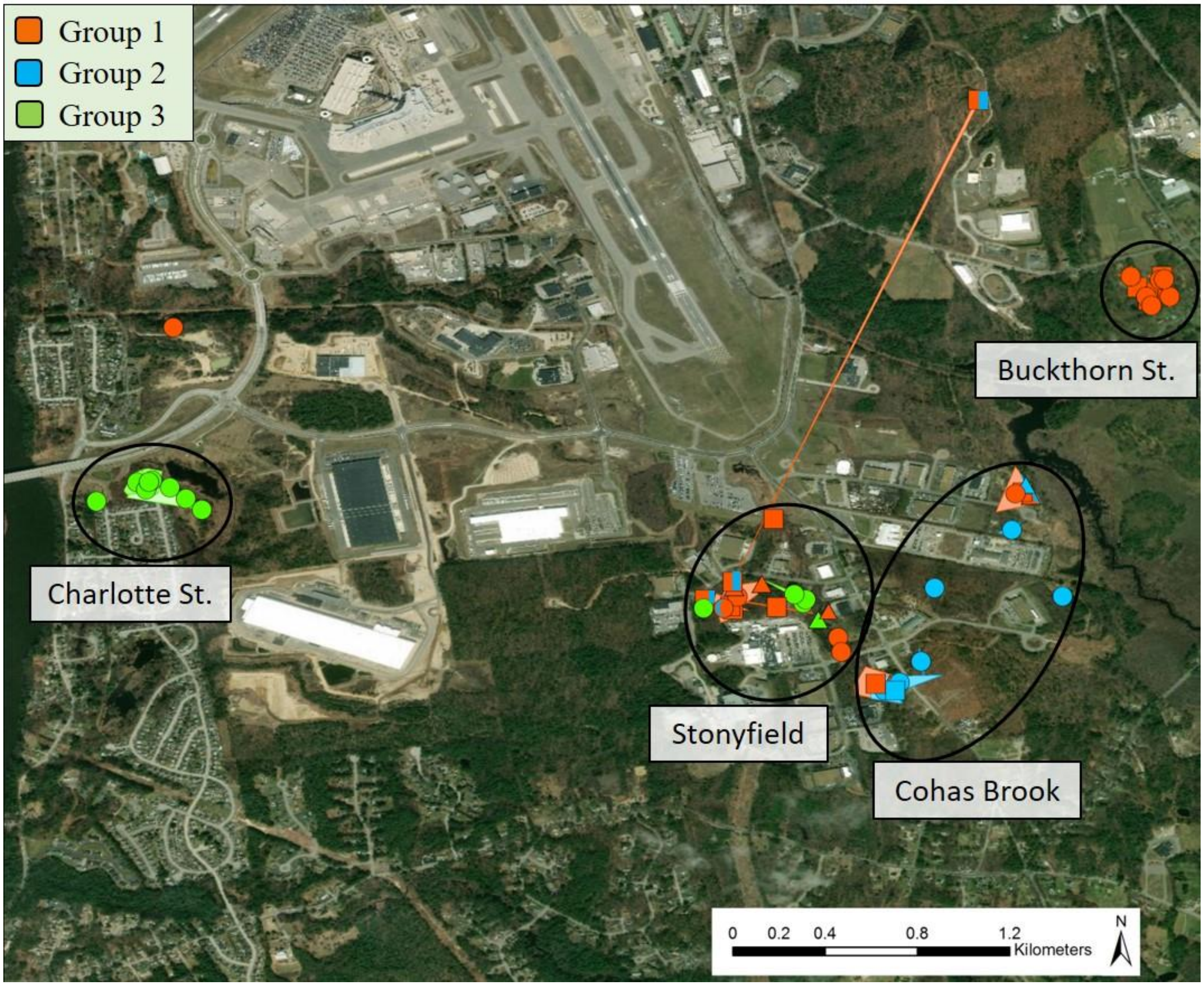


Fig. 1.5 New England cottontail individuals in Londonderry, New Hampshire color-coded by their assignment to one of three genetic groups identified in STRUCTURE analyses. Ancestry indicates three genetic groups segregated geographically, with admixture in the central Stonyfield patch. Shapes indicate the year the individual was sampled (circle=2017, square=2016, triangle=2015). Individuals sampled multiple times are mapped with polygons surrounding their detected movement range (note one individual dispersed 2.2 km northeast of Stonyfield in 2016).

Table 1.4 Genetic diversity metrics for New England cottontails in each geographic patch in Londonderry, New Hampshire, and for the population as a whole. Samples were collected from the Londonderry patches in the following years: Stonyfield 2015-2017, Buckthorn Street 2016-2017, Cohas Brook 2015-2017, Charlotte Street 2017. No. individuals: number of unique individuals detected, H_O: observed heterozygosity, *r*: relatedness calculated in ML-Relate.

Population	Patch size (acres)	No. individuals	No. of alleles	Allelic richness	H_O	*r*
Stonyfield	21	21	3.3	3.2	0.549	0.080
Buckthorn St.	14	11	3.3	2.6	0.524	0.122
Cohas Brook	20	16	3.0	2.9	0.453	0.136
Charlotte St.	11	8*	2.9	2.7	0.450	0.127
TOTAL:	66	57**	3.7	3.7	0.503	0.098

*Charlotte Street metrics include data for two F1 hybrid New England cottontail-eastern cottontail individuals from tissue samples collected in 2017.

**TOTAL includes one additional sampled individual from a location outside of these four patches, north of Charlotte Street.

Table 1.5 New England cottontail population size estimates for patches in Londonderry, New Hampshire and for the population as a whole, calculated in *capwire* for years with sufficient capture history data (2016-2018).

Patch	Year	No. pellet samples (No. NEC samples)	No. individuals (Additional individuals identified only through tissue samples)	*Capwire* abundance estimate (95% CI)	Avg. pellet captures per individual	Range of captures per individual
Stonyfield	2016	15	8	10 (8-16)	1.9	1-3
	2017	22	4 (4)	13	3.6	1-10
	2018	52 (25*)	2	2	11.0	8-14
Cohas Brook	2016	21	2 (2)	4	5.3	1-16
	2017	29	8 (1)	10 (9-12)	3.7	1-7
	2018	8	3	3	2.7	1-4
Charlotte St.	2017	25	5 (3**)	5	4.0	1-11
	2018	20 (16***)	3***	3	5.0	4-8
Buckthorn St.	2016	15	4	4	3.8	1-9
	2017	26	8	8	3.3	2-5
	2018	32	13	14 (13-16)	2.5	1-6
TOTAL:	2016	51	16	18 (16-24)		
	2017	104	33****	36 (35-38)		
	2018	112 (81)	21	22 (21-24)		

*2018 samples from Stonyfield included 25 New England cottontail samples and 27 snowshoe hare samples.

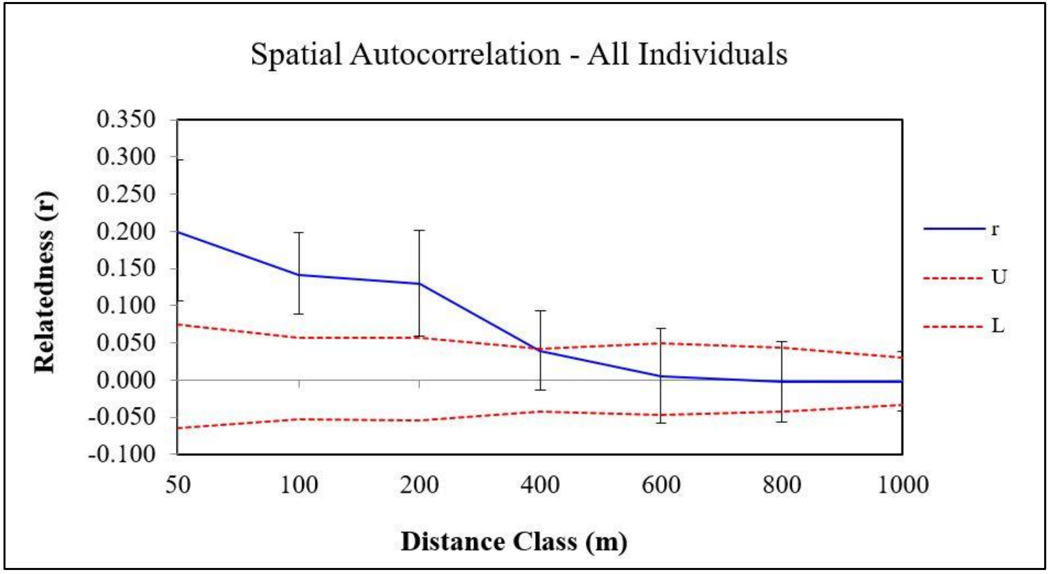
**2017 individuals identified through tissue samples included two New England cottontail-eastern cottontail F1 hybrids.

***2018 samples from Charlotte St. included four samples from a female New England cottontail, four samples from a male eastern cottontail, and 12 samples from two F1 hybrid individuals.

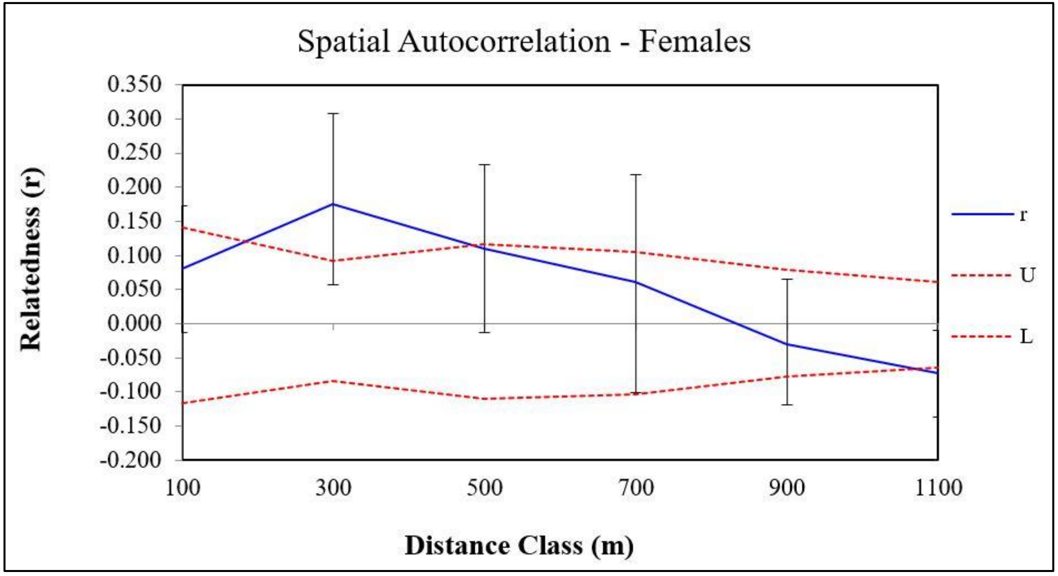
****TOTAL for 2017 excludes one additional sampled individual from a location outside of these four patches, north of Charlotte Street.

Spatial autocorrelation analyses identified fine scale genetic structure and limited dispersal in the Londonderry population. All individuals were highly related to a distance of 400 m (Fig. 1.6a) indicating restricted dispersal, females were related to a distance of 500 m (Fig. 1.6b), and males were related to a distance of 75 m (Fig. 1.6c). The shorter distance to which males were highly related in this population compared to females indicates male-biased dispersal (i.e. males are more connected with local relatedness extending to a shorter geographic distance).

a)



b)



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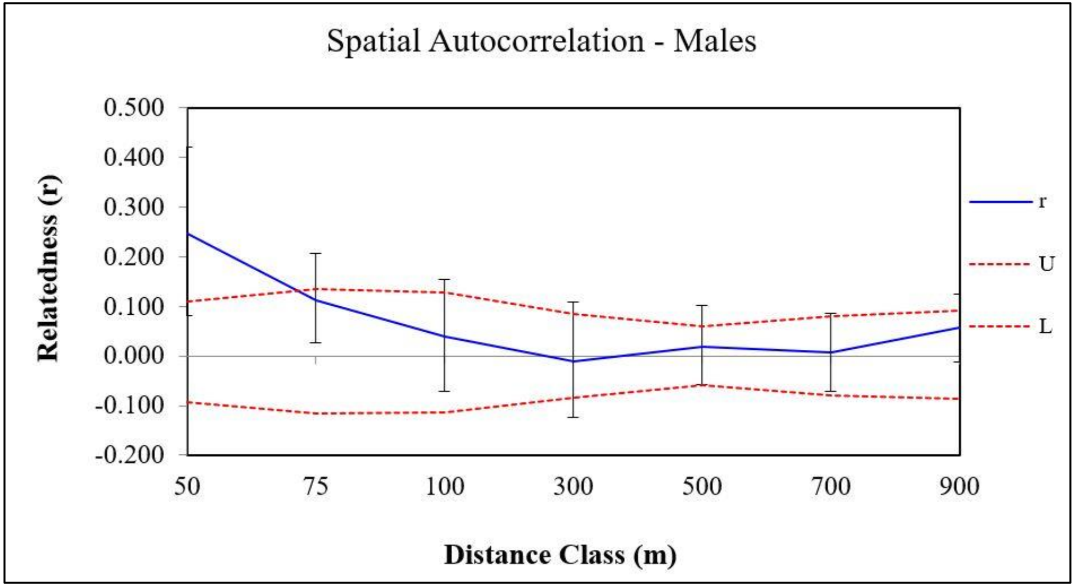


Fig. 1.6 Spatial autocorrelation for New England cottontail individuals in Londonderry, New Hampshire indicating fine-scale spatial structure and relatedness extending over a short distance on the landscape; **a)** spatial autocorrelation of all Londonderry individuals, **b)** spatial autocorrelation of females, **c)** spatial autocorrelation of males. r indicates relatedness and U and L bound the confidence interval for the null hypothesis of no population structure.

Univariate resistance surface modeling

In the optimization of cost values for landscape features in Londonderry, models trialing different cost values produced results within 10 ΔAIC_C for most landscape variables in both the 2012 landscape and 2015 landscape with additional digitized development. Beta coefficients were significant for the cost values with the lowest AIC_C (95% CIs excluded 0). However, given the small extent over which pairwise genetic distances and pairwise geographic distances were compared in these models (all occupied patches are within less than 4 km in Londonderry), I determined there was insufficient power to identify exact cost values with empirical data. To determine cost values for use in a multivariate surface for connectivity mapping, I compared values with the lowest AIC_C from empirical Londonderry data to optimized cost values from previous landscape genetic research in southern Maine and Cape Cod (Papanastassiou 2015, Amaral et al. 2016; Table 1.6). Where there was disagreement among studies, or where the landscape in Londonderry warranted a different consideration, I incorporated expert opinion (H. Holman, B. Ferry, NHFG; A. Kovach UNH) to assign cost values that ranked landscape variables in terms of their relative barrier values, or as facilitators. For example, given expert opinion, I did not buffer roadsides as facilitators in this landscape due to the minimal shrubby habitat along small residential roads and mowed roadsides along interstates. To address uncertainty in cost values for the small geographic extent of the Londonderry landscape, I evaluated a set of conservative barrier values and high barrier values in connectivity maps (Table 1.6, Figs. 1.7b, 1.7c).

Table 1.6 Comparison of the best supported Londonderry cost values (lowest AIC_C) to optimized values from previous landscape genetic research for New England cottontail populations in Maine and Cape Cod landscapes (Papanastassiou 2015, Amaral et al. 2016), and consensus values used for connectivity mapping in the Londonderry landscape. Kittery is a more fragmented landscape with a high proportion of forest and roads, and Cape Elizabeth is a less fragmented landscape but with a high proportion of forest and development. The Cape Cod landscape has a low proportion of development and has a high proportion of a unique habitat feature – pitch pine-scrub oak. NA: not applicable in the study area, NS: not significant in the study area.

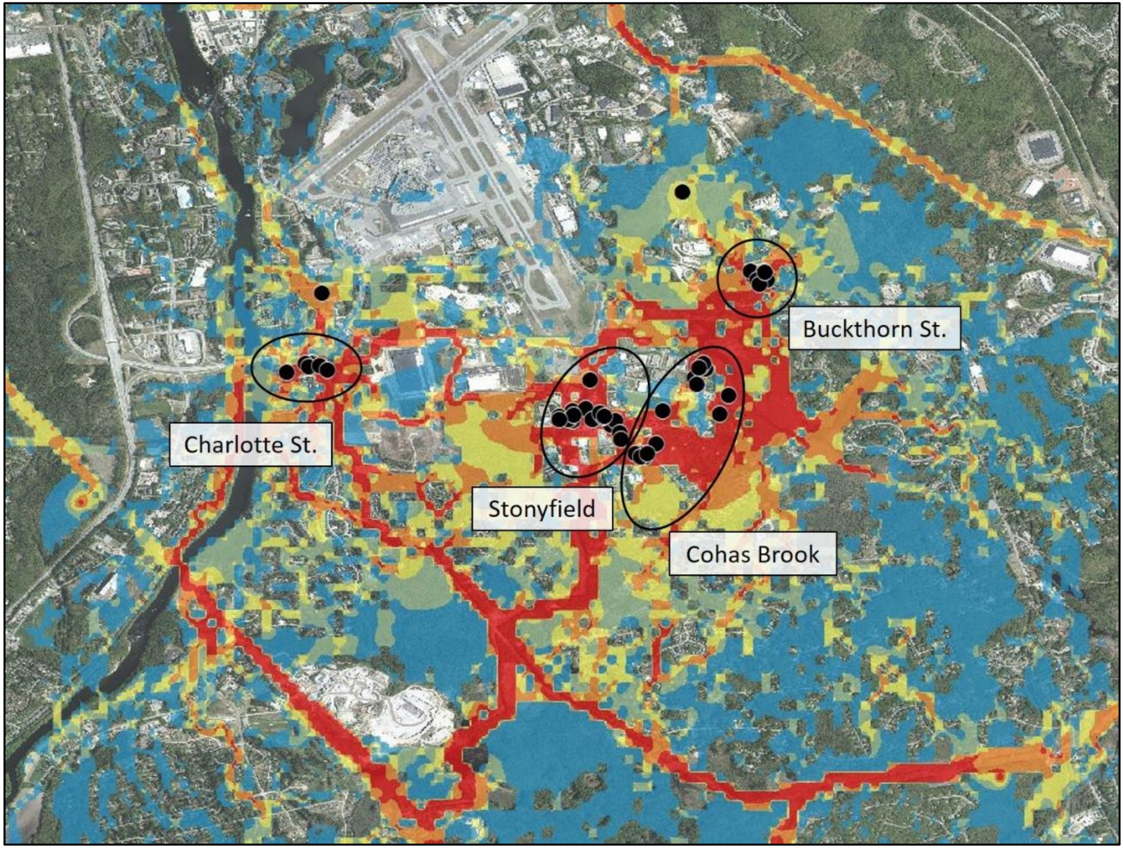
Landscape variable	Londonderry, NH	Kittery, ME	Cape Elizabeth, ME	Cape Cod, MA	Consensus cost values for connectivity mapping	
					Conservative	High
Shrub*	1	1	1	1 (NS)	1	1
Shrub wetland	2 (2012), 25 (2015)	1	1	NA	1	1
Herbaceous wetland	750**	1	1	1	1	1
Linear facilitators*	1	1	1	1	1	1
Development	2	2	50	500 (NS)	50	500
Forested wetland	1,000***	100	250	NS	100	250
Forest	2	2	5	25	5	25
Fields/ agriculture	2	10	2	NS	5	5
Water	50	10	2	NS	50	500
High-volume roads†	2	10	NA	50, 25	100	500, 100
Moderate-volume roads‡	2	10, 5, 5	50, 25, 25	10, 5, 2	5	25
Low-volume roads	2	2	NA	NA	5	5

*Shrub and linear facilitators not modeled as barriers in Londonderry.
**Small amount of herbaceous wetland land cover on the landscape.
***10 cost models within 2 ΔAIC_C for forested wetland.
† Multi-lane highways, statewide corridors.
‡ Secondary roads, improved roads, unimproved roads.

Connectivity mapping

CIRCUITSCAPE analyses corroborated STRUCTURE and genetic diversity analyses, highlighting the isolation of the Charlotte Street patch and also the potential connectivity between Stonyfield and nearby patches on the landscape. Given the high barrier values of forest and development and their extent on the landscape, CIRCUITSCAPE models highlighted powerline rights-of-ways, the shrub wetland near the Cohas Brook patch, and a railway between Cohas Brook and Buckthorn Street as potential areas of connectivity (Fig. 1.7a). Current flow outside of the landscape immediately surrounding occupied patches was low and was concentrated in powerline rights-of-ways (Figs. 1.7b, 1.7c). There were minimal differences between maps generated with conservative versus high barrier values for Londonderry. The higher barrier values (Fig. 1.7c) indicated slightly lower connectivity in the landscape immediately surrounding occupied patches, and placed greater emphasis on powerline corridors as facilitators.

a)



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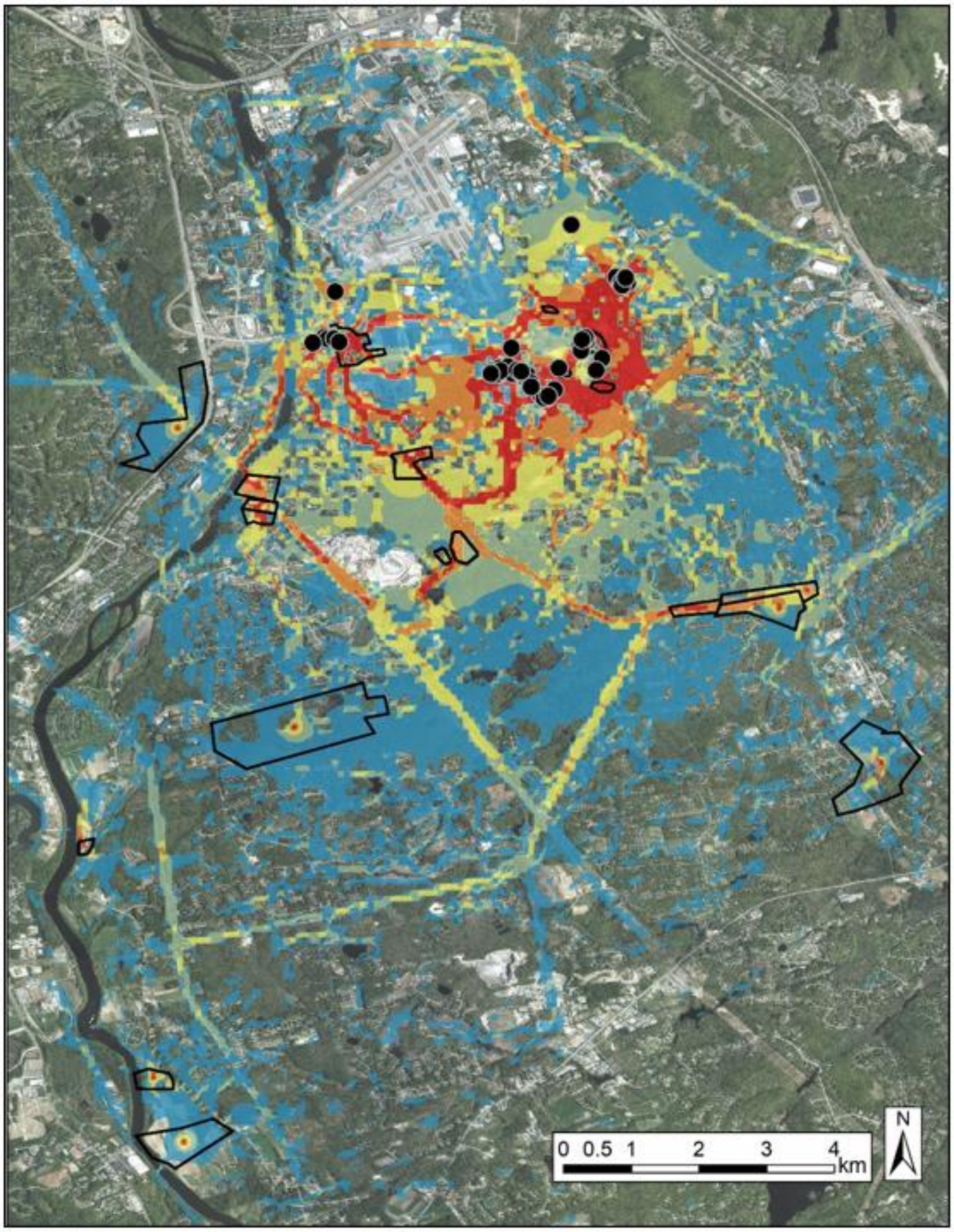

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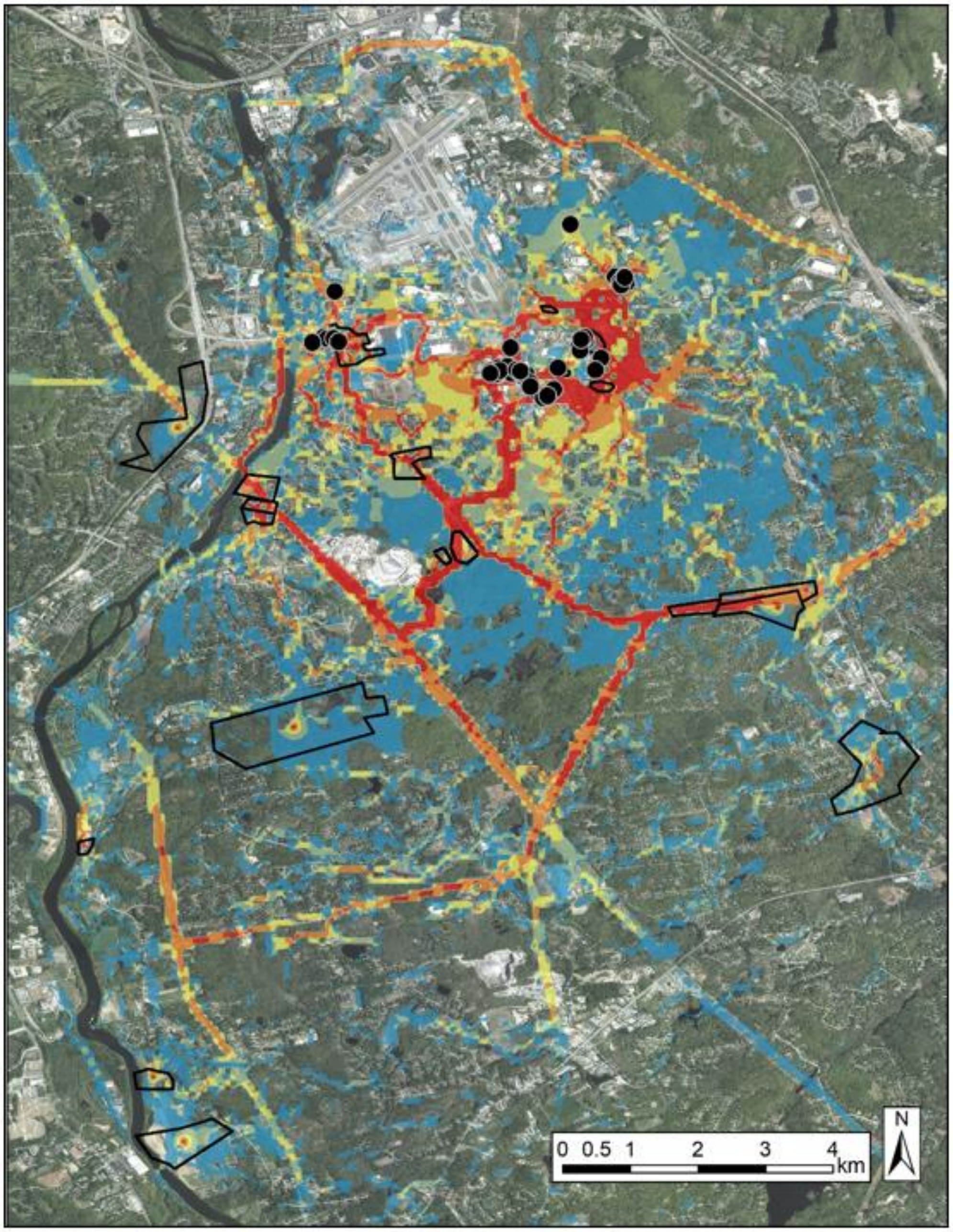


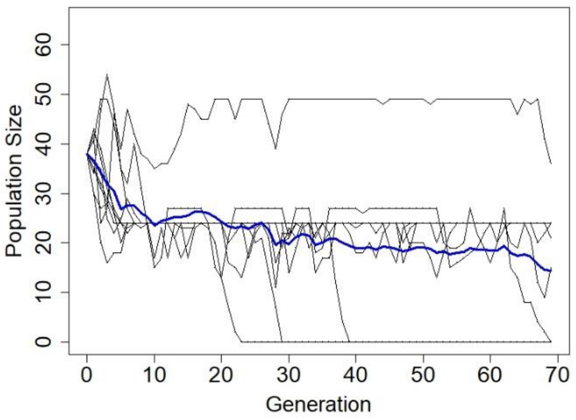
Fig. 1.7 Connectivity mapped with a resistance surface between sampled cottontail individuals and completed habitat projects within a larger restoration landscape in Londonderry. Red indicates high connectivity through the landscape and cooler colors indicate limited connectivity through the landscape; black polygons outline completed habitat projects in the larger restoration landscape; **a)** connectivity with high cost values at the smaller spatial extent of the occupied Londonderry patches, **b)** connectivity given conservative cost values (Table 1.6), **c)** connectivity given high cost values.

Parameterization of a spatially explicit, individual-based simulation framework

CDPOP model performance was highly sensitive to the amount of dispersal specified in the model. When parameterized such that average dispersal distances were set to reflect the low rate of realized dispersal events observed in recent studies (250 m, a distance threshold exceeding home range sizes and considered the minimum movement distance for cottontail dispersal in a telemetry study in New York; Cheeseman 2017), baseline model input generated simulated cottontail populations persisting at an average of 20 individuals for 70 generations after an initial decline from the starting population size of 38 individuals during the first ~7 generations (Fig. 1.8a). This parameterization, however, produced ~30% individuals dispersing in the model, a higher percentage of individuals dispersing than observed in empirical populations (Cheeseman 2017, B. Ferry unpublished data). When the model was parameterized with a higher average dispersal distance of 500 m, cottontail populations grew from the initial size of 38 for ~5 generations and persisted at an average of 50 individuals over 70 generations (Fig. 1.8h). Populations were highly stable with few fluctuations over Monte Carlo replicates given the larger 500 m average dispersal distance (Fig 1.8h). This also produced a greater frequency of dispersal than observed in empirical populations, with ~40% individuals dispersing in the modeled population. Population size was sensitive to both recruitment and adult mortality, with populations stabilizing at a greater size of 36 individuals with a higher recruitment input of 4.05 offspring/female (Fig. 1.8c) compared to the baseline input of 2.25 offspring/female, and populations declining and becoming extinct after 50 generations given a higher adult mortality of 0.70 (Fig. 1.8d) compared to the baseline mortality of 0.40. Higher initial population sizes did not produce larger stable population sizes, given the limited area of suitable habitat surrounding currently occupied patches (Figs. 1.8f, 1.8g).

The number of alleles in the population and observed heterozygosity declined in all model parameterizations from the initial values of 43.9 alleles and 0.49 observed heterozygosity, which were based on observed allele frequencies in the sampled Londonderry population. In the baseline model, the average number of alleles declined to 18.0 and observed heterozygosity declined to 0.03 (excluding Monte Carlo replicates where populations went extinct and number of alleles and heterozygosity were 0; Figs. 1.9a, 1.10a) as populations declined from 38 to 20 individuals. This indicates a projected severe decline in genetic diversity in this small modeled population. The sharpest decline in number of alleles and observed heterozygosity corresponded to the model parameterization with a high adult mortality of 0.70 that produced the sharpest population decline (Figs. 1.9i, 1.10i, green projection line).

a)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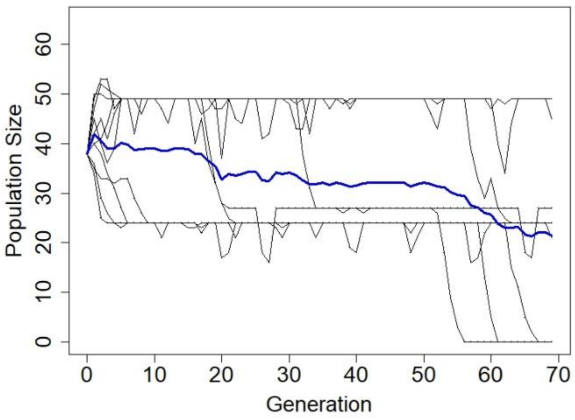
Generation

b)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Generation

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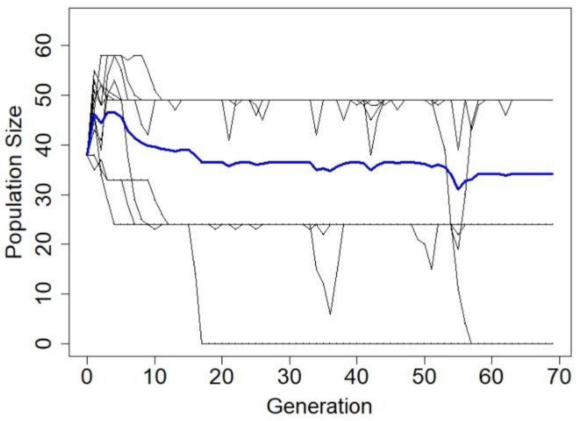
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Generation

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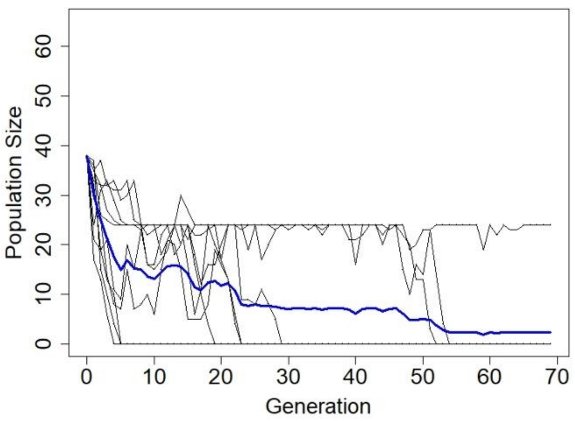
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Generation

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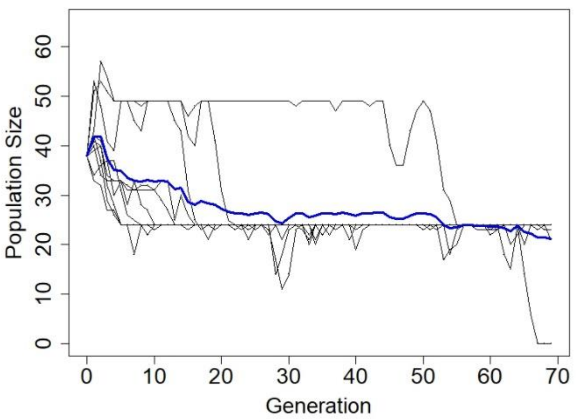
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Generation

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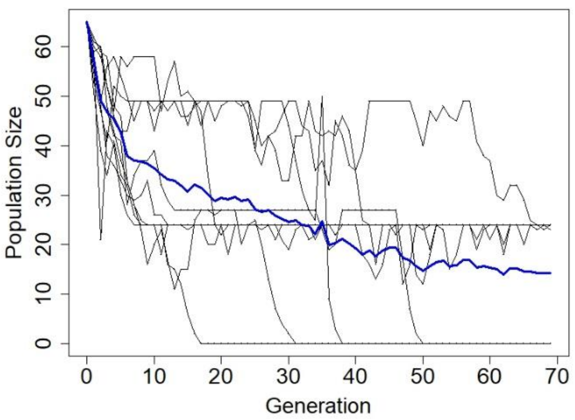
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Generation

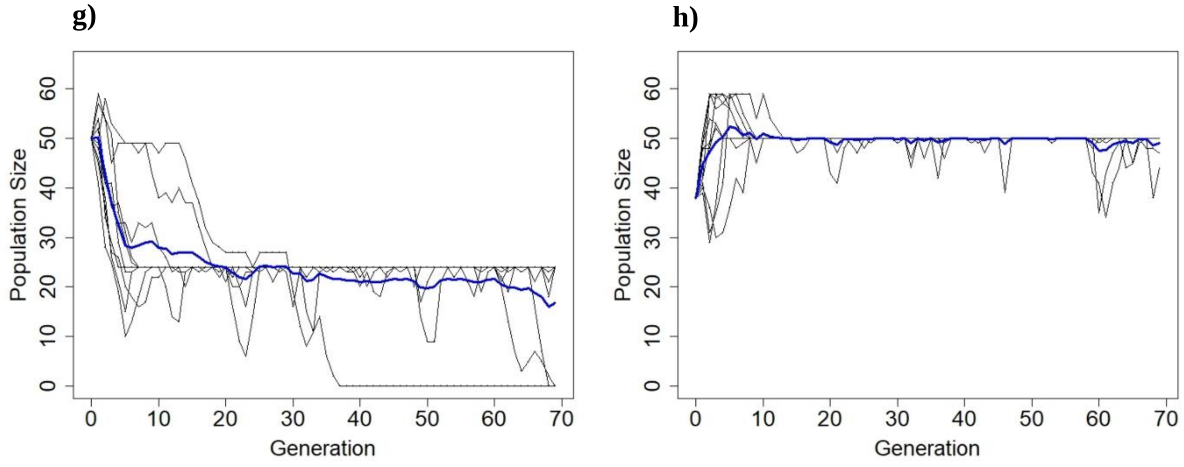


Fig. 1.8 Projected New England cottontail population size in Londonderry, New Hampshire over 70 simulated generations in CDPOP. Blue lines indicate the average values over 10 Monte Carlo replicates for the following scenarios: **a)** baseline model input; **b)** 0.80 juvenile mortality (recruitment 3 offspring/female); **c)** 0.73 juvenile mortality (recruitment 4.05 offspring/female); **d)** 0.70 adult mortality; **e)** 0.23 adult mortality; **f)** initial population size at carrying capacity of 65 individuals; **g)** initial population size of 50 individuals; **h)** baseline model input with 500 m average dispersal distance.

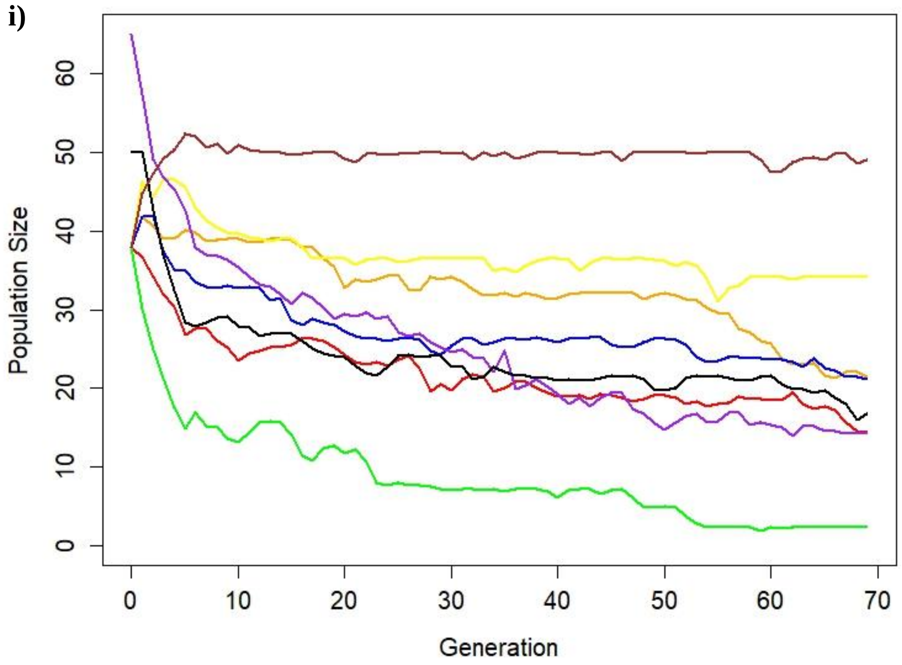
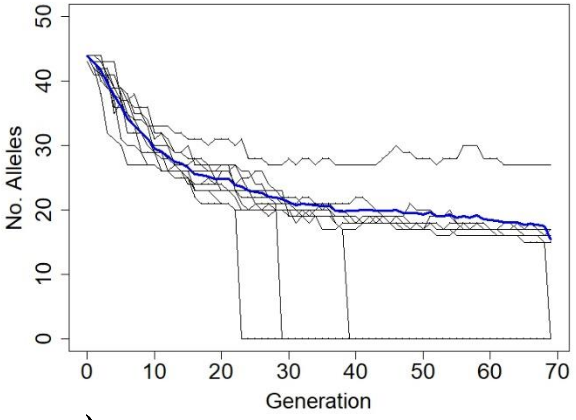


Fig 1.8i CDPOP model averages for cottontail population size over 10 Monte Carlo replicates for models: **a)** RED, baseline model input; **b)** ORANGE, 0.80 juvenile mortality (recruitment 3 offspring/female); **c)** YELLOW, 0.73 juvenile mortality (recruitment 4.05 offspring/female); **d)** GREEN, 0.70 adult mortality; **e)** BLUE, 0.23 adult mortality; **f)** PURPLE, initial population size at carrying capacity of 65 individuals; **g)** BLACK initial population size of 50 individuals; **h)** BROWN baseline model input with 500 m average dispersal distance.

a)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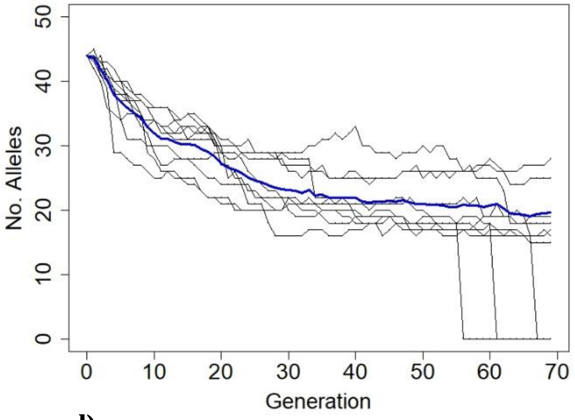
Generation

b)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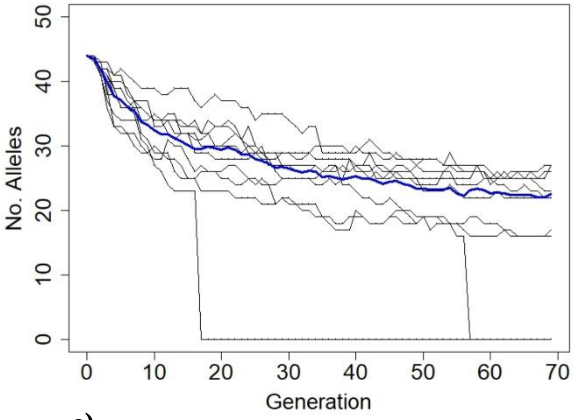
Generation

c)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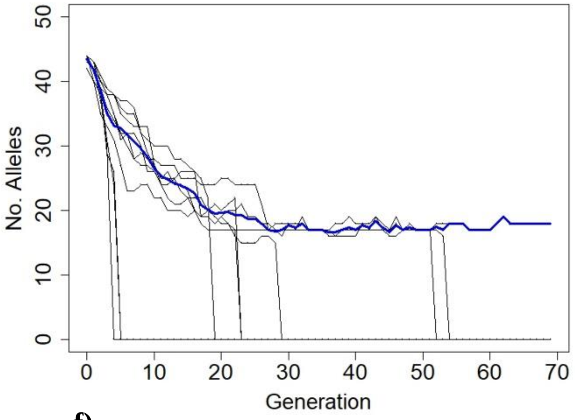
Generation

d)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Generation

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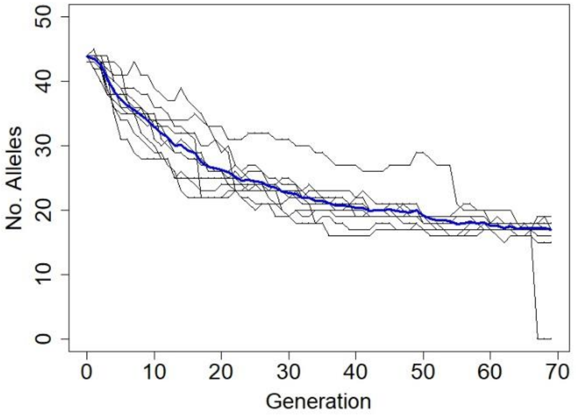
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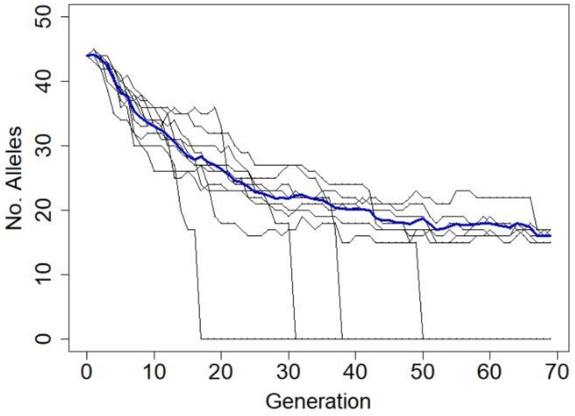
Generation

f)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Generation

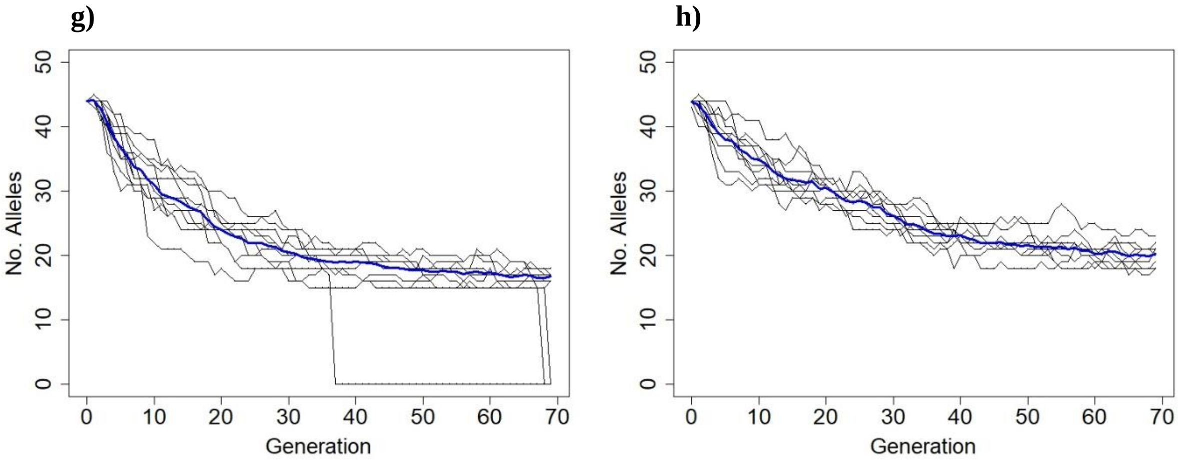


Fig. 1.9 Projected number of alleles in the simulated New England cottontail population in Londonderry, New Hampshire over 70 generations in CDPOP. Blue lines indicate the average values over 10 Monte Carlo replicates for the following scenarios: **a)** baseline model input; **b)** 0.80 juvenile mortality (recruitment 3 offspring/female); **c)** 0.73 juvenile mortality (recruitment 4.05 offspring/female); **d)** 0.70 adult mortality; **e)** 0.23 adult mortality; **f)** initial population size at carrying capacity of 65 individuals; **g)** initial population size of 50 individuals; **h)** baseline model input with 500 m average dispersal distance.

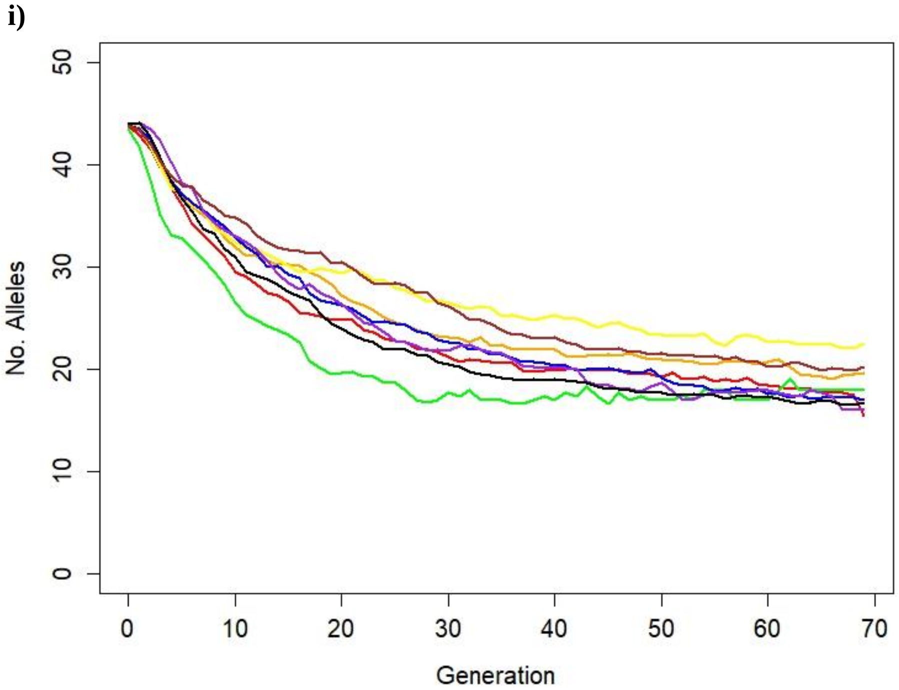
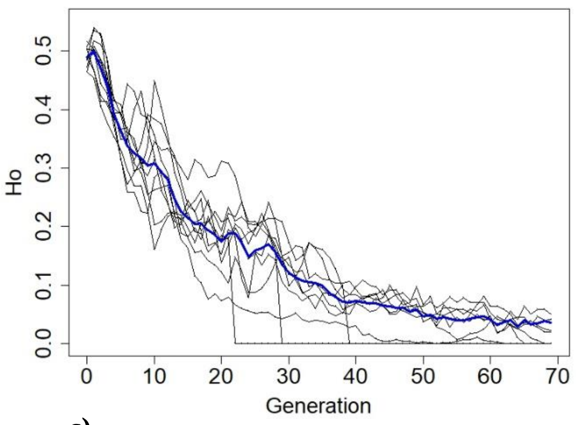


Fig 1.9i CDPOP model averages for number of alleles in the simulated Londonderry, New Hampshire New England cottontail population over 10 Monte Carlo replicates for models: **a)** RED, baseline model input; **b)** ORANGE, 0.80 juvenile mortality (recruitment 3 offspring/female); **c)** YELLOW, 0.73 juvenile mortality (recruitment 4.05 offspring/female); **d)** GREEN, 0.70 adult mortality; **e)** BLUE, 0.23 adult mortality; **f)** PURPLE, initial population size at carrying capacity of 65 individuals; **g)** BLACK initial population size of 50 individuals; **h)** BROWN baseline model input with 500 m average dispersal distance.

a)


Ho
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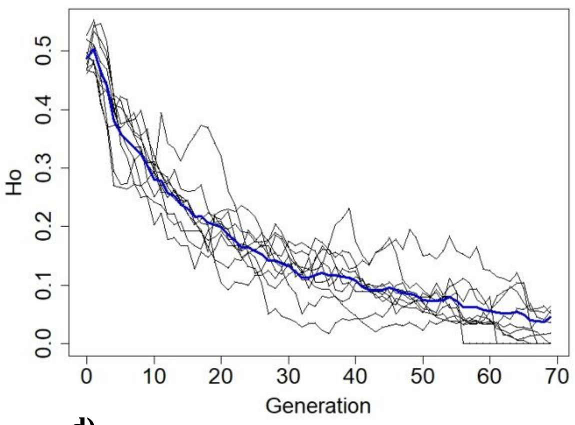
Generation

b)


Ho
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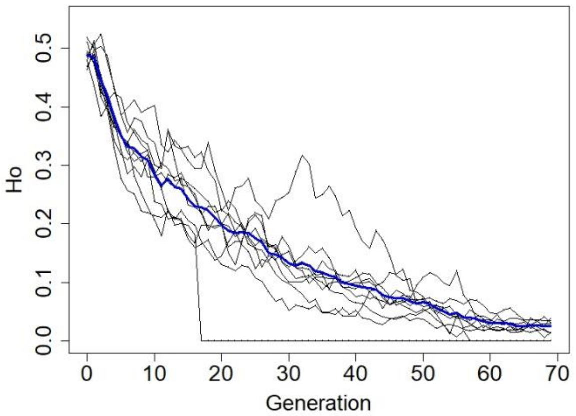
Generation

c)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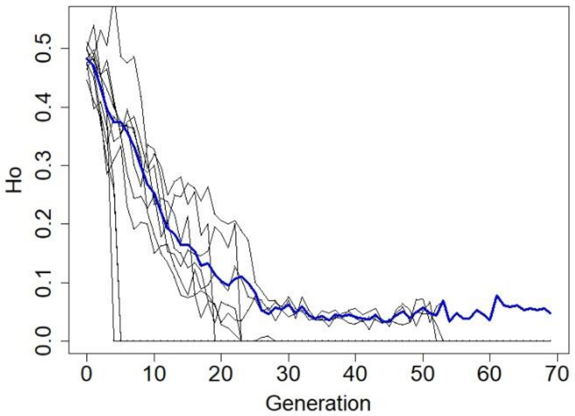
d)


Ho
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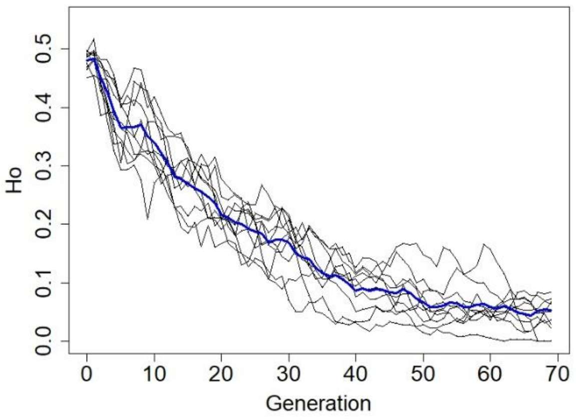
Generation

e)


Ho
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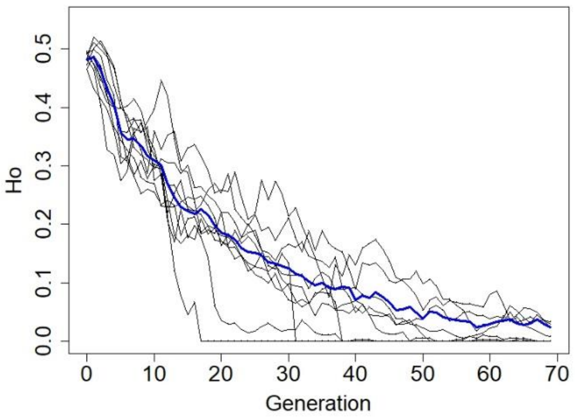
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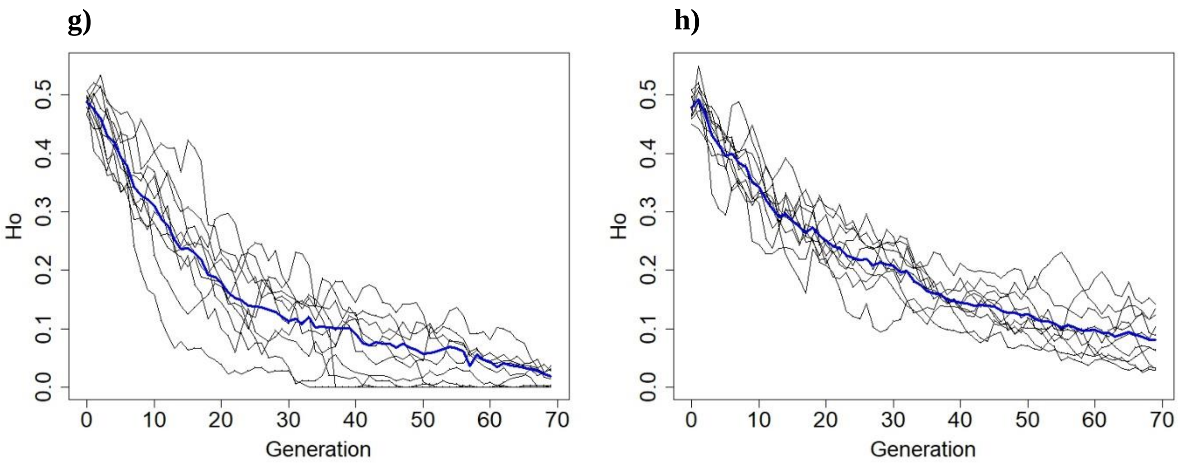

Fig. 1.10 Projected observed heterozygosity (H_O) in the simulated New England cottontail population in Londonderry, New Hampshire over 70 generations in CDPOP. Blue lines indicate the average values over 10 Monte Carlo replicates for the following scenarios: **a)** baseline model input; **b)** 0.80 juvenile mortality (recruitment 3 offspring/female); **c)** 0.73 juvenile mortality (recruitment 4.05 offspring/female); **d)** 0.70 adult mortality; **e)** 0.23 adult mortality; **f)** initial population size at carrying capacity of 65 individuals; **g)** initial population size of 50 individuals; **h)** baseline model input with 500 m average dispersal distance.

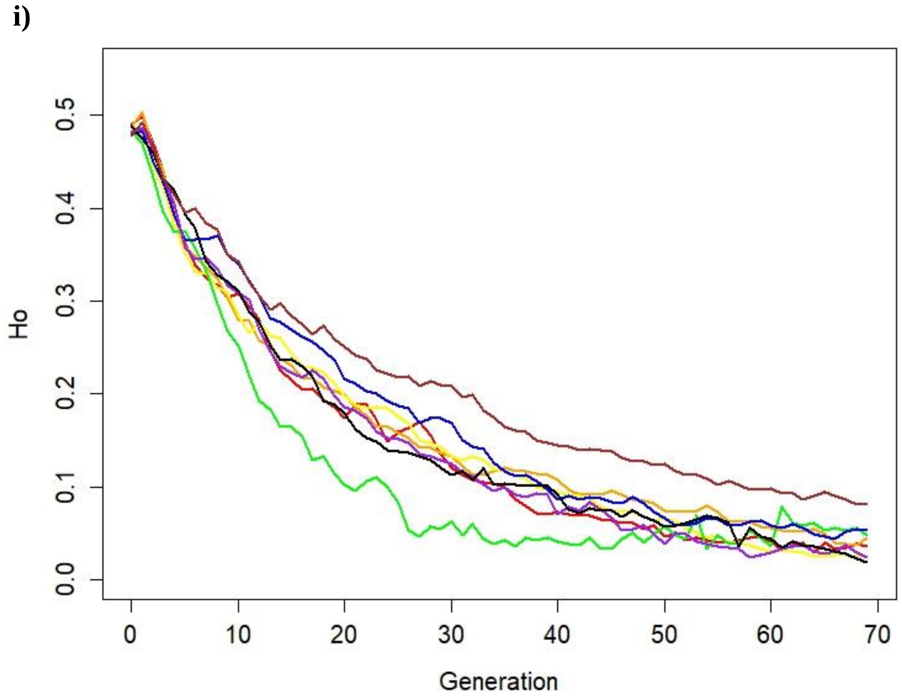

Fig 1.10i CDPOP averages for observed heterozygosity (H_O) in the simulated Londonderry, New Hampshire New England cottontail population over 10 Monte Carlo replicates for models: **a)** RED, baseline model input; **b)** ORANGE, 0.80 juvenile mortality (recruitment 3 offspring/female); **c)** YELLOW, 0.73 juvenile mortality (recruitment 4.05 offspring/female); **d)** GREEN, 0.70 adult mortality; **e)** BLUE, 0.23 adult mortality; **f)** PURPLE, initial population size at carrying capacity of 65 individuals; **g)** BLACK initial population size of 50 individuals; **h)** BROWN baseline model input with 500 m average dispersal distance.

Tracking predicted population response to a restoration scenario

When the Londonderry, New Hampshire New England cottontail population was simulated across 70 generations in a landscape reflecting restored habitat on managed parcels, population size and stability increased from the baseline scenario (Fig. 1.11i). The population size fluctuated between 35 and 40 individuals compared to a baseline scenario where populations persisted at about 20 individuals. Cottontail locations output at generations 10, 25, 50, and 70 indicated that individuals dispersed into restored parcels within the immediate vicinity of occupied patches to a distance of about 500 m, but did not disperse to restored parcels that were farther away from occupied patches (parcels that were >1200 m from occupied patches) given the high cost of the underlying landscape. Genetic diversity metrics remained low in this small projected population, with number of alleles and observed heterozygosity only slightly higher than the baseline projections (Figs. 1.11b, 1.11c).

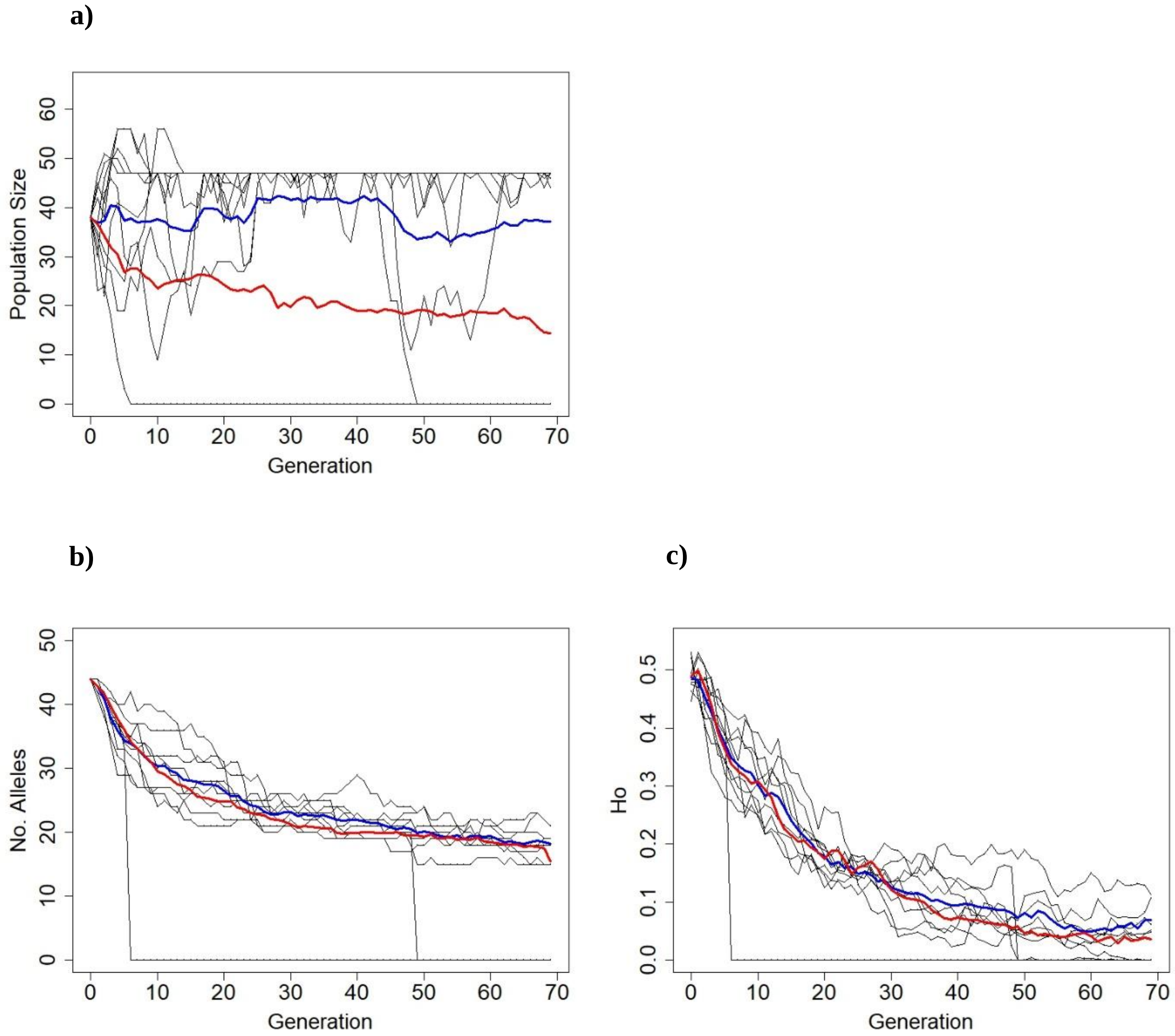

Fig. 1.11 a) Projected population size in the simulated New England cottontail population in Londonderry, NH on a restoration landscape over 70 generations in CDPOP. The restoration landscape includes managed habitat parcels reflected as suitable habitat for cottontail occupancy (see Fig. 1.2); **b)** projected number of alleles in the simulated New England cottontail population under the restoration scenario; **c)** projected observed heterozygosity (H_O) in the simulated New England cottontail population under the restoration scenario. Blue lines indicate the average value over 10 Monte Carlo replicates. Red lines provide a comparison to baseline averages over 10 Monte Carlo replicates.

Discussion

The effects of isolation due to habitat loss and fragmentation were evident for this population of New England cottontails in Londonderry, New Hampshire, including small effective population sizes (Bohonak 1999, Keyghobadi 2007), low genetic diversity (Brook et al. 2002, O'Grady et al. 2006), stochastic decline on certain patches (Stacey & Taper 1992), and break down of metapopulation function (Chandler et al. 2015). I observed three genetically distinct groups through STRUCTURE analyses over a small geographic extent of only 4 km, indicating limited connectivity among patches in this developed landscape. The three genetic groups were isolated on separate patches, with admixture in the central Stonyfield patch. The isolation of genetically distinct groups in separate patches reflects a history of low dispersal and barriers to gene flow in the landscape. For example, construction of a large industrial facility between the Stonyfield and Charlotte Street patches in 2013 likely isolated the Charlotte Street patch from the nearest source of potential immigrants in the metapopulation. Patterns of genetic structure that show multiple genetic groups on the central Stonyfield patch indicate that this patch may have historically served as a source patch for this metapopulation and exhibited some connectivity with surrounding patches. Management to restore young forest on the large, 20-acre Stonyfield patch took place in 2008, creating high quality habitat several years later that supported a greater abundance of cottontails than other patches in the metapopulation. Though the Stonyfield patch historically may have been more connected with surrounding patches reflecting a genetic signature of a source patch, I only detected one dispersal event out of Stonyfield during this three-year study.

Abundance on the Stonyfield patch declined in 2018, indicating the habitat may be aging to the point where it no longer provides ideal cover, and management is needed to restore the patch as a stronghold in this metapopulation. In 2018, abundance declined on the Cohas Brook patch, likely due to recent cutting that will ultimately create higher quality habitat, and abundance also declined severely on the Charlotte Street patch. Genetic diversity was low across all patches in the Londonderry metapopulation, but higher in the central Stonyfield patch and lower on isolated peripheral patches. Relatedness was high in each patch and for the metapopulation as a whole. Relatedness was lowest at 0.08 in the central Stonyfield patch where admixture had taken place and the population size was larger than on other patches. Relatedness was relatively high on more isolated patches, ranging from 0.122 to 0.136 (relatedness in the range of cousins).

Charlotte Street had the lowest observed number of alleles and heterozygosity, and high relatedness. Allelic richness would have been lower and relatedness higher on this patch prior to colonization by a male eastern cottontail and the production of hybrid offspring in 2017 by two female New England cottontails with this male (A. Kovach, M. Bauer in prep.). This documented hybridization was an apparently rare event resulting from recent expansion of the eastern cottontail range into parts of Londonderry, and the small and isolated New England cottontail populations persisting in this landscape. It was likely that there were no surviving adult males on the Charlotte Street patch in the summer of 2017 when the hybridization took place (A. Kovach, M. Bauer, H. Holman, B. Ferry unpublished data).

Spatial autocorrelation indicating low dispersal and fine-scale relatedness over a small distance of 400 m adds to the picture of isolated patches that have lost metapopulation function. Low dispersal rates in this population were further supported by the detection of only one dispersal event through pellet surveys, and one additional dispersal event out of 37 collared rabbits through a telemetry study (B. Ferry, NHFG, personal communication). In such a fragmented landscape, when dispersal does occur, it may not contribute toward sustaining populations or increasing gene flow if the dispersing individual moves to an unoccupied patch and cannot breed, as was the case with the dispersers detected in this study. For the Londonderry New England cottontail population, low dispersal rates between populations isolated by development indicate that, in the current landscape, populations are likely to remain small, isolated, and at risk of stochastic decline. Low dispersal in fragmented landscapes and resulting fine-scale population structure are significant challenges to New England cottontail population restoration range-wide (Fenderson et al. 2014, Amaral et al. 2016, Cheeseman 2017). The low dispersal capabilities of New England cottontails in fragmented landscapes emphasize the importance of restoring corridors to promote connectivity between occupied patches. Because the New England cottontail is a shrubland obligate, both within its home range (Barbour & Litvaitis 1993, Litvaitis 2003) and during dispersal (Amaral et al. 2016), linear corridors and large persistent patches (whether managed or natural shrublands such as wetlands), could be key in promoting dispersal to maintain metapopulations. Additional strategies such as reintroductions may also be necessary to create functional metapopulations (Chapter 2, Fischer & Lindenmayer 2000, DeMay et al. 2017).

Given the small geographic extent (4 km) of the occupied Londonderry patches, I lacked statistical power to robustly optimize cost values for a resistance surface reflecting landscape influences on gene flow in this population. Empirically optimized cost values in Londonderry were higher than in other previously studied landscapes for shrub wetlands, herbaceous wetlands, and forested wetlands. These values could have been an artefact of the complex history of isolation and admixture that formed the genetic structure of this metapopulation, related to factors such as development between patches. Therefore, I compared our empirical results from univariate resistance surface modeling with previously optimized values from landscapes in Maine and on Cape Cod, incorporated expert opinion, and used both a set of conservative and high cost values in multivariate resistance surfaces for connectivity mapping. CIRCUITSCAPE output highlighted the current isolation of occupied patches, with few paths of connectivity between Stonyfield and Charlotte Street, and few direct paths between Stonyfield and Buckthorn Street, other than the shrub wetland corridor passing through the Cohas Brook patch. CIRCUITSCAPE maps highlighted powerline rights-of-ways as the most prominent connecting feature in the Londonderry landscape, a feature which has also been highlighted as a potential focal point for restoration to connect cottontail populations across larger landscapes (Amaral et al. 2016). Powerline rights-of-ways have also been noted as potentially important movement corridors for other shrubland wildlife, such as shrubland specialist birds (M. Tarr, R. Shoe, unpublished data). Additional connectivity was highlighted through a shrub wetland area near the Cohas Brook patch, and along a railway between the Cohas Brook and Buckthorn Street patches that continues along the eastern edge of the landscape. Connectivity maps are a valuable tool to highlight potential focal areas for restoration to connect occupied patches or create habitat within the dispersal capabilities of the species. Patches or corridors identified as potentially valuable restoration sites can be explored further in simulations to predict the effect of restoration on population connectivity and persistence.

Simulation modeling of populations with CDPOP revealed that dispersal had a strong influence on population size and persistence. This insight highlighted the importance of restoring patches and corridors within the dispersal distance of sites occupied by New England cottontails. In the current baseline parameterization of the model, the frequency of dispersal is greater than is observed in empirical populations. With the input of a baseline average dispersal distance of 250 m (the minimum dispersal distance found to exceed within-home-range movements in empirical studies), ~30% of simulated cottontails were dispersing. This rate of dispersal is much higher than observed dispersal frequencies, with one dispersal event out of 37 collared New England cottontails observed in a telemetry study in Londonderry, NH (2.7% dispersal, B. Ferry, NHFG, personal communication) and 19 dispersal events observed out of 204 collared eastern and New England cottontails in a telemetry study in New York (9.3% dispersal, Cheeseman 2017). The high proportion of individuals dispersing in the current parameterization of the model is likely the factor that enables small populations of ~20 individuals to persist for a simulated 70 generations. However, long-term persistence of a population of 20 individuals is unrealistic. Populations this small will exhibit low genetic diversity, high relatedness, and be vulnerable to stochastic decline from sources such as severe winters, predation, skewed sex ratios that decrease mating opportunities, and declines in habitat quality over time. The low dispersal rates exhibited in natural populations could not be modeled with the dispersal functions available in the current CDPOP framework. Future research will evaluate outcomes with more realistic dispersal rates, however, this will require parameterizing a new version of CDPOP that allows users to input probability surfaces that provide more control over specifying dispersal (E. Landguth, personal communication).

The sensitivity of the model to high adult mortality was also an important finding. Survival is highly variable annually in New England cottontail populations, ranging from approximately 15-77% annual survival in New York and New Hampshire (A. Cheeseman, B. Ferry, personal communication). The impact of high mortality on modeled populations and variable empirical survival estimates highlight the vulnerability of small populations to stochastic decline given years of high mortality. Recruitment was also an influential parameter.

The highest recruitment tested (4.05 offspring per female) increased the size at which the population could persist. However, there is high uncertainty regarding recruitment values in wild populations and recruitment as high as 4 surviving offspring for every female in a population is likely higher than rates that would occur in natural populations. As such, a high adult mortality, within the range of values observed in empirical populations, is a more influential parameter than high recruitment to explore with future modeling. Overall, this model shows that dispersal is key to maintaining small populations in fragmented landscapes, high mortality such as that from stochastic events can threaten small populations, and genetic diversity declines dramatically in critically small populations.

The restoration scenario I simulated in Londonderry with restored management parcels did allow the population to persist at a higher size of between 35-40 individuals compared to the baseline outcome of a population of about 20 persisting in the landscape. Restoration of habitat patches within the immediate vicinity of occupied patches allowed the population to persist at a higher size than the baseline scenario. However, individuals did not disperse to restored patches farther from occupied patches given the high cost of forest and development between occupied and restored patches in the larger landscape. This indicates that translocations may be necessary to facilitate dispersal to restored patches to rebuild a metapopulation on a larger geographic scale.

Given the small abundance estimates fluctuating at and below 38 individuals in the Londonderry population, and observed declines on three of four patches in 2018, the population may be declining too quickly to respond to habitat restoration scenarios alone. To maintain this population, reintroductions could be needed to bolster and maintain populations on currently occupied patches while habitat patches are restored, starting with habitat restoration close to occupied patches and then restoring patches in the larger landscape. The Stonyfield patch will be managed in the near future to set back succession and restore shrubland habitat. Restoring parcels adjacent to a powerline corridor near occupied habitat could also provide a promising means to increase connectivity in this population. If natural dispersal does not occur from occupied patches to newly created habitat in the larger restoration landscape, for example if rights-of-ways are mowed too frequently to provide suitable dispersal habitat, reintroductions or translocations may be needed to initiate populations on restored patches.

Spatially explicit simulation modeling with a resistance surface approach provides a powerful means to compare the effects of alternate restoration scenarios on cottontail population size, population persistence, genetic diversity, and connectivity. By altering the underlying resistance surface to reflect changes in connectivity from the addition of habitat patches or restoration of corridors, managers can investigate the outcomes of alternate scenarios on cottontail population growth and dispersal. Managers can also use this simulation framework to investigate the outcomes of restoration strategies such as population reintroductions or augmentations. This modeling framework will be used to investigate management scenarios to determine the configuration and acreage of natural and managed habitat needed to sustain New England cottontail metapopulations in Londonderry, New Hampshire, and is a proof-of-concept which can be implemented in other parts of the species' range. Spatially-explicit simulations will provide insight to guide restoration efforts, e.g., if a particular management scenario is revealed to be substantially more successful than others, or if given the best-case restoration scenario, a population is unlikely to persist in a given landscape. This predictive knowledge will help managers balance competing management priorities and make justifiable decisions if triage is necessary to maintain a population.

Conclusion

I documented three distinct genetic groups in the Londonderry, New Hampshire New England cottontail population over a small geographic extent of only 4 km indicating limited dispersal and a loss of metapopulation function in this population. Spatial autocorrelation analyses, genetic diversity and relatedness metrics for isolated patches, and connectivity maps corroborated the finding of limited dispersal and gene flow in this highly developed landscape. Loss of metapopulation function has resulted in cottontails in the Londonderry population being isolated on certain patches. Cottontails persist at low abundances on isolated patches, and are at risk of decline from stochastic and demographic processes. Without connectivity between patches in this metapopulation, the population is unlikely to persist long-term. These findings highlight the importance of dispersal for maintaining New England cottontail populations in fragmented landscapes. The simulation framework I applied for this cottontail population serves as a proof-of-concept to compare alternate restoration strategies that aim to increase dispersal and rebuild a functioning metapopulation through the restoration of managed shrubland in combination with persistent shrublands such as shrub wetlands and powerline rights-of-ways. This modeling framework will allow managers to project population size and genetic diversity to identify restoration scenarios that best promote connectivity, and to predict the ability of cottontail populations to persist in a developed landscape with a mosaic of natural and managed shrub habitats.

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CHAPTER 2

TRACKING THE SUCCESS OF A NEW ENGLAND COTTONTAIL POPULATION REINTRODUCTION WITH GENETIC MONITORING[1]

Abstract

Intensive 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 habitat specialist, the New England cottontail, in southeastern New Hampshire. We monitored the apparent survival and breeding success of founder individuals and tracked changes in population size and genetic diversity for five years following an initial reintroduction in 2013. We released 42 rabbits, documented 30 unique offspring in years following releases through noninvasive surveys, identified 6 founder individuals that bred, and documented variable apparent survival of founders from the release period to the winter survey period ranging from 0 to 62.5 percent. The population size remained relatively stable during the first three years of the introduction, declined in 2017, and rebounded slightly in 2018. Genetic diversity increased as population size increased and additional founders with diverse genetic backgrounds were released and bred. Newly recruited juveniles were identified each year, and dispersal of juveniles to a restored patch 700 m from the release site was documented. Genetic diversity declined after the population declined in 2017 and remaining individuals on the patch were highly related. For New England cottontail reintroductions to be successful in the long term, releases will be needed

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[1] Melissa L. Bauer, Brett Ferry, Heidi Holman, Adrienne I. Kovach. Manuscript in preparation for the *Wildlife Society Bulletin*.

at multiple patches within dispersal distance, and habitat corridors need to be created or restored between patches to create a functioning metapopulation. For small or isolated reintroduced populations that are not yet functioning as metapopulations, continued intensive monitoring is needed to detect stochastic declines in population size or changes in sex ratios and react accordingly with subsequent reintroductions. Noninvasive genetic sampling is a valuable tool to monitor reintroductions of the New England cottontail and other threatened species and provide managers with detailed information to inform decision-making in an adaptive management framework.

Key Words: reintroduction, monitoring, New England cottontail, noninvasive genetic sampling

Introduction

Reintroductions are an important strategy to conserve small and endangered wildlife populations (Fischer & Lindenmayer 2000, Fritz et al. 2001, Whittaker et al. 2004, Seddon et al. 2007, Jachowski & Lockhart 2009). There are many challenges to consider when recovering small populations. 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 & 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, DeMay et al. 2017).

We used noninvasive genetic monitoring to track a local reintroduction of a threatened habitat specialist, the New England cottontail (*Sylvilagus transitionalis*). The New England cottontail, New England's only native rabbit, 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 & Litvaitis 1993, Litvaitis 2003), and during dispersal (Fenderson et al. 2014, Amaral et al. 2016). Although patchy and ephemeral by nature, these shrubland habitats have declined in area and experienced extensive fragmentation in the northeastern United States due to forest maturation, widespread development, and suppression of natural disturbance regimes that maintain early successional habitat (Litvaitis 1993, Litvaitis 2003, Schlossberg & King 2007). Today, New England cottontails are isolated into five geographically (Litvaitis et al. 2006) and genetically (Fenderson et al. 2011) distinct regional populations (Fig. 2.1). 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 2011, 2014, Cheeseman 2017).

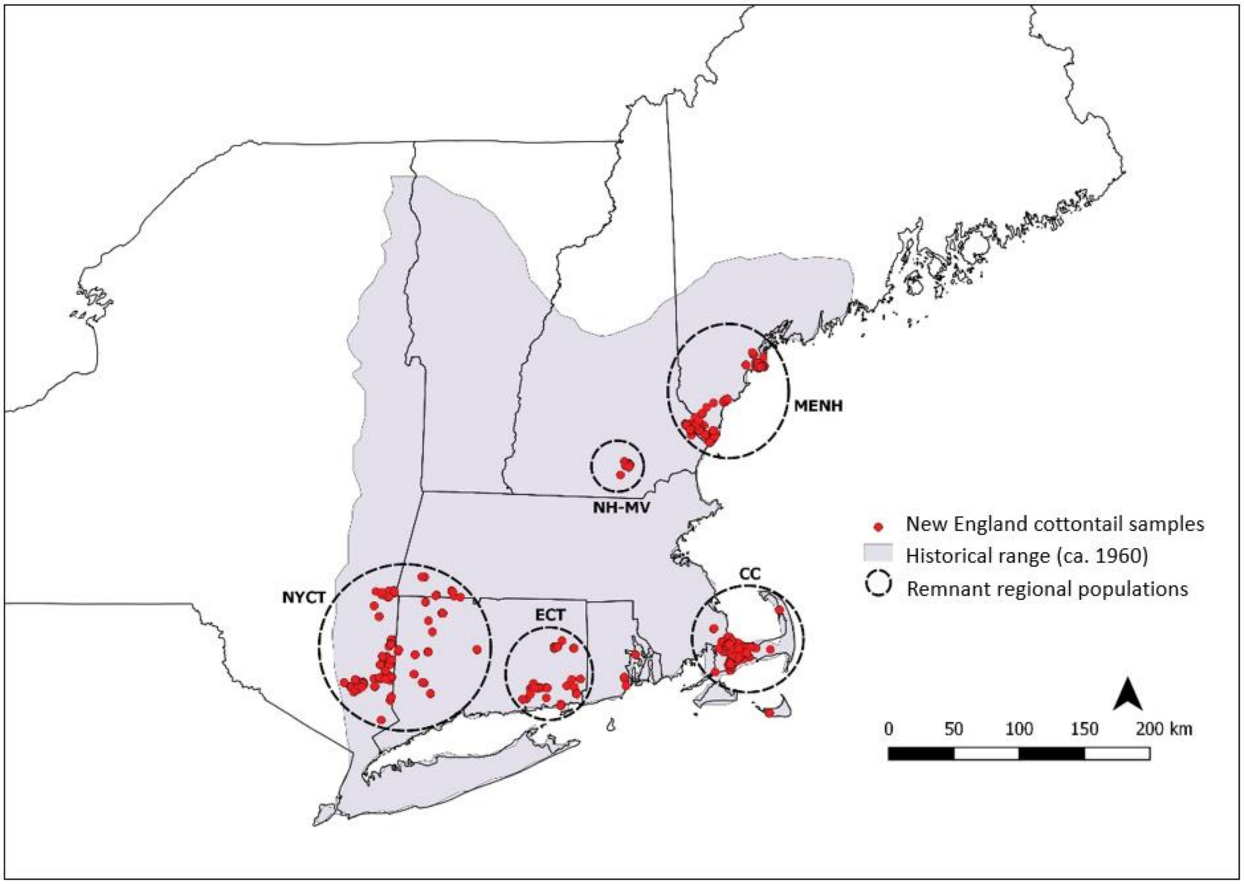

Fig. 2.1 Five geographically and genetically distinct New England cottontail populations, following Fenderson et al. (2011): MENH – southern Maine and seacoast New Hampshire; NH-MV – Merrimack River Valley region of New Hampshire; NYCT – southeastern New York, western Connecticut, and southwestern Massachusetts; CC – Cape Cod, Massachusetts; ECT – eastern Connecticut; with remnant and reintroduced populations in Rhode Island, including a captive island colony. Samples in the figure indicate New England cottontails detected from 2011 through 2017 (locations obtained from New England Cottontail Technical Committee regional monitoring data, unpublished).

Loss and fragmentation of shrubland habitat have impeded dispersal within New England cottontail metapopulations; historically dispersal movements would have offset patch extinctions in stably persisting metapopulations. Within each metapopulation, cottontails persist on remnant patches of shrubland habitat surrounded by an inhospitable landscape matrix, with roads, development, and mature forest serving as dispersal barriers (Amaral et al. 2016). New England cottontails exhibit low dispersal capabilities in these landscapes. A telemetry study in New York documented a median movement distance of approximately 50 m. Movements greater than 250 m were exceedingly rare, and for New England cottontails that did disperse, the median dispersal distance was 512 m (Cheeseman 2017). Further, New England cottontails in that study 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 documented 1 dispersal event out of 37 collared New England cottontails, and the dispersing cottontail moved 900 m before being predated (B. Ferry, NHFG, personal communication).

In response to declining New England cottontail populations and their nine-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 8,600 acres restored or maintained across the range of the New England cottontail as of 2017 (New England Cottontail Technical Committee 2018), and the development of a captive breeding program. 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 and an outdoor breeding pen in New Hampshire. Releases of captively-reared individuals from zoos, the island colony, and outdoor breeding pen 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 in 2017. The goal of this study was to use noninvasive genetic sampling to monitor the success of the first reintroduction at Bellamy River WMA from 2013-2018. 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. 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 suitable 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.

Methods

Study area

Bellamy River WMA (43.156030, -70.857880) is a 400-acre property in Dover, New Hampshire comprised of a variety of habitats including mature forest, wetlands, fields, and shrublands. Approximately 113 acres of habitat projects have been completed to create shrubland habitat on this property, and about half of those acres have grown into the dense shrub habitat required by New England cottontails. Two key shrubland patches include a 25-acre release site on the northern portion of the property, and an additional 25-acre 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 did not identify any individuals remaining on the patch. Bellamy River WMA and the surrounding landscape is 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 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 ~156 acres have been completed at nearby sites within a 3 km distance from Bellamy, and of that 23 acres of dense shrubland habitat have been restored that could support cottontails.

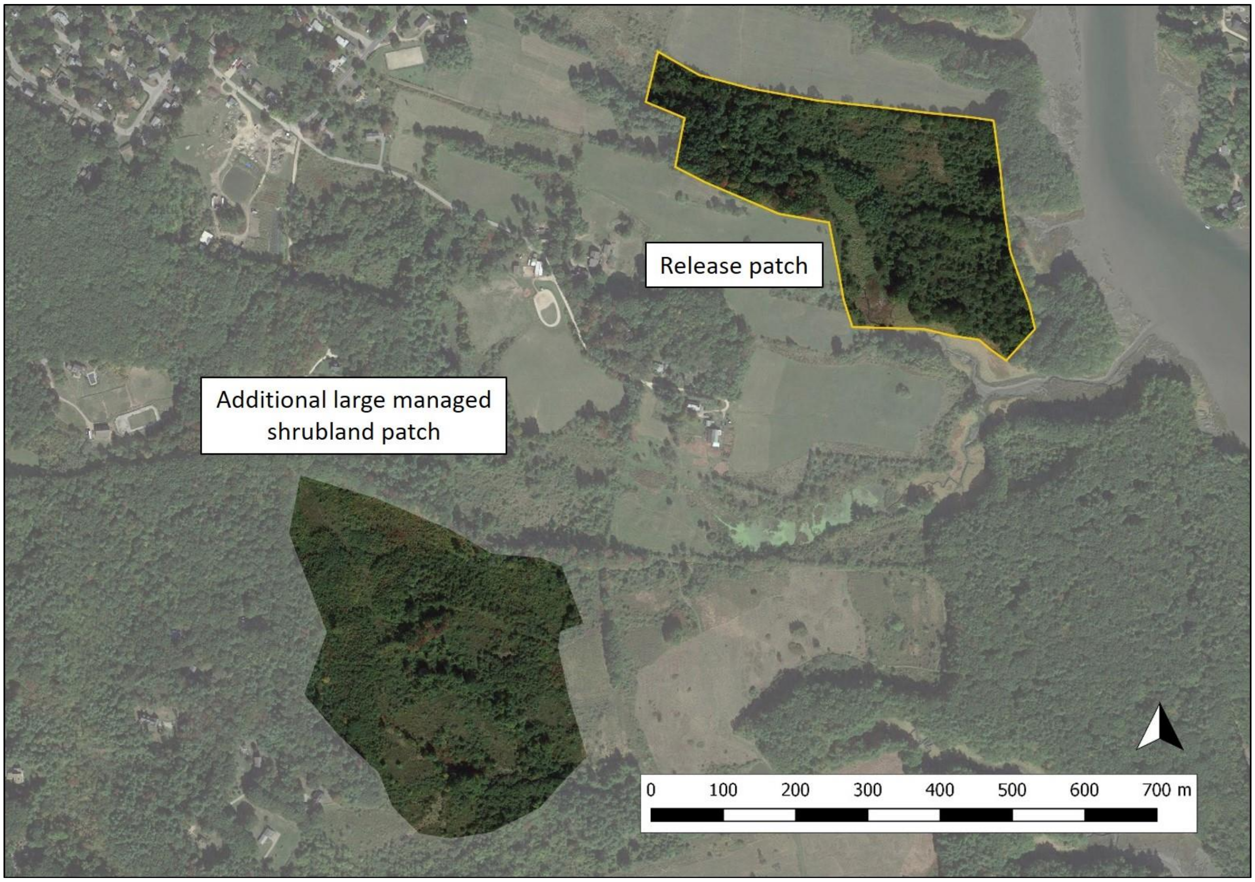

Fig. 2.2 Bellamy River Wildlife Management Area. Release patch for founder New England cottontails is outlined in yellow and a second large managed shrubland patch south of the release patch is highlighted.

Founder individuals were released 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 Refuge (Charlestown, RI, USA). Founders were released in the center of the release patch (Fig. 2.2) primarily in the fall, but some were released in the spring and late summer (Table 2.1). Pellet surveys were conducted in the winter.

Table 2.1 Number of founder New England cottontails released at Bellamy River WMA each year, and month of release.

Year	Month	No. founders released
2013	July	5
2013	Oct.	3
2014	Apr.	2
2014	Sept.	5
2014	Oct.	8
2014	Nov.	3
2015	July	2
2015	Oct.	5
2017	Aug.	4
2017	Oct.	2
2017	Nov.	3

Winter pellet surveys and samples from founders

Spatially referenced (Garmin GPSMAP 64s, Olathe, KS) cottontail fecal pellet samples were collected during winter surveys conducted from 2014 through 2018 under optimal survey conditions to detect New England cottontails (snow depth <30.5 cm, wind speed <40 km/h; Brubaker et al. 2014) in a fine-scale sampling scheme, following the methods of Kristensen & Kovach (2018) (two independent surveys 3-5 d after snowfall, with 30 m spacing between search transects). Prior to release, a tissue biopsy was collected from the ear of founder individuals. Fecal pellets were stored in 15-mL conical tubes at -20 ºC and tissue samples were stored in 100% ethanol until DNA extraction. Founder individuals were outfitted with radio-collars (Advanced Telemetry Systems M1555, Isanti, MN) with a mortality signal to track survival and monitored 1-5 times weekly. For all mortalities, date and cause of mortality were recorded.

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.

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, Valencia, CA, USA). DNA was amplified in three multiplex polymerase chain reactions (PCR) (see Appendix A 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 locus developed for sex identification in the European rabbit (Vašíček et al. 2011). PCR products were electrophoresed on a 3730*xl* 96-capillary DNA Analyzer at the Yale DNA Analysis Facility (New Haven, CT, USA). Alleles were manually scored in PeakScanner (Applied Biosystems, Foster City, CA, USA).

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. For quality control of low copy DNA, we used a multiple tubes approach (Frantz et al. 2003, Waits & Paetkau 2005). We required two replicate allele observations for heterozygous loci, and three replicate observations for homozygous loci to determine a consensus genotype (Frantz et al. 2003). We quantified the per allele and per locus genotyping error by comparing the genotypes of all replicates to the consensus genotype (Pompanon et al.

2005). Samples missing data at three or more loci were excluded from analyses. 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 multi-locus matches option in GenAlEx 6.5 (Peakall & Smouse 2006, 2012). We re-evaluated samples differing at only one to two loci and considered samples with mismatches that appeared to be due to allelic dropout the same individual. We calculated the probability of identity of siblings ($P_{ID\text{-}SIBs}$), the probability that two 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 & 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). COLONY settings included 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 & Smouse 2006, 2012). We calculated allelic richness corrected for sample size in FSTAT 2.9.3.2 (Goudet 1999, 2002). 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 four patches in the Londonderry population (Chapter 1). We estimated census population size using a single session mark-recapture method in the R package *capwire* 1.1.4 (Pennell & Miller 2015) for years with sufficient recapture data. We estimated effective population size, the number of breeding individuals in a population, in NeEstimator 2.01 (Do et al. 2014) using the linkage disequilibrium method, with the combined data from all years of the study.

Results

Survey detection and founder survival

We surveyed 50 acres with intensive annual fecal pellet surveys in suitable habitat surrounding the release site. We collected a total of 191 pellet samples during the five winter survey seasons (2014-2018), successfully genotyped 175 samples, and identified 36 unique individuals, 5 of which were detected over multiple years (Table 2.2). Of the unique individuals detected, 6 were released founders and 30 were offspring recruited into the population. Genotyping success varied across years from 87.2 to 100.0 percent, and a range of 1 to 21 samples were collected per individual (Table 2.2). The probability of identity for siblings was 3.5×10^{-5} for this population, meaning that there was a one in 28,571 chance that two siblings share the same genotype at these genetic markers. Molecular sex identification agreed with field sex for all founder individuals.

Of the 42 founders that were released overall, 9 survived long enough to breed or until at least the following winter (detected through telemetry, in winter fecal pellet surveys, or as breeders through parentage analyses), including 5 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 2.3). Apparent annual survival of founders ranged from 62.5 percent in 2013 to 0 percent in 2014 (Table 2.3).

Detection of surviving founders was high overall, but imperfect and varied by year. 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), one founder was not detected each year from the 2013, 2015, and 2017 releases. Parentage analyses were useful in identifying individuals that were not detected in pellet surveys. One founder from the 2013 release was not detected in the winter but identified as a breeder the following summer. One adult present during the 2017 winter surveys (offspring of founder reproduction in prior years), but not detected, was identified as a parent of offspring born the following summer and was subsequently detected in 2018 winter surveys.

Table 2.2 Number of New England cottontail fecal pellet samples collected during winter surveys at Bellamy River WMA each year of the reintroduction, number of pellets successfully genotyped, percent genotyping success, number of unique individuals identified, and range of number of samples collected (i.e. captures) per individual.

Year (winter surveys)	No. pellet samples collected	No. pellet samples genotyped	Genotyping success (%)	No. unique individuals	Range of captures
2014	20	18	90.0	10	1-5
2015	23	21	91.3	8	1-8
2016	78	68	87.2	12	1-21
2017	18	18	100.0	4	2-8
2018	52	50	96.2	7*	1-15
TOTAL:	191	175	**AVG:** 92.9	8.2	4.1

*This does not account for the few cases in which founders were known not to be detected (see text).

Table 2.3 Number of founder New England cottontails released each year at Bellamy River WMA, number of mortalities within a month of the release date, number of founders detected surviving through the winter survey period or identified as breeders, percent apparent annual 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. founders released	No. mortalities within 1 month	No. survived (detected through telemetry, winter surveys, or breeding)	Apparent survival (%)	Founder detection (%)
2013	8	0	5	62.5	80.0
2014	18	5	0	0	NA
2015	7	3	1	14.3	0
2017	9	5	3	33.3	66.6

Population size and genetic diversity

The population remained relatively stable for the first three years after the initial reintroduction in 2013, experienced a decline in 2017 (Fig. 2.3, Fig. 2.5), and began to stabilize again in 2018 (Fig. 2.3, Fig. 2.6). For years where capture histories were sufficient to produce population size estimates, abundance ranged from 8-13 individuals, largely consistent with the number of unique individuals detected (Table 2.4). The effective population size estimate was 3.7 breeding individuals (95% CI 3.3-5.1) across all years. This estimate is slightly lower than the average of 5.4 breeding individuals identified each year through parentage analyses in COLONY (range 2-7 breeding individuals per year). Allelic richness and heterozygosity of the population increased as founder alleles were incorporated into the population. Allelic richness decreased with a population decline from 12 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, allelic richness and heterozygosity were higher than the only other remnant population in New Hampshire, located in Londonderry (M. Bauer and A. Kovach, unpublished data, Chapter 1). Table 2.5 shows genetic diversity metrics for the remnant Londonderry population for comparison to the reintroduced Bellamy population.

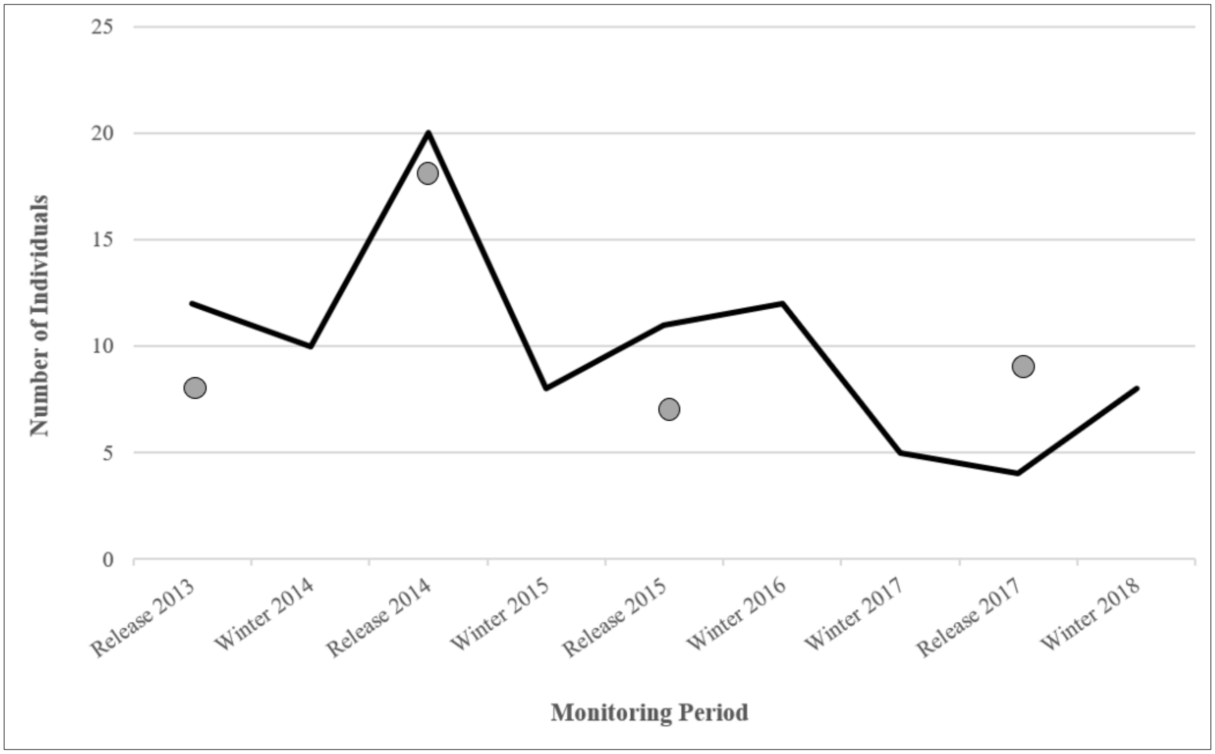

Fig. 2.3 Number of individuals known to be present in the release site and surrounding patches on Bellamy River WMA during each spring through fall release period or winter monitoring period. Number of individuals (black line) was calculated from number of founders released and surviving longer than one month, number of individuals detected during winter pellet surveys, and number of individuals known to be present through parentage analyses. Gray circles indicate the number of founders released each year. *All founders released in 2014 had confirmed mortality through telemetry prior to winter pellet surveys.

Table 2.4 Population size estimates of the reintroduced Bellamy River WMA population calculated in *capwire* for years with sufficient capture history data.

Year (winter surveys)	No. pellet samples collected	No. unique individuals	*Capwire* abundance estimate (95% CI)	Avg. pellet captures per individual	Range of captures per individual
2014	20	10	11 (10-13)	2.2	1-5
2016	78	12	12 (12-14)	5.6	1-21
2018	52	8	8	6.3	1-15

Table 2.5 Genetic diversity metrics of the reintroduced Bellamy River WMA population including individuals detected as breeders or alive on the patch during winter pellet surveys. Metrics for the Londonderry, New Hampshire population are provided for comparison. In Londonderry, the Stonyfield patch is a source patch, Buckthorn Street and Cohas Brook are patches close to the source patch, and Charlotte Street is a more isolated patch with lower genetic diversity. Samples were collected from the Londonderry patches in the following years: Stonyfield 2015-2017, Buckthorn Street 2016-2017, Cohas Brook 2015-2017, Charlotte Street 2017. No. individuals: number of individuals identified as breeders plus offspring for Bellamy, and number of individuals detected for Londonderry; H_O: observed heterozygosity; r: relatedness calculated in ML-Relate.

Year or population	Patch size (acres)	No. individuals	No. of alleles	Allelic richness	H_O	r
Bellamy						
2014	50	10	2.7	2.8	0.514	0.134
2015	50	14	3.1	3.0	0.567	0.143
2016	50	14	3.5	3.4	0.569	0.170
2017	50	6	2.7	2.6	0.569	0.083
2018	50	6	2.0	2.0	0.400	0.147
Londonderry patches						
Stonyfield	21	21	3.3	3.2	0.549	0.080
Buckthorn St.	14	11	3.3	2.6	0.524	0.122
Cohas Brook	20	16	3.0	2.9	0.453	0.136
Charlotte St.	11	8	2.9	2.7	0.450	0.127
Londonderry TOTAL:	66	57*	3.7	3.7	0.503	0.098

**Londonderry TOTAL includes 1 additional isolated individual not grouped with any of the 4 patches.*

Founder reproduction and dispersal

The number of recruited offspring detected during each year's winter surveys ranged from three to nine. During each of the first three years of the reintroduction, there were seven breeding individuals, but only one breeding pair following the population decline in 2017 (Table 2.6). Two males and one female were detected breeding over two consecutive years. One male successfully sired offspring with four different females in 2013 and three different females in 2014. Females often bore offspring with two separate males during a season, but not with more than two males. Females produced as many as four surviving offspring per season (i.e. the offspring were born in the preceding summer and to be detected had to survive at least ~6 months until the following winter), with an average of 2.1 recruited offspring per season.

Individuals born in the wild were also documented breeding, producing second generation wild-born individuals. Four individuals were detected surviving through two winter survey seasons, and one male was detected in pellet surveys for three consecutive years. In the second winter survey season, individuals were detected 700 m southwest of the release site in another 25-acre patch of shrubland, indicating that dispersal occurred (Figs. 2.4-2.6).

Table 2.6 Number of offspring identified in the reintroduced Bellamy River WMA population each winter survey season, number of males and females identified as parents, number of breeding founders, number of parents identified that were not detected in pellet surveys, and number of offspring with full and half sib relationships.

Year (winter surveys)	No. offspring	No. males breeding	No. females breeding	No. founders breeding	No. unsampled parents	No. full sibs	No. half sibs
2014	6	3	4	3	4	0	6
2015	8	4	3	4	1	4	8
2016	9	3	4	2	3	4	10
2017	3	2	2	0	1	0	2
2018	4	1	1	0	0	4	0

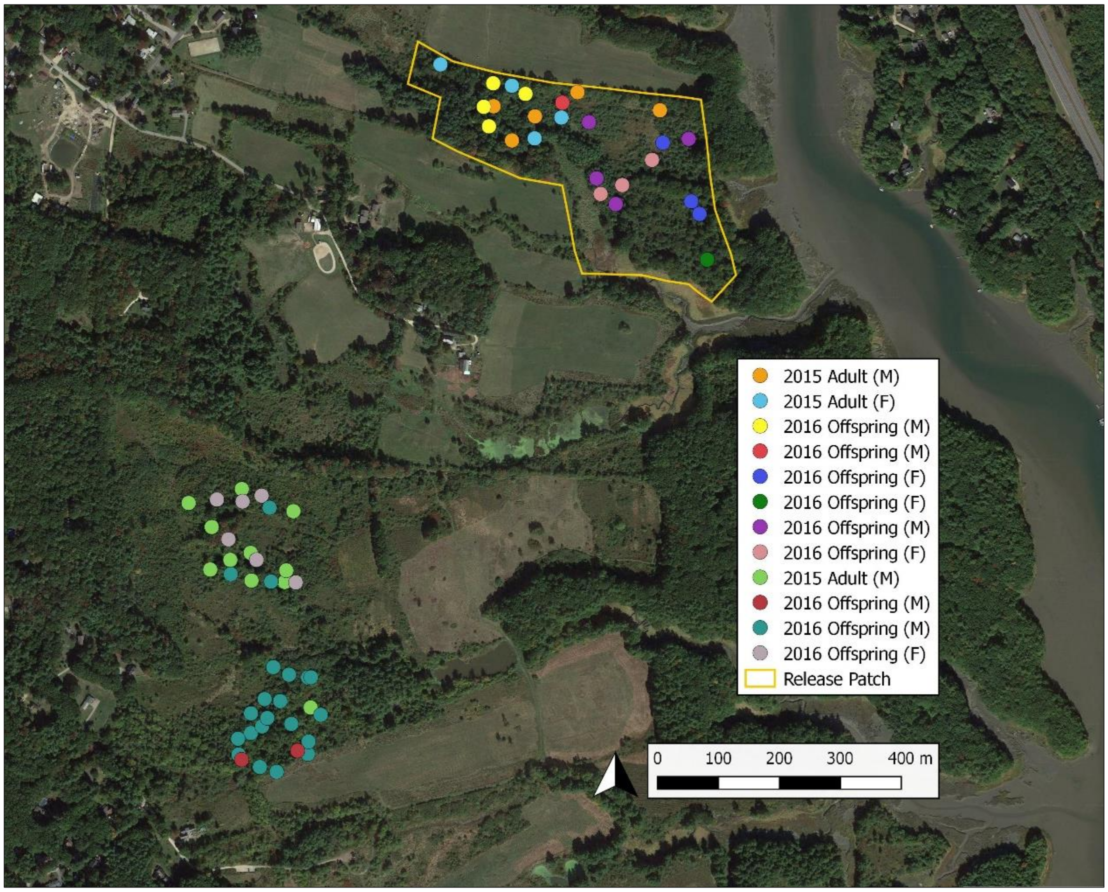

Fig. 2.4 Individual New England cottontail adults and presumed offspring (M=male, F=female) identified in pellet surveys at the Bellamy River WMA release site and surrounding locations during the winter 2015-2016 survey period showing dispersal from the release site (outlined in yellow) to another managed shrubland patch to the southwest. Adults were present on the patch in summer 2015 and survived to be detected in winter 2016, and presumed offspring were born in summer 2015 and first detected in winter 2016.

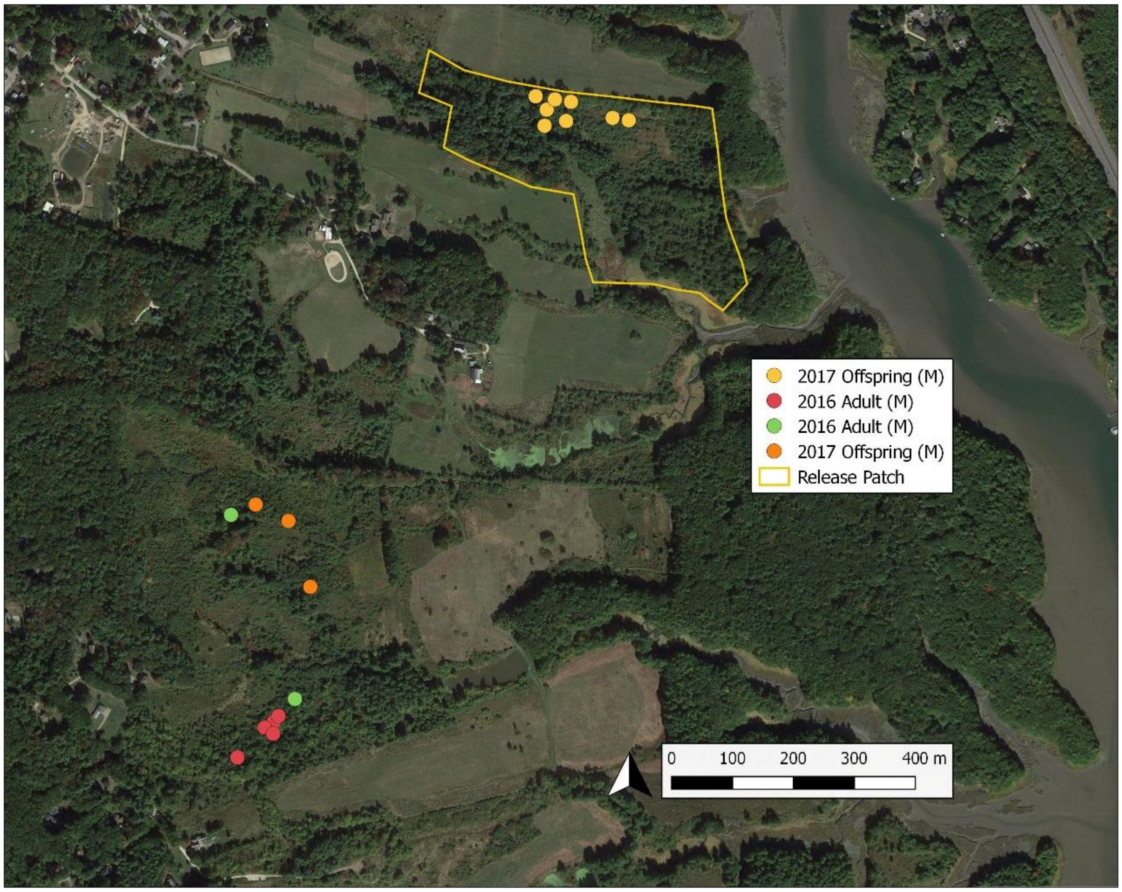

Fig. 2.5 Individual New England cottontail adults and presumed offspring (M=male, F=female) identified in pellet surveys at the Bellamy River WMA release site and surrounding locations during the winter 2016-2017 survey period showing a decline in the population from the previous winter survey period (winter 2015-2016). Adults were present on the patch in summer 2016 and survived to be detected in winter 2017, and presumed offspring were born in summer 2016 and first detected in winter 2017.

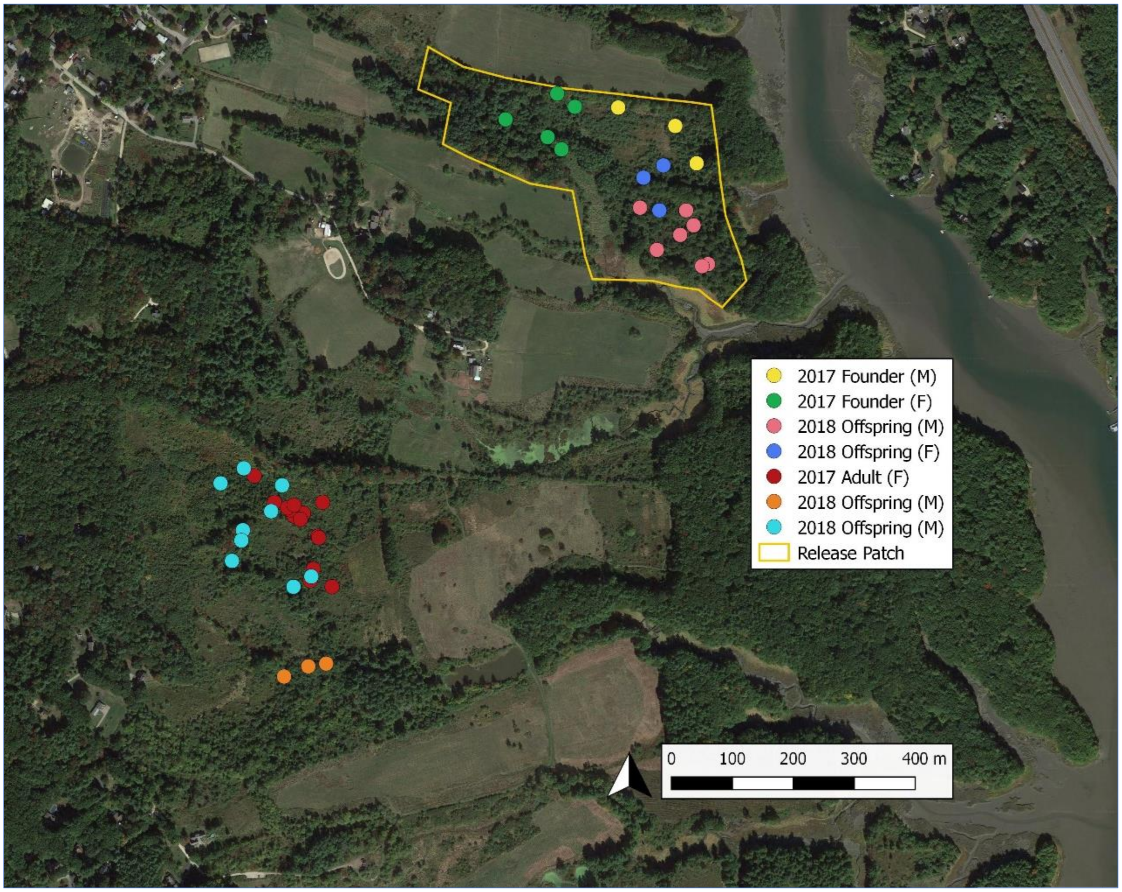

Fig. 2.6 Individual New England cottontail adults and presumed offspring (M=male, F=female) identified in pellet surveys at the Bellamy River WMA release site and surrounding locations during the winter 2017-2018 survey period showing an increase in population size following the decline of the previous year. Adults were present on the patch in summer 2017 and survived to be detected in winter 2018, and presumed offspring were born in summer 2017 and first detected in winter 2018. Founders were released in fall of 2017, after the breeding season. All offspring are full siblings from a male and female present on the patch prior to 2017 founder releases.

Discussion

Genetic monitoring is a valuable tool to evaluate the success of population reintroductions (Schwartz et al. 2007, Johnson et al. 2010, Olson et al. 2013). Noninvasive genetic sampling provides critical insight into the viability and recovery of populations of rare or cryptic species (Waits & Paetkau 2005, 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, for five years post-reintroduction. We tracked the survival and reproduction of founder rabbits and quantified changes in population size and genetic diversity following releases. Annual monitoring revealed changes in the population status that influenced the subsequent management response, allowing for adaptive reactions in this conservation effort.

Apparent survival of founder individuals was variable annually, ranging from 0 percent of founders surviving through the winter survey period to 62.5 percent surviving. In winter 2015, the 0 percent apparent survival of founders was due to extensive deep snowfall. There were three two-foot snowstorms over a less than two-month period between the end of January 2015 and March 2015, and starvation and predation were causes of mortality. Other recent studies incorporating information on New England cottontail survival indicate extremely variable survival annually, with estimates ranging from approximately 10 to 75% survival (A. Cheeseman, B. Ferry, unpublished data). New England cottontail survival has been found to be lower on small patches (Barbour & Litvaitis 1993, Litvaitis & Villafuerte 1996) with estimates of 23% survival on sink patches and 45% survival on source patches (Litvaitis & Villafuerte 1996). Of all individuals detected, including wild-born offspring, only four of the 36 were detected surviving through two winter survey periods, and one male was detected in pellet surveys for three years, indicating low survival past age one. It is thought that New England cottontails generally don't live longer than two to three years (Fuller & Tur 2012).

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 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 & 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 (*Oryctolagus cuniculus*) (Calvete et al. 1997, Letty 1998, Letty et al. 2002) and 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 two days following release, and 69% within the first month due to predation by mammalian predators. Mortality following heavy snowfall was documented in 2015 both directly from predation, and with predation resulting following a decline in body condition of individuals. In 2016 a mortality was documented five days after a 6-inch snow event and in 2018 a mortality was documented two days after an 11-inch snow event. This trend has been noted in other studies, with increased mortality from predation documented with an increase in the number of days of snow cover for New England cottontails (Brown & Litvaitis 1995), and increased predation documented with an increase in both snow depth and persistence for eastern cottontails (Boland & Litvaitis 2008). As has been suggested with other lagomorph reintroductions with high post-release mortality, releasing larger groups of individuals simultaneously may be necessary to ensure stable breeding populations following the acclimation period (Armstrong & Seddon 2008, Hamilton et al. 2010). More research is needed on the number of individuals that need to be released to combat post-release mortality, but decisions should take into consideration both mortality rates of released individuals and the carrying capacity of the reintroduction landscape. For example, presuming a density of 2 cottontails/ha and ~10 ha of habitat in each of the two patches at Bellamy WMA, and the survival we observed for the first year of the release of 62.5%, 64 cottontails would need to be released to fill the estimated carrying capacity of this landscape of 40 cottontails. Additional releases in subsequent years would need to take into account the number of individuals detected on the patch and higher mortality rates exhibited after the first year of a release to determine the number of individuals to release. Reintroductions of other lagomorphs have required releasing a large number of individuals, for example 100-800 individuals per year for a reintroduction of pygmy rabbits with survival rates ranging from 39% in the first year to 10% in the third year of the release (DeMay et al. 2017).

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 (*Sylvilagus bachmani riparius*) (Hamilton et al. 2010). This trend 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), competition with established rabbits, or differences in release methodology between years (Hamilton et al. 2010). Competition could have been a factor in this reintroduction as aggressive interactions between males have been documented in New England cottontails (Tefft & Chapman 1987) and eastern cottontails (*Sylvilagus floridanus*; McKinney 1970, Brenner & 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 up to 8 individuals on the patch from the previous winter and survival was 14.3%, and when founders were released in 2017 there were only 5 individuals on the patch from the previous winter, and survival was slightly higher at 33.3%.

Successful breeding was documented for founder individuals as well as wild-born offspring. Parentage analyses support a promiscuous breeding strategy. Males produced offspring with one to four females per season, and females often produced offspring sired by two different males in a season. Not all contributing breeders were sampled in each year (i.e. in some years, unsampled individuals had the highest parentage probability in COLONY analyses). This led to gaps in the pedigree in later years of the study. Similarly, in a study that used winter fecal pellet surveys to monitor the reintroduction of a pygmy rabbit population, DeMay et al. (2017) did not sample all parents leading to gaps in the pedigree later in the study. Conducting two independent surveys (Kristensen & Kovach 2018) with closer spacing than the currently implemented 30 m between transects, and avoiding surveying after heavy snowfall events could increase detection and improve the ability to track founder survival and reproduction in cottontail reintroductions.

Population size remained relatively constant for the first three years of the reintroduction, declined substantially in 2017, and rebounded slightly in 2018. Estimated effective population size over all years combined was low (mean 3.7 breeding individuals, 95% CI 3.3-5.1), and slightly lower than the average number of breeding individuals identified through parentage analyses (5.4 individuals, range 2-7). Given the extremely small population size, without further monitoring and possible additional reintroductions, this population remains at high risk of decline due to 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 skewed sex ratio, with four males and one female in the population. In 2018, remaining individuals were highly related, including the 2017 female and her offspring, plus two unrelated surviving founder males released in fall of 2017 that had not yet bred. The release of founder rabbits over several years successfully bolstered genetic diversity in this population, but genetic diversity markedly declined following the 2017 population decline. After only two breeding seasons, the observed heterozygosity in this reintroduced population surpassed that of the largest remnant population in New Hampshire (Londonderry, NH population). After three breeding seasons, allelic richness surpassed that of the Londonderry population as additional founder alleles were incorporated into offspring in the reintroduced Bellamy population. To buffer the potential impacts of stochasticity, continued monitoring is needed, with additional reintroductions following population declines. 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 that can provide dispersers to offset patch extinctions, maintain sufficient population sizes, and prevent bottlenecks.

Dispersal was documented from the release site to another high quality shrubland patch 700 m away within the wildlife management area, exemplifying the potential for a reintroduced population to occupy a landscape in a metapopulation context through reproduction and dispersal. That patch remained occupied each year after the initial dispersal event. This location in the Bellamy 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 present on patches surrounding the release site to breed with.

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 each of three of the four years that individuals were released. Parentage analyses identified one founder and one wild-born individual that were present at the time of pellet surveys, not detected, but identified breeding the summer after winter surveys. Similar detection results were documented for a reintroduction at Wells National Estuarine Research Reserve in Wells, Maine. Two intensive winter pellet surveys were conducted, and of the seven radio-collared rabbits known to be on the site at the time, only six were detected (M. Bauer and A. Kovach, unpublished data). A collared rabbit known to be on the site was not detected during surveys at Bellamy in 2018 following heavy snowfall. Decreased detection following heavy snow events could be due to subnivean behavior (Katzner & Parker 1997, Brubaker et al. 2014), decreased cottontail movement, or snow falling off branches and covering pellets following a heavy storm (J. Tash, C. Stearns, personal communication). Surveys conducted at Wells in 2018, however, were not following heavy snow events, indicating that variation in individual cottontail movement ranges or other factors may require more intensive surveying to detect all rabbits on a patch, for example by decreasing the spacing between search transects. Multiple surveys per patch within a window of population closure is also necessary to improve detection given varying environmental conditions (Kristensen & Kovach 2018).

Tracking this reintroduction with genetic monitoring has produced insights to guide future reintroductions of New England cottontails. First, importantly, our findings show that reintroductions of New England cottontails can be successful. We documented successful breeding by both founder individuals and wild-born individuals, with some individuals reproducing and surviving over multiple years. Genetic diversity increased with the addition of breeding founders. However, we found that survival was variable, and may be related to stochastic events, predator response, or competition for territory with established individuals. A population decline and skewed sex ratio four years post-reintroduction highlighted that stochastic events can have dramatic implications for both demography and genetic diversity. To address concerns about low survival, more research is needed on methods to increase survival following a release (e.g. season of release, hard versus soft release methods, or age at which individuals are released). Current timing of releases are based on availability of cottontails from the captive breeding program. Releasing juveniles from the breeding program earlier in the season may correlate to increased survival (H. Holman, personal communication), and could give founders released as juveniles a better opportunity to breed in their first summer. If adults are available for release, releasing them earlier in the season would allow time for multiple breeding attempts and litters. Additional research is also needed on methods to increase productivity in the captive breeding program and recently piloted outdoor breeding pens. This will allow for larger numbers of individuals to be released to combat high post-release mortality.

Trends identified by studying the first New England cottontail reintroduction at Bellamy WMA can inform management for successful cottontail reintroductions. Successful reintroductions in the short term would produce high survival of released individuals, reproduction by both founders and wild-born individuals, and dispersal into additional patches of suitable 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. Key recommendations for a successful reintroduction of a small cottontail population vulnerable to stochastic decline include: **1)** restoring a self-sustaining metapopulation that includes multiple occupied patches within dispersal distance and shrubland corridors connecting patches; **2)** annual monitoring to track population size, sex ratios, number of breeders, and genetic diversity; and **3)** repeated reintroductions over time and reintroducing larger numbers of individuals, distributed spatially (i.e. releasing individuals throughout the patch instead of at one location) to avoid exceeding carrying capacity and to combat high post-release mortality (Armstrong & Seddon 2008, Hamilton et al. 2010). Additional research is needed to improve our understanding of the factors influencing founder survival and how best to supplement reintroductions after the initial release to prevent collapse of a small population. Releasing founders early in the season could increase survival and reproduction. Strategically supplementing populations after the initial reintroduction may require releasing rabbits into unoccupied habitat in the patch (as determined by telemetry and genetic monitoring), determining how many individuals need to be released accounting for mortality and the size of the existing population, and tracking sex ratios to release more individuals of the rarer sex if necessary.

Restoring multiple connected and occupied patches is the key for successful reintroductions of New England cottontails, which historically persisted in a metapopulation system. With a limited number of cottontails available for release from the captive breeding program, the most feasible way to establish introduced rabbits within a functioning metapopulation is to release rabbits into restored patches within dispersal distance from currently occupied patches. With the low dispersal rates documented in studied populations (Cheeseman 2017, B. Ferry unpublished data, Chapter 1), and the importance of dispersal for maintaining cottontail populations, especially small populations, expanding existing metapopulations will likely be more successful than establishing new populations. If reintroducing populations to areas where cottontail populations have recently become extirpated is a goal, a large number of individuals will need to be released into multiple patches within dispersal distance to restore a metapopulation, and additional releases will likely be needed for years after the initial reintroduction. Reintroducing individuals to additional patches within dispersal distance will provide colonists that can bolster populations in years of stochastic decline and counteract potential negative demographic effects. Ensuring sufficient habitat connectivity between occupied patches will promote dispersal and help limit negative population growth and sink patches (Hanski & Gilpin 1997). The current challenge with releasing additional individuals into satellite populations to rebuild a metapopulation is the low number of New England cottontails available for release. Until more individuals are available for release and functioning landscapes are restored, continued monitoring and annual augmentation of reintroduced populations are needed. Genetic monitoring through intensive noninvasive pellet surveys is a valuable tool for making decisions about New England cottontail reintroductions in an adaptive management framework.

Authors' contributions

AK and HH developed the study and secured funding for research. HH and BF coordinated and implemented New England cottontail releases, conducted winter pellet surveys and telemetry monitoring, and provided tissue samples from the Great Bay NWR breeding pen. MB helped collect pellet samples, extracted and genotyped samples, conducted analyses, and wrote the manuscript with help from AK. All authors read and approved the final manuscript.

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CHAPTER 3

SHRUBLAND BIRD OCCUPANCY ON NEW ENGLAND COTTONTAIL MANAGED SITES: EXPLORING THE REPRESENTATIVE SPECIES CONCEPT[2]

Abstract

Species that rely on shrubland habitat are declining throughout the Northeast due to habitat loss from development, succession, and restriction of natural disturbances. Species of particular conservation concern include shrubland birds and the New England cottontail. Though these species all require shrubland habitat types, each species' specific habitat associations influence how they will fit into landscape-level management in the Northeast. The goal of this study was to assess the value of habitat management for the New England cottontail as representative of conservation design for shrubland bird specialists. The specific objectives were to **1)** determine microhabitat and patch-level influences on shrubland bird occupancy at sites occupied by or managed for New England cottontails; and **2)** identify shrubland bird specialists that are indicative of the specific habitats required by New England cottontails. Point counts were conducted at 44 survey points in 2015 and 66 survey points in 2016 on sites in thicket, coastal and wetland shrub, young forest, pitch pine-scrub oak, edge, and old field habitats in southern Maine, coastal New Hampshire, and on Cape Cod in eastern Massachusetts. We developed occupancy models for Chestnut-sided Warblers, Yellow Warblers, Black-and-white Warblers, Prairie Warblers, and Eastern Towhees and identified Yellow Warblers and Prairie Warblers as species that would benefit most from management that creates microhabitat

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conditions suitable for cottontails. Through indicator species analyses, we identified 11 shrubland specialist bird species detected frequently either on sites occupied by cottontails or in microhabitat conditions associated with cottontail occupancy, including: Prairie Warblers, Yellow Warblers, Brown Thrashers, Field Sparrows, Blue-winged Warblers, Alder Flycatchers, Gray Catbirds, Song Sparrows, Indigo Buntings, American Goldfinches, and Black-and-white Warblers. Additional associations between certain shrubland bird species and herbaceous vegetation and low shrubs indicates that shrubland habitat managed for cottontails, but not yet dense enough to provide suitable cover for cottontails, will benefit an additional suite of shrubland birds. Our findings support the notion that the New England cottontail serves an indicator species role for shrubland habitat management and provides managers with information on bird species that will benefit from restoration aimed at improving and increasing habitat for cottontails.

Key Words: New England cottontail, shrubland birds, occupancy modeling, representative species

Introduction

Species that rely on shrubland habitat are declining throughout the Northeast, including 52 birds, mammals, and reptiles listed as Species of Greatest Conservation Need (Gilbart 2012). Species of particular management interest include shrubland birds, for which declining species outnumber increasing species three to one (Schlossberg & King 2007), and the New England cottontail, which is absent from over 86% of its historical range (Litvaitis et al. 2006, Fenderson et al. 2014, Brubaker et al. 2014) and persists on remnant, isolated patches. Declines in populations of shrubland species in New England are primarily due to habitat loss from development, restriction of natural disturbances that maintain early successional habitat, succession of abandoned farmlands, and reductions in even-aged silviculture (Cronon 1983, Litvaitis 1993, Trani et al. 2001). As such, active habitat management is essential to restore populations of specialist species in these ephemeral habitats.

Extensive resources and collaboration between federal, state, and private organizations and landowners have been invested in creating and restoring shrubland and young forest habitats in the Northeast (Fuller & Tur 2012, NFWF Early Successional Forest Keystone Initiative Report 2015, Fuller et al. 2016). Many of these shrubland restoration projects are focused on restoring habitat for the New England cottontail in response to its nine-year (2006-2015) candidate listing status under the Endangered Species Act. The New England Cottontail Conservation Initiative is pursuing a habitat restoration goal of 27,000 acres of shrubland and young forest. Nearly 18,700 acres were maintained or restored as of 2017, including 10,000 acres of self-sustaining habitat (New England Cottontail Executive Committee 2018).

To manage habitat to provide the greatest benefit for the most species with available resources, the U.S. Fish and Wildlife Service relies on representative species designations for part of its strategic planning process. A representative species is one that, because of its habitat use, ecosystem function, or management response, typifies life cycle or habitat requirements for a larger group of species (USFWS 2012). The USFWS is responsible for the management of Federal trust species, including migratory birds, threatened and endangered species, and interjurisdictional species of conservation concern. With limited resources, higher priority is often given to one or more trust species due to management need or vulnerability. The primary objective of this study was to assess the feasibility of designating the New England cottontail a representative species for shrubland habitat management and planning purposes, with a focus on shrubland specialist birds.

The New England cottontail is a shrubland obligate, requiring dense thicket habitat for cover, forage, and thermoregulation, both within its home range (Barbour & Litvaitis 1993, Litvaitis 2003), and during dispersal (Fenderson et al. 2014, Amaral et al. 2016). Recent studies comparing bird abundances across shrubland habitat types have documented that many species show distinct habitat preferences (Bulluck & Buehler 2006, Fink et al. 2006, King et al. 2009). Associations have been identified between shrubland specialist birds and vegetation structure and succession post-harvest (DeGraaf & Yamasaki 2001, Schlossberg et al. 2007, Grodsky et al. 2016), forage and nesting substrate (DeGraaf & Yamasaki 2001), and specific plant species (Schlossberg et al. 2010). Given the specific habitat preferences of New England cottontails, and the extensive management underway to create and maintain shrubland habitat with a focus on cottontails, it is likely that certain species of shrubland specialist birds with similar microhabitat associations will benefit from shrubland management focused on the New England cottontail as a representative species.

The specific objectives of this study were to **1)** determine microhabitat and patch-level influences on shrubland bird occupancy at sites occupied by or managed for New England cottontails; and **2)** identify shrubland specialist birds that are indicative of the specific microhabitats required by New England cottontails. We conducted point counts three times during the breeding season in 2015 and 2016 on sites in thicket, coastal and wetland shrub, old field, edge, young forest, and pitch pine-scrub oak habitats in southern Maine, coastal New Hampshire, and on Cape Cod in eastern Massachusetts. We modeled shrubland bird occupancy in relation to habitat covariates, related shrubland bird specialist richness across sites to microhabitat conditions, identified multivariate influences structuring the shrubland specialist bird community, and conducted indicator species analyses to identify shrubland birds detected with high frequency in microhabitat conditions that are associated with New England cottontail occupancy.

Methods

Study area

In 2015, we surveyed 44 point count locations on 18 sites in southern Maine and seacoast New Hampshire. Additional sites were added in 2016, for a total of 66 point count locations on 28 sites in Maine, New Hampshire, and Massachusetts on Cape Cod. Point counts were conducted on sites occupied by New England cottontails, sites managed for New England cottontails with microhabitat conditions capable of supporting cottontails, and on sites managed for New England cottontails but not yet capable of supporting cottontails. Surveyed patches ranged from 2.3 to 98.0 ha on a variety of shrubland habitat types including thicket, coastal and wetland shrub, old field, edge habitat, young forest, and pitch pine-scrub oak barrens.

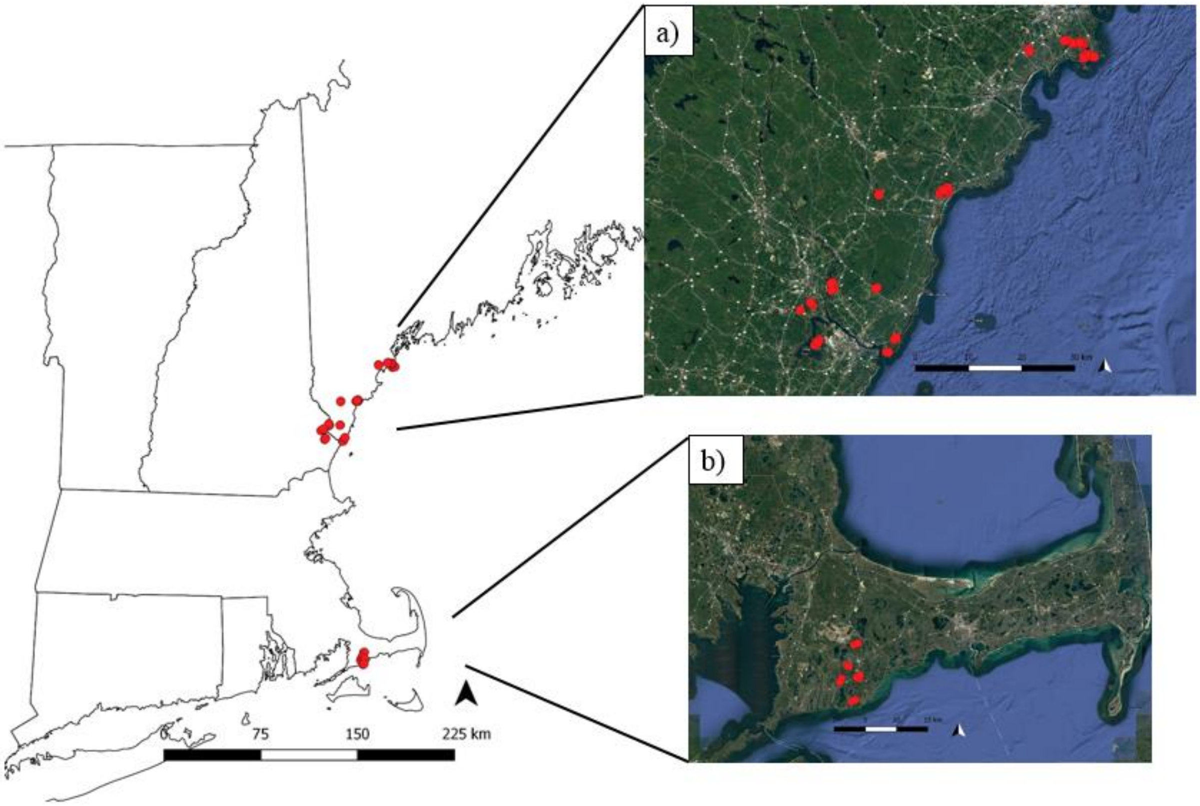

Fig. 3.1 Locations where point counts were conducted for shrubland bird occupancy in 2015 and 2016 in **a)** southern Maine and seacoast New Hampshire, and **b)** in 2016 in Massachusetts on Cape Cod.

Point counts

We conducted 10-minute point counts on three separate visits during the breeding season (late May to early July) following U.S. Fish and Wildlife Service landbird monitoring standard operating procedures (Knutson et al. 2008). Surveys were conducted by an experienced observer between 0.5 hr before sunrise and 6 hr after sunrise and efforts were made to rotate the order of points surveyed so as not to bias detection rates at any survey point due to time of day. Point counts were not conducted under conditions of high wind, rain, or excessive background noise. During each point count, the observer recorded all birds detected at distance bins of 0-25 m, 26-50 m, 51-100 m, and >100 m. The type of detection (auditory, visual, both, or flyover) was also recorded.

Habitat surveys

In 2017, we collected habitat data at each point count location for three categories of covariates (vegetation structure, patch-level characteristics, and non-living structure) pertinent to both New England cottontail and shrubland bird habitat suitability. Data were collected at two spatial scales: vegetation structure and non-living structure covariates were collected within 50 m of the point count location to assess the microhabitat in which birds were detected, and patch-level covariates were recorded reflecting characteristics that could influence cottontail occupancy on a larger scale, including patch area, plant species richness, and proportion of invasive shrubs. Habitat data was collected every 10 m in each cardinal direction from the bird point count location to a distance of 50 m, for a total of 20 vegetation sampling points surrounding each point count location (Fig. 3.2). Stem count data was collected from a 1x2 m plot (Brubaker et al. 2014) in the center of each 50 m cardinal direction transect, for a total of four stem density plots for each bird point count location.

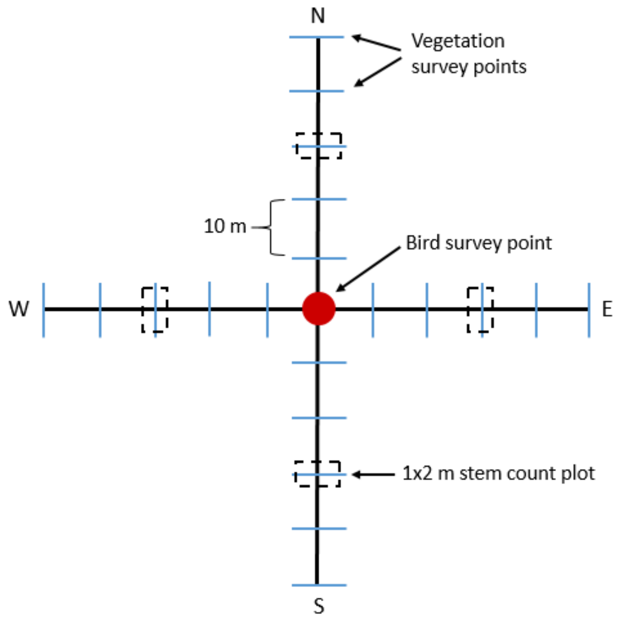

Fig. 3.2 Plot layout for 20 vegetation survey points and four 1x2 m stem count plots surrounding each bird point count location.

The vegetation structure category consisted of measurements of vegetation density, understory height, stem density, and canopy cover. Understory vegetation density at various heights is an important factor in habitat selection for different species of shrubland birds (Keller et al. 2003, Schlossberg et al. 2010). Vegetation density was quantified by recording the number of stem and leaf hits on a 3.0 m telescoping pole in 0.2 m height increments (Vitz & Rodewald 2006, 2007). The species of each stem or leaf hit was recorded, as were hits for categories of grass, ferns, forbs, and herbaceous vegetation. In addition to recording vegetation density at height categories on the telescoping pole, we also recorded the representative understory height within 1 m^2 of the telescoping pole. Understory height is an important habitat feature for New England cottontail escape cover from aerial and terrestrial predators (Litvaitis & Jakubas 2004, Arbuthnot 2008), and for certain shrubland bird species that prefer taller understory vegetation for nesting substrate, foraging, and protective cover (Nolan 1978, Schlossberg & King 2007, Schlossberg et al. 2010). Stem density, a key metric for assessing habitat suitability and escape cover for New England cottontails (Barbour & Litvaitis 1993, Fuller & Tur 2012, NEC Regional Technical Committee 2013, Warren et al. 2016), was collected by recording the species and number of woody stems ≥0.5 m tall and ≤7.5 cm diameter at breast height (dbh) (New England Cottontail Conservation Initiative 2009). Canopy cover has also been found to be an important metric for both New England cottontails (Buffum et al. 2015) and shrubland bird species (King & DeGraaf 2000, Schlossberg et al. 2010). We recorded canopy cover at each site using a concave spherical densiometer. Measured habitat variables were averaged over the 20 sampling points.

Patch-level covariates included area of the management unit patch, woody vegetation species richness, and proportion of woody invasives. Species richness and proportion of invasives were determined from species data and number of hits collected using the telescoping pole. Non-living structure covariates included woody debris and snags weighted by size, and number of brushpiles. Species-specific relationships between shrubland birds and coarse woody debris (Lanham & Guynn 1996, Lohr et al. 2002, Grodsky et al. 2016) and snags (Lohr et al. 2002, Johnson 2014) have been documented, and may be important for communication, cover, foraging, and nesting in regenerating stands. At each habitat sampling point, we recorded the number of small (dbh ≥7.5 cm to 30 cm), medium (dbh >30 cm to 60 cm), and large (dbh >60 cm) pieces of woody debris and snags ≥ 1 m in length or height. Number of brushpiles was recorded because brushpiles may be important for New England cottontail cover (Warren et al. 2016), and are an emphasized habitat component for land management for cottontails (NEC Regional Technical Committee 2013).

Data preparation

We retained point count data from the 0-25 m and 26-50 m distance bins to correspond to the distance surveyed for microhabitat covariate data, and excluded flyover detections. We modeled occupancy for species detected at a moderate number of points, because these species showed variation in occupancy that could be related to surveyed habitat covariates. The five shrubland specialist species for which we modeled occupancy included: Chestnut-sided Warbler, Yellow Warbler, Black-and-white Warbler, Prairie Warbler, and Eastern Towhee. Continuous habitat variables were *z*-transformed and patch area was log-transformed in order to improve normality and equality of variances, and to improve performance with the PRESENCE occupancy modeling software (MacKenzie 2012).

Shrubland bird occupancy models

We identified relationships between shrubland bird specialist species and habitat covariates with single-species, multi-season implicit dynamics occupancy models (MacKenzie et al. 2006) using Program PRESENCE (version 12.7, http:// www.mbr-pwrc.usgs.gov/software/presence.html, accessed 1 Nov. 2017). While accounting for imperfect detection, implicit dynamics models effectively apply a single-season model to data collected in each season (i.e. year), while allowing occupancy to change at a site between, but not within, seasons (MacKenzie et al. 2006).

Using a multi-stage approach, we first modeled detection (*p*) for each species while holding occupancy covariates at a global structure (MacKenzie 2012). For detection, we considered linear models for the covariates time of survey, temperature, cloud cover, wind speed, background noise level, and survey day (i.e. days since the start of the survey season), plus a null model. We also considered quadratic models for temperature and survey day because it was possible that birds were detected with greater frequency at moderate temperatures and that detection might vary non-linearly throughout the breeding season with different breeding activities such as territory establishment, incubation, and feeding nestlings. Model fit was assessed by AIC_C and model weight (Burnham & Anderson 2002). Model selection under an AIC_C framework makes retention of covariates based on 85% confidence intervals more appropriate than 95% confidence intervals (Arnold 2010). We therefore considered models within 2 AIC_C competitive (Burnham & Anderson 2002), and parameters informative for inference if 85% confidence intervals of covariate coefficient estimates excluded 0 (Arnold 2010). The best supported detection model was retained for modeling occupancy covariates.

In modeling occupancy (Ψ) covariates, we followed a multi-stage approach (Olson et al. 2005, Dugger et al. 2011) to retain informative parameters from each of the three habitat covariate groups. We first retained the best supported model (lowest AIC_C) from the vegetation structure group. Covariates were only retained if they were informative based on 85% confidence intervals of the coefficient estimates. We then modeled the covariate(s) retained from the vegetation structure stage, adding each covariate in the patch-level group. Similarly, we retained the best model from the vegetation structure + patch-level stage and assessed additive models including covariates from the non-living structure stage. Again, model selection was based on AIC_C and model weights and additive models of covariates at each stage were considered competitive if the addition of a covariate improved model fit by >2 AIC_C or the model with the additional covariate was within 2 AIC_C and the additional covariate was informative based on 85% confidence intervals of the coefficient estimates. Detection and yearly occupancy estimates were obtained by model-averaging within 2 AIC_C.

Table 3.1 Covariates considered in occupancy models for five shrubland specialist bird species, covariate descriptions, and modeling stage at which covariates were assessed.

Covariate	**Description**	**Modeling stage**
.	Null model	p and Ψ
TIME	Continuous; survey end time	p
TEMP	Continuous; temperature at survey start	p
TEMP^2	Continuous; quadratic form of TEMP	p
SKY	Categorical; cloud cover and precipitation	p
WIND	Categorical; wind speed	p
NOISE	Categorical; background noise	p
SURVEYDAY	Continuous; day within survey period on which point count was conducted	p
SURVEYDAY^2	Continuous; quadratic form of SURVEYDAY	p
HITS0TO1	Continuous; average number of stem and leaf hits from 0 to 1 m	Ψ, Vegetation structure
HITS1TO2	Continuous; average number of stem and leaf hits from >1 to 2 m	Ψ, Vegetation structure
HITS2TO3	Continuous; average number of stem and leaf hits from >2 to 3 m	Ψ, Vegetation structure
UNDERSTORYHT	Continuous; representative understory height within 1 m^2 of the telescoping pole	Ψ, Vegetation structure
STEMDENSITY	Continuous; average number of stems in a 1x2 m plot	Ψ, Vegetation structure
CANOPYCOVER	Proportion; proportion of overstory canopy	Ψ, Vegetation structure
RICHNESS	Continuous; Number of woody species	Ψ, Patch-level
PATCHAREA	Continuous; Area (ha) of management unit patch	Ψ, Patch-level
PROPORTIONINVASIVES	Proportion; proportion of woody vegetation classified as invasive	Ψ, Patch-level
DEBRIS	Continuous; (avg. # small pieces debris * 1) + (avg. # med. pieces debris * 2) + (avg. # large pieces debris * 3)	Ψ, Non-living structure
SNAGS	Continuous; (avg. # small pieces debris * 1) + (avg. # med. pieces debris * 2) + (avg. # large pieces debris * 3)	Ψ, Non-living structure
BRUSHPILES	Continuous; average number of brushpiles	Ψ, Non-living structure

Shrubland bird specialist richness

For richness and multivariate analyses, we included only species designated as shrubland specialists following Schlossberg and King (2007). Analyses were conducted using R 3.4.3 (R Core Team 2017). We tested for differences in shrubland bird specialist richness, the number of shrubland specialist bird species detected at a point, across point count locations in relation to sampled habitat covariates using Poisson generalized linear models and the glm function in R base software.

Multivariate indicator species analyses

We used canonical correlation analysis in PC-ORD v.7 (McCune & Mefford 2016) to identify multivariate relationships between shrubland specialist bird species and habitat conditions (McGarigal et al. 2000, King et al. 2009). Only species detected at ≥15% of sites were retained for canonical correlation analysis to improve skewness and kurtosis of the data and ability to detect effects of habitat variables.

We used indicator species analyses (Dufrêne & Legendre 1997) in PC-ORD to identify shrubland specialist birds that were detected with high frequency and exclusivity in microhabitat conditions characteristic of sites occupied by New England cottontails. An indicator species analysis identifies species that are indicative of a particular habitat type or group based on the frequency and exclusivity with which the species occurs at surveyed points in the habitat group. We classified point count locations into habitat groups with microhabitat conditions suitable for cottontails (Table 3.2) and identified indicator species for each defined habitat group. Microhabitat conditions we considered suitable for cottontail occupancy included: points with high stem density, tall understory height, dense vegetation between 1-2 or 2-3 m, large number of brushpiles present, and moderate canopy cover. We also conducted indicator species analyses for shrubland habitat types characteristic of cottontail occupancy including thickets and coastal and wetland shrub, and for points where cottontails had been detected within 50 m of the bird point count location in the past five years or on the surveyed patch within 200 m of the point count location. Cottontail occupancy was determined using winter fecal pellet detections from range-wide monitoring efforts. All shrubland specialist bird species were retained for indicator species analyses except Ruby-throated Hummingbird and Ruffed Grouse which had a lower probability of detection because they did not vocalize.

Table 3.2 Habitat groupings assessed in indicator species analyses for shrubland specialist bird species. Habitat groups considered characteristic of conditions suitable for New England cottontails (NEC) that were the focus of indicator species analyses are indicated in bold.

Habitat group	**Description**
NEC present/absent within 50 m	***NEC present***: NEC have been detected within 50 m of the point count location within the past five years (winter 2012/2013 – winter 2016/2017) *NEC absent*: NEC have not been detected within 50 m of the point count location in the past five years
NEC present/absent on patch	***NEC present***: NEC have been detected on the patch within 200 m of the point count location within the past five years *NEC absent*: NEC have not been detected on the patch within 200 m of the point count location within the past five years
Habitat	*1)* **Thicket**, *2)* **coastal/wetland shrub**, *3)* old field, *4)* edge, *5)* young forest, *6)* pitch pine-scrub oak
Stem density	*Low*: <15,000 stems/acre *Moderate*: 15,000 to <20,000 stems/acre *High*: **≥20,000 stems per acre** (NEC Regional Technical Committee 2013)
Understory height	*Low*: <1 m *Moderate*: 1 to <1.5 m *High*: **≥1.5 m** (Schlossberg et al. 2010)
Stem and leaf hits between 1-2 m	Lower third of vegetation density at point count locations, middle third of vegetation density at point count locations, **upper third of vegetation density at point count locations**
Stem and leaf hits between 2-3 m	Lower third of vegetation density at point count locations, middle third of vegetation density at point count locations, **upper third of vegetation density at point count locations**
Brushpiles	Lower third of brushpiles at point count locations, middle third of brushpiles at point count locations, **upper third of brushpiles at point count locations**
Canopy cover	*Low*: 0 to 0.33 canopy cover *Moderate*: **>0.33 to 0.66 canopy cover** *High*: >0.66 canopy cover

Results

We detected 19 shrubland bird specialist species on the 44 points surveyed in 2015 and 22 shrubland bird specialist species on the 66 points surveyed in 2016 (range 1-17 shrubland specialist species per point count location, median 9 shrubland specialist species per point count location) (Appendix C, Table C.1).

Shrubland bird occupancy models

Chestnut-sided Warbler

We detected Chestnut-sided Warblers (CSWA) on 16 of 44 point count locations in 2015 and 16 of 66 point count locations in 2016. CSWA occupancy probability was estimated to be 0.35 (95% CI 0.20 – 0.54) in 2015 and 0.27 (95% CI 0.17 – 0.40) in 2016 with an overall detection rate of 0.64 (95% CI 0.48 – 0.77). The best supported detection model indicated that CSWA detection decreased with SurveyDay throughout the breeding season. The best supported occupancy model indicated that CSWA occupancy decreased with an increase in UnderstoryHt. No other habitat covariates were informative in explaining occupancy. Although in subsequent steps of the multi-stage modeling approach, the StemDensity, Hits0to1, and PatchArea variables appeared in models with delta AIC_C<2 from the best supported model, the covariate estimates were uninformative based on 85% confidence intervals (i.e. confidence intervals of the estimates spanned zero).

Table 3.3 Chestnut-sided Warbler occupancy models after completing the multi-stage modeling with vegetation structure + patch-level + non-living structure covariate groups. UNDERSTORYHT was the only informative vegetation covariate; no covariates in the patch-level and non-living structure group were within 2 AIC$_C$ of the top model while also having coefficient estimates with 85% confidence intervals that did not span zero. Informative parameters (85% CI does not span 0) are shown in bold.

DETECTION

Model	**AIC$_C$**	**ΔAIC$_C$**	***ωi***	***K***	**-2 log (ƀ)**
Ψ (global), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	277.98	0.00	0.2577	16	234.88
Ψ (global), gam(.), eps=1-gam, *p*(.)	278.27	0.29	0.2229	15	238.67
Ψ (global), gam(.), eps=1-gam, *p*(**TIME**)	278.82	0.84	0.1693	16	235.72
Ψ (global), gam(.), eps=1-gam, *p*(SURVEYDAY+SURVEYDAY^2)	279.58	1.60	0.1158	17	232.83
Ψ (global), gam(.), eps=1-gam, *p*(NOISE)	281.28	3.30	0.0495	16	238.18
Ψ (global), gam(.), eps=1-gam, *p*(TEMP+TEMP^2)	281.32	3.34	0.0485	17	234.57
Ψ (global), gam(.), eps=1-gam, *p*(TEMP)	281.33	3.35	0.0483	16	238.23
Ψ (global), gam(.), eps=1-gam, *p*(WIND)	281.35	3.37	0.0478	16	238.25
Ψ (global), gam(.), eps=1-gam, *p*(SKY)	281.69	3.71	0.0403	16	238.59

VEGETATION STRUCTURE

Model	**AIC$_C$**	**ΔAIC$_C$**	**ω_i**	***K***	**-2 log (ƀ)**
Ψ (**UNDERSTORYHT**), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	259.08	0.00	0.2383	5	248.08
Ψ (.), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	259.28	0.20	0.2156	4	250.62
Ψ (STEMDENSITY), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	259.78	0.70	0.1679	5	248.78
Ψ (HITS0TO1), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	259.86	0.78	0.1613	5	248.86
Ψ (HITS2TO3), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	261.32	2.24	0.0778	5	250.32
Ψ (HITS1TO2), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	261.47	2.39	0.0721	5	250.47
Ψ (CANOPYCOVER), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	261.62	2.54	0.0669	5	250.62

VEGETATION STRUCTURE + PATCH-LEVEL

Model	**AIC$_C$**	**ΔAIC$_C$**	**ω_i**	***K***	**-2 log (ƀ)**
Ψ (**UNDERSTORYHT**), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	259.08	0.00	0.3340	5	248.08
Ψ (.), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	259.28	0.20	0.3023	4	250.62
Ψ (**UNDERSTORYHT**+PATCHAREA), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	260.60	1.52	0.1562	6	247.18
Ψ (**UNDERSTORYHT**+RICHNESS), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	261.35	2.27	0.1074	6	247.93
Ψ (**UNDERSTORYHT**+PROPORTIONINVASIVES), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	261.49	2.41	0.1001	6	248.07

VEGETATION STRUCTURE + PATCH-LEVEL + NON-LIVING STRUCTURE

Model	**AIC$_C$**	**ΔAIC$_C$**	**ω_i**	***K***	**-2 log (ƀ)**
Ψ (**UNDERSTORYHT**), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	259.08	0.00	0.3541	5	248.08
Ψ (.), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	259.28	0.20	0.3204	4	250.62
Ψ (**UNDERSTORYHT**+DEBRIS), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	261.40	2.32	0.1110	6	247.98
Ψ (**UNDERSTORYHT**+BRUSHPILES), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	261.47	2.39	0.1072	6	248.05
Ψ (**UNDERSTORYHT**+SNAGS), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	261.47	2.39	0.1072	6	248.05

Table 3.4 Covariate summary data for the best supported occupancy model for Chestnut-sided Warblers.

Covariate	Estimated coefficient	SE	85% CI	
			Lower	Upper
SURVEYDAY	-0.39	0.20	-0.67	-0.10
UNDERSTORYHT	-0.53	0.34	-1.02	-0.03

Table 3.5 Chestnut-sided Warbler detection and occupancy estimates. Models produced an overall estimate for detection, and yearly estimates for occupancy. 95% confidence intervals were calculated using the delta method.

Parameter	Estimate	SE	95% CI	
			Lower	Upper
p	0.64	0.08	0.48	0.77
Ψ 2015	0.35	0.09	0.20	0.54
Ψ 2016	0.27	0.06	0.17	0.40

Yellow Warbler

We detected Yellow Warblers (YEWA) on 34 of 44 point count locations in 2015 and 41 of 66 point count locations in 2016. YEWA occupancy was estimated to be 0.96 (95% CI 0.60 – 0.99) in 2015 and 0.67(95% CI 0.53 – 0.78) in 2016 with an overall detection rate of 0.76 (95% CI 0.66 – 0.84). The best supported detection model indicated that YEWA detection decreased with SURVEYDAY throughout the breeding season. The best supported occupancy model indicated that YEWA occupancy increased with an increase in STEMDENSITY. Models including PATCHAREA, PROPRTIONINVASIVES, and SNAGS were within 2 AIC_C of the best supported model during the multi-stage process, but were uninformative because the 85% confidence intervals of the coefficient estimates spanned zero.

Table 3.6 Yellow Warbler occupancy models after completing the multi-stage modeling with vegetation structure + patch-level + non-living structure covariate groups. STEMDENSITY was the only informative covariate from the vegetation structure group and no patch-level covariates were found to be informative by AIC$_C$ and coefficient estimates. In this final modeling stage, an additive model including SNAGS was within 2 AIC$_C$ of the top model, but was uninformative based on the 85% CI of the estimate 1.06 (-0.23 – 2.35).

DETECTION

Model	**AIC$_C$**	**ΔAIC$_C$**	***ωi***	***K***	**-2 log (ѣ)**
Ψ (global), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	403.06	0.00	0.8501	16	359.96
Ψ (global), gam(.), eps=1-gam, *p*(SURVEYDAY+SURVEYDAY^2)	406.66	3.60	0.1405	17	359.91
Ψ (global), gam(.), eps=1-gam, *p*(TEMP)	413.00	9.94	0.0059	16	369.90
Ψ (global), gam(.), eps=1-gam, *p*(.)	415.97	12.91	0.0013	15	376.37
Ψ (global), gam(.), eps=1-gam, *p*(TEMP+TEMP^2)	416.33	13.27	0.0011	17	369.58
Ψ (global), gam(.), eps=1-gam, *p*(SKY)	419.05	15.99	0.0003	16	375.95
Ψ (global), gam(.), eps=1-gam, *p*(WIND)	419.15	16.09	0.0003	16	376.05
Ψ (global), gam(.), eps=1-gam, *p*(NOISE)	419.18	16.12	0.0003	16	376.08
Ψ (global), gam(.), eps=1-gam, *p*(TIME)	419.37	16.31	0.0002	16	376.27

VEGETATION STRUCTURE

Model	**AIC$_C$**	**ΔAIC$_C$**	***ω$_i$***	***K***	**-2 log (ѣ)**
Ψ (**STEMDENSITY**), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	392.25	0.00	0.9980	5	381.25
Ψ (UNDERSTORYHT), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	406.33	14.08	0.0009	5	395.33
Ψ (HITS0TO1), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	407.04	14.79	0.0006	5	396.04
Ψ (CANOPYCOVER), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	408.49	16.24	0.0003	5	397.49
Ψ (HITS1TO2), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	410.53	18.28	0.0001	5	399.53
Ψ (.), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	412.11	19.86	0.0000	4	403.45
Ψ (HITS2TO3), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	414.45	22.20	0.0000	5	403.45

VEGETATION STRUCTURE + PATCH-LEVEL

Model	**AIC$_C$**	**ΔAIC$_C$**	***ω$_i$***	***K***	**-2 log (ѣ)**
Ψ (**STEMDENSITY**), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	392.25	0.00	0.4192	5	381.25
Ψ (**STEMDENSITY**+PATCHAREA), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	393.14	0.89	0.2686	6	379.72
Ψ (**STEMDENSITY**+PROPORTIONINVASIVES), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	393.97	1.72	0.1774	6	380.55
Ψ (**STEMDENSITY**+RICHNESS), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	394.52	2.27	0.1347	6	381.10
Ψ (.), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	412.11	19.86	0.0000	4	403.45

VEGETATION STRUCTURE + PATCH-LEVEL + NON-LIVING STRUCTURE

Model	**AIC$_C$**	**ΔAIC$_C$**	***ω$_i$***	***K***	**-2 log (ѣ)**
Ψ (**STEMDENSITY**), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	392.25	0.00	0.4312	5	381.25
Ψ (**STEMDENSITY**+SNAGS), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	393.00	0.75	0.2963	6	379.58
Ψ (**STEMDENSITY**+DEBRIS), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	394.51	2.26	0.1393	6	381.09
Ψ (**STEMDENSITY**+BRUSHPILES), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	394.60	2.35	0.1332	6	381.18
Ψ (.), gam(.), eps=1-gam, *p*(**SURVEYDAY**)	412.11	19.86	0.0000	4	403.45

Table 3.7 Covariate summary data for the best supported occupancy model for Yellow Warblers.

Covariate	Estimated coefficient	SE	85% CI	
			Lower	Upper
SURVEYDAY	-0.57	0.15	-0.78	-0.36
STEMDENSITY	5.74	2.38	2.32	9.17

Table 3.8 Yellow Warbler occupancy and detection estimates. Models produced an overall estimate for detection, and yearly estimates for occupancy. 95% confidence intervals were calculated using the delta method.

Parameter	Estimate	SE	95% CI	
			Lower	Upper
p	0.76	0.044	0.66	0.84
Ψ 2015	0.96	0.058	0.60	0.99
Ψ 2016	0.67	0.066	0.53	0.78

Black-and-white Warbler

We detected Black-and-white Warblers (BAWW) on 15 of 44 point count locations in 2015 and 23 of 66 point count locations in 2016. BAWW occupancy was estimated to be 0.22 (95% CI 0.06 – 0.57) in 2015 and 0.54 (95% CI 0.34 – 0.73) in 2016 with an overall detection rate of 0.33 (95% CI 0.23 – 0.45). The best supported detection model was a null model (suggesting that detection was not influenced by any measured variable), and the best supported occupancy model indicated that BAWW occupancy increased with a decrease in HITS0TO1. During the multi-stage modeling process, HITS0TO1 was the only informative vegetation structure covariate. Patch-level covariates of PROPORTIONINVASIVES and PATCHAREA as well as the non-living vegetation covariate of DEBRIS were within 2 AIC_C of the top model, but the 85% confidence intervals of the coefficient estimates spanned zero, suggesting they were not informative variables for BAWW occupancy.

Table 3.9 Black-and-white Warbler occupancy models after the final step of multi-stage modeling with vegetation structure + patch-level + non-living structure covariate groups. HITS0TO1 was the only informative vegetation structure covariate and no patch-level covariates were found to be informative by AIC_C and examination of 85% confidence intervals of the coefficient estimates. In the final modeling step, an additive model including DEBRIS was within 2 AIC_C of the top model, but was uninformative based on the 85% CI of the estimate 0.31 (-0.38 – 0.99).

DETECTION

Model	**AIC_C**	**ΔAIC_C**	***ωi***	***K***	**-2 log (ƀ)**
Ψ (global), gam(.), eps=1-gam, *p*(.)	303.74	0.00	0.3506	15	264.14
Ψ (global), gam(.), eps=1-gam, *p*(TEMP+TEMP^2)	304.00	0.26	0.3078	17	257.25
Ψ (global), gam(.), eps=1-gam, *p*(SURVEYDAY)	306.81	3.07	0.0755	16	263.71
Ψ (global), gam(.), eps=1-gam, *p*(SKY)	306.99	3.25	0.0690	16	263.89
Ψ (global), gam(.), eps=1-gam, *p*(TEMP)	307.10	3.36	0.0653	16	264.00
Ψ (global), gam(.), eps=1-gam, *p*(NOISE)	307.12	3.38	0.0647	16	264.02
Ψ (global), gam(.), eps=1-gam, *p*(WIND)	308.64	4.90	0.0303	16	265.54
Ψ (global), gam(.), eps=1-gam, *p*(TIME)	309.23	5.49	0.0225	16	266.13
Ψ (global), gam(.), eps=1-gam, *p*(SURVEYDAY+SURVEYDAY^2)	310.15	6.41	0.0142	17	263.40

VEGETATION STRUCTURE

Model	**AIC_C**	**ΔAIC_C**	**ω_i**	***K***	**-2 log (ƀ)**
Ψ (**HITS0TO1**), gam(.), eps=1-gam, *p*(.)	293.96	0.00	0.4967	4	285.30
Ψ (CANOPYCOVER), gam(.), eps=1-gam, *p*(.)	296.12	2.16	0.1687	4	287.46
Ψ (.), gam(.), eps=1-gam, *p*(.)	297.21	3.25	0.0978	3	290.82
Ψ (STEMDENSITY), gam(.), eps=1-gam, *p*(.)	297.56	3.60	0.0821	4	288.90
Ψ (HITS2TO3), gam(.), eps=1-gam, *p*(.)	297.69	3.73	0.0769	4	289.03
Ψ (HITS1TO2), gam(.), eps=1-gam, *p*(.)	298.77	4.81	0.0448	4	290.11
Ψ (UNDERSTORYHT), gam(.), eps=1-gam, *p*(.)	299.39	5.43	0.0329	4	290.73

VEGETATION STRUCTURE + PATCH-LEVEL

Model	**AIC_C**	**ΔAIC_C**	**ω_i**	***K***	**-2 log (ƀ)**
Ψ (**HITS0TO1**), gam(.), eps=1-gam, *p*(.)	293.96	0.00	0.3609	4	285.30
Ψ (**HITS0TO1**+ PROPORTIONINVASIVES), gam(.), eps=1-gam, *p*(.)	994.87	0.91	0.2290	5	283.87
Ψ (**HITS0TO1**+ PATCHAREA), gam(.), eps=1-gam, *p*(.)	294.96	1.00	0.2189	5	283.96
Ψ (**HITS0TO1**+ RICHNESS), gam(.), eps=1-gam, *p*(.)	296.16	2.20	0.1201	5	285.16
Ψ (.), gam(.), eps=1-gam, *p*(.)	297.21	3.25	0.0711	3	290.82

VEGETATION STRUCTURE + PATCH-LEVEL + NON-LIVING STRUCTURE

Model	**AIC_C**	**ΔAIC_C**	**ω_i**	***K***	**-2 log (ƀ)**
Ψ (**HITS0TO1**), gam(.), eps=1-gam, *p*(.)	293.96	0.00	0.4511	4	285.30
Ψ (**HITS0TO1**+ DEBRIS), gam(.), eps=1-gam, *p*(.)	295.87	1.91	0.1736	5	284.87
Ψ (**HITS0TO1**+ BRUSHPILES), gam(.), eps=1-gam, *p*(.)	296.22	2.26	0.1457	5	285.22
Ψ (**HITS0TO1**+ SNAGS), gam(.), eps=1-gam, *p*(.)	296.29	2.33	0.1407	5	285.29
Ψ (.), gam(.), eps=1-gam, *p*(.)	297.21	3.25	0.1245	3	290.82

Table 3.10 Covariate summary data for the best supported occupancy model for Black-and-white Warblers.

Covariate	Estimated coefficient	SE	85% CI	
			Lower	Upper
HITS0TO1	-1.10	0.55	-1.89	-0.31

Table 3.11 Black-and-white Warbler occupancy and detection estimates. Models produced an overall estimate for detection, and yearly estimates for occupancy. 95% confidence intervals were calculated using the delta method.

Parameter	Estimate	SE	95% CI	
			Lower	Upper
p	0.33	0.06	0.23	0.45
Ψ 2015	0.22	0.14	0.06	0.57
Ψ 2016	0.54	0.10	0.34	0.73

Prairie Warbler

We detected Prairie Warblers (PRAW) on 8 of 44 point count locations in 2015 and 6 of 66 point count locations in 2016. PRAW occupancy was estimated to be 0.11 (95% CI 0.03 – 0.34) in 2015 and 0.09 (95% CI 0.04 – 0.19) in 2016 with an overall detection rate of 0.76 (95% CI 0.47 – 0.92). The best supported detection model indicated that detection increased with an increase in NOISE. This was likely an artefact of the fact that PRAW were most commonly detected in a transmission line right-of-way near a major road. Accordingly, and because our study did not include additional rights-of-ways, we retained a null model of detection for occupancy modeling stages. The best supported occupancy model indicated that PRAW occupancy increased with an increase in HITS2TO3, decreased with an increase in PATCHAREA, and decreased with an increase in BRUSHPILES.

Table 3.12 Prairie Warbler occupancy models after completing the multi-stage modeling with vegetation structure + patch-level + non-living structure covariate groups.

DETECTION

Model	**AIC$_C$**	**ΔAIC$_C$**	***ωi***	***K***	**-2 log (ѣ)**
Ψ (global), gam(.), eps=1-gam, *p*(**NOISE**)	127.56	0.00	0.5300	16	84.46
Ψ (global), gam(.), eps=1-gam, *p*(.)	129.77	2.21	0.1756	15	90.17
Ψ (global), gam(.), eps=1-gam, *p*(TEMP)	131.54	3.98	0.0725	16	88.44
Ψ (global), gam(.), eps=1-gam, *p*(WIND)	132.03	4.47	0.0567	16	88.93
Ψ (global), gam(.), eps=1-gam, *p*(SURVEYDAY+SURVEYDAY^2)	132.03	4.47	0.0567	17	85.28
Ψ (global), gam(.), eps=1-gam, *p*(SURVEYDAY)	132.41	4.85	0.0469	16	89.31
Ψ (global), gam(.), eps=1-gam, *p*(SKY)	132.71	5.15	0.0404	16	89.61
Ψ (global), gam(.), eps=1-gam, *p*(TEMP+TEMP^2)	134.73	7.17	0.0147	17	87.98
Ψ (global), gam(.), eps=1-gam, *p*(TIME)	136.34	8.78	0.0066	16	93.24

VEGETATION STRUCTURE

Model	**AIC$_C$**	**ΔAIC$_C$**	**ω_i**	***K***	**-2 log (Ѣ)**
Ψ (**HITS2TO3**), gam(.), eps=1-gam, *p*(.)	136.22	0.00	0.4069	4	127.56
Ψ (.), gam(.), eps=1-gam, *p*(.)	137.85	1.63	0.1801	3	131.46
Ψ (HITS1TO2), gam(.), eps=1-gam, *p*(.)	138.92	2.70	0.1055	4	130.26
Ψ (CANOPYCOVER), gam(.), eps=1-gam, *p*(.)	138.93	2.71	0.1050	4	130.27
Ψ (HITS0TO1), gam(.), eps=1-gam, *p*(.)	139.33	3.11	0.0859	4	130.67
Ψ (UNDERSTORYHT), gam(.), eps=1-gam, *p*(.)	140.10	3.88	0.0585	4	131.44
Ψ (STEMDENSITY), gam(.), eps=1-gam, *p*(.)	140.11	3.89	0.0582	4	131.45

VEGETATION STRUCTURE + PATCH-LEVEL

Model	**AIC$_C$**	**ΔAIC$_C$**	**ω_i**	***K***	**-2 log (Ѣ)**
Ψ (**HITS2TO3**+**PATCHAREA**), gam(.), eps=1-gam, *p*(.)	132.54	0.00	0.7338	5	121.54
Ψ (**HITS2TO3**), gam(.), eps=1-gam, *p*(.)	136.22	3.68	0.1165	4	127.56
Ψ (**HITS2TO3**+PROPORTIONINVASIVES), gam(.), eps=1-gam, *p*(.)	137.49	4.95	0.0618	5	126.49
Ψ (.), gam(.), eps=1-gam, *p*(.)	137.85	5.31	0.0516	3	131.46
Ψ (**HITS2TO3**+RICHNESS), gam(.), eps=1-gam, *p*(.)	138.55	6.01	0.0364	5	127.55

VEGETATION STRUCTURE + PATCH-LEVEL + NON-LIVING STRUCTURE

Model	**AIC$_C$**	**ΔAIC$_C$**	**ω_i**	***K***	**-2 log (Ѣ)**
Ψ (**HITS2TO3**+**PATCHAREA**+**BRUSHPILES**), gam(.), eps=1-gam, *p*(.)	131.65	0.00	0.4062	6	118.23
Ψ (**HITS2TO3**+**PATCHAREA**), gam(.), eps=1-gam, *p*(.)	132.54	0.89	0.2603	5	121.54
Ψ (**HITS2TO3**+**PATCHAREA**+DEBRIS), gam(.), eps=1-gam, *p*(.)	133.78	2.13	0.1400	6	120.36
Ψ (**HITS2TO3**+**PATCHAREA**+SNAGS), gam(.), eps=1-gam, *p*(.)	133.87	2.22	0.1339	6	120.45
Ψ (**HITS2TO3**), gam(.), eps=1-gam, *p*(.)	136.22	4.57	0.0413	4	127.56
Ψ (.), gam(.), eps=1-gam, *p*(.)	137.85	6.20	0.0183	3	131.46

Table 3.13 Covariate summary data for the best supported occupancy model for Prairie Warblers.

Covariate	Estimated coefficient	SE	85% CI	
			Lower	Upper
HITS2TO3	1.12	0.65	0.18	2.05
PATCHAREA	-2.72	1.29	-4.58	-0.87
BRUSHPILES	-0.89	0.53	-1.65	-0.12

Table 3.14 Prairie Warbler model-averaged occupancy and detection estimates. Models produced an overall estimate for detection, and yearly estimates for occupancy. 95% confidence intervals were calculated using the delta method.

Parameter	Estimate	SE	95% CI	
			Lower	Upper
p	0.76	0.12	0.47	0.92
Ψ 2015	0.11	0.07	0.03	0.34
Ψ 2016	0.09	0.04	0.04	0.19

Eastern Towhee

We detected Eastern Towhees (EATO) on 14 of 44 point count locations in 2015 and 18 of 66 point count locations in 2016. EATO occupancy was estimated to be 0.48 (95% CI 0.28 – 0.68) in 2015 and 0.30 (95% CI 0.20 – 0.43) in 2016 with an overall detection rate of 0.48 (95% CI 0.13 – 0.85). The best supported detection model indicated that EATO detection decreased with an increase in TIME, suggesting that EATO were more likely to be detected earlier in the morning. The best supported occupancy model indicated that EATO occupancy increased with an increase in PATCHAREA, decreased with an increase in SNAGS, and decreased with an increase in DEBRIS. During the modeling process, the additional vegetation covariates HITS0TO1, HITS1TO2, HITS2TO3, CANOPYCOVER, and UNDERSTORYHT, and the patch-level covariate PROPORTIONINVASIVES were in models with $\Delta AIC_C<2$ but were uninformative based on 85% confidence intervals.

Table 3.15 Eastern Towhee occupancy models after completing the multi-stage modeling with vegetation structure + patch-level + non-living structure covariate groups.

DETECTION

Model	**AIC$_C$**	**ΔAIC$_C$**	$\boldsymbol{\omega i}$	***K***	**-2 log (ƀ)**
Ψ (global), gam(.), eps=1-gam, *p*(**TIME**)	287.49	0.00	0.5702	16	247.88
Ψ (global), gam(.), eps=1-gam, *p*(.)	289.86	2.37	0.1743	15	250.26
Ψ (global), gam(.), eps=1-gam, *p*(SURVEYDAY)	291.43	3.94	0.0795	16	248.33
Ψ (global), gam(.), eps=1-gam, *p*(WIND)	292.04	4.55	0.0586	16	248.94
Ψ (global), gam(.), eps=1-gam, *p*(NOISE)	293.04	5.55	0.0355	16	249.94
Ψ (global), gam(.), eps=1-gam, *p*(TEMP)	293.35	5.86	0.0304	16	250.25
Ψ (global), gam(.), eps=1-gam, *p*(SKY)	293.36	5.87	0.0303	16	250.26
Ψ (global), gam(.), eps=1-gam, *p*(SURVEYDAY+SURVEYDAY^2)	295.08	7.59	0.0128	17	248.33
Ψ (global), gam(.), eps=1-gam, *p*(TEMP+TEMP^2)	295.95	8.46	0.0083	17	249.20

VEGETATION STRUCTURE

Model	**AIC$_C$**	**ΔAIC$_C$**	$\boldsymbol{\omega_i}$	***K***	**-2 log (ƀ)**
Ψ (.), gam(.), eps=1-gam, *p*(**TIME**)	267.51	0.00	0.2661	4	258.85
Ψ (HITS2TO3), gam(.), eps=1-gam, *p*(**TIME**)	268.66	1.15	0.1497	5	257.66
Ψ (HITS0TO1), gam(.), eps=1-gam, *p*(**TIME**)	268.81	1.30	0.1389	5	257.81
Ψ (CANOPYCOVER), gam(.), eps=1-gam, *p*(**TIME**)	268.92	1.41	0.1315	5	257.92
Ψ (HITS1TO2), gam(.), eps=1-gam, *p*(**TIME**)	269.03	1.52	0.1244	5	258.03
Ψ (UNDERSTORYHT), gam(.), eps=1-gam, *p*(**TIME**)	269.39	1.88	0.1039	5	258.39
Ψ (STEMDENSITY), gam(.), eps=1-gam, *p*(**TIME**)	269.78	2.27	0.0855	5	258.78

VEGETATION STRUCTURE + PATCH-LEVEL

Model	**AIC$_C$**	**ΔAIC$_C$**	$\boldsymbol{\omega_i}$	***K***	**-2 log (ƀ)**
Ψ (**PATCHAREA**), gam(.), eps=1-gam, *p*(**TIME**)	266.78	0.00	0.4335	5	255.78
Ψ (.), gam(.), eps=1-gam, *p*(**TIME**)	267.51	0.73	0.3010	4	258.85
Ψ (PROPORTIONINVASIVES), gam(.), eps=1-gam, *p*(**TIME**)	268.72	1.94	0.1643	5	257.72
Ψ (RICHNESS), gam(.), eps=1-gam, *p*(**TIME**)	269.69	2.91	0.1012	5	258.69

VEGETATION STRUCTURE + PATCH-LEVEL + NON-LIVING STRUCTURE

Model	**AIC$_C$**	**ΔAIC$_C$**	$\boldsymbol{\omega_i}$	***K***	**-2 log (ƀ)**
Ψ (**PATCHAREA**+**SNAGS**), gam(.), eps=1-gam, *p*(**TIME**)	265.48	0.00	0.3565	6	252.06
Ψ (**PATCHAREA**+**DEBRIS**), gam(.), eps=1-gam, *p*(**TIME**)	266.25	0.77	0.2425	6	252.83
Ψ (**PATCHAREA**), gam(.), eps=1-gam, *p*(**TIME**)	266.78	1.30	0.1861	5	255.78
Ψ (.), gam(.), eps=1-gam, *p*(**TIME**)	267.51	2.03	0.1292	4	258.85
Ψ (**PATCHAREA**+BRUSHPILES), gam(.), eps=1-gam, *p*(**TIME**)	268.33	2.85	0.0857	6	254.91

Table 3.16 Covariate summary data for the best supported occupancy model for Eastern Towhees.

Covariate	Estimated coefficient	SE	85% CI	
			Lower	Upper
TIME	-0.37	0.24	-0.71	-0.02
PATCHAREA	1.39	0.78	0.26	2.51
SNAGS	-0.76	0.43	-1.38	-0.15
DEBRIS	-0.78	0.49	-1.49	-0.06

Table 3.17 Eastern Towhee model-averaged occupancy and detection estimates. Models produced an overall estimate for detection, and yearly estimates for occupancy. 95% confidence intervals were calculated using the delta method.

Parameter	Estimate	SE	95% CI	
			Lower	Upper
p	0.48	0.23	0.13	0.85
Ψ 2015	0.48	0.11	0.28	0.68
Ψ 2016	0.30	0.06	0.20	0.43

Shrubland bird specialist richness

Several measured microhabitat and patch-level variables were associated with the species richness of shrubland specialist birds (Table 3.18). The strongest relationship identified with shrubland bird specialist richness was a positive association with the proportion of woody invasives (χ^2=16.42, GLM, p<0.0001). Shrubland bird richness was highest at points with a moderate proportion of woody invasives, and lower at points with few invasives and heavily invaded points (Fig. 3.3).

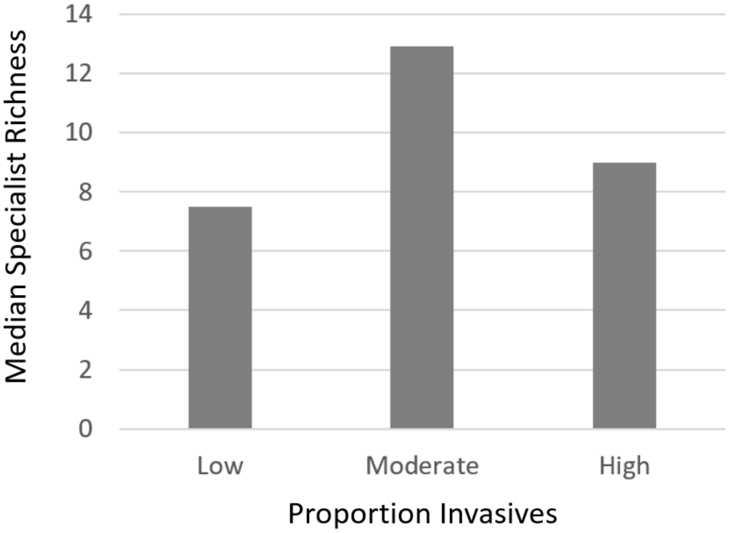

Fig. 3.3 Median species richness for shrubland specialist birds at point count locations with low, moderate, and high proportion of woody invasive shrubs. The lower third of points surveyed ranged from 0-0.13 proportion invasives, the moderately invaded points ranged from 0.18-0.53 proportion invasives, and the upper third of points contained 0.55 to 0.93 proportion invasives.

Specialist richness was positively associated with the number of vegetation hits from 0-1 m (χ^2=8.46, GLM, p<0.01), and negatively associated with number of snags (χ^2=9.32, GLM, p<0.01), and number of pieces of woody debris (χ^2=5.56, GLM, p<0.05) (Table 3.18). Canopy cover showed a marginal negative trend with shrubland specialist richness (χ^2=3.16, GLM, p<0.1).

Table 3.18 Results of GLM Poisson regression models assessing relationships between measured covariates and shrubland bird specialist richness.

Covariate	**χ^2**	***p*-value**	**Estimate**	**SE**	**95% CI**	
					Lower	**Upper**
PROPORTIONINVASIVES	16.42	<0.0001	0.59	0.15	0.31	0.88
SNAGS	9.32	<0.01	-0.028	0.0093	-0.046	-0.010
HITS0TO1	8.46	<0.01	0.024	0.0082	0.0083	0.040
DEBRIS	5.56	<0.05	-0.017	0.0075	-0.032	-0.0027
CANOPYCOVER	3.16	0.075	-0.28	0.16	-0.60	0.029

Multivariate analyses

Canonical correspondence analysis of shrubland specialist bird species with measured habitat variables showed strong relationships between certain species (e.g. Yellow Warbler, Song Sparrow, and Black-and-White Warbler) and habitat covariates (Fig. 3.4). Other species did not show strong habitat associations, either because they were not sufficiently abundant to detect effects of measured habitat covariates, or because they are associated with covariates that were not measured (e.g. forage availability). The proportion of invasives, presence of brushpiles, snags, canopy cover, and patch area were the most informative of the habitat and patch-level variables (Fig. 3.4). Yellow Warbler, Gray Catbird, Northern Cardinal, and Chestnut-sided Warbler showed positive relationships with invasives, Black-and-white Warbler showed a positive relationship with canopy cover, Yellow Warbler showed a positive relationship with vegetation height, and Indigo Bunting and American Goldfinch showed negative relationships with brushpiles. Vegetation density at 0-1 m and 2-3 m were also informative, albeit to a lesser degree, in this analysis, with Song Sparrow showing a positive relationship with vegetation density at both 0-1 m and 2-3 m. These findings reflect the sampled variables that structure the shrubland specialist bird community and the variation in fine-scale habitat preferences among species.

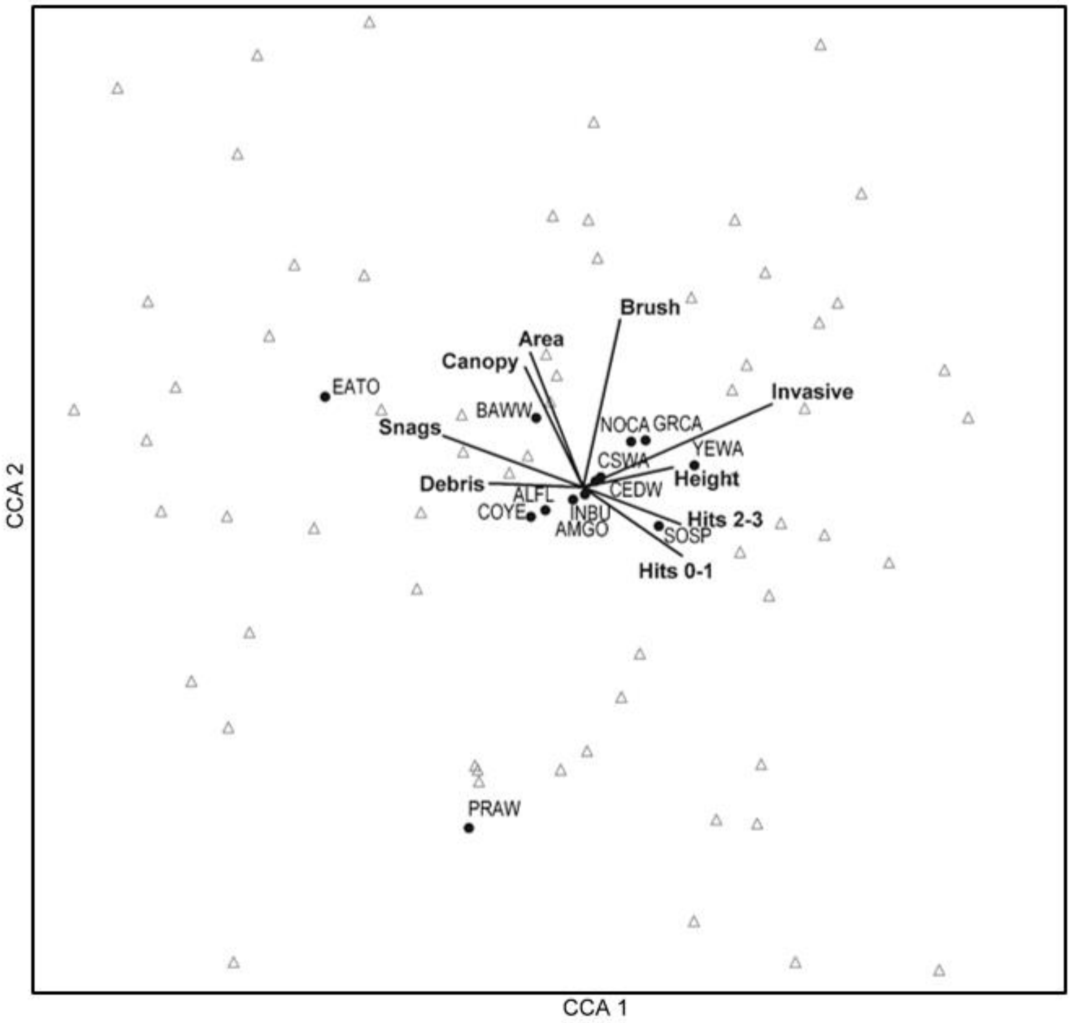

Fig. 3.4 Canonical correspondence analysis of abundance (number of detections per visit) of shrubland bird specialist species and measured habitat variables with $R^2 \geq 0.1$. Data were combined from 2015 and 2016 and species represented were detected on at least 15% of points sampled. Species close together occur in similar habitat conditions. Length of lines corresponding to habitat variables indicates the importance of each variable in influencing shrubland bird community structure. Location of each species point along habitat lines indicates the relative importance of the habitat variable on abundance of the species. Triangles indicate point count locations that were sampled. ALFL Alder Flycatcher, AMGO American Goldfinch, BAWW Black-and-white Warbler, CEDW Cedar Waxwing, CSWA Chestnut-sided Warbler, COYE Common Yellowthroat, EATO Eastern Towhee, GRCA Gray Catbird, INBU Indigo Bunting, NOCA Northern Cardinal, PRAW Prairie Warbler, SOSP Song Sparrow, YEWA Yellow Warbler.

Indicator species analyses identified 11 species ($p<0.05$) that occurred with high frequency in microhabitat conditions suitable for New England cottontail occupancy (Table 3.19). These 11 species include: Brown Thrasher (*Toxostoma rufum*), Field Sparrow (*Spizella pusilla*), Blue-winged Warbler (*Vermivora cyanoptera*), Alder Flycatcher (*Empidonax alnorum*), Gray Catbird (*Dumetella carolinensis*), Yellow Warbler (*Setophaga petechia*), Prairie Warbler (*Setophaga discolor*), Song Sparrow (*Melospiza melodia*), Indigo Bunting (*Passerina cyanea*), American Goldfinch (*Spinus tristis*), and Black-and-White Warbler (*Mniotilta varia*). In addition, a number of species were associated with microhabitat conditions suitable for New England cottontails at a *p*-value of <0.1 (Table 3.20).

Table 3.19 Results of indicator species analyses identifying shrubland specialist birds identified with high frequency and exclusivity in habitat suitable for New England cottontails (NEC) ($p<0.05$). Habitat groups suitable for New England cottontails are listed, along with indicator bird species for each habitat group, indicator values for the species (100 meaning a species is a perfect indicator of a habitat group), and *p*-values. FISP Field Sparrow, BRTH Brown Thrasher, YEWA Yellow Warbler, BAWW Black-and-white Warbler, BWWA Blue-winged Warbler, ALFL Alder Flycatcher, GRCA Gray Catbird, SOSP Song Sparrow, INBU Indigo Bunting, PRAW Prairie Warbler, AMGO American Goldfinch.

Indicator habitat group	Indicator species	Indicator values	*p*-values
NEC present at point count location	FISP, BRTH	22.0, 15.0	<0.05, <0.05
NEC present on patch	YEWA, BAWW	53.2, 42.6,	<0.05, <0.05
Thicket/shrub habitat	BWWA	25.0	<0.05
Coastal/wetland shrub habitat	YEWA, ALFL, GRCA	43.0, 26.7, 25.2	<0.001, <0.05, <0.05
Stem density ≥ 20,000 stems per acre	SOSP, INBU, PRAW	41.1, 36.3, 24.3	<0.05, <0.05, <0.05
Understory height ≥ 1.5 m	YEWA, GRCA, AMGO SOSP, FISP	48.8, 45.9, 41.7, 41.4, 30.2	<0.01, <0.01, <0.05, <0.05, <0.01
Vegetation density between 1-2 m, upper third of sites	YEWA, GRCA, PRAW	44.7, 41.4, 21.7	<0.05, <0.05, <0.05
Vegetation density between 2-3 m, upper third of sites	GRCA	41.4	<0.05
Brushpiles, upper third of sites	GRCA	45.4	<0.01

Table 3.20 Results of indicator species analyses identifying shrubland specialist birds associated with habitat suitable for New England cottontails ($p<0.1$). Habitat groups suitable for New England cottontails are listed, along with indicator bird species for each habitat group, indicator values for the species (100 meaning a species is a perfect indicator of a habitat group), and *p*-values. AMGO American Goldfinch, BAWW Black-and-White Warbler, CEDW Cedar Waxwing, SOSP Song Sparrow, INBU Indigo Bunting, BWWA Blue-winged Warbler, NOCA Northern Cardinal, FISP Field Sparrow.

Indicator habitat group	Indicator species	Indicator values	*p*-values
NEC present at point count location	AMGO, BAWW	54.6, 37.8	0.090, 0.094
NEC present on patch	AMGO	54.6	0.066
Coastal/wetland shrub habitat	CEDW, SOSP, AMGO	28.2, 24.1, 23.3	0.080, 0.084, 0.065
Edge habitat	INBU	24.1	0.062
Vegetation density between 2-3 m, upper third of sites	BWWA	17.3	0.067
Brushpiles, upper third of sites	NOCA	36.4	0.091
Moderate canopy cover, 0.33 – 0.66	AMGO, FISP	39.5, 15.5	0.064, 0.081

Discussion

We found significant relationships between species of shrubland specialist birds and vegetation structure, patch-level habitat attributes, and non-living structure habitat attributes. For each of the five focal species modeled, different habitat and patch-level covariates were found to influence occupancy, reflecting the variation in fine-scale habitat preferences of these shrubland species. Of the shrubland bird species for which we modeled occupancy in relation to habitat covariates, Yellow Warbler and Prairie Warbler showed higher occupancy at sites with microhabitat conditions associated with New England cottontail occupancy. Yellow Warblers occupied sites with high stem densities and Prairie Warblers occupied sites with dense vegetation between 2-3 m.

The associations that we observed between Yellow Warblers and Prairie Warblers and microhabitat conditions typical of occupied cottontail sites are supported by prior studies relating these bird species to vegetation characteristics. In addition to the association of Yellow Warblers with stem density in occupancy models, canonical correlation analysis showed that Yellow

Warblers were also associated with tall understory height and proportion of invasive vegetation, and indicator species analyses showed that Yellow Warblers were often found in coastal and wetland shrub habitat, at points with an understory height ≥1.5 m, at points with a high vegetation density between 1-2 m, and at sites with New England cottontails present on the patch. Yellow Warblers breed most commonly in wet, deciduous thickets (Lowther et al. 1999, Schlossberg & King 2007) and build nests at a mean height of 1.2 to 2.8 m (Graber et al. 1983, Peck & James 1987, Campbell et al. 2001). Yellow Warblers forage between heights of 1.2 to 4.9 m (Hutto 1981), with males foraging at greater heights and more conspicuously than females during territory establishment (Busby & Sealy 1979). Cottontail management on wet sites that increases stem and vegetation density and height will likely benefit Yellow Warblers.

The association we found between Prairie Warbler occupancy and vegetation density between 2-3 m is also supported by other research on the species. Prairie Warblers exhibit a preference for areas with low trees and shrubs (DeGraaf & Yamasaki 2001). They have been documented nesting at a mean height of 2.3 m and the heights of successful nests increase throughout the breeding season (Nolan 1978). Indicator species analyses further support the association between Prairie Warblers and vegetation density, with Prairie Warblers frequently detected at points with stem densities ≥20,000 stems per acre, and at points with dense vegetation between 1-2 m. The negative association we found with patch area in Prairie Warbler occupancy models is likely an artefact of our sampling regime and the low occupancy rate of Prairie Warblers on our sites. Prairie Warblers were detected most frequently on a 6.4 ha site in a transmission line right-of-way in Maine, near the northern extent of the species' breeding range. This was a small site in our study area, with surveyed patches ranging from 2.3-98.0 ha. In a study on area requirements for shrubland birds, Roberts and King (2017) found that Prairie

Warblers frequently occupied openings close to large patches of habitat, even if those openings were small in size including rights-of-ways. In the southern portion of our study area on Cape Cod, we detected Prairie Warblers on large patches of pine barrens habitat. This species is commonly associated with xeric upland habitat and conifers in much of its range (Nolan 1978, King et al. 2009). The negative association we detected between Prairie Warbler occupancy and brushpiles and the species' outlier position in canonical correspondence ordination may be artefacts of the small number of sites at which Prairie Warblers were detected at in our study area. Alternatively, the negative relationship with brushpiles may be due to the fact that brushpiles were not common in the pitch pine scrub-oak and right-of-way habitats in which Prairie Warblers were detected. It is also possible that brushpiles could provide refuge for nest predators that may negatively impact Prairie Warbler occupancy. A camera study of Common Yellowthroat and Prairie Warbler nests in New Hampshire found that the primary predators of nestlings were eastern chipmunks and garter snakes (M. Tarr, personal communication), species which could be more abundant in the vicinity of brushpiles. Sperry and Weatherhead (2010) documented radio-tracked snakes using brushpiles during 10% of their study period despite brushpiles comprising less than 0.2% of the study habitat, presumably because of increased small mammal abundances documented at brushpiles. Sperry and Weatherhead (2010) did not document increased avian nest predation in the first year following brushpile creation, but caution that brushpiles should be created away from avian nesting habitat due to their use by snakes and small mammals.

Through occupancy modeling, we found that Chestnut-sided Warblers were negatively associated with understory height. Roberts and King (2017) also found a negative association between Chestnut-sided Warbler occupancy and median vegetation height in forest openings.

This relationship is indicative of the Chestnut-sided Warbler's preference for complex vegetation structure for nesting substrate. Chestnut-sided Warblers have been documented nesting in shrubs at heights between 0.3 to 1.2 m (DeGraff & Yamasaki 2001). The complex vegetation structure preferred by this species is found in regenerating stands 3 to 10 years post-harvest, and occupancy declines steadily as stand development reduces available nesting habitat (DeGraff & Yamasaki 2003).

Occupancy models showed that Black-and-white Warblers were negatively associated with vegetation density between 0-1 m. Black-and-white Warblers are found in later successional habitats such as young forests, and abundance of the species has been found to increase linearly on a site up to 20 years after a clearcut (Schlossberg & King 2009). Black-and-white Warblers will use habitat with dense sapling to pole sized trees in semi-open and mature deciduous and mixed forests with shrubby understories (DeGraaf & Yamasaki 2001, Schlossberg & King 2007). The negative association we found with vegetation density at low heights could indicate selection against habitat in the early stages of shrubland succession, and is corroborated by an association with increased canopy cover in canonical correspondence analysis.

We found Eastern Towhee occupancy increased with an increase in patch area, and decreased with an increase in snags and woody debris. Canonical correspondence analysis also showed a negative association with snags. In our study area, snags and woody debris were more abundant on older successional sites or on sites that had recently been clearcut. Eastern Towhee abundances are low in years immediately following logging, increase for approximately 10 years post-logging, and then decline (Schlossberg & King 2009). We hypothesize the negative association between Eastern Towhee occupancy and sites with high numbers of snags and debris in our study area is indicative of Eastern Towhees selecting against recently clearcut and older successional sites. In a study on shrubland bird response to removal of harvest residues (i.e. coarse woody debris) from plots, Grodsky et al. (2016) found that Eastern Towhees did not frequently use coarse woody debris for foraging or cover and that successional trajectory rather than availability of harvest residues primarily influenced use of regenerating stands for most shrubland bird species. In contrast, Lohr et al. (2002) found that Eastern Towhees had fewer breeding territories on plots where downed wood had been experimentally removed in pine forests in the southeastern United States. The positive relationship between Eastern Towhee occupancy and patch area in our study area reflects the fact that Towhees were frequently detected at larger management sites ranging from 40 to 90 ha in Maine and New Hampshire, and in large pitch pine-scrub oak sites on Cape Cod. These sites were also typical of the relatively dry, open shrubby habitats with few trees associated with Eastern Towhee occupancy (Schlossberg & King 2007).

Collectively, findings from occupancy modeling inform us about the fine-scale habitat preferences of these five avian specialists and provide insights into their likely overlap with habitats managed for New England cottontails. Habitat management that creates dense, tall vegetation suitable for cottontail cover will provide valuable nesting and foraging substrate for Prairie Warblers and, on wet sites, Yellow Warblers. Complex vegetation structure lower to the ground that is not yet tall enough for cottontail cover will provide valuable nesting substrate for Chestnut-sided Warblers. Managing for young forest, which can provide suitable habitat for cottontails in some areas, for example dense alder stands under moderate canopy in coastal Maine, could provide valuable habitat for Black-and-white Warblers. Restoring pitch pine scrub-oak habitats characteristic of occupied cottontail habitat on Cape Cod will increase the amount of drier open habitat preferred by Eastern Towhees.

Poisson regression identified a strong positive relationship between the number of shrubland specialist bird species detected at a point and the proportion of woody invasive shrubs. This trend was also shown through the positive association of several species with proportion invasive vegetation in canonical correspondence analysis. However, notably, shrubland bird species richness was highest at sites with a moderate proportion of invasive shrubs and began to decline in heavily invaded sites with 55-93% invasive shrubs. This specific trend of higher shrubland bird richness in moderately invaded sites was documented in a recent study of native, mixed, and invaded sites in transmission line rights-of-ways in southeastern New Hampshire (Tarr 2017), suggesting there is a threshold at which a high proportion of invasive shrubs reduces available resources that can increase bird species richness. Other recent studies of shrubland birds in New England have also shown positive associations with invasive vegetation (King et al. 2009, Schlossberg et al. 2010), though negative impacts of invasive vegetation have been documented for shrubland birds, including a decline in forage resources (Tarr 2017) and an increase in nest predation (Borgmann & Rodewald 2004).

In a synthesis of shrubland bird habitat associations from recent studies across New England, Schlossberg et al. (2010) found that different suites of shrubland birds select for two distinct shrubland habitat types. Some shrubland birds prefer areas of tall (>1.5 m) vegetation with dense shrub cover, and other species prefer lower vegetation, fewer shrubs, and habitat with more abundant forbs and herbaceous vegetation. Our regression analyses showed that shrubland bird richness was positively associated with vegetation density between 0-1 m. High vegetation density between 0-1 m indicates suitable habitat for the suite of birds needing younger shrubland habitat or persistent shrublands with low vegetation and open areas with grasses and forbs, such as coastal shrublands or shrub habitats on dry or wet soils that impede forest succession. Hence, management that creates shrub habitats that are open and lack the cover needed by cottontails will benefit a different suite of shrubland birds such as ground nesters and foragers that require abundant grass and forb cover. Poisson regression showed a negative relationship between shrubland specialist richness and snags and woody debris. Sites in our study area that had a high number of snags and woody debris included recently clearcut sites, young forest sites, and pitch pine-scrub oak sites maintained with fire. The negative relationship between shrubland bird richness and snags and woody debris may be indicative of relationships with these specific habitat types rather than snags and woody debris specifically. In other words, in our study area richness was lower on young sites such as recent clearcuts, later successional habitats like young forest, and habitats with specific plant assemblages such as pitch pine-scrub oak.

Specialist birds requiring dense shrub cover and taller vegetation typical of habitats suitable for New England cottontails, were identified through indicator species analyses. Indicator species analyses identified 11 shrubland birds that were detected with high frequency in microhabitat conditions associated with New England cottontail occupancy. In addition to Yellow Warbler and Prairie Warbler, which were associated with habitat covariates pertinent to New England cottontails in occupancy models, indicator species analyses identified Brown Thrasher, Field Sparrow, Blue-winged Warbler, Alder Flycatcher, Gray Catbird, Song Sparrow, Indigo Bunting, American Goldfinch, and Black-and-white Warbler as species characteristic of microhabitat conditions suitable for New England cottontails.

Field Sparrows and Brown Thrashers were identified at points where New England cottontails had been detected within 50 m of the bird point count location (the area surveyed for vegetation structure and microhabitat conditions) within the past five years (winter 2012-2013 through winter 2016-2017). In our study area, Field Sparrows and Brown Thrashers were detected primarily on coastal shrubland sites that had areas of dense shrubs and interspersed open areas with grasses and forbs. The dense shrubs on these sites provide important cottontail cover. The open areas with grasses and forbs are important resources for Field Sparrows and Brown Thrashers which are ground foragers, and also provide nesting substrate for the ground nesting Field Sparrow. Herbaceous forage is also important for New England cottontails during the growing season (Dalke and Sime 1941, Smith and Litvaitis 2000), but dense escape cover must be present nearby. With New England cottontail occupancy considered as a patch-level metric, Yellow Warblers and Black-and-white Warblers were detected frequently at point count locations where cottontails were present within the larger patch (within 200 m of the point count location). These patches occupied by cottontails included wet coastal or riparian sites suitable for Yellow Warblers and young forests such as alder stands under a moderate canopy suitable for Black-and-white Warblers.

Yellow Warblers, Alder Flycatchers, and Gray Catbirds were detected at wet coastal sites and wetland thickets, habitat types associated with New England cottontail occupancy in Maine and seacoast New Hampshire. The Blue-winged Warbler was an indicator species of shrubland and thicket habitat not designated as coastal or wetland shrub. Yellow Warblers, American Goldfinches, Song Sparrows, and Field Sparrows were detected frequently on sites with an understory height of >1.5 m and would benefit from habitat management that increases vegetation height. These species have all been documented nesting in shrubs, besides the Field Sparrow which is a ground nester, and Yellow Warblers and American Goldfinches nest particularly high in shrubs at about 3.0 m (Lowther et al. 1999). Field Sparrows were detected at sites with complex vegetation structure including both areas of tall shrubs and open areas with herbaceous vegetation. Song Sparrows, Indigo Buntings, and Prairie Warblers were detected frequently on sites with stem densities >20,000 stems per acre, densities capable of providing escape cover for cottontails (NEC Regional Technical Committee 2013). Occupancy models showed that in addition to these species, Yellow Warblers could also benefit from management to increase stem densities on wet sites. Yellow Warblers, Gray Catbirds, and Prairie Warblers were detected frequently at points with high vegetation density between 1-2 m which provides suitable nesting substrate and tall enough cover for cottontails. Gray Catbirds were also an indicator species for dense vegetation between 2-3 m, and brushpiles, and as such would benefit from New England cottontail habitat management aimed at a number of microhabitat metrics. Gray Catbirds have shown increased abundances with increased vegetation height (Schlossberg et al. 2010), and nest in shrubs at least 1.2 m off the ground (Smith et al. 2011). Because Gray Catbirds forage for insects on the ground, brushpiles could increase forage resources for this species.

In addition to these 11 species that were significant indicators of habitat attributes related to New England cottontail occupancy ($p<0.05$), several species were also associated with these habitat attributes ($p<0.1$). Black-and-white Warblers were detected frequently at points with cottontails detected within 50 m, American Goldfinches were detected frequently on sites with cottontails present in the larger patch, Cedar Waxwings were detected in coastal and wetland shrub habitats, and Northern Cardinals were detected frequently at sites with a large number of brushpiles.

Conclusion

Efforts to restore New England cottontail populations in fragmented landscapes face many challenges, including the extremely limited dispersal exhibited by New England cottontails (Cheeseman 2017; M. Bauer, A. Kovach, B. Ferry, H. Holman, Chapter 1), and lack of corridors with dense cover connecting shrubland patches. However, given the extensive habitat management efforts underway to create and restore shrubland habitat for cottontails, there are a number of species that will likely benefit from this management before cottontail populations respond. Through occupancy modeling for shrubland specialist species and regression analyses relating shrubland bird richness to habitat covariates, we determined that shrubland birds associated with dense herbaceous vegetation and low shrubs will benefit from shrubland habitat management that creates microhabitat conditions that are not yet dense enough to provide cover for cottontails. Through occupancy modeling and indicator species analyses, we identified shrubland bird specialists that are indicative of the specific microhabitats required by New England cottontails and would benefit from shrubland habitat management focused on increasing vegetation density and height for cottontails. Eleven species of shrubland specialist birds are associated with sites that have been occupied by New England cottontails within the past five years, or sites with microhabitat conditions associated with New England cottontail occupancy. Of these 11 specialist birds, 9 are listed as Species of Greatest Conservation Need by at least one New England state (Gilbart 2012). We recommend that designating the New England cottontail an indicator species for shrubland habitat management would be a valuable classification. Significant resources are currently invested in New England cottontail management and outreach to create habitat for cottontails. These efforts will also benefit Prairie Warblers, Yellow Warblers, Brown Thrashers, Field Sparrows, Blue-winged Warblers, Alder Flycatchers, Gray

Catbirds, Song Sparrows, Indigo Buntings, American Goldfinches, and Black-and-white Warblers. Designating the New England cottontail a representative species would be an efficient conservation strategy for the management of multiple shrubland habitat specialists. Younger shrublands not yet suitable for New England cottontails or the shrubland birds associated with cottontail habitat would likely benefit an additional suite of shrubland birds requiring more open habitats. Shrubland bird species that are particularly rare, or experience unique management issues will require individual conservation planning.

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CONCLUSION

Shrubland and young forest habitats have declined in New England as a result of succession, suppression of natural disturbance regimes, and development (Cronon 1983, Litvaitis 1993), with concomitant declines in populations of shrubland specialist species, including the New England cottontail and shrubland birds (Litvaitis et al. 2006, Schlossberg & King 2007). Significant conservation efforts have been implemented to create and restore shrubland habitat with a focus on the New England cottontail, but with the goal that other species will also benefit (National Fish and Wildlife Foundation Keystone Initiative, Fuller & Tur 2012). Additional conservation efforts have been implemented to increase monitoring efforts for New England cottontail populations and increase reproduction in the captive breeding program to support ongoing reintroduction efforts (Fuller & Tur 2012, New England Cottontail Technical Committee 2018). Evaluation of shrubland specialist populations is needed to guide adaptive management and evaluate implemented restoration efforts. I used genetic monitoring to assess the connectivity of a small, isolated New England cottontail population in an urban landscape, and to track the success of a New England cottontail reintroduction. I modeled shrubland bird occupancy and frequency of detection in microhabitat conditions suitable for New England cottontails to determine if designating the New England cottontail a representative species as part of strategy to manage for multiple species would benefit shrubland birds, and which bird species would benefit most from management aimed at creating habitat for cottontails.

I demonstrated that genetic monitoring is a valuable tool to track the response of small New England cottontail populations to management. In the urban landscape of Londonderry, New Hampshire, I documented attributes of small populations that pose conservation challenges, including loss of metapopulation function, low genetic diversity, high relatedness, stochastic decline on isolated patches, skewed sex ratios on patches that had declined to a few individuals, and limited dispersal in a fragmented landscape. Genetic monitoring of a reintroduced population at Bellamy River Wildlife Management Area indicated that reintroductions can be successful and can bolster population sizes and genetic diversity. However, isolated reintroduced populations lacking connectivity to occupied patches within dispersal distance face the same threats as other small populations, including unstable demographics (Murrow et al. 2009), skewed sex ratios (Tella 2001, Clout et al. 2002), inbreeding depression (Brook et al. 2002, O'Grady et al. 2006), stochastic decline (Stacey & Taper 1992), and loss or lack of metapopulation function (Chandler et al. 2015). Populations of New England cottontails range-wide are experiencing similar conservation challenges, exhibiting fine-scale population structure and limited dispersal in fragmented landscapes (Fenderson et al. 2011, 2014, Cheeseman 2017).

Restoring multiple connected and occupied patches is key for successful reintroductions and restoration of existing populations of New England cottontails, which historically persisted in a metapopulation system. The importance of restoring multiple patches that facilitate dispersal indicates that expanding existing metapopulations will likely be more successful than restoring a new metapopulation isolated from existing populations in the landscape. Additional recommendations for a successful reintroduction of a small cottontail population vulnerable to stochastic decline include: annual monitoring to track population size, sex ratios, number of breeders, and genetic diversity; repeated reintroductions over time; and reintroducing a large number of individuals, distributed spatially to avoid exceeding carrying capacity and to combat high post-release mortality (Armstrong & Seddon 2008, Hamilton et al. 2010).

I also demonstrated that spatially explicit predictive modeling is a valuable tool for guiding restoration of cottontail populations. I parameterized a framework to simulate the effects of alternate restoration scenarios on cottontail population size, persistence, genetic diversity, and connectivity by reflecting restoration scenarios on a cost surface. For example, this model can predict how a population will respond to a particular management scenario, such as adding additional habitat patches or augmenting the population through translocations. This modeling framework will provide valuable information if a management scenario is revealed to be substantially more successful than others, or if given the best-case restoration scenario, a population is unlikely to persist in a given landscape. This predictive knowledge will help managers balance competing management priorities and make justifiable decisions if triage is necessary to maintain a population. In addition to providing a proof-of-concept for using predictive modeling as a management tool, the model I parameterized clearly shows the importance of dispersal for maintaining small cottontail populations in fragmented landscapes. Successful conservation will incorporate a focus on dispersal and functioning metapopulations.

Given the habitat creation efforts underway, information was needed on what other species benefit from the specific habitats managed for cottontails. I modeled shrubland specialist bird occupancy, assessed shrubland bird richness, and conducted indicator species analyses for birds detected on multiple shrubland habitat types in southern Maine, seacoast New Hampshire, and Massachusetts on Cape Cod. I identified 11 species of shrubland specialist birds associated with sites that have been occupied by New England cottontails within the past five years, or sites with habitat that could support, and is being managed for, New England cottontails. These include: Prairie Warblers, Yellow Warblers, Brown Thrashers, Field Sparrows, Blue-winged Warblers, Alder Flycatchers, Gray Catbirds, Song Sparrows, Indigo Buntings, American

Goldfinches, and Black-and-white Warblers. Of these 11 specialist birds, nine are listed as Species of Greatest Conservation Need by at least one New England state (Gilbart 2012). Designating the New England cottontail a representative species would be an efficient conservation strategy for the management of multiple shrubland habitat specialists.

Insights gained from studying the New England cottontail populations in Londonderry, New Hampshire and at the first reintroduction site on Bellamy River Wildlife Management Area highlight the challenges of restoring small populations in fragmented landscapes. Creating landscapes that facilitate dispersal between multiple patches in close proximity to one another is critical to restoring metapopulations that can persist long-term in stochastic environments. Ongoing restoration efforts focused on restoring habitat for New England cottontails will benefit a suite of shrubland specialist birds with similar habitat requirements and could effectively support populations of multiple declining specialist species.

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APPENDIX A:
MICROSATELLITE PRIMERS AND MULTIPLEX PCR CONDITIONS FOR THE ANALYSIS OF NEW ENGLAND COTTONTAIL PELLET AND TISSUE SAMPLES

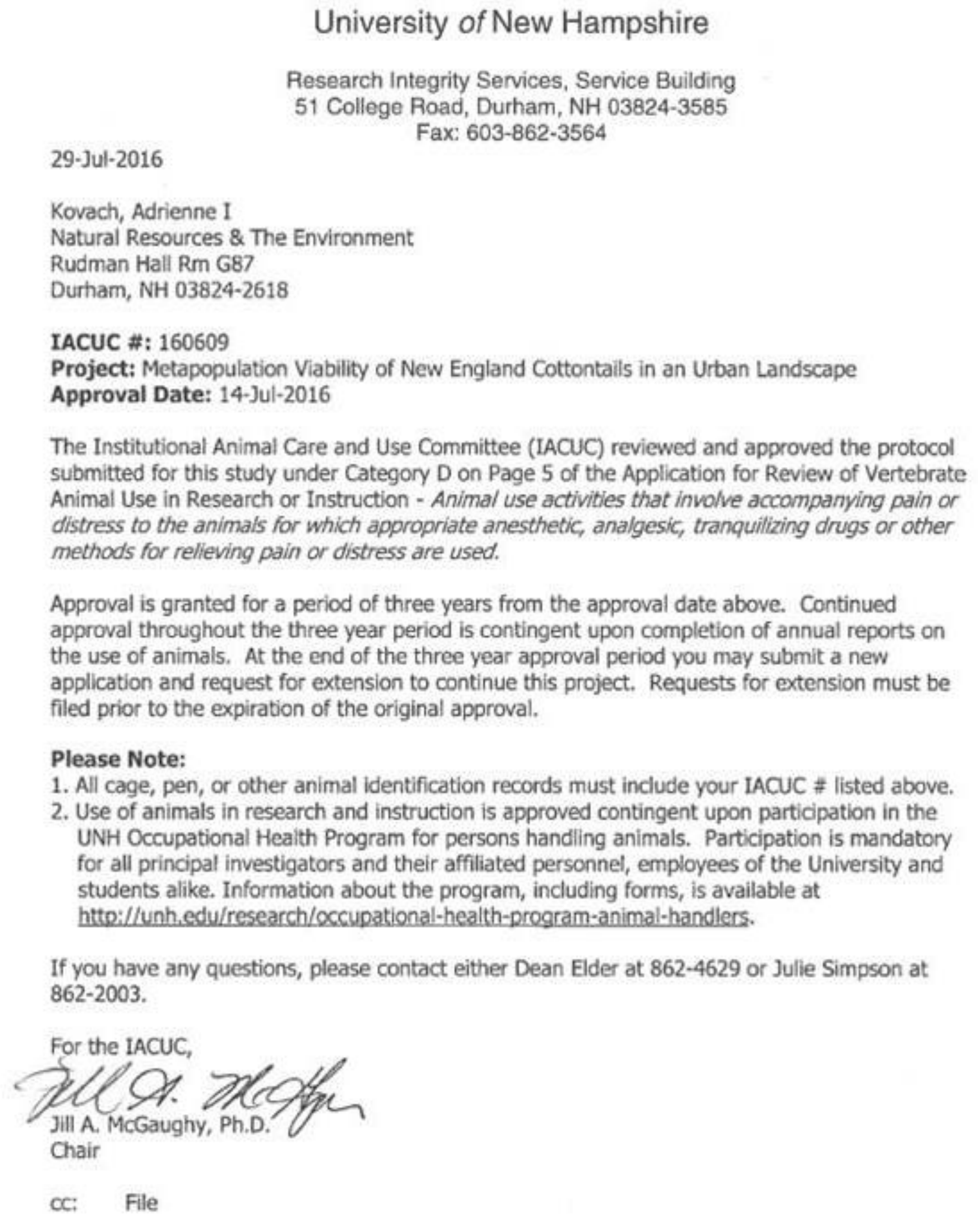
Table A.1 Microsatellite primers and multiplex PCR conditions for three multiplexes used in the analysis of New England cottontail pellet and tissue samples.

Primer	Multiplex
StrQ25	A
StrQ41	A
StrQ43	A
StrQ49	A
StrQ08	A
StrQ46	B
StrQ18	B
StrQ02	B
StrQ32	B
StrQ15	B
StrQ30	C
StrQ10	C
StrQ24	C
StrQ26	C
Sfl014	C
OcSRY21-F/ OcSRY23-R	C

Multiplex A and B PCR conditions included an initial denaturation at 95 °C for 10 min; 35 cycles of 30-s denaturation at 94 °C, 30-s annealing at 58 °C for Multiplex A and 59 °C for Multiplex B, and 30-s extension at 72 °C; and a final extension at 72 °C for 7 min. We used 25 uL reactions with 4 uL of DNA for pellet samples and 3 uL of DNA for tissue samples. Multiplex A and B PCRs contained primers (between 0.16 and 0.52 µM each), 1x buffer, 2.0 mM $MgCl_2$, 0.2 mg/mL of BSA, 200 µM DNTPs, and 1 unit of AmpliTaq Gold® 360 DNA polymerase from Applied Biosystems.

Multiplex C PCR conditions included an initial denaturation at 95 °C for 15 min; 35 cycles of 30-s denaturation at 95 °C, 90-s annealing at 58 °C, and 60-s extension at 72 °C; and a final extension at 60 °C for 30 min. We used 16 uL reactions with 4 uL of DNA for pellet samples and 3 uL of DNA for tissue samples. Multiplex C PCRs contained primers (between 0.07 and 0.2 µM each), and Qiagen Type-it Multiplex PCR Master Mix 1x.

APPENDIX B:
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) APPROVAL



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IACUC #: 160609
Project: Metapopulation Viability of New England Cottontails in an Urban Landscape
Approval Date: 14-Jul-2016

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category D on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *Animal use activities that involve accompanying pain or distress to the animals for which appropriate anesthetic, analgesic, tranquilizing drugs or other methods for relieving pain or distress are used.*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:

1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. Information about the program, including forms, is available at http://unh.edu/research/occupational-health-program-animal-handlers.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,

Jill A. McGaughy, Ph.D.
Chair

cc: File

APPENDIX C: POINT COUNT DETECTIONS OF SHRUBLAND BIRD SPECIALISTS IN ME, NH, AND MA IN 2015 AND 2016

Table C.1 Shrubland bird specialists detected within 50 m of the survey point by site in 2015 and 2016. Sites surveyed only in 2016 are listed in italics.

Region	**Site**	**ALFL**	**AMGO**	**BAWW**	**BRTH**	**BWWA**	**CEDW**	**COYE**	**CSWA**	**EATO**	**FISP**	**GRCA**	**HOWR**	**INBU**
Cape Elizabeth, ME	Crescent East	‘16	‘15, ‘16	‘15, ‘16			‘15, ‘16	‘15, ‘16			‘15	‘15, ‘16		‘16
Cape Elizabeth, ME	Crescent West	‘16	‘15, ‘16	‘16			‘15, ‘16	‘15, ‘16	‘15, ‘16			‘15, ‘16		
Cape Elizabeth, ME	Gull Crest	‘16	‘15, ‘16	‘15, ‘16			‘15, ‘16	‘15, ‘16	‘15, ‘16	‘16	‘15	‘15, ‘16		‘16
Cape Elizabeth, ME	Kettle Cove	‘16	‘15, ‘16	‘15, ‘16	‘16		‘15, ‘16	‘15, ‘16		‘15	‘15	‘15, ‘16	‘16	‘16
Cape Elizabeth, ME	Libby Field	‘16	‘15, ‘16	‘15			‘15, ‘16	‘15, ‘16	‘16	‘15		‘15, ‘16	‘16	‘16
Cape Elizabeth, ME	Barber Easement	‘16	‘15, ‘16	‘15, ‘16			‘15, ‘16	‘15, ‘16	‘15			‘15		
Cape Elizabeth, ME	*Kelly Field*	‘16	‘16	‘16			‘16	‘16				‘16		‘16
Cape Elizabeth, ME	*Willey Field*	‘16	‘16	‘16			‘16	‘16				‘16		
Cape Elizabeth, ME	*Scarborough Marsh*	‘16	‘16				‘16	‘16				‘16		‘16
Wells, ME	*Perkinstown Clearcut*		‘16	‘16			‘16	‘16		‘16		‘16		‘16
Wells, ME	Foss Property		‘15, ‘16	‘15, ‘16			‘15, ‘16	‘15, ‘16	‘16	‘15	‘15	‘16		
Wells, ME	Litchfield		‘15, ‘16	‘15, ‘16			‘16	‘15, ‘16	‘15, ‘16			‘16		
Wells, ME	Spiller	‘16	‘15, ‘16	‘15, ‘16			‘15, ‘16	‘15, ‘16				‘16		‘16
Wells, ME	Wells Reserve		‘15, ‘16	‘15, ‘16	‘16		‘15, ‘16	‘15, ‘16	‘15, ‘16	‘15, ‘16		‘15, ‘16	‘16	‘16
Kittery/York/Eliot, ME	Cutts Island		‘15, ‘16				‘15, ‘16	‘15, ‘16	‘15			‘15, ‘16		‘16
Kittery/York/Eliot, ME	Fort Foster		‘15, ‘16				‘15, ‘16	‘15, ‘16				‘15, ‘16	‘15	
Kittery/York/Eliot, ME	Highland Farm	‘16	‘15, ‘16	‘15, ‘16		‘16	‘15, ‘16	‘15, ‘16	‘15, ‘16	‘15		‘15, ‘16		
Kittery/York/Eliot, ME	Houde Powerlines		‘15, ‘16			‘16	‘15, ‘16	‘15, ‘16	‘15, ‘16	‘15, ‘16	‘16	‘15, ‘16	‘16	‘16
Kittery/York/Eliot, ME	Savage Preserve		‘15, ‘16	‘15			‘16	‘15, ‘16				‘16		
New Hampshire	Bellamy	‘16	‘15, ‘16	‘15, ‘16		‘16	‘15, ‘16	‘15, ‘16	‘15, ‘16	‘16		’15, ‘16		‘16
New Hampshire	Bunker Lane		‘15, ‘16	‘15		‘15, ‘16	‘15, ‘16	‘15, ‘16	‘15, ‘16			‘15, ‘16		‘16
New Hampshire	*Great Bay*	‘16	‘16	‘16	‘16	‘16	‘16	‘16		‘16	‘16	‘16	‘16	‘16
Cape Cod, MA	*Childs River*		‘16				‘16	‘16		‘16		‘16		
Cape Cod, MA	*Gravel Pit*		‘16					‘16		‘16				
Cape Cod, MA	*Orenda Land Trust*		‘16					‘16		‘16				
Cape Cod, MA	*Pine Barrens*		‘16				‘16	‘16		‘16		‘16		
Cape Cod, MA	*Quashnet River*		‘16					‘16		‘16		‘16		
Cape Cod, MA	*South Cape Beach*		‘16				‘16	‘16		‘16		‘16		

Region	Site	MAWA	NOCA	NOMO	PRAW	RTHU	RUGR	SOSP	WIFL	WTSP	YEWA	TOTAL Specialists
Cape Elizabeth, ME	Crescent East		‘15, ‘16					‘15, ‘16			‘15, ‘16	11
Cape Elizabeth, ME	Crescent West	‘16	‘15, ‘16			‘15	‘16	‘15, ‘16			‘15, ‘16	13
Cape Elizabeth, ME	Gull Crest	‘15	‘15, ‘16				’15, ‘16	‘15, ‘16			‘15, ‘16	15
Cape Elizabeth, ME	Kettle Cove		‘15, ‘16					‘15, ‘16			‘15, ‘16	14
Cape Elizabeth, ME	Libby Field		‘15, ‘16					‘15, ‘16			‘15, ‘16	13
Cape Elizabeth, ME	Barber Easement			‘15				‘15, ‘16			‘15, ‘16	10
Cape Elizabeth, ME	*Kelly Field*		‘16									8
Cape Elizabeth, ME	*Willey Field*							‘16			‘16	8
Cape Elizabeth, ME	*Scarborough Marsh*		‘16					‘16			‘16	9
Wells, ME	*Perkinstown Clearcut*				‘16			‘16				9
Wells, ME	Foss Property		‘15, ‘16					‘15, ‘16			‘16	11
Wells, ME	Litchfield		‘15, ‘16								‘16	8
Wells, ME	Spiller		‘15					‘15, ‘16			‘15	10
Wells, ME	Wells Reserve		‘15, ‘16	‘15, ‘16		‘16		‘15, ‘16		‘15	‘15, ‘16	16
Kittery/York/Eliot, ME	Cutts Island		‘15, ‘16	‘15	‘15			‘15, ‘16			‘15, ‘16	11
Kittery/York/Eliot, ME	Fort Foster	‘16	‘15, ‘16	‘15	‘15	‘16		‘15, ‘16			‘15, ‘16	12
Kittery/York/Eliot, ME	Highland Farm		‘15		‘15			‘15, ‘16	‘16		‘16	14
Kittery/York/Eliot, ME	Houde Powerlines		‘15, ‘16		‘15, ‘16			‘15, ‘16			‘15, ‘16	14
Kittery/York/Eliot, ME	Savage Preserve		‘15, ‘16					‘15, ‘16				7
New Hampshire	Bellamy	‘15	’15, ‘16					’15, ‘16			’15, ‘16	14
New Hampshire	Bunker Lane		‘16					‘16			’15, ‘16	11
New Hampshire	*Great Bay*		‘16			‘16		‘16			‘16	16
Cape Cod, MA	*Childs River*		‘16			‘16		‘16				8
Cape Cod, MA	*Gravel Pit*											3
Cape Cod, MA	*Orenda Land Trust*					‘16						4
Cape Cod, MA	*Pine Barrens*		‘16					‘16				7
Cape Cod, MA	*Quashnet River*				‘16							5
Cape Cod, MA	*South Cape Beach*		‘16									6

Table C.2 Four-letter American Ornithological Union Alpha codes for shrubland bird specialists detected in Maine, New Hampshire, and Massachusetts at 66 point count locations from 2015-2016.

Alpha code	Shrubland bird specialist species
ALFL	Alder Flycatcher
AMGO	American Goldfinch
BAWW	Black-and-white Warbler
BRTH	Brown Thrasher
BWWA	Blue-winged Warbler
CEDW	Cedar Waxwing
COYE	Common Yellowthroat
CSWA	Chestnut-sided Warbler
EATO	Eastern Towhee
FISP	Field Sparrow
GRCA	Gray Catbird
HOWR	House Wren
INBU	Indigo Bunting
MAWA	Magnolia Warbler
NOCA	Northern Cardinal
NOMO	Northern Mockingbird
PRAW	Prairie Warbler
RTHU	Ruby-throated Hummingbird
RUGR	Ruffed Grouse
SOSP	Song Sparrow
WIFL	Willow Flycatcher
WTSP	White-throated Sparrow
YEWA	Yellow Warbler