DISTRIBUTION AND COARSE-SCALE HABITAT ASSOCIATION OF SNOWSHOE HARES IN PENNSYLVANIA

Final Report

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Executive Summary

The snowshoe hare (*Lepus americanus*) is a charismatic species of interest to hunters and non-hunting wildlife enthusiasts. The hare is a lagomorph named for its disproportionately large hind feet (11–14 cm), which with dense fur and stiff hairs form "snowshoes" well adapted for locomotion in deep, powdery snow. It is also called varying hare because it has a pure white pelage in winter, except for black eyelids and ear tips, which changes to a black-peppered rusty brown or grayish summer pelage. In Pennsylvania, hares likely are distributed patchily and associated with specific habitat types. However, Pennsylvania is within the southern periphery of the hare's range, and habitat use by this species in Pennsylvania is not well understood. Important hare habitat in northern portions of its range is dense, young, regenerating stands of hardwoods and conifers, as well as scrub-shrub wetlands. In contrast, conifer cover is scarce in the unglaciated regions of Pennsylvania, which may comprise the largest portion of the range of hares in the Commonwealth.

A variety of non-invasive techniques, such as identifying the presence of animal sign, to monitor rare or elusive species have been developed. However, any monitoring program for rare or elusive species must (1) sample usually because of the large spatial size of the range of the species and (2) account for the probability of detecting the presence of a species at a sampling site because of imperfect detection probabilities. One method of confirming presence of a species is by conducting surveys for fecal sign. However, this method is limited only to species that have distinct fecal morphological characteristics from other species. For species whose feces have similar morphological characteristics, DNA analysis has been identified as a means of differentiating among species, and has been developed for lagomorphs.

The goals of this project were to delineate the geographic distribution and identify coarse-scale habitat associations of snowshoe hares across northern Pennsylvania. Randomly selected sites from across northern Pennsylvania were visited and lagomorph fecal pellets collected as well direct evidence of the presence of hares, such as tracks in snow and visual observations. Fecal pellets were identified whether from hares by extracting and analyzing DNA and habitat characteristics of each sampling site was documented.

We sampled 213 of 240 selected sites (56 conifer, 56 deciduous, 50 mixed, and 51 transitional) and 34 additional woody transitional sites during January–April 2004. Transitional habitat was areas in which tree vegetation was regenerating. Eighteen sites were discarded because they fell outside defined habitats of the study area. Nine sites could not be sampled because of treacherous terrain, permission was not granted by landowners, or deep snow. Lagomorph sign was undetected at 144 sites. Lagomorph pellets, tracks, or both were detected at 62 sites. Snowshoe hare sign was positively identified at 18 of these 62 sites. Sign (primarily pellets) at 44 sites either was cottontail sign, or could not be distinguished in the field between hares or cottontails. Sign at 7 sites was too indistinct to classify as lagomorph or non-lagomorph. Lagomorph sign was detected at 12 (including positively identified hare sign at 3) of the 34 additional transitional sites. To estimate detection probabilities, 24 sites with lagomorph sign were resampled by different technicians 1–3 times, with and without snow cover. Some sign, either pellets, tracks, or direct observation, of snowshoe hares were observed in 47 sites, or 18.73% of the sites visited.

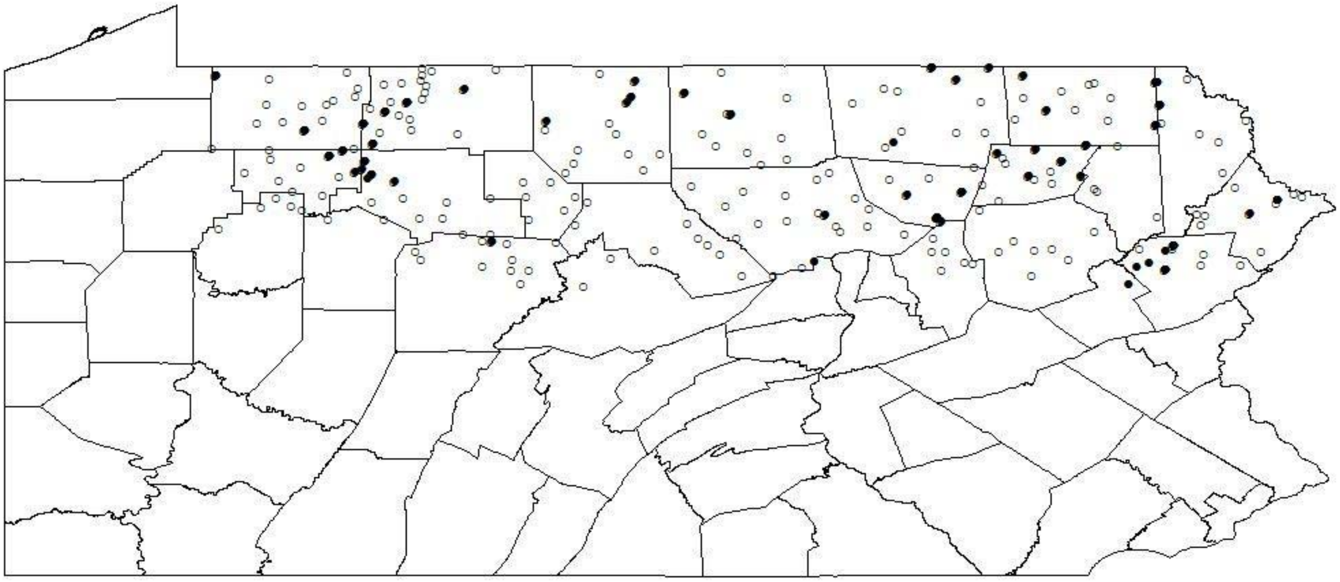
Snowshoe hare pellets were most easily detected in transitional habitat; the detectability of snowshoe hare pellets in transitional habitat is estimated to be more than three times that in the forested habitats. Snowshoe hare tracks were most detectable in full snow, and were not easily detected when there was no or partial snow on the ground. Not surprisingly, the detectability of this elusive animal by direct sighting is very small, at about 2%.

Estimates of detectability of snowshoe hare pellets, tracks, and direct sightings.

Sign	Condition	Percent detectability	Standard error
Pellets	Transitional habitat	65.91	9.54
	Forested habitat	20.96	5.54
Tracks	Full snow	38.13	5.94
	No or partial snow	9.27	9.00
Direct sighting		2.00	1.41

The estimated occurrence of snowshoe hares in conifer habitat was 15.9% (SE = 6.57), in deciduous habitat was 5.7% (SE = 3.28), in mixed deciduous-conifer habitat was 38.1% (SE = 10.44), and in transitional habitat 26.8% (SE = 6.31).

Based on harvest data from the Pennsylvania Game Commission (Figure 1), the range of snowshoe hares in Pennsylvania includes counties of the northern tier of the state and extends south to Maryland in counties that encompass the Laurel Highlands. The largest concentrations of sites where hares were detected were distributed similarly to the harvest data (Figure 6), which were Warren, McKean, Forest, and Elk counties in the west and the Poconos in the east.



Location of randomly selected sampling sites for the presence of snowshoe hares based on DNA testing of fecal pellets. Open circles represent sites where the species was not detected, solid circles indicate snowshoe hares were detected, Pennsylvania, 2004.

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Introduction

The snowshoe hare (*Lepus americanus*) is a charismatic species of interest to hunters and non-hunting wildlife enthusiasts. The hare is a lagomorph named for its disproportionately large hind feet (11–14 cm), which with dense fur and stiff hairs form “snowshoes” well adapted for locomotion in deep, powdery snow. It is also called varying hare because it has a pure white pelage in winter, except for black eyelids and ear tips, which changes to a black-peppered rusty brown or grayish summer pelage. In Pennsylvania, hares likely are distributed patchily and associated with specific habitat types. However, Pennsylvania is within the southern periphery of the hare’s range, and habitat use by this species in Pennsylvania is not well understood. Important hare habitat in northern portions of its range is dense, young, regenerating stands of hardwoods and conifers, as well as scrub-shrub wetlands. In contrast, conifer cover is scarce in the unglaciated regions of Pennsylvania, which may comprise the largest portion of the range of hares in the Commonwealth.

Snowshoe hares are herbivores that during summer feed on grasses, wild berries, wild flowers, clover, horsetails, and new growth of trees and shrubs. In winter, their diet consists of twigs, bark, and evergreen needles. Hares are found in areas with dense vegetation that provides food, thermal cover, and protection from predators. Dense understory vegetation provides protection from predators; thus, clearcuts are avoided until woody vegetation reaches a height >2 m (Litvaitis et al. 1985, Wolfe et al. 1982). Ten to 30-year-old regenerating conifer stands provide excellent hiding cover for hares in Maine (Monthey 1986), but hares were more abundant in 30-year-old stands than in 20-year-old stands in Labrador (Newbury and Simon 2005). Because of the longer growing season and faster growing tree species, in Pennsylvania suitable habitat for hares is likely to exist 5-15 years after clearcutting in northern hardwoods (Brown 1984) and mixed-oak forests (Storm et al. 2003). Edge habitats between mature forest and regenerating clearcuts have potential to be high-quality habitat because both cover and food are in close proximity (Meslow and Keith 1968, Litvaitis et al. 1985, Forsey and Baggs 2001); however, these edge habitats may have greater predator densities (Forsey and Baggs 2001).

In Pennsylvania, snowshoe hares are associated with forested habitats at higher elevations (>450 m) and scrub-shrub type wetlands (Glazer 1959, Doutt et al. 1966, Brown 1984). The key characteristic of quality habitat for snowshoe hares is high stem densities of woody vegetation that provides browse and visual isolation from predators (Brown 1984). Consequently, in Pennsylvania, hares often are associated with regenerating clearcuts 5–15 years old. Scott and Yahner (1989) suggested that hardwood clearcut stands in Pennsylvania may be suitable, but marginal, habitat for snowshoe hares. This is because coniferous habitats are generally considered preferred habitat and Pennsylvania has little of this type of forest habitat (<10% of total biomass, U.S. Forest Service, Forest Inventory and Analysis Program).

However, Brown (1984) and Scott and Yahner (1989) found that stem densities in regenerating hardwood stands (>10,000 stems/ha) provided sufficient browse and protective cover, and Brown (1984) noted that areas with mountain laurel (*Kalmia latifolia*) or eastern hemlock (*Tsuga canadensis*) were used less than 5-15 year old clearcuts when all three habitat types were in close proximity. Scott and Yahner (1989) noted that the proximity (<0.5 km) of other clearcut stands was important to hares because these nearby habitats provided alternate sources of food and cover. They noted that habitat availability may be critical to the population viability of snowshoe hares in Pennsylvania. Home ranges are generally 3–6 ha and typically <10 ha. Brown (1984) recommended maintaining 16% of a forest management unit (~900 ha) in the optimal age class for hares using a 60-year rotation period to maintain suitable habitat.

In Pennsylvania, snowshoe hares browse *Rubus* spp., striped maple (*Acer pennsylvanicum*), and yellow birch (*Betula alleghaniensis*) more than expected and browse American beech (*Fagus grandifolia*), pin cherry (*Prunus pennsylvanica*), black cherry (*Prunus serotina*), red maple (*Acer rubrum*), and sugar maple (*Acer saccharum*) less than expected in northern hardwood forests (Brown 1984, Scott and Yahner 1989). However, areas with a high density of *Rubus* spp. are avoided, supposedly because of the lack of overhead cover (Scott and Yahner 1989). Extensive browsing by white-tailed deer (*Odocoileus virginianus*) may have important negative consequences for snowshoe hares (Glazer 1959, Brown 1984, Scott and Yahner 1989) because of their effect on forest regeneration.

An 8–11-year cycle of abundance exists in northern snowshoe hare populations (Keith 1963) followed by a similar, but lagged, cycle in predator abundance. Although many hypotheses for these abundance cycles have been proposed, a coarse-scale, long-term study by Krebs et al. (1995) indicated that population cycles of the snowshoe hare in the boreal forest are best explained as a result of a three trophic-level interaction among food supplies, predators, and prey. However, in southern parts of its range, where ecosystems are more stable and diverse, hare populations are weakly cyclic, or irruptive, or stable. Population densities in non-cyclic populations tended to be greater than cyclic populations at numerical lows (Murray 2000). In Pennsylvania, hare populations likely are non-cyclic (Fergus 1976), and have been reported as being non-cyclic in other mid-Atlantic and northeastern states (Murray 2000).

The global ranking of the snowshoe hare is one of security and abundance (NatureServe 2004). The following states and provinces rank the snowshoe hare as secure: Colorado, Wyoming, Montana, Idaho, Washington, Wisconsin, Michigan, New York, Vermont, New Hampshire, Maine, Massachusetts, British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Quebec, Labrador, Prince Edward Island, and Nova Scotia. The following states have ranked the snowshoe hare apparently secure: Oregon, West Virginia, and Connecticut. Pennsylvania, Rhode Island, New Mexico, California, Nevada, and Utah have ranked the hare as vulnerable. Unranked or under review states include North Dakota, Minnesota, Alaska, and the provinces of Yukon and the Northwest Territories. The only state with a possibly extirpated population is Maryland. Snowshoe hare populations are presumed extirpated in Virginia, New Jersey, and Ohio.

The Pennsylvania population may be important to genetic flow along the Allegheny Mountain Range as a link between extant populations in New York and West Virginia, as well as possibly extirpated populations in Maryland and Virginia. Reported harvests from the Pennsylvania Game Commission's (PGC) annual Game Take Survey provide information on the current distribution of snowshoe hares at the county scale (Figure 1). Based on data from 1997-2003, the greatest harvest occurs in northwestern counties of the Allegheny National Forest and from

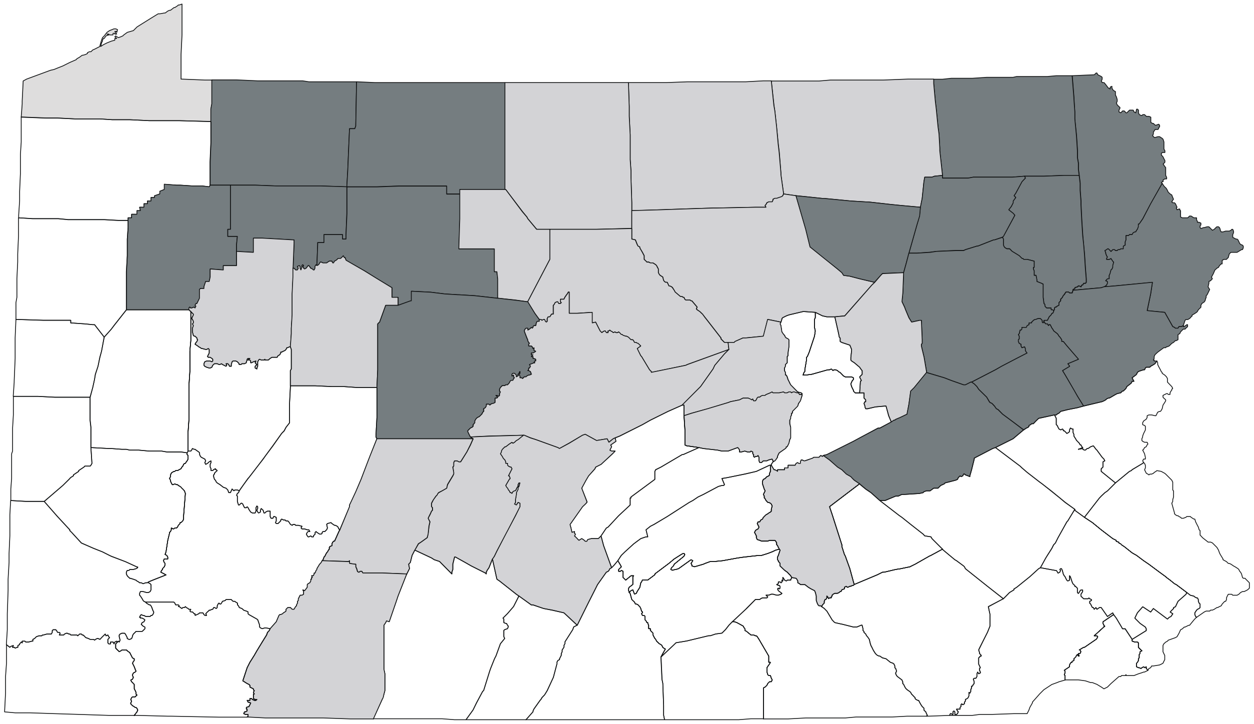

Figure 1. Harvest of snowshoe hares reported by county from the Pennsylvania Game Commission's annual Game Take Survey, 1997-2003 (raw counts of responses from approximately 10,000 randomly-selected license buyers each year). Dark gray counties had an eight-year total reported harvest of $\geq$6 hares and light gray counties had <6 hares.

the Poconos extending south into Schuylkill County. Fewer harvests are reported in the northcentral counties and south along the counties encompassing the Laurel Highlands.

Snowshoe hare populations in Pennsylvania likely increased in the early 20th century in response to the forest regeneration that occurred following the massive deforestation of the late 19th and early 20th centuries. Since this peak in the amount of early successional forest habitat in the early- to mid-20th century, the amount of suitable habitat for snowshoe hares in Pennsylvania has been declining as Pennsylvania's second- and third-growth forest matures (Alerich 1993). Hunter harvest surveys suggest a low, but constant, abundance of hares throughout the 1983-2003 hunting seasons, and no dramatic changes since the 1930s (Figure 2). Hunter harvest of hares has been low, but highly correlated with the number of hunters ($r = 0.83$, $n = 25$). Consequently, harvest per hunter indicates no trends in hunter success rates (Figure 2), which suggests that Pennsylvania hare populations are non-cyclic and have been fairly stable.

A variety of non-invasive techniques, such as identifying the presence of animal sign, to monitor rare or elusive species have been developed (e.g., Wilson and Delahay 2001). However, any monitoring program for rare or elusive species must (1) sample usually because of the large spatial size of the range of the species and (2) account for the probability of detecting the presence of a species at a sampling site because of imperfect detection probabilities (MacKenzie et al. 2002, 2004).

One method of confirming presence of a species is by conducting surveys for fecal sign. However, this method is limited only to species that have distinct fecal morphological characteristics from other species. Consequently, depending on the species, fecal surveys have proven to be both successful (e.g., Zuercher et al. 2003) and unreliable (e.g., Davison et al. 2002, Reed et al. 2004). For species whose feces have similar morphological characteristics, DNA analysis has been identified as a means of differentiating among species, and has been developed for such species groups as mustelids (Gomez-Moliner et al. 2004), canids (Reed et al. 2004), and lagomorphs (Kovach et al. 2003).

Imperfect detection of fecal sign at sampling sites can be estimated and used to obtain unbiased occupancy rates (e.g., MacKenzie et al. 2002). However, using DNA from feces to genotype species has its own associated errors, which are independent of the sampling design. If genotyping error rates result in a probability of <1.0 of detecting the presence of species given that a fecal sample is obtained, then occupancy rates will be negatively biased.

Genotyping errors can arise from limited amounts of DNA in the fecal sample as well as degraded DNA. Amount and quality of DNA material can be limited by the amount of fecal matter collected. Quality is also affected by contamination through mixing of feces from multiple individuals or species, and degradation caused by environmental or storage conditions (Taberlet et al. 1996, Lucchini et al. 2002). Litvaitis and Litvaitis (1996) found that genotyping errors from fecal DNA analysis resulted in approximately 18% of fecal pellets from New England cottontails and approximately 52% from eastern cottontails being correctly identified. However, Kovach et al. (2003) developed techniques to differentiate 98% ($n = 133$) of fecal pellets that amplified successfully among 3 sympatric lagomorphs: snowshoe hare, New England cottontail, and eastern cottontail.

The techniques developed by Kovach et al. (2003) modified a commercially available kit to amplify a specific gene sequence coding tRNA proline and the first 310 base pairs of the D-loop using the polymerase chain reaction (PCR). The PCR products were further incubated with restriction enzymes to take advantage of restriction fragment length polymorphisms among the three species. This method produces fragments specific for each species; however, it is time-consuming because it requires two steps and is only moderately sensitive.

Recently developed is a faster, more sensitive method using real-time PCR methodology. This technique requires less DNA to successfully genotype a fecal sample, reducing the need for repeated amplifications of the DNA material. Consequently, real-time PCR methodology reduces the time required to analyze samples and reduces costs, which can make the use of DNA technology feasible and allow researchers to increase the number of samples for analyses.

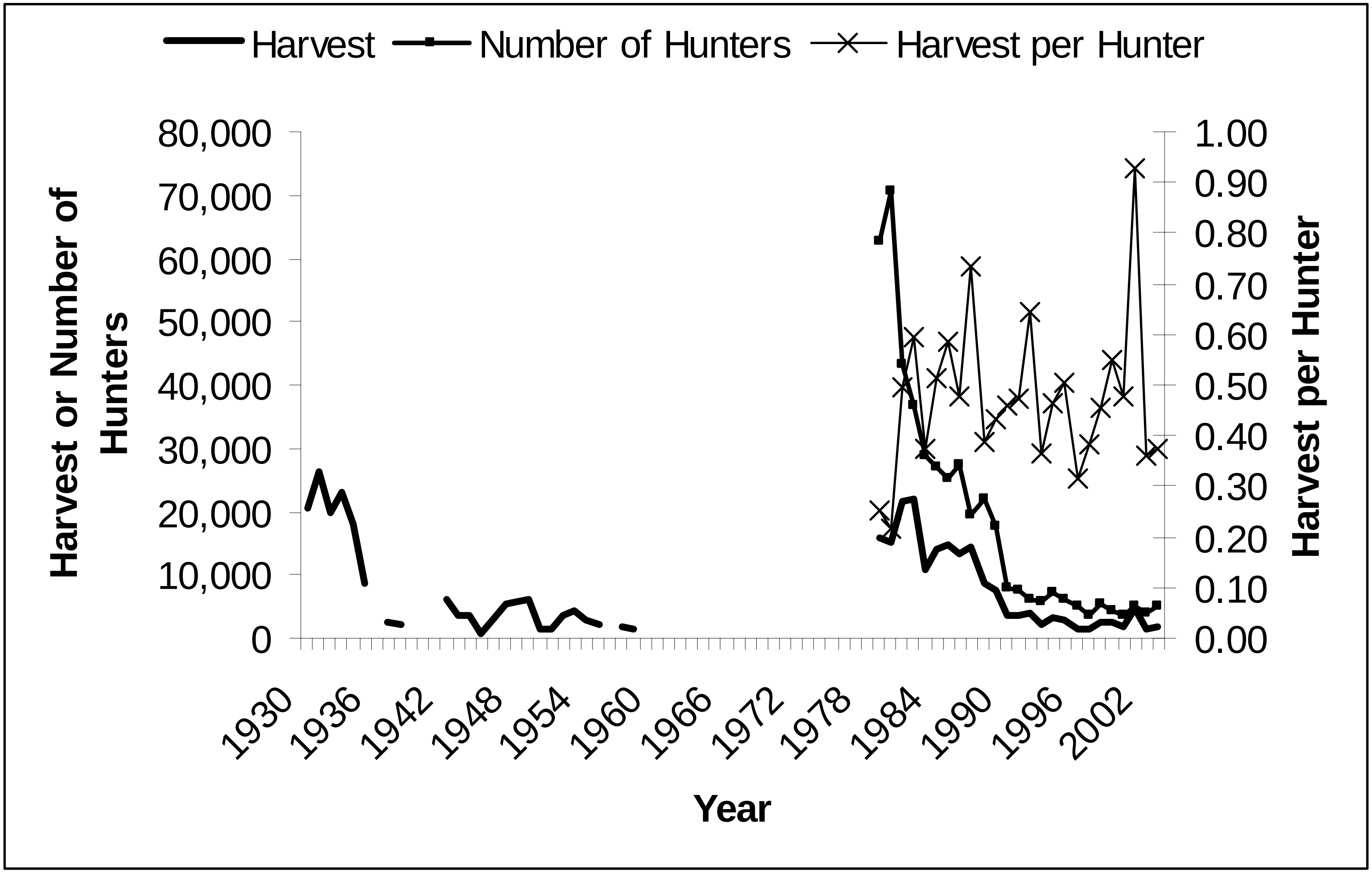

Figure 2. Harvest, number of hunters, and harvest per hunter for snowshoe hares in Pennsylvania, 1930-2003 (Pennsylvania Game Commission, unpublished data).

Goals and Objectives

The goals of this project were to delineate the geographic distribution and identify coarse-scale habitat associations of snowshoe hares across northern Pennsylvania. Randomly selected sites from across northern Pennsylvania were visited and lagomorph fecal pellets collected as well direct evidence of the presence of hares, such as tracks in snow and visual observations. Fecal pellets were identified whether from hares by extracting and analyzing DNA and habitat characteristics of each sampling site was documented.

The objectives of this project were to:

1. Estimate the probability of detecting the presence of snowshoe hares based on sign (tracks or visual sightings) and DNA analysis of fecal pellets
2. Estimate the proportion of area, by habitat type, occupied by snowshoe hares,
3. Estimate the distribution of snowshoe hares in northern Pennsylvania, and
4. Identify coarse-scale habitat features associated with the presence of snowshoe hares.

Study Area

We reviewed recent data from the annual Game Take Survey conducted by the PGC to determine where hunters reported harvesting snowshoe hares. Data from seven years (1997-2003) were pooled to assess where hunter effort occurred and hunter success. Annually, the PGC receives approximately 10,000 responses to their survey. Respondents reported harvesting hare in 34 counties in Pennsylvania, most of which were in counties that encompassed or were north of Interstate 80 (Figure 1). Moreover, nearly all the counties with the greatest harvests encompassed or were north of Interstate 80. Consequently, we restricted our study to the area north of Interstate 80 from Warren, Forest, and Clarion counties to the New York and New Jersey borders.

The study area included all or part of 27 counties in northern Pennsylvania (Figure 3). We used a geographic information system (GIS) with vegetative data from Pennsylvania State University, Pennsylvania Spatial Data Access (http://www.pasda.psu.edu) to exclude all habitat types that were not woody transitional, deciduous forest, mixed forest, or coniferous forest.

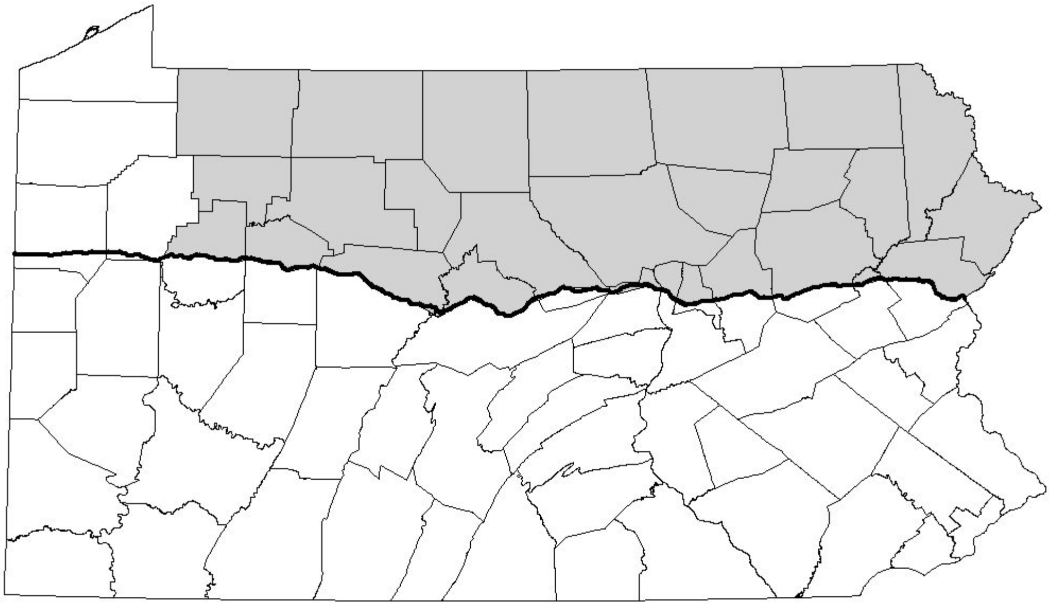

Figure 3. Study area (shaded gray) encompassing all or part of 27 counties in northern Pennsylvania in which snowshoe hare track and pellet surveys were conducted, 2004.

Methods

This section details the design of the snowshoe hare sampling program and the subsequent statistical analyses of the resulting data. Sample sites were selected to estimate the occurrence of snowshoe hares in different habitats, and to describe the range of distribution of snowshoe hares in northern Pennsylvania. At each of the selected sites, the sampling protocol required searching for lagomorph sign. Sign could include pellets, tracks, or visual observations, or a combination of the three along a 1-km transect. With fresh snow on the ground, tracks and pellets could be easily sighted. However, when there was no fresh snow on the ground, field biologists had to search closely for lagomorph pellets, and not all lagomorph sign may have been detected. In such conditions, the detectability, defined to be the probability of detecting hares when hares are present (Thompson 1992) is likely to be <100%. Estimates of the proportion of each habitat occupied by snowshoe hares must be corrected for detectability; otherwise that proportion could be underestimated. Detectability was estimated using data from repeat visits of a subset of sites where hares were previously detected from the presence of tracks.

Sampling Design

Northern Pennsylvania is divided into 8 habitat types (Table 1). Snowshoe hares may be assumed to be absent in water and urban/barren habitats. Moreover, snowshoe hares are expected in less than 1% of annual herbaceous and perennial herbaceous habitats.

Table 1. Habitats of northern Pennsylvania, their areas, and expected occurrence of snowshoe hares.

Habitat	Area (km^2)	Expected percent of sites occupied
Transitional	3,076	5-20%
Conifer	776	2-10%
Mixed Forest	4,112	1-5%
Deciduous Forest	24,896	1-5%
Annual Herbaceous	1,532	<1%
Perennial Herbaceous	3,960	<1%
Water	468	0%
Urban/Barren	696	0%

Therefore, herbaceous, water, and urban habitats were excluded from the study region. A stratified random sampling design was used to select sample sites from the remaining four habitats. If our primary objective were to estimate the proportion of all sites occupied by snowshoe hares, then the optimal sampling design would call for an allocation of sampling effort to each stratum proportional to the product of the stratum area and the standard deviation of the presence/absence data. However, the primary objective of this study is to delineate the distribution of snowshoe hares, which can be partly explained by habitat use. Under the null model that the proportion of sites occupied does not depend on habitat, equal allocation is optimal.

Any large scale sampling design must address the trade-off between how intensive to sample at a site versus the number of sites to visit. Generally, it is preferred to visit more sites at the expense of less intensive sampling at any given site. We decided to search 1,000 meter transects for hare sign because we estimated 240 sample sites then could be visited by two technicians during a 12-week sampling period. Sites were selected via stratified random sampling, but allocated equally (60 sites) among the four habitat types. When the primary habitat being sampled was coniferous, mixed, or deciduous forest, an attempt was made to locate and sample a 1,000-m transect of woody transitional habitat (among the four habitat types, expected to contain greater incidence of hares) within 5 km of the original habitat patch.

Sampling was conducted by 2 field crews over a 12-week period. Each crew could sample approximately two sites per day for five days of each week; thus, 252 sites were sampled. Under equal allocation, this yielded approximately 60 sites per habitat stratum. To estimate detectability, a portion of this sampling effort was allocated to repeat visits to sites where lagomorphs were previously detected. The optimal allocation n_3 to repeat visits in each habitat is obtained by finding n_3 that minimizes the variance of $\hat{p}$. The number n_1 of sites sampled in each habitat on snow days is limited by the number of available snow days, and the number of non-snow sample sites is $n_2 = 60 - n_1 - n_3$. Based on county-by-county records, a randomly selected site in Pennsylvania was expected to have snow cover for 43 days between January and March.

To illustrate the optimal allocation of sampling effort to repeat visits, Figure 4 shows a plot of the variance of $\hat{p}$ against n_3 for the special case where the hare frequency is $p = 0.125$, the detectability is $q = 0.4$, and the number of snow days sampled per stratum is $n_1 = 40$. This case corresponds to the median frequency of snowshoe hares expected for the scrub/shrub habitat, and assumes unequal detectability among habitat types. The variance of $\hat{p}$ increases with decreasing detectability. The selection of $q = 0.4$ corresponds to the lowest expected detectability, yielding a conservative estimate for the optimal allocation. Here, the optimal design calls for only $n_3 = 6$ repeat visits in a stratum with $p = 0.125$. Table 2 gives the optimal allocation of repeat visits per stratum for values of p ranging across those expected for the four habitats (Table 1). Note that the optimal number of repeat visits increases with increasing hare frequencies. Conversely, the variance of $\hat{p}$ decreases with increasing hare frequencies. The results suggest that only a few repeat visits are required to obtain optimal estimates of hare frequencies. However, precise estimates of detectability are not possible with so few repeat visits.

More precise estimates of detectability can be achieved under the assumption that detectability does not depend on habitat type. Table 3 presents the total optimal allocation to repeat visits under this more parsimonious assumption. Here, all repeat visit sites are used to estimate the common detectability. Comparing the results in Tables 2 and 3, we see that the use of the pooled estimate of detectability results in a substantial reduction in variance. Again, the optimal allocation increases and the variance of $\hat{p}$ decreases with increasing hare frequencies.

Table 2. Optimal allocation of repeat sampling effort per stratum for different snowshoe hare frequencies, together with the optimal variance. Hare frequencies are estimated under the assumption that detectability depends on habitat type.

p	$\hat{n}_3$	$\mathrm{var}(\hat{p})$
0.010	2	0.000174
0.020	2	0.000350
0.060	4	0.001062
0.100	5	0.001755
0.125	6	0.002175
0.200	7	0.003336

Table 3. Optimal total allocation of repeat sampling effort for different snowshoe hare frequencies, together with the optimal variance. Hare frequencies are estimated under the assumption that detectability does not depend on habitat type.

p	$\hat{n}_3$	$\mathrm{var}(\hat{p})$
0.010	6	0.000091
0.020	8	0.000189
0.060	14	0.000605
0.100	18	0.001041
0.125	20	0.001319
0.200	25	0.002152

Since greatest variances occur when hare frequencies are large, it was decided that the optimal allocation to repeat visits should be based on a hare frequency of $p = 0.2$, the highest likely hare frequency to be observed. Therefore, we attempted to visit 24 sites for repeat visits. Optimal spatial predictions are achieved for regular sampling designs, for which no location in the study region is far away from a sample site. A total of 60 sample sites were selected from each of the strata, under the constraint that no pair of sample sites is located within 10 km of one another. An extra 6 sites per stratum were included as alternates. These sites were intended to replace sample sites, where permission to sample was not granted. Figure 5 is a map of the locations of the sample sites. Note that the sample sites are evenly dispersed throughout northern Pennsylvania. Moreover, the samples from the various habitat strata are well mixed.

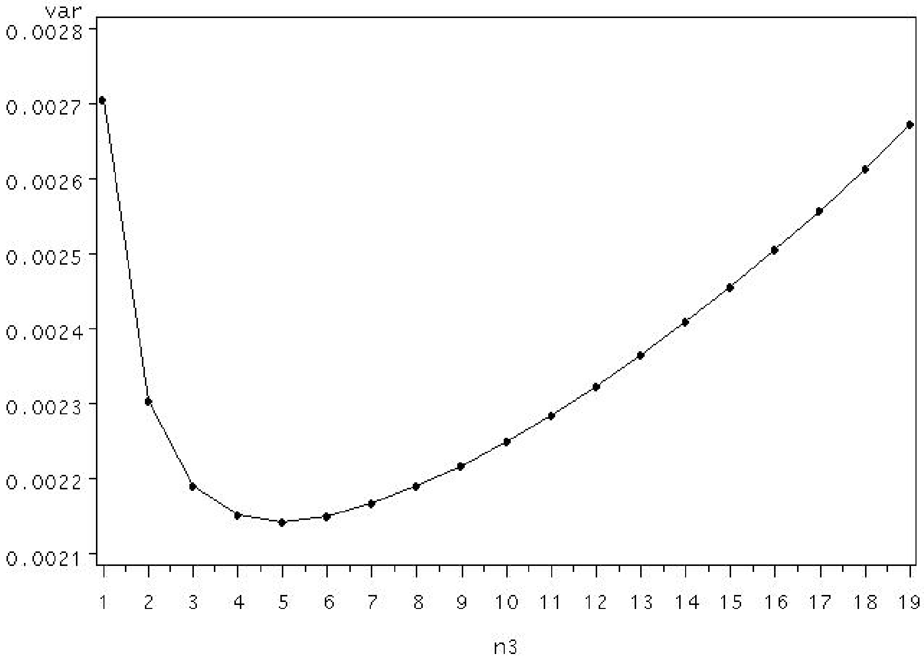

Figure 4. Plot of the variance of $\hat{p}$ against n_3 for $p = 0.125, q=0.5,$ and $n_1 = 40$.

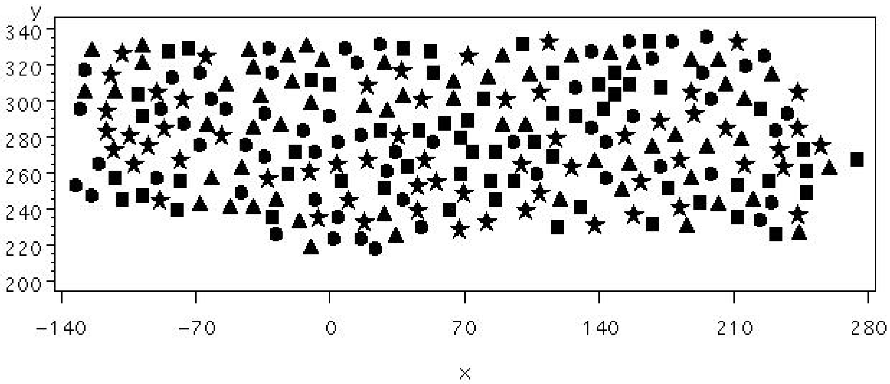

Figure 5. Distribution of sample sites. The circles are in deciduous forest, the squares in mixed forest, the triangles in transitional, and the stars in evergreen forest.

The absence of snowshoe hares in a particular location may be due to dispersal limitation and/or habitat unsuitability. It is thought that snowshoe hares favor the scrub/shrub habitat, so the distribution of snowshoe hares in scrub/shrub habitats may yield better information regarding the distribution of snowshoe hares in northern Pennsylvania. Therefore, at each of the non-scrub/shrub habitat sites, a search will be conducted for any scrub/shrub habitat that may occur within 1 km of the sample site. A second, paired sample site will be established where such habitats are available. If snowshoe hares are absent in both sites, then the absence of hares in the non-scrub/shrub site could be attributed to dispersal limitation. If hares are present in the paired scrub/shrub site, then their absence may be attributed to habitat unsuitability.

Field Protocols

Field sampling protocols were developed to maintain consistency across the study area. Each technician was provided a Compaq palm computer (iPAQ) with an integrated GPS receiver (Diefenbach et al. 2002), DeLorme Pennsylvania gazetteer, aerial photographs and/or topographic maps, Whirl-pac bags for pellet samples, permanent markers, surgical gloves, clipboards and datasheets, a picnic cooler and coolpaks for storing pellet samples, tire chains, a shovel, a pair of snowshoes, an a compass. The iPAQ was prepared to run ArcPad programming. Coordinates of sample sites were uploaded to the iPAQ prior to the field season. Each of the 240 randomly generated sites were uniquely numbered. However, upon a visit to sites, technicians were to give each a unique name using topographic or named locations nearby. The site name was listed on the log sheet and coordinates were provided for the starting point of the transect. This served as a backup in case data was lost from the iPAQ. Also provided on the log sheet was the gazetteer map number for reference with a corresponding mark in the gazetteer for the position of the sampling site. Pellet samples were to be kept cool or frozen in picnic coolers during time in the field. Samples could be kept outside if temperatures permitted; however, coolpaks or ice may have been required if temperatures were too warm. Track surveys and pellet collections, when possible, were conducted ≥12 hours after a snow event. Pellet collections were best conducted after fresh snow events, but could be conducted at any time, even without snow cover. Track surveys will provide the best data immediately after a fresh snow event.

We were granted permission to access Department of Conservation and Natural Resource (DCNR) state forest lands at our discretion and could obtain access to gated roads if we contacted the District Office prior to arrival. Permission for access to State Game Lands, State Parks, and Allegheny National Forest was not necessary. Permission to access private property needed to be obtained by technicians at the time of site visits. We provided landowners a brochure explaining the technicians were from Penn State conducting a track survey and collecting pellets of snowshoe hares and rabbits, they were taking nothing besides pellets from the property, were leaving nothing behind, and the entire time spent on property shouldn't exceed 2-4 hours.

Many sites were remote and access was from poorly maintained roads. Four-wheel drive and tire chains were necessary in many situations. We returned at a later date when roads were too icy or deep with snow. At the end of each day, technicians checked in via phone to confirm they had safely left the field and reported which sites were planned for the next day. Conducting surveys required parking as near as possible to the starting coordinates. Technicians then navigated to the start of the transect using the iPAQ unit or a GPS. Transects were one kilometer long, although not all transects were same shape or orientation to make the entire 1 km of each transect fit within a single habitat type. Transects were walked as displayed on the iPAQ, as closely as possible. Heavy conifer canopy cover or steep hillsides obscured satellite signals, causing loss of location precision in which case more deviation was expected. If satellite reception were poor, technicians may have waited until satellite orientation provided greater precision.

The spatial data from which habitat type was derived is nearing 10 years old, which may lead to differences between cover type as indicated by the iPAQ and what was found on the ground. At the starting point of a transect, the habitat type surrounding the surveyor was verified to be the same as that recorded in the iPAQ. Particularly for evergreen and transitional habitats, if they were unable to confirm the habitat type, additional hiking and, if necessary, more driving was performed to locate a stand or patch of the same habitat type within 5 km capable of containing a transect. A survey was then conducted in the located patch. Visiting habitat types equally throughout the survey season was important, but one of each type does not need to be done each two days or so. Throughout the season how many plots in each habitat type had been visited was reevaluated and visitation rates were adjusted accordingly.

Along the transect all sign was recorded in real time using the iPAQ stylus and data forms for tracks and pellets observed. Investigation of probable tracks, seen from the transect, to confirm species was permitted but wandering throughout the stand was not. Keeping transect lengths approximately equal among habitat types was critical, so individual transect lengths were kept as close to 1 km as possible. Keeping vigilant for pellets was imperative since hare and rabbit pellets typically are not deposited in groups (as in white-tailed deer). Rather they are deposited individually so will be solitary and more difficult to locate. Hare and rabbit pellets are morphologically indistinguishable so all pellets were collected.

When encountering pellets, surgical gloves were donned to pick up pellets and place them in a Whirl-pac bag. Multiple pellets were placed within a single Whirl-pac, but only from the same site. Those within a few yards of each other likely are from the same animal, but pellets hundreds of meters apart could be from different species. When in doubt, more Whirl-pac bags were used for fewer pellets per bag. A permanent marker clearly indicated on each Whirl-pac bag the site name and number from which they were collected and the date they were collected. This was also recorded on the paper data sheet. Collected pellets were to be delivered to State College regularly (preferably weekly) for storage in lab freezers.

At each incidence of tracks or pellets, recorded data included site number, type of sign located, sample number, general habitat characteristics of the stand, and comments. Habitat characteristics were: cover type (one of the 4 major types), dominant tree species (e.g., hemlock, white oak, red maple), approximate low and high range of diameters at breast height of dominant stems (e.g., 1-3 inches in recent clearcuts, 10-12 inches, etc.), approximate low and high spacing of dominant stems (stem density; e.g., 10-20 feet, 1-3 feet in recent clearcuts) in mature forest types, presence of laurel, presence water (particularly riparian zones, forested wetlands, etc.), and a general cardinal direction for aspect.

DNA Analytical Techniques

Conditions to isolate genomic DNA from hare fecal pellets and amplify a specific gene sequence were determined. Between 1 and 5 µg of DNA can be isolated from single hare fecal pellets using a QIAamp DNA Stool Mini Kit with modifications. Approximately 40 to 50 ng of DNA was amplified in a PCR reaction using a modified protocol from A. I. Kovach (University of New Hampshire). The primers used in the PCR reaction anneal to the threonine tRNA gene and the conserved sequence block of the D-loop and have been described in Litvaitis et al. (1997). The forward primer sequence, CCC TGG TCT TGT AAG CCA GAA ATG G, and the reverse, GGA CCT CAT CCT TGG TCT AC, amplified a 561 base pair product. A gradient of annealing temperatures from 45 to 55 ºC was used to determine which annealing temperature gave greatest specificity and amounts of product.

The forward primer also was synthesized with a 6-FAM at the 5' end in the lab using a Beckman Oligo 1000M synthesizer. Addition of this primer to the PCR reaction in place of unlabeled forward primer produced labeled product, which could be visualized using the ABI 3100 Genetic Analyzer (Foster City, California). Approximately 10 ng of the labeled PCR product was incubated with the restriction enzyme Nla III (New England Biolabs) and BSA at 37 ºC for 2 h. Deionized formaldehyde and ROX (X-Rhodamine) standard (Bioventures, Murfeesboro, Tennessee, USA) was added to 1 µl of the cut products and fragments were separated on a 36 cm capillary array in the 3100. Fragments were analyzed using GeneScan version 3.7.1. Work is ongoing to maximize amplification of the fragments.

Methods for PCR analysis.-- Real Time PCR technology uses a fluorescent reporter that combines amplification of a gene target along with hybridization of a specific probe. The Taqman assay (Applied Biosystems) which uses the 5' nuclease activity of Taq Polymerase is one example of this technology (Livak et al. 1995). This method uses forward and reverse primers of traditional PCR but also requires a fluorescent-labeled probe which anneals to the gene sequence between the two primers. The probe is synthesized with a 5' fluorescent reporter and a 3' quencher molecule. As long as the probe is intact, the presence of the 3' quencher eliminate the emission of the 5' fluorescent molecule. As the amplification of the gene sequence proceeds with the forward primer being extended by Taq Polymerase, the probe which anneals to the strand downstream of the 3' primer binding sequence is displaced by the growing strand. An inherent nuclease activity of the Taq degrades the probe and the fluorescent reporter molecule is released. The release of the reporter molecule from the quenching of the 3' molecule and subsequent excitation of the reporter causes emission of light. The light is detected in instrument used and the amount of light produced is directly proportional to the amount of amplified sample.

Because there are three strands of DNA which must bind, specificity is very high and more than one or two mismatches preclude any amplification or release of the fluorescent reporter. The design of the probe and primers with a higher temperature for the probe than the primers also ensures the specificity.

Primer/Probes.-- The sequences used to design the primer/probe sets for the three lagomorphs were from consensus sequences for the mitochondrial DNA (mtDNA) gene sequences coding tRNA proline and the first 310 base pairs of the D-loop. Primer Express version 1.0 (Applied Biosystems, Foster City, California, USA) was used to select the primer/probe sets. Primers were synthesized using phosphoramidite chemistry in the MerMade 12 (Bioautomation, Plano, Texas, USA) at the Penn State University Nucleic Acid Facility (The Pennsylvania State University, Huck Institute, University Park, Pennsylvania, USA). The snowshoe hare and New England/Allegheny cottontail probe was synthesized with a 5' VIC reporter and 3' TAMRA quencher by Applied Biosystems (Foster City, CA). The eastern cottontail probe was synthesized by Biosearch Technologies (Novato, CA) with a 5' FAM and 3' BHQ1.

Extraction of DNA.-- Fecal pellets were maintained in the freezer of a standard refrigerator until they were brought to the PSU Nucleic Acid Facility where they were stored at -20 F until DNA was extracted. A modified procedure was devised using the QIAamp DNA stool Mini kit.

PCR Protocol.-- DNA extracted from the fecal pellets was added to a master mix containing 10X TaqMan Buffer, $MgCL_2$, dNTPS, moles of primer, moles of probe, and units of TaqMAN Gold polymerase. Samples were assayed in duplicate in a 96 well thin-walled PCR plate in an Applied Biosystems 7300 (Foster City, California, USA). The cycling protocol was separated between the cottontails and hares. The eastern cottontail and the New England/Appalachian cottontail primer/probe sets could be multiplexed and combined in the same assay mix due to the same probe. The snowshoe hare primer/probe set was assayed separately with its own probe.

Maximum Likelihood Estimation of Detectability and Snowshoe Hare Frequencies

This section outlines the statistical procedure for jointly estimating the detectability of snowshoe hare pellets, tracks, and direct observation, as well as snowshoe hare frequencies as a function of habitat type. Let p_i denote the probability that a randomly selected site in habitat type *i* contains snowshoe hares. In addition, let q_{1i} denote the detectability of snowshoe tracks in habitat type *i*, let q_{2j} denote the detectability of snowshoe hare tracks under snow cover type *j*, and let q_3 denote the detectability of snowshoe hares by direct observation. The log likelihood of these model parameters is given by

$$\begin{aligned} L(p,q) = {} & \sum_i (n_{i\cdots} - n_{i\cdot 0000})\log(p_i) + \sum_i \sum_j n_{ij0000} \log\{p_i(1-q_{1i})(1-q_{2j})(1-q_3) + 1 - p_i\} \\ & + \sum_i (n_{i\cdot 1\cdot\cdot} + m_{i\cdot 1\cdot})\log(q_{1i}) + \sum_i (n_{i\cdot 011} + n_{i\cdot 010} + n_{i\cdot 001} + m_{i\cdot 0\cdot})\log(1-q_{1i}) \\ & + \sum_j (n_{\cdot j\cdot 1} + m_{\cdot j\cdot 1})\log(q_{2j}) + \sum_j (n_{\cdot j101} + n_{\cdot j100} + n_{\cdot j001} + m_{\cdot j\cdot 0})\log(1-q_{2j}) \\ & + n_{\cdots\cdot 1}\log(q_3) + (n_{\cdot\cdot 110} + n_{\cdot\cdot 100} + n_{\cdot\cdot 010} + m_{\cdots\cdot})\log(1-q_3), \end{aligned}$$

where n_{ijklm} is the frequency during initial visits to habitat i under snow cover j in which pellets were observed ($k = 1$) or were not observed ($k = 0$), snowshoe hare tracks were observed ($l = 1$) or not observed ($l = 0$), or snowshoe hares were directly observed ($m = 1$), or not observed ($m = 0$). Similarly, m_{ijkl} is the frequency during site revisits to habitat *i* under snow cover *j* in which pellets were observed ($k = 1$) or were not observed ($k = 0$), snowshoe hare tracks were observed ($l = 1$) or not observed ($l = 0$). Note that snowshoe hares were not directly observed during any of the site revisits. The sums $\sum_i$ and $\sum_j$ are over all habitat types, and snow cover classes, respectively.

In the first term in the log likelihood, the log probabilities of snowshoe hare presence in each habitat type are multiplied by the number of initial site visits in which some sign of snowshoe hares (either pellets, tracks, or direct observation); the results are then summed over the habitat types. In the second term, $p_i (1 - q_{1i}) (1 - q_{2j}) (1 - q_3)$ is the probability that snowshoe hares are present in a randomly selected site in habitat i and snowcover type j, but no signs of snowshoe hares were observed, and $1 - p_i$ denotes the probability that there are no snowshoe hares at that site. Adding these probabilities together yields the probability that no signs of snowshoe hares are detected; the log of this probability is then multiplied by the number of sites in which no snowshoe hare signs were detected. In the third term in the log likelihood, the log of the detectability of snowshoe hare pellets in habitat type i is multiplied by the number of visits (either initial or revisits) in which pellets were detected in habitat i. In the fourth term, the number of visits during which no pellets were detected in habitat i, but snowshoe hares are known to be present (either through tracks or direct sighting) is multiplied by the log of 1 minus the detectability of pellets in habitat i. In the fifth term, the number log detectability of snowshoe hare tracks is multiplied by the number of visits during which tracks were observed. In the sixth term, the number of visits during which no tracks were detected under snow cover j, but snowshoe hares are known to be present (either through pellets or direct sighting) is multiplied by the log of 1 minus the detectability of tracks under snow cover j. In the seventh term, the log detectability of snowshoe hares by direct sighting is multiplied by the number of times that snowshoe hares were directly sighted. Finally, in the last term, the number of sites in which no snowshoe hares were directly sighted, but snowshoe hares are known to be present (either through pellets or tracks) is multiplied by the log of 1 minus the detectability of snowshoe hares by sight.

The Newton-Raphson algorithm was used to obtain maximum likelihood estimates of the model parameters. The corresponding, large-sample standard errors were obtained from the inverse of the Hessian matrix.

Results

Hare Sign

We sampled 213 of 240 selected sites (56 conifer, 56 deciduous, 50 mixed, and 51 transitional) and 34 additional woody transitional sites during January–April 2004. Eighteen sites were discarded because they fell outside the defined study area. Nine sites could not be sampled because of treacherous terrain, permission was not granted by landowners, or deep snow. Lagomorph sign was undetected at 144 sites. Lagomorph pellets, tracks, or both were detected at 62 sites. Snowshoe hare sign was positively identified at 18 of these 62 sites. Sign (primarily pellets) at 44 sites either was cottontail sign, or could not be distinguished in the field between hares or cottontails. Sign at 7 sites was too indistinct to classify as lagomorph or non-lagomorph. Lagomorph sign was detected at 12 (including positively identified hare sign at 3) of the 34 additional transitional sites. To estimate detection probabilities, 24 sites with lagomorph sign were resampled by different technicians 1–3 times, with and without snow cover.

Pellets containing snowshoe hare DNA were found in 30 sites, snowshoe hare tracks were sighted in 33 sites, and snowshoe hares were sighted in 2 sites. Hares were directly observed in one of the transitional habitat sites, and one of the mixed conifer/deciduous forest sites. Some sign, either pellets, tracks, or direct observation, of snowshoe hares were observed in 47 sites, or 18.73% of the sites visited.

Pellet frequencies were not independent of habitat (χ^2_3 = 10.03; *P* = 0.0211; unless stated otherwise, *P* values were obtained using Fisher's exact test). Snowshoe hare tracks were most frequently observed in transitional habitats, and least frequently observed in conifer and deciduous forests; intermediate frequencies were found in mixed conifer/deciduous forests (Table 4).

Table 4. Contingency table for snowshoe hare pellets by habitat. Percent of sites where pellets containing snowshoe hare DNA were present are given in parentheses. Note that the habitat was not identified for one of the sites and no pellets containing snowshoe hare DNA were found in this missing site.

	Habitat				
Pellets	Conifer	Deciduous	Mixed	Transitional	Total
Absent	43	55	49	74	221
Present	2	3	7	18	30
	(4.44%)	(5.17%)	(12.50%)	(19.57%)	(11.95%)
Total	45	58	56	92	251

There was only weak evidence to suggest that snowshoe track frequencies depended on habitat (χ^2_3 = 6.84; *P* = 0.0755). Snowshoe hare tracks were most frequently observed in mixed forests, and least frequently in deciduous forests; conifer forests and transitional habitats had intermediate frequencies of snowshoe hare tracks (Table 5).

Snowshoe hare sign (pellets, tracks, or direct observations) were not independent of habitat (χ_3^2 = 10.21; $P = 0.0125$). Sign was most frequent in mixed forests and transitional habitats, and least frequent in deciduous forests; intermediate frequencies of sign were found in conifer forests (Table 6).

Table 5. Contingency table for snowshoe hare tracks by habitat. Percent of sites where snowshoe hare tracks were present are given in parentheses. Note that the habitat was not identified for one of the sites and no pellets containing snowshoe hare DNA were found in this missing site.

	Habitat				
Pellets	Conifer	Deciduous	Mixed	Transitional	Total
Absent	40	55	44	79	218
Present	5 (11.11%)	3 (5.17%)	12 (21.43%)	13 (14.13%)	33 (13.15%)
Total	45	58	56	92	251

Table 6. Contingency table for snowshoe signs by habitat. Percent of sites where snowshoe hare signs were present are given in parentheses.

	Habitat				
Sign	Conifer	Deciduous	Mixed	Transitional	Total
Absent	39	54	41	70	204
Present	6 (13.33%)	4 (6.90%)	15 (26.79%)	22 (23.91%)	47 (18.73%)
Total	45	58	56	92	251

The results summarized above do not take into account sites where no sign of snowshoe hares were detected even though snowshoe hare were present. Therefore, the estimates of frequencies of snowshoe signs (regardless of type) are underestimates that may be considerably below the actual frequencies of snowshoe hares in these habitats. Moreover, differences in the detectability of pellets containing snowshoe hare DNA, and the detectability of snowshoe hare tracks among habitats may account for differences in results obtained in Tables 5 and 6. It should also be noted that snowfall was not independent of habitat (χ_6^2 = 25.02; $P = 0.0003$). Full snow was most frequently observed in transitional and mixed forest habitats, and least frequently in conifer and deciduous forests (Table 7). Neither pellets nor snowshoe hare tracks were observed at any site in which no snow was present. The low frequencies at which snowshoe hare sign was detected in decidous forests might be attributed to the higher frequencies at which no snow was observed in those forests. Among the 31 sites where partial snow was present, only three had pellets and one had snowshoe hare tracks.

Table 7. Contingency table of snow cover type by habitat. Snow cover was not recorded at nine sites.

	Habitat				
Snow	Conifer	Deciduous	Mixed	Transitional	Total
Full snow	29	55	44	79	218
	(65.91%)	(63.16%)	(81.48%)	(85.06%)	(75.62%)
No snow	5	15	6	2	28
	(11.36%)	(26.32%)	(11.11%)	(2.30%)	(11.57%)
Partial snow	10	6	4	11	31
	(22.73%)	(10.53%)	(7.41%)	(12.64%)	(12.81%)
Total	44	57	54	87	242

Detectability

To assess the effects of habitat type and snow cover on the detectability of snoeshoe hare signs, a total of 42 site revisits were conducted. Each of the sites selected for resvits, had at least one sign that hares were present in the initial visit. Among these site revisits, pellets with snowshoe hare DNA were detected in 14 revisits, and snowshoe hare tracks were detected in only 6 revisits. No snowshoe hares were directly observed during the site revisits. Some sign of snowshoe hares, either pellets or tracks, was observed in 16 revisits, or 33.33% of the site revisits.

There was no statistically significant evidence suggesting that the detection of pellets depended on snow conditions (χ_2^2 = 1.94; P = 0.55). However, detectabiltiy of pellets did depend on habitat type (χ_3^2 = 12.29; P = 0.0058). The highest detectability of pellets occurred in the transitional habitat (Table 8). No pellets with snowshoe hare DNA were detected in the seven site revisits in deciduous forests. Moreover, detectability did not differ among the conifer, mixed, and deciduous forest types (χ_2^2 = 2.13; P = 0.36). Therefore, to obtain a reasonably precise estimate, these latter forest types were pooled to obtain a common estimate of detectability (Table 9).

Table 8. Contingency table giving frequencies at which pellets with snowshoe hare DNA were detected as a function of habitat type. Percent detectability by habitat type is given in parentheses.

	Habitat				
Pellets	Conifer	Deciduous	Mixed	Transitional	Total
Absent	5	7	12	4	28
Present	1	0	4	9	14
	(16.67%)	(0.00%)	(25.00%)	(69.23%)	(33.33%)
Total	6	7	16	13	42

Table 9. Estimated detectability as a function of habitat type. Estimates of detectability were pooled over conifer, deciduous, and mixed forests.

Habitat	Detectability	SE
Transitional	0.6923	0.1280
Forest (conifer, deciduous, or mixed)	0.1724	0.0701

There was no statistically significant evidence that detectability of snowshoe hare tracks depends on habitat type (χ_3^2 = 0.0823; P = 1.0). However, there is weak evidence suggesting that detectability of snowshoe tracks depends on snow condition (χ_2^2 = 5.94; P = 0.0798). Tracks were most frequently detected during site revisits when there was full snow on the ground (Table 10). Tracks were only detected once when there was no snow, and were never detected under partial snow conditions. Table 11 presents preliminary estimates of detectability of snowshoe hare tracks as a function of snow conditions, pooling the data over partial and no snow.

The estimates of detectability given in Tables 9 and 11 do not take into account information on detectability available from the initial site visits. The following section considers methods for jointly estimating detectability and snowshoe hare frequencies.

Table 10. Contingency table giving frequencies at which pellets with snowshoe hare DNA were detected as a function of snow conditions. Percent detectability by habitat type is given in parentheses.

	Snow condition			
Tracks	Full snow	Partial snow	No snow	All conditions
Absent	4	3	26	33
Present	2	0	1	3
	(33.33%)	(0.00%)	(3.70%)	(8.33%)
Total	6	3	27	36

Table 11. Estimated detectability of snowshoe hare tracks as a function of snow cover type.

Snow condition	Detectability	SE
Full snow	0.3333	0.1925
No or partial snow	0.0333	0.0328

The estimated detectabilties are presented in Table 12. Snowshoe hare pellets were most easily detected in transitional habitat; the detectability of snowshoe hare pellets in transitional habitat is estimated to be more than three times that in the forested habitats. Snowshoe hare tracks were most detectable in full snow, and were not easily detected when there was no or partial snow on the ground. Not surprisingly, the detectability of this elusive animal by direct sighting is very small, at about 2%.

Table 12. Maximum likelihood estimates of detectabilities of snowshoe hare pellets, tracks, and direct sightings.

Sign	Condition	Percent detectability	S.E.
Pellets	Transitional habitat	65.91	9.54
	Forested habitat	20.96	5.54
Tracks	Full snow	38.13	5.94
	No or partial snow	9.27	9.00
Direct sighting		2.00	1.41

Occurrence by Habitat Type

When corrected for detectability, snowshoe hares are estimated to be most frequent in mixed forests, with an estimated percent frequency of approximately 38% (Table 13). Snowshoe hares are also common in transitional forests, where they are estimated to occur in approximately 27% of such sites. Snowshoe hares are least abundant in deciduous and conifer forests.

Table 13. Maximum likelihood estimates of the percent frequencies at which snowshoe hares occur in different habitat types.

Habitat	Percent Occupied	S.E.
Conifer	15.93	6.57
Deciduous	5.66	3.28
Mixed	38.09	10.44
Transitional	26.77	6.31

Distribution

Based on harvest data from the Pennsylvania Game Commission (Figure 1), the range of snowshoe hares in Pennsylvania includes counties of the northern tier of the state and extends south to Maryland in counties that encompass the Laurel Highlands. The largest concentrations of sites where hares were detected were distributed similarly to the harvest data (Figure 6), which were Warren, McKean, Forest, and Elk counties in the west and the Poconos in the east.

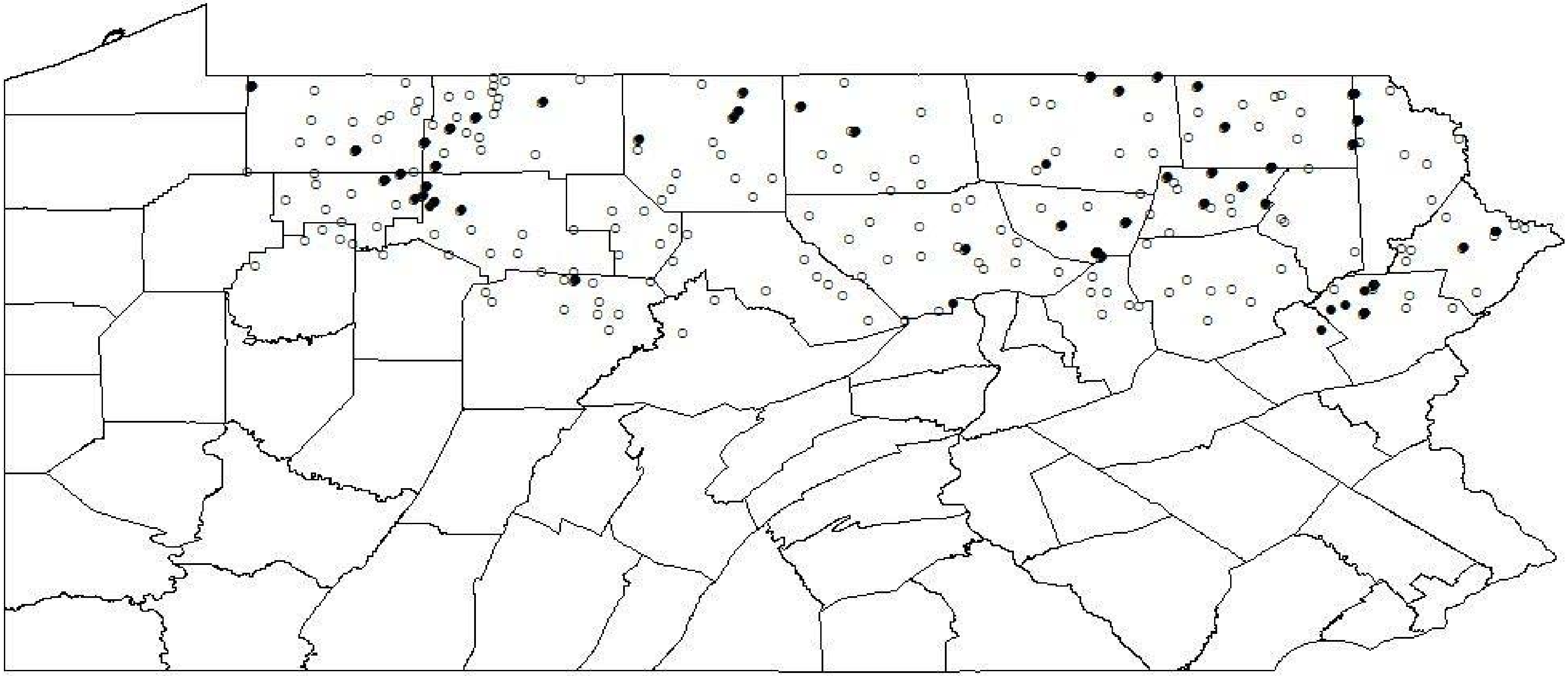

Figure 6. Location of randomly selected sampling sites for the presence of snowshoe hares based on DNA testing of fecal pellets. Open circles represent sites where the species was not detected, solid circles indicate snowshoe hares were detected, Pennsylvania, 2004.

Discussion

Our study shows that it is feasible to use fecal pellet surveys to conduct a statistically-based survey of the distribution of snowshoe hares. However, such surveys still require estimating the probability of detecting the presence of hares whether it is via pellets or other sign. We found that the detection of fecal pellets was dependent on habitat type and varied from 0 – 69% (Table 8).

We were able to estimate the percent of habitat occupied by snowshoe hares, corrected for detection probability, and found that occupancy rates were greater than expected for some habitat types. We anticipated that occupancy rates would be $\leq$20% for the best habitat types, and generally <10% (Table 1), but estimated occupancy rates of 5.6–38.1% among the four habitat types investigated (Table 13).

Despite the greater than expected occupancy rates, however, snowshoe hares are patchily distributed. Moreover, they are much more patchily distributed than indicated by the PGC's Game Take Survey data (Figure 1). Although our results indicated greatest occurrence in northwestern Pennsylvania and the Poconos (Figure 6), similar to Figure 1, the occurrence of hares in the northwest appears to be much more clustered (see Forest, McKean, Warren, and Clarion counties).

Based on our findings that snowhoe hares are patchily distributed and associated with certain habitat types, especially mixed deciduous forest and transitional habitats, we believe we can identify several key threats to the long-term viability of this species in Pennsylvania. These factors are (1) the distribution and connectivity of early successional (transitional) habitat, (2) regeneration problems associated with Pennsylvania's forests, (3) climatic changes, and (4) loss of northern hemlock (*Tsuga canadensis*) to the hemlock woolly adelgid, an Asian insect that causes mortality in northern hemlocks.

The primary threat influencing the existence of Snowshoe Hare in Pennsylvania is loss of early successional habitat and corresponding lack of suitable habitat connectivity (e.g., for dispersal). The amount of early successional forest habitat has been decreasing for decades (Alerich 1993) in Pennsylvania because timber harvest rates have not kept pace with succession of forest vegetation. As a result, the remaining early successional habitats are becoming fragmented. Fortunately, this type of fragmentation does not have to be permanent and can be addressed through proper forest planning and the application of appropriate silvicultural techniques.

Permanent fragmentation of habitat caused by human development is another problem for Snowshoe Hares. For example, counties in the Poconos are now considered part of the New York City metropolitan area because of the number of commuters who reside in these counties, and are experiencing some of the largest population growth and housing development rates in Pennsylvania. These changes are resulting in permanent fragmentation of forested habitats.

Poor forest regeneration caused by acid deposition (Sharpe and Drohan 1999) and browsing by white-tailed deer (Scott and Yahner 1989) likely is having an adverse effect on snowshoe hares.

Glazer (1959) noted that introduced hares were less likely to become established in areas where white-tailed deer reduced available browse. In addition, poor silvicultural practices can adversely affect habitat quality for hares. Properly applied silvicultural practices ensure forest regeneration with high stem densities (e.g., clearcuts or shelterwood cuts), but these techniques often are not applied on private lands. Instead, exploitative logging oftentimes results in too much shade such that tree regeneration is limited or out-competed by invasive species (e.g., ferns). Further, regenerating tree species are usually shade tolerant and may not be preferred browse species.

Finally, climatic changes and invasive species could have significant effects on the viability of snowshoe hares in Pennsylvania. Global warming has already reduced winter severity in Pennsylvania (e.g., currently lakes are ice-covered for about 2 weeks less each winter). With milder winters will come less snow cover, which puts Snowshoe Hares at greater risk of predation because of their white pelage in winter. Some subspecies in the southern range have evolved to forego pelage coloration change (Dalquest 1942 in Murray 2003), but it is unlikely other subspecies would be able to adapt fast enough in response to global warming. The spread of the hemlock woolly adelgid could result in the total loss of the primary species providing conifer cover in Pennsylvania, and has potential consequences for snowshoe hares because they are associated with mixed conifer-deciduous forests. Especially in the Poconos, loss of hemlocks could result in degradation of existing habitats used by snowshoe hares.

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