Detecting New England Cottontails During Winter Pellet Surveys
Executive Summary of a Report by
Adrienne Kovach & Daniel Brubaker

We conducted a systematic investigation of New England cottontail detection rates to evaluate the effectiveness of presence/absence winter survey protocols and determine the influence of survey conditions on cottontail detectability. We surveyed 50 range-wide sites of known New England cottontail occupancy in two to six visits in the winters of 2010 and 2011. Thirty sites yielded detections during at least one survey visit, facilitating their use in detection models. We modeled detection probabilities in the program PRESENCE to explore the influence of a reduced set of six covariates (determined by preliminary statistical analyses) on NEC detection: prior knowledge of cottontail activity, snow depth, patch size, average patch-specific stem density, and pellet deposition days (as measured by the number of days since snowfall with wind <40 kl/hr or with temperature >-10ºC). We also evaluated effects of search effort, by modeling detections that occurred during a threshold search time of 20 minutes and by exploring the effects of reduced subplot sampling on detection rates. Across the 30 sites with known occupancy, the probability of detecting a New England cottontail during a single visit was 0.73. Prior knowledge of New England cottontail activity was the single most influential factor, with a strong positive effect on detection. Snow depth <12 inches and increasing pellet deposit days also increased detection. High winds appeared to be more limiting for cottontail activity than low temperature, and stem density and snow condition (powder or other) had no statistically significant effects on detection. Eighty-two percent of detections occurred within the first 20 minutes of a survey. Limiting surveys to a 20-minute search threshold reduced the naïve detection rate to 0.60. The reduction in detection for the time-limited surveys was most severe for large patches. For large patches that were surveyed with multiple subplots, we found that reducing the number of subplots substantially reduced detection. The consequence of reduced search area is most severe on large sites with low cottontail densities and/or sympatric occurrence of eastern cottontails. Overall, we found that detection probabilities are relatively high (72-90%) when surveys are conducted in optimal or near optimal conditions: snowpack <12" and with one to three pellet deposition days. Under these conditions, two to three surveys should yield reliable presence/absence data with 95% confidence in detection. We provide the following procedural recommendations for optimal detection and high confidence determination of patch-specific occupancy status:

- Conduct surveys with a snowpack <12" and within two to four days (without high winds) following a snowfall event.
- Survey the patch systematically and intensively, following loose continuous transects with approximately 30-m spacing, focusing on suitable habitat.
- To verify occupancy by genetic species identification, collect pellet samples from three to five distinct locations within the patch or each subplot (five is advised for sympatric sites).
- Allow an unlimited search time for surveys, bearing in mind that the benefit of extended search time is minimal beyond 40 minutes, but may be warranted in certain contexts.
- For small to moderate sized patches (up to 6-10 acres), survey the entire patch. For larger patches, survey multiple 2-acre subplots totaling at least 20% of the total patch area.
- Conduct two or three independent survey visits after separate snowfall events, if optimal snow depth and deposit time can be achieved. If snow pack exceeds 12", allow at least three days for pellet deposition and conduct additional surveys (four total). Avoid surveying in deep snow with fewer than three pellet deposition days.
- For large, low-density patches and/or patches with sympatric occurrence of eastern cottontails, consider increasing the proportion of the patch searched >20%, increasing search time, and collecting pellets from five distinct locations in each subplot.

Detecting New England Cottontails During Winter Pellet Surveys: An Evaluation of Current Practices and Recommendations for an Improved Monitoring Protocol for Maximal Detection of Occupancy

A Product of the RCN Project, titled "Development of Noninvasive Monitoring Tools for New England Cottontail Populations: Implications for Tracking Early Successional Ecosystem Health"

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Objective

The overarching goal of this project was to develop new monitoring tools for measuring the effectiveness of conservation actions for the New England cottontail (NEC), a Species of Greatest Conservation Need in the Northeast and a candidate for federal listing under the Endangered Species Act. In this report, we focus on the objective of occupancy monitoring via winter pellet surveys. Specifically, our objective was to

- ❑ *Evaluate the effectiveness of the current presence/absence winter survey monitoring protocol through a systematic investigation of detection rates; use this knowledge to develop an improved protocol for maximal detection of NEC on occupied patches.*

Problem Statement and Background

Once widespread throughout the New England states and eastern New York, the New England cottontail (NEC) has suffered severe population decline in recent decades, due at least in part to loss and fragmentation of the early successional (thicket) habitats on which it depends. Remaining 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). In light of this significant range contraction and the uncertainty for long-term viability of the NEC, the species is under review as a candidate for federal listing under the Endangered Species Act and has generated much interest among local state and federal agencies for conservation and management efforts. Critical to the success of these efforts will be the development of an adaptive management program that utilizes appropriate biological monitoring tools.

In the case of rare and threatened species, such as the New England cottontail, population monitoring is a challenge, and is best accomplished by noninvasive, genetic methods. Current monitoring efforts for New England cottontails rely exclusively on genetic analysis of fecal pellets collected noninvasively during winter surveys. A diagnostic mitochondrial DNA (mtDNA) test is used for species identification (Litvaitis and Litvaitis 1996, Kovach et al. 2003), as the New England cottontail is sympatric with either eastern cottontail (*Sylvilagus floridanus*, hereafter EC) or snowshoe hare (*Lepus americanus,* hereafter SSH) throughout its range, and the pellets of the different species are not distinguishable in the field, although in fresh snow conditions the tracks of NEC can typically be distinguished from those of SSH. The result of these efforts is a determination of the occupancy status (presence/absence) of each surveyed site, typically based on a single site visit. While this method has proven very effective in broad-scale survey efforts, such as the recent range-wide survey assessing the distributional status of the NEC (Litvaitis et al. 2006), it may suffer from shortcomings on a local/patch-specific level, where finer scale data are needed.

A critical issue with presence/absence-based surveys is the problem of detectability. That is, nondetection does not imply that a species is absent, unless the probability of detection is 1 (MacKenzie et al. 2002). If detection probabilities are <1 and vary with site characteristics, time or environmental variables, then a seemingly unoccupied patch may not be truly vacant, but may appear so due to poor detection, i.e. a "false absence". Therefore, it may be misleading to draw conclusions about a monitoring index without accounting for detectability. To this end, relatively simple models have been developed by MacKenzie et al. (2002) to estimate site occupancy rates when detection probabilities are <1. These occupancy models enable the identification of covariates that are the most influential in detection. With this knowledge, patch occupancy can be more accurately assessed and guidelines can be developed for conducting monitoring under ideal conditions for maximal detection.

Concerns about detection are particularly relevant to monitoring efforts for NEC, as surveys are conducted in the wintertime, when environmental conditions can be highly variable from day to day. Furthermore, deep snow and/or cold temperatures may force rabbits into subnivean behavior, precluding their detection for potentially long periods of time during the winter. These concerns are serious in light of evidence of continued population decline (Fenderson 2010, Kovach and Fenderson 2010, Fenderson et al. 2011) and the rapid pace of restoration efforts. Monitoring for performance and adaptive management will require protocols that incorporate knowledge of detection rates. We addressed this concern through a range-wide, systematic study of detection. Using the results of the detection study, we developed a standardized protocol dictating the number and circumstances of site visits needed to accurately determine patch occupancy.

Detection Study

Approach

Study Sites: To investigate detection rates and their influence on ascertaining patch occupancy for NEC, we conducted a series of systematic, repeated surveys on carefully selected sites. Our goal was to target 50 sites throughout the species' range, encompassing a range of sizes. Because we were not interested in estimating occupancy, but rather in determining the factors that influence detection on occupied sites, we focused on sites of known or highly probable occupancy; a few sites of unknown occupancy were also included. 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 and Massachusetts.

In total, 60 sites, ranging in size from 2 – 26 ha, were surveyed range-wide for this effort in either 2010 or 2011. Due to poor survey conditions in 2010 (infrequent snowfall), sufficient surveys were not obtained for most sites and they were resurveyed the following year. For sites that were surveyed in both years, we used the data from the year with the greater number of surveys, which was usually 2011 (see Appendix 1 for a summary of all survey efforts in the 2-year study). For five sites, the 2010 data were retained because resurvey efforts were unsuccessful in 2011, due to either logistical reasons or changes in occupancy. Sites that did not yield detections during the surveys were excluded from detection modeling, unless occupancy could be verified by other means (e.g. pellets found during non-detection surveys of the site). In Maine, 26 sites were surveyed within Cape Elizabeth (which includes over 300 acres of nearly contiguous habitat within Crescent

Beach, Kettle Cove and Two Lights State Parks and adjacent private lands), Wells (located at the Wells National Estuarine Research Reserve and Rachel Carson lands), and southern York County (south of Rte 91, along the coast and I-95 corridor and in the Berwicks). Ten of these sites yielded no detections in either year. Given the typically small size and isolation of eight of these sites, they were presumed to be no longer occupied and therefore not be used in the model; for two other sites, occupancy was confirmed during additional surveys outside of the detection window, enabling us to use these sites in the model. Two additional sites yielded detections in 2010 (with two or three visits), but no detections in 2011 (with five visits), suggesting they were no longer occupied. For those sites, 2010 data were used in the model. In New Hampshire, eight sites were surveyed in the Durham-Dover area and one site in the Merrimack River Valley (Londonderry). Six of the nine NH sites yielded sufficient data for detection modeling. Ten sites were surveyed in Connecticut (fie in western and five in eastern CT). Six of these sites yielded no detections across four visits; detection modeling was run both with and without these six sites, given the strong prior evidence of occupancy. In New York, ten sites were surveyed in Putnam, Duchess and Columbia Counties. Six of these sites yielded no detections across four or five visits; given uncertainty about occupancy status, these sites were not included in the detection models. Five sites were surveyed in Cape Cod, Massachusetts. Due to logistical constraints and poor survey conditions, none of the MA sites yielded sufficient data for inclusion in the occupancy models. In total, 30 sites yielded sufficient surveys and occupancy data to warrant inclusion in the detection models (see Figure 1).

Surveys: The survey protocol used in this study was similar to that of Litvaitis et al. (2002, 2006), but with more systematic search strategies within a patch. Survey sites were delimited by patches of continuous suitable habitat that a cottontail may utilize without venturing into a risky open area (>30 feet wide), such as highly unsuitable vegetation, a high traffic road, or a water body. Sites were surveyed in the wintertime, with snow on the ground, and at least 12-24 hours after a snowfall event. Patches were surveyed systematically using loose, continuous transects, winding back and forth across the patch with approximately 30-meter spacing (see Appendix 2, Figure A2-1). Search paths were delineated within the patch boundaries using the "track" feature on a GPS unit or drawn approximately on a map of the site. Searches focused on suitable habitat within a patch and continued until either a cluster of pellets was found (or 3-5 pellet clusters in the case of sites with sympatric NEC and EC) or until all suitable habitat had been exhaustively searched. For patches ≤6 acres, the search area included the entire patch. For patches >6 acres, the search area was restricted to 2-acre subplots within the patch (see Appendix 2, Figure A2-2). 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 (Appendix 2).

Sites were visited two to six times. As 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), we aimed for five visits per site, whenever logistically feasible, with a minimum of three (MacKenzie and Royle 2005). Of the 36 sites that entered the detection models (including the six CT sites of questionable occupancy), 32 sites had four or five visits, two sites had six visits and one site each with three and two visits were also used. 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). Given irregular snowfall and different snowfall patterns across the study area, it proved logistically challenging to restrict all searches to the same window of time across all of the geographically dispersed study sites. Surveys occurred between December 23 and March 25 across all sites. The average survey window across all sites was 43 days (range: 22 – 70). Samples sizes were insufficient to separate surveys into different time periods and we did not attempt to address seasonality.

When pellets were found, their collection was restricted to an area approximately 5 x 5 ft or smaller to minimize the chance of obtaining samples from >1 rabbit per vial. For small patches known to contain only NEC (e.g., some sites in Maine and New Hampshire), only one vial of pellets was collected per patch. For larger patches, or any site where NEC may share habitat with other lagomorph species (EC or SSH), a total of 3-5 vials of pellets were collected per site, from 3-5 distinct locations within the patch, separated by at least 100 m, or farther, depending on patch size (e.g., from 3-5 disjunct subplots of a large patch). The location of each pellet collection was recorded using a GPS.

Covariates: To explore potential sources of variation in detection probability, the following additional data were recorded by the surveyor for each site and survey visit: observer, patch size, search time, snow conditions (no snow, powder, wet snow, crusted snow, melted out), snow depth (none, trace-6", 6-12", 12-24", 24-36", >36), days since last snow fall, whether high winds or rain had occurred over the last three days, current temperature, temperature range over the past three days, and patch suitability based on habitat features. Due to subjectivity associated with the latter parameter, we replaced it with average stem density. We estimated patch stem density by averaging counts of all woody stems at a height of half a meter obtained for up to 30 evenly spaced 1x2 meter plots per patch. Following site visits, we calculated the area searched during each survey by buffering a fixed distance (3 or 5 meters depending on high or low stem density, respectively) from the search path. A predation index was also scored for each site (1 = no sign of predation, 2 = some sign of predation evident, e.g. tracks, 3 = heavy predation evident, e.g. many tracks or an actual predator sighting). Finally, we identified if the surveyor had prior knowledge of cottontails for each visit. We considered prior knowledge as known location of pellets or rabbit sign from a previous visit that field season or from information provided by the landowner.

For detection modeling, some covariates were classified into too many categories for meaningful statistical inference, given the sample of survey visits and sites used. These covariates were either reclassified into fewer categories or dropped from the analysis if meaningful categories could not be identified. Snow condition and snow depth covariate categories were reduced to powder/no powder and depth < or > 12". Predation index was not used, due to difficulty identifying predator activity and lack of surveys without predation. Observer could also not be used, due to the large number of individuals who conducted surveys relative to the number of sites surveyed and lack of a meaningful way to categorize different observers. We were also unable to model a meaningful proxy for search effort, as neither search time nor search area was deemed suitable. Given that searches always concluded once pellets were detected, surveys were not conducted in a manner that allowed for modeling the influence of search area or time. In addition, search time was highly influenced by patch size, observer, stem density, and rabbit density, and therefore could not be isolated as a unique, meaningful factor in detection. Search time was used, however, to explore the effects of a maximum threshold search time on detection, by regressing the cumulative number of successful surveys (during which NEC were detected) over increasing search time. For the weather covariates, in the analysis we used data collected from a single weather data set (http://www.weather.com/), instead of the data collected during surveys for increased consistency. These parameters included average daily low temperature and daily high wind speed since snow fall, the number of days since snow fall when temperature remained above -15 and -10 °C, respectively, and the number of days since snow fall during which high winds did not exceed 40 kl/h. Temperature and wind speed thresholds were based on expert opinion.

Variable reduction: Initially, our full set of covariates included 13 factors suspected of influencing cottontail detection, which we reduced using preliminary statistical testing before further analysis (see Table 1 for complete list of covariates). Several of the original covariates included slight variations of the same factor (e.g., multiple measures of pellet deposition days were tested as iterations of days since snowfall with or without accounting for influence of temperature or wind) or 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). We used nominal logistic regression to select the most influential factor from each of these correlated sets. We then used partition modeling to identify uninformative factors and removed them from further analysis. We performed a final simple linear regression on the remaining factors, retaining those with significant effect likelihood scores (Table 1). Based on this analysis, snow condition was not found to be an informative parameter in detection and therefore removed from further analysis. Patch size and stem density were retained despite non-significant effect likelihood scores because their effects on detection were of particular interest. Days since snowfall with temperature >10°C was also included in the modeling because we were interested in potential effects of temperature on detection.

Detection modeling: We evaluated the effect of survey conditions on NEC detection on the 30 sites of known occupancy by modeling detection probabilities as logit functions of the six, selected covariates in the program PRESENCE 2.0 (Hines 2006). 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 2006). Candidate models were ranked according to Akaike's information criteria corrected for small sample size (*AICc*) and model weights calculated (Burnham and Anderson 2002). Models with the lowest *AICc* were considered the most parsimonious. 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). Detection probability was also modeled including the six CT sites of questionable occupancy (36 sites total); in this analysis occupancy was not fixed at one. To explore the effects of a threshold search time, we also modeled detection probabilities on the 30 known sites 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. 2003, 2006) and because we found that 82% of detections in this study occurred within this time period (see Figure 2).

Results & Interpretation

Occupancy

Of the 50 sites for which sufficient survey and covariate data were collected range-wide, 20 yielded no detections in a minimum of three visits, including eight sites in ME, one in NH, five in NY and six in CT. An additional three sites (two in ME and one in NY) yielded detections in 2010, but not in 2011. A lack of detection on these sites may be due to changes in occupancy status, insufficient prior knowledge of occupancy, or an artifact of the sampling protocol.

The ten sites without detections in ME and NH were of small size (<5 ha) and in relative isolation. They were last known to be occupied in 2007 – 2009 and likely only supported one or two rabbits at that time. Given the severe fragmentation of patches in this region and their low cottontail densities (Fenderson 2010, A. Kovach unpublished data), these sites are likely no longer occupied. Changes in cottontail occupancy are not unexpected, given the ephemeral nature of early successional habitat. Nonetheless, these findings warrant consideration, given that site selection was based on a high level of knowledge of the last occupied sites in this region. These changes in occupancy therefore are of conservation significance as they are representative of the continued, ongoing decline of New England cottontails in Maine and New Hampshire (Fenderson 2010, Kovach and Fenderson 2010, Weidman and Litvaitis 2011).

In NY, the site with NEC detection in 2010 but not 2011, Indian Brook Rd, was a small (<2 ha) site; similar to the ME/NH sites described above, it may have experienced a change in occupancy. For the other five NY sites without detection, prior knowledge of occupancy was not strong, as it was based on surveys that occurred during the 2000-2004 range-wide inventory of Litvaitis et al. (2006). Since that time, occupancy status has changed and NEC populations have declined in portions of the species' range (Kovach and Fenderson 2010); therefore, these historically occupied sites may have been vacant at the time of our study. Alternately, New England cottontail may have been present but gone undetected due to a high density of sympatric eastern cottontails on these sites. For two sites, intensive population surveys conducted across the entire patch, in addition to the detection surveys, yielded no indication of occupancy by NEC. In absence of additional data, the most likely explanation is that these sites are currently unoccupied by NEC.

Lack of detection on the six CT sites was somewhat unexpected, given very recent confirmation of their occupancy (H. Kilpatrick, unpublished data). The logistics of subplot subsampling may have influenced the outcome of surveys on these large sites that are co-occupied by eastern cottontails. Results of a radiotelemetry study suggest that the home ranges of the two species of cottontail overlap, but that core areas are spatially segregated on these patches, and that the subplots surveyed in this study were primarily utilized by eastern cottontails (H. Kilpatrick personal communication). Intensive sampling throughout the patch was conducted, however, for one of these six sites, Bluff Point West, during which New England cottontail were also not detected, suggesting a lack of occupancy or potentially low abundance.

Important Factors Influencing Detection – Results of Detection Modeling
Detection modeling for the 30 sites with known occupancy showed a naïve detection probability of 0.73 (the average probability of detecting a cottontail on an occupied site in a single survey visit) and indicated that prior knowledge, snow depth and days since last snowfall with wind <40 mph (DaysWind) were the most influential factors influencing detection probabilities for New England cottontails. These three factors comprised the most parsimonious model. All additional models with $\Delta AICc$ <2 contained these three variables and one or two additional variables. Following Arnold (2010), these hierarchically more complex (nested) models were not considered competitive and the additional variables in these models were interpreted as having poor explanatory power (uninformative parameters); the weights of these nested models were combined into the weights of the more parsimonious model. All models in the 95% confidence set contained knowledge and snow depth. A model consisting of knowledge, snow depth and days since snowfall with temperature >-10°C (DaysTemp) was also in the 95% confidence set with $\Delta AICc$ <4, however, its similar rank by *AICc* score and weight to the next highest ranking model consisting of only knowledge and snow depth gave further support for the greater importance of these two parameters and indicated that DaysTemp was not an informative parameter. The low *AICc* weights of these two models (0.056 and 0.55) suggested they were not competitive relative to the top model that also contained DaysWind. The remaining models in the 95% confidence set were hierarchically complex versions of these two models with very low *AICc* weights and therefore noncompetitive (see Table 2 for the full 95% set of candidate models). Similar to DaysTemp, stem density and patch size only appeared in nested models and therefore were deemed as uninformative parameters with respect to detection probability of NEC.

Similar results were obtained when we included the six sites from CT with unknown but probable occupancy and no detections. In this analysis, however, the top model contained only knowledge and snow depth and all other parameters only appeared in nested models of these two variables (not shown). Not surprisingly, the inclusion of these sites increased the importance of knowledge in detection probability. Given uncertainty in the occupancy of the surveyed portions of these patches, we suggest it is more appropriate to draw inference from the smaller set of 30 sites with known occupancy, including DaysWind as an informative parameter influencing cottontail detection. The naïve detection rate dropped to 0.62 for this analysis, due to the addition of six sites without detections.

Influence of Search Time

Regression of NEC detections by survey search time showed that the benefit of increasing search time decreases significantly 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. These results are consistent with previous findings, which led to the adoption of a 20-minute survey protocol by Litvaitis et al. (2006). Our results indicate that for a broad-scale survey of occupancy, a 20-minute survey period may be of practical value and provide reasonably accurate course-grain presence/absence results. The missed detections, however, reduce confidence in occupancy results for time-restricted searches relative to those with unlimited search time; in our study this was manifest in a reduction of the naïve detection rate from 0.73 to 0.60. For finer-scale, e.g. patch-specific knowledge of occupancy, where a higher degree of certainty is required, longer (potentially unrestricted) search times are warranted. Patch size and cottontail density likely both have a strong influence on the importance of search time. While evaluating effects of cottontail density was beyond the scope of our study, our modeling results confirmed the importance of patch size on detection probability for searches of ≤ 20 minutes duration. For the 20-minute search threshold model set, the two top-ranking models (*ΔAICc<2* and 85% of model weights*)* included knowledge, patch size and DaysWind and knowledge and DaysWind, respectively (Table 3). Snow depth and DaysTemp also occurred in lower ranking models with summed weights of only 11% and 5%, respectively. These results combined with those of our full model set suggest that prior knowledge and DaysWind are overall the two most influential factors influencing cottontail detection in winter surveys. Given unrestricted search time, snow depth has additional, significant impact on detection, while, given a 20- minute search limit, patch size has a greater effect.

Influence of Survey Conditions on Cottontail Detection & Inferences for Optimal Surveys

Prior knowledge of location of cottontail activity and snow <12 inches had a strong positive influence on detection (Table 4). Prior knowledge of NEC activity increased detection substantially by providing the observer with known areas to focus the search within the patch, and on several occasions even the location of NEC burrows. Observers were more likely to have prior knowledge on sites with high rabbit densities (e.g. sites located in Cape Elizabeth) or on small sites where the activity patterns of a single rabbit could be easily observed. Another potential influence of prior knowledge is the effect it might have on the search behavior of observers: we noticed a tendency for some observers to search more intently and more exhaustively on sites where they expected to find NEC relative to sites where they had no prior detections nor anecdotal knowledge of NEC activity.

Detection rates also increased when snowpack was below 12 inches, a finding that fit our expectations for a number of reasons. Most significantly, reduced snowpack provides easier travel for both NEC and observers. Ease of travel increases NEC activity, thereby providing additional sign for detection (both tracks and pellets). Ease of travel for observers means they are able to cover a greater search area in a given time period and may thereby increase the thoroughness of their search effort. Deep snow may decrease detectible NEC activity by promoting subnivean travel and foraging, both of which are hard for observers to detect. Finally, low snowpack is more likely to occur during times of the year with advantageous weather for NEC (generally early and late winter with mild temperature and reduced wind), allowing them to be more active. In summary, both prior knowledge and low snow depth have a direct positive impact on detection, but both are also positively correlated with other factors that promote NEC detection.

Detection probability also increased slightly with days since snowfall with wind <40 kl/hr, which was a proxy for days available for pellet deposition. Although less important than the preceding factors, deposition days are clearly important and likely more so on a small site that supports only one or two individuals, although sufficient sample sizes were lacking to test that assumption. Limitations exist for the positive effect of increasing deposition days, however. As time passes and weather conditions change, pellets may become harder to detect, for example if they melt into the snow, or as debris accumulates and both pellet and track visibility decreases. Further, with increasing exposure to environmental conditions, pellet DNA degradation can occur (Kovach et al. 2003), precluding genetic confirmation of species of origin for pellets collected under these circumstances. Our results suggest that on average three days (range: 2 –4) provide an optimal time period for pellet deposition. If other conditions for detection are poor, then we suggest allowing for the longer pellet deposit time. We found, however, that waiting much longer than four days did not improve detection, but rather tipped the balance toward the negative effects of reduced DNA quality and decreased visibility of sign.

Patch size had a slight negative effect on detection, which was only statistically significant for the 20-minute restricted search efforts (Table 5). Patch size likely has confounding effects on detection. It can decrease detection probabilities due to the greater effort required to search large patches. Additionally, the subsampling scheme that we used may have focused some surveys into unoccupied portions of the patch. These negative effects of increasing patch size are likely influential on sites with low rabbit density and may be more pronounced on sympatric sites. On large, low-density patches, longer search times may be desirable for optimal detection. In contrast, large patches may support higher cottontail densities, such as on several patches in Cape Elizabeth, Maine, for which detections were achieved on every survey visit, typically with minimal effort. Similar to patch size, stem density had confounding effects on detection, likely resulting in its nonsignificant, but slightly positive, influence. The confounding effects of stem density are likely due to the variability in cottontail densities in relation to stem density. Higher stem density can lower detection probability by reducing visibility of pellets and increasing search effort. In contrast, higher stem density sites may support higher densities of cottontails, thereby increasing their likelihood of detection.

DaysTemp also had a slightly positive, albeit not significant effect on detection. This factor was based on low daily temperatures, which generally occur at night, but often change significantly during the day. Therefore, even when night temperatures are well below the -10°C threshold, effective daytime temperatures in the sun, particularly on calm days, may be moderate enough to allow NEC activity. We observed that locations with southern exposures had higher activity during particularly cold weather. Lastly, although extremely cold temperature likely does have a negative influence on cottontail behavior and detection, its effect may not have been statistically significant in our analyses due to multicolinearity of the DaysTemp covariate with DaysWind (R^2= 0.31), *i.e.* DaysTemp did not provide enough additional variation to be a significant factor once we accounted for DaysWind.

Influence of Search Area
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 consequences will be most severe on large sites with low rabbit density (such as the Wells Reserve) and on sympatric sites (such as in CT), on which occupancy is highly heterogeneous.

Influence of Sympatry with Eastern Cottontails
New England cottontails occur sympatrically with eastern cottontails on all the sites we surveyed in both NY and CT. We originally intended to address the effect that sympatry has on NEC detection, but due to low detection on many sympatric sites, sample sizes were insufficient to isolate sympatry as a covariate for detection modeling. We addressed effects of sympatry on the 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 NEC in 2010. We used the proportion of NEC to EC 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 NEC. 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 NEC were detected. On average we needed to analyze two pellet samples before finding NEC; 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 NEC 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 CT without NEC detections suggests that in some cases, searching 20% of the patch may be insufficient in the absence of prior knowledge of patch-specific space use by New England vs. eastern cottontails.

Survey Scenario Predictions – Detection Probabilities & Number of Required Visits
The top detection model for the overall data set showed that prior knowledge, < 12 inches of snow, and increased days since snowfall without high wind have significant positive effects on NEC detection. As a general rule, our model suggests that detection rates increase dramatically if surveys are conducted with either prior knowledge or low snow depth. Prior knowledge alone, irrespective of snow depth, and even with a single deposit day following a snow, yields a detection probability of at least 0.85, while under the most optimal survey conditions (prior knowledge, snow <12" and 3 deposition days) a 0.99 detection rate is obtainable (Table 6). We recognize that these optimal conditions will be rare and particularly prior knowledge of NEC activity will not occur in the context of most monitoring efforts. Surveys conducted in the absence of prior knowledge, with snow depth <12 inches, and after one deposit day yield a detection rate of 0.72, while allowing three deposit days raises the detection rate to 0.90. Poorest detection occurs for surveys without prior knowledge and with snow depth >12", resulting in detection rates from 0.22-0.49, with one and three pellet deposition days, respectively. Predicted detection probabilities and number of surveys needed to obtain 95% certainty of detection are provided for a number of scenarios in Table 6.

Effectively, our results indicate that, if surveys are conducted with prior knowledge and/or snow depth <12 inches, two to three surveys are needed for NEC to be detected with 95% certainty if they are occupying a particular patch (Table 6). Even without knowledge, given ideal snow depth and three pellet deposition days, two surveys may be sufficient for confident detection. Although model results would suggest theoretically that simply increasing the number of deposit days should continually raise the detection rate, we caution that this is not a realist scenario. Rather, as noted above, negative factors that could not be modeled, such as DNA degradation, and decreased visibility caused by snow melt out and accumulation of snow surface debris will negate the added benefit of additional deposit days beyond three or four days, and may even result in an overall detriment for longer deposition time. This is an important consideration for sympatric sites, where quality DNA is critical for successful genetic species determination. Additionally, our model results show that surveying in deep snow without prior knowledge may have limited utility, as it will generally result in poor detection and will require several visits. If, under these suboptimal conditions, three days are allowed for pellet accumulation, detection rates can increase from 0.22 (with one deposit day) to 0.49. A detection rate of 0.49 would yield the 95% confidence of detection with four visits.

Detection models for the 20-minute search threshold dataset showed that prior knowledge and DaysWind again had a strong positive influence on detection, while increasing patch size reduced detection. We used the top model to generate predictions for a number of survey scenarios given presence or absence of prior knowledge, one or three deposition days and two extremes of patch size (3 and 25 ha; see Table 7). On small sites (3 ha) with prior knowledge and an optimal number of deposit days (3 days), a detection rate of 0.87 was predicted. Performing the same survey on a large patch (25 ha) reduced detection to 0.68, while removing prior knowledge dropped rates to 0.62 and 0.33, for small and large patches, respectively. Detection rates are even lower with less than three deposition days (Table 7). These results suggest that the reduction in naïve detection rate incurred from limiting the search time has a significant impact on survey logistics by increasing the number of visits required for confident detection to a minimum of three visits in the absence of prior knowledge of NEC activity. The negative effects of patch size are more pronounced in limited time surveys, further reducing detection probabilities and increasing the number of visits required for confident detection on large patches (a minimum of four visits in the absence of knowledge).

Finally, we note that these are theoretical predictions and caution against their strict interpretation. Particularly, although we found a 99% detection rate with optimal survey conditions (including prior knowledge of NEC activity), we would not recommend placing such high confidence in a single survey visit and suggest that a second visit may be a useful precaution even in the best of survey conditions. Further, the effect of increased deposition days may not be in reality as strong as suggested by model predictions. For example, in the absence of knowledge with snow <12", the actual increase in detection probabilities for three vs. one deposition day may not in effect be sufficient to warrant confidence in two rather than three surveys.

Summary & Conclusion

This study yields important insight into several aspects of range-wide New England cottontail occupancy and detection, which can inform future monitoring efforts integral to the range-wide conservation initiative. We identified three easily measured factors that have significant effects on NEC detection and found that when surveys are conducted in conditions that maximize these factors, NEC can be detected with high confidence (95%) in one to three survey visits. Prior knowledge of the location of NEC activity has an overwhelmingly strong positive influence on detection probability, and provides the only context in which one survey visit may be sufficient to yield confident presence/absence determination. Given that prior knowledge will rarely be available for most monitoring efforts, snow depth is the single most controllable factor influencing NEC detection during winter surveys. Snow pack less than 12 inches has a strong positive influence on detection rates, and whenever possible surveys should be conducted under these conditions for optimal success and confidence in occupancy status, as well as for logistical efficiency. The number of days since snowfall without high winds (over 40kl/h) also increases detection by providing more time for pellet deposition, but this benefit is only realized up to about four days, beyond which negative effects of reduced pellet and track visibility and DNA quality likely outweigh any added benefit of increased deposition time. Patch size is also influential in cottontail surveys; it has a slight overall negative effect on detection, which is more pronounced when occupancy is unknown, and a substantial negative effect when the search time is limited to 20 minutes. The lack of a stronger effect of patch size is likely due to the confounding effects of size and rabbit density: detection may be high on large patches that support high densities of NEC, but reduced for low density patches. Large patches with low rabbit densities present the most challenging conditions for NEC detection and may require searching a greater percentage of the patch or a larger number of survey visits. Average stem density and snow conditions (powder *vs*. other) do not have a significant, predictable effect on NEC detection. Cold temperature is not as important as high wind in decreasing detectability through a reduction in cottontail activity.

Overall, our results suggest that detection probabilities are relatively high (72-90%) when surveys are conducted in optimal or close to optimal conditions: snowpack <12" and with one to three pellet deposition days, even in absence of prior knowledge. Under these circumstances, a single survey visit may yield occupancy data of sufficient confidence (if 90% is acceptable) on a broad scale. If greater certainty is required for broad-scale inventories (95%), two survey visits are warranted. For finer scale objectives, such as determining patch-specific occupancy or monitoring site-specific effects of management actions for performance evaluation, a higher number of survey visits will be required to obtain results with high certainty. In the latter case, two to three surveys conducted in optimal or near optimal conditions should yield presence/absence data with at least 95% confidence in detectability. Lastly, the lack of detections in this study on 20 of 50 sites, for which at least four survey visits were conducted, provides additional evidence that NEC occupancy continues to decline, particularly on small isolated sites located in fragmented habitat. We recommend therefore that future monitoring efforts focus not only on evaluating population responses to newly restored and managed habitats, but also continue to monitor and actively manage known remnant patches of NEC.

Recommendations for an Improved Monitoring Protocol

The survey protocol employed in this detection study was largely successful in yielding relatively high single-visit detection rates. Based on the results of our detailed evaluation of the factors that influence New England cottontail detection, we provide specific procedural recommendations for the future conduction of winter surveys to yield optimal detection and high confidence (95%) determination of occupancy status.

Survey conditions: Surveys should be conducted with a snowpack less than 12 inches and within two to four days following a snowfall event. If high winds and extremely low temperatures have occurred since snowfall, allow additional days for deposition of pellets and other rabbit sign, up to a maximum of four days since snowfall with winds <40 kl/hr.

Search path: Patches should be searched systematically and intensively, following loose, continuous transects that wind back and forth across the patch with approximately 30-meter spacing, focusing on suitable habitat. This method is easy to perform, even by novice surveyors, requires minimal logistical preparation, and is systematic while still allowing flexibility to adapt to patch-specific habitat characteristics or to follow cottontail tracks and other sign. A handheld GPS can be used to facilitate execution of the survey transect and, while not necessary, may prove helpful on moderate to large-sized patches.

How to verify occupancy: At this time, the only reliable way to confidently verify occupancy status is to perform genetic species identification from fecal pellets collected during winter surveys. While tracks remain useful for distinguishing New England cottontails from snowshoe hare in portions of the range unoccupied by eastern cottontail (primarily Maine), the confidence from DNA testing will always be higher, especially given potential complications with track identification from snow melt out or other suboptimal tracking conditions. For small to moderate patches, we recommend collecting pellet samples from three distinct locations in a patch; if eastern cottontails are known to occur sympatrically, and on large patches, we suggest collecting five distinct pellet samples.

How long to search: In general, we recommend allowing an unlimited search time for surveys, as long as the surveyor is progressing at a reasonable pace through the patch. Allowing unlimited search time accounts for differences in observer search strategies, which cannot otherwise be controlled for, including walking speed and observational skill. Importantly, it also substantially increases detection on large patches. For broad-scale monitoring purposes, a 20-minute search limit may be feasible for most observers and will likely result in detection of >80% of occupancies; whether this reduced detection rate is acceptable will depend on the context of the monitoring application. The cost of missed detections and decreased certainty in occupancy status with a restricted search time likely cannot be afforded in finer scale applications and certainly not in the context of determining high confidence patch-specific occupancy. The benefit of extended search time is minimal beyond 40 minutes, and in most contexts, except the largest patches, this could be considered a realistic upper limit to search time. For large search areas and highly heterogeneous patches, searching beyond 40 minutes may be warranted.

How much to search: For fine-scale monitoring applications, we assume that the patch is the sampling unit. For small to moderate sized patches (≤6 acres, although potentially up to 10 acres), we recommend searching the entire patch, using the above described loose transect approach. For larger patches, subsampling is necessary. We found that 2-acre subplots were of a viable size that provided ease of relatively rapid and efficient search, while covering enough area and potentially diverse habitat to serve as representative subsamples of the patch. Given the decrease in detection associated with a reduction in effective search area, we cannot recommend use of smaller than 2-acre subplots. We also recommend the use of multiple subplots placed in areas of suitable habitat equivalent in total area to at least 20% of the total patch area. Searching less than 20% of the patch results in missed detections, with greatest consequence for sites with low rabbit densities and sympatric eastern cottontail occurrence. For sympatric sites, it may be warranted to search an area greater than 20% of the patch. We also do not recommend random placement of sampling units, but rather suggest careful placement in areas of suitable habitat.

How many visits: Given that surveys are conducted with snowpack <12 inches, two or three visits, after separate snowfall events, should be made to each site. If both surveys occur three or four days after a snowfall event (without high winds), then two surveys should be sufficient. If fewer days were allowed for pellet deposition, then three visits are recommended per site. These are guidelines to ensure high confidence patch-specific occupancy determination. If broader-scale monitoring objectives are intended, then fewer site visits may be warranted, with the possibility of obtaining 90% detection rates from single visits under ideal conditions (snowpack <12" and three pellet deposition days). Notwithstanding, for any objective requiring finer resolution than a course-grain occupancy inventory, we recommend a minimum of two visits per site.

Special considerations for large patches: Large patches present the greatest challenges for occupancy surveys, particularly if NEC occur at low density and in sympatry with EC. In these situations, NEC occupancy may be highly heterogeneous across the patch, making detection problematic. For these reasons, it is important to search representative portions of suitable habitat across the patch through a systematic subsampling effort (see above). To increase detection rates, at least 20% and potentially a greater amount of the patch area should be surveyed, with unlimited search time.

Special considerations for sites with sympatric eastern cottontails: Due to the potentially unpredictable spatial segregation of NEC and EC co-occupying a patch, we recommend the collection of pellet samples from five distinct locations within a patch or subplot. Genetic species determination can be conducted on these pellets sequentially and terminated once a NEC sample is confirmed, for maximal efficiency of resources, time and labor.

Lastly, we emphasize that our survey and sampling recommendations in this document apply specifically for the purpose of simple presence/absence (occupancy) determination. Addressing other monitoring objectives, such as mark-recapture population estimation, or estimates of proportional occupancy by New England and eastern cottontails, will require modifications to this protocol.

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TABLES

Table 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. 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).

Variable Name	Description	P-value
Observer	Identity of individual(s) conducting survey	
Search time	Amount of time until pellet/tracks detected	
Area searched	Area of search path with buffer	
Predation index	1,2 or 3 with increasing intensity	
Days	Days since snowfall	
DaysWind	**Days since snowfall with wind <40kl/h**	**0.023**
DaysTemp>-10C	**Days since snowfall with temperature >-10°C**	**NA**
DaysTemp>-15C	Days since snowfall with temperature >-15°C	NA
SnowDepth	**Snow depth < or > 12 "**	**<0.001**
SnowPowder	Snow conditions - powder or not	0.752
Stem density	**Average stem density for patch**	**0.251**
Knowledge	**Prior knowledge of cottontail location on patch**	**<0.001**
Size	**Patch area in ha**	**0.099**

Table 2: 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 (*AICc*), the difference in *AICc* (*ΔAICc*), *AICc* weight (w_i), the number of parameters (K), and the maximized log likelihood (-2 log (£)) is given.

Model	AICc	ΔAICc	w_i	K	-2 log (£)
Knowledge + SnowDepth + DaysWind	111.4	0	0.89	5	100.94
Knowledge + SnowDepth + DaysTemp	115.2	3.79	0.06	5	104.73
Knowledge + SnowDepth	115.3	3.89	0.05	4	106.98

Table 3: 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 (*AICc*), the difference in *AICc* (*ΔAICc*), *AICc* weight (w_i), the number of parameters (K), and the maximized log likelihood (-2 log (£)) is given.

Model	AICc	ΔAICc	w_i	K	-2 log (£)
Knowledge + Size + DaysWind	170	0	0.52	5	159.54
Knowledge + DaysWind	171.11	1.11	0.33	4	162.8
Knowledge + Size + SnowDepth	173.03	3.03	0.07	5	162.57
Knowledge + Size + DaysTemp	173.57	3.57	0.03	5	163.11
Knowledge + SnowDepth	174.03	4.03	0.04	4	165.72
Knowledge + DaysTemp	174.49	4.49	0.02	4	166.18

Table 4: Untransformed linear logit parameter estimates, standard errors and 95% confidence intervals for explanatory variables from the best supported model of New England cottontail detection probability, in which 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 kl/hr; DaysWind).

Parameter	Coefficient estimate	SE	Lower CI	Upper CI
Intercept	-1.87	0.58	-3	-0.74
Knowledge	3	0.57	1.9	4.11
SnowDepth	2.23	0.63	0.99	3.46
DaysWind	0.61	0.27	0.09	1.13

Table 5: Untransformed linear logit parameter estimates, standard errors and 95% confidence intervals for explanatory variables from the best supported model of New England cottontail detection probability for the 20-minute restricted survey period, in which 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 kl/hr; DaysWind).

Parameter	Coefficient estimate	SE	Lower CI	Upper CI
Intercept	-0.70	0.47	-1.62	0.21
Knowledge	1.45	0.39	0.68	2.21
DaysWind	0.45	0.19	0.07	0.83
Size	-0.05	0.03	-0.11	0.00

Table 6: 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 is modeled as either one or three days since snowfall with winds <40 kl/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.

Scenario	Knowledge	SnowDepth<12”	DaysWind	Det Prob	# visits for 95%
1	1	1	1	0.98	1
2	1	1	3	0.99	1
3	1	0	1	0.85	2
4	1	0	3	0.95	1
5	0	1	1	0.72	3
6	0	1	3	0.90	2
7	0	0	1	0.22	>6
8	0	0	3	0.49	4

Table 7: 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 kl/hr), and patch size (which was 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.

Scenario	Knowledge	DaysWind	Size	Det Prob	# visits for 95%
1	1	1	25	0.46	4
2	1	1	3	0.74	3
3	1	3	25	0.68	3
4	1	3	3	0.87	2
5	0	1	25	0.17	>6
6	0	1	3	0.40	6
7	0	3	25	0.33	>6
8	0	3	3	0.62	4

FIGURES

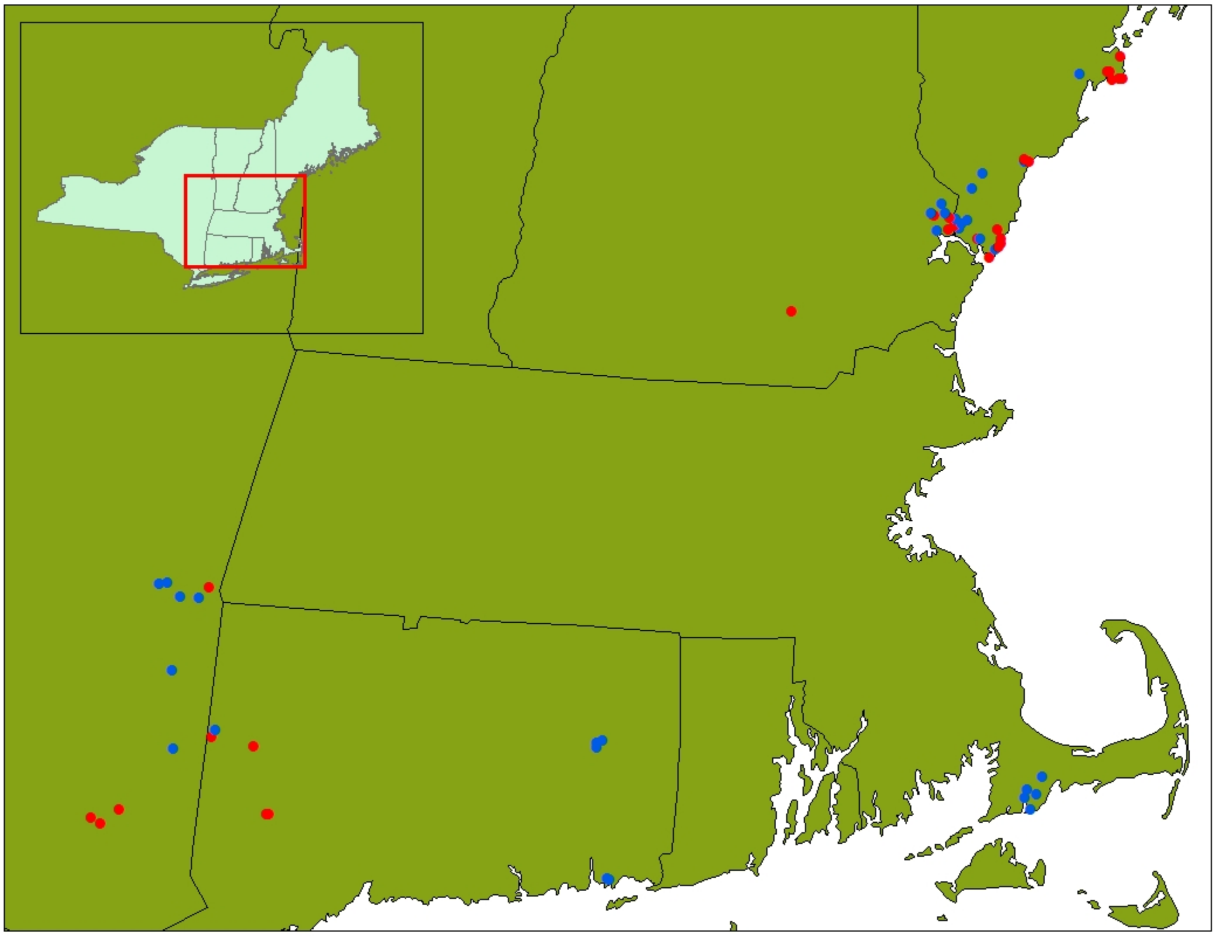

Figure 1: Locations of 60 range-wide sites surveyed for New England cottontail detectability during the winters of 2010 and 2011. Sites in red met criteria for use in detection modeling (see text).

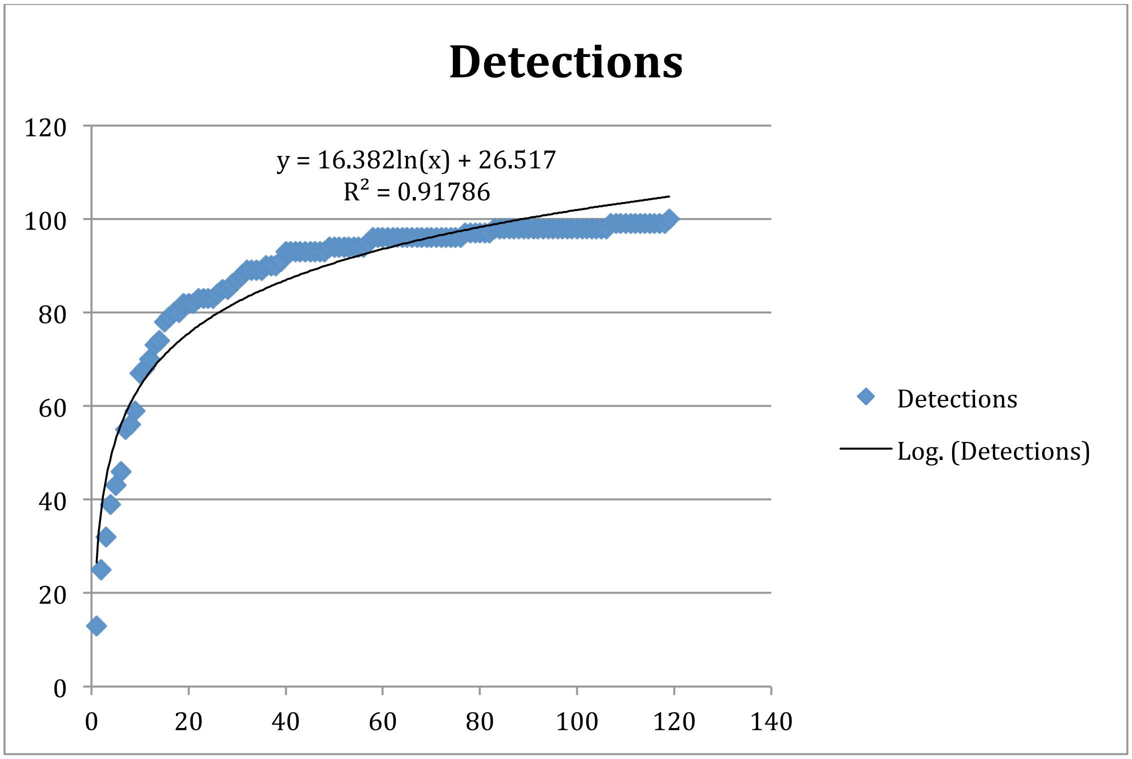

Figure 2: The best fit curve for the number of New England cottontail detections by survey search time. Positive gains of increased search time begin to diminish at 20 minutes (82% of detections), with only slight gain for increased effort beyond 40 minutes (93% of detections).

APPENDICES

Appendix 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 –relevant data are shown for the year used in detection modeling

Site Name	State	Size (Hect)	Sub-plots	Visits	Detec -tions	Date of First Survey	Date of Last Survey	Survey Length
Aldo Leopold A	CT	7.59	3	4	4	1/11/2011	3/22/2011	70
Aldo Leopold B	CT	3.67	0	4	4	1/11/2011	3/22/2011	70
Arno Farm	CT	2.69	3	4*	0	12/29/2010	1/31/2011	33
Averill Farm	CT	9.68	0	4	4	1/11/2011	3/22/2011	70
Bluff Point East	CT	2.66	0	4*	0	12/28/2010	2/10/2011	44
Bluff Point West	CT	3.28	0	4*	0	12/28/2010	2/10/2011	44
McAvoy Property	CT	0.87	0	4*	0	1/14/2011	3/25/2011	70
Pudding Hill Road	CT	7.47	0	4*	0	1/14/2011	3/25/2011	70
Scotland DOT	CT	1.48	0	4*	0	1/20/2011	3/25/2011	64
Silverstone Farm	CT	5.5	3	4	3	12/29/2010	1/31/2011	33
Childs River	MA	4	2	2*	1	1/28/2011	2/4/2011	7
Crane WMA	MA	40	0	2*	0	1/5/2010	1/7/2010	2
Santuit Pond	MA	4	2	2*	0	1/28/2011	2/4/2011	7
Greenwood	MA	4	2	2*	1	1/28/2011	2/4/2011	7
Qwuashent River	MA	4	2	2*	0	1/28/2011	2/4/2011	7
Bartlett Lane	ME	1.24	0	5*†	0	12/30/2010	2/10/2011	42
Braveboat Harbor	ME	1.37	0	5*†	0	12/28/2010	2/11/2011	45
Braveboat Spit	ME	4.2	0	3†	3	1/5/2010	1/21/2010	47
Cardinal Drive	ME	3.16	0	6†	2	12/30/2010	3/4/2011	64
Crescent Beach West	ME	12.43	3	5†	5	12/31/2010	2/14/2011	45
Depot Road	ME	2.23	0	5*†	0	12/30/2010	2/10/2011	42
Drake Island Road	ME	2.85	0	5*†	0	12/29/2010	2/10/2011	43
Dyer Point	ME	3.28	0	4†	4	12/29/2010	1/29/2011	31
Fort Foster	ME	4	0	5†	4	12/28/2010	1/28/2011	31
Fort William Park	ME	1.5	0	5†	5	12/29/2010	2/10/2011	43
Frieze Property	ME	0.73	0	5†	2	12/28/2010	1/28/2011	31
Haley East	ME	2.04	0	5*†	0	12/30/2010	2/11/2011	43
Haley West (Farm)	ME	7.42	3	5†	4	12/30/2010	2/11/2011	43
Houde Road	ME	1.21	0	5*†	0	12/30/2010	2/10/2011	42
Kettle Cove State Park	ME	20.4	5	4†	4	12/29/2010	2/11/2011	44

Scarborough WMA	ME	3.75	0	5*†	0	12/31/2010	2/14/2011	45
North Berwick	ME	1.61	0	5*†	0	12/29/2010	2/10/2011	43
River Road	ME	1.84	0	4	4	1/9/2010	1/29/2010	51
Libby Field	ME	8.61	3	5†	2	12/31/2010	2/14/2011	45
South Berwick	ME	3.18	0	5*†	0	12/29/2010	2/10/2011	43
Barber Easement	ME	4.2	0	5†	5	12/31/2010	2/14/2011	45
Wells Reserve	ME	18.9	5	5†	0	12/29/2010	2/11/2011	44
Wells Reserve West	ME	2.27	0	5†	3	12/29/2010	2/11/2011	44
Western Point Rd-E	ME	1.1	0	5†	4	12/28/2010	2/11/2011	45
Western Point Rd-W	ME	1.43	0	4	4	1/15/2010	2/18/2010	34
York Middle School	ME	4.71	0	2†	2	1/24/2010	2/18/2010	25
Bellamy	NH	13.21	0	5†	0	12/30/2010	2/9/2011	41
Bunker Hill	NH	3.97	0	5*†	0	12/30/2010	2/9/2011	41
Bus Depot	NH	5.37	0	5†	3	12/30/2010	2/9/2011	41
Clifford	NH	5.1	0	1*	0	1/21/2010	1/21/2010	1
Dover Radio Station	NH	2.41	0	5†	3	12/30/2010	2/9/2011	41
Martineau	NH	15	0	5*	1	1/22/2010	3/2/2010	40
Progress Drive	NH	3.01	0	4†	1	1/14/2011	2/9/2011	26
Rollinsford	NH	12	0	4*	0	2/12/2010	3/3/2010	51
Stonyfield	NH	4.93	0	6	5	1/13/2011	3/1/2011	47
CFSP-HPCA	NY	10.26	3	4†	4	12/10/2010	1/24/2011	45
Doodletown Road	NY	5.5	0	6*	0	1/28/2011	3/9/2011	40
Drowned Land Swp	NY	12	0	6*	0	1/15/2010	3/25/2010	70
Indian Brook Road	NY	1.86	0	4	4	12/23/2010	1/14/2010	22
Lake Taghkanic 1	NY	1.3	0	5*	0	1/15/2010	3/19/2010	64
Lake Taghkanic 2	NY	1.8	0	5*	0	1/15/2010	3/3/2010	48
Shuman Road	NY	6.5	0	5*	0	1/11/2011	2/17/2011	37
TSP-301	NY	26.32	6	5†	5	1/11/2011	3/16/2011	64
Weed Mine	NY	10.24	3	5	3	1/11/2011	2/10/2011	30
West Mtn. St. Forest	NY	2.5	0	3*	0	2/2/2010	3/16/2010	45

Appendix 2
New England Cottontail Detection Study -Survey Field Protocol

Overview
The overall goal of this study is to evaluate detection rates in our current winter pellet surveying approach. That is, when we don't find any sign of rabbits on a patch is it due to the fact that rabbits are really absent, or is it because we just didn't find them that day, "false absence"? The study also seeks to identify sources of variation in detection so that ideal monitoring conditions can be developed and standardized for the future.

- To evaluate detection rates, each site will be visited at least 4 times to determine occupancy (presence/absence based on whether or not pellets are found).

- To meet the assumption of population closure with respect to patch occupancy, initial searches will be restricted to the first half of the winter (December – mid February), ideally within a 5-6 week window, pending snow conditions.

- If feasible, an additional 2-3 surveys may be conducted on some sites in the late winter (late February - late March/early April). Please contact Daniel and Adrienne to confirm the appropriate time frame for surveys.

- To identify factors that influence detection, the following patch-specific information will be collected on a datasheet during each survey: date of survey, weather conditions within the last 3 days (rain, high winds, temperature), snow depth, snow conditions (powder, wet snow, crusted, melted out), time since last snow, predation index (1=none, 2=some or 3=high predation), patch size, distance searched, habitat, and observer.

- So that data from all states and observers can be incorporated into a single model of detection, sites should be surveyed by the specific, systematic search protocol detailed in this document. Documentation of the patch delineation and search path on a map is needed.

- If pellets are found, they should be collected from 3-5 separate locations in a patch potentially occupied by both NEC and EC. For sites known to contain only NEC, 1 vial of pellets from a single location in the patch is sufficient. No more than 5 vials of pellets are needed from any single site per visit.

The study design and search protocol reflect the specific study objective, which is to answer the question: what is the probability of detecting a New England cottontail on an occupied patch? As this is a practical objective, aimed at aiding managers and landowners, we have adopted a realistic approach that can be effectively implemented by such individuals. We assume that individuals who will conduct searches will have a basic ability to recognize cottontail habitat; even an inexperienced volunteer can learn to do so with minimal instruction. Therefore, we focus our searches on patches known to have New England cottontail and in suitable habitat, rather than adopting a randomized search protocol, which would be inefficient and impractical.
This protocol differs from the typical pellet survey protocols. Because we are interested in detectability, recording the search path along with the other covariates is vital. This means that finding pellets at a site is only helpful if the survey protocol is followed as closely as possible.

Site selection
For the purposes of a survey, a site will be defined as a patch of suitable habitat that a cottontail may utilize without venturing into a risky open area (>30 feet wide) such as highly unsuitable vegetation, a high traffic road, or a water body. Delineate the site on a topographic or aerial photograph, including the area that you searched (if less than the whole patch). If pellets are collected indicate the location on the map and record GPS coordinates whenever possible. If no pellets are found, record GPS coordinates in center of site (if possible). Bring a map of the site with you.

Site Visit & Search Protocol
Although data is being collected by multiple observers across the range of NEC, it ultimately will all be incorporated into a single detection model. For this to happen, it is important that every participant follow the same protocol. We have developed a systematic but flexible search protocol, outlined below.

When to search: The best time to search for sign of New England cottontails is shortly after a fresh snow. If snowstorms are frequent, restrict searches to at least 12-24 hours after the most recent snowfall to maximize accumulation of fresh tracks and pellets; surveys can be performed as long as at least a full night has been allowed to pass after a snow. Sites may vary in size and suitability and consequently in the amount of time required to search them thoroughly. The search will be considered completed when pellets are found and collected, or once all suitable habitat has been exhaustively searched, according to the search protocol below.

Search using a loose transect approach: Search the patch systematically using a continuous transect that winds its way back and forth across the patch keeping approximately 30 meters between passes (*see Figure 1*). Focus your search on suitable habitat most likely to be used by NEC (e.g. dense escape cover, browse species, such as blackberry, viburnum, low bush blueberry). Due to the nature of thicket habitat, the search transects need only serve as a rough guide from which one may need to deviate to facilitate movement around denser thickets. If cottontail tracks are discovered they should be followed until either pellets are found or the tracks are lost (due to crusted snow or dense vegetation). If the tracks are lost, or more samples are required (e.g. sites with NEC and EC present) the survey should continue from the point where the tracks were first discovered. To the extent possible, delineate your search path on the map of the site; this procedure can be facilitated by using the "track" feature on a GPS unit. If a GPS is not available, draw the approximate path searched on a map of the site.

How much of the patch to search: Survey protocol will vary depending on the size of the patch. For patches ≤6 acres, search the patch completely using the above protocol. For patches >6 acres, the search will be restricted to subplots within the patch (*see Figure 2*). To ensure similar search effort, each subplot will be 2 acres and the total area searched will be 6 acres or 20% of the total patch area, whichever is greater. To designate subplots, divide the patch into units approximately 2 acres each, such that individual units will be as easy as possible to identify in the field (e.g., minor habitat changes or oddly shaped portions of the site). Then, select three nonadjacent units (subplots) that look to have the best habitat and search them following the above protocol. Selection of subplots should reflect a balance between covering spatially disjunct portions of the patch and searching in the most appropriate habitat. For example, a 21-acre patch would be divided into 11 2-acre units and three of them would be selected as subplots to be searched in the survey. For patches >30 acres, additional 2-acre subplots should be searched until 20% of the patch has been surveyed.

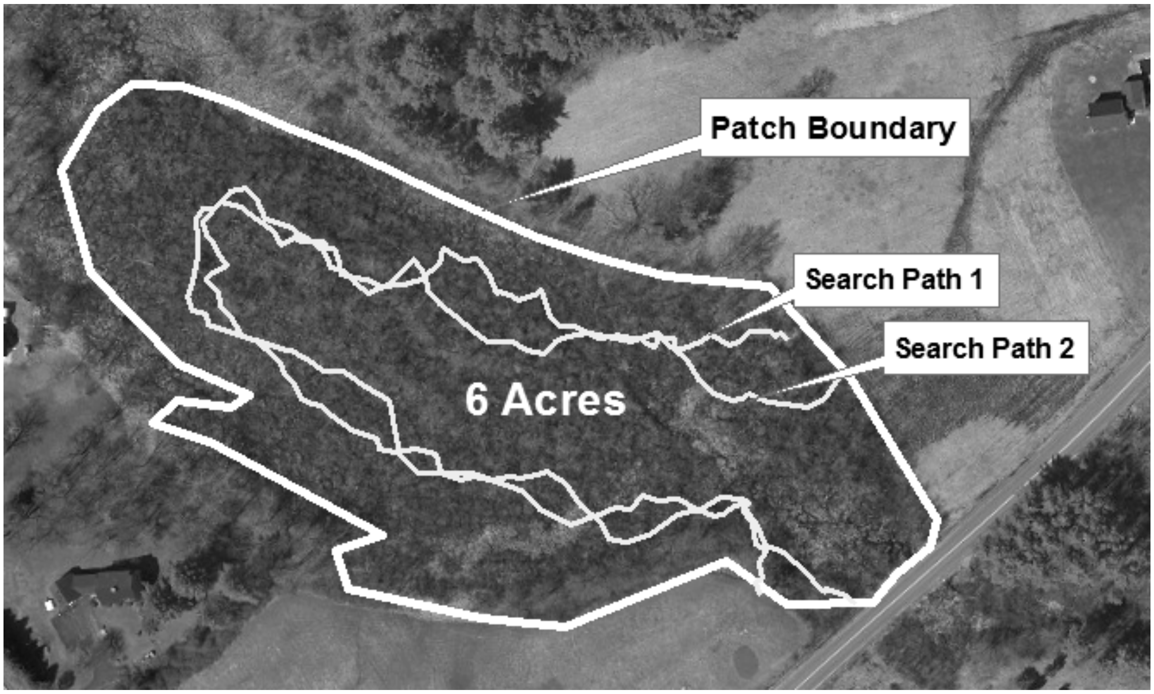

Figure A2-1: Shows a small 6 acre patch with search paths from two different surveys.

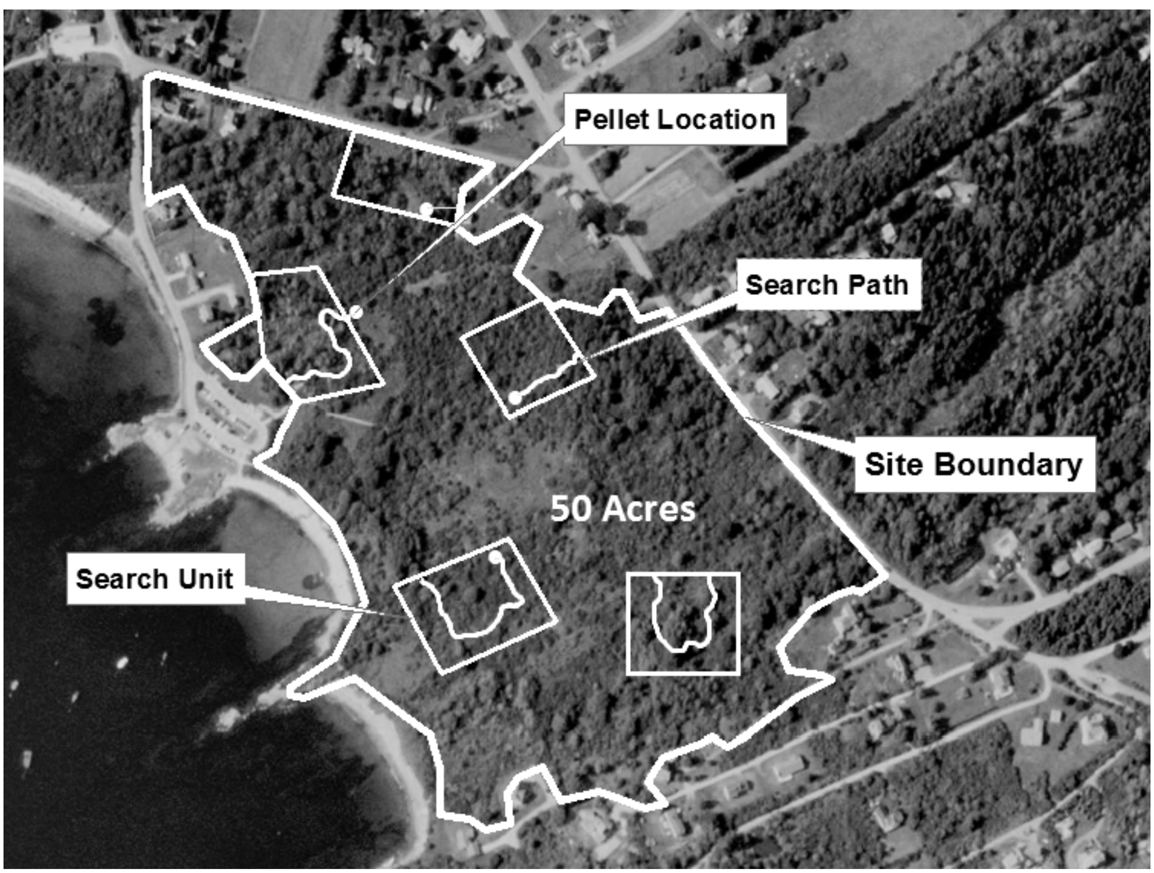

Figure A2-2: A large 50 acre plot showing the 5 search units of ~2 acres each. The search path is indicatedin each search unit and a white circle signifies the location where pellets were found.

While searching: Record the time the search is initiated. While searching, look for signs of lagomorph activity, including browse and tracks. Twigs clipped by rabbits and hares can be identified by a clean, 45° cut rather than a ragged edge that may be left by another herbivore. Cottontail tracks may be similar to snowshoe hare and squirrel tracks, so be sure to familiarize yourself with key differences in size, shape, and track patterns (information on Yahoo Groups page). Once clipped twigs, girdled stems, or tracks have been found, search the ground for fecal pellets. Once pellets are identified, record the time on the datasheet. Take a photograph at the pellet collection site and towards the 4 cardinal directions.

Additional data collection: Record environmental conditions including snow depth, days since last snow, temperature, and if wind or rain has occurred within the past 3 days. Look for sign of other non-lagomorph species and make a determination about the level of predation apparent in the patch. Use the predation index on the datasheet (1 = no sign of predation, 2 = some sign of predation evident, e.g. tracks, 3 = heavy predation evident, e.g. many tracks or an actual predator sighting). Record these and all variables others (e.g. observer, patch size) on the datasheet, for use in the detection model. Complete the back of the datasheet with information about site habitat characteristics to the extent possible.

Pellet Collection

Pellets may be found within a distinct or slightly diffuse clump, or alternately, several pellets may be scattered individually along a short trail. The objective is to collect several pellets from the same individual rabbit; these pellets will be placed together in a unique vial (ideally 2-10 pellets per vial, but if you can only find one pellet, please collect it!). Restrict collection for each vial to an area approximately 5 x 5ft or smaller to minimize the number of individuals represented in each vial (ideally, one rabbit per vial). Use disposable sterile latex gloves and place the pellets in a unique vial; try to keep snow out of the vial but avoid rubbing the pellet as it may remove DNA. Do not touch the pellets with your bare hands, as this may cause contamination, and use a fresh glove for each vial.

For small patches known to contain only NEC (e.g., some sites in Maine and New Hampshire), only *one vial* of pellets need be collected per patch; the survey is complete once collected. For larger patches, or any site where NEC may share habitat with other lagomorph species (EC or SSH), a total of 3-5 vials of pellets should be collected per site, if possible, from 3-5 distinct locations within the patch. Collection points should be separated by at least 100 m and farther if appropriate, depending on patch size (e.g., from 3-5 disjunct subplots of a large patch). *No more than 5 vials of pellets need to be collected.*

Use a GPS to record the exact location of pellets collected. It is important that the location of each collection is as specific as possible (i.e., not just patch coordinates, but collection point of the vial). Record the latitude and longitude at the point of collection (NAD 83) and the format of the coordinate (decimal degrees preferred, but degrees minutes seconds or UTM acceptable, if format is specified). Label each vial with the date, site name, your initials, coordinate, and the vial number (if more than one vial is collected at that site). Label multiple vials from the same patch with sequential numbers and also record this in the space on the datasheet (e.g., Libby Field #1, Libby Field #2, Libby Field #3). Given multiple site visits, it is imperative to record the collection date on each vial. Because DNA can degrade quickly if not frozen, return the vial(s) to a cooler upon returning to your vehicle and deposit in a freezer as soon as possible. A good trick is to temporarily store your pellets in a ziplock bag filled with snow while you are in the field (e.g., to prevent them from heating up in your pocket), and transfer them to a cooler once you get to your vehicle. Once a patch has been exhaustively searched, record the time that the survey was completed. Submit samples, datasheets and site maps to Dr. Adrienne Kovach and/or Daniel Brubaker at the University of New Hampshire.