Monitoring occupancy and abundance of New England cottontails using non-invasive genetic tools

Daniel Brubaker

University of New Hampshire, Durham

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Monitoring occupancy and abundance of New England cottontails using non-invasive genetic tools

Abstract
The New England cottontail (Sylvilagus transitionalis) is a species of conservation concern. Efficient monitoring methods are needed to guide and assess conservation decisions in an adaptive management framework. I used genetic tools and non-invasively collected fecal DNA to determine New England cottontail detection rates during presence/absence surveys and to identify the environmental and behavioral factors that influence detection.

I found New England cottontail detection rates to be high (>90%) when surveys were conducted under ideal conditions. Prior knowledge of cottontail activity, low snow depth, and allowing 2-4 days without high winds following a snowfall are the most important factors positively associated with cottontail detection. I also found that increased patch size reduces detection when search efforts are limited to 20 minutes.

I used genetic mark-recapture methods to produce baseline abundance estimates for New England cottontail populations across their range. I used microsatellite genotyping in conjunction with single session mark-recapture models in the program CAPWIRE to estimate New England cottontail abundance on 17 occupied patches in Maine, New Hampshire, and New York. Precision of estimates was reasonable for most small sites and several large sites, but decreased with increasing subsampling distance. I also evaluated the methodology used and recommended changes to future survey efforts to improve efficiency and precision. These recommendations include allowing at least three days to pass following a snowfall before conducting a population survey, and sampling pellets intensively on sites to provide a better chance of obtaining an adequate number of recaptures. The tools developed herein will be useful in future occupancy monitoring and abundance estimation needed for the adaptive management of New England cottontail populations.

Keywords
Biology, Genetics, Agriculture, Wildlife Conservation, Biology, Ecology

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MONITORING OCCUPANCY AND ABUNDANCE OF NEW ENGLAND COTTONTAILS USING NON-INVASIVE GENETIC TOOLS

BY

Daniel Brubaker

Baccalaureate Degree (BS), Eastern Mennonite University, 2005

THESIS

Submitted to the University of New Hampshire
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In
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This thesis has been examined and approved.

Thesis Director, Adrienne Kovach
Research Assistant Professor
Natural Resources

Mark Ducey, Professor of Forest Biometrics and Management

Walter J. Jakubas, Mammal Group Leader
ME Dept. of Inland Fisheries and Wildlife

Kate O’Brien, Refuge Biologist
USFWS, Rachel Carson
National Wildlife Refuge

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Date
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ABSTRACT

MONITORING OCCUPANCY AND ABUNDANCE OF NEW ENGLAND COTTONTAILS USING NON-INVASIVE GENETIC TOOLS

By

Daniel Brubaker

University of New Hampshire, March, 2012

The New England cottontail (*Sylvilagus transitionalis*) is a species of conservation concern. Efficient monitoring methods are needed to guide and assess conservation decisions in an adaptive management framework. I used genetic tools and non-invasively collected fecal DNA to determine New England cottontail detection rates during presence/absence surveys and to identify the environmental and behavioral factors that influence detection.

I found New England cottontail detection rates to be high (>90%) when surveys were conducted under ideal conditions. Prior knowledge of cottontail activity, low snow depth, and allowing 2-4 days without high winds following a snowfall are the most important factors positively associated with cottontail detection. I also found that increased patch size reduces detection when search efforts are limited to 20 minutes.

I used genetic mark-recapture methods to produce baseline abundance estimates for New England cottontail populations across their range. I used microsatellite genotyping in conjunction with single session mark-recapture models in the program CAPWIRE to estimate New England cottontail abundance on 17 occupied patches in Maine, New Hampshire, and New York. Precision of estimates was reasonable for most
small sites and several large sites, but decreased with increasing subsampling distance. I also evaluated the methodology used and recommended changes to future survey efforts to improve efficiency and precision. These recommendations include allowing at least three days to pass following a snow fall before conducting a population survey, and sampling pellets intensively on sites to provide a better chance of obtaining an adequate number of recaptures. The tools developed herein will be useful in future occupancy monitoring and abundance estimation needed for the adaptive management of New England cottontail populations.
Introduction

Wildlife monitoring is a vital part of species conservation, providing management agencies the ability to determine the impacts that management decisions have on target populations. Large scale habitat loss has placed increased pressure on many habitats and species (Sodhi et al. 2008; Underwood et al. 2009) and long-term monitoring programs are needed to provide reliable data for future management. This is especially true for threatened and fragmented species whose conservation may depend on annual data concerning their status. This can be time and resource intensive, creating the need for efficient monitoring techniques. The use of genetic tools, particularly from non-invasively collected DNA, provides one such method. The purpose of this study was to develop and evaluate non-invasive genetic tools and methodologies to effectively monitor New England cottontail populations across their range.

For species like the New England cottontail that inhabit fragmenting landscapes, large patches become less contiguous and lower quality edge habitat increases, leading to an overall decrease in healthy core habitat decrease (Temple & Wilcox 1986; Vergara & Hahn 2009). An equally serious side effect of fragmentation is that patches may lose connectivity within the landscape, isolating plant and animal species in patches that are too small to persist over time.

As habitat degradation and patch isolation increase, stochastic factors may raise extinction probabilities within remaining populations. Stochastic forces can be demographic, genetic, or environmental and have the greatest impact on small
populations (May 1973; Roughgarden 1975). Demographic forces include mortality and fecundity rates, as well as random fluctuations in the breeding success in small populations. Genetic stochasticity can result from loss of variability due to inbreeding, founder effects, and random fixation through drift (Melbourne & Hastings 2008). The effects of genetic stochasticity have been shown in a number of small or declining populations (Gottelli et al. 1994; Lacy & Lindenmayer 1995; Broders et al. 1999; Larson et al. 2002). Environmental stochasticity includes fires, floods, severe storms, drought, and other natural disasters. Management for declining populations must take these factors into account when determining a minimum viable population for a particular species and when reconstructing habitat. Species must be capable of persisting not only during ideal conditions, but also through infrequent but catastrophic stochastic events.

Fragmentation caused by natural disturbances is generally temporary while anthropogenic disturbances often create permanent alterations and loss of both habitat and connectivity. The permanent and expansive nature of human development makes it vital to identify and preserve movement corridors and maintain connectivity between existing populations (Bolger et al. 2001). In most situations restoring habitat to its historic levels is not feasible because either those levels are not known, or unalterable changes have occurred since those levels were attained.

Early successional habitat has been extensively impacted by anthropogenic disturbance or lack thereof. Early successional habitat is often referred to as “thicket” or “shrubland” and is highly ephemeral. Without periodic disturbance, shrublands will mature into forest stands. These disturbances may be caused by natural or anthropogenic
events. Natural events include coastal salt spray, high winds, diseases, beaver activity, and stand-replacing disturbances to forests due to insects, hurricanes, and wildfire. Anthropogenic forces prior to European settlement were generally associated with prescribed burning by Native Americans (Lorimer & White 2003). Prior to migrating westward, European farmers cleared large tracts of land for agriculture and livestock grazing. During the early 1900’s these abandoned farms underwent succession, greatly increasing the amount of thicket habitat available (Litvaitis 2003). Subsequent human development and the maturation of thickets into closed stand forests began to erode this early successional habitat during the 1930’s, and declines rose sharply by the middle and later parts of the twentieth century (Trani et al. 2001). During this time land ownership shifted from a handful of large plots to a greater number of smaller ones. Excluding Maine, 88% of forested land in New England is privately owned (Litvaitis 1993).

There has been a visible reduction in the population size of animal species that mirrors the loss of early successional habitat following the middle of the twentieth century. Populations of many avian species that rely partially or entirely on thicket habitat, e.g. the yellow-breasted chat (*Icteria virens*) (Annand & Thompson 1997), are in decline. It is suggested that shrubland bird communities should receive a “high degree of conservation attention within the northeastern US (Detmers 2003).” Other taxa, including mammals (Fuller & DeStefano 2003) and insects (Wagner et al. 2003) are also negatively impacted by the loss and fragmentation of thicket habitat.

The New England cottontail is one of the mammal species negatively impacted by the loss of thicket habitat. New England cottontails were first identified as a unique
species by H.E. Holden and were reported to range from Maine south along the Appalachian Mountains to Alabama (Holden & H.S. 1970). Subsequently, variation of the chromosome number between northern and southern populations of New England cottontail (52 in northern and 46 in the southern) was used as the basis for dividing the taxon into two sister species (Ruedas et al. 1989; Chapman et al. 1992). The Hudson River became a line of demarcation with populations to the north and east remaining New England cottontail, and populations south and west of the river becoming Appalachian cottontails (Sylvilagus obscures). The Integrated Taxonomic Information System maintains this distinction and New England cottontails continue to be managed as a distinct species, although analysis of mitochondrial DNA has suggested that the differences between the two species may not be great enough to warrant the separation (Litvaitis et al. 1997).

A recent range-wide inventory of New England cottontail occupancy by Litvaitis et al. (2006) concluded that the species is only present in 14% of its historic range. Potential causes for their decline include loss and fragmentation of habitat, and indirect competition with eastern cottontails (Sylvilagus floridanus) (Litvaitis et al. 2008). The potential for competition between New England and eastern cottontails began during the 1920s when hunters released >200,000 eastern cottontails into the northeast (Chapman & Morgan 1973). Chapman and Morgan’s (1973) “niche width-introduction hypothesis” suggests that eastern cottontails have the ability to survive in a wider array of habitat types than New England cottontails. Eastern cottontails are also approximately 20% larger than New England cottontails (Litvaitis et al. 2008), but despite these factors, no
significant interference competition was found in a study between the two species in artificial enclosures (Probert & Litvaitis 1996). Support for potential hybridization has been reported in captive cottontails (Fay & Chandler 1955), but is not supported by mtDNA analysis (Litvaitis et al. 1997). Therefore, currently it seems unlikely that hybridization is having an impact on New England cottontails.

New England cottontail populations exist and function as a metapopulation mirroring that of the early successional habitat on which they rely. Metapopulation theory was first introduced by Levin’s classic model which portrayed populations inhabiting a static number of habitat patches with the ratio of patches occupied fluctuating over time (Levins 1969). Populations of New England cottontails, though, are functionally more similar to the mainland-island metapopulation model (Litvaitis & Villafuerte 1996). This model contains one or more large stable patches (mainland) where extinction is unlikely, connected to numerous smaller patches (islands), each with varying probabilities of local extinction, but which also have the potential to be recolonized by the mainland population (Ross 2006). For New England cottontails, mainland/source populations are often found in the coastal shrublands (Litvaitis 2003) which are less prone to closed canopy succession, providing more temporally stable habitat. Source populations are also found inland in habitat with frequent fires or poor soil, preventing forest succession (Latham 2003). Island/sink patches include the many smaller patches that regularly undergo forest succession. Roads, along with residential and commercial development have effectively contracted many source populations while
further isolating sink patches. The consequence is greater isolation and higher extinction rates on island patches because there are fewer migrants available for recolonization.

Effective long term monitoring has become important for New England cottontails because of their naturally ephemeral metapopulation structure and significant habitat loss over recent decades. These factors have made many populations unstable, accelerating changes in patch occupancy and increasing the risk of regional extirpation (Litvaitis et al. 2006; Fenderson et al. 2011). Adaptive management has become crucial for species like New England cottontails because it provides the ability to tailor conservation strategies in response to changes in cottontail population health at local and regional scales. These changes can only occur if monitoring methods are in place to provide accurate information on New England cottontail status. It is difficult to collect this information for New England cottontails due to the poor visibility in thicket habitat, small numbers of occupied patches, and their naturally secretive behavior. Another challenge is their co-occurrence with eastern cottontails (Sylvilagus floridanus) across much of their range. These challenges make traditional monitoring techniques expensive and inefficient. Noninvasive genetic sampling provides a solution, and has been used successfully on a variety of other rare or elusive animal species (Woods et al. 1999; Hájková et al. 2009; Kendall et al. 2009). It requires less time than traditional methods and does not impact the sampled species (Waits & Paetkau 2005).

This study investigated two important aspects of New England cottontail monitoring using DNA extracted from non-invasively collected fecal pellets. Chapter One addresses issues of detectability as they relate to determining New England
cottontail patch occupancy. This includes determining detection rates for New England cottontail surveys, as well as understanding the environmental and behavioral factors that affect those rates. I accomplish this through systematic repeated surveys of 30 patches occupied by New England cottontails and I present recommendations for future occupancy monitoring surveys. In Chapter Two I use single session mark recapture models to make population estimates for New England cottontails on patches across their range. I also present methods for optimizing future population surveys with regards to pellet collection and analysis.
CHAPTER 1

Detection Rates and Factors Influencing the Detection of New England Cottontails

Daniel R. Brubaker, Adrienne I. Kovach, Mark J. Ducey, Kathleen M. O’Brien, and Walter Jakubas

Abstract

The New England cottontail (Sylvilagus transitionalis) is a species of conservation concern across its range due primarily to extensive loss of its preferred early successional habitat. To facilitate efficient broad scale occupancy monitoring efforts we conducted repeat presence/absence surveys on 30 sites occupied by New England cottontails to determine what environmental and behavioral factors have the most influence on cottontail detection.

We modeled cottontail detectability in the program PRESENCE and found that detection rates are high (>90%) when surveys are conducted under ideal conditions and found that prior knowledge of cottontail activity, low snow depth, and an increased number of days without high winds following a snowfall as the most important factors associated with cottontail detection. We also found that large patch size reduces detection when search efforts are limited to 20 minutes. We recommend conducting occupancy surveys in snow less than 12 inches deep 2-4 days (without high winds) after a snowfall. We also recommend surveying large sites without a restricted search time.
Our findings show that under the recommended survey conditions, New England cottontail occupancy can be determined on a broad scale in 1-2 visits.
Introduction

The monitoring of biological resources is of major importance in conservation biology (Marsh & Trenham 2008) and a key component of successful, active conservation management (Nichols & Williams 2006). Monitoring has the capacity for generating ecological knowledge about the behavior and dynamics of a system as well as guiding and evaluating the effectiveness of management actions (Yoccoz et al. 2001). Site occupancy modeling can be used to identify spatial and temporal factors in occupancy and is an effective technique for monitoring rare, cryptic, and endangered species in a landscape context (MacKenzie et al. 2005; Nielsen et al. 2008). Trends in occupancy status can indicate population trends and occupancy data can be used to make inferences about abundance (MacKenzie 2005). Occupancy monitoring objectives can be incorporated into adaptive management programs, such that the results of monitoring are used to drive management as well as to assess the response of the system to management actions in an iterative process (Nichols & Williams 2006).

Occupancy status is determined from presence/absence data collected from suitable habitat patches in the landscape. As is true for all monitoring data, detectability is a primary source of variation that generates error in presence/absence data (Yoccoz et al. 2001). The use of single surveys makes the assumption that the species of interest will be detected if it is present at a given site. In many cases, however, a species’ detection rate may be well below one (Gu & Swihart 2004), and failure to account for the lowered detection rate can result in incorrectly identifying occupied sites as vacant, leading to misinformed management decisions (MacKenzie 2006). Modified occupancy models have been developed to account for imperfect detection (MacKenzie et al. 2002;
Mackenzie 2003; Royle & Nichols 2003). These models call for repeated surveys of target sites, during which the species’ presence/absence is recorded within a period of time that allows for assumptions of population closure to be met. A cumulative detection history is built for each site, from which is calculated the probability that an animal is actually detected when present. This observed detection rate can then be applied as a correction factor in estimating occupancy (MacKenzie et al. 2002). This approach is particularly useful for monitoring rare and cryptic species for which detection rates are typically low (Heard et al. 2006; Roughton & Seddon 2006; Durso et al. 2011; Scharine et al. 2011).

One rare and cryptic species for which accurate occupancy monitoring is needed is the New England cottontail (Sylvilagus transitionalis). Once widespread throughout the New England states and eastern New York, populations of New England cottontail have declined dramatically in recent decades, and continue to do so today, due to loss and fragmentation of critical habitats upon which the species depends (Litvaitis et al. 2006; Fenderson et al. 2011). New England cottontails rely on dense thicket habitats in the form of early successional or coastal shrubland (Barbour & Litvaitis 1993; Litvaitis et al. 2003). These habitats are often ephemeral, due to their dependence upon mature forest stand disturbance. The loss of many historic disturbances (fire, beaver activity, agricultural clearing) combined with land use change have precipitated a steep decline in these habitats in recent decades, along with a decline in populations of a suite of species that depend on them (Trani et al. 2001; Brooks 2003; Litvaitis 2003; Lorimer & White 2003; King et al. 2009). Many of the remaining suitable habitat patches are small, precluding them from sustaining significant cottontail populations, and therefore making
them highly susceptible to local and regional extinction (Litvaitis & Villafuerte 1996). Decreased connectivity of the landscape exacerbates this problem by impeding recolonization of increasingly isolated patches (Fenderson 2010). Remaining New England cottontail populations today occur in five geographically and genetically distinct regions within less than 14% of the species’ historic range (Litvaitis et al. 2006; Fenderson et al. 2011). As a result of this extensive decline, range contraction, and uncertainty for long-term viability of the New England cottontail, the species is a candidate for federal listing under the Endangered Species Act and is listed as endangered in Maine and New Hampshire (MDIFW 2007; NHFG 2008; USFWS 2008).

Occupancy monitoring provides guidance for adaptive management of New England cottontails. The most efficient method for monitoring cottontails is by noninvasive fecal pellet surveys conducted after fresh snowfall in the winter (Litvaitis et al. 2006). A diagnostic mitochondrial DNA (mtDNA) test is used to determine the species of origin of the pellets (Kovach et al. 2003), as the New England cottontail occurs sympatrically with either eastern cottontail (*Sylvilagus floridanus*) or snowshoe hare (*Lepus americanus*) throughout portions of its range, and the pellets of the different species are not distinguishable in the field. In fresh snow conditions the tracks of cottontails can typically be distinguished from those of snowshoe hare, and track surveys can be used to aid occupancy determination (Litvaitis et al. 2006). The outcome of this effort is a determination of patch-specific presence/absence based on a single survey visit. This approach was used in a recent range-wide survey to determine occupancy status across the historic range of the species (Litvaitis et al. 2006), whereby New
England cottontails were only found in 7% of the 2,301 patches of suitable habitat searched.

Presence/absence winter pellet surveys continue to be used to track occupancy status of New England cottontails on suitable patches within occupied portions of the species’ range. On such a local scale, however, given the scarcity and ephemeral occupancy status of the suitable patches, this approach is likely fraught with issues of imperfect detection. The ability to determine detection rates and uncover the environmental factors and survey conditions that influence detection of New England cottontails during winter pellet surveys is important for optimizing the reliability of current occupancy monitoring approaches. Factors likely to influence detection of New England cottontails during winter surveys include those that affect cottontail activity and those that affect the efficiency of observational success of the surveyor. Temperature and other weather conditions may affect cottontail behavior by limiting their movement around a patch, effectively reducing the amount of detectible sign. Factors like patch size and vegetation characteristics may impact survey efficiency and visibility of pellets to the observer. Some factors may have an effect on both rabbit behavior and survey effort, and it would be useful to distinguish which influence is more important in the survey outcome. For example, deep snow limits the mobility of both cottontails and surveyors while dense vegetation may be linked with higher cottontail density, thereby presumably increasing detection rates, but may also reduce surveyor visibility and mobility.

To address these issues of detectability, we conducted a systematic study of detection of New England cottontails during presence/absence surveys. Our specific objectives were to 1) estimate the probability of detecting New England cottontails on
occupied sites, 2) identify the factors that influence detection, 3) determine optimal
survey conditions and survey effort for reliable inference of occupancy, and 4) develop
recommendations for improved occupancy monitoring to facilitate the adaptive
management of this threatened species.

**Methods**

**Study Sites**

In 2010 and 2011, we conducted a series of systematic, repeated presence/absence
surveys of 60 sites across the New England cottontail’s range (Appendix A, Table 1, and
Figure 1). New England cottontails were detected at least once on 30 sites and results
from those sites were used for this study (Figure 1.1). Sites were chosen based on known
occupancy from 2007-2009 winter survey efforts in Maine, New Hampshire, and
Connecticut and based on the most recent occupancy data (Litvaitis et al. 2006) in New
York. Because our objective was to determine the factors that influence detection on
occupied sites, we focused only on sites of known or highly probable occupancy. Sites
ranged in size from 2-26 ha. Sites in Maine and New Hampshire were generally more
isolated, often surrounded by development, open fields, or, in the case of coastal sites,
rocky coastline and open water. Sites in New York and Connecticut were predominantly
early successional shrub land or wetlands surrounded by mature forest. Maine and New
Hampshire sites contained only New England cottontail while New York and
Connecticut sites were co-occupied by New England and eastern cottontails. Sites were
comprised of patches of continuous suitable habitat that a cottontail may utilize without
venturing into a risky open area (>30 feet wide), and were delimited by areas of highly
unsuitable vegetation (open fields or open forest), major roads, or water bodies.
**Presence-absence surveys**

Surveys occurred in the wintertime, with snow on the ground, and at least 12-24 hours after a snowfall event, following Litvaitis et al. (2006). Patches were surveyed systematically using loose, continuous transects, winding back and forth across the patch with approximately 30-meter spacing. We considered utilizing fixed straight line transects but the dense vegetation structure makes following straight transects difficult and inefficient. In addition, loose transects allow the surveyor to focus on the highest quality habitat and to quickly search the edges of dense thickets where pellets are most visible and often located. Searches continued until a cluster of pellets was found, or until all suitable habitat had been exhaustively searched. For sites with both cottontail species, searches continued until three to five distinct pellet clusters separated by at least 100 meters were detected. Once detected, pellets were collected for later genetic species identification. To maximize the likelihood that each cluster of pellets originated from a single rabbit, they were collected from an area of no more than 5 x 5 ft. For patches >6 acres, the search area was restricted to 2-acre subplots within the patch. To ensure similar search effort, the total area searched for each patch was equivalent to six acres or 20% of the total patch area, whichever was greater.

Increasing the number of visits per site improves the precision of the estimated occupancy rate as well as the accuracy of the estimate when detection probabilities are low (MacKenzie et al. 2002). Our target was to visit each site five times whenever logistically feasible, with a minimum of three visits (Mackenzie & Royle 2005). Twenty-nine of the 30 sites were visited at least three times: one 3 times, 11 four times, 15 five times, and 2 sites six times. To meet the assumption of population closure with respect to
patch occupancy, we attempted to complete the majority of searches within a 6-week window of time, ideally within the first half of the winter (late December – mid February). Surveys occurred between December 23 and March 25 across all sites. The average survey window across all sites was 43 days. All but two sites were surveyed during the winter of 2011. Two sites had unconfirmed occupancy in 2011 so for these we used surveys completed in 2010.

**Species identification from fecal pellets**

We used diagnostic genetic assays to determine the identity of the species that deposited the pellets. We extracted DNA from pellets using QIAamp® DNA Stool Mini Kits (Qiagen, Valencia, California) following the methods of Kovach et al. (2003). We amplified an approximately 560 base pair segment of the mitochondrial control region and used a combination of two diagnostic RFLP tests, one using the restriction enzyme \textit{Nla III} (Kovach et al. 2003) and one with \textit{Bfa I} (Litvaitis & Litvaitis 1996), to distinguish pellets of New England cottontails from those of the two sympatric lagomorph species, eastern cottontails and snowshoe hares. On sympatric sites, we assayed pellet samples until we identified a New England cottontail or exhausted all collected samples.

**Covariates and variable reduction**

During each site visit surveyors collected data on the following covariates: observer, patch size, search time, snow condition (no snow, powder, wet snow, crusted snow, melted out), snow depth (categorized as < or > 12 inches), and days since last snow fall (a measure of the number of pellet deposition days). We recorded the time spent searching at each patch and calculated the area searched during each survey by buffering a fixed distance, based on average patch stem density, from the recorded search path.
Sites with lower stem densities were given a larger buffer distance due to the increased line of site in less dense vegetation. For each visit we also identified if the surveyor had prior knowledge of cottontail activity. We considered prior knowledge to be known locations of pellets or rabbit sign from a previous visit that same field season, or from information provided by the landowner concerning specific rabbit locations within the patch. To account for differences in habitat suitability we measured the average stem density at each patch by averaging estimated counts of all woody stems at a height of half a meter, obtained for up to 30 evenly spaced 1x2 meter plots per patch with a minimum of 10 plots on the smallest patches. Finally, we collected temperature, wind, and precipitation data from Weather Underground (weatherunderground.com) for all potential pellet deposit days, which we identified as any day after the last snowfall but prior to each respective survey. We then used these data to further categorize the number of pellet deposition days as the total number of days since snowfall without high winds above 40 km/h and the total number of days since snowfall with temperatures > than -10°C. High winds have been shown to negatively affect lagomorph activity (Fletcher et al. 1999; Ballinger & Morgan 2002) and this may be true for New England cottontails particularly in the winter. High winds decrease temperatures through wind chill, and severely cold temperatures may limit cottontail activity.

We collected data on 11 different factors (see Table 1.1 for factor descriptions and abbreviations), which we then reduced using preliminary statistical testing to obtain a reduced set of informative factors for detection modeling. We removed Observer from consideration because the logistics of surveying sites across New England produced too many observers to be statistically viable with our sample size. We then used nominal
logistic regression to select the most influential factor for correlated sets. These sets included several of the original covariates that were measured as slight variations of the same factor (e.g., multiple measures of pellet deposition days evaluated as iterations of days since snowfall with or without accounting for influence of temperature or wind) and covariates that were evaluated as both continuous and nominal variables (e.g., total number of days since snowfall and greater than or less than two days since snowfall).

From the remaining uncorrelated set of factors, we then used partition modeling to identify uninformative factors and removed them from further analysis. This resulted in the removal of DaysTemp>-15°C, SnowPowder, and both SearchTime and SearchArea.

We performed a final simple linear regression on the remaining factors and retained those with significant effect likelihood scores (Table 1.1). PatchSize and StemDensity were retained despite non-significant effect likelihood scores because their effects on detection were of particular interest. Despite some multicolinearity with Days and DaysWind, DaysTemp was also included in the modeling because we were interested in potential effects of temperature on detection. We retained six factors for further analysis through detection modeling: PatchSize, StemDensity, Knowledge, SnowDepth, and two measures of the number of days since last snow fall, DaysWind and DaysTemp.

**Detection modeling**

We modeled New England cottontail detectability as a logit function of the six selected covariates in the program PRESENCE 2.0 (Hines 2006). The logit link function states that

$$\theta = \frac{\exp(XB)}{1+\exp(XB)},$$
where $\theta$ represents the detection probability, $X$ is the covariate value, and $B$ is the model parameter. We constructed 36 a priori models based on our knowledge of cottontail biology and survey logistics. Models held occupancy constant at one and allowed detection to be a function of covariates. Given our exclusive use of occupied sites, this approach enabled us to evaluate directly the influence of survey covariates on detection without confounding influence of occupancy status (Mackenzie et al. 2006). To explore the effects of a threshold search time, we also modeled detection probabilities using only detections that occurred within the first 20 minutes of a survey. The 20-minute threshold was chosen because it has been used in past protocols for cottontail occupancy surveys (Litvaitis et al. 2006) and because we found that 82% of detections in this study occurred within this time period (Fig. 2)

Candidate models were ranked according to Akaike’s information criteria corrected for small sample size ($\text{AIC}_c$). The variance inflation factor, ($\hat{c}$), calculated from a goodness-of-fit test on our global model, was not greater than 1 and didn’t require a quasi-likelihood modification (Burnham & Anderson 2002).

Models with the lowest $\text{AIC}_c$ were considered to have the best combination of fit and parsimony. We used Akaike weights to evaluate the probability that a particular model was the best in our candidate set of models. To evaluate the relative influence of covariates, model weights were summed for all candidate models in the 95% confidence set (all models whose summed weights represented at least 95% of the total weight of the candidate set of models) with the given covariate ($w^+(i)$; Burnham and Anderson 2002).
Results

We completed 137 surveys over the 30 study sites and detected New England cottontail during 100 of those surveys, resulting in a detection probability of 0.73. The best-fitting model indicated that Knowledge ($w_{+}(i) = 1$), SnowDepth ($w_{+}(i) = 1$), and DaysWind ($w_{+}(i) = .89$) were the most influential factors influencing cottontail detection probabilities (Table 1.2). All additional models with $\Delta AICc < 2$ contained these three variables (Table 1.3). These hierarchically more complex (nested) models were not considered competitive and the additional variables in them were interpreted as having poor explanatory power; the weights of these nested models were combined into the weights of the more parsimonious model (Arnold 2010).

Regression of New England cottontail detections by survey search time showed that the benefit of increasing search time decreased dramatically beyond 20 minutes. Eighty two percent of the detections during our study occurred prior to 20 minutes. Increased search time only increased detections slightly, with 87% of total detections occurring within 30 minutes and 93% within 40 minutes. Beyond 40 minutes, the added search time provided very little return in additional detections (Fig. 2). Restricting searches to 20 minutes decreased detection probability to 0.62. In this model set, Knowledge ($w_{+}(i) = 1$) and DaysWind ($w_{+}(i) = .85$) remained significant factors, but PatchSize ($w_{+}(i) = .62$) replaced SnowDepth as an influential factor in the top model (Table 1.2). These three covariates were also the only ones included in the top model (Table 1.4). The covariate coefficients for Knowledge and DaysWind were similar for both model sets and showed a strong positive relationship with detection. SnowDepth
also had a positive relationship in the overall model while PatchSize had a slightly negative effect in our 20-minute model (Table 1.7, 1.8).

Three covariates were not significant in each of the two model sets: DaysTemp, PatchSize, and StemDensity in the full model set, and DaysTemp, SnowDepth, and StemDensity in the 20-minute model set. Each of these factors had summed weights below .5 implying they were not informative for cottontail detection (Table 1.2). Several of these factors were present in one or more of the top models but only because they were associated with other more significant covariates in the model.

We used our models to generate predicted detection rates for different combinations of the influential factors (see Table 1.55 for predicted scenarios). These predictions showed that for surveys conducted without a time limit detection rates are high, from 0.85 to 0.99 with prior knowledge, but decrease to a maximum of 0.49 when searches are conducted in the absence of prior knowledge and in deep snow (Table 1.5). The detection rate for surveys of a 25 ha patch with three wind free deposit days ranges from 0.68 to 0.33 with and without prior knowledge (Table 1.6). Detection rates on large patches with limited search times are quite low and such surveys will require 3-6 visits, depending on deposition days and prior knowledge, for confident occupancy determination.

**Discussion**

**Detection Rates**

We found that detection of New England cottontails during presence/absence surveys can be high under ideal conditions, but that detection must be accounted for to obtain high confidence in occupancy monitoring. The overall detection rate in our study
was 0.73 across all sites. This is higher than detection rates for other lagomorph species in similar dense habitats (e.g. eastern cottontail and swamp rabbit, *Sylvilagus aquaticus*, Scharine et al. (2011); marsh rabbit, *Sylvilagus palustris*, Eaton et al. (2011), and comparable to detection of species in more open habitats with greater visibility (e.g., European rabbit *Oryctolagus cuniculus*, van Strien et al. (2011). The higher detection rates in our study, despite the dense habitat, may be due to increased visibility afforded by the winter survey approach. Winter pellet surveys may provide enhanced opportunity for cottontails to be detected by allowing tracks and pellets to accumulate on top of snow for several days. In comparison, Scharine et al. (2011) performed live capture surveys and detected eastern cottontails at a rate of only 0.44 and swamp rabbits at a rate of 0.12. Eaton et al. (2011) did utilize pellet surveys, but the environmental conditions did not allow for surveys on snow and their lower detection rates likely reflected the difficulty of detecting pellets in marsh rabbit habitat, typically consisting of subtropical salt marsh transition zones and upland freshwater marshes. Our detection rates were also high compared to several other studies of rare or cryptic species (Roughton & Seddon 2006; Durso et al. 2011; Olea & Mateo-Tomas 2011). The use of sign, in the form of scat and tracks, on top of snow provides a broader detection window per visit compared to surveys where the target species must be actively seen, or heard each site visit. Even detection via track plates, camera traps, or live traps is limited to the location of the plate or trap. This is particularly important for New England cottontails because of the reduced visibility in their preferred thicket habitat. Higher detection rates in this study may also be a result of the positive effect of prior knowledge of occupancy, which we had for most surveys on sites following the first detection.
Detection decreased from 0.73 to 0.60 when the search time was limited to a 20-minute threshold. This reduced detection was negatively associated with increased patch size and suggests that 20 minutes may not be adequate to thoroughly search larger patches. Nonetheless, 82% of all detections occurred within the first 20 minutes of a survey, with minimal additional gains from increasing search effort on most sites. This is consistent with previous findings (Litvaitis et al. 2006). These results suggest a trade-off in balancing survey efficiency with the need for certainty in the occupancy determination. The optimal solution will depend on the survey objective. Efficiency (time-limited search) may be more important for a broad-scale monitoring effort, where regional trends in occupancy are sufficient. On a local scale, where patch-specific knowledge of occupancy is required, the need for a higher degree of certainty will dictate an unlimited search time.

**Factors that influence detection**

Two factors, prior knowledge of cottontail activity and increased pellet deposition days had a positive influence on detectability for both model sets. Having some knowledge of where cottontails have been active on a site had the strongest effect on each model set. Prior knowledge provides the observer with known areas to focus their search within the patch, sometimes even providing specific locations of cottontail burrows or runs. We also noticed that observers had a tendency to search more intently and more exhaustively on sites where they expected to find rabbits relative to sites where there was no such expectation. Most surveys conducted on sites for future monitoring will likely lack prior knowledge, but the strong positive effect it provides suggests that it may be helpful for surveyors to talk with individuals living on or around potential survey sites.
This could be particularly true for large sites where anecdotal information could greatly improve search efficiency.

We found that allowing an increased number of days without high winds had a positive effect on detection. This was expected because these days reflect the amount of time available for pellets and other sign to accumulate. While somewhat important on all sites, deposition days are likely more important for small sites (< 3 ha) where occupancy determination may rely upon detecting just one or two individuals on a patch. Measuring deposition time by simply counting the number of days since the last snowfall is not as effective, as it does not account for the potential reduction in cottontail activity caused by poor weather. Theoretically one day without wind or cold weather has the potential to allow more activity than three days of high winds and subzero temperatures. The number of days without high winds was more influential than the number of days without extreme cold. This may be because cold windy weather may limit cottontails more than cold, calm weather. Also, even if nighttime temperatures are below -10° C, effective daytime temperatures in the sun, particularly on calm days, may be moderate enough that cottontail activity is not limited. We also observed that locations with southern exposures had proportionally higher cottontail activity during cold mornings suggesting that even on extremely cold days cottontails may be able to utilize microhabitats where temperatures are moderated. It is also likely that noise caused by high winds limits predator detection by cottontails lowering their activity level. Some studies have found decreased lagomorph activity due to high wind (Fletcher et al. 1999; Ballinger & Morgan 2002) while others found no decrease in activity (Wallagedrees 1989; Twigg et al. 1998). It is likely that wind affects lagomorphs differently depending on the climate, season, and
species. Our study occurred during the winter when high winds and poor weather may have a more direct impact on movement and survival.

Although our model results theoretically suggest that increasing the number of deposit days should continually raise the detection rate, this is not the case. There are negative factors, which could not be modeled, associated with increased deposit days. They include DNA degradation, decreased visibility caused by snow melt out, and accumulation of snow surface debris. These all act to negate the added benefit of additional deposit days beyond three or four days. This is a particularly important consideration for sympatric sites, where quality DNA is critical for successful genetic species determination.

Snowpack below 12 inches increased detection rates, a finding that fit our expectations. Reduced snowpack provides easier travel for both cottontails and observers. Ease of travel increases cottontail activity, thereby providing additional sign for detection, and allows the observer to cover a greater search area in a given time period, thereby increasing the thoroughness of their search effort. Conversely, deep snow decreases cottontail movement and may promote subnivean travel and foraging, which have both been documented in pygmy rabbits (Katzner & Parker 1997). We observed large open air pockets below the snow on several patches and cottontail runs were seen connecting these areas, so it is likely that a certain amount of subnivean activity occurs on sites with dense vines or other vegetation that folds over under snow creating air pockets. It may be possible that increased subnivean activity could decrease cottontail detectability. Anecdotally, the site with increased subnivean movement, WPRE, did not have lower detection and still had large numbers of pellets distributed across the patch.
Finally, low snowpack is more likely to occur in the early winter and late spring when weather conditions are generally milder, promoting increased cottontail activity.

Additional factors

We expected that patch stem density would affect detectability but it was not a major factor in any of our models. Stem density may have had multiple confounding effects. Increased stem density is generally associated with increased cottontail density (Barbour & Litvaitis 1993; Litvaitis 2003) which should theoretically improve detection. Dense vegetation, however, reduces visibility of rabbit sign to observers and makes traveling through a patch more difficult. Both of these reduce search efficiency and decrease the likelihood of detection. While our study was not designed to incorporate cottontail density, we expect that cottontail detection will be reduced on sites with low rabbit densities. Anecdotally we observed that even large sites had high detection rates as long as they also had relatively large cottontail populations. Conversely, large sites with low rabbit densities, determined from subsequent population surveys, had extremely poor detection. It would be beneficial for future studies to isolate possible impacts that both vegetation density and cottontail density could have on detectability.

Two other factors that most likely influence cottontail detectability but were not specifically modeled in this study are search area and sympatry of New England cottontails and eastern cottontails. We conducted post hoc analyses (detailed in Appendix A) to evaluate the potential effects of these two factors. These analyses suggested that both sympatry and reduced search area may decrease detection and may require more intensive surveying of a greater proportion of a patch, and potentially the collection of larger numbers of samples on sympatric sites. We also modeled a data set
that incorporated six additional sites from Connecticut that were originally excluded because of questions concerning the occupancy status of those sites – no New England cottontails were detected on any of four visits to each of these sites, despite prior occupancy in previous years. The results of these analyses indicated that sympatry may negatively influence detection. See Appendix A for a more thorough treatment of these analyses.

**Optimal survey conditions and required visits**

Our results indicate that a survey will have the best chance of detecting New England cottontails on a site if the surveyor has some prior knowledge of cottontail activity, the survey is conducted in less than 12 inches of snow, and if 2-4 days without high winds are allowed to pass following a new snow event. Given these conditions, detection rates will be well over 95% (Table 1.5). If surveys are time limited, an ideal scenario includes prior knowledge and 3-4 days for pellet deposition, but detection will be affected by patch size. On small patches (<3 ha), detection rates still approach 90% but are only 70% on patches larger than 25 hectares (Table 1.6). Generally survey detection rates of 80% or higher will provide 95% confidence of detection after only two site visits. When detection rates range from 65-80%, at least 3 visits are required to achieve the same detection confidence.

More realistically, surveys will be conducted without prior knowledge. In this case, surveying with low snow depth and 3-4 pellet deposition days without high winds is important. With these conditions overall detection rates for a single visit may be as high as 90%, still requiring only 2 visits for confident determination of occupancy. If cottontails are detected on the first visit, a second visit is not needed. Surveying with
fewer pellet deposition days decreases the detection rate to as low as 73%, while
detection rates for surveys in deep snow are 22% and 49% for 1 and 3 pellet deposition
days, respectively. For time limited surveys a lack of prior knowledge reduces ideal
detection more significantly, with maximal detection of 62% on small sites and only 33%
on large sites. Time-limited surveys on sites without prior knowledge would require 4-6
visits for high confidence occupancy determination. This suggests that searching large
sites with restricted search times may not be an efficient protocol.

**Recommendations**

We recommend searching patches systematically and thoroughly, following loose,
continuous transects that wind back and forth across the patch, focusing on preferred
New England cottontail habitat. While the traditional 20 minute search limit may be
sufficient on small sites (< 3 ha), we do not recommend limiting search time for patch-
specific occupancy surveys. While we recognize that prior knowledge typically will not
be available for the initial search of a patch, once cottontails have been detected,
subsequent visits in the same season will benefit from the knowledge gained during the
first detection. Surveys should also be conducted with snow depth below 12 inches, and
surveyors should allow 2-4 days to pass following a snow fall before searching. On sites
co-occupied by New England and eastern cottontails we recommend collecting samples
from at least 5 distinct pellet clusters, well distributed throughout the patch, to maximize
the chances of sampling a New England cottontail if it is present. Lastly, to adhere to
assumptions of population closure, we recommend conducting multiple searches, when
needed, in as short a time span as possible.
Limitations

Sporadic snowfall made it difficult to complete multiple surveys during the winter of 2010 and many sites had to be revisited in 2011. Due to poor snow conditions in parts of southern New England, we were unable to incorporate several sites from Massachusetts. Inconsistent snowfall also forced our survey windows to be slightly wider than planned, increasing the risk of violating closure assumptions for cottontail populations. Detection rates did not change throughout the winter suggesting that the increased survey windows were not detrimental. In addition, any bias produced by an increased survey window in this study would be a conservative bias.

The presence of prior knowledge was the most prominent factor affecting detection from our study. The strength of the prior knowledge covariate has the potential to overshadow other factors in our models and we recognize that it generally will not be available for most monitoring surveys. We included it as a covariate primarily because we identified that it might have a strong effect on detection and wanted it to be accounted for rather than have it provide an unknown but strong effect on the model.

Conclusion

This study found that when surveys are conducted in ideal conditions, New England cottontails can be detected with high confidence (>95%) in one to three surveys. We also identified three easily measured factors that have significant effects on New England cottontail detection. Prior knowledge of cottontail activity had a very strong positive influence on detection probability and provided the only context in which a single survey visit may be sufficient to yield confident presence/absence determination. Detection also improved for surveys conducted with a snowpack below 12 inches. We
found that it is important to wait an adequate number of days without high winds (>40km/hr) following a snowfall to allow pellets and sign to accumulate. This benefit is only realized up to about four days, beyond which negative effects of reduced pellet and track visibility and increased DNA degradation likely outweigh any added benefit of increased deposition time. For surveys limited to 20 minutes we found that increased patch size has a negative effect on detection, and the effect of size is likely even greater on large sites with low cottontail densities. We anticipate that these findings will facilitate more effective and reliable occupancy monitoring of New England cottontails.
Table 1.1. Full set of variables considered in New England cottontail detection and their descriptions. Effect likelihood P-values from simple linear regression are given for a reduced set of variables retained from preliminary statistical testing. Observer, Search time, Area searched, and Days were removed during this preliminary testing. NA indicates the variable could not be tested due to co-linearity with another factor. Bold font indicates the final set of variables used in detection modeling (see text for explanation).

<table>
<thead>
<tr>
<th>Variable Name</th>
<th>Description</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observer</td>
<td>Identity of individual(s) conducting survey</td>
<td></td>
</tr>
<tr>
<td>SearchTime</td>
<td>Amount of time until pellet/tracks detected</td>
<td></td>
</tr>
<tr>
<td>AreaSearched</td>
<td>Area of search path with added buffer distance</td>
<td></td>
</tr>
<tr>
<td>Days</td>
<td>Days since snowfall</td>
<td></td>
</tr>
<tr>
<td>DaysWind</td>
<td>Days since snowfall with wind &lt;40km/h</td>
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</tr>
<tr>
<td>DaysTemp&gt;-10°C</td>
<td>Days since snowfall with temperature &gt;-10°C</td>
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</tr>
<tr>
<td>DaysTemp&gt;-15°C</td>
<td>Days since snowfall with temperature &gt;-15°C</td>
<td>NA</td>
</tr>
<tr>
<td>SnowDepth</td>
<td>Snow depth &lt; or &gt; 12 &quot;</td>
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<tr>
<td>SnowPowder</td>
<td>Snow conditions - powder or not</td>
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<td>StemDensity</td>
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<td>Knowledge</td>
<td>Prior knowledge of cottontail location on patch</td>
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<tr>
<td>PatchSize</td>
<td>Patch area in ha</td>
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</table>

Table 1.2. Summed Akaike information criterion weights ($w_+(i)$) for all variables in the full model set and the model set with search times limited to 20 minutes. Variables are defined in Table 1.1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Full Model $w_+(i)$</th>
<th>20 min. Model $w_+(i)$</th>
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<td>Size</td>
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</tr>
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<td>Density</td>
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<tr>
<td>DaysTemp</td>
<td>0.29</td>
<td>0.33</td>
</tr>
</tbody>
</table>
Table 1.3. The 95\% confidence set of candidate detection models from PRESENCE for the full dataset of 30 sites with confirmed New England cottontail occupancy. For each model, the Akaike information Criterion adjusted for sample-size (\(AIC_c\)), the difference in \(AIC_c\) (\(\Delta AIC_c\)), \(AIC_c\) weight (\(w_i\)), the number of parameters (\(K\)), and the maximized log likelihood (\(-2 \log(\ell)\)) is given.

<table>
<thead>
<tr>
<th>Model</th>
<th>(AIC_c)</th>
<th>(\Delta AIC_c)</th>
<th>(w_i)</th>
<th>(K)</th>
<th>(-2 \log(\ell))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge - SnowDepth - DaysWind</td>
<td>111.4</td>
<td>0</td>
<td>0.89</td>
<td>5</td>
<td>100.94</td>
</tr>
<tr>
<td>Knowledge - SnowDepth - DaysTemp</td>
<td>115.2</td>
<td>3.79</td>
<td>0.06</td>
<td>5</td>
<td>104.73</td>
</tr>
<tr>
<td>Knowledge - SnowDepth</td>
<td>115.3</td>
<td>3.89</td>
<td>0.05</td>
<td>4</td>
<td>106.98</td>
</tr>
</tbody>
</table>

Table 1.4. The 95\% confidence set of candidate detection models from PRESENCE for the reduced set of sites with New England cottontail detections that occurred within 20 minutes of searching. For each model, the Akaike Information Criterion adjusted for sample-size (\(AIC_c\)), the difference in \(AIC_c\) (\(\Delta AIC_c\)), \(AIC_c\) weight (\(w_i\)), the number of parameters (\(K\)), and the maximized log likelihood (\(-2 \log(\ell)\)) is given.

<table>
<thead>
<tr>
<th>Model</th>
<th>(AIC_c)</th>
<th>(\Delta AIC_c)</th>
<th>(w_i)</th>
<th>(K)</th>
<th>(-2 \log(\ell))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge + Size + DaysWind</td>
<td>170</td>
<td>0</td>
<td>0.52</td>
<td>5</td>
<td>159.54</td>
</tr>
<tr>
<td>Knowledge + DaysWind</td>
<td>171.11</td>
<td>1.11</td>
<td>0.33</td>
<td>4</td>
<td>162.8</td>
</tr>
<tr>
<td>Knowledge + Size + SnowDepth</td>
<td>173.03</td>
<td>3.03</td>
<td>0.07</td>
<td>5</td>
<td>162.57</td>
</tr>
<tr>
<td>Knowledge + Size + DaysTemp</td>
<td>173.57</td>
<td>3.57</td>
<td>0.03</td>
<td>5</td>
<td>163.11</td>
</tr>
<tr>
<td>Knowledge + SnowDepth</td>
<td>174.03</td>
<td>4.03</td>
<td>0.04</td>
<td>4</td>
<td>165.72</td>
</tr>
<tr>
<td>Knowledge + DaysTemp</td>
<td>174.49</td>
<td>4.49</td>
<td>0.02</td>
<td>4</td>
<td>166.18</td>
</tr>
</tbody>
</table>
Table 1.5. Predictions from survey scenarios based on the top model of New England cottontail detection, which includes the influence of Knowledge (1 signifies presence of prior knowledge, 0 signifies absence of knowledge), SnowDepth, (1 signifies snow pack <12", 0 signifies snowpack >12"), and DaysWind (modeled as either one or three days since snowfall with winds <40 km/hr). All three variables have a positive influence on detection. Predicted responses are the detection probability for a single survey visit (DetProb) and the number of visits required for 95% confidence in detection.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Knowledge</th>
<th>SnowDepth&lt;12&quot;</th>
<th>DaysWind</th>
<th>Det Prob</th>
<th># visits for 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.98</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0.99</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.85</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0.95</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.72</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0.90</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.22</td>
<td>&gt;6</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0.49</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 1.6. Predictions from survey scenarios based on the top model of New England cottontail detection for the 20-minute restricted survey period, which includes the influence of Knowledge (1 signifies presence of prior knowledge, 0 signifies absence of knowledge), DaysWind (either one or three days since snowfall with winds <40 km/hr), and patch size (modeled for the two extremes of sizes in this study, 3 ha and 25 ha). In this model, knowledge and DaysWind have a positive influence on detection, while patch size has a negative influence. Predicted responses are the detection probability for a single survey visit (DetProb) and the number of visits required for 95% confidence in detection.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Knowledge</th>
<th>DaysWind</th>
<th>Size</th>
<th>Det Prob</th>
<th># visits for 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>25</td>
<td>0.46</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0.74</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
<td>25</td>
<td>0.68</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>0.87</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1</td>
<td>25</td>
<td>0.17</td>
<td>&gt;6</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>0.40</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>3</td>
<td>25</td>
<td>0.33</td>
<td>&gt;6</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>0.62</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 1.7. Untransformed linear logit parameter estimates, standard errors and 95% confidence intervals for explanatory variables from the best supported model of New England cottontail detection. Detection probability was modeled as a function of prior knowledge of cottontail occurrence (knowledge or no knowledge), snow depth (< 12 inches or >12 inches), and deposition days (number of days since last snowfall with winds <40 km/hr; DaysWind).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient estimate</th>
<th>SE</th>
<th>Lower CI</th>
<th>Upper CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-1.87</td>
<td>0.58</td>
<td>-3</td>
<td>-0.74</td>
</tr>
<tr>
<td>Knowledge</td>
<td>3</td>
<td>0.57</td>
<td>1.9</td>
<td>4.11</td>
</tr>
<tr>
<td>SnowDepth</td>
<td>2.23</td>
<td>0.63</td>
<td>0.99</td>
<td>3.46</td>
</tr>
<tr>
<td>DaysWind</td>
<td>0.61</td>
<td>0.27</td>
<td>0.09</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Table 1.8. Untransformed linear logit parameter estimates, standard errors and 95% confidence intervals for explanatory variables from the best supported model of New England cottontail detection for the 20-minute restricted survey period. Detection probability was modeled as a function of prior knowledge of cottontail occurrence (knowledge or no knowledge), patch size (ha), and deposition days (number of days since last snowfall with winds <40 km/hr; DaysWind).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient estimate</th>
<th>SE</th>
<th>Lower CI</th>
<th>Upper CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.70</td>
<td>0.47</td>
<td>-1.62</td>
<td>0.21</td>
</tr>
<tr>
<td>Knowledge</td>
<td>1.45</td>
<td>0.39</td>
<td>0.68</td>
<td>2.21</td>
</tr>
<tr>
<td>DaysWind</td>
<td>0.45</td>
<td>0.19</td>
<td>0.07</td>
<td>0.83</td>
</tr>
<tr>
<td>Size</td>
<td>-0.05</td>
<td>0.03</td>
<td>-0.11</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Figure 1.1. Map of study area with points indicating the locations of the 30 New England cottontail detection sites.
Figure 1.2. The best fit curve for the number of New England cottontail detections by survey search time. Positive gains of increased search time begin to diminish at 20 minutes (82%), with only slight gain for increased effort beyond 40 minutes (93%).
CHAPTER 2

New England Cottontail Abundance Estimation

Abstract

Effective adaptive management of New England cottontails would benefit from accurate abundance estimates at a regional and patch specific scale. I used single session mark-recapture modeling and non-invasively collected DNA from fecal pellets to perform population estimates for New England cottontails on 17 patches in Maine, New Hampshire, and New York. Precision of estimates was high for most small sites and several large sites. I found multiple small patches containing only several individuals but also found sites in Maine (Crescent Beach, and Kettle Cove), New Hampshire (Stonyfield), and New York (CFSP) with much larger populations.

I also evaluated the methodology used and recommend using a single well timed survey for future population surveys. Surveys should occur 2-4 days following a snowfall to allow adequate time for pellets and sign to accumulate, but waiting too long will deteriorate DNA quality and decrease survey efficiency. Sites should be surveyed with a minimum distance between pellets of 30 m or less.
**Introduction**

Effective adaptive management requires accurate and reliable population estimates. Obtaining these population numbers for many animal species is a challenge, particularly rare and elusive ones. Mark-recapture approaches are commonly employed for estimating abundances while accounting for imperfect detection (Otis et al. 1978; Pollock et al. 1990). Traditional mark-recapture techniques involve an initial live-trapping session where individuals are caught, marked with ear tags, leg bands, or other identifier, and then released. Subsequent trapping sessions are used to recapture a percentage of the originally marked individuals, and the ratio of recaptures to new captures can be used to estimate minimum population numbers (Nichols 1992). Less invasive methods using photographs from camera traps (Karanth 1995) and other identifiable “sign” have been developed, but such techniques are better suited for large animals that inhabit extensive home ranges.

These methods pose several challenges for studying rare and elusive species such as the New England cottontail (*Sylvilagus transitionalis*). Using field observation of sign is difficult for New England cottontails because they exist sympatrically in portions of their range with eastern cottontails (*Sylvilagus floridanus*) and snow shoe hare (*Lepus americanus*). It is difficult to differentiate between the two species based on sign alone (Litvaitis & Litvaitis 1996), and distinguishing pelage differences is exceedingly difficult in the field. The time and cost involved with trapping, marking, and recapturing an adequate number of rabbits is prohibitive and limits the number of patches that can be accurately surveyed. Another problem with traditional live-capture techniques is that they have the potential to negatively impact the target species (Arnemo et al. 2006;
Schutz et al. 2006). Additionally, due to differences in individual behavior and trap susceptibility, the use of live-trapping for mark-recapture surveys may provide biased results (Woods et al. 1999; Mills et al. 2000).

Non-invasive genetic tagging techniques provide an alternative approach for population estimation that is suitable for rare species and eliminates the risks associated with capture and handling. An accurate individual genetic profile can be obtained from trace amounts of genetic material. This enables genetic analysis from noninvasively collected tissues such as feathers, hair follicles (Woods et al. 1999; Kendall et al. 2008) and feces (Kohn et al. 1995; Palomares et al. 2002; Kovach et al. 2003). These genetic samples can be used to identify individual animals by their unique genotypes through the use of microsatellite markers. With an appropriate sampling scheme, multiple unique and re-sampled genotypes can be “captured” from a study area. Population estimates can then be performed using the same mark-recapture algorithms originally developed for live-trapping studies (Palsboll et al. 1997; Woods et al. 1999).

Several models exist for abundance estimation from genetic mark-recapture data. Traditional models, including closed capture models (Otis et al. 1978), can be evaluated using the program MARK (White & Burnham 1999). Additional models can be developed in MARK to test the effects of study-specific covariates (Moore & Swihart 2005) and individual encounter histories (Kendall et al. 2008). A disadvantage of this traditional closed capture models is that they require multiple capture sessions to produce estimates and only incorporate one sample, or capture, per session. Collection of genetic samples, however, allows for the same individual to be genetically marked and then “recaptured” multiple times within the same session. Traditional closed capture models
are unable to take advantage of more than one capture per individual per session meaning that the data is lost for sessions with multiple recaptures (Miller et al. 2005).

To address this weakness of traditional mark-recapture approaches, several single-session models have been developed. These models enable the use of multiple captures within the same trapping (or genetic tagging) session, making them more efficient than multi-session models when working with genetic data (Petit & Valiere 2006). Working with single session models requires several assumptions: (1) the study population must be closed; (2) the capture probability must be equal to the recapture probability; and (3) individuals must have equal capture probability (Puechmaille & Petit 2007). The simplest single session method is rarefaction, or accumulation curve modeling (Kohn et al. 1999; Eggert et al. 2003), which fits new captures to the equation of an asymptotic curve for the population (Petit & Valiere 2006). Essentially, rarefaction provides approximate population estimate by modeling the decrease in new captures as the survey effort increases. Eggert et al. (2003) found versions of this model to perform well but in simulated tests Miller et al. (2005) showed that the program CAPWIRE outperformed rarefaction on small populations. It also outperformed two other closed-capture heterogeneity estimators, the Chao estimator (Chao 1988) and jackknife estimator (Burnham & Overton 1979). Since we expected all of our sites to have small populations (N<100) we chose to use CAPWIRE.

CAPWIRE implements a maximum likelihood approach that enables the incorporation of heterogeneity in capture probability by modeling the population as a mixture of individuals with two different innate capture probabilities. The null model, also called the even capture model (ECM), assumes equal capture rates for all
individuals. The two innate rates model (TIRM), assumes there are two distinct capture probabilities within the population. Several studies have shown that CAPWIRE provides accurate estimates, especially for scenarios of low abundance (Puechmaille & Petit 2007; Hájková et al. 2009).

The goal of this study was to investigate the feasibility of using genetic mark-recapture approaches to estimate New England cottontail abundance from single session pellet surveys. The specific objectives were 1) to determine the most practical and robust sampling scheme for New England cottontail population estimation, and 2) employ a suite of polymorphic microsatellite loci to produce population estimates for habitat patches across the species’ range.

**Methods**

**Study Sites**

I performed population surveys on 19 sites, 10 in Maine, five in New Hampshire, three in New York, and one in Connecticut. I visited three of these sites, Western Point Road East (WPRE), River Road, and Coast Bus, on two separate occasions to evaluate potential benefits of multiple capture sessions and to explore potential variation in capture rates and population estimates between separate survey visits. I selected most of the population sites from sites surveyed in the detection study (Chapter 2; Figure 1.1). In addition to using several detection sites, I also surveyed three new sites located on the Sprague property in Cape Elizabeth, ME. Sites ranged in size from 0.73 to 26.3 hectares (Table 2.1) and were selected to represent a variety of patch sizes, habitats, and cottontail densities. I surveyed sites in Maine; sites in New York, Connecticut, and New Hampshire were surveyed by state agency biologists. Only eastern cottontail pellets were
found at Bunker Lane in New Hampshire, and at Bluff Point in Connecticut, and therefore, both sites were removed from further analysis.

The protocol for population surveys followed the same general outline as the detection survey protocol described in Chapter 2, with a few changes. Surveys were conducted at least three days following a snow event to provide ample opportunity for pellets to accumulate. Population surveys were also conducted more thoroughly than detection surveys, and patches were searched exhaustively, regardless of how many distinct samples were found (Figure 2.3). The goal was to sample thoroughly enough such that most/all unique individuals from a particular site were sampled at least once, and a subset of them were resampled to serve as recaptures in the modeling process.

Pellet samples were collected systematically throughout the entire patch, whenever encountered, given a minimum distance of 30 to 50 meters from any other sample. The sampling distance (minimum distances between distinct samples) was dependent on the overall density of pellets on the patch and the size of the site. On a few small sites or sites with high pellet densities I collected samples with a minimum distance less than 30 meters. On the densest sites I even sampled from neighboring distinct piles of pellets in case more than one individual was active in the same area.

Most small and moderately sized sites, up to about six hectares, were surveyed by a single surveyor. Multiple surveyors conducted searches on larger sites. On those occasions, we searched as a group in one of two ways. On large cohesive sites, surveyors each walked a loose transect parallel to a neighboring surveyor. Alternatively, if the patch was oddly shaped it was split up into sections with individual surveyors searching a respective section (Figure 2.4). Once detected, pellets were collected with sterile gloves...
and stored in sterile 15 ml tubes. Tubes were stored in bags with snow while in the field and transferred to -20°C freezers for permanent storage. To maximize the likelihood that each cluster of pellets originated from a single rabbit, individual vials of samples were collected from an area of no more than 5 x 5 ft.

**Subsampling**

To verify that samples were deposited by New England cottontails, I performed two diagnostic restriction fragment length polymorphism (RFLP) tests on an approximately 560 base pair segment of the mitochondrial control region to differentiate New England cottontails from eastern cottontails and snowshoe hares (following Litvaitis and Litvaitis [1996] and Kovach et al. [2003]; see Chapter 1 for more details). I removed eastern cottontail and snowshoe hare samples from further analysis. Samples collected from sites only occupied by New England cottontails, in Maine and New Hampshire did not require species confirmation because they had been surveyed prior to this study and found to only contain New England cottontails. To evaluate the effect of sampling effort, I subsampled to obtain sets of samples corresponding to varying spatial sampling intensities. I evaluated three different minimum distances between samples – 30 m, 50 m, and 75 m. (Figure 2.2). The smallest minimum distance was 30 m for all but the five smallest sites. Small sites often had only a few samples, and even for sites where I collected an adequate number of pellets, subsampling with a 30-m minimum distance removed a significant number of individual from the population. Two of those sites, Weed Mine and Bellamy, only had five and six samples collected respectively, making any sample reduction impractical. I chose to genotype all, or the majority of the samples from three other sites, WPRE, River Road and Coast Bus, to evaluate the potential
benefit of sampling exhaustively (Appendix B, Table 1). I performed the subsampling in the GIS software ArcMap (ESRI 2010).

**DNA Extraction and Microsatellite Genotyping.**

I extracted DNA from selected samples using QIAamp® DNA Stool Mini Kits (Qiagen, Valencia, California) following the methods of Kovach et al. (2003). I originally amplified DNA at 11 multiplexed microsatellite markers following the protocols of Fenderson et al. (2011) with slight modification (Tables 2.2, 2.3, 2.4 for primer and protocol information). The Sat12 locus did not amplify consistently during preliminary testing and was not used for analysis. DNA was amplified using fluorescent dye-labeled primers and multiplex PCR. Amplified products were electrophoresed using an automated DNA sequencer (ABI 3130, Applied Biosystems, Foster City, CA). I then scored all alleles manually using Peak Scanner 1.0 (Applied Biosystems, Foster City, CA). I used a positive control in conjunction with the program Allelogram (Morin et al. 2009) to standardize allele sizes across different electrophoretic runs. I manually binned the resulting normalized allele sizes produced by Allelogram.

DNA extracted from fecal samples is often of lower quantity and quality than that extracted from tissue samples. I employed a modified multiple-tubes approach to detect and account for genotyping errors, false alleles and allelic dropout, which often result from genotyping with low quantity and quality DNA (Taberlet et al. 1996). I initially amplified each sample four times and used these replicates to create a consensus genotype. I accepted heterozygote genotypes if each allele amplified at least twice (Gervasi et al. 2010) and as long as both alleles amplified together in at least one replicate. If three distinct alleles each amplified at least twice the consensus was based
on the two alleles that occurred most often. If two such alleles could not be identified then I discarded the locus for that particular sample. I only accepted homozygote genotypes if they amplified as homozygotes at least four times and if no allele co-amplified more than once across all replicates.

I performed an additional 2-4 replicates for samples that amplified poorly in order to achieve 4 positive amplifications. In order to improve amplification for these samples, I re-extracted DNA, using two pellets, instead of just one, in an attempt to extract larger amounts of DNA. For 3.6% of samples I accepted a consensus homozygote after only 3 replicates, but only if all three replicates were identical, and only after attempting additional amplifications. Samples with >3 loci that failed to produce a consensus genotype were removed from the dataset.

**Genotyping Error**

I calculated genotyping error rates by manually comparing replicate genotypes to the consensus genotype for each sample. I categorized differences between a replicate and the consensus as either an allelic dropout (ADO) or a false allele (FA). Allelic dropout occurs when a particular allele fails to amplify in one or more replicates while false alleles occur when an allele of different size than in the consensus genotype amplifies (Taberlet et al. 1996). I identified a false allele as any allele that was different from either consensus allele and that only amplified once among all replicates. I calculated per locus and per allele error rates for both classes of error, ADO and FA. Per locus error, or genotype error, is the ratio of the number of individual genotypes containing at least one mismatched allele to the total number of genotypes across all replicates. It is calculated as:
\[ e_g = \frac{m_g}{nt}, \]

where \( m_g \) is the number of single-locus genotypes that contain at least one allelic mismatch, and \( nt \), the number of replicated single-locus genotypes. The per allele error rate is the ratio of all allelic mismatches to the total number of alleles and is defined as:

\[ e_a = \frac{m_a}{2nt}, \]

where \( m_a \) is the total number of allelic mismatches, and \( 2nt \) is the number of replicated alleles (Pompanon et al. 2005).

**Individual Identification**

I used the program DROPOUT (McKelvey & Schwartz 2005) to identify unique samples with identical genotypes, indicating they were deposited by the same individual, as well as those mismatched at one, two, or three loci. I then manually compared each of these samples and determined if they were similar enough to any other sample to be considered a recapture of that sample (i.e. if the minor discrepancies could be accounted for by genotyping or scoring error), or if they should be considered a unique individual.

To account for the uncertainties associated with genotyping samples of low quality and low genetic diversity, I made two estimates for the total number of unique individuals on each site, a minimum and a maximum estimate. The minimum was inclusionary with respect to mismatched genotypes (conservative), while the maximum estimate was exclusionary (minimizing the contributions of genotyping error). For the minimum estimate I attempted to group samples into as few individuals as possible, allowing for a greater number of mismatches (up to 3) between samples. Similar genotypes were considered the same individual unless they differed by at least three instances of ADO, or at least one FA and one ADO. For the maximum estimate, similar genotypes were
considered unique individuals if they differed by more than a single case of ADO. In general, due to its high error rates, both ADO and FA, I never used mismatches from the Sat13 locus as the single distinguishing factor in differentiating two individuals.

To determine the discriminatory ability of my loci, I calculated both the probability of identity ($PI$) (Paetkau et al. 1995) and the more conservative probability of identity for related individuals ($PISib$) (Waits et al. 2001). The $PI$ statistic for a given set of loci indicates the chance that any two distinct individuals could have identical multi-locus genotypes for the suite of markers used, and therefore be classified as the same individual. I used $PISib$ for my analysis because it is more appropriate for small or closely related populations (Waits et al. 2001).

**Population Estimation**

I performed population estimates in CAPWIRE. I allowed the program to use a likelihood ratio test to select ECM or TIRM model, rather than imposing one or the other on the data set. For the three sites with two separate visits, I analyzed the first and second visits independently and also evaluated the overall sampling effort by combining the two visits into one session. I also conducted estimates for each site using both the minimum and the maximum number of unique individuals. On sites where subsampling was performed, I estimated abundance separately for datasets comprised of the 30-m, 50-m, and 75-m subsampling intervals. I also mapped the locations of each individual to facilitate visualization of cottontail distribution and activity within a patch (Appendix B, Figure 1). I did not estimate abundance for four sites, Bellamy, Radio Station, Frieze, and Weed Mine, for which an insufficient number of samples were available (Appendix B, Table 1). Three and four samples were collected from Bellamy and Frieze,
respectively, which originated from just one individual on each patch. Alternatively, the three samples processed from Weed Mine were from three different rabbits. The poor DNA quality from the Radio Station samples prevented me from determining anything more than that there was at least one New England cottontail present.

**Results**

A total of 427 individual pellet samples were collected from 20 visits to 17 sites (Table 2.7, see Appendix B, Table 1 for site specific pellets collected). All the pellets collected from Maine were deposited by New England cottontails. Population surveys on the three New York sites, which were assumed to be occupied by both species, yielded only New England cottontails at TSP-301, and both species at the other two sites, CFSP and Weed Mine. Of the 42 samples collected at CFSP, only eight were from eastern cottontails while at Weed Mine 41 of 46 samples were easterns. I selected 290 of the 427 samples, based on the subsampling scheme, and obtained usable genotypes for all but 40 of them. Samples that failed to provide usable genotypes were primarily from the Radio Station, Coast Bus, CFSP and TSP-301 sites (Appendix B, Table 1).

Genotyping error varied considerably among loci. Average false allele error rates across all loci were 0.036 per genotype and 0.020 per allele (Table 2.5), similar to a previous study of New England cottontail using these loci (Fenderson 2010). Average allelic drop out error rates were 0.086 per genotype and 0.043 per allele (Table 2.6). The highest ADO rates were for Sat13 and Sol03 at 0.208 and 0.152 per genotype respectively. Average female error rates were only marginally different from males (Table 2.8).
$PI_{sib}$ values below 0.05 are generally considered adequate to correctly distinguish individuals from each other in a population (Waits et al. 2001). The $PI_{sib}$ for the majority of sites in this study were below 0.05. Average $PI_{sib}$ for males, 2.03E-2, was lower than that of females, 2.62E-2, because the Y marker is only found in males providing an additional locus for distinguishing male individuals (Table 2.7). Two sites, Wells Reserve and WPRE, had male and female $PI_{sib}$ values above 0.05 while two additional sites, Fort Williams and River Road, had female $PI_{sib}$ above 0.05. (See Appendix B, Table 2 for site specific $PI$ and $PI_{sib}$), indicating lower discriminatory power on these sites.

Using the minimum estimates as a conservative measure, I identified 88 unique individuals across the 17 sites. Of those 88, the majority, 57, were male and 39 were female, giving a gender ratio of 1M:0.66F. There were 31 occasions where an individual was captured three or more times and in 20 of those cases the individual was male. On eight of the 17 sites, 13 individuals were captured at least five times. Nine of the 13 were males and on six of the eight sites the individual captured most often was a male.

Site-specific population estimates are provided in Table 2.10. Width of the confidence intervals varied across sites. Estimates for small and medium sized sites generally had more narrow confidence intervals than larger sites. Confidence intervals also varied based on sampling scheme. River Road, WPRE, and Coast Bus were visited twice. Each independent visit at River Road and WPRE produced similar estimates to those of the combined estimate. In contrast the two visits at Coast Bus captured different sets of individuals and provided different estimates (Table 2.10a). The minimum and maximum point estimates for the first visit were 11 and 15 while for the second visit they
were both five. When the two visits were combined into a single session the estimates were closer to the first visit at 15 and 21 for the minimum and maximum, respectively.

Population estimates were generated for 10 sites using the 30-m minimum sampling distance (Table 2.10a, b). Fort Williams, Coast, and Sliver are small sites, which generated small estimates (3-7 individuals), as expected. The estimates for Coast site had wide confidence intervals while confidence intervals for both Fort Williams and Sliver estimates were narrow. Wells Reserve, Kettle Cove, and CFSP were three large sites that varied in their abundance estimates. Only four samples were processed from Wells Reserve, with estimates ranging from 2-5. The estimate for Kettle Cove was 10–11 (min–max) and 5-8 for CFSP, with narrow confidence intervals. Estimates for the four remaining sites, Stonyfield, Orchard, Crescent Beach, and TSP-301 were large (13-77) with wide confidence intervals.

I also generated estimates for Stonyfield, Kettle Cove, Sliver, Crescent Beach, and CFSP, with subsampling using the 50-m and 75-m minimum, sampling distances (Tables 2.10a, b). The subsampling affected point estimates differently. The minimum and maximum point estimates for Stonyfield decreased as the minimum distance increased, but the opposite was true for Kettle Cove and Sliver (Table 2.11). The estimates doubled, or more, for the latter two sites with the 75-m subsampling. For Crescent Beach and CFSP, the point estimates increased for the 50-m subsampling, relative to the 30-m sampling, and decreased for the 75-m subsampling. Generally, even with the reduced sample sizes, the confidence intervals on the Sliver, CFSP, and Kettle Cove estimates remained fairly consistent. The confidence intervals for the Stonyfield estimates decreased at both the 50-m and 75-m sampling distances while those for
Crescent Beach increased dramatically for the 50-m subsampling and decreased dramatically at the 75-m sampling distance.

Several patterns in allele frequency and distribution emerged between states and specific sites (Table 2.9). Alleles from most loci were generally shared by individuals in all three states, or by only two of the three, Maine and New Hampshire or New York and New Hampshire, but never by only Maine and New York. Many alleles were also unique to populations within each state. Three sites contained multiple alleles from several loci that were site-specific. Stonyfield had seven unique alleles across five loci and Coast Bus had six unique alleles across four loci. TSP-301 in New York had two unique alleles in two loci.

**Discussion**

This study showed that reliable population estimates can be made with noninvasive genetic tagging using microsatellite genotyping from DNA extracted from fecal pellets of New England cottontails. I found several methodological considerations to be important in influencing the precision and reliability of the estimates, including sampling scheme and effort, timing of survey relative to the last snowfall, genetic diversity of cottontails, and cottontail density. I also identified some of the challenges that must be addressed in a large-scale monitoring effort and give recommendations for future implementation in a monitoring program for New England cottontails.

**Population estimation**

We found that reasonably precise New England cottontail population estimates can be obtained from surveys conducted during a single site visit with an adequate sampling scheme. Confidence intervals for estimates in this study varied but were
narrow for eight of the 13 estimated sites. River Road, WPRE, Sliver, Coast and Fort Williams are small sites for which proportionally large sample sizes were obtained, with high capture and recapture rates, and therefore, precise estimates. The estimate from Wells Reserve had high precision, despite only four samples collected from a large site, because all four samples were localized within the patch and came from only two individuals. Kettle Cove and CFSP were the only large sites that generated population estimates with high precision. The population estimate for Kettle Cove was comparatively small for the size of the site (20.4 ha). A potential explanation for this is that pellets found were only located in half of the site. Other parts of the patch may not have been sampled as intensively as other sites resulting in the lower estimate. Two other large sites, Crescent Beach and Stonyfield, also had large sample sizes, but reduced precision (wide confidence intervals) for the maximum estimates due to low recapture rates. The estimate for TSP-301 also had reduced precision, likely resulting from the small sample size. Poor pellet DNA quality precluded the use of eight of 19 samples collected at this site, resulting in a low sample size for estimation. These results suggest that large sample sizes are important and that an adequate number of recaptures is required to achieve precise estimates.

Precision was also affected by pellet subsampling. I subsampled five sites at the 30-m, 50-m, and 75-m distances and confidence intervals for four of the five sites widened as the sampling distance increased and, therefore, sample size decreased. The Sliver site was unique in that I collected a proportionally large number of samples for its moderate size and also estimated its population with and without subsampling. Due to the large sample size on this site, precision was not lost between the estimates made with
a 30-m sampling distance and those made without any subsampling. Stonyfield was the only site where precision increased as the sampling distance decreased. This occurred because Stonyfield had a high proportion of single captures and reduced sampling distances removed these samples more proportionally than those from recaptured individuals. Generally, sample size reduction, caused by subsampling, had the most impact on sites with large numbers of single captured individuals.

In mark-recapture studies, all individuals will not always be captured at the same rate. This capture heterogeneity can be caused by a variety of factors (Huber 1962; Otis et al. 1978). CAPWIRE accommodates heterogeneity within a population by using a two innate rates model (TIRM), which allows for two capture rates within the population. Model choice (TIRM vs. ECM) varied primarily according to sampling effort, but with some notable exceptions. Most of the smaller sites had large sample sizes, due to high collection rates and limited subsampling, providing multiple recaptures for most individuals on the site and thereby meeting the assumptions of the ECM. Sample sizes at WPRE and Coast Bus were large relative to site size, similar to other small sites, but with capture heterogeneity due to a single individual that was recaptured multiple times for each site, fitting the TIRM. Stonyfield was the only site on which TIRM was selected for the 30-m subsampling effort. This is because Stonyfield had the highest proportion of single captures of any site.

Maximum estimates were made by splitting individuals based on as little as only one or two allelic mismatches and genotyping error may have had a larger affect on the final estimates, leading to the decreased precision. This means that the maximum estimates generally showed more bias than the minimum estimate. The estimates
produced from the minimum number of individuals had greater precision (narrower confidence intervals. Genotyping error is a potential explanation for the wider confidence intervals in the maximum estimates because increased error rates create false individuals which have no chance of being “recaptured” unless an identical error occurs in a second sample (Waits & Leberg 2000). Population estimates that have low recapture rates and low precision may indicate that genotyping error is affecting the estimate, generally resulting in an overestimation in the final abundance estimate (Marucco et al.; Lukacs & Burnham 2005).

Given the insight generated from the min/max estimates, I reevaluated the number of unique individuals with a single set of criteria designed to minimize spurious identification of individuals resulting from genotyping error. The criteria are as follows: Any time samples that differed by four or more mismatches were always considered unique individuals. If three mismatches occurred, the two samples were considered identical if all three mismatches were a result of ADO and if the gender marker matched or was uncertain. If one of the mismatches could not be explained by ADO (i.e. three different alleles across putative individuals), only one additional mismatch, of any type, was needed to differentiate the two samples. I then used CAPWIRE to generate a revised set of best estimates using individuals discriminated by these guidelines (Tables 2.14, 2.15, 2.16).

Using these criteria for individual discrimination resulted in population estimates with increased precision relative to those generated with the minimum/maximum criteria above. I conclude these criteria therefore are the most appropriate for discriminating
individuals for population estimation with the suite of microsatellite loci used in this study.

**Individual distribution and gender**

In this study, males were sampled and resampled much more frequently than females. The distribution of pellet samples across the patch suggests that males range over larger portions of patches than females, allowing them to be resampled at greater frequencies. These gender differences in pellet distribution may be a result of differences in the behavior of male and female cottontails, a male-biased sex ratio, or errors in the genetic gender identification.

Pellet distributions from this study suggest that male cottontails may have larger home ranges, and therefore utilize a greater proportion of the patch than females, but there is limited ecological data from the species to support this. Data from two congeneric species are conflicting, as male home ranges have been found to be larger in eastern cottontails (Trent & Rongstad 1974; Bond et al. 2001), while no differences in home range size were found in Appalachian cottontails (*Sylvilagus obscurus*) (Stevens & Barry 2002; Boyce & Barry 2007). Evidence from observations on New England cottontails in enclosures suggests that males, and particularly dominant males, explore more often and range further than females (Tefft & Chapman 1987). Pellet distribution patterns in this study are consistent with this finding and suggest that males are willing to travel in poorer quality habitat than females. This can be seen in the sample distribution maps for several sites (Appendix B, Figure 1b, g, h, j, k, i), where male samples are found in lesser quality habitat and in some instances even outside the patch boundary, while female samples are clustered in and around dense vegetation within a patch. Pellets from
individual females are also distributed over smaller areas than those of males. Tefft & Chapman (1987) also found that New England cottontails did not defend territories in the traditional sense, allowing individuals to cohabitate portions of a patch. The pellet distributions in this study support this finding as well. While I collected samples during the winter, the observations of New England cottontails by Tefft & Chapman (1987), in addition to the studies referenced on eastern and Appalachian cottontails, were carried out primarily during leaf on months. Therefore, there may be seasonal differences in cottontail behavior not captured by these previous studies.

Pellet distributions on differently sized sites may provide insight into habitat use. Samples from individuals on small sites tended to occur across a higher percentage of the patch, regardless of habitat quality, compared to those on large sites. I found that samples from males generally occurred in a greater proportion of the patch than females and were also found in poor quality habitat more often than pellets from females. The sample distribution pattern from River Road indicates a single male visiting all corners of the patch, including wet open areas in the southern portion of the patch, and a female remaining in the densest areas in the northern half of the patch (Appendix B, Figure 1g). WPRE shows a similar scenario with the lone female staying in the densely vegetated areas, which cover most of the site, while the two males traveled west of the patch into forested habitat with sparse understory (Appendix B, Figure 1j). Both male and female movement within the boundaries of WPRE may have been aided by subnivean travel provided by open spaces under the snow created by particularly dense vegetation. The distribution of pellet samples from the four individuals on Sliver shows all four inn distinct areas of the patch, but with the males occupying the poorer western habitat
(Appendix B, Figure 1h). Sliver is a small, long, and narrow patch, and its configuration may account for the greater segregation of the four individuals. On large sites, pellet distribution patterns suggest that individuals ventured into poor habitat less often, but similar to small sites, males were more likely to occupy marginal habitat than females (Appendix B, Figure b, e, k).

**Evaluation of Methodology**

Noninvasive genetic sampling from fecal pellet surveys has previously been used to monitor occupancy and identify population structure of New England cottontails (Kovach et al. 2003; Litvaitis et al. 2006; Fenderson et al. 2011). This study is the first to use noninvasive genetic sampling to make site-specific population estimates. To this end, an important objective of this study was to evaluate the effectiveness of the methodology and identify areas for improvement in future applications. Below I discuss methodological considerations with respect to survey effort, and timing, DNA quality, genotyping error and genetic marker selection, and issues surrounding potentially low genetic diversity on isolated patches in portions of the species’ range.

**Logistical considerations of surveys**

Decisions concerning the timing of surveys, how many samples to collect during a survey, and the ideal number of site visits are important logistical considerations for population surveys. With respect to the timing of surveys, it is important to provide time for pellet deposition in order to obtain a sufficient sampling of unique and recaptured individuals. However, increasing deposition days decreases DNA quality, lowering genotyping success. The conflicting effects of increased deposition time on capture rates and sample quality (DNA degradation) must be balanced. This is more challenging for
New England cottontails because their fecal DNA is known to degrade rapidly (Kovach et al. 2003). Kovach et al. (2003) utilized mtDNA, which is more prevalent than the nuclear DNA used for genotyping in this study, suggesting that DNA degradation may be more detrimental for population studies. To ensure sufficient quality of DNA, fecal pellets must be collected before weather conditions, primarily high temperature, rain, and sunlight, significantly deteriorate the DNA. Results from Kovach et al. (2003) show that amplification success for New England cottontail pellets declines steeply once pellets are deposited, with success rates of only 10% a week after pellet deposition. This highlights the need to perform population surveys within a few days after a snowfall event.

Three of the 17 population sites were surveyed on two separate occasions. For two of these sites (River Road and WPRE) the two surveys provided similar estimates. At Coast Bus, however, the two visits generated similar numbers of samples, but the first survey resulted in a greater number of unique individuals and a larger population estimate. This is most likely because the second survey only searched approximately half the patch, potentially missing individuals sampled during the first survey. Overall, conducting multiple surveys may not be worth the additional effort compared to the value of a single well timed survey, which balances deposition time with potential DNA degradation. On the other hand, conducting two surveys may provide insight into individual rabbit activity and movements within a patch without the need for live capturing or time intensive telemetry.

**Sampling effort**

The ideal sampling effort (distance between collected samples) for population surveys was difficult to deduce definitively but generally intensive sampling (Exhaustive
for small sites and 30-m sampling distance for all other sites) is ideal on most sites. This is particularly important for surveys on sympatric sites where an unknown number of samples may originate from eastern cottontails. Collecting too many, rather than too few, samples makes sense from a practical standpoint, because the time spent collecting pellets during a survey is minimal compared with other aspects of the survey. Subsampling can be used to reduce the number of pellets analyzed in the lab if deemed necessary.

A sampling distance of 50 m may be sufficient in some cases, but sampling at 30 m is recommended for all sites as a conservative measure. I found that on the five sites where I sampled at 30 m and 50 m, increasing the minimum distance from 30 m to 50 m reduced the number of samples analyzed by 25% (Table 2.12). Precision generally decreased (wider confidence intervals) due to this 25% sample size reduction, but the extent of this change varied by site, with minor loss of precision at Kettle Cove and Stonyfield, and larger loss at Crescent Beach. This variation was influenced by the number of recaptured individuals on each site. For sites with high recapture rates, the precision of the estimate was maintained despite sample size reduction better than sites with fewer recaptures. Sampling at 30 m was always more precise for minimum estimates. Maximum estimates were more variable. On most sites, the 75-m sampling distance did not provide an adequate number of recaptures for confident population estimation. The goal of sample selection is to provide an adequate ratio of single captures and recaptures on a site without processing samples unnecessarily. Collecting pellets at a minimum sampling distance of 30-m, followed by additional subsampling
during processing will prevent oversampling while still providing an adequate number of individual recaptures.

**DNA quality and genotyping error**

Poor quality DNA extracted from fecal pellets lead to some uncertainty in the construction of consensus genotypes and comparison of genotypes for individual discrimination. Issues of DNA quality and genotyping error are a challenge in all non-invasive genetic studies, for which DNA sample quality is typically low (Taberlet & Luikart 1999). In this study, 250 of the 290 (86%) genotyped samples were of sufficient quality to generate multi-locus genotypes with no more than three missing loci. This success rate compares favorably to other fecal genotyping studies with a range of 71-88% (Hájková et al. 2009; Cullingham et al. 2010; Harris et al. 2010; Stenglein et al. 2010). Of the 40 poor quality samples, 27 (66%) came from four sites, suggesting that site-specific, suboptimal survey conditions were responsible. Two of those sites, Coast Bus and the Radio Station, were surveyed with groups of volunteers. These surveys were scheduled in advance according to volunteer availability, rather than with respect to ideal survey conditions, resulting in surveys that occurred greater than five days after the most recent snowfall. These suboptimal survey conditions were likely responsible for poor sample quality, as DNA in fecal pellets degrades after five days of exposure to the environment (see Chapter 2 and Kovach et al. 2003). Only about half of the 19 collected samples at a third site, TSP-301, yielded successful genotypes. The survey at TSP-301 occurred nine days after a snowfall which likely resulted in degraded DNA for many of the samples. This reduction in usable samples resulted in a small sample size and low
proportion of recaptured individuals on this site. These sampling issues are consistent with the large confidence interval for the population estimate for TSP-301.

The loci used in this study were developed for other lagomorph species; eastern cottontail, European wild rabbit (*Oryctolagus cuniculus*), and South African Hare (*Lepus saxatilis*). Using non species-specific loci likely contributed to the error rates for some of the loci. The three loci that were developed for the most closely related species (eastern cottontail), SFL008, SFL011, and SFL015 had among the lowest error rates for both FA and ADO. These loci also amplified far more consistently (Table 2.13). The use of non species-specific markers also likely increased the proportion of null alleles in this study. Fenderson et al (2010) found evidence of null alleles in five of the loci used in this study, including those with the highest error rates. Null alleles produce homozygote excess, lowering heterozygosity and potentially biasing estimates (Van Oosterhout et al. 2004; Hoffman & Amos 2005). Developing polymorphic, species-specific loci for New England cottontails would likely improve amplification and potentially reduce null allele and genotyping error rates for noninvasive DNA samples.

Several sites used in this study had relatively low power discriminating individuals (Appendix B, Table 2). This could be a result of reduced power from using non species-specific loci or a result of low genetic diversity among individual cottontails. When loci have low polymorphism a greater number are needed to obtain sufficient discriminatory power. Genotyping error, however, increases with the number of loci used (Waits et al. 2003). The Sat13 and Sol03 loci had the highest genotyping errors in this study and were the most difficult loci to score. It is likely that poor amplification in these two loci produced the increased error. On average, per locus error rates for this
study (FA = 3.9% and ADO = 8.9%) are comparable to other non-invasive studies (Broquet & Petit 2004)

**Probability of identity**

The average $PI_{sib}$ across all sites was 3.33E-02 for males and 4.01E-02 for females meaning that, at a minimum, for every 30 closely related males and 25 related females, each individual should have a unique multi-locus genotype. $PI_{sib}$ for nine of the 13 sites was below 0.05. Low $PI_{sibs}$ combined with overall small populations makes it unlikely that these population estimates are biased by potential “shadow effect,” which is when two unique individuals share identical multi-locus genotypes and are therefore incorrectly categorized as a single individual rather two (Mills et al. 2000). The occurrence of shadow genotypes gives a downward bias to population estimates. The four sites with $PI_{sib}$ above 0.05 (Wells Reserve, River Road, Fort Williams, and WPRE) all had low numbers of alleles (Table 2.17) and individuals. River Road, Fort Williams, and WPRE were also small sites.

**Recommendations**

In light of the above methodological considerations, I provide recommendations for survey and analytical approaches in future population estimation surveys. I recommend waiting 3-4 days without high winds or rain after a snowfall before conducting population surveys. Given optimal survey conditions, a single site visit is sufficient, but if conditions are poor, or if information regarding patch use and movement of individual rabbits is desired, multiple visits may be needed. Additional visits could provide insight about the behavior of individuals on a patch over the course of the winter.
Sampling effort should vary based on site size. Small sites (<4 ha) should generally be sampled exhaustively unless performed on sites known to have a large number of pellets. All other patches, including sympatric sites, should be sampled at a 30-m distance. Finally, the development of species-specific markers for New England cottontails will improve scoring efficiency, lower error rates, and increase estimate precision. It may also provide insight into whether the observed low genetic diversity on sites is restricted to small isolated and inbred populations or characterizes New England cottontail populations on a broader scale.
Table 2.1 Sites surveyed for New England cottontail population estimation.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>State</th>
<th>Size (ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frieze Property</td>
<td>ME</td>
<td>0.73</td>
</tr>
<tr>
<td>Sliver</td>
<td>ME</td>
<td>0.98</td>
</tr>
<tr>
<td>WPRE</td>
<td>ME</td>
<td>1.1</td>
</tr>
<tr>
<td>Fort Williams</td>
<td>ME</td>
<td>1.5</td>
</tr>
<tr>
<td>River Road</td>
<td>ME</td>
<td>1.84</td>
</tr>
<tr>
<td>Radio Station</td>
<td>NH</td>
<td>2.41</td>
</tr>
<tr>
<td>Coast</td>
<td>ME</td>
<td>3.27</td>
</tr>
<tr>
<td>Orchard</td>
<td>ME</td>
<td>3.76</td>
</tr>
<tr>
<td>Stonyfield</td>
<td>NH</td>
<td>4.93</td>
</tr>
<tr>
<td>Coast Bus</td>
<td>NH</td>
<td>5.37</td>
</tr>
<tr>
<td>Weed Mine</td>
<td>NY</td>
<td>10.24</td>
</tr>
<tr>
<td>CFSP</td>
<td>NY</td>
<td>10.26</td>
</tr>
<tr>
<td>Crescent Beach</td>
<td>ME</td>
<td>12.43</td>
</tr>
<tr>
<td>Bellamy</td>
<td>NH</td>
<td>13.21</td>
</tr>
<tr>
<td>Wells Reserve</td>
<td>ME</td>
<td>18.9</td>
</tr>
<tr>
<td>Kettle Cove</td>
<td>ME</td>
<td>20.4</td>
</tr>
<tr>
<td>TSP-301</td>
<td>NY</td>
<td>26.32</td>
</tr>
</tbody>
</table>

Table 2.2. References for each of the microsatellite markers used in this study.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sol03</td>
<td>(Rico et al. 1994)</td>
</tr>
<tr>
<td>Sol44</td>
<td>(Surridge et al. 1997)</td>
</tr>
<tr>
<td>Sat3, Sat13</td>
<td>(Mougel et al. 1997)</td>
</tr>
<tr>
<td>INRA016, INRAACCDDV0326 (Y)</td>
<td>(Chantry-Darmon et al. 2005)</td>
</tr>
<tr>
<td>LSA1</td>
<td>(Kryger et al. 2002)</td>
</tr>
<tr>
<td>SFL008, SFL011, SFL015</td>
<td>(Berkman et al. 2009)</td>
</tr>
</tbody>
</table>
Table 2.3. Multiplex PCR conditions for 10 microsatellite loci optimized for this study. All PCRs used identical initial denaturation steps of 95 °C for five minutes and 95 °C for 30 seconds.

<table>
<thead>
<tr>
<th>Locus/Multiplex</th>
<th>Annealing (°C)</th>
<th>Extension (°C)</th>
<th>Final Extens. (°C)</th>
<th>Time (°C)</th>
<th>Time (°C)</th>
<th>Cycles</th>
<th>Size (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sat13, Lsa1</td>
<td>60</td>
<td>52</td>
<td>60</td>
<td>90&quot;</td>
<td>30&quot;</td>
<td>40</td>
<td>15</td>
</tr>
<tr>
<td>Sol03</td>
<td>52</td>
<td>58</td>
<td>60</td>
<td>90&quot;</td>
<td>72</td>
<td>30'</td>
<td>12</td>
</tr>
<tr>
<td>INRA16, Sol44, Y</td>
<td>60</td>
<td>58</td>
<td>60</td>
<td>90&quot;</td>
<td>58</td>
<td>60</td>
<td>12</td>
</tr>
<tr>
<td>Sat3</td>
<td>58</td>
<td>58</td>
<td>60</td>
<td>30&quot;</td>
<td>30&quot;</td>
<td>30&quot;</td>
<td>35</td>
</tr>
</tbody>
</table>

Table 2.4. PCR concentrations for each multiplex of loci. All reactions used 1X BSA, 1X buffer, and 0.75 U Taq polymerase.

<table>
<thead>
<tr>
<th>Locus/Multiplex</th>
<th>Primer Conc. (µM)</th>
<th>MgCl₂ (mM)</th>
<th>dNTP (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sat13, Lsa1</td>
<td>0.2, 0.17</td>
<td>2.5</td>
<td>0.20</td>
</tr>
<tr>
<td>Sol03</td>
<td>0.25</td>
<td>1.87</td>
<td>0.20</td>
</tr>
<tr>
<td>INRA16, Sol44, Y</td>
<td>0.4, 0.53, 0.53</td>
<td>1.5</td>
<td>0.20</td>
</tr>
<tr>
<td>Sat3</td>
<td>0.25</td>
<td>1.87</td>
<td>0.20</td>
</tr>
<tr>
<td>SFL008, SFL011, SFL015</td>
<td>0.33, 0.27, 0.33</td>
<td>1.25</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Table 2.5. False allele error rates per genotype and per allele.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Genotype</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSA1</td>
<td>0.017</td>
<td>0.009</td>
</tr>
<tr>
<td>Sat13</td>
<td>0.116</td>
<td>0.060</td>
</tr>
<tr>
<td>Sol03</td>
<td>0.063</td>
<td>0.031</td>
</tr>
<tr>
<td>Sat3</td>
<td>0.087</td>
<td>0.044</td>
</tr>
<tr>
<td>INRA16</td>
<td>0.013</td>
<td>0.007</td>
</tr>
<tr>
<td>Sol44</td>
<td>0.029</td>
<td>0.015</td>
</tr>
<tr>
<td>SFL008</td>
<td>0.004</td>
<td>0.002</td>
</tr>
<tr>
<td>SFL011</td>
<td>0.014</td>
<td>0.007</td>
</tr>
<tr>
<td>SFL015</td>
<td>0.008</td>
<td>0.004</td>
</tr>
<tr>
<td>Y</td>
<td>0.012</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Table 2.6. Allelic dropout error rates per genotype and per allele.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Genotype</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSA1</td>
<td>0.033</td>
<td>0.017</td>
</tr>
<tr>
<td>Sat13</td>
<td>0.208</td>
<td>0.104</td>
</tr>
<tr>
<td>Sol03</td>
<td>0.152</td>
<td>0.076</td>
</tr>
<tr>
<td>Sat3</td>
<td>0.084</td>
<td>0.042</td>
</tr>
<tr>
<td>INRA16</td>
<td>0.083</td>
<td>0.041</td>
</tr>
<tr>
<td>Sol44</td>
<td>0.091</td>
<td>0.046</td>
</tr>
<tr>
<td>SFL008</td>
<td>0.056</td>
<td>0.028</td>
</tr>
<tr>
<td>SFL011</td>
<td>0.045</td>
<td>0.022</td>
</tr>
<tr>
<td>SFL015</td>
<td>0.049</td>
<td>0.024</td>
</tr>
<tr>
<td>Y</td>
<td>0.063</td>
<td>0.031</td>
</tr>
</tbody>
</table>
Table 2.7. Summary by state of the total number of samples collected, genotyped, and the number of usable samples for population modeling.

<table>
<thead>
<tr>
<th>State</th>
<th>#Samples</th>
<th>#Processed</th>
<th>#Genotyped</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maine</td>
<td>298</td>
<td>180</td>
<td>172</td>
</tr>
<tr>
<td>New Hampshire</td>
<td>72</td>
<td>61</td>
<td>46</td>
</tr>
<tr>
<td>New York</td>
<td>57</td>
<td>56</td>
<td>32</td>
</tr>
<tr>
<td>Total</td>
<td>427</td>
<td>290</td>
<td>250</td>
</tr>
</tbody>
</table>

Table 2.8. The average false allele and allelic dropout rates per genotype for male and female cottontails. Male error rates include Y locus while female rates do not.

<table>
<thead>
<tr>
<th>Column1</th>
<th>False Allele</th>
<th>Allelic Dropout</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>0.039</td>
<td>0.089</td>
</tr>
<tr>
<td>Males</td>
<td>0.036</td>
<td>0.086</td>
</tr>
</tbody>
</table>

Table 2.9. The site probability of identity for males and probability of identity among siblings for males and females.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Overall PI</th>
<th>PIsib Male</th>
<th>PIsib Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coast Bus</td>
<td>2.57E-09</td>
<td>2.71E-04</td>
<td>6.45E-04</td>
</tr>
<tr>
<td>Stonyfield</td>
<td>4.66E-09</td>
<td>3.49E-04</td>
<td>7.83E-04</td>
</tr>
<tr>
<td>TSP-301</td>
<td>1.19E-05</td>
<td>5.27E-03</td>
<td>5.27E-03</td>
</tr>
<tr>
<td>Coast</td>
<td>3.44E-05</td>
<td>9.05E-03</td>
<td>9.05E-03</td>
</tr>
<tr>
<td>Crescent Beach</td>
<td>1.82E-05</td>
<td>5.99E-03</td>
<td>9.61E-03</td>
</tr>
<tr>
<td>Orchard</td>
<td>5.13E-05</td>
<td>9.83E-03</td>
<td>1.31E-02</td>
</tr>
<tr>
<td>Kettle Cove</td>
<td>2.12E-04</td>
<td>1.54E-02</td>
<td>2.55E-02</td>
</tr>
<tr>
<td>CFSP</td>
<td>6.43E-04</td>
<td>6.65E-03</td>
<td>2.66E-02</td>
</tr>
<tr>
<td>Sliver</td>
<td>1.82E-03</td>
<td>4.57E-02</td>
<td>4.57E-02</td>
</tr>
<tr>
<td>Wells Reserve</td>
<td>2.61E-03</td>
<td>5.26E-02</td>
<td>5.26E-02</td>
</tr>
<tr>
<td>River Road</td>
<td>1.39E-03</td>
<td>3.50E-02</td>
<td>5.89E-02</td>
</tr>
<tr>
<td>Fort Williams</td>
<td>2.07E-03</td>
<td>4.39E-02</td>
<td>6.97E-02</td>
</tr>
<tr>
<td>WPRE</td>
<td>3.52E-02</td>
<td>2.04E-01</td>
<td>2.04E-01</td>
</tr>
</tbody>
</table>
Table 2.10. Population estimates from Capwire for samples analyzed with no minimum distance (exhaustive sampling), 30-meter, 50-meter, and 75-meter sampling distances. Survey = is the visit number or both visits combined. Sample size is the total number of pellet samples genotyped. = Individuals Min Max is the number of unique individuals for the minimum and maximum estimates respectively. Minimum N (LCI-HCI) and Maximum N (LCI-HCI) are Capwire's point estimate (N) with the lower and upper confidence limits in parenthesis, for the minimum and maximum estimates. Model describes the model type selected by Capwire (TIRM = Two innate rates model, ECM = Equal capture model).

a. Maine sites.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Size (ha)</th>
<th>Survey #</th>
<th>Sample Size</th>
<th># Individuals Min / Max</th>
<th>Minimum N (LCI-HCI)</th>
<th>Maximum N (LCI-HCI)</th>
<th>Model</th>
<th>Sub-sample (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coast</td>
<td>3.27</td>
<td>1</td>
<td>8</td>
<td>5.5</td>
<td>7 (5-11)</td>
<td>7 (5-11)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Crescent Beach</td>
<td>12.43</td>
<td>1</td>
<td>49</td>
<td>20 (20-25)</td>
<td>22 (19-32)</td>
<td>102 (53-213)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Crescent Beach</td>
<td>12.43</td>
<td>1</td>
<td>36</td>
<td>19 (8-29)</td>
<td>12 (3-3)</td>
<td>15 (9-27)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Crescent Beach</td>
<td>12.43</td>
<td>1</td>
<td>12</td>
<td>8 (8-29)</td>
<td>12 (19-32)</td>
<td>102 (53-213)</td>
<td>TIRM</td>
<td>50</td>
</tr>
<tr>
<td>Fort Williams</td>
<td>1.5</td>
<td>1</td>
<td>9</td>
<td>3.4</td>
<td>3 (3-3)</td>
<td>3 (9-27)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Kettle Cove</td>
<td>20.4</td>
<td>1</td>
<td>21</td>
<td>9 (9-27)</td>
<td>10 (9-11)</td>
<td>15 (9-27)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Kettle Cove</td>
<td>20.4</td>
<td>1</td>
<td>16</td>
<td>9 (9-27)</td>
<td>10 (9-11)</td>
<td>15 (9-27)</td>
<td>TIRM</td>
<td>50</td>
</tr>
<tr>
<td>Kettle Cove</td>
<td>20.4</td>
<td>1</td>
<td>10</td>
<td>8 (9-27)</td>
<td>10 (9-11)</td>
<td>15 (9-27)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Orchard</td>
<td>3.76</td>
<td>1</td>
<td>7</td>
<td>6 (6-19)</td>
<td>19 (6-19)</td>
<td>19 (6-19)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>River Road</td>
<td>1.84</td>
<td>1</td>
<td>9</td>
<td>2.2</td>
<td>2 (2-2)</td>
<td>2 (2-2)</td>
<td>ECM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>River Road</td>
<td>1.84</td>
<td>2</td>
<td>13</td>
<td>3 (3-3)</td>
<td>5 (5-5)</td>
<td>5 (5-5)</td>
<td>ECM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>River Road</td>
<td>1.84</td>
<td>Combined</td>
<td>35</td>
<td>5 (5-7)</td>
<td>2 (2-2)</td>
<td>2 (2-2)</td>
<td>ECM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>Sliver</td>
<td>0.98</td>
<td>1</td>
<td>9</td>
<td>4 (4-4)</td>
<td>5 (4-4)</td>
<td>5 (4-4)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Sliver</td>
<td>0.98</td>
<td>1</td>
<td>7</td>
<td>4 (4-4)</td>
<td>5 (4-4)</td>
<td>5 (4-4)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Sliver</td>
<td>0.98</td>
<td>1</td>
<td>5</td>
<td>4 (4-4)</td>
<td>5 (4-4)</td>
<td>5 (4-4)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Sliver</td>
<td>0.98</td>
<td>1</td>
<td>16</td>
<td>4 (4-4)</td>
<td>5 (4-4)</td>
<td>5 (4-4)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Wells Reserve</td>
<td>18.9</td>
<td>1</td>
<td>4</td>
<td>2 (2-2)</td>
<td>2 (2-2)</td>
<td>2 (2-2)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>WPRED</td>
<td>1.1</td>
<td>1</td>
<td>13</td>
<td>NA* 2</td>
<td>NA*</td>
<td>2 (2-2)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>WPRED</td>
<td>1.1</td>
<td>2</td>
<td>19</td>
<td>2 (2-2)</td>
<td>2 (2-2)</td>
<td>2 (2-2)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>WPRED</td>
<td>1.1</td>
<td>Combined</td>
<td>32</td>
<td>2 (2-2)</td>
<td>2 (2-2)</td>
<td>2 (2-2)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
</tbody>
</table>

* This subset of samples resulted in only one individual with a recapture and eight single captures making modeling it impractical.
b. New Hampshire and New York sites.

<table>
<thead>
<tr>
<th>Site Name (State)</th>
<th>Size (ha)</th>
<th>Survey #</th>
<th>Size</th>
<th># Individuals Min / Max</th>
<th>Minimum N (LCI-HCI)</th>
<th>Maximum N (LCI-HCI)</th>
<th>Model</th>
<th>Sub-sample (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coast Bus (NH)</td>
<td>5.37</td>
<td>1</td>
<td>10</td>
<td>5.6</td>
<td>11 (5-21)</td>
<td>15 (6-30)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>Coast Bus (NH)</td>
<td>5.37</td>
<td>2</td>
<td>13</td>
<td>3.3</td>
<td>5 (3-8)</td>
<td>5 (3-8)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>Coast Bus (NH)</td>
<td>5.37</td>
<td>Combined</td>
<td>23</td>
<td>7.8</td>
<td>15 (7-21)</td>
<td>18 (8-25)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>Stonyfield (NH)</td>
<td>4.93</td>
<td>1</td>
<td>18</td>
<td>10:12</td>
<td>18 (10-30)</td>
<td>30 (12-50)</td>
<td>TIRM</td>
<td>30</td>
</tr>
<tr>
<td>Stonyfield (NH)</td>
<td>4.93</td>
<td>1</td>
<td>12</td>
<td>7.8</td>
<td>9 (7-18)</td>
<td>12 (8-29)</td>
<td>ECM</td>
<td>50</td>
</tr>
<tr>
<td>Stonyfield (NH)</td>
<td>4.93</td>
<td>1</td>
<td>9</td>
<td>4.6</td>
<td>4 (4-4)</td>
<td>9 (6-33)</td>
<td>ECM</td>
<td>75</td>
</tr>
<tr>
<td>CFSP (NY)</td>
<td>10.26</td>
<td>1</td>
<td>20</td>
<td>5.7</td>
<td>5 (5-5)</td>
<td>8 (7-12)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>CFSP (NY)</td>
<td>10.26</td>
<td>1</td>
<td>16</td>
<td>5.7</td>
<td>5 (5-5)</td>
<td>11 (7-19)</td>
<td>TIRM</td>
<td>50</td>
</tr>
<tr>
<td>CFSP (NY)</td>
<td>10.26</td>
<td>1</td>
<td>9</td>
<td>4.6</td>
<td>4 (4-4)</td>
<td>9 (6-33)</td>
<td>ECM</td>
<td>75</td>
</tr>
<tr>
<td>TSP-301 (NY)</td>
<td>26.32</td>
<td>1</td>
<td>9</td>
<td>6.7</td>
<td>6 (9-33)</td>
<td>15 (7-33)</td>
<td>ECM</td>
<td>30</td>
</tr>
</tbody>
</table>
Table 2.11. Differences in point estimates from CAPWIRE. Min and Max are the minimum and maximum estimates entered into the model along with the 30, 50, and 75 minimum distance categories.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Min 30m</th>
<th>Max 30m</th>
<th>Min 50m</th>
<th>Max 50m</th>
<th>Min 75m</th>
<th>Max 75m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stonyfield</td>
<td>18</td>
<td>9</td>
<td>4</td>
<td>30</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Kettle Cove</td>
<td>10</td>
<td>15</td>
<td>30</td>
<td>11</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Sliver</td>
<td>4</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Crescent Beach</td>
<td>22</td>
<td>24</td>
<td>12</td>
<td>67</td>
<td>102</td>
<td>12</td>
</tr>
<tr>
<td>CFSP</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>8</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 2.12. Percent of pellets retained from a survey when the minimum distance was increased from 30 m to 50 m or 75 m. The 30-m column contains the number of samples analyzed with the 30-m subsampling scheme, while the 50-m and 75-m columns show the proportion of samples that were retained.

<table>
<thead>
<tr>
<th>Site</th>
<th>State</th>
<th>30m</th>
<th>50m</th>
<th>75m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crescent Beach</td>
<td>ME</td>
<td>49</td>
<td>0.73</td>
<td>0.36</td>
</tr>
<tr>
<td>Kettle Cove</td>
<td>ME</td>
<td>21</td>
<td>0.76</td>
<td>0.48</td>
</tr>
<tr>
<td>Sliver</td>
<td>ME</td>
<td>9</td>
<td>0.78</td>
<td>0.56</td>
</tr>
<tr>
<td>Stonyfield</td>
<td>NH</td>
<td>18</td>
<td>0.67</td>
<td>0.5</td>
</tr>
<tr>
<td>CFSP</td>
<td>NY</td>
<td>21</td>
<td>0.8</td>
<td>0.43</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>0.75</td>
<td>0.48</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.13. The proportion of samples that failed to produce a consensus genotype for each of the 10 loci.

<table>
<thead>
<tr>
<th>Loci</th>
<th>No Consensus</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSA</td>
<td>0.04</td>
</tr>
<tr>
<td>Sat13</td>
<td>0.29</td>
</tr>
<tr>
<td>Sol03</td>
<td>0.26</td>
</tr>
<tr>
<td>Sat3</td>
<td>0.27</td>
</tr>
<tr>
<td>I16</td>
<td>0.09</td>
</tr>
<tr>
<td>Sol44</td>
<td>0.09</td>
</tr>
<tr>
<td>Y</td>
<td>NA</td>
</tr>
<tr>
<td>SFL008</td>
<td>0.06</td>
</tr>
<tr>
<td>SFL011</td>
<td>0.09</td>
</tr>
<tr>
<td>SFL015</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Table 2.14. Population estimates from Capwire based on the most likely number of unique individuals per site derived from the most exhaustive sampling conducted at each site (Exhaustive or 30 m).

<table>
<thead>
<tr>
<th>Site Name</th>
<th>State</th>
<th>Size (ha)</th>
<th># Samples Processed</th>
<th># Successful Genotypes</th>
<th># Unique Individuals</th>
<th>Population Estimate N (LCI-HCI)</th>
<th>Model</th>
<th>Sub-sample (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frieze</td>
<td>ME</td>
<td>0.73</td>
<td>4</td>
<td>4</td>
<td>4+</td>
<td>NA</td>
<td>NA</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>Sliver</td>
<td>ME</td>
<td>0.98</td>
<td>17</td>
<td>16</td>
<td>4</td>
<td>4 (4-4)</td>
<td>ECM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>WPRE</td>
<td>ME</td>
<td>1.1</td>
<td>34*</td>
<td>33</td>
<td>3</td>
<td>3 (3-3)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>Fort Williams</td>
<td>ME</td>
<td>1.5</td>
<td>9</td>
<td>9</td>
<td>4</td>
<td>4 (4-4)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>River Road</td>
<td>ME</td>
<td>1.84</td>
<td>22*</td>
<td>22</td>
<td>4</td>
<td>4 (4-6)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>Coast</td>
<td>ME</td>
<td>3.27</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>7 (5-11)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Orchard</td>
<td>ME</td>
<td>3.76</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>9 (6-19)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Wells Reserve</td>
<td>ME</td>
<td>18.9</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>2 (2-2)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>Kettle Cove</td>
<td>ME</td>
<td>20.4</td>
<td>22</td>
<td>21</td>
<td>7</td>
<td>7 (7-7)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Crescent Beach</td>
<td>ME</td>
<td>12.43</td>
<td>49</td>
<td>49</td>
<td>21</td>
<td>30 (21-41)</td>
<td>TIRM</td>
<td>30</td>
</tr>
<tr>
<td>Stonyfield</td>
<td>NH</td>
<td>4.93</td>
<td>19</td>
<td>18</td>
<td>10</td>
<td>18 (10-30)</td>
<td>TIRM</td>
<td>30</td>
</tr>
<tr>
<td>Coast Bus</td>
<td>NH</td>
<td>5.37</td>
<td>28*</td>
<td>23</td>
<td>7</td>
<td>15 (7-21)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>Bellamy</td>
<td>NH</td>
<td>13.21</td>
<td>4</td>
<td>3</td>
<td>1†</td>
<td>NA</td>
<td>NA</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>Weed Mine</td>
<td>NY</td>
<td>10.24</td>
<td>5</td>
<td>3</td>
<td>3†</td>
<td>NA</td>
<td>NA</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>CFSP</td>
<td>NY</td>
<td>10.26</td>
<td>26</td>
<td>20</td>
<td>4</td>
<td>4 (4-4)</td>
<td>TIRM</td>
<td>30</td>
</tr>
<tr>
<td>TSP-301</td>
<td>NY</td>
<td>26.32</td>
<td>17</td>
<td>9</td>
<td>7</td>
<td>15 (7-33)</td>
<td>ECM</td>
<td>30</td>
</tr>
</tbody>
</table>

* Denotes sites where two capture sessions were conducted and the population estimate is based on the combined samples from both visits.
† Denotes sites where no population estimate was made because there were either too few unique individuals or no recaptures.
Table 2.15. A comparison of population estimates for the three sites where two capture sessions were performed.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>State</th>
<th>Size (ha)</th>
<th>Visit</th>
<th>Sample Size</th>
<th># Unique Individuals</th>
<th>Population Estimate N (LCI-HCI)</th>
<th>Model</th>
<th>Sub-sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coast Bus</td>
<td>NH</td>
<td>5.37</td>
<td>1</td>
<td>10</td>
<td>5</td>
<td>11 (5-21)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>Coast Bus</td>
<td>NH</td>
<td>5.37</td>
<td>2</td>
<td>13</td>
<td>3</td>
<td>5 (3-8)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>Coast Bus</td>
<td>NH</td>
<td>5.37</td>
<td>All</td>
<td>23</td>
<td>7</td>
<td>15 (7-21)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>River Road</td>
<td>ME</td>
<td>1.84</td>
<td>1</td>
<td>9</td>
<td>2</td>
<td>2 (2-2)</td>
<td>ECM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>River Road</td>
<td>ME</td>
<td>1.84</td>
<td>2</td>
<td>13</td>
<td>4</td>
<td>4 (4-6)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>River Road</td>
<td>ME</td>
<td>1.84</td>
<td>All</td>
<td>22</td>
<td>4</td>
<td>4 (4-6)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>WPRE</td>
<td>ME</td>
<td>1.1</td>
<td>1</td>
<td>13</td>
<td>2</td>
<td>2 (2-2)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>WPRE</td>
<td>ME</td>
<td>1.1</td>
<td>2</td>
<td>19</td>
<td>2</td>
<td>2 (2-2)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>WPRE</td>
<td>ME</td>
<td>1.1</td>
<td>All</td>
<td>32</td>
<td>3</td>
<td>3 (3-3)</td>
<td>TIRM</td>
<td>Exhaustive</td>
</tr>
</tbody>
</table>
Table 2.16. Comparison of population estimates for sites using different sub-sampling efforts.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>State</th>
<th>Size (ha)</th>
<th>Sample Size</th>
<th># Unique Individuals</th>
<th>Population Estimate N (LCI-HCI)</th>
<th>Model</th>
<th>Sub-sample (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crescent Beach</td>
<td>ME</td>
<td>12.43</td>
<td>49</td>
<td>21</td>
<td>30 (21-41)</td>
<td>TIRM</td>
<td>30</td>
</tr>
<tr>
<td>Crescent Beach</td>
<td>ME</td>
<td>12.43</td>
<td>36</td>
<td>20</td>
<td>26 (20-36)</td>
<td>ECM</td>
<td>50</td>
</tr>
<tr>
<td>Crescent Beach</td>
<td>ME</td>
<td>12.43</td>
<td>18</td>
<td>13</td>
<td>24 (13-45)</td>
<td>ECM</td>
<td>75</td>
</tr>
<tr>
<td>Kettle Cove</td>
<td>ME</td>
<td>20.4</td>
<td>21</td>
<td>7</td>
<td>7 (7-7)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Kettle Cove</td>
<td>ME</td>
<td>20.4</td>
<td>16</td>
<td>7</td>
<td>10 (7-18)</td>
<td>TIRM</td>
<td>50</td>
</tr>
<tr>
<td>Kettle Cove</td>
<td>ME</td>
<td>20.4</td>
<td>10</td>
<td>7</td>
<td>12 (7-42)</td>
<td>ECM</td>
<td>75</td>
</tr>
<tr>
<td>Sliver</td>
<td>ME</td>
<td>0.98</td>
<td>16</td>
<td>4</td>
<td>4 (4-4)</td>
<td>ECM</td>
<td>Exhaustive</td>
</tr>
<tr>
<td>Sliver</td>
<td>ME</td>
<td>0.98</td>
<td>9</td>
<td>4</td>
<td>4 (4-4)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>Sliver</td>
<td>ME</td>
<td>0.98</td>
<td>7</td>
<td>4</td>
<td>5 (4-8)</td>
<td>ECM</td>
<td>50</td>
</tr>
<tr>
<td>Sliver</td>
<td>ME</td>
<td>0.98</td>
<td>5</td>
<td>4</td>
<td>8 (4-8)</td>
<td>ECM</td>
<td>75</td>
</tr>
<tr>
<td>Stonyfield</td>
<td>NH</td>
<td>4.93</td>
<td>18</td>
<td>10</td>
<td>18 (10-30)</td>
<td>TIRM</td>
<td>30</td>
</tr>
<tr>
<td>Stonyfield</td>
<td>NH</td>
<td>4.93</td>
<td>12</td>
<td>7</td>
<td>9 (7-18)</td>
<td>ECM</td>
<td>50</td>
</tr>
<tr>
<td>Stonyfield</td>
<td>NH</td>
<td>4.93</td>
<td>9</td>
<td>4</td>
<td>4 (4-4)</td>
<td>ECM</td>
<td>75</td>
</tr>
<tr>
<td>CFSP</td>
<td>NY</td>
<td>10.26</td>
<td>20</td>
<td>4</td>
<td>4 (4-4)</td>
<td>ECM</td>
<td>30</td>
</tr>
<tr>
<td>CFSP</td>
<td>NY</td>
<td>10.26</td>
<td>16</td>
<td>4</td>
<td>4 (4-4)</td>
<td>TIRM</td>
<td>50</td>
</tr>
<tr>
<td>CFSP</td>
<td>NY</td>
<td>10.26</td>
<td>9</td>
<td>4</td>
<td>4 (4-4)</td>
<td>ECM</td>
<td>75</td>
</tr>
</tbody>
</table>
Table 2.17. Table showing by site the Observed (Ho) and Expected (He) Heterozygosity, Fis, Total Alleles, and Allelic Richness.

<table>
<thead>
<tr>
<th>Population</th>
<th>State</th>
<th>Patch Size</th>
<th>Sample Size (n)</th>
<th># Unique Individuals</th>
<th>Ho</th>
<th>He</th>
<th>Fis</th>
<th>Total Alleles (Avg per locus)</th>
<th>Allelic Richness (Avg per Locus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bellamy</td>
<td>NH</td>
<td>13.21</td>
<td>3</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>12 (1.2)</td>
<td>11 (1.2)</td>
</tr>
<tr>
<td>CFSP</td>
<td>NY</td>
<td>10.26</td>
<td>20</td>
<td>4</td>
<td>0.51</td>
<td>0.44</td>
<td>-0.2</td>
<td>22 (2.2)</td>
<td>13 (1.4)</td>
</tr>
<tr>
<td>Coast</td>
<td>ME</td>
<td>3.27</td>
<td>8</td>
<td>5</td>
<td>0.45</td>
<td>0.49</td>
<td>0.1</td>
<td>23 (2.3)</td>
<td>13.5 (1.4)</td>
</tr>
<tr>
<td>Coast Bus</td>
<td>NH</td>
<td>5.37</td>
<td>23</td>
<td>7</td>
<td>0.42</td>
<td>0.78</td>
<td>0.5</td>
<td>48 (4.8)</td>
<td>15.7 (1.6)</td>
</tr>
<tr>
<td>Crescent Beach</td>
<td>ME</td>
<td>12.43</td>
<td>49</td>
<td>21</td>
<td>0.53</td>
<td>0.47</td>
<td>-0.1</td>
<td>32 (3.2)</td>
<td>13.2 (1.3)</td>
</tr>
<tr>
<td>Fort Williams</td>
<td>ME</td>
<td>1.5</td>
<td>9</td>
<td>4</td>
<td>0.32</td>
<td>0.39</td>
<td>0.1</td>
<td>21 (2.1)</td>
<td>12.7 (1.3)</td>
</tr>
<tr>
<td>Frieze</td>
<td>ME</td>
<td>0.73</td>
<td>4</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>13 (1.3)</td>
<td>12 (1.3)</td>
</tr>
<tr>
<td>Kettle Cove</td>
<td>ME</td>
<td>20.4</td>
<td>21</td>
<td>7</td>
<td>0.41</td>
<td>0.42</td>
<td>0.0</td>
<td>22 (2.2)</td>
<td>12.7 (1.3)</td>
</tr>
<tr>
<td>Orchard</td>
<td>ME</td>
<td>3.76</td>
<td>7</td>
<td>6</td>
<td>0.47</td>
<td>0.46</td>
<td>0.0</td>
<td>26 (2.6)</td>
<td>13.3 (1.3)</td>
</tr>
<tr>
<td>Radio Station</td>
<td>NH</td>
<td>2.46</td>
<td>1</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>11 (1.1)</td>
<td>11 (1.2)</td>
</tr>
<tr>
<td>River Road</td>
<td>ME</td>
<td>1.84</td>
<td>22</td>
<td>4</td>
<td>0.5</td>
<td>0.33</td>
<td>-0.5</td>
<td>19 (1.9)</td>
<td>12.1 (1.2)</td>
</tr>
<tr>
<td>Sliver</td>
<td>ME</td>
<td>0.98</td>
<td>16</td>
<td>4</td>
<td>0.3</td>
<td>0.35</td>
<td>0.2</td>
<td>18 (1.8)</td>
<td>12.3 (1.2)</td>
</tr>
<tr>
<td>Stonyfield</td>
<td>NH</td>
<td>4.93</td>
<td>18</td>
<td>10</td>
<td>0.53</td>
<td>0.74</td>
<td>0.2</td>
<td>48 (4.8)</td>
<td>15.5 (1.6)</td>
</tr>
<tr>
<td>TSP-301</td>
<td>NY</td>
<td>26.32</td>
<td>9</td>
<td>7</td>
<td>0.49</td>
<td>0.56</td>
<td>0.2</td>
<td>28 (2.8)</td>
<td>14.0 (1.6)</td>
</tr>
<tr>
<td>Weed Mine</td>
<td>NY</td>
<td>10.24</td>
<td>3</td>
<td>3</td>
<td>0.68</td>
<td>0.68</td>
<td>0.0</td>
<td>23 (2.3)</td>
<td>14.4 (1.6)</td>
</tr>
<tr>
<td>Wells Reserve</td>
<td>ME</td>
<td>18.9</td>
<td>4</td>
<td>2</td>
<td>0.5</td>
<td>0.38</td>
<td>-0.3</td>
<td>18 (1.8)</td>
<td>13.2 (1.3)</td>
</tr>
<tr>
<td>WPRE</td>
<td>ME</td>
<td>1.1</td>
<td>33</td>
<td>3</td>
<td>0.14</td>
<td>0.12</td>
<td>-0.1</td>
<td>13 (1.3)</td>
<td>10.2 (1.0)</td>
</tr>
</tbody>
</table>
Figure 2.1. Locations of 19 sites surveyed for population estimation (see Appendix A for specific site locations).
Figure 2.2. Figure showing how samples were subsampled for genotyping. Stars and crosses represent the initial sample size with a minimum distance of 30 m (moderate and dark grey circles). Alone, stars represent the samples that remain following subsampling at a distance of 50 m (light gray circles). Several sites included an additional subsampling distance at 75 m (not shown on this map) that further reduced the sample size.
Figure 2.3. Map showing an example search path, in red, for population surveys on small to moderate sized sites. River road is 1.84 hectares in size.
Figure 2.4 Map showing examples of both search methods for large sites. The eastern portion shows multiple surveyors searching in parallel paths while the less contiguous parts in the western portion of the patch show individual surveyors searching specific areas. Different colored lines represent paths used by different surveyors. Wells Reserve is 18.9 hectares in size.
CHAPTER 3

Conclusion

The goal of this research was to use non-invasive genetic tools to guide the development of optimal monitoring protocols for New England cottontail occupancy and abundance. I conducted a study to investigate the environmental and behavioral factors that influence the detectability of New England cottontails during winter pellet surveys. I also conducted a study of to evaluate the methodology for mark-recapture population estimation and made preliminary, baseline estimates for 17 occupied patches across the species’ range.

Detection modeling showed that prior knowledge of cottontail activity, snow depth < 12 inches at the time of survey, and allowing a sufficient number of pellet deposition following a snowfall, are positively associated with New England cottontail detection. Patch size also influences detection when surveys are limited to 20-minute time periods, with detection decreasing on large patches. Overall, detection probabilities were high when surveys were conducted under ideal conditions.

I made recommendations for improved efficiency and reliability of future occupancy surveys. I suggest surveyors allow at least 2-4 days without winds over 40 km/hr to pass following a fresh snowfall prior to conducting a survey, to facilitate sufficient accumulation of pellets and other cottontail sign. Optimal survey conditions are when
snowpack is below 12 inches. While most detections will occur within the first 20 minutes, I do not recommend limiting search time, unless very broad-scale monitoring objectives make it appropriate. Surveys of large patches in particular should be allowed unlimited time to be completed if necessary. On patches with sympatric eastern cottontail occupancy, there may be additional challenges resulting from unknown population ratios of the two species and the potential for spatial segregation of home ranges within a patch. To address these challenges, I suggest collecting at least five pellet samples from distinct locations in the patch or subplot/sampling unit. Even without prior knowledge of cottontail activity, the models show that detection rates for surveys conducted following the above recommendations will be high enough to provide 95% confidence in occupancy determination in 2-4 surveys. These results provide valuable guidance for future monitoring for New England cottontail occupancy on regional and patch specific scales.

The results from the population study provide the first patch-specific population estimates for New England cottontails across their range. I found that the field methodology was effective at identifying and systematically sampling pellets from multiple individuals within a patch, and I identified effective minimum sampling distances between pellets needed to provide adequate data to estimate population size using single session mark-recapture models. I identified 30 meters as an adequate minimum sampling distance for most patches, but some small patches (<3 ha) may benefit from more exhaustive sampling.

Several small patches in Maine apparently only supported one or two individuals, while several sites in the Cape Elizabeth area (and Stonyfield in NH, and CFSP in NY)
appear to have healthier populations. Population estimates had relatively high precision for most of the small to medium sites, in addition to several large ones, suggesting effective sampling and genotyping conditions. I also identified potential changes for future surveys that could significantly improve estimate precision. Allowing too many days for pellets to accumulate may actually hinder survey efforts by decreasing both DNA quality (through increased degradation with extended environmental exposure) and detectability of pellets and tracks (through increased litter and snow melt out).

Both the detection and population estimation aspects of this study provide valuable information that can be used to develop more effective monitoring efforts for the conservation management of New England cottontails. These tools can be used to increase the efficiency of occupancy surveys as well as to track and manage existing populations on a regional and site specific scale. This study also provides methodology allowing, for the first time, for management decisions to be made based on cottontail population numbers on patches across their range. This study also found several previously occupied patches to be uninhabited suggesting continued decline in New England cottontail populations, emphasizing the need for continued conservation efforts.
Literature Cited


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Appendix A

Search Area
In addition to New England cottontail density survey search was not modeled directly for this study but that could affect detectability. We quantified search area as the effective area of the patch searched, using buffered search paths (representing a conservative estimate of pellet visibility as a function of stem density). Our intention originally was to use the estimated effective search area as another proxy for search effort. Similar to search time, however, we found that search effort varied considerably with observer. Further, termination of surveys upon pellet detection biased the dataset toward low search time and area (i.e., short search times and small search areas only occurred for surveys with successful detections), precluding the validity of accounting for search effort in our detection modeling.

Although we could not model search area directly, we used our estimates of effective search area and also conducted randomized subplot resampling to qualitatively evaluate the influence of surveying a reduced portion of the patch on detection. Sufficient data for this assessment were only available for five sites (all in ME) that were large enough with effective search area data for multiple subplots. For these sites, we systematically dropped one subplot from each site from the detection results and reassessed detection rates accordingly; we then did the same dropping two and three subplots. We found that raw detection rates for these sites dropped from 0.55 when all subplots were used to 0.43 when surveys were decreased by one subplot and 0.22 when surveys were decreased by two subplots. Conservative estimates of effective search areas for these sites indicated that although the area within the subplots comprised 19 – 28% of the area of the whole patch, the area of the patch effectively visible to the observer comprised only 28% of the subplot and only 5.5 - 8.0 % of the entire patch. These findings suggest that reducing the proportion of the patch surveyed below 20% will result in missed detections and thereby underestimate patch occupancy. These most severe consequences will be on large sites with low rabbit density (such as the Wells Reserve in Maine) and on sympatric sites (such as in southern New England), on which occupancy is highly heterogeneous.

We addressed these potential effects on survey protocol by evaluating the minimal number of samples required to detect New England cottontails on sympatric sites. This post hoc analysis was conducted using 14-28 samples collected during five survey visits on three NY sites occupied by New England cottontails in 2010. We used the proportion of New England to eastern cottontail pellets found in all pellet samples, per site, to calculate how many samples need be collected to be confident that at least one of them would originate from a New England cottontail. For these sites we determined that 3-5 pellets per site is sufficient to have a high probability (93-98%) of detecting New England cottontail if present. In 2011, to minimize the laboratory effort associated with the large number of samples collected, we did not conduct genetic analyses of all pellets (3-5) collected during each survey visit, unless it was necessary to do so to confirm detection success; rather we terminated analyses once a New England cottontail was
detected. On average we needed to analyze two pellet samples before finding a New England cottontail; however, the analysis of four or five pellets was required for confirmation on five different occasions, suggesting that collecting multiple samples from sympatric sites is warranted to determine New England cottontail occupancy with high certainty. Further, our results suggest that on sympatric sites, the proportion of the patch searched may have a critical impact on detection. Anecdotal evidence from six sites in Connecticut without New England cottontail detections suggests that in some cases, searching 20% of the patch may be insufficient in the absence of prior knowledge concerning potential differences in patch-specific space use by New England vs. eastern cottontails (Howard Kilpatrick, personal communication, 2011).

**Detection rates for full 36 site models**

We did not include six detection sites from CT in our original analysis because there were no detections on these sites for any of the detection visits and we could not confirm New England cottontail occupancy. The questionable occupancy for these sites meant they were not well suited for our model. Subsequent surveys have found New England cottontails on one of the six sites but not on two others; data are lacking for the remaining three sites. Given the uncertainty in occupancy, and our strong interest in potentially valuable data from sympatric sites, we also modeled a data set that included these six sites with the original 30 sites to see how their inclusion might affect the results. We found that overall, prior knowledge of cottontail activity, low snow depth, and increased deposition days without high winds were still the most important factors toward successful detection. Including the additional sites decreased the importance of each factor but the order of importance remained the same. There was, though, an overall decrease in detection rates (Table 2). Our general recommendations do not change when considering the additional six sites.

**Sympathy**

New England cottontails occur sympatrically with eastern cottontails on all the sites we surveyed in both New York and Connecticut. We originally intended to address the effect that sympathy has on New England cottontail detection, but sample sizes were insufficient to isolate sympathy as a covariate. Following our formal analysis we did incorporate site sympathy into our PRESENCE models to see, despite the small sample sizes, what effect it may have. When we incorporated the six additional sites from Connecticut they had a negative effect on detection. Based on this limited modeling and on simple probabilities of pellet deposition, it’s likely that co-occupancy with eastern cottontails will make New England cottontails more difficult to detect consistently. Or, at the least, it will increase the effort needed to establish their occupancy with confidence.
Table 1. Description of all sites surveyed for cottontail detectability during the winters of 2010 and 2011. * denotes a site not used in detection modeling due to insufficient survey or occupancy data and † denotes that the site was surveyed in both years—with relevant data are shown for the year used in detection modeling.

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Table 2. Detection rate predictions for scenarios modeled using the additional 6 sites from CT.

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Figure 1. Locations of 60 range-wide sites surveyed for New England cottontail detectability during the winters of 2010 and 2011. Sites labeled in blue were removed from our study because we were unable to confirm New England cottontail occupancy during the time we conducted our presence/absence surveys.
Table 1: Summary of samples for individual population sites. = Samples are the total samples collected at the site. = Processed are the samples selected from the subsampling process for genotyping. = Usable are all the processed samples that amplified cleanly enough to be used for individual IDs and population modeling. The 30, 50, and 75 meter sampling distance columns show how many samples were included in the abundance estimation for those subsampling distances. Samples collected but deposited by eastern cottontails are not included.

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* denotes sites where the #Samples is compiled from two different site visits.
† denotes sites where samples were processed even when collected below the 30-meter sampling distance.
Table 2. PI and $\text{PI}_{\text{Sib}}$ output from DROPOUT

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c. Coast

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Figure 1. Locations of individual New England cottontail pellets surveyed on 17 sites and genotyped for population estimation. The yellow line denotes the patch boundary. Triangles indicate male cottontails and circles females. Colors designate distinct individuals on each patch. The assignment of individuals is based on the minimum estimate for all sites. White symbols are each their own individual but were each only captured one time.

a)
d.

Frieze

Meters

0 15 30 60 90

N E S W
m.