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DEVELOPMENT OF NONINVASIVE MONITORING TOOLS FOR NEW ENGLAND COTTONTAIL POPULATIONS: IMPLICATIONS FOR TRACKING EARLY SUCCESSIONAL ECOSYSTEM HEALTH

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Problem Statement & Purpose

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. Of the many species that prefer or utilize early successional habitats, the New England cottontail is among the most critically threatened. Once abundant throughout the New England states and eastern New York, the New England cottontail has suffered a significant range contraction and severe recent and ongoing population decline; as a result, it is currently under review as a candidate for federal listing under the Endangered Species Act. The development of appropriate monitoring tools is critical for successful adaptive management of this species. To this end, the objectives of our project were two-fold, including the development of optimal methods for estimation of patch-specific occupancy and abundance.

Objectives

The stated objectives of this project were to:

1. *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.*
2. *Develop a noninvasive genetic monitoring technique for estimating the numbers of individuals occupying a habitat patch; apply this tool to measure baseline population levels on multiple sites throughout the range of NEC.*
3. *Use the population estimates in (2) to develop a population index based on pellet counts; establish guidelines for implementing this tool throughout the range of NEC.*

Part I – Objective 1 - Detection Study for Development of Occupancy Monitoring Protocol

We conducted a systematic investigation of New England cottontail detection rates during presence/absence pellet surveys, to address the following specific objectives related to occupancy monitoring:

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;
4. develop recommendations for improved occupancy monitoring to facilitate the adaptive management of the New England cottontail.

A detailed report of our methods and results was provided and distributed to stakeholders; herein we provide a summary. 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 km/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.

We found that 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 provided procedural recommendations for optimal detection and high confidence determination of patch-specific occupancy status.

Recommendations for Patch-Specific Occupancy Monitoring

- 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. Time visits within as narrow window as possible, to avoid closure violation (2-3 weeks ideal).
- 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 more than five distinct locations in each subplot.

Part II – Abundance Estimation – Objectives 2 & 3

Objective 2 – Development of a noninvasive genetic monitoring technique to estimate patch-specific abundance.

We developed an approach for abundance estimation of New England cottontails from noninvasive genetic sampling of fecal pellets collected during a single, well-timed, systematic (thorough and exhaustive) site survey. Addressing this goal required evaluation of several aspects of the methodology, for which we developed the following specific objectives:

1. *Develop the laboratory protocols and evaluate the suitability and power of the genetic markers for discriminating individual cottontails.*
2. *Identify the most appropriate sampling scheme with respect to the number of independent surveys and distance between collected pellets.*
3. *Determine the most appropriate mark-recapture algorithms and study design, by comparing rarefaction approaches with single and multi-session capture-mark-recapture approaches.*
4. *Make recommendations for the optimal approach and protocol for range-wide population estimation.*
5. *Apply this protocol to obtain baseline abundance estimates for range-wide sites.*

The details of our approach and methodology have been provided in a report to stakeholders; herein we summarize key findings. For this portion of the study, we focused on a subset of 20 range-wide sites, on which, with the help of partners, systematic population estimation surveys were conducted. Our search protocol requires exhaustive sampling and pellet collection during a single visit. We identified individuals by genotyping using a suite of 10 microsatellite markers and rigorous quality control procedures in the laboratory. To identify the most appropriate sampling scheme, we evaluated the effects of varying sampling intensity and survey visits on the consistency and precision of the population estimates. We evaluated the effects of sampling intensity by comparing estimates generated from data sets resulting from subsampling at three different sampling intervals – exhaustive or 30-m distance between pellets, and 50-m and 75 – m sampling intervals. We evaluated the effects of the potential for temporal variation in capture rates on small patches by sampling three sites on two separate occasions and comparing the specific individuals identified and resulting population estimates from on each visit and the combined sample from both visits. We generated estimates using a single session mark-recapture algorithm implemented in the program CAPWIRE. For comparison, on seven sites with sufficient sample sizes, we also generated estimates using a rarefaction approach.

We determined that a single, well-timed survey visit with intensive sampling could provide robust estimates with reasonable precision, using the CAPWIRE estimation algorithm. Precision of estimates varied by site and, except for the smallest sites, appeared to be more strongly influenced by density than by patch size. On small patches (<5 ha), unique individuals were consistently identified with high recapture rates, yielding high precision in estimates. On patches >5 ha, both rabbit densities and the precision of the estimates varied. On high-density sites, the most significant challenge is to obtain significantly high recapture rates. To this end, intensive sampling (exhaustive or with 30-m sampling intervals) is required. Although individual

heterogeneity in deposition rates was evident on some sites, especially small ones, we found no benefit from conducting multiple visits, provided the single visit was conducted thoroughly and with optimal timing (allowing 4-5 pellet deposition days after a snowfall event). In comparison of the population estimation algorithms, we found that the rarefaction approach performed less robustly than CAPWIRE with small sample sizes and low recapture rates. With respect to the genetic protocols, we encountered challenges posed by the limitations of using markers adapted from other lagomorph species. We developed strict quality control protocols to address these challenges. We recognize that these challenges will likely be significantly decreased with the development of species-specific microsatellite markers, currently underway in ongoing collaborative research. We anticipate that species-specific markers will yield higher discriminatory power, lower genotyping error rates, and higher amplification success for poor quality samples. These improvements might further increase the precision of the estimates. Therefore, we recommend that future genetic tagging studies adapt our laboratory protocols for use with a new suite of species-specific markers.

Recommendations for Patch-Specific Abundance Estimation:

- Conduct population surveys 4-5 days after a snowfall event.
- In a single site visit, survey the patch thoroughly using systematic, loose transects with approximately 30-m spacing between transects, focusing on suitable habitat throughout the entire patch.
- Collect pellets intensively, either exhaustively or every 30 meters, throughout the patch.
- If pellet density is moderate and logistics allow, analyze all samples in the laboratory.
- If pellet density is high and/or a large number of samples were collected from a large site, analyze a subsample of the total pellets with a 30-m or maximally 50-m sampling interval. If population estimates cannot be obtained with reasonable precision, analyze additional samples to increase capture and recapture rates needed for precise estimates.
- If on a small site a single survey does not yield an estimate with high precision and identifies significant heterogeneity of individuals, conduct a second independent survey.
- Use stringent quality control protocols in the laboratory, including multiple replicate amplifications (minimum of 4) of each sample and evaluation of samples that mismatch by only a few alleles (up to 3 mismatching loci, depending on genotyping error rates and the number of markers used).
- Utilize knowledge of site-specific genetic diversity and probability of identity statistics in evaluating similar genotypes.
- Adapt the laboratory protocols developed in this document for use with species-specific microsatellite markers as soon as they become available.
- When developing a suite of species-specific markers, select a set of no more than 6-8 loci with high polymorphism that yield sufficient power for individual discrimination (*PIsib* <0.05 or preferably <0.001) and which can be co-amplified in 2 multiplexed reactions.

Objective 3 – Development of a Population Index

This third and final objective was developed to address practical concerns that a monitoring tool that relies solely on genetic analysis may have limitations in its application, given the need for agencies to contract for the services and the expense of the effort. To address these concerns, we conducted several pilot efforts to evaluate the feasibility of adapting a pellet count index, similar to what is used for snowshoe hares, to monitoring New England cottontails. Our efforts were frought with challenges. First, with the help of partners, we established pellet plots on Stage Island and initiated a pilot study evaluating the utility of using pellet counts for a population index. However, due to high mortality, this pilot study had to be aborted. We also trialed pellet plots on sites in Cape Elizabeth, ME. We encountered a remarkably low rate of pellets (despite a reasonable cottontail density). We also encountered significant logistical difficulties of establishing plots in the dense thicket habitat. Given these challenges, we determined that pellet plots were not a viable approach for monitoring NEC, and given support of our partners, we did not pursue this objective any further. It is important to note, that even if these challenges could be overcome, a pellet count index would only have utility in limited portions of the species range (Maine and portions of New Hampshire), where eastern cottontails are absent. The confounding effects of sympatric lagomorphs (potentially nonneglible with respect to sympatric snowshoe hares in Maine) likely preclude the feasibility of this approach for New England cottontails.

Outcomes & Deliverables

The following products resulted from this project:

- Protocols for monitoring occupancy and abundance of New England cottontails
- Detection rates for New England cottontails during presence/absence surveys
- Baseline population estimates from 17 range-wide sites
- A new collaboration among scientist from USGS, URI and UNH to develop species-specific genetic markers with greater power for discriminating individuals
- A report detailing methods and outcomes of the detection study and recommendations for survey conditions for confident occupancy determination
- A report detailing methods and outcomes of the population estimation study, with recommendations for robust abundance estimation
- A manuscript for publication in a scientific journal on the detection study
- A Masters Thesis (Daniel Brubaker, UNH, 2012)