Evaluating the Prey Base for Lynx: Snowshoe Hare Distribution and Abundance in Glacier National Park

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Ellen Cheng [1]
Dr. L. Scott Mills [1]
Dr. Karen E. Hodges [2]

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[1] University of Montana, Wildlife Biology Program, Department of Ecosystem and Conservation Sciences Missoula, MT 59812
[2] University of British Columbia Okanagan, 3333 University Way, Kelowna, B.C. V1V 1V7, Canada

BACKGROUND AND MOTIVATING QUESTIONS

In 2000, the Canada lynx *(Lynx canadensis)* was awarded protection under the ESA as a Threatened species throughout its range in the lower 48 US states. Glacier National Park (Glacier NP) contains a population of lynx, and is one of the few US Parks known to do so. Because of its location within the Northern Rockies ecosystem, regional efforts to protect lynx will require effective protection of lynx in Glacier NP. In addition to serving as a potential reservoir and corridor for lynx populations beyond the Park, Glacier NP is an ecosystem untouched by logging and still sculpted by wildfires. As such, it can provide insight into how lynx dynamics are influenced by historically important natural disturbance processes, compared to the managed logging and prescribed burns that have replaced wildfires in many US forests.

Currently, data are insufficient to identify important habitat or develop a management plan for Canada lynx in Glacier NP. Personnel have been monitoring the Park's lynx population through informal track surveys for the past 15+ years, with limited results. Sightings of lynx have been infrequent in the Park. However, lynx have occasionally been captured in wolverine traps around Many Glacier, on the Park's east side. In 2000, the National Lynx Survey also identified at least 4 unique lynx individuals in the northeast section of Glacier NP and at least 2 unique individuals in its southeast corner. These data, though sparse, have suggested that lynx in the Park primarily occur east of the Continental Divide and in the 1988 Red Bench burn area in the Park's northwest corner (Fig. 1).

Throughout their range, lynx prevalence coincides with densities of snowshoe hares *(Lepus americanus),* their primary prey (Koehler 1990; Parker et al. 1983; Ruggiero et al. 2000; Squires and Ruggiero 2007). The fundamental link between snowshoe hare densities and Canada lynx abundance, survival, and productivity was recognized by the US Fish and Wildlife Service with its inclusion of snowshoe hares as an essential component of critical habitat for Canada lynx. Thus, to understand lynx distribution and dynamics requires knowledge of hare distribution and dynamics.

In addition to their role as primary prey of Canada lynx, snowshoe hares are also important as a strongly interacting species in boreal forests (Ruesink et al. 2002). In addition, hares have become a sentinel for evaluating climate change effects, due to the potential mismatch between their seasonal coat color change and a reduction in snow cover (Mills et al. In Prep; Huang 2009).

From 2005 to 2007, we conducted an intensive field survey of snowshoe hares in Glacier NP. We had three primary objectives. The first was to identify the distribution and abundance of snowshoe hares in the Park, and what factors may influence where they occur. In particular, we were interested in understanding whether infrequent reporting of lynx on Glacier NP's west side may be due to low densities of snowshoe hares there. Forests on the west side are primarily mixed conifer stands. Regenerating stands from the 1988 Red Bench fire occur in a mosaic with mature multistory stands. Forests on the east side are patchier, with multistory conifer stands interspersed among shrub fields, aspen stands, fescue grasslands, and subalpine meadows. Our research investigated whether snowshoe hare densities are higher in the east than in the west, and addressed related questions of how landscape patterns affect snowshoe hare abundance and distribution in Glacier NP.

Our second objective was to assess how wildfires and post-fire regeneration affect hares. Large-scale wildfires occur regularly on Glacier NP's west side, with an average 2,000 hectares burned each year since 1988. In 2003, the largest fire year for Glacier NP since 1910, approximately 13% of the Park's more than 410,000 hectares burned in wildfires. Understanding how snowshoe hares respond to post-fire regenerating habitats would allow the Park to assess likely impacts of this and future fires on snowshoe hares and lynx. Our research focused on hare use of regeneration from the 1988 Red Bench Fire, another major fire that had burned over 11,000 hectares of primarily lodgepole pine *(Pinus contorta)* forest in the Park's northwest corner.

Our third objective was to develop a cost-effective non-invasive genetic approach to abundance estimation that could be of benefit to national parks and other areas surveying snowshoe hares and other moderately abundant species. The field and laboratory work for this component of the study continued into 2011, with additional funding provided by Animal Welfare Institute. We present the results of this method development and cost-benefit analysis as Appendix I to this report.

Although little was known about snowshoe hares in Glacier NP when this study began, many studies have examined snowshoe hare habitat use in other parts of the species' range, especially in relation to forestry practices. From these studies, we know that snowshoe hares use a variety of habitat types, with understory cover being one of the most important elements to them (Hodges 2000a, 2000b). Researchers have found that stands with shrubs, stands that are densely stocked, and stands at ages where branches have more lateral cover are more heavily used by hares (Black 1965; Dolbeer and Clark 1975; Koehler 1990; Litvaitis et al. 1985; Monthey 1986; Walker et al. 2011; Wolfe et al. 1982). Overstory cover is sometimes correlated with hare habitat use patterns, but typically in cases where it is also significantly correlated with understory cover (Orr and Dodds 1982; Parker et al. 1983; Richmond and Chien 1976; Rogowitz 1988). The species composition in a stand appears to be less correlated with hare habitat use than is understory structure (Ferron and Ouellet 1992, Griffin and Mills 2007). Stand age per se does not appear to be critical, again because of the importance of stand structure.

Particularly in their southern range, where habitats are patchy and fragmented, landscape-level factors and metapopulation dynamics may be important for persistence of snowshoe hare populations (Griffin and Mills 2003, 2009; Wirsing et al. 2002). Habitat interspersion may be valuable to hares by providing them access to habitats with different protective abilities and food availabilities (Koehler and Brittell 1990; Krenz 1988; Thomas et al. 1997). In Minnesota, hare habitat use was more correlated with habitat interspersion than with stand type, and hares used the edges more than centers of the heavily used stand types (Conroy et al. 1979). Habitat patchiness can also create source-sink dynamics, dampening landscape-scale population growth (Griffin and Mills 2009), and landscape structure around a patch can influence local dynamics (Walker et al. 2011).

Although there are few field studies of hares' use of burned stands, several authors have suggested that fire contributes to the cycling of hare populations, by transiently providing dense stands of regenerating forest rich in food and cover for hares (Ferron and St- Laurent 2007; Fox 1978; Grange 1965; Howell 1923). Our recent work on snowshoe hares in Yellowstone National Park (Hodges et al. 2009) found three of our five highest hare density

sites occurred in lodgepole pine stands regenerating from Yellowstone's 1988 fires. Hare densities in regenerating stands were positively correlated with sapling density and understory cover.

Much of our understanding of hare-habitat relationships comes from studies of forests managed at least in part for timber production. We investigated whether hares would be more abundant in the more patchy forests of Glacier NP's east side, compared to the more closed canopy forests west of the Continental Divide. Also, we tested for effects of understory structure and amount of forest edge habitat in the unharvested landscape of Glacier NP. Finally, we tested whether hares would have higher densities in the regenerating forest from the 1988 Red Bench Fire.

METHODS

Site Selection

We surveyed three types of study sites: Random Unburned, Targeted Unburned, and Red Bench Fire sites. All study sites met these criteria: 1) at least 85% forest cover, 2) did not occur in areas burned since 2001, 3) at least 500 m from the nearest road, 4) did not overlay Park hiking trails, and 5) at least 300 m from the nearest study site. We set a minimum threshold of 85% forest cover because snowshoe hares are obligate forest species infrequently found in large open areas (Hodges 2000a, 2000b). For the same reason, we did not survey recent burns. Remaining criteria were set to minimize potential impacts of anthropogenic disturbances, and to maximize independence of sites.

For Random Unburned sites, we used a tessellated random sampling approach to ensure unbiased selection of sites, but fairly even coverage of the full extent of the Park. We used an existing geographic information system (GIS) layer that gridded the Park into 46 large sampling blocks corresponding to USGS Digital Orthophoto Quadrangle maps. Each year of the study (2005–2007), we used Hawth's Analysis Tools (www.spatialecology.com/htools/tooldesc.php) to randomly allocate 3 points to each of the 46 sampling blocks. Using GIS, we superimposed a 20-ha study site (500 m X 400 m) centered on each of these points. We then excluded all sites that did not meet the five site criteria listed above. With this method, the number of sites per sampling block was correlated with forest cover, producing a relatively even distribution of sites across Glacier NP's forested habitats, which comprise 55% of the Park.

Targeted Unburned sites were identified by Park officials as areas likely to support snowshoe hares, based on previous sightings. We ground surveyed these areas and selected sites with high densities of snowshoe hare pellets. These sites were used to pilot test a protocol for non-invasive genetic abundance estimation of snowshoe hares (Appendix I), and to provide additional data for characterizing high hare density habitats in Glacier NP.

Red Bench Fire sites were randomly selected points within the area delineated by GIS as the 1988 Red Bench Fire. To ensure a sufficiently large sample size for characterizing hare use of regeneration, we sampled these sites at a higher density than Random Unburned sites.

We attempted to survey as many pre-selected sites as possible each year, and to cover the Park widely. However, for logistical and safety reasons, we excluded sites that required

potentially dangerous river crossings as well as sites that required more than a two days' hike to access and could not be combined with nearby sites in a multiple day backcountry survey trip. Within a field season, we could only complete a subset of pre-selected sites. We prioritized sites that could be more easily accessed as well as sites that filled spatial sampling gaps. Except for sites that were live-trapped, each site was surveyed a single time.

When we arrived at a site, we conducted a ground reconnaissance to verify the site met all criteria (in particular, 85% forest cover could not be accurately determined from GIS at the scale of our study site). When necessary, we used a predefined field protocol to objectively shift sites (by <500m) that did not meet criteria.

Fecal Pellet Counts and Habitat Measurements

A detailed field protocol is provided in Appendix II. We conducted field work May to August in 2005, 2006 and 2007. Each 20-ha study site was divided into an 8 X 10 grid, with 80 sampling points and 50-m spacing between points. We averaged fecal pellet counts across 80 sampling points per site to index hare abundance (Hodges and Mills 2008; Krebs et al. 2001; Krebs et al. 1987; Mills et al. 2005). At every sampling point, a 0.155 m^2 rectangular transect was laid due north from a random start. All pellets that fell at least one-half within the transect area were counted.

Along three internal grid rows of each site (2^{nd}, 4^{th}, and 7^{th} rows), we conducted vegetation surveys at 50-m spacing, for a total of 30 vegetation sampling points. Vegetation surveys focused on overstory and understory habitat variables well-established as correlates with hare densities. For overstory habitat, we recorded the dominant canopy species as the species for which the canopy covered the largest percentage of our fish-eye view at each point. We estimated canopy closure as % view obstructed (viewed through a vertical PVC tube) at the sampling point and at 6 m east and 6 m west of the sampling point. For understory habitat, we measured understory cover, sapling density, and an index of downed logs. We used a 0.532-m^2 cover board, vertically divided into four blocks, to measure understory cover. A person standing at the sampling point estimated % view obstructed at 0.5-m increments above the ground, while the cover board was held at 6 m east and also at 6 m west of the sampling point. Sapling density was calculated as the number of live saplings with trunk centerline within a 2-m radius of the sampling point. Saplings, defined as trees with <5 cm diameter-at-breast-height, were recorded to species and categorized into three height categories: 0.5 – 1.5 m, 1.5 – 3 m, and >3 m. Our index of downed logs was the number of downed logs >5 cm diameter encountered along a 12 m east-west transect centered on the sampling point.

Characteristics of the surrounding landscape may influence snowshoe hare density within a study site (Shick 2003; Thomas et al. 1997; Walker et al. 2011). As a rough index of landscape heterogeneity around hare study plots, we used Glacier NP's 2007 GIS vegetation coverage to calculate linear distance of edge habitat (defined as the boundary between NVC2_L1X forest and all other habitats) within a 600 m and a 1000 m radius circle centered on each study site (Hop et al. 2007).

Statistical Analyses

Average pellet count for each site was converted to hare density, using a standard regression equation modified for single-survey (i.e., uncleared) pellet transects (Hodges and Mills 2008; Hodges et al. 2009). Because pellet counts are a relatively coarse index of hare

abundance, especially at densities below 0.3 hares/ha (Mills et al. 2005), we used pellet counts to categorize sites as low (< 0.3 hares/ha) or moderate (≥ 0.3 hares/ha) hare density. In this context, the <0.3 hares/ha threshold is used to describe areas where hares are functionally absent, persisting well below a threshold likely to support lynx (e.g., 0.5 hares/ha; Ruggiero et al. 2000). Likewise, our designation of "moderate" density as ≥ 0.3 hares/ha is a relative term about where lynx might be supported in conjunction with alternate prey. For comparison purposes to show that ≥0.3 is a moderate density for this part of the hare's range, we considered our density estimates based on capture-mark-recapture of 33 sites within 200 km of GNP that we have sampled for up to 12 years between 1998 and 2010 (for a total of N = 198 site-years of density estimates) (Mills et al. 2005; Mills and Hodges, unpublished data). For these 198 site-years across a variety of climate conditions, land uses, structure types, and landscape contexts, hare densities ranged from 0.03 to 3.38 hares/ha, with mean = 0.69 (SE = 0.043) and median = 0.53.

With the GIS data available when this study commenced, it was difficult to identify forest stands by dominant canopy species during the initial GIS-based site selection process. Therefore, we used GIS simply to identify forested habitats, and subsequently used field-collected data to classify sites into broad categories of dominant canopy species for analyses:

1) Subalpine fir-Engelmann spruce (ABLA_PIEN*, Abies lasiocarpa-Picea engelmannii*)—Sites predominantly comprising one or a mixture of both species.

2) Lodgepole pine (PICO, *Pinus contorta*)—Lodgepole pine was the dominant canopy species at 70% or more of sampling points in the site.

3) Lodgepole pine saplings (PICOsap) —Regenerating lodgepole pine forests with few canopy trees. All PICOsap sites were burned in the 1988 Red Bench Fire, but not all Red Bench Fire sites fell in this category because some regenerating stands had achieved mixed canopies or the canopies were dominated by a species other than lodgepole.

4) Mixed canopy (MIXED) —Sites were MIXED if no single species was recorded as the dominant canopy species at 70% (or more) of sampling points in the site.

5) Other single-species stands (OTHERS) —Sites dominated by a single canopy species other than lodgepole pine, subalpine fir, or Engelmann spruce. Primarily, these stands were dominated by either Douglas fir *(Pseudotsuga menziesii)* or western hemlock *(Tsuga heterophylla)*.

To explore hare habitat use, we conducted univariate comparisons of habitat variables versus hare density category (moderate or low). We used Fisher's Exact Test to determine if moderate versus low hare density sites differ by dominant canopy category, site type (Random Unburned, Targeted Unburned), or by location east versus west of the Continental Divide. The Wilcoxon Rank Sum Test was used to determine if moderate versus low hare density sites differ in habitat characteristics measured as continuous variables: % canopy, downed logs, understory cover, and forest edge habitat (600 m and 1000 m radius).

We also combined habitat variables in a logistic multiple regression with hare density category as the response variable. Based on results of univariate analyses, which suggested PICOsap was the only dominant canopy category that influenced hare density, we included

dominant canopy as a single variable coded as 1 if the site was PICOsap and 0 if the site was not PICOsap. We compared information criteria (Akaike's Information Criterion and Bayesian Information Criterion) and two cross-validation approaches (delete-d and K-fold) for selecting the best model, as implemented in the R statistical package, bestglm (McLeod and Xu 2010). Bayesian Information Critierion (BIC; Schwarz 1978) applies a higher penalty to parameters, so often selects more parsimonious models than Akaike's Information Criterion (AIC; Akaike 1974).

Cross-validation methods identify the best model for each of p = 0 (intercept only) to the maximum number of model parameters, then use cross-validation to select the predictive model with the smallest deviance. Delete-d uses a much larger validation set than typically used in K-fold cross-validation, and (unlike K-fold) its predictive ability appropriately converges to 1 as the number of observations increases (Shao 1993). For delete-d cross-validation, our data were analyzed with a training set of 31 sites and a validation set of 89 sites, as recommended for a sample size of N = 120 sites (Shao 1997). Results were based on 1000 replications. For K-fold cross-validation, our data were analyzed with a training set of 90 sites (K = 4) and a validation set of 30 sites. One study site was excluded from logistic regression and model selection analysis because sapling density data were not available.

RESULTS

We conducted pellet counts and vegetation surveys at 121 sites in Glacier NP. Of these, 20 were regenerating lodgepole pine forest from the Red Bench Fire, 92 were Random Unburned sites, and 9 were Targeted Unburned sites. Almost one-third (N = 42) of the 121 sites were MIXED canopy sites, with no single canopy species dominating. Almost a quarter (N = 28) were dominated by lodgepole pine. We distinguished between mature lodgepole pine forests (PICO, N = 16) and regenerating forests primarily comprising saplings <10 m tall (PICOsap, N = 12) because habitat characteristics were very different for these categories. Over a quarter (N = 28) of sites were mature subalpine fir-Engelmann spruce forests. The remaining 13 sites were categorized as OTHER—these sites were dominated by Douglas fir *(Pseudotsuga menziesii)*, western hemlock *(Tsuga heterophylla)*, or other less represented species. PICOsap was our only category of regenerating forest; all other categories were mature forest with extensive canopy cover. Our PICOsap sites occurred only in the Red Bench Fire area in the northwestern corner of the Park. Other dominant canopy categories were well distributed throughout the Park (Fig. 2).

Hare densities were low in much of the Park. We found no snowshoe hare pellets on 14% of study sites. Only 18% (N = 22 of 121) of sites had estimated hare densities greater than 0.3 hares/ha. Less than 7% (N = 8 of 121) of sites had hare densities exceeding 0.5 hares/ha, a commonly cited minimum threshold for an area to sustain a lynx population (Ruggiero et al. 2000). Five of the 8 sites that exceeded 0.5 hares/ha occurred in regenerating lodgepole forests of the Red Bench Fire; the other 3 sites were Random Unburned sites east of the Continental Divide (Fig. 3).

Among Random Unburned sites, the proportion of moderate density sites (≥ 0.3 hares/ha) was greater east of the Continental Divide (20% of 35 sites) than west of the Continental Divide (7% of 57 sites; Fisher's Exact Test, N = 92, p = 0.10). All Red Bench Fire sites occurred

west of the Continental Divide, and had a relatively high proportion (35% of 20 sites) of moderate hare density sites. When Random Unburned and Red Bench Fire sites were combined in analysis, we found no significant difference in hare density category east or west of the Continental Divide (Fisher's Exact Test, N = 112, p = 0.58).

The proportion of moderate hare density sites was significantly greater for Red Bench Fire sites than for Random Unburned sites (35% compared to 12%, Fig. 4; Fisher's Exact Test, N = 112, p = 0.018). Not surprisingly, Targeted Unburned sites were also more likely than Random Unburned sites to support moderate hare densities (Fisher's Exact Test, N = 101, p = 0.026).

The frequency of moderate hare density sites differed significantly among dominant canopy categories (Kruskal-Wallis Rank Sum Test, $\chi^2 = 17.2$, p = 0.0015), with PICOsap stands supporting a higher percentage of moderate hare densities than any other stand type (Fig. 5). PICOsap was the only dominant canopy category with moderate hare densities at more than half of sites surveyed. With PICOsap excluded, we found no significant differences in the frequency of moderate hare densities among dominant canopy categories (Kruskal-Wallis Rank Sum Test, $\chi^2 = 3.4$, p = 0.33).

All PICOsap sites were regenerating lodgepole forests from the Red Bench Fire, but the Red Bench Fire also included sites categorized as MIXED and OTHERS (Fig. 6). Among Red Bench Fire sites, only those categorized as PICOsap had moderate hare densities (Fig. 7). Subalpine fir-Engelmann spruce (ABLA_PIEN) sites made up more than one-third of the Unburned (Random and Targeted) sites surveyed. For Unburned sites, the distribution of dominant canopy categories did not clearly differ between moderate and low hare density sites (Fig. 8).

We used univariate analyses to examine differences in vegetation characteristics among dominant canopy categories, with particular focus on how PICOsap differed from other categories. The three categories of understory cover above 0.5 were highly correlated ($r > 0.78$), so were collapsed into a single variable (understory cover 0.5 – 2 m) for all analyses. We also combined all sapling data (species and size classifications) into a single measure of average sapling density per site. The regenerating lodgepole pine forests that characterized PICOsap had lower % canopy, more downed logs, higher sapling density, higher understory cover at 0.5 - 2 m above ground, and more edge habitat than other dominant canopy categories (Table 1). Most striking was the difference in sapling density—which was, on average, more than an order of magnitude greater in PICOsap than in other dominant canopy categories.

We explored differences in vegetation characteristics between moderate and low hare density sites, separately for Red Bench Fire and Unburned sites. Based on univariate analyses of Red Bench Fire sites, we found significantly higher sapling density and forest edge in moderate- compared to low-hare density sites (Fig. 9, Table 2, Wilcoxon Rank Sum Test, p = 0.0005 - 0.046). Among Unburned (Random and Targeted) sites, understory cover at 0 – 0.5 m above ground was significantly lower in moderate- compared to low-hare density sites (Fig. 10, Table 3, Wilcoxon Rank Sum Test p = 0.005). Sapling density tended to be higher in moderate hare density sites (p = 0.083).

Multivariate analyses confirmed the overriding importance of Red Bench Fire regenerating lodgepole forests in predicting moderate hare density sites in Glacier NP. Whether or not a site was categorized as PICOsap was the one habitat variable that was identified by all multivariate methods as an important predictor of hare density category (Table 4). It was the only variable included in the best-fit models based on cross-validation. BIC model selection identified two additional variables—understory cover 0 – 0.5 m and East-West category—as important predictors. Sites with low understory cover 0 – 0.5 m and sites east of the Continental Divide were more likely to have moderate hare densities. AIC model selection included these three variables as well as % canopy and sapling density in the best-fit model, but the only statistically significant variables were those shared with the BIC model.

DISCUSSION

Snowshoe hares occur at low densities and are patchily distributed in Glacier National Park, with highest densities found in regenerating lodgepole forests of the 1988 Red Bench Fire. Red Bench Fire sites were three times more likely to support densities ≥ 0.3 hares/ha than were randomly selected unburned sites. Given the well-known association between snowshoe hares and early- to mid-stage lodgepole pine regeneration (recently reviewed in Ellsworth and Reynolds 2006) we expect regenerating forests of the Red Bench Fire to continue supporting moderate hare densities and lynx over the next decade. Indeed, during our 3-year study, our field crew reported two lynx sightings, both in Red Bench Fire study sites.

Overall, hare densities in the Park were higher east of the Continental Divide. This pattern was not significant in univariate analyses, but the (east-west) location of a site was included in best-fit AIC and BIC models for predicting hare density. However, as regenerating forests from Glacier NP's west side 2003 fires (which burned forty times more area than the Red Bench Fire) mature to stages favorable for hares and lynx over the next several decades, Glacier NP may observe a shift in distribution of its hare and lynx populations to take advantage of transiently favorable food and cover conditions west of the Continental Divide.

Within Glacier NP's unburned forested habitats, moderate hare densities occurred most frequently in mature conifer forests along the eastern and southern boundaries of the Park—particularly in the areas of Many Glacier, Two Medicine, and Railroad Creek. The regions of moderate hare density identified by our random sampling of the Park corresponded well with areas of lynx occurrences and also matched areas Park biologists had independently identified as snowshoe hare hotspots.

In univariate analyses, hare densities in the Park were positively associated with sapling densities. This association was statistically significant in Red Bench Burn sites and nearly significant in Unburned (both Random and Targeted) sites. In some analyses we also found a significant negative association between hare densities and understory cover at 0 – 0.5 m (the height at which a hare would travel) and a positive association between hare densities and forest edge within a 600 m and 1000 m site radius. However, high sapling density and classification as a Red Bench Fire site were the two variables that most consistently predicted moderate hare densities.

The strong association of snowshoe hares with regenerating lodgepole pine in Glacier NP is consistent with findings from many other studies (Koehler et al. 1979; Malloy 2000; Miller 2005; Sullivan 1984; McKelvey and McDaniel 2001). More surprising was the lack of strong positive association between hare densities and understory cover, as is often reported (reviewed in Hodges 2000a, 2000b). In this study, the lack of a strong positive association between hare densities and understory cover, as measured by horizontal coverboards, may be due in part to changes in shrub and other deciduous foliage density during the course of each field season (May – August). These seasonal changes meant a study site could yield different understory cover measurements depending on which month of the growing season it was surveyed. In comparison, sapling density (which was significantly associated with hare density in this study) was a potentially more stable metric of year-round understory cover, as well as an important measure of winter food availability.

Overall, the range of hare densities recorded in Glacier National Park are within the range of other parts of the hare's southern range (e.g., 0 to 2.7 hares/ha for US Rocky Mountains; Ellsworth and Reynolds 2006), but somewhat lower than other areas we have studied in the Northern Rockies region. In this study, we find in GNP that only 18% (22/121) of sites had hare densities >0.3 hares/ha, and less than 7% (8/121) had densities >0.5 hares/ha; notably, 9 of the 121 sampled sites were subjectively chosen to represent the best hare habitat in the Park (based on observations by Park biologists). By contrast, we find in our other study area in Montana and Washington that at least 50% of sites tend to have hare densities exceeding 0.3 hares/ha (e.g., medians 0.3 or greater). From 1998 to present, at multiple sites in 3 regions within 300 km of Glacier National Park, we have estimated abundance using either live trapping or pellet plots converted to density as in this study. If we average across multiple years of sampling at each site to calculate a site mean, including site/year combinations with 0 hares, we find summer densities of:

- Okanagan National Forest (north-central Washington), 2003 and 2004: median = 0.67; N = 76 sites; pellet sampling (Walker et al. 2011).
- Seeley Lake Ranger District, Montana, 1998–2010; median = 0.28 hares/ha; N = 25 sites; live trapping (Mills et al. 2005; Mills unpublished data).
- Tally Lake Ranger District, Montana, 2001–2009; median = 0.9 hares/ha; N = 8 sites, live trapping (Mills and Hodges, unpublished data).

In short, Glacier National Park's relative hare density appears well below the densities in complexes of sites we have sampled in WA and MT within 200km of GNP. On the other hand, Glacier hare densities are well above what we found in Yellowstone National Park (Hodges et al. 2009), an area of poor hare habitat, where 38% of sampled stands had no pellets at all (nearly 3 times the rate of zeros as in this GNP study).

The high variability in hare densities at Red Bench Fire sites primarily reflects differences in forest regeneration rates, which vary at a fine scale due to topographic relief, soil moisture, and other habitat factors (Baumgartner et al. 1984; Koch 1996). In a study concurrent with our Glacier work, we found a similar patchiness in hare distribution among lodgepole pine forests burned in Yellowstone National Park's 1988 fires (Hodges et al. 2009). At a landscape scale, differences in forest regeneration rates can translate to differences in optimal post-disturbance age for supporting hares and lynx. Studies in the western US have found highest

hare densities correlated with regenerating forests ranging from 20 to 67 years old (Koehler et al. 1979; Zimmer 2004; Miller 2005). Regardless of when the optimum sapling densities and understory conditions are reached, snowshoe hares' association with regenerating lodgepole pine forests is transient. As lodgepole pine forests mature, the trees self-prune their lower branches, creating a more open understory. Thus, the distribution of snowshoe hares in Glacier NP will change over time, particularly with the changing mosaic of post-fire succession on the Park's west side.

Notably, a history of fire suppression throughout the west may be expected to reduce the availability of young post-fire stands. To the extent that GNP maintains fire return intervals more characteristic of background levels, then post-fire stands, and the higher densities of hares they support, will serve as yet another example of an important ecological process maintained within the Park.

Finally, we reiterate that Appendix I contains a draft manuscript in preparation describing our work to compare the performance of non-invasive genotyping of hare pellets to traditional trap-based capture-mark-recapture sampling as methods of estimating abundance. This project was funded in part by this project, and some of the sampling was conducted in Glacier NP. We found that abundance estimates based on non-invasive genetic sampling were comparable to traditional live capture studies; more importantly for Park management, when we incorporated logistics and costs, we found that non-invasive genetic sampling was more efficient than live-trapping for all but the highest density hare sites, especially as distance from roads increased (see especially Fig. 10, Appendix I). Thus, we provide both a methodology and a rationale for increasing the use of non-invasive genetic sampling for estimating hare abundance, especially at backcountry sites.

OTHER DELIVERABLES

In addition to this report and the Appendices already discussed in this report (Appendix I: draft manuscript on non-invasive genotyping to estimate hare abundance; Appendix II: Glacier Project Field Manual), we also include three other products. Appendix III describes modifications made to the project from start to finish. Appendix IV mentions key outreach and education outcomes of the project. Appendix V includes the Raw GIS and data files (to be transmitted to Park personnel separately due to their size).

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FIGURE 1. LYNX OCCURRENCES IN GLACIER NATIONAL PARK, 2001 – 2005.

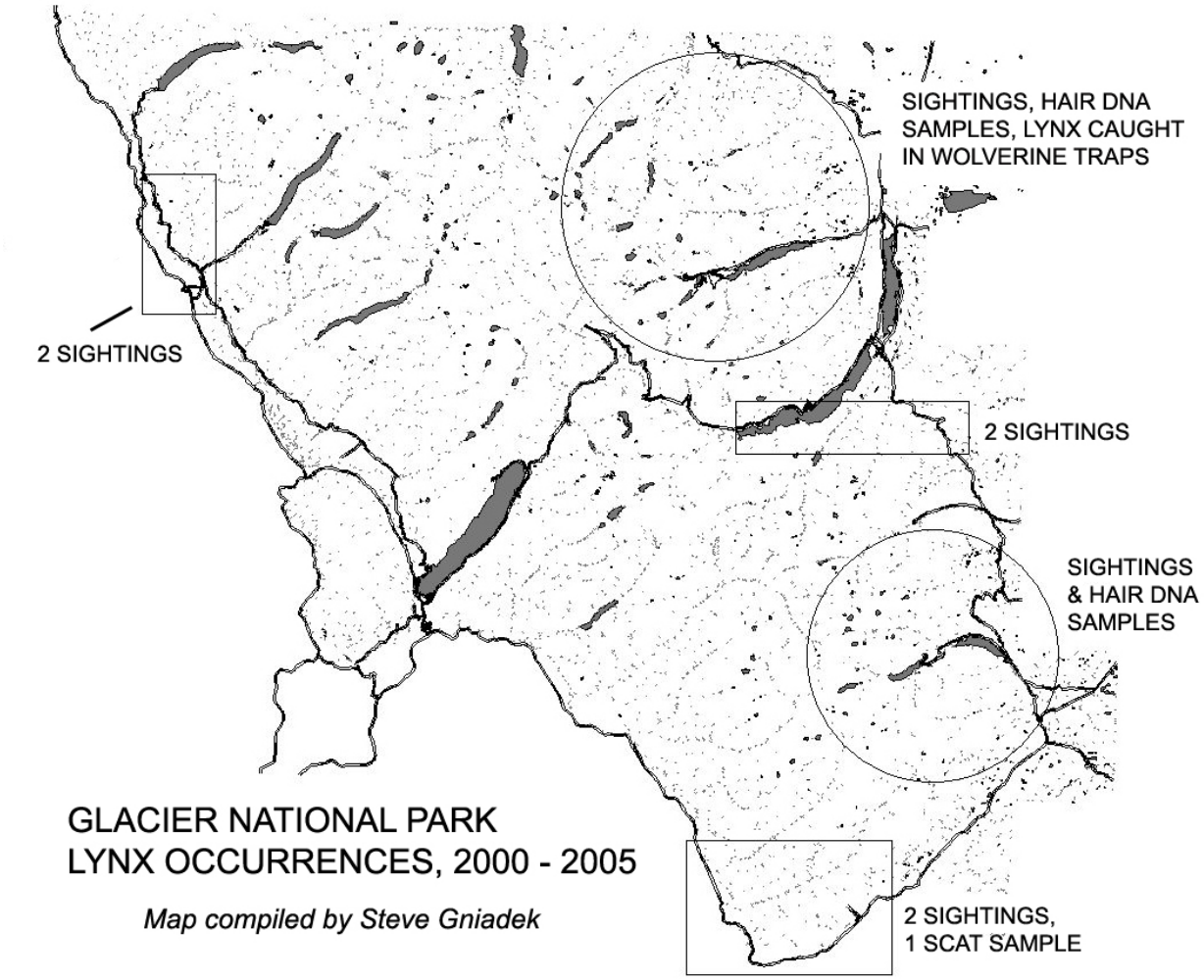


FIGURE 2. DISTRIBUTION OF SNOWSHOE HARE STUDY SITES AND DOMINANT CANOPY CATEGORIES.

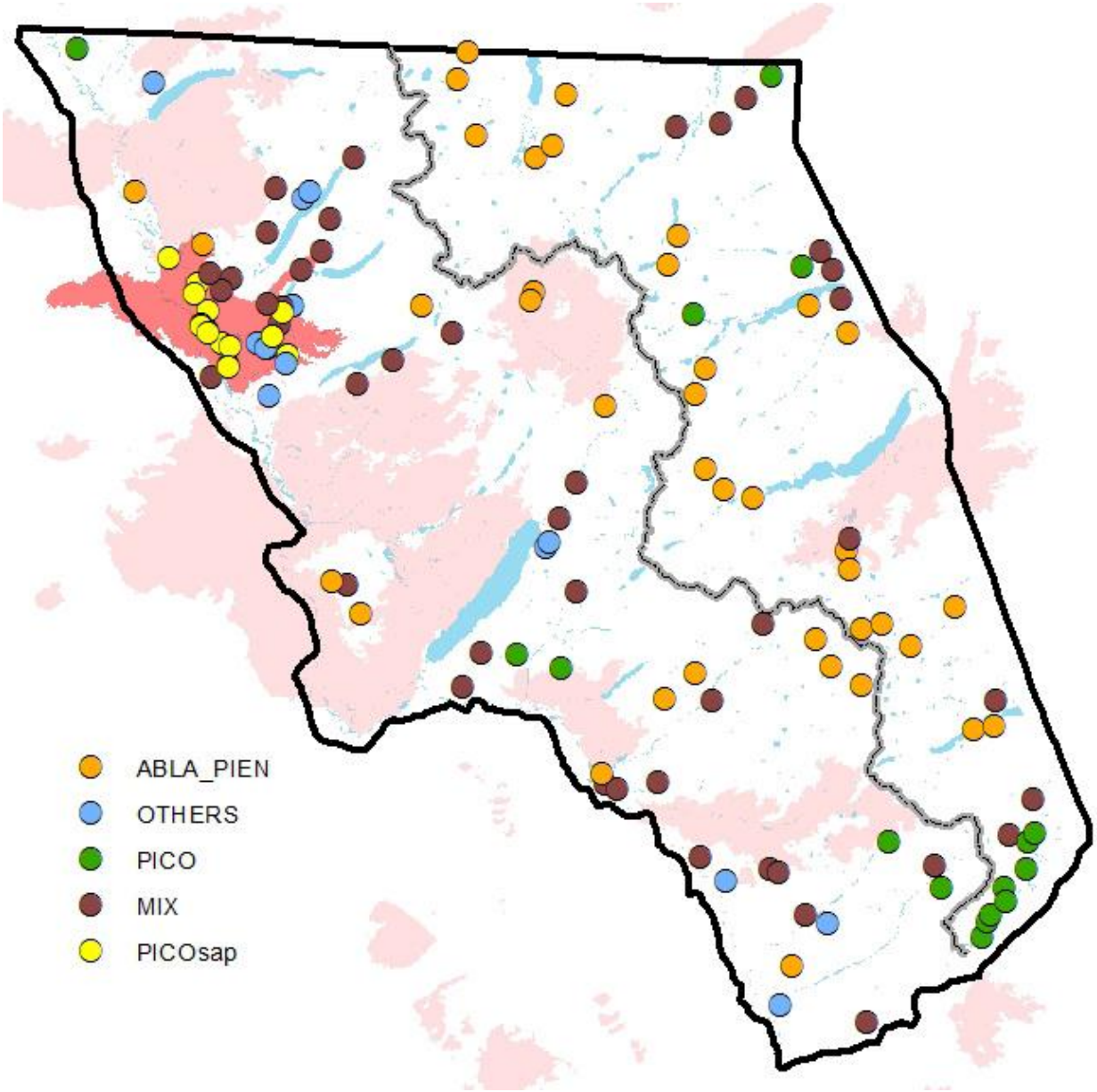


FIGURE 3. DISTRIBUTION OF HARE DENSITIES IN GLACIER NATIONAL PARK

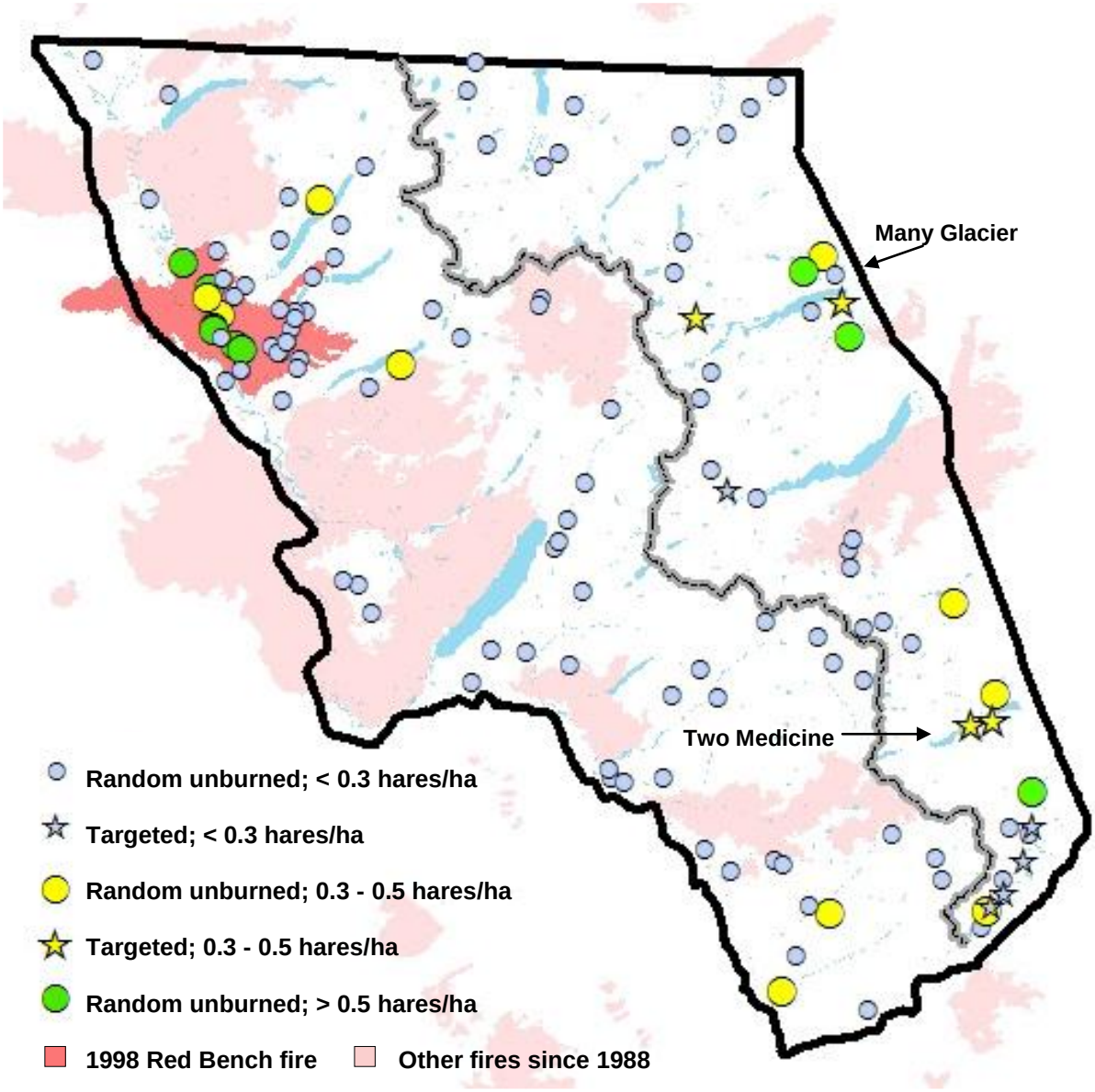


FIGURE 4. BOXPLOTS OF HARE DENSITY BY SITE TYPE. RED BENCH FIRE SITES (= 'BURN88') (N = 20) CONTAINED THE GREATEST PROPORTION OF MODERATE-DENSITY HARE SITES, FOLLOWED BY TARGETED UNBURNED SITES (N = 9) AND RANDOM UNBURNED SITES (N = 92).

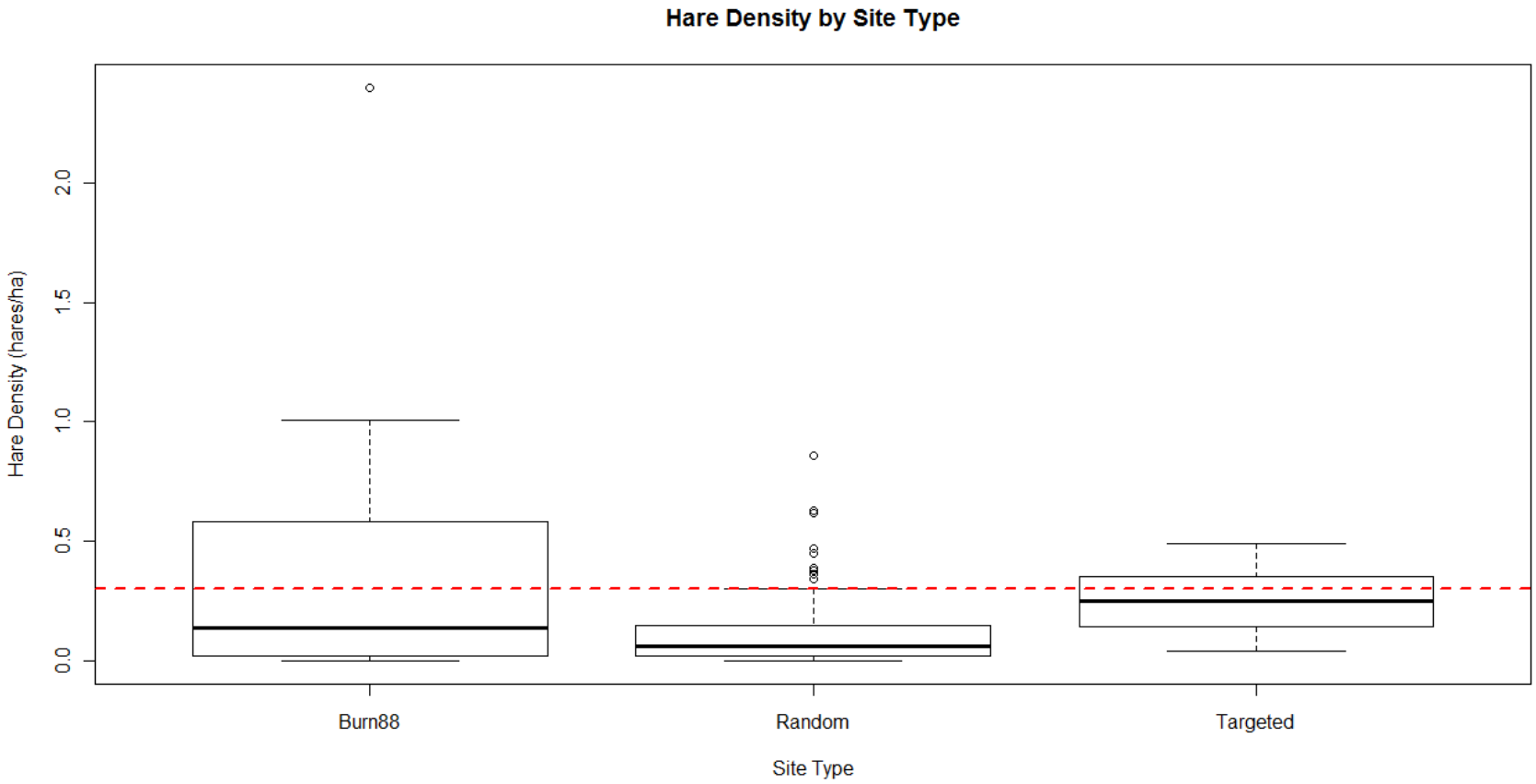


FIGURE 5. BOXPLOTS OF HARE DENSITY BY DOMINANT CANOPY HABITAT TYPE.

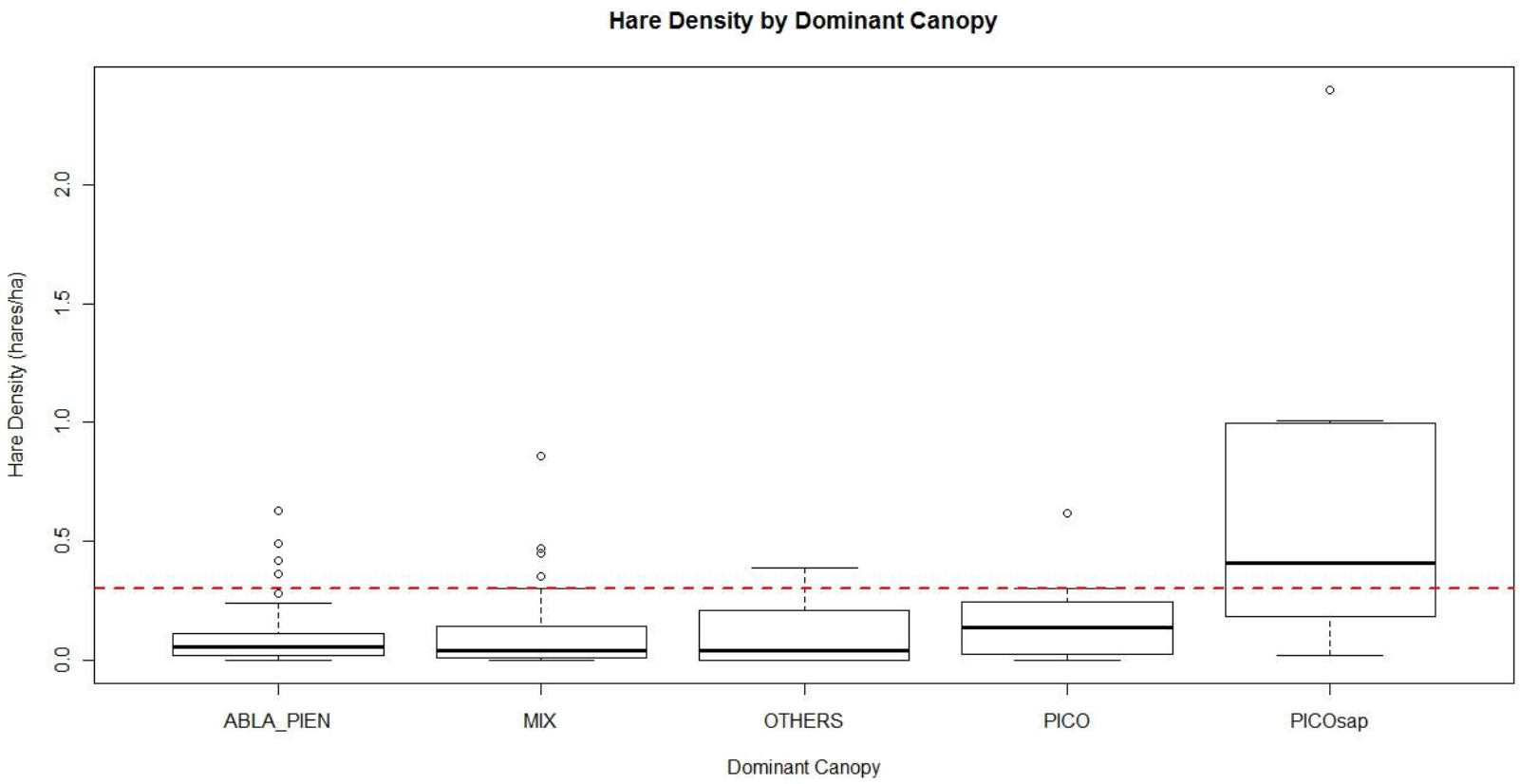


FIGURE 6. PERCENT OF RED BENCH FIRE SITES (A) AND UNBURNED SITES (B; BOTH TARGETED AND RANDOM SITES) COMPRISING DIFFERENT STAND STRUCTURE TYPES.

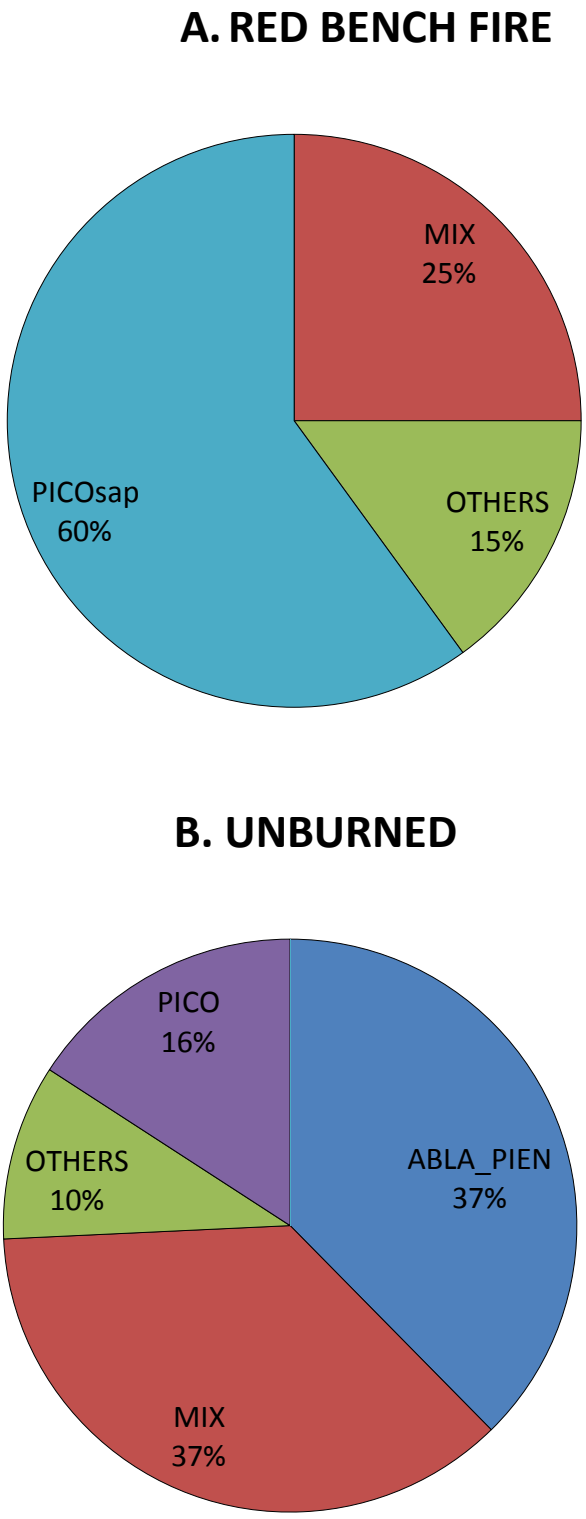


FIGURE 7. FOR THE RED BENCH FIRE SITES, % STAND STRUCTURE FOR MODERATE VS LOW HARE AREAS.

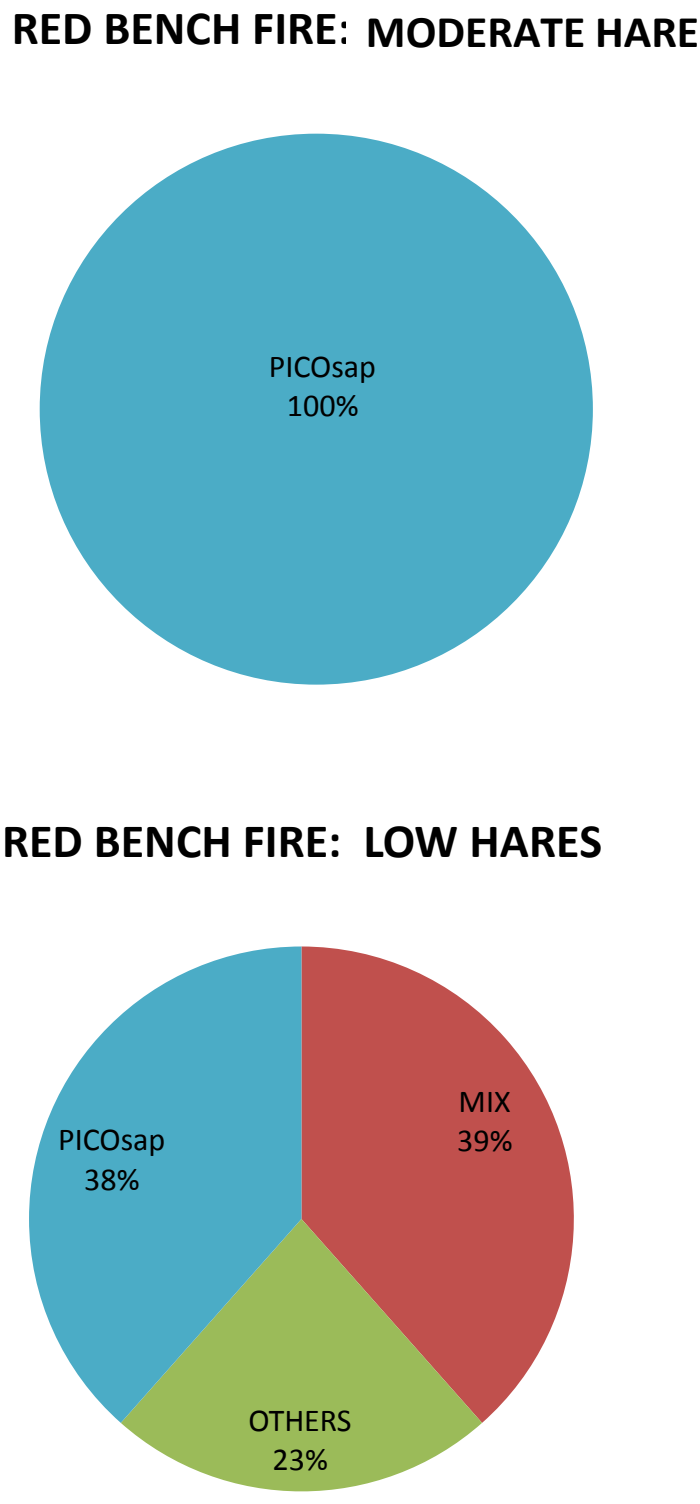
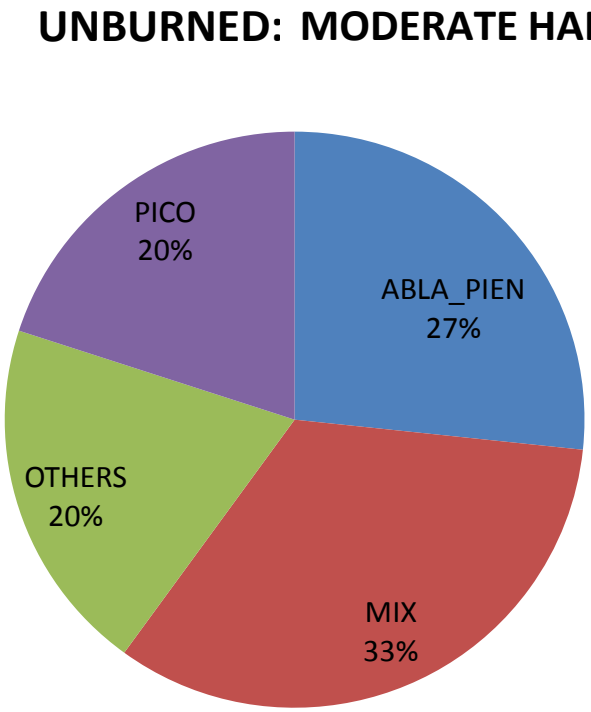


FIGURE 8. FOR THE UNBURNED SITES, % STAND STRUCTURE FOR MODERATE VS LOW HARE AREAS.



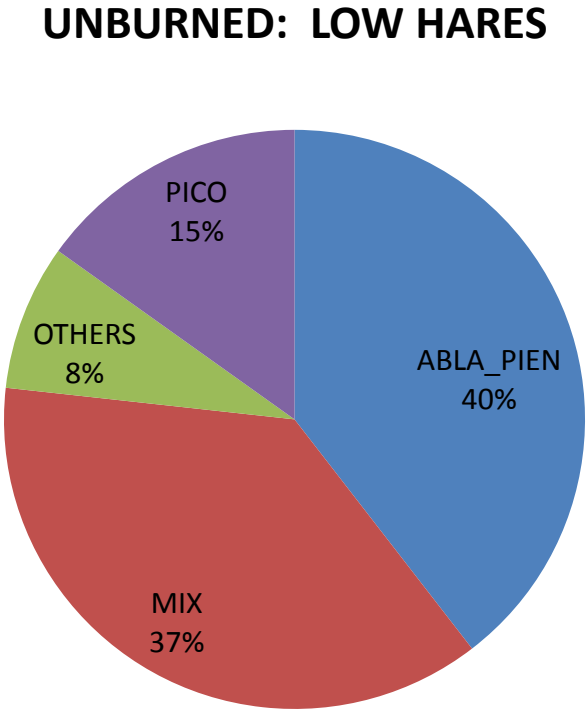


FIGURE 9. VEGETATION CHARACTERISTICS FOR MODERATE (≥0.3 HARES/HA) AND LOW (<0.3 HARES/HA) DENSITY RED BENCH FIRE SITES.

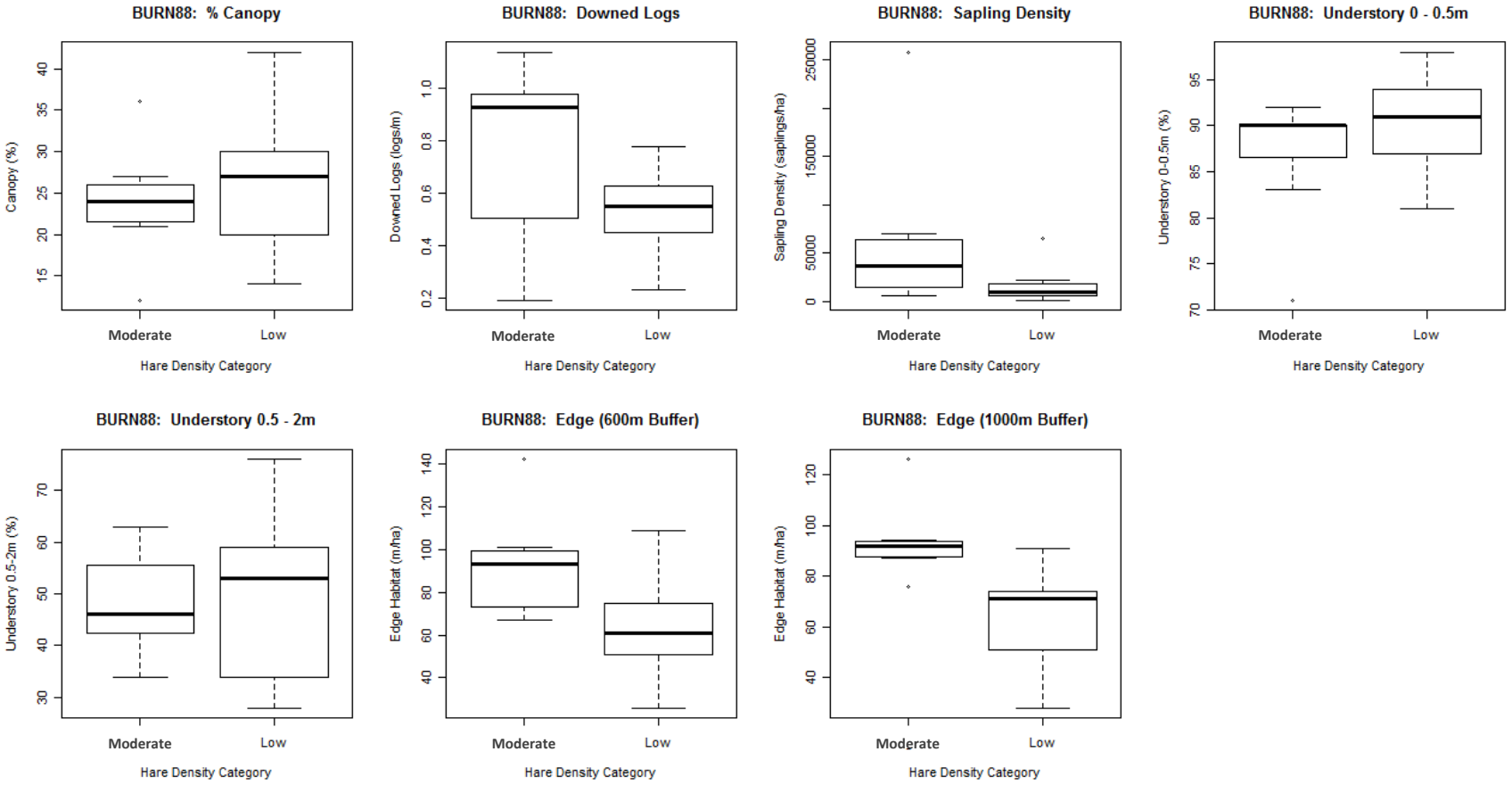


FIGURE 10. VEGETATION CHARACTERISTICS FOR MODERATE (≥0.3 HARES/HA) AND LOW (<0.3 HARES/HA) DENSITY UNBURNED SITES.

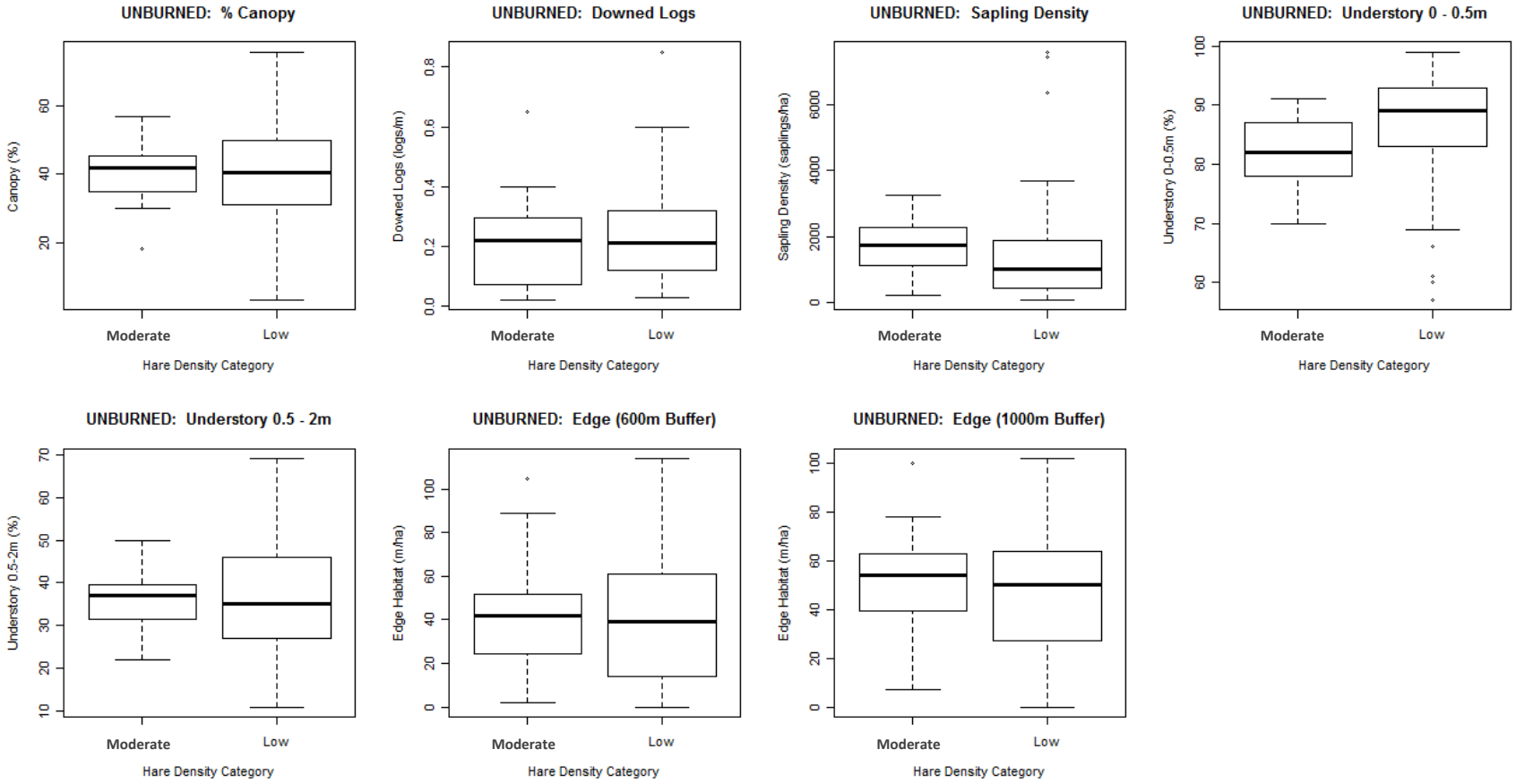


TABLE 1. STRUCTURE CHARACTERISTICS ACROSS VARIOUS FOREST STAND TYPES. REGENERATING LODGEPOLE PINE FORESTS THAT CHARACTERIZED PICOSAP, AND THAT CONTAINED HIGHEST DENSITIES OF HARES AND WERE FOUND ONLY IN THE RED BENCH FIRE SITES, DIFFERED FROM OTHER STAND TYPES IN SEVERAL STRUCTURAL CHARACTERISTICS (MOST NOTABLY, SAPLING DENSITY).

	ABLA_PIEN (N = 38)	PICO (N = 16)	PICOsap (N = 12)	MIXED (N = 42)	OTHERS (N = 13)	χ^2	p
% Canopy	0.34 ± 0.13	0.37 ± 0.09	0.24 ± 0.07	0.43 ± 0.13	0.44 ± 0.18	10.6	0.014 *
Downed Logs	0.22 ± 0.13	0.18 ± 0.20	0.65 ± 0.30	0.30 ± 0.20	0.31 ± 0.17	10.6	0.014 *
Sapling Density	1145 ± 815	781 ± 843	47368 ± 70513	2666 ± 3567	4877 ± 5812	20.1	0.0005 ***
Understory 0 - 0.5m	0.88 ± 0.08	0.84 ± 0.10	0.88 ± 0.07	0.88 ± 0.07	0.82 ± 0.13	3.8	0.28
Understory 0.5 - 2m	0.44 ± 0.10	0.29 ± 0.10	0.49 ± 0.14	0.36 ± 0.11	0.32 ± 0.14	22.5	< 0.0001 ***
Edge (600m buffer)	51 ± 29	37 ± 26	84 ± 30	40 ± 30	33 ± 23	5.4	0.14
Edge (1000m buffer)	56 ± 22	46 ± 21	85 ± 19	44 ± 28	44 ± 20	5.2	0.16

TABLE 2. UNIVARIATE ANALYSES OF RED BENCH FIRE SITES. W IS THE WILCOXON RANK SUM STATISTIC AND P IS THE P-VALUE FOR THAT TEST. SEE FIGURE 9 FOR DATA SUMMARY PLOTS.

	W	p
% Canopy	50	0.75
Downed Logs	28.5	0.19
Sapling Density	20	0.046 *
Understory 0 - 0.5m	58	0.33
Understory 0.5 - 2m	45.5	1
Edge (600m buffer)	18	0.03 *
Edge (1000m buffer)	5	0.0005 ***

TABLE 3. UNIVARIATE ANALYSES OF UNBURNED (RANDOM + TARGETED) SITES. W IS THE WILCOXON RANK SUM STATISTIC AND P IS THE P-VALUE FOR THAT TEST. SEE FIGURE 10 FOR DATA SUMMARY PLOTS.

	W	p
% Canopy	646	1
Downed Logs	738.5	0.38
Sapling Density	457.5	0.083
Understory 0 - 0.5m	936	0.005 **
Understory 0.5 - 2m	658.5	0.9
Edge (600m buffer)	625.5	0.86
Edge (1000m buffer)	591	0.61

TABLE 4. MULTIVARIATE ANALYSES OF PREDICTORS OF MODERATE HARE DENSITY SITES IN GLACIER NP. CV='CROSS VALIDATION' MODEL.

	ESTIMATE	SE	Z	P
AIC BEST MODEL, likelihood-ratio test X = 29.6, p = 0.00002 * **				
(Intercept)	4.24	2.79	1.52	0.13
East-[West]	-2.29	0.78	-2.93	0.003 **
PICOsap	3.31	1.23	2.69	0.007 **
Sapling Density	0.00003	0.00003	1.13	0.26
% Canopy	3.6	2.48	1.45	0.14
Understory 0 - 0.5m	-7.77	3.08	-2.52	0.012 *
BIC BEST MODEL, likelihood-ratio test X = 25.1, p = 0.00001 * **				
(Intercept)	5.47	2.61	2.1	0.036 *
East-[West]	-1.76	0.66	-2.68	0.007 **
PICOsap	3.4	0.84	4.04	0.00005 ***
Understory 0 - 0.5m	-7.69	3.05	-2.52	0.012 *
CV BEST MODEL, likelihood-ratio test X = 11.0, p = 0.0009 * **				
(Intercept)	-1.82	0.28	-6.56	< 0.00001 ***
PICOsap	2.16	0.65	3.33	0.0009 ***

LIST OF APPENDICES

A Comparison of Non-Invasive and Traditional Mark-Recapture Estimates of Abundance
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INTRODUCTION

Animal abundance and density are central to most wildlife management and conservation decisions, including assessment of trend, evaluation of response to natural and anthropogenic habitat disturbances or human activities, and measurement of population persistence and habitat quality. Capture-mark-recapture (CMR) statistics applied to live-trapping data have long been the gold standard for obtaining robust estimates of animal abundance. CMR can correct animal count data for differences in animal detection probability over space and time (e.g., due to weather conditions or site-specific habitat characteristics) by using recapture histories of marked individuals to estimate detection probabilities and adjust population estimates (Mills 2007, Williams et al. 2002). When statistically rigorous estimates of abundance or density is of interest—as it often is when managing high profile species or making politically sensitive decisions based on wildlife surveys—CMR methods should be used. However, for logistical (terrain, topography, road access) reasons, live-trapping to mark animals for abundance estimation is daunting to implement in remote areas, including wilderness areas and National Parks. In addition, researchers and land managers are increasingly interested in applying wildlife research techniques that are minimally invasive to the animal.

As an alternative to trap-based abundance estimation, indices based on animal sign (e.g., pellet or track counts) or on sightings are sometimes used to non-invasively assay relative population abundance. Compared to trap-based CMR, index data are typically easier and cheaper to collect, allowing researchers to quickly survey a large number of sites for broadly characterizing species distribution over space and time. But spatial or temporal differences in an index count may be due to unanticipated factors (e.g., higher rates of pellet decomposition in wetter microhabitats or years) unrelated to population abundance. Because individuals are not marked and recaptured in index surveys, population estimates cannot be corrected for unknown sources of detection bias.

Over the past decade, non-invasive genetic sampling (NGS) has rapidly gained popularity as a new way to obtain individual count data amenable to CMR analysis, with the ease and non-invasive benefits of index-based sampling. Instead of live-trapping animals to mark unique individuals for population estimation, NGS can identify unique individuals through the genotypes obtained from scat, hair, or other non-invasively collected samples. NGS coupled with a statistically rigorous CMR framework has been used to estimate densities for many rare or elusive species, including wombats (Banks et al. 2002), gray wolves (Creel et al. 2003), bears (Bellemain et al. 2005; Stetz et al. 2010), and wolverines (Mulders et al. 2007). For these difficult-to-capture species, NGS has yielded larger sample sizes and proven more cost-effective than traditional live-trapping methods for CMR-based abundance estimation. But what are the limits of efficiency of NGS for estimating abundance of wildlife populations? Would NGS also

be cost-effective for estimating abundances of relatively common species, widely sampled with traditional trap-based CMR estimation methods?

This critical question has, to date, hampered the broad application of non-invasive genetic sampling as a replacement to live-trapping in wildlife surveys. NGS often incurs lower field costs than live-trapping because scat and other sources of genetic data are relatively easy to collect. However, in addition to field work, genetic sampling requires laboratory processing to obtain reliable consensus genotypes from samples. Laboratory costs can be high when working with the low quality and quantity of DNA typically available from non-invasive genetic samples, and costs increase almost linearly with number of genetic samples and density of surveyed populations.

This study evaluates the cost-effectiveness of a non-invasive CMR genetic sampling method developed for estimating snowshoe hare abundances in the remote backcountry of Glacier National Park, Montana. Compared to most species monitored with non-invasive genetic sampling, snowshoe hares are moderately abundant and relatively trap happy. But the logistics of trapping hares in Glacier—and many other National Parks and wilderness areas in the United States—can be daunting due to the lack of road access. With this study, we develop a NGS abundance estimation protocol for snowshoe hares, and compare both abundance estimates and cost efficiency with those from conventional live-trapping approaches for a range of conditions.

METHODS

Pilot Study

Our first objective was to develop and test an NGS sampling approach that maximized collection of hare fecal pellets sufficiently fresh for genetic analysis, while minimizing time, labor, and sampling costs in the field.

We used a simple modification of a design used for live-trapping snowshoe hares (Mills et al. 2005, Hodges et al. 2009). A 20-hectare (400 m X 500 m) rectangular study site was gridded into 80 (50 m X 50 m) square plots. In each plot, we lay out a baited ground cloth. Ground cloths were left in the field for several days to accumulate fresh fecal pellets from hares attracted to the bait.

In summer 2006, we conducted pilot tests of this NGS sampling method at five 20-ha sites in Glacier National Park. We compared the effectiveness of three baits (apples, oats, alfalfa), two plot sizes (0.5 and 1.0 m^2), and different sampling durations (1 to 6 days) for "capturing" fresh hare pellets. We used a Wilcoxon Rank Sum Test to determine if bait type and plot size significantly influenced the proportion of sampling plots that yielded pellets.

Pellet Decomposition Study

Concurrent with the pilot study, we conducted a pellet decomposition study. The purpose of the decomposition study was to determine how quickly hare pellet DNA degrades in the field so we could identify the optimal sampling duration for ground cloths to accumulate hare pellets at

a site (i.e., long enough to collect many pellets, but short enough to ensure sufficiently high DNA amplification success and relatively low genotyping error rates).

From each unique hare that we captured via live-trapping at two pilot study sites in Glacier National Park, we collected an ear tissue sample as well as pellets the hare had deposited in the bottom of the cage. We transferred these pellets to a forested area near our base camp. At 0, 2, 4, and 6 days post-capture, we collected 1 – 3 pellets from the pellet pile corresponding with each previously trapped hare. Pellets were stored in vials of 95% alcohol, transported to the lab, and stored in a -20°C laboratory freezer until extraction.

At our laboratory at The University of Montana, we used Qiagen DNeasy Blood & Tissue Kits to extract DNA from hare tissue samples. Pellet samples were extracted with QIAamp DNA Stool Mini Kits, in a separate laboratory designated exclusively for low quality DNA samples collected non-invasively. All samples were genotyped at 8 highly variable microsatellite loci originally developed in the European rabbit, *Oryctolagus cuniculus*, and successfully used with snowshoe hares (Burton et al. 2002; Cheng 2010; Schwartz et al. 2007). PCR amplifications of the 8 loci were combined into three multiplex reactions, and each sample was amplified across all loci four times. PCR amplifications were run on an ABI 3130xl Genetic Analyzer (Murdoch DNA Sequencing Facility; Missoula, MT) and scored with GENEMAPPER v. 3.7 (Applied Biosystems Inc., Foster City, CA). We manually checked all microsatellite genotypes to confirm allele calls. Tissue and pellet extraction and PCR conditions were based on protocols extensively optimized in our laboratory during the year prior to commencing genetic analysis (**Appendix I**).

We used Program GIMLET (Valiere 2002) to calculate amplification success and genotyping error rates for each pellet age class. Because genotyping error rates are typically much lower for tissue compared to fecal samples, we used the consensus genotype (i.e., the genotype confirmed across 4 PCR amplifications) for each tissue sample as the “correct” genotype against which to evaluate 0, 2, 4, and 6-day old pellet samples from the same hare.

Amplification rate is the proportion of times a sample-locus generates a genotype in a PCR run, whether or not that genotype is correct. Amplification success for each pellet age class was averaged across all loci, samples and PCRs. For example, a 37.5% amplification success for 2-day old pellets meant that, on average, a 2-day old pellet amplified at 3 of 8 loci (3/8=37.5%) in a single PCR run.

We also calculated allelic dropout rates and false allele rates, separately for each pellet age class. Allelic dropout is defined as when a heterozygote (based on the tissue consensus genotype) is typed as a homozygote. A false allele is defined as when a homozygote (based on the tissue consensus genotype) is typed as a heterozygote. Five additional error types calculated by Program GIMLET were combined in the category “Other Error Types”. These additional error types represented various types of base pair shifts, and were rare in our samples. Genotyping error rates for each pellet age class were averaged across all loci, samples and PCRs in the specified pellet age class.

Comparing Trap- and Genetic-Based CMR Estimates of Hare Abundance

We compared hare population estimates obtained from NGS and live-trapping at 2 study sites in Glacier National Park in 2006 (B88-1 in the 1988 Red Bench Fire area and MG3 in Many Glacier). In 2009, we conducted this study at an additional 3 study sites in the adjacent Tally Lake National Forest. At each of our 5 study sites, live-trapping and genetic sampling occurred sequentially within a 2-week time period and in random order (i.e., at some sites, live-trapping was conducted before genetic sampling; at other sites, vice versa).

LIVE-TRAPPING

At each 20-hectare (400 m X 500 m) site, we placed 80 Tomahawk live-traps in the same 8 X 10 grid configuration used for genetic sampling. Traps were opened every evening and baited with apple and certified weed-free alfalfa, following approved protocol. Traps were checked every morning, then closed for the duration of the day. Captured hares were weighed, sexed, and ear tagged. A small piece of ear tissue (for genetic analysis) was collected from each captured hare, using a sterile 3-mm veterinary biopsy punch. Tissue samples were stored in silica gel until return from the field, at which point they were frozen to -20°C.

NON-INVASIVE GENETIC SAMPLING

Non-invasive genetic sampling followed a refined protocol developed from our pilot pellet collection and decomposition studies. At each sampling plot in a 20-ha site, we baited 80 small (0.5 m^2) ground cloths with alfalfa, and returned 4 days later to collect pellets for genotyping.

At our 3 Tally Lake National Forest sites, we additionally placed a sampling plot between each of the 80 original plots, for a total of 160 plots per site. We collected pellets from these additional plots in order to evaluate if population estimates were influenced by the use of 80 compared to 160 sampling plots per site.

In cases with multiple pellets per plot, sample size for the CMR analysis would potentially increase by genotyping multiple pellets, but at higher cost, and therefore lower cost efficiency if the pellets tended to be from the same individual. To evaluate how population estimates were influenced by the number of pellets genotyped per plot, for all sites we genotyped up to 4 pellets per plot and compared population estimates based on sampling a maximum of 1, 2, 3, and 4 pellets per plot.

Pellets were genotyped at 8 microsatellite loci, using laboratory protocols described above. Samples with less than 40% amplification success in the first two PCR runs were automatically discarded from further analysis. All other samples were amplified up to six times, in order to confirm a sample consensus genotype across 8 loci. We used a 3-stage approach for confirming sample genotypes, modified from Morin et al. (2001) and Waits and Paetkau (2005):

1) A sample was designated a likely homozygote at a locus if it amplified as a clear homozygote in at least 3 PCRs, and a confirmed homozygote if it amplified as a clear homozygote in at least 4 PCRs of that locus with no discrepancies (i.e., no other alleles amplified in any PCR run). A genotype was designated a likely heterozygote at a locus if each allele amplified clearly in at least one PCR of that locus, and a confirmed

heterozygote if each allele amplified clearly in at least two PCRs of that locus with no discrepancies (i.e., no third allele amplified an any PCR run).

2) A sample genotype (i.e., the genotype across all 8 loci) was confirmed if a likely or confirmed genotype was designated at each of the 8 loci AND the full sample genotype exactly matched that of another pellet or tissue sample collected from the same site.

3) Program GIMLET was used to identify all sample pairs whose genotypes differed at only one or two loci. We evaluated all PCR runs of these near-match sample pairs to determine if the samples were probably from the same individual. For example, allelic dropout rates are often high for non-invasive samples. Therefore, if two pellets collected from the same sampling plot differed at only one locus, where one sample had a genotype of 123/125 and the other sample had a genotype of 123/123, we assumed the samples were actually from the same individual and the true genotype at the differing locus was heterozygous.

For final abundance estimation, we retained all samples with likely or confirmed genotypes across all loci after evaluation by this 3-stage approach.

CALCULATING ABUNDANCE

We used different guidelines for allocating trap- and NGS-based data to capture sessions for CMR abundance estimation, because these sampling methods differ in how individuals are captured. With CMR based on traditional live-trapping, a capture history is derived from two or more capture sessions, with animals released between capture episodes. In contrast, with NGS, a "capture" occurs for any individual leaving a fecal pellet or other genetic signature at any sampling plot during the sampling interval. An individual can leave its genetic signature at multiple plots without having to be "released" by the researcher between captures. Therefore, capture data can be collected in a single episode (i.e., one return trip to a site), and the capture history built from different sampling plots within the site rather than different collection visits to the site.

We used the simplest possible CMR estimator, based on the two-sample Lincoln-Petersen method corrected for small sample size (Mills 2007). With live trapping data, captures from the first two nights of live-trapping at each site were combined into the first capture session (n_1), and captures during the remaining 2 or 3 trap nights were combined into the second capture session (n_2). The number of individuals captured in both sessions was the number of recaptures (m_2). Abundance ($\hat{N}$) and its variance were estimated as:

$$\hat{N} = \left[\frac{(n_1+1)(n_2+1)}{(m_2+1)} \right] - 1.$$

$$\text{var}\left(\hat{N}\right) = \frac{(n_1+1)(n_2+1)(n_1-m_2)(n_2-m_2)}{(m_2+1)^2(m_2+2)}$$

The square root of the variance gives the SE of the estimate.

With genetic sampling, individual hares (identified by their pellet genotypes) were assigned to the two Lincoln-Peterson capture sessions according to the sample plots from which their pellets were collected. For each site, we randomly allocated each of the 80 original sampling plots to one of two capture sessions. With this approach, a hare was "recaptured" only if it deposited pellets on at least one plot allocated to capture session 1 and also on at least one plot allocated to capture session 2. With each random allocation of sampling plots to the two capture sessions, we obtained one population estimate for a site. The final genetically based capture estimate for each site was averaged across 500 population estimates, determined from 500 independent randomizations of sampling plots, as coded in Program R. Abundance standard deviation represented the square root of variances in these 500 population estimates.

For each site, separate abundance estimates were calculated based on genotyping up to 1, 2, 3, or 4 pellets per plot. At our Tally Lake National Forest sites we additionally estimated abundance based on 160 sampling plots, for comparison to estimates based on 80 plots. We used paired t-tests to determine if NGS-based CMR estimates of $\hat{N}$ differed substantially from trap-based CMR estimates of $\hat{N}$.

Cost-Benefit Analysis

We estimated costs of live-trapping and genetic sampling for two types of sites characteristic of our study area, and of many unroaded areas: easy sites located close to roads and more difficult, but still feasible, sites requiring a day's hike from the nearest road (Table 1).

Both live-trapping and genetic sampling have costs associated with obtaining field data, i.e., costs of trapping hares and of collecting pellets for genotyping. We assumed first-time visits to all field sites, so field set-up time includes flagging site grids as well as putting out traps or genetic sampling plots. Difficult sites, due to their distance from roads, typically require camping near sites for the 5-day duration of trapping or the 4-day duration of genetic sampling, but we only accounted for person-hours directly involved in survey work and travel to-and-from sites (making our costs conservative for difficult sites).

Genetic sampling has laboratory costs associated with extracting and amplifying DNA from pellets, and person-hour costs of analyzing chromatogram results and determining consensus genotypes. We assumed 19% of pellets would have low amplification rates (based on our study), so would be excluded from further analysis after the first 2 PCR runs. Pellets retained for analysis would average 4.6 PCR runs to yield a reliable consensus genotype.

Genetic sampling costs for a site depend on the number of pellets genotyped, which is influenced by hare abundance. Therefore, we used our field data to generate a regression equation relating number of hares at a site (the average of abundance estimates from live-trapping and genetic sampling) and the likely number of pellets that would be genotyped, when sampling 1 or 2 pellets per plot. From these calculations, we developed general guidelines for determining when live-trapping or genetic sampling should be more cost-effective, as a function of site type (easy or difficult) and the expected number of hares at a site.

RESULTS

Pilot Study

The three baits (apples, oats and alfalfa) performed equally well for attracting hares to sampling plots (Wilcoxon Rank Sum tests p = 0.20 to 0.86; Fig. 1). Therefore, our final protocol used alfalfa bait, which is easiest to handle in the field. Larger sample plots (1 m^2) captured pellets, on average, at 11% more plots than did smaller sample plots (0.5 m^2)(Wilcoxon Rank Sum test p = 0.10; Fig. 2), but smaller plots were much easier to work with; therefore our final protocol used 0.5 m^2 sampling plots. Pellets accumulated most rapidly on sample plots during the first 3 – 4 days after plots were placed (Fig. 3). In the first day of sampling, an average 10% of plots collected pellets. By the fourth day, 26% of plots had at least one pellet.

Pellet Decomposition Study

Non-amplification and genotyping error rates increased quickly and steadily with pellet age (Fig. 4). Non-amplification was the primary difficulty in this study, reaching almost 50% in-4 day-old pellets. Among the loci evaluated, SAT03 consistently exhibited the lowest amplification rates, ranging from 70% (compared to mean 93%) for 0 day-old pellets to 14% (compared to mean 29%) for 6 day-old pellets.

When genotypes amplified, false alleles were the most common type of genotyping error, consistent with other non-invasive genetic studies(reviewed in Broquet and Petit 2004). Genotype success rate (proportion of loci that amplified with correct genotypes) for 0 day-old hare pellets averaged 76%. Genotype success rate declined at a fairly consistent rate of ~22% for every one day increase in pellet age, from 0 to 6 days. Our final protocol used a sampling duration of 4 days, to ensure a sufficient number of pellets could be collected for abundance estimation. The non-amplification rate for 4-day-old pellets is high (48%), but a majority of the pellets collected after a 4-day sampling period would actually be less than 4-days old.

Comparing Trap- and Genetic-Based CMR Estimates of Hare Abundance

FIELD SAMPLING

We captured 78 hares across 5 study sites (Table 2). Averaged across sites, 36% (±16%) of individuals were captured during both Lincoln-Peterson capture sessions. We collected 522 pellets across 5 study sites (Table 3). A majority of sampling plots (80%±15%) did not accumulate any pellets over the 4-day sampling period (Fig. 5).

OBTAINING CONSENSUS GENOTYPES

From the 522 pellets collected in this study, we randomly selected up to 4 pellets per plot for genotyping. From plots that accumulated less than 4 pellets over the 4-day sampling period, we genotyped all pellets.

A total of 260 pellets were genotyped, of which 210 (81%) yielded consensus genotypes (Table 4). We conducted an average of 4.6 PCR runs per sample. Eighty-seven percent of pellet samples had genotypes that could be matched to another pellet or to a tissue genotype from a hare live-trapped at the same site.

We identified 85 unique hare genotypes across 5 study sites. On plots with more than one pellet, the number of individuals identified was usually less than the number of pellets genotyped (Fig. 6). Averaged across sites, 42% (±6%) of genotyped individuals were captured on more than one sampling plot (Fig. 7). Fifty-five percent (±17%) of hares that were live-trapped at a site were also identified from genetic sampling. Forty-two percent (±10%) of hares identified from genetic sampling were also live-trapped at a site.

ESTIMATED ABUNDANCE WITH EACH METHOD

Trapping and genetic sampling generated similar hare abundance estimates at study sites (Fig. 8). Sampling pellets from 160, rather than 80, plots per site approximately doubled the number of pellets collected, but did not influence abundance or variance estimates with any consistent pattern. Similarly, we could not identify any trend in estimates as we increased the number of pellets genotyped per plot from 1 to 4. Tally Plume was a notable exception. At this site, genetic-based CMR estimates more than doubled trap-based estimates, and increased with number of pellets genotyped per plot.

Cost-Benefit Analysis

Although the regression equation was heavily influenced by our highest hare density site, we found a tight relationship between estimated number of hares at a site (based on the average of live-trapping and NGS abundance estimates) and the number of pellets collected when genotyping 1 or 2 pellets per plot (Fig. 9). Using these equations (for genotyping 1 and 2 pellets per plot) and the cost parameters determined from our study, we identified threshold hare abundances below which genetic sampling is cheaper than live-trapping (Fig. 10). For easy field sites, this threshold was 23 hares when genotyping 1 pellet per plot, and 14 hares when genotyping up to 2 pellets per plot. For difficult field sites, this threshold was 57 and 36 hares when genotyping 1 or up to 2 pellets per plot. As the number of hares increases at a site, so does the number of pellets collected for genotyping, and also the costs of genetic sampling to estimate abundance. Note that the cost analysis cut-offs must be evaluated in terms of abundance, not density, because the cost of analysis depends on numbers of samples analyzed. Thus, the 23 hare cut-off remains the same regardless of whether the site is 20 hectares (density = 1.15 hares/ha) or 10 hectares (density =2.3 hares/ha).

DISCUSSION

We found non-invasive genetic sampling (NGS) to be more efficient for estimating abundance in a capture-mark-recapture framework than traditional live-trapping, under a wide range of conditions for a moderately common, medium-sized herbivore (snowshoe hares). If general, this broadens the scope where NGS may be the method of choice for rigorous estimation of abundance and density distribution.

NGS has become widely adopted for estimating abundance for species such as carnivores (Schwartz and Montfort 2008, Kelly et al 2011) whose numbers or trappability are so small that live-trapping would produce inaccurate or biased estimates. NGS not only has potential to

increase capture probability compared to live trapping, but also it can reduce number of required visits to study sites, stress and mortality for the animals, and capture bias due to trap response (Lukacs and Burnham 2005b; Mills et al. 2000; Petit and Valiere 2006). However, a chronic concern with NGS is that abundance estimates may be inflated by high genotyping error rates arising from the low-quality, low-quantity DNA characteristic of NGS samples (Taberlet et al. 1999, Waits and Leberg 1999, Kalinowski et al. 2006) Genotyping error rates can be reduced by following strict field and laboratory protocols to minimize contamination and DNA degradation (reviewed in Broquet and Petit 2004; Paetkau 2003). However, it is still usually necessary to conduct multiple PCR amplifications of each sample to obtain a reliable genotype—a practice that greatly increases the costs of this method and that has limited its application, so far, to surveying the rare and elusive species that are most costly to monitor using traditional live-trapping.

Surprisingly few comparisons have been made between traditional trap based and non-invasive estimates of abundance and density, and to our knowledge none have asked if non-invasive genetic methods can be cost-efficient for surveying relatively common and easily trappable species. These questions are increasingly relevant because the downsides of non-invasive genetic sampling have been rapidly decreasing with improved laboratory and analytical techniques, including more and cheaper markers and a better understanding of how genotyping error can be incorporated into abundance estimates (Lukacs and Burnham 2005a).

We demonstrate a non-invasive genetic sampling approach that produces abundance estimates comparable to those from live-trapping, and can be cost-efficient even for surveying relatively common species like snowshoe hares. In places such as Glacier National Park, where it is logistically challenging (and sometimes not even possible) to live-trap in much of the Park, genetic sampling offers an opportunity to apply a statistically rigorous survey method for estimating species abundance, avoiding the common fall-back of convenience sampling a subset of easily accessible sites.

We obtained similar abundance estimates from genetic sampling and live-trapping, for a wide range of snowshoe hare densities (0.35 – 2.65 hares/ha) represented by our study sites. Tally Plume was an exception—genetic-based estimates (15.5 – 34 hares) at this site were much higher than the trap-based estimate of 8.3 hares. However, with the same sampling duration and number of genetic sampling plots, we collected more pellets from Tally Plume than we did from Tally Pigskin, which had an estimated 20.6 hares averaged across methods, suggesting that the Tally Plume trap-based abundance estimate may be biased low.

Genotyping more than one pellet per plot did not clearly improve abundance estimates relative to trap-based estimates or relative to genotyping just one pellet. This finding is not too surprising, since multiple pellets on a single plot generally arose from just one or two different individuals. Because costs of abundance estimation with NGS scales directly with number of samples analyzed, our results imply that only one pellet per plot would need to be sampled for an efficient CMR-based estimate of abundance.

The resounding success of NGS using just one pellet per plot in estimating abundance has potentially profound implications for the most efficient method of sampling even common species such as hares. We found that for difficult-to-trap sites, NGS was more efficient at live trapping for all hare abundances less than 57; for easy-to-trap sites, NGS was more efficient for abundances less than 23 hares. To place these numbers in context, we note that hare abundances in the Northern Rocky Mountains region are almost always below these thresholds. Between 1998 and 2010 we have live-trapped snowshoe hares in the summer at 33 sites within 200km of Glacier National Park (Mills 2005, Mills unpublished data, Hodges and Mills In Prep.); most of these would be considered 'easy' sites, with road access). Based on $\hat{N}$ estimated for these 183 site-years using the same CMR methods used in this paper (and not counting sites with 0 hares captured), we find that 81% of the site-years had abundances <23 and 99% had <57 hares. Thus, it appears that for monitoring purposes, under most circumstances and regardless of access, NGS methods would be more efficient estimators of snowshoe hare abundance than traditional trap-based approaches.

Low amplification success and high genotyping error rates were the most problematic issues with our genetic sampling protocol. Almost a fifth of samples we extracted did not yield consensus genotypes. This problem may be reduced by shortening the sampling period to 2 or 3 days in future studies. With a shorter sampling period, increasing the number of sampling plots may improve abundance estimates. Although more field time is required to place 160 plots per site, the additional field time is minimal compared to the time and money invested in the laboratory to genotype pellets. We did not find that 160 plots per site instead of 80 appreciably changed abundance estimates, but if sampling period were shortened the higher number of plots would increase sample size and likely improve estimates. Reducing non-amplification and genotyping error rates should reduce per-sample laboratory costs, as fewer PCR runs will be required to obtain reliable consensus genotypes. Eliminating the most problematic loci (those with the lowest amplification success and highest error rates) can also reduce costs while potentially improving abundance estimates, provided the remaining loci are sufficiently variable to minimize error due to the 'shadow effect' (Mills et al. 2000).

Although live trapping will continue to have an important role in ecological studies – for example for physiological and disease measurements, body condition, attaching radiocollars for space use studies, and outreach (e.g. the public seeing and touching wild animals) – we find that non-invasive genetic sampling produced equivalent abundance estimates for snowshoe hares at lower cost than live-trapping under a wide variety of field conditions. For monitoring trend or other objectives requiring abundance estimates, non-invasive genetic sampling may be a cost efficient alternative even for relatively common species that are widely and conventionally sampled with live trapping.

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FIGURE 1. COMPARISON OF BAITS FOR GENETIC SAMPLING. Response variable is the proportion of sampling plots with accumulated hare pellets, for each bait type. Baited ground cloths were left in the field for four days. Error bars represent ± 1 SD. Differences among baits were not statistically significant (Wilcoxon Rank Sum test p = 0.20 – 0.86).

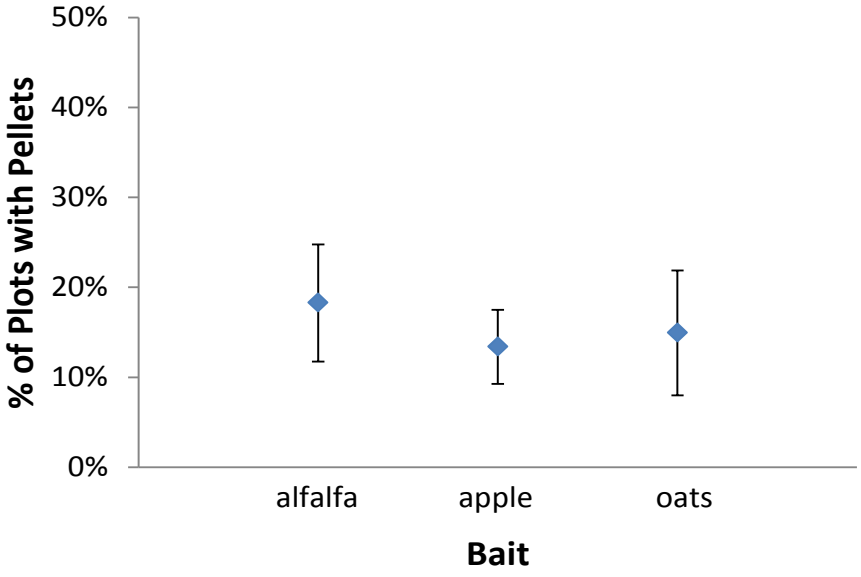


FIGURE 2. COMPARISON OF PLOT SIZE FOR GENETIC SAMPLING. Response variable is the proportion of sampling plots with accumulated hare pellets, for small (0.5 m^2) versus large (1.0 m^2) plots. Baited ground cloths were left in the field for four days. Error bars represent ± 1 SD. Differences between plot sizes were not statistically significant (Wilcoxon Rank Sum test p = 0.10).

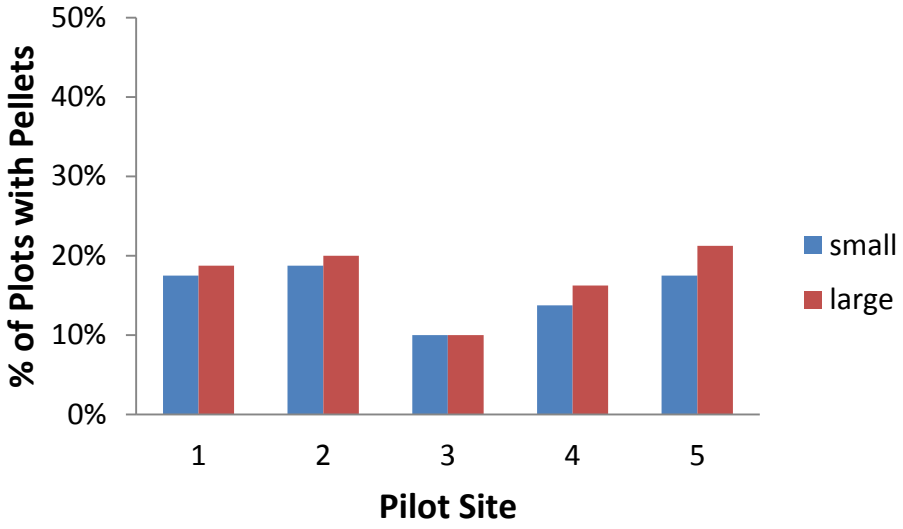


FIGURE 3. ACCUMULATION OF PELLETS AS A FUNCTION OF SAMPLING DURATION for 5 study sites in and around Glacier National Park. Response variable is the proportion of sampling plots with accumulated hare pellets, for sampling durations of 1 – 5 days.

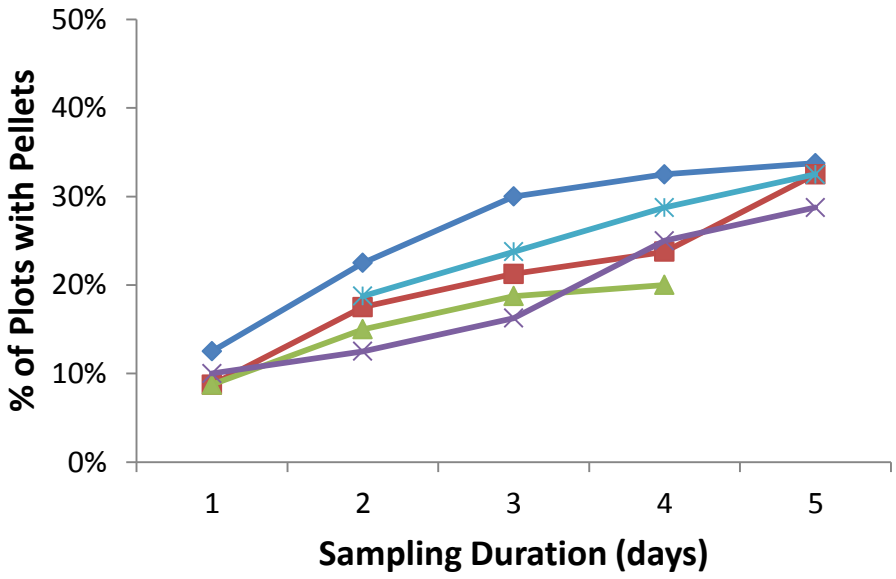


FIGURE 4. PROPORTION OF LOCI WITH MISSING OR INCORRECT GENOTYPES AS A FUNCTION OF PELLET AGE.

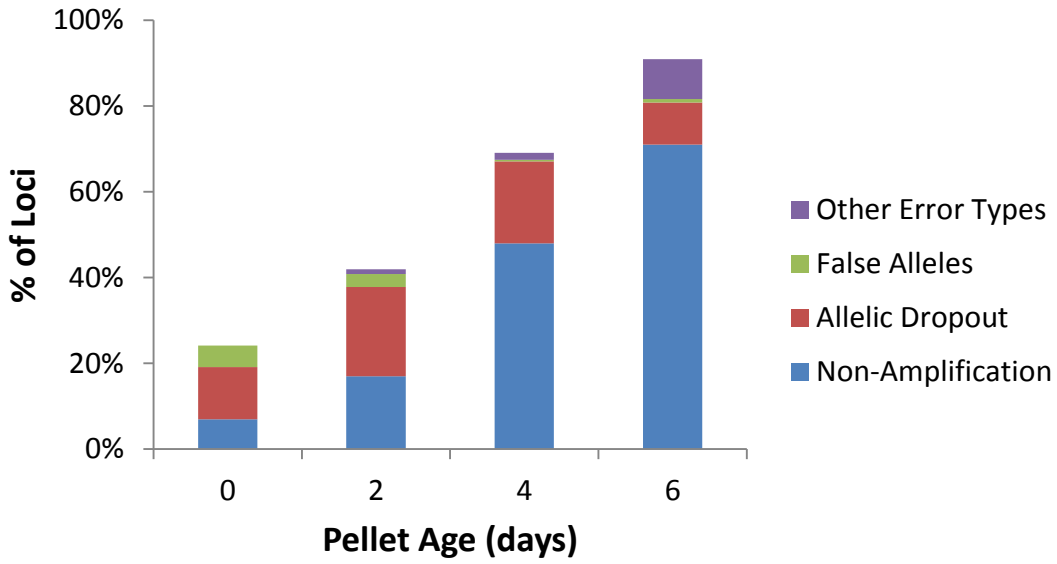


FIGURE 5. DISTRIBUTION OF PELLETS ACROSS GENETIC SAMPLING PLOTS.

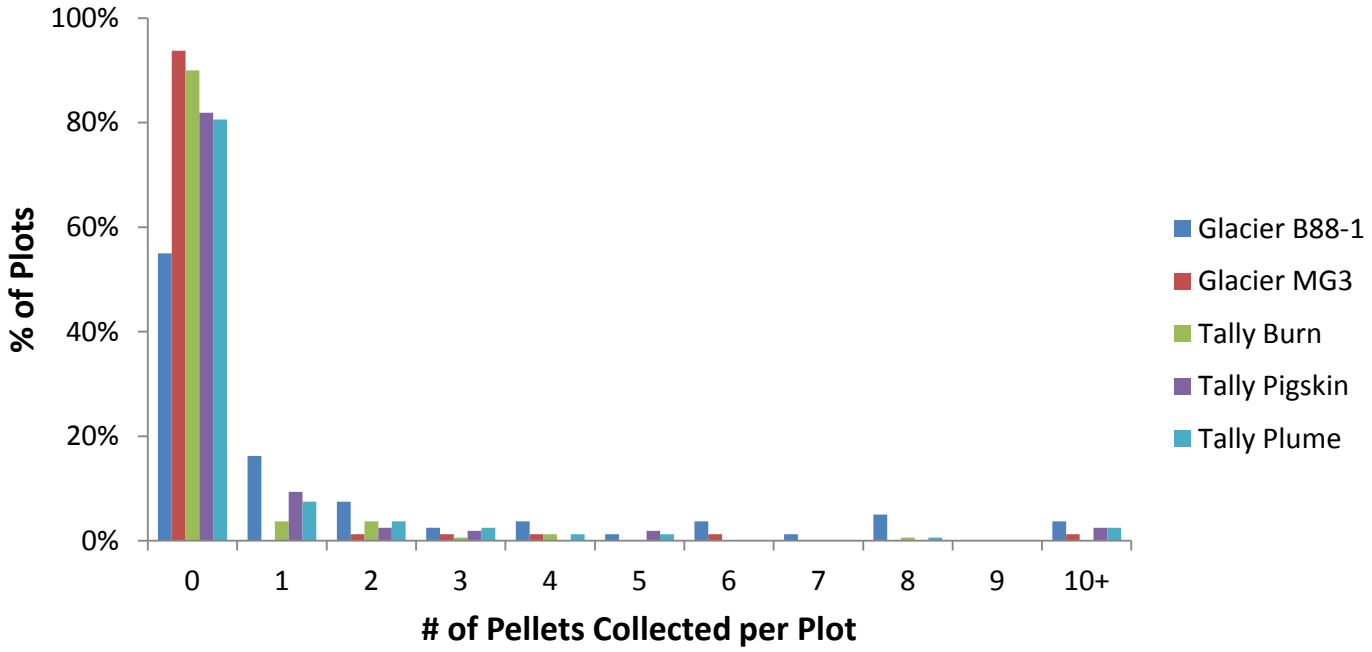


FIGURE 6. NUMBER OF INDIVIDUALS REPRESENTED ON GENETIC SAMPLING PLOTS.

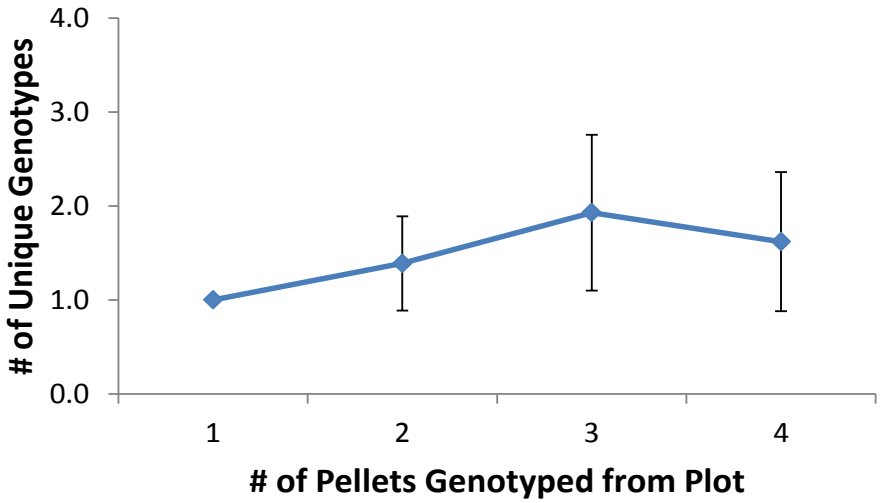


FIGURE 7. DISTRIBUTION OF INDIVIDUALS ACROSS GENETIC SAMPLING PLOTS.

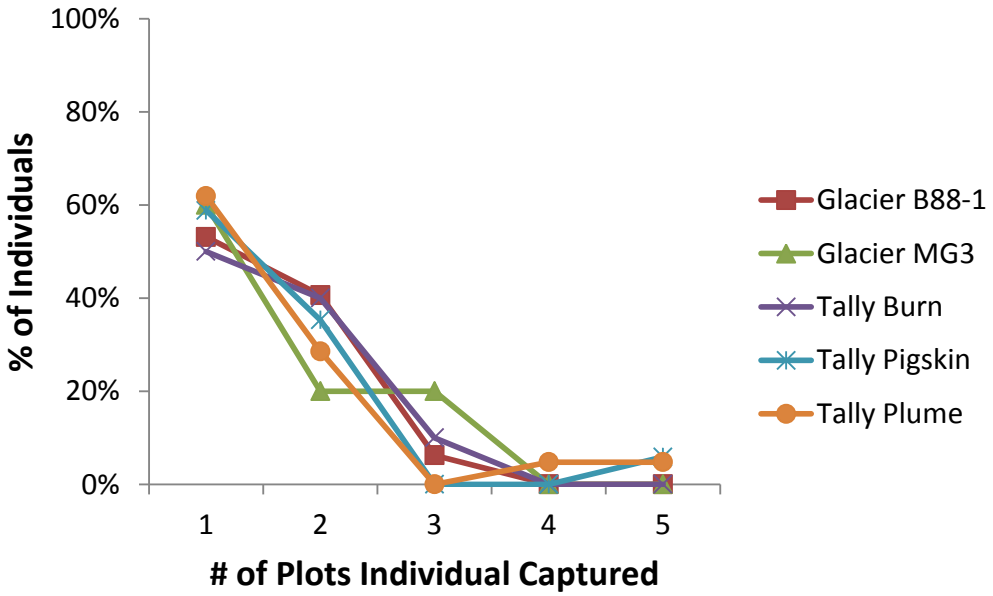


FIGURE 8. LINCOLN-PETERSON ESTIMATES OF SNOWSHOE HARE ABUNDANCE AT 5 STUDY SITES. Error bars represent ± 1 SD. Trap-based estimates are gray columns; all other columns are estimates based on genetic sampling, calculated separately for (maximum) 1 – 4 pellets genotyped per plot. For 3 sites in Tally Lake National Forest (Burn, Pigskin and Plume), separate estimates were calculated for 80 sampling plots per site and 160 sampling plots per site as indicated on X-axis.

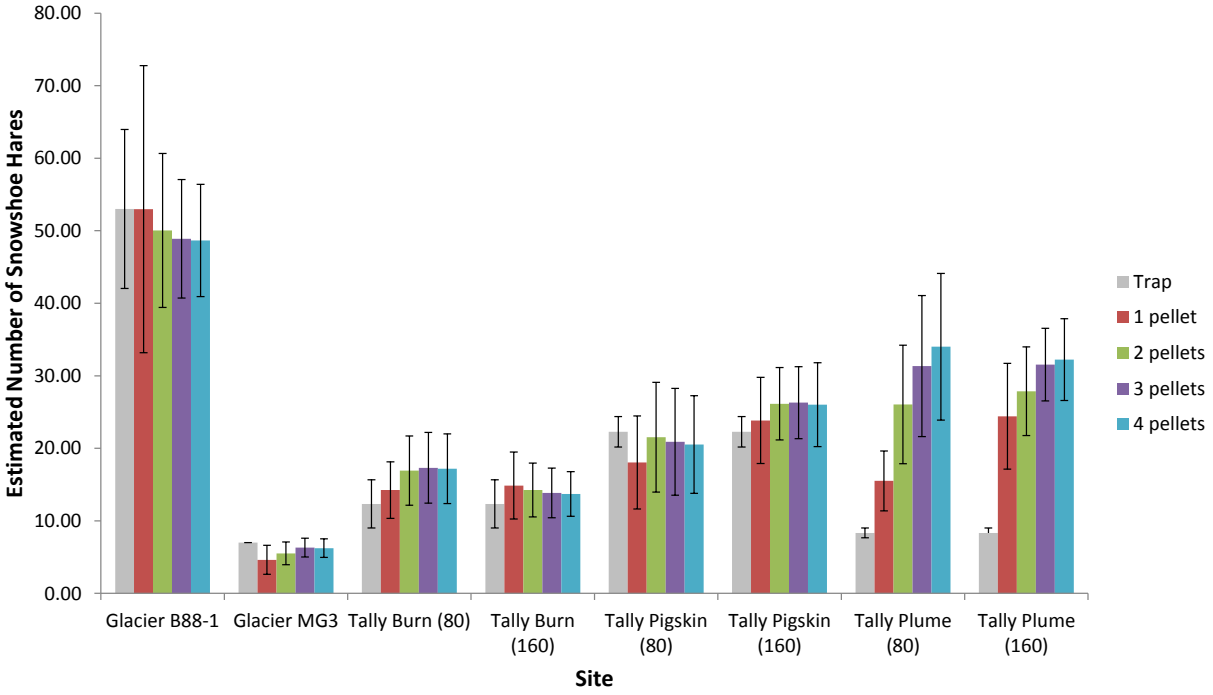
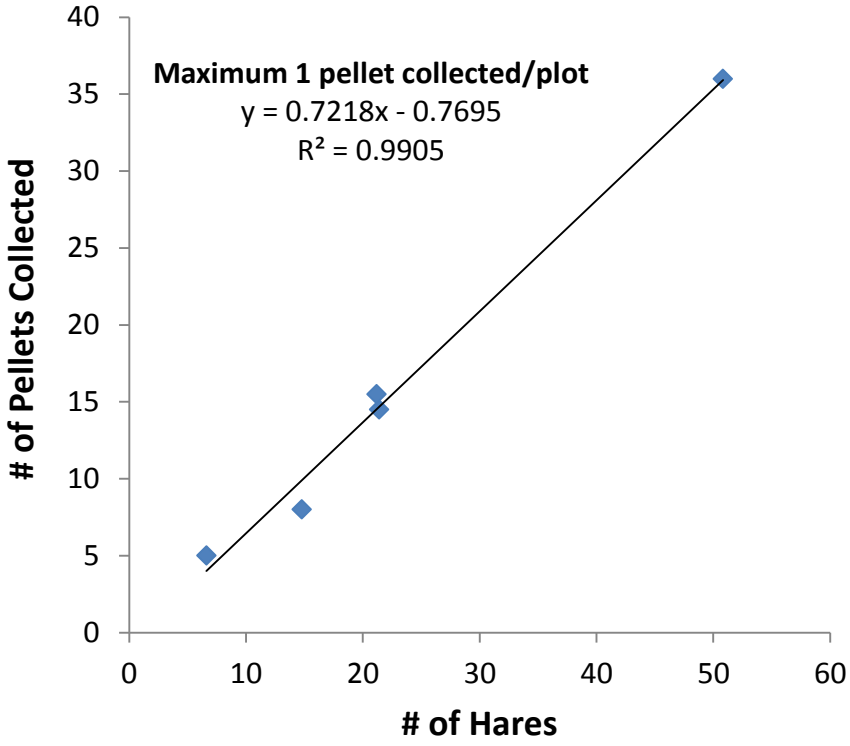


FIGURE 9. RELATIONSHIP BETWEEN ESTIMATED NUMBER OF HARES AND NUMBER OF PELLETS COLLECTED FOR GENETIC ANALYSIS.



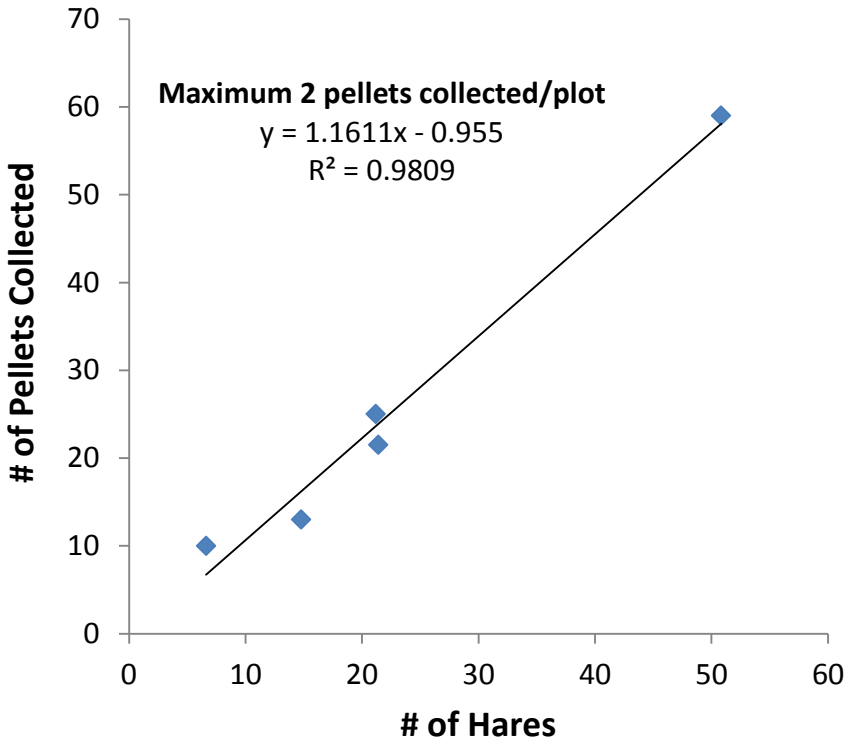


FIGURE 10. COSTS OF LIVE-TRAPPING AND NON-INVASIVE GENOTYPING FOR ABUNDANCE ESTIMATION, AS A FUNCTION OF NUMBER OF HARES AT A SITE. NON-INVASIVE GENOTYPING IS CHEAPER THAN TRAPPING FOR ALL BUT THE HIGHEST DENSITY HARE SITES WHEN LOGISTICS ARE DIFFICULT, AND FOR MANY ABUNDANCES WHEN LOGISTICS ARE EASIER.

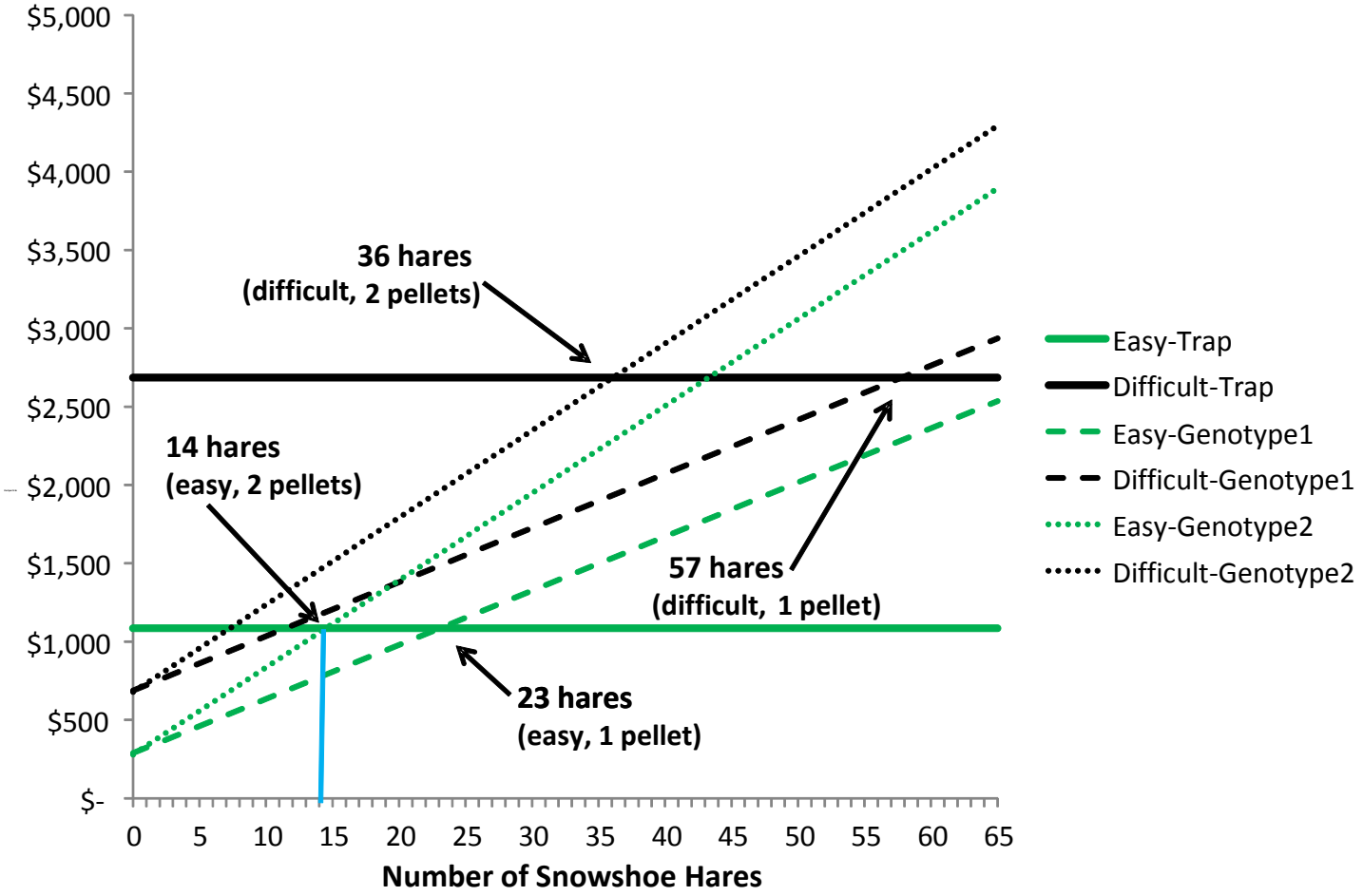


TABLE 1. PARAMETERS FOR COST-BENEFIT ANALYSIS OF LIVE-TRAPPING AND GENETIC SAMPLING.

		LIVE-TRAPPING		GENETIC SAMPLING	
FIELD COSTS		**Easy Site**	**Difficult Site**	**Easy Site**	**Difficult Site**
A	Field supplies (bait)	$5	$5	$5	$5
B	Set up site grid, put out 80 traps/ground cloths	4 people, 8 hrs	8 people, 14 hrs*	2 people, 8 hrs	2 people, 18 hrs
	Live-trap hares	5 days, 4 people, 3 hrs/day	5 days, 8 people, 1.5 hrs/day		
	Collect traps/pellets	4 people, 4 hrs	8 people, 12 hrs**	2 people, 8 hrs	2 people, 18 hrs**
LABORATORY COSTS					
C	Per-pellet supplies and fragment analysis[+]			$31.7/pellet	$31.7/pellet
D	Laboratory technician			1.35 person-hrs/pellet[++]	1.35 person-hrs/pellet[++]
	TOTAL COSTS[+++]	**$1085**	**$2685**	**$325 + $47.9/pellet**	**$725 + $47.9/pellet**

* maximum per-person load of 10 traps and camp gear, requiring crew of 8 people for initial 10 hr hike in to site

** includes 10 hr hike out of site

\+ 8 loci combined in 3 multiplexes. We assumed 19% of pellets would have low amplification rates (based on our study), so would be excluded from further analysis after the first 2 PCR runs. Pellets retained for analysis would average 4.6 PCR runs to yield a reliable consensus genotype. All of these assumptions were incorporated into the per-pellet laboratory cost.

++ includes extraction, PCR, analyzing chromatogram results, and determining consensus genotypes

+++ assumes field technician rate of $10/hr and laboratory technician rate of $12/hr. For live-trapping, the cost equation is: A + $10×B. For genetic sampling, the cost equation is: A+ 10×B + C×pellets + $12×D×pellets.

TABLE 2. ESTIMATED NUMBER OF SNOWSHOE HARES AT STUDY SITES, BASED ON LIVE-TRAP CMR. Captures were allocated to two trap sessions for Lincoln-Peterson abundance estimation. In this table, total captures = number of captures summed over all trap nights, unique individuals = number of unique hares captured, n1 = number of individuals trapped in first capture session (data combined from first two trap nights at each site), n2 = number of individuals trapped in second capture session (data combined from remaining trap nights), m2 = number of individuals trapped in both capture sessions, N = Lincoln-Peterson estimate of abundance, SD = standard deviation of abundance estimate.

SITE	Total Captures	Unique Individuals	n1	n2	m2	N	SD
Glacier B88-1	43	33	23	17	7	53.00	10.95
Glacier MG3	12	7	7	2	2	7.00	0.00
Tally Burn	11	9	4	7	2	12.33	3.33
Tally Pigskin	38	20	15	15	10	22.27	2.10
Tally Plume	24	9	6	7	5	8.33	0.67

TABLE 3. PELLETS COLLECTED AND GENOTYPED FOR GENETIC SAMPLING. In this table, total plots = number of sampling plots distributed at each site, total pellets = number of pellets collected from plots.

SITE	Total Plots	Total Pellets
Glacier B88-1	80	172
Glacier MG3	80	26
Tally Burn	160	37
Tally Pigskin	160	138
Tally Plume	160	149

TABLE 4. INDIVIDUALS IDENTIFIED BY GENETIC SAMPLING. In this table, pellets genotyped = number of pellets genotyped per site (up to 4 pellets per plot), consensus genotypes = number of genotyped pellets that yielded consensus genotypes, unique individuals = number of unique hares represented by consensus genotypes. The last two columns in this table refer only to individuals identified from genetic sampling, not all individuals known at site (e.g., from live-trapping).

SITE	Pellets Genotyped	Consensus Genotypes	Unique Individuals	% Individuals Identified by >1 Pellet	% Individuals Captured on >1 Plot
Glacier B88-1	85	79	32	59%	44%
Glacier MG3	16	12	5	60%	40%
Tally Burn	31	29	10	50%	50%
Tally Pigskin	59	41	17	41%	41%
Tally Plume	69	49	21	48%	33%

APPENDIX II. GLACIER HARE PROJECT FIELD MANUAL

University of Montana and University of British Columbia Okanagan Snowshoe Hare Research Network

2007 Glacier Hare Project Manual

Ellen Cheng
L. Scott Mills
Karen E. Hodges

Wildlife Biology Program

University of Montana

and

University of British Columbia Okanagan

IMPORTANT PHONE NUMBERS

UNIVERSITY OF MONTANA FOLKS

Ellen Cheng
(h) (406) 251-7269
(c) (406) 370-8709

Scott Mills
(w)(406) 243-5552
(h) (406) 549-5188
(c) (406) 370-4052

Jeanne Franz (UM Forestry Administration)
(406) 243-5292

Vanetta Burton (Co-op Unit Administration, for things related to co-op truck)
(406) 243-4388

Patti Loewen (Forestry Accounting)
(406) 243-5591 // ploewen@forestry.umt.edu

Jodi Todd (Forestry Accounting)
(406) 243-4480 // jodi.todd@umontana.edu

GLACIER NATIONAL PARK FOLKS

Steve Gniadek (GNP Biologist, @ W. District Headquarters)
(406) 888-7833

Pat Suddath (W. side District Ranger)
(406) 888-7831

Ann Marie Chytra (E. side District Ranger, @ St. Mary Headquarters)
(406) 732-7730

Traci Kolc (Front Country—W. side campgrounds, @ W. District Headquarters)
(406) 888-7825

Angie Terrell-Wagner (Front Country—E. side campgrounds, @ St. Mary Headquarters)
(406) 732-7703

Brian McKeon (Backcountry campgrounds, @ Apgar)
(406) 888-7857

Glacier Communications Center
(406) 888-7804

WELCOME

We are glad you have chosen to work with us this summer! This is the third field season for the Glacier research project on snowshoe hares, with the primary goals of better understanding the distribution and abundance of hares in the Park, what habitats they use, how fires impact their populations, and their relationship with lynx distribution in the Park. Snowshoe hares are interesting both because of their ten-year population cycle and because they are an important prey species for many forest carnivores, including the federally threatened Canada lynx. Wildlife biologists in Glacier National Park will be directly applying our results to Park decisions about lynx and other carnivore populations.

To address questions about snowshoe hare population dynamics and habitat use in Glacier National Park, we will use fecal pellet counts in different habitat types. We have selected study sites to represent a random mix of forested habitat types evenly distributed throughout the Park. Additional sites have been chosen to target previously burned areas that are in a regeneration stage likely to support hare populations (i.e., 1986 – 1996 fires).

This study forms one of a family of studies that Dr. L. Scott Mills and Dr. Karen E. Hodges have ongoing, with research on snowshoe hares in Lolo National Forest (NW Montana), Flathead National Forest (NW Montana), and Okanogan National Forest (central Washington). Thus the data collected in Glacier will be used not only to address hare dynamics within Glacier, but will also contribute to broader questions about how landscape patterns affect hare dynamics, population cycles, and possible synchrony in population dynamics among regions.

It is our hope that you enjoy the work you undertake this summer on this research. This manual outlines most of the information you will need to perform the fieldwork. Please feel free to ask Ellen any questions about the actual fieldwork or the research questions we're addressing.

PERSONAL SAFETY AND RESPONSIBILITY

The success of a field season depends on the field crew working safely and trouble-shooting problems that arise. Ideally, each season is injury- and accident-free and people enjoy their time in the field. To that end, in this section we outline the major safety concerns that relate to our work, as well as describing the major responsibilities each person should take for safety.

Vehicle use

The following are requirements.

- **OBEY ALL TRAFFIC LAWS AND SPEED LIMITS.** When driving a work vehicle, do not exceed the speed limit. This means that the drive-5-over-the-limit rule does not apply when you are driving a work vehicle.
- **WEAR SEATBELTS AT ALL TIMES.**
- **Always lock doors when work vehicles are unattended.** Even though it is unlikely anything will happen, it only takes seconds to lock the work vehicle up, to minimize chances of losing expensive equipment. Hide keys behind the vehicle gas tank cover instead of taking them in the field with you--this step prevents loss of keys, and saves trouble if someone is injured or incapacitated.

- **It is illegal to have any alcohol or alcohol containers (even empty ones) in a project work vehicle.**
- Trade drivers if you are tired, or tired of driving.
- Check fuel levels at the end of each work day. Refuel when there is 1/3 – 1/4 tank of gas remaining. Remember to fill out the fuel log and save the gas receipt in the vehicle binder.
- Report any vehicle problems to Ellen ASAP so they can be remedied immediately.

Team leaders will have the primary responsibility of transporting crews to and from field sites. It is very important that drivers pay attention and drive responsibly when in a vehicle. Passengers are also expected to speak up when drivers are speeding or driving recklessly in any way.

Wildlife: bears, mountain lions, moose

These animals usually want to avoid you, but all of these animals can kill you. The most dangerous situation is when you suddenly and silently come upon a female with offspring, because mothers aggressively defend their offspring. Here are some things you can do to minimize the risk that you will have a negative encounter with one of these animals.

1) Make noise, particularly when you are walking through dense vegetation or approaching blind corners. This option may not be possible when you are handling snowshoe hares because you are trying to be calm and quiet to minimize stress to them.

2) Keep your eyes and ears open and if you hear crashing, chewing, vocalizations, or other signals, be careful. Or if you go from a place with lots of squirrel and bird noise into a very quiet zone, or vice versa. . .pay extra attention.

4) If you notice the animal before it notices you, do not draw attention to yourself. Keep your eyes on it and back away from it. Once you safely can, warn other people in the area. Avoid direct eye contact, which can be perceived as a threat.

5) If you and the animal notice each other at the same time, see if you can back away. Talk in a normal voice—that may be enough to scare the animal off.

6) Always carry bear spray with you and make sure it is readily accessible. If you leave your pack to check out an off-trail site, take a picture, or for any other reason, remember to bring your bear spray with you. This rule applies whenever you are in the field and not in a vehicle.

Grizzly bears. If you are a distance away, the bear may not know what you are. If it stands up or ambles closer or peers at you, talk in a normal voice and raise your arms slowly—let the bear know you are human. If that doesn't send the bear running away, try to walk away yourself, or climb a tall tree. Do not run away unless safety (car, etc.) is nearby and you are sure you can reach it quickly. Most charges are mock charges. If a bear charges you and gets within 8 m., make sure you are not downwind of the bear (shift your position as necessary) and discharge your bear spray for a couple seconds. If the bear continues to charge, discharge your spray again. If you are attacked, lie face down, protect your neck with your hands, and spread your legs shoulder-width apart. Do not move until the attack is over unless it is clear the bear intends to eat you—in that case, fight like mad.

Black bears. If it hasn't seen you, walk away. If it has seen you, walk away if you can,

making sure it knows what you are. Make yourself look large if the bear threatens you (wave arms, make noise), and stand firm rather than running. If a bear charges you and gets within 8 m., make sure you are not downwind of the bear (shift your position as necessary) and discharge your bear spray for a couple seconds. If the bear continues to charge, discharge your spray again. As a last resort, fight like mad and make lots of noise. Do not lie down if you are attacked—when black bears attack, they often aim to kill. Do not climb a tree—black bears are excellent tree climbers.

Mountain lions. Do not run or turn your back on a mountain lion. Shout, wave your arms, and make yourself look as big as possible. If you are attacked by a mountain lion, fight back.

Moose. More people are killed by moose than by black or grizzly bears. If none of the avoidance techniques have worked and a moose is looking hostile, run or climb a tree, or at least keep a tree between yourself and the animal. These animals run faster than you do, but are generally not interested in chasing you.

Communication with each other

Part of our personal safety will come from each other.

- When we are in Glacier, we will generally work in teams of 4 or more people per site per day. At the site we will usually work in pairs (or at least in pairs within shouting distance of each other).
- Make sure other people know your destination and route.
- When working in the backcountry, each 4-person team will have a Park radio, but note that in some areas these radios will not work. When working in the front country, teams may leave their Park radio in the work truck while in the field. Use them responsibly. Park personnel can hear everything you say on Park radios. Use project walkie talkies—rather than Park radios--for communicating with your teammates.
- **The ultimate responsibility for safety lies in your hands**. Be aware of your surroundings, the weather, your location, and your physical state at all times. Here are some items you should carry with you when you're in the field: bear spray, compass, headlamp, water, emergency food, extra clothing, rain gear, walkie talkie, first aid kit, extra batteries.

Heat, cold, and high elevation

We will work in a range of temperatures and weathers and at high elevations. Dress appropriately and carry water and safety gear. Heat stress is especially possible. To reduce the risk of heat stress: wear a hat and sunscreen, drink a lot, work in the shade if possible, wear sunglasses, and wear light-coloured clothing. Communicate with each other if you are experiencing disorientation, unusual fatigue, weakness, difficulty walking or talking, headaches, or muscle cramps. To recover, lie down in the shade, drink, put water on your skin, and elevate your feet.

Reporting injuries

Report all injuries to Ellen as soon as possible. We need to file a report that an incident happened within 24 hours of the event.

WORKING AND LIVING IN GLACIER NATIONAL PARK

Working in a national park requires care and courtesy, both for the places in which we will work and the way in which we will live. To develop this research, we have had to go through an extensive grant-writing and permitting process, involving collaboration with many park personnel. This project is one of very few research projects that is supported financially by the Park—thus, we represent the scientific community in the eyes of the Park. The Park is extending us the courtesy of providing campsites, providing maps and expertise, and much more. Without their support and encouragement, work in Glacier would not be possible. Because we are visible to the public in the Park, we need to take extra care. Assume that the public is watching and judging us at all times. We will need to present ourselves and our research clearly and professionally at all times. Similarly, our field operations must be kept litter-free and minimally invasive. **The reputation of our crew, and our ability to continue work in the Park, rests on each one of us. It only takes one person to give us a bad reputation.**

Living in the Park

We are living in subsidized Park housing and must follow Park rules even on days off. In particular, pay heed to these:

- **Quiet time is 9 PM every night**—do not bring loud parties to the Glacier hare house.
- No illegal activities—this is grounds for immediate removal from the project.

Driving in the Park

- **OBEY ALL TRAFFIC LAWS AND SPEED LIMITS.**
- We will be issued entrance passes for the Park. Carry a copy with you whenever you are working: it will allow you access to the Park.
- If you drink, drink responsibly and do not drive while under the influence.
- **Park only in designated areas**, unless we have obtained special permission from the Park to do otherwise. In cases where there are not many pull-outs near a field site, this means we may have to walk some distance along the road to get to our vehicles.

Camping in the Park

- At least two weeks prior to using a front country or backcountry camp or cabin, we will request camp/ cabin access from the Park.
- When you arrive at a Park campground, let the camp host know that you are the Glacier Snowshoe Hare research crew, and tell him/her the expected duration of your stay. Fill out campground permits for the nights you will be staying at a Park campground. In the Fee section, write “Glacier Snowshoe Hare Research Crew—Park supervisor, Steve Gniadek, Park Wildlife Biologist”.
- Keep campsites clean. When leaving a campsite, even if only for a 15-minute errand run, we need to put everything away in tents or vehicles. This includes water bottles, coolers (empty or otherwise), clothing items, and especially food items and kitchenware. The Park will fine us for any items left out when we are not attending the campsite and they are quick to

notice.

- Respect neighbors and be as quiet as possible while leaving, especially during early mornings. **Quiet time for our crew in Park campgrounds begins at 9 PM—NO EXCEPTIONS!**
- When using backcountry cabins, pack out all trash including biodegradable food garbage.
- During fire season, no smoking in the backcountry. At any time, no cigarette butts left at field or camp sites except in fire pit.

Hiking and Conducting Research in the Park

- Observe all wildlife closure areas and times. Of particular concern are Bear Management and Wolf Denning Areas.
- When hiking, try not to enlarge trails—if muddy or water-logged and your boots are waterproof, walk through the mud. Particularly when we go off-trail, we need to minimize the impact of our activities.
- We are an important set of eyes and ears for the Park. Report significant wildlife sightings on the forms provided to us by the Park. Backcountry crews should report on trail and campsite conditions to local Park rangers upon return from each trip.

CARING FOR GEAR AND THE VEHICLE

Much of the gear you will use is new and expensive. Protect items from weather, handle them gently, and keep work vehicles clean. Be especially careful not to leave equipment behind as you move around and between study sites. This applies to items as minor as sharpies and flagging, as well as to more expensive gear such as GPS units and compasses. Report any faulty equipment as soon it is noticed. If we are running low on supplies (e.g., pin flags, datasheets, etc.), take the initiative to tell Ellen with ample advance notice.

Vehicles—drive carefully and responsibly!! Work vehicles may not be used to transport any alcohol. Work vehicles are not to be used for driving to the bar, parties, etc. We pay $0.49 per mile to drive the co-op truck and $0.45 per mile to drive Blue Betty—vehicles are to be used primarily for driving to and from field sites.

GENERAL PROJECT RULES

- **Regular work days begin at 7:30 AM (this is the time we are in the truck, ready to go).**
- Work days end only after datasheets have been collected & filed, data have been organized and properly stored, the next day's equipment prepared, and log book entry completed.
    - Team leaders are responsible for collecting, checking, and storing data at the end of each work day, and also for updating site map & site chart regularly. This applies during backcountry hitches as well.

- Each person is responsible for ensuring his/her equipment are in good order and ready for the next day—this includes recharging batteries and fixing Krebs ropes as necessary.
- One person will be put on log book-duty each week. The log book is our best way to keep track of everything we do on this project. Every log entry should begin with the date, person's name, and start/end time for that work day. Describe what your team did that day and any suggestions, unusual circumstances, sightings, etc. Only one person per team needs to write a full entry, but all team members should contribute information to the daily report. This information will be used to keep track of what each team is doing in the field, since we will be working different sites. When working backcountry, full letter-sized paper can be used in lieu of bringing the log book. Make sure these sheets are stapled into the log book upon return from the backcountry.
- One person each week will be responsible for completing site surveys and filling out a grid UTM sheet for the next day.

DATA COLLECTION AND MANAGEMENT

Data integrity is essential to the whole scientific endeavor. If you are unclear about what to record or how to record it, ask. Never falsify records or disregard the written protocols. Substantial amounts of funding have been provided for the work we are undertaking and we have a moral obligation to uphold our end of the contract by recording data accurately.

If you make a mistake, report it immediately. We all make mistakes and almost all of them are minor and correctable. **The worst mistake you can make is to hide a mistake.**

The whole purpose for being in the field is to collect meaningful field data to answer ecological questions. We have datasheets for all of the kinds of data you will collect: be sure to follow the protocols, write clearly, and **make notes of any unusual happenings**. The data become unusable if we cannot read your writing, or if you don't write down enough information, or if you do not follow the protocols. Please note the following points about recording data:

- **Write your name** or initials on all data sheets.
- Write the full date, **including year,** on all data sheets.
- When working with a partner, **repeat numbers** before writing them down. This particularly applies to surveying vegetation cover—this is a good way to double check that entries are for % OBSTRUCTED, not % visible.
- **Write legibly.**
- **When in doubt, ask.**
- **Record values for all grid stakes or sample points.** If a value is 0, write 0 (i.e., do not leave blank).
- **Before leaving a site, the team leader should ensure that all transect lines and points have been completed.**

FINANCIAL ACCOUNTING AND RECEIPTS

Make sure you ask permission before purchasing anything for which you will ask to be reimbursed from project funds. University of Montana requires a complete paper trail for purchases: note that receipts must display what the items are that you bought (i.e., credit card receipts need to be accompanied by the actual receipt showing the items). This means that a receipt line of "misc." or "supplies" is not sufficient for reimbursement. If your receipt does not specifically say what item you purchased, ask the clerk for a handwritten receipt that does detail the purchase. **Purchases without receipts are absolutely not reimbursable!**

FIELD PROTOCOLS

Side note on hare sampling

If you find a road kill hare in Glacier and can safely do so, please grab a biopsy sample from the ear and write the UTM coordinates of the location, along with a note indicating it is a road kill. Keep in freezer until Ellen can bring it to Missoula. If the road kill hare is in good condition, grab it for a museum sample and let Ellen know ASAP. The Park or the University will be happy to supplement their collections.

SITE RECONNAISSANCE

UTMS are in NAD83

Site reconnaissance equipment

Datasheets:
- Map of site with UTM coordinates
- Site survey form

Pencil
Camera
GPS unit
Compass

Getting to the site

We will use GPS units and topographic maps to get to our sites. Before leaving the truck and/or trail to bushwhack, GPS the location so you can find it again when hiking out. When you arrive at the site and before your team splits to separate tasks:

- check that walkie talkies are working
- confirm site objectives & task assignments
- confirm site name, date, and other datasheet header information

All sites meet the criterion of 85% forested based on Glacier's GIS vegetation layer. However, in some cases we will arrive at a site and find that the site is unsuitable for our work. If a site seems questionable upon arrival, use your GPS and/or pacing (with a compass) to walk the perimeter and at least one diagonal (preferably two) to get a general idea of the site's suitability for our work.

A site is unsuitable if >15% of the site (equivalent to approximately 18 of the 80 site points) is unforested (e.g., talus slope, large river running through site, large meadows). This may be difficult to determine from the ground. For example, there may be a tendency to perceive an open meadow as covering a larger portion of the site than it does in reality. Remember that 20 ha is a large area and that almost two full lines (e.g., all of lines A&B) would have to be in open meadow for a site to be >15% unforested. It makes no difference if the unforested area is one large open meadow in one corner of the site, or if it is several small meadow patches dispersed throughout the site.

A site is also unsuitable if doing a stand would be physically dangerous to access or work in due to extreme cliffs, etc. If a stream or a steep rocky cliff intersects a site, this is not a reason to

reject the site unless the stream or unforested cliff covers >15% (i.e., >18) of the site points. It may be necessary for half the team to access the site from one side of the stream or cliff and the other half to access from the other side.

If there are large patches of snow on the site, note this on the Site Survey. We will return to these sites later in the season after much of the snow melts. We cannot conduct Krebs counts over snow patches because all pellets under snow cover will be hidden from view.

Sites should not be intersected by trails or roads. An exception may be made if the intersecting trail is no longer maintained and therefore little used—but make note of this on the Site Survey form. Sites falling on trails should be shifted in the field--we don't want to disturb the public's perception of Glacier as "untouched".

In some cases we will have to cross water bodies to get to sites. Since many sites will only be accessed once (for one day of Krebs & vegetation work), this should not be used as a reason to reject a site except under extenuating circumstances (e.g., accessing the site would require a 5-km bushwhack). **Under no circumstances should you cross a stream under unsafe conditions.** Instead, look for bridges you can use or places where stream crossing would be safe—this may require a few kilometers of additional hiking.

Choosing alternative sites

In general, the need to reject a site should be rare. If you do reject a site, however, check alternative site possibilities by using the (formerly) SW corner of the original site as the SE corner, NE corner, or NW corner of potential grids. If all of these "corner" alternatives fail, choose an alternative by shifting the site just beyond the problem area (e.g., if the northern half of the original site is open meadow, shift the site just south of the meadow). If an alternative is found, make sure you note the new site UTM's on the Site Survey datasheet. The site should be referred to as "XX-alt" on all datasheets (e.g., Site# 26-alt). Whether you find an alternative or not, a Site Survey should be completed on the original (rejected) site as well as the new (alternative) site (when applicable). The Site Survey for the new alternative site should include details on prior alternatives that were attempted and why they were unsuccessful—i.e., we should show that we tried alternative corner sites and had valid reasons for not using these alternatives (difficult site conditions such as high shrub densities or downed logs are not valid reasons for rejection) before simply shifting sites beyond the problem area.

Site survey forms

For each site, complete a Glacier Site Survey data form. These forms will be scanned and linked to the Park's GIS database, so fill them out responsibly and thoroughly. On these forms provide information on potential logistical difficulties presented by the site (either accessing the site or working on the site), suitability of the site for our work (e.g., does it meet the 85% forested criterion?, did you see hare pellets during your reconnaissance?, etc.), and a detailed description of site habitat. For the latter we are primarily interested in dominant canopy tree species, nature of understory (open vs. dense? shrubby? herbaceous?), and presence of water bodies, open meadows, cliffs, or other non-forest habitat characteristics, and any unusual signs of tree mortalities, etc. Please be detailed in your notes, e.g., describe the extent of open patches rather than just saying there are open patches on the site. Also make note of wildlife signs (e.g., did you

see lots of deer pellets?, fresh bear scat?, many squirrel middens?). Get information from other crew members to inform your site survey, since other transects may look quite different from the transects you walked.

Site photos

Team leaders are responsible for taking photos at each study site. These photos will be linked to the Park's GIS database. The purpose of these photos is to give the Park a general idea of the habitat at each of these sites. Most of our sites will be in areas that Park personnel have not visited recently, so our photo documentation will provide useful information to the Park about the area. Take a photo of the site looking North from the 1- and 10-points of two different transects (preferably not adjoining transects) per site, and record the information on the Krebs sheet photo section--camera# and photo point, in the order photos were taken. If a tree is in your way when you try to take the picture, move to the side to get a photo of the general habitat from the sampling point. If you see anything unusual on a site (e.g., wolf den, large-scale tree mortalities, etc.), take a photo for the Park and record the photo information on the Krebs sheet photo section.

KREBS PELLET COUNTS & VEGETATION SAMPLING

Krebs pellet count & vegetation sampling equipment

Datasheets:

- Map of site with UTM coordinates
- Grid UTM's
- Krebs
- Veg.-understory
- Veg.-saplings

Pencil
Compass
GPS unit
Krebs measuring rope (doubles as 5 cm. DBH tape for saplings)
6 m. vegetation rope
Canopy (PVC) tube
Coverboard

Overview

At all of our study sites we will conduct Krebs pellet counts and vegetation sampling. Krebs pellet counts provide a quick way to track snowshoe hare relative abundance—a stand can be sampled in less than a day. The Krebs plots we will be using are 10 feet by 2 inches; this size was initiated by researchers in Yukon, and proves to be better than other shapes and sizes at providing reasonable estimates of pellet density on a stand.

To maximize project efficiency, we will not set up formal site grids prior to conducting Krebs pellet counts. Instead we will use pacing, compasses, and GPS units to approximate our 50-m. grid points.

KREBS PELLET COUNTS

NOTE: KREBS DATA ARE COLLECTED IN INCHES AND ONE DECIMAL POINT OUT!

When you arrive at the site and before your team splits to separate tasks:
- check that all walkie talkies are working
- confirm site objectives & task assignments
- confirm site name, date, and other datasheet header information

- We will establish 20 ha grids that are 8 x 10 with 50 m spacing, labeled from A1 to H10. Grid lines will run north-south (1 at south end, 10 at north end), with A1 at the SW corner of the grid.
- Use a GPS to find your starting point on a grid line (either the 1-end or the 10-end of a line). If the accuracy of the GPS is large (e.g., worse than 15 meters), pace out to your grid line from the A1 point, rather than using the GPS. You will need to adjust your pacing to account for rough terrain (e.g., climbing over downed logs, steep slopes, etc.). It is important that you do not bias your steps toward areas that are easier to walk through, etc. Do your best to walk a straight line to your sampling point. **Check your compass frequently.** If you come across water bodies, cliffs, etc., do your best to estimate the number of paces you would have taken, had you walked through / across that obstacle. Also note where you should be coming out on the other side of the obstacle, and pick up your remaining paces from that end.
- When you hit your last step, spin your compass in one direction for a random bearing. Without looking up, throw your Krebs rope in the direction of the compass bearing, and use the landing point of the Krebs nail as the southern end of your Krebs transect.
- Use your backpack or some other item to mark your grid point before walking to your Krebs point. From the Krebs nail landing point, stretch the Krebs measure rope out due north as straight and close to the ground as possible. **Make sure you don't step in the path of the Krebs rope!**
- Working from the north end of the Krebs rope (opposite the Krebs nail) and pushing the Krebs rope to the ground in front of you as you go along it, run the 2" slider along the measure rope and count all pellets that fall at least half within the bounds of the 2" slider. Only count pellets that are at least partially visible on the surface of the ground—do not dig through leaf litter to search for underlying pellets. NOTE: If you are unsure if some pellets are hare or squirrel pellets, count and collect them (for verification later in camp), asterisk the datum and indicate in your notes how many of those were questionable.
- **Be careful of inclusion bias!!** For many people there is a tendency to count a pellet as "in" when it is close to the Krebs transect, even if it not at least half within the bounds of the 2" slider. This is especially true at low pellet density sites. Avoid this temptation!! Inclusion bias is potentially a serious problem with Krebs plots because these plots have a high edge: area ratio—be aware of this bias and make a conscious effort to avoid it.
- On your datasheet, write the total number of pellets you counted in the Krebs plot.
- If your Krebs plot intersects an area that hares physically cannot inhabit (i.e., water bodies or through the trunk of standing trees) or in which we cannot accurately count pellets (e.g., snow patches that may "hide" pellets), estimate the length of the plot that would cross through the non-habitat or snow patch. Add up the number of combined transect **inches**

(rounded to the nearest half inch) that fall in each of these non-habitats or snow patches and enter this on your datasheet under "Water", "Tree Basal", or "Snow". Keeping the same Start Point, rotate the Krebs transect clockwise until you can pull the transect out to its full 10' extent without intersecting non-habitat or snow. Count pellets in this new (shifted) Krebs transect instead of the original transect (which intersected non-habitat or snow). Basically, your Krebs pellet count assumes it is possible to find pellets along the entire length of the transect. This means that the Krebs transect on which we count pellets should not intersect even a few inches of water, tree basal, or snow. However, we do want to keep track of how much of each site is non-habitable so we can incorporate this information in our calculation of site-specific hare densities.

- Wet meadow, patches of wet forest, and downed logs are not considered areas that hares physically cannot inhabit, so do not necessitate any shift in the Krebs transect. When possible, pass your Krebs transect under downed logs rather than over downed logs, to keep your transect as close to the ground as possible.
- The only time you should shift your Krebs Start Point is if it falls in water, tree basal, or on snow. In this case, estimate the length of the plot that would cross through the non-habitat or snow patch. Add up the number of combined transect inches (rounded to the nearest half inch) that fall in each of these non-habitats or snow patches and enter this on your datasheet under "Water", "Tree Basal", or "Snow". Move your Start Point to the nearest point outside of the water, tree basal or snow, and conduct your pellet count from this new Start Point (with Krebs transect running north unless a north transect would intersect non-habitat or snow).
- To get to your next sampling point, pace out 50 meters, as above. At approximately the 1, 4, 7, and 10 lines you should use your GPS to check your location and conduct your Krebs pellet count from the GPS-corrected location unless GPS error is high. In this way you will periodically correct your location to ensure you don't get too far off course from the 50-meter grid points.

VEGETATION SAMPLING

On all 10 points of lines B, D, and G of site grids we will obtain estimates on the following site characteristics. Vegetation sampling should be conducted from the Krebs Start Point. If the Krebs Start Point has been moved due to water, tree basal, or snow, vegetation sampling should be conducted from the new (rather than original) Krebs Start Point. If 6 m. east or 6 m. west from the Krebs Start Point would land you in water or beyond a cliff for canopy & understory cover readings, rotate to the closest location (not in water) for these readings, but maintain a 6-ft. distance from the Krebs Start Point. Make note of the circumstances on your datasheet Notes column.

Canopy cover

For canopy cover, use the PVC tube directly at the Krebs point and at one point 6 m. east and one point 6 m. west from the Krebs point. When possible, have your partner verify that you are holding the PVC tube vertical before each reading. Record to the nearest 5% (or, from 0-5% or 95-100%, to the nearest 1%). **You should be recording % OBSTRUCTED.** Because it is easy to forget that we are recording % obstruction rather than % visibility**, call readings out loud** so your partner can catch potential mistakes.

Understory cover
For understory cover, one person should move 6 m. east of the Krebs point and hold up the cover board for the other person to read. If the cover board holder is facing perpendicular to a slope, (s)he should hold the cover board so the horizontal center of the coverboard is on the ground—this means one side of the cover board will be slightly above ground, while the other side will run into the ground. The cover board is read per 0.5 m height increment: move your eyes to the CENTER of each block to approximate what an animal at that height would see. If the coverboard is up- or downslope from the cover board reader, the reader's eyes should be the same height above ground as the center of each block—i.e., the line of sight to the coverboard should be parallel to the slope rather than horizontal.

If there is a tree or downed log just where the cover board should be, the cover board should be held behind the obstruction. Do not move the cover board around obstacles to improve visibility!! If there is a tree directly in front of the cover board reader, (s)he should not move to improve visibility.

Record % OBSTRUCTED. Because it is easy to forget that we are recording % obstruction rather than % visibility**, the cover board reader should call his/her readings out loud,** so his/her partner can catch potential mistakes.

Repeat the cover board readings at 6 m. west of the Krebs point.

Note: Prior to beginning vegetation work, each person should use the cover board to determine the sitting / standing position appropriate for reaching eye level for each height category.

Downed logs
Count the number of downed logs >5 cm DBH along the 6 m transect path (both east & west) from the Krebs point. If a log is partially buried, it should be counted if >5 cm of the log diameter is above ground.

Dominant canopy tree species
From the Krebs Start Point, record the dominant canopy tree species in your fish eye view. Your fish eye view is what you see when you look straight up without moving your eyeballs. The dominant canopy tree species is the one species whose canopy covers the largest percentage of area in your fish eye view. Canopy trees are defined as trees >10 m. tall. When recording data, use the tree codes in your vegetation identification packet. If there are no trees in your fish eye view >10 m. tall, record "none" on your datasheet.

Definitions: TREE – Though there is no set definition regarding minimum size, the term generally applies to plants at least 6 m (20 ft) high at maturity and, more importantly, having secondary branches supported on a single main stem or trunk with clear apical dominance. SHRUBS-- Distinguished from a tree by its multiple stems and lower height, usually less than 6 m tall. A large number of plants can be either shrubs or trees, depending on the growing conditions they experience.

Canopy trees (RELASCOPE DATA COLLECTED ONLY IN 2005)
Note: dead trees do not get counted. One person should use the relascope at the center point. Start by setting the relascope to 0. Look through the relascope (while turning full circle) at all trees at DBH to determine which ones have DBH greater than your basal area factor (BAF). Try to get 10-12 trees per site. Use basal area factor 10 (the two left bars) first, and if that gives too many trees, use 20; if it gives too few, use 5. Be sure to record your BAF on the data sheet! Record each included tree by species and DBH. The other person can do the measuring (at DBH) and identification. NOTES:

- If a tree DBH is exactly as wide as the BAF, the tree should be included in your count.
- If a tree splits into two trunks below 4.5' height, consider each trunk as a separate tree and look through the relascope at 3.5' above where the tree splits. If a tree splits into two trunks above 4.5' height, consider it one tree and look through the relascope at a distance below where the tree splits (so you don't get the part of the tree that widens just before the split).
- If a tree is hidden behind another one, step to the side so you can see the hidden tree and check its DBH against the relascope. Do not skip a tree simply because it is hidden!

Sapling density
Note: dead saplings do not get counted. Count live saplings that have their trunk centerline within a 2 m radius of the Krebs point. Record saplings by species into the following categories: 0.5 to 1.5 m tall, 1.5 to 3 m tall, >3 m tall. Saplings have DBHs <5 cm. When recording data, use the tree codes in your vegetation identification packet. Use hash marks to keep track of counts in each category, then write and circle the final tally for each category. If you count >150 saplings in 1/4 of the sampling circle, multiply by 4 to get the total density. If you count >150 saplings in 1/2 of the sampling circle, multiply by 2 to get the total density. In all other cases, you must conduct a full count of the entire circle.

Note: To collect this data efficiently, determine where on your body each height category falls, and use this as a coarse gauge of sapling height. Determine how a 5 cm DBH tree fits in your cupped hand and use this as a coarse gauge of to determine if trees fulfill the <5 cm DBH criterion for saplings. Only measure out saplings that are borderline height or DBH.

BACKCOUNTRY WORK

Backcountry work is the same as front country work, except our sites are farther in, so we will use backcountry campsites and spend more time traveling to sites.

Before embarking on a backcountry trip, fill out a backcountry travel plan and give it to the Communications Center (or Ellen can fax it to 888-7808, or email glac_com_center@nps.gov). This form will tell the Park what time/date you're leaving and arriving on your trip and where you will be each night. You must check in with the Comm Center just before and just after your trip (call or visit). IF YOU DO NOT CHECK IN WITH THE COMM CENTER THE DAY YOU WERE EXPECTING TO RETURN, WE WILL HAVE RANGERS OUT LOOKING FOR YOU. Therefore, if you cannot return on your expected date, you should call in on the Park

radio. If you can't get reception with the Park radio, plan to return as soon as it is safely possible and call the Comm Center the first chance you get.

LIVE-TRAPPING HARES

Live traps that are improperly handled are kill traps. Take time to position, set and trap carefully. Ask questions. Double check everything. For you, it is inconvenient; for the animal, it is life or death.

Trap set-up

Datasheet:
  Trap set-up
Traps
Frame pack to carry traps
Alfalfa bait
White flagging to mark trap locations

Trapping equipment (ingredients of a trapping kit)

Datasheet:
  Trap datasheet
Pencil
Sharpie
Writing board
Eartags (should have 20 minimum)
Eartagging pliers
Biopsy punches
DNA vials with silica
Alcohol (for washing biopsy punches)
Needle nose & cutting pliers—useful for tweaking traps as needed, removing eartags, etc.
Handling bag
Sugar water and dropper
Pesola scale
Tweezers
Neosporin

General trapping suggestions

One key issue when trapping is to make sure all traps are dealt with. Traps can get missed when you are tired, when your mind wanders, when people are meeting each other on a grid, etc. For this reason, you should record an entry on the datasheet for every trap, whether or not a hare is captured. For traps that are closed but do not have hares, record CBE (Closed But Empty). Record if bait has been removed from a trap, whether the trap is closed or open.

- When you arrive at the site and before your team splits to separate tasks:
  - check that all walkie talkies are working
  - confirm site objectives & task assignments
  - confirm site name, date, and other datasheet header information

- We typically set traps in the evening, after 6 PM. That will minimize captures of squirrels and will also reduce the time hares are in traps.
- We will check traps starting at 6:00 a.m. or at first light, whichever is later. We will do so to minimize the time hares are in traps, thus reducing their stress levels.
- Before leaving a site, meet with your group and call out each grid line and the person who completed that line. This will help ensure that the entire grid was completed.

Trap set-up

- Keywords: stable, level, and sheltered.
- Lock back door shut with both hooks; the back door should be in the last row of the bottom of the trap. Failure to do so can allow animals to escape, or, more likely, to injure themselves.
- Put down the big metal U bar to stabilize the trap sides.
- Be sure the lever attaching the treadle to the hook on the door moves easily. Test the hook and treadle: if the mechanism is too tight or too loose, adjust the hook, using pliers if necessary.
- Place the trap under shelter: under branches of a tree, or under thick shrub stems. Also, break off pine branches, grass, etc. to provide additional cover for traps. The shelter helps protect them if weather turns nasty, and (we think) helps them feel more secure from predators.
- Make sure no twigs or branches are sticking into the trap (bottom, sides, top). Twigs sticking into a trap can make the trap malfunction or can injure an animal.
- The bottom of the trap should be stable: if it is uneven, animals may not go in. It is OK if there are small hollows under the trap so long as the mesh is generally flat and mostly supported. If the trap is on sloping ground, try to position the door end of the trap uphill: hares are more likely to go down into a trap than up into one. But if this means the trap door will be facing into a tree trunk or other obstruction, face the trap door downhill to keep a clear entry area. The slope should be less than ~10º. Feel free to engineer the ground to reduce the slope or to make the trap sit evenly on the ground.
- Make sure the door area is clear: hares aren't going to walk through tall grass, shrubs, branches, fireweed etc. to get into a trap.
- If you see a hare runway, or lots of pellets under some trees, those are good places to place traps.
- Check that the squirrel escape is present, the wire is well anchored, and the wire is curved/bent towards one side.
- Put 1-2 alfalfa chunks and some apple <u>behind</u> the treadle. Make sure none of the bait rolls forward under the treadle.
- Use white flagging on a nearby tree or bush to mark the trap location.

Checking traps

Trap still set: Simply close the door. A <u>gentle</u> kick often will do this.

Trap closed but empty: If you come to a trap that is closed but empty, record the stake as CBE on your data card.

Other animal species: record trap point, species, and any other notes. Release the animal without handling.

Snowshoe hare:

- Remember that the hare is scared. Move quietly and softly. When you first see a hare in a trap, keep your distance and try to determine if the hare is new or a recapture (look for an eartag). Stay several meters away to set up your equipment for processing the hare (e.g., pull out necessary equipment and lay them on the ground, put an eartag in the eartag pliers if it is a new hare & record eartag # on datasheet, etc.).
- Hares can injure themselves more easily inside the trap than inside the handling bag, so your next priority is to get it into the bag quickly and safely. Put handling bag over door end of trap with at least 10 cm of bag overlapping front of trap; gather loose material in your hand so it fits tightly. Open door. Blowing on the hare from back of trap often encourages it to move into handling bag. Take your time—don't get the hare riled up and anxious. If it is ballistic, do what you can to get it in the bag fast. DO NOT hold the trap vertical and try to shake the hare into the bag—you can seriously injure the hare if its foot gets caught in the process.
- Weigh the hare. If the hare is wet, note it on the datasheet.
- Check BOTH ears for eartags. Read the tag if there is one. Note torn ears, especially if the tear is where eartags are usually put.
- If there is no eartag, put an eartag in. The eartag should be in the hare's right ear. Feel for the cartilage in the lower quarter of the outside of the ear: the point of the tag should sit just behind that for maximum stability. Tag numbers should be on the inside of the ear.
- Take a tissue sample. Use a new biopsy punch or one that has been cleaned in alcohol and is dry. Use the data board as a brace behind the top of the right ear. Try to avoid blood vessels in the ear. The punch should not require twisting, just a quick careful push. Once you have the tissue sample, keep it in the biopsy punch until you are done handling the hare, then you can store and label the sample. Blow through the tube into the top of the sample vial or use tweezers (gently) to remove the tissue and place it in the vial. Try to work over your handling bag: it is easy to lose these valuable samples on the forest floor! Label the vial on both the top and the side with the date, site, and eartag#. Wash your punch with alcohol, then flame sterilize it with a lighter. If you do NOT obtain a tissue sample, write 'no DNA' on your data card.
- Check age, sex and reproductive condition. It is easiest to flip the hare by positioning it securely at the bottom of the bag and flipping the entire bag. Position the animal on its back, with your right hand anchoring both hind feet. If you see scrotal testes or prominent nipples, those are sure signs of males and females, respectively. But if you do not see these signs, you need to evert the genitals. Both males and females have tissue that will become visible as you press gently on either side of the genitals with your fingertips. Males are identifiable because there is a ring of tissue around the base of the penis that is speckled with white.

Females are distinguishable because the white-speckled tissue at the base of the genitals does not go all the way around, and there is a slit that opens towards the anus. The tissue must be fully everted to see all of this detail! Record on the data card whether testes are scrotal or abdominal (for males) and if the hare is lactating (for females--you will see wet or matted fur around base of nipple if the hare is lactating. Gray but dry area around nipples does not indicate lactation).

- Record any other comments—e.g. injuries or odd fur patterns.
- If you need to replace an eartag (i.e. taking out a poorly placed or infected one, or it is obvious a tag ripped out before), do this last. Note on the comments part of the trapping card what happened—be sure to record the numbers of any tag you take out.
- To release the hare, it is generally least stressful to have the hare comfortable in the bag, then open the bag so it can see out. Then it can decide when to leave the bag. Position the hare/bag on the ground in a manner that will allow the hare to leave in an unobstructed way.

Injured hares

If an animal is shaky or in shock, give it some sugar water. If it is bleeding or has exposed raw areas, use neosporin to reduce chance of infection. If an animal is so badly injured it is unlikely to survive, it is best to mercy kill it by snapping its neck or hitting its head with a rock (make sure its head is against another rock rather than on soft ground). Be sure to record the circumstances on your datasheet.

IMPORTANT: Never hold a hare only by its legs—it can snap its back or legs in trying to escape. Keep a firm hold on the body of the hare at all times as you are processing it. Keep hare's eyes covered as much as possible, to reduce stress on the hare.

Back at camp

When you are back at base after trapping, it is your responsibility to store samples in the appropriate places, replace anything you need in your trapping kit, and put away data cards. Report any trap or hare handling problems to your supervisor and enter them in the log book.

Rain / snow protocol

Heavy, prolonged rain and snow can cause hypothermia or death for hares. We will try to avoid trapping in these conditions. If we accidentally get caught out by the weather, we will modify the trapping procedure to get hares out of traps more quickly. Modifications could be as extreme as recording no data at all—more likely, we will do eartags but not weight or sex.

APPENDIX III. PROJECT MODIFICATIONS

Our original proposal included some elements that were subsequently modified or removed, with Park approval, for cost-efficiency or logistical reasons. We had proposed to radio collar 30-45 snowshoe hares, to estimate survival rates and causes of death, and to obtain coarse movement information. In our first field season we found that hare densities are low and patchily distributed in much of Glacier NP, and radio collaring sufficient hares for survival and movement estimates would be a time-intensive activity with relatively little return in information gained and would prevent a sufficient sampling of hare abundances across the park. Therefore, we removed this component of the study and instead put additional effort (including finding supplemental funding from other sources) into developing and testing our non-invasive genetic approach for abundance estimation (see Appendix I). Consequently, the genetic approach has proven to be especially suitable for monitoring hares in Glacier NP, because its cost-effectiveness as an alternative to live-trapping is greatest at low hare densities and with difficult-to-access field sites.

In 2006, we live-trapped snowshoe hares at seven study sites that were chosen for their relatively high densities of hare pellets. We attempted to compare trap-based capture-mark-recapture (CMR) density estimates with density estimates from pellet counts, as initially proposed. Although we had targeted relatively high hare density sites, we were able to recapture sufficient hares at only four of the seven sites, for trap-based density estimation (Table A3.I). Although trap- and pellet-based density estimates seem comparable at these four sites, we have insufficient data for a rigorous comparison of these methods.

TABLE A3.I. COMPARISON OF HARE DENSITIES FROM LIVE-TRAPPING AND PELLET COUNTS. ESTIMATED NUMBER OF SNOWSHOE HARES AT STUDY SITES, BASED ON LIVE-TRAP CMR.

Captures were allocated to two trap sessions for Lincoln-Peterson abundance estimation. In this table, unique individuals = number of unique hares captured, n1 = number of individuals trapped in first capture session (data combined from first two trap nights at each site), n2 = number of individuals trapped in second capture session (data combined from remaining trap nights), m2 = number of individuals trapped in both capture sessions, N_{CMR} = Lincoln-Peterson estimate of abundance from live-trap data ('NA' means estimate could not be calculated because no hares were recaptured), N_{PELLET} = SD = Regression-based estimate of abundance from pellet counts.

SITE	LOCATION	UNIQUE INDIVIDUALS	n1	n2	m2	N_{CMR}	N_{PELLET}
B88-1	1988 Red Bench Fire	33	23	17	7	2.65	2.40
TM16	Two Medicine	11	10	3	2	0.68	0.42
B88-3	1988 Red Bench Fire	8	6	3	1	0.65	0.72
MG3	Many Glacier	7	7	2	2	0.35	0.30
TM14	Two Medicine	7	3	4	0	NA	0.49
RRC2	Railroad Creek	5	3	2	0	NA	0.25
B88-4	1988 Red Bench Fire	3	2	1	0	NA	0.22

We had originally proposed to conduct annual surveys to assess temporal changes in hare densities at a subset of study sites. However, at the low hare densities observed in much of the Park (below 0.3 hares/ha), live-trapping and pellets counts have little power to distinguish year-to-year changes in population numbers (Mills et al. 2005), and the biological importance of hares as prey for sustaining lynx populations is also limited (Ruggiero et al. 2000; Steury and Murray 2004). Therefore, we refocused our research efforts to conduct pellet surveys across as many different sites as possible, to identify snowshoe hare hotspots in Glacier NP.

Finally, our original proposal called for indexing lynx abundance via hair snares and genetic analysis, to evaluate the relationship between lynx and snowshoe hare distribution. With the relatively low hare densities in the Park, it seemed unlikely this approach would yield new information on lynx occurrences, unless implemented at a spatial scale and intensity that was beyond the capabilities of this study.

APPENDIX IV. OUTREACH AND EDUCATION

Two important components of our work in Glacier NP were a public outreach and education program, and training of undergraduate and recent graduate students in wildlife research and field techniques. Over 3 years, we employed 23 undergraduates and recent graduates as field research assistants. We also trained 6 short-term volunteers (1 -2 weeks) on this project:

2005 Field Crew	2006 Field Crew	2007 Field Crew	Volunteers
Michael Clark	Adam Copp	Kelsey Bauer	Paulo Celio Alves
Emily Colgate	Amy Crumb	Jonathan Lewis	Daniel Eacker
Brenda Johnson	John Fothergill	Jason Massarone	Michael McDonald
Derf Johnson	Steph Kurikka	Meryl Mims	Ben Sutphen
Aira Kidder	Adrienne Ross	Michael Porco	Jeffrey Rudd
Gina Morgan	Matthew Strauser	Rob Saltmarsh	Tshering Tempa
Kevin Scully	Patty Ten Boom	Matthew Strauser	
Roxanne Vistocci	Elizabeth Williams	Roxanne Vistocci	
Shana Weber			

In 2006 and 2007 we conducted 3 evening campfire talks to teach Park visitors about the importance of wildlife research in National Parks and about our research on snowshoe hares in Glacier NP. These talks were well-attended and warmly received by the public and, importantly, provided valuable outreach training for our young crew members who presented the talks. In addition to campfire talks, on invitations from Matt Graves (West District Interpreter) in 2007 and 2008, Ellen Cheng or Scott Mills spent a half-day at Glacier National Park all-interpreter training, giving talks and discussing with interpreters about wildlife issues – including snowshoe hare ecology – relevant to the Park:

MILLS, L. S. *Climate change and winter coats.* Half-day training lectures to all Interpreters/Naturalists at Glacier National Park, June 10, 2008.

CHENG, E. *Population dynamics of Canada lynx and snowshoe hares.* Half-day training lectures to all Interpreters/Naturalists at Glacier National Park, 2007.

Results of our work in Glacier NP were also disseminated more broadly through several conference presentations, scientific talks, and one magazine article:

CHENG, E. 2009. Non-invasive genetic sampling: a kinder approach to population estimation. AWI Quarterly, summer issue.

CHENG, E., L.S. MILLS, and K.E. HODGES. March 2008. A comparison of non-invasive genetic and traditional approaches to estimating animal abundance. Donana National Park Research Center (oral; Seville, Spain)

CHENG, E., L.S. MILLS, and K.E. HODGES. March 2008. A comparison of non-invasive genetic and traditional approaches to estimating animal abundance. Northwest Section of The Wildlife Society (oral; Spokane, WA)

CHENG, E., L.S. MILLS, and K.E. HODGES. February 2008. A comparison of non-invasive genetic and traditional approaches to estimating animal abundance. Montana Chapter of The Wildlife Society (oral; Missoula, MT)

CHENG, E., L.S. MILLS, and K.E. HODGES. February 2007. Distribution and abundance of snowshoe hares in Glacier National Park. Montana Chapter of The Wildlife Society (oral; Bozeman, MT)

CHENG, E., L.S. MILLS, and K.E. HODGES. February 2006. Evaluating the prey base for lynx: snowshoe hare abundance, habitat use and population dynamics in Glacier National Park. Montana Chapter of The Wildlife Society (oral; Helena, MT)

We are currently preparing two manuscripts on our research in Glacier NP, for publication in peer-reviewed scientific journals. The first manuscript will report our findings on snowshoe hare distribution and abundance in the Park; the second will detail the cost-benefit analysis of non-invasive versus traditional mark-recapture estimates of abundance (Appendix I of this report).

APPENDIX V. GIS AND RAW DATA

GIS products and all raw data files from this research have been transmitted to the Park. This Appendix provides a brief description of these products.

GIS FILES

- **GLACIER SSH GIS.mxd (file) & GLACIER SSH GIS.gdb (folder) —** GIS shape files mapping study site locations and the 600 and 1000 m site buffer zones used to analyze landscape-level edge effects on snowshoe hare densities. The attributes table associated with the study sites shape file includes all summary habitat data used in analyses. In addition, the attributes table includes the following information:
    - HareDensity = Hare density (# of hares/hectare) estimated from Krebs pellet counts
    - HareCategory = 'Low' for sites with <0.3 hares/ha, 'Moderate' for sites with ≥0.3 hares/ha
    - Analyzed? = 'Y' indicates sites that were included in data analysis. Some sites were surveyed, but not included in final analysis because they did not fulfill site requirements (e.g., minimum 85% forest cover) or because data collection was incomplete.
    - East_West = 'East' for sites east of the Continental Divide; 'West' for sites west of the Continental Divide
    - SitePhotos = hyperlink to photos of the study site
    - SiteSurvey = hyperlink to a scanned copy of the Site Survey form completed for the study site
    - UTM_ZONE = 11 or 12, depending on which of Glacier NP's two UTM zones the study site was located in
    - COORDX / COORDY = NAD83 UTM coordinates for study site at the site corner specified in column labeled UTM_CORNER
    - COORDX_Z12 / COORDY_Z12 = For study sites in UTM Zone 11, this column contains the UTM coordinates converted for Zone 12, so all study sites are presented in the same UTM Zone
    - CENTER_X / CENTER_Y = The Zone 12 UTM coordinate for the center of each study site

  NOTE: To switch between Site Photos and Site Surveys for the Hyperlink tool, file document properties need to be set to the relevant hyperlink base and the properties for the GLACIER_FINAL_GIS need to be set to the relevant attributes table field.

- **Glacier Site Photos (folder) —** This folder contains digital photographs of study sites, taken from multiple grid points per site. Photos are not available for all sites.

- **Glacier Site Surveys (folder) —** This folder contains scanned copies of Site Surveys completed for most study sites. Each Site Survey includes directions for accessing the study site, descriptions of vegetation, animal signs, and other features of the study site, and the dates that pellet counts, vegetation surveys, and live-trapping (when applicable) were completed at the site.

RAW DATA

All raw data are provided in a single Excel file (GLACIER SSH RAW DATA 08_01_2011). Data columns are explained by 'comments' embedded in the Excel file. Data are split into the following worksheets, by data type:

- **TRAP —** Live-trap data for 2006 (trapping was not conducted in other years)
- **PELLETS —** Krebs pellet count data for 2005 – 2007
- **VEGETATION —** Vegetation data for 2005 – 2007, excluding sapling and relascope data. Some vegetation data were collected only in 2006 & 2007
- **SAPLINGS —** Sapling species and densities data for 2005 – 2007
- **RELASCOPE—** Canopy tree species data collected using relascopes in 2005
- **GENETICS —** Genotypes at 8 highly variable microsatellites, for a subset of live-trapped hares