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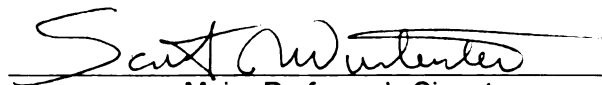
**ESTIMATION OF BLACK BEAR (*URSUS*
AMERICANUS) ABUNDANCE IN THE NORTHERN
LOWER PENINSULA OF MICHIGAN USING
MICROSATELLITE DNA MARKERS.**

presented by

Brian P. Dreher

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Fisheries and Wildlife



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By

Brian P. Dreher

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ABSTRACT

ESTIMATION OF BLACK BEAR (*URSUS AMERICANUS*) ABUNDANCE IN THE NORTHERN LOWER PENINSULA OF MICHIGAN USING MICROSATELLITE DNA MARKERS.

By

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The estimation of black bear population abundance has long been a difficult task, however valuable when justifying harvest quotas and managing populations. Recent advancements in molecular genetics have provided a means to identify bears using DNA contained in tissue and hair samples. We collected hair samples non-invasively using barbed wire hair snares and tissue samples from harvested bears to genetically identify individuals and estimate bear abundance in the Northern Lower Peninsula (NLP) of Michigan using a capture-recapture methodology. In both 2002 and 2003 we derived hair snare locations using a stratified random design and collected hair samples over a number of sampling intervals. As an additional sampling occasion we collected tissue samples from hunter harvested bears. We genetically analyzed hair and tissue samples with 5 microsatellite loci and quantified genotyping errors using samples from harvested bears. We estimated the population of yearling and adult black bears in the NLP with models that account for genotyping error to be 1,882 bears (95% CI 1,389-2,551 bears). We created a simulation model to quantify the effects of genotyping error and sub-sampling hair samples and found that genotyping error dramatically biases population estimates and the selection of 3 hair samples, when multiple hair samples were available, reduced the variance of population estimates.

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CHAPTER 1 – GENERAL INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Black bears (*Ursus americanus*) are a publicly owned resource managed in the state of Michigan in the public trust by the Michigan Department of Natural Resources (MDNR). In 1996 the public decided how black bears should be managed in Michigan using the public referendum process. Two proposals (D and G) were placed on the ballot. Proposal D was a statutory initiative lobbied by Citizens United for Bear (CUB) to prohibit the use of bait piles and dogs for hunting black bears in Michigan (CRC Memorandum 1996). Proposal G was a legislative referendum on Public Act 377 of 1996 that would give the exclusive authority of all hunting regulations to the Natural Resources Commission (NRC) using “principles of sound scientific management” (CRC Memorandum 1996). In the statewide vote, Proposal D was supported by 38 percent of voters and proposal G was supported by 72 percent of voters (Peyton 1998). Bear hunting using bait and dogs is still the primary means to manage the Michigan bear population and the Natural Resources Commission must utilize “principles of sound scientific management” in the decision-making process.

Management of Black Bear in Michigan

Bear hunting in Michigan occurs in fall in both the Upper and northern Lower Peninsulas. Interest in black bear hunting is increasing as evidenced by the number of people applying to hunt bear in the northern Lower Peninsula (NLP: 5,365 in 1995 and 14,674 in 2001). The number of bears harvested in the NLP increased from 136 bears in 1995 to 357 bears in 2002 (Frawley 2002). Black bear hunting provides a significant

source of revenue, estimated at \$5.3 million in the 1995 hunting season, to the state through small local businesses and license fees (USDI 1998).

It is a stated goal of the MDNR Black Bear Management Program “to maintain a healthy black bear population that provides Michigan residents with a diversity of bear-related recreational opportunities. A bear population that provides viewing and hunting opportunities, yet does not create excessive nuisance bear problems for people, is crucial for the success of this program” (1998, MDNR bear hunting guide). Additionally, Proposal G mandates that the Natural Resources Commission and the MDNR utilize “principles of sound scientific management” in the decision-making process for black bear management. A population estimation or survey could be considered one means of obtaining requisite data to conduct “sound scientific management” (Bailey 1984). A precise population estimate of black bears in the NLP would help to simultaneously ensure the maintenance of recreational hunting opportunities and a viable black bear population by providing a means to evaluate and justify harvest quotas and satisfy public desire to know “how many bears there are.”

The bear population in the NLP is ecologically and socially important with a wide array of human placed values (Peyton et al. 2001). According to the MDNR, the bear population in the NLP is increasing and expanding geographically and this increases the likelihood of human-bear conflicts (Etter et al. 2002). Peyton et al. (2001) surveyed public attitudes towards bear in the NLP recognizing that the bear population in the NLP is increasing and expanding into areas that were not previously occupied by the species in recent history. Peyton et al. (2001) identified the areas of the NLP where tolerance for black bears is expected (e.g. traditional bear range) and found an increase in bear

intolerance as residents were surveyed from north to south in the NLP. A precise population estimate could be used to gauge public opinion about bears as the population continues to expand its range in the Lower Peninsula (Peyton et al. 2001). This estimate could allow the MDNR to relate actual black bear numbers with social carrying capacity, and possibly proactively manage the population to avoid potential negative human-bear interactions.

Recent research into NLP bear population demography (1991–2001) included the use of radio-telemetry techniques, which enhanced the MDNR's understanding of bear in this region (Etter et al. 2002). Data collected from 126 radio-collared black bears provided estimates of age- and sex-specific survivorship, recruitment, movements and home range. Causes of non-harvest bear mortality in Michigan included collision with automobiles, illegal killing, nuisance bear control, and unknown causes (Etter et al. 2002). Non-harvest mortality is low for both subadults (bears ≤ 2 years) and adults (bears > 2 years) with both sex and age classes exhibiting seasonal and annual mortality rates $< 10\%$. Legal harvest is the single largest cause of mortality (59% annual deaths) for bears from the NLP (Etter et al. 2002). Home range estimates were some of the largest reported for the species including 867 km^2 for adult males and 131 km^2 for adult females (Etter et al. 2002).

Currently, the MDNR uses a sex- and age-specific population model developed for bears from Minnesota to estimate population size and set hunting tag quotas (Garshelis and Snow 1988). The five components of the model include initial population size, birth rates, death rates, immigration, and emigration. For the NLP, empirical data collected from 1991–2001 provide direct estimates of births, deaths, and movements

(Etter et al. 2002). However, without an accurate estimate of initial population size, the MDNR model is not complete. Additionally, the MDNR model has not been validated because indirect or direct estimates of black bear population abundance are currently not available. Rather, the model has been verified using population indices such as a bait station index.

In 1990 the MDNR created Bear Management Units (BMU's) and limited the number of licenses allocated in each unit (Frawley 2001). The NLP is comprised of three units open to hunting: Red Oak, Baldwin and Gladwin BMU's (Figure 1.1). Hunters that are successful in harvesting a bear are required to present the entire bear or head at a MDNR office or designated registration location within 72 hours of the kill. Information collected upon registration includes: hunter name and address, date of the kill, BMU in which the bear was taken, location of the kill (township, range and section), county of the kill, sex of the bear, method of take, and comments about the weight of the bear and general body condition. Additionally, the MDNR attempts to collect teeth from all harvested bears and reproductive tracts from female bears are voluntarily collected. Teeth from individual bear are aged using the cementum method (Hildebrandt 1976).

Overview of Population Estimation Techniques

Currently, the MDNR uses an index of bear sightings, an annual bait survey, nuisance bear reports, and number of bears harvested to survey populations (Etter et al. 2002). These methods provide only population trends rather than an estimate of population size. Population estimation techniques are difficult to use on carnivores because of their secretive nature, large home-range size and difficulty of capture (Woods et al. 1999;

Mills et al. 2000; Taberlet et al. 2001). Capture-recapture is one method that has been used to survey large carnivores. Individuals are captured and outfitted with a recognizable mark and released into the population. A number of individuals are subsequently recaptured and examined for the previously placed marks. Information about the number of individuals initially marked and the number of animals in the recapture with and without marks can provide an estimate of the population (White et al. 1982). Mace et al. (1994) estimated the population size of grizzly bears (*Ursus arctos horribilis*) by capturing and marking bears with colored ear tags and streamers. Researchers then used remote cameras to recapture individuals to obtain a population estimate. Although this method is feasible for use on large carnivores, capturing and handling of individuals can result in small sample sizes and logistical difficulties. Garshelis and Visser (1997) used baits laced with the ingested biomarker tetracycline to non-invasively mark bears in the Upper Peninsula (UP) of Michigan. Individual bears were then recaptured in the bear harvest when hunters submit tooth samples upon registration. By examining the teeth or bone, the “mark” was revealed using ultraviolet light, which fluoresces the tetracycline biomarker. This method can be an effective means to mark many individuals non-invasively thereby increasing the sample size, and has proved to be cost-effective in the UP for estimating bear population size. However, one disadvantage of the tetracycline marking technique is the limited biological information that can be collected when marking an individual (e.g. sex). Furthermore, this method provides no means for positively identifying recaptured individuals, which limits the models that can be applied to the data.

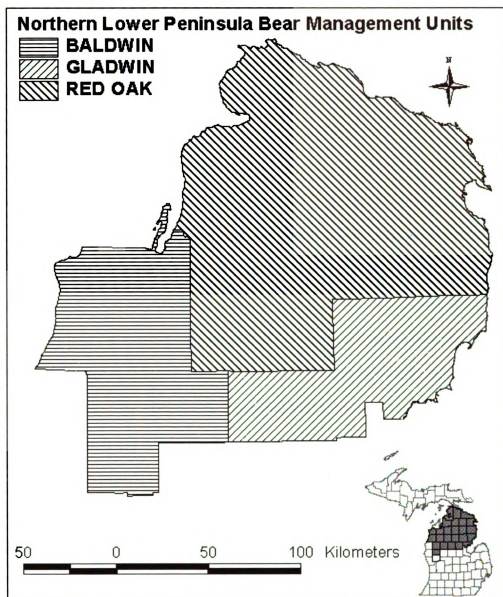


Figure 1.1. Study area (36,848 km²) comprised of the Baldwin, Gladwin and Red Oak bear management units.

Advancements in molecular genetics have made it possible to conduct a mark-recapture study non-invasively using hair and tissue as a DNA source. Utilization of this method involves three major parts: 1) field collection of a DNA source, 2) lab analysis of the DNA source to produce genotypes of individuals, and 3) estimation of the population size using mark-recapture models.

Field Data Collection

Many different sources and methods exist for the non-invasive collection of DNA. Gerloff et al. (1995) extracted DNA from feces of wild, free ranging bonobos (*Pan paniscus*) for individual identification. Kohn et al. (1999) used DNA extracted from feces as a non-invasive means to estimate the size of a coyote (*Canis latrans*) population in California. Pearce et al. (1997) used eggshells and feathers collected non-invasively in nests as a DNA source for a conservation genetics study of common eiders (*Somateria fischeri*) in both Russia and Alaska. In a population survey of the endangered Pyrenean brown bear (*Ursus arctos*), Taberlet and Bouvet (1992) documented the use of hair as a non-invasive source of DNA. This technique for brown bears included the collection of hair samples from barbed wire nailed to trees and was a better alternative to handling individuals because of the risk associated with capture and anesthesia of an endangered species.

Woods et al. (1999), Mowat and Strobeck (2000), Poole et al. (2001), and Boerson et al. (2003) successfully captured both black and brown bear hair using a snaring device made from barbed wire. The basic configuration of the hair snare used a single strand of four-pronged barbed wire stretched around 3 or more trees at a uniform

height forming an enclosure around an attractant or lure. As bears pass over or under the barbed wire, hair is deposited on the wire for subsequent collection.

Lab Analysis of DNA

Recent advancements in molecular genetics have provided a means to identify individuals using small quantities of DNA collected from hair or feces (Higuchi et al. 1988). Extracted DNA in conjunction with microsatellite markers can be amplified using the polymerase chain reaction (PCR). PCR results in millions of copies of a target section of DNA, and occurs in the repetition of three phases including denaturation, annealing, and extension (Oste 1988). Using microsatellite DNA markers, a specific target section of DNA can be isolated (Parker et al. 1998). Microsatellite markers are composed of forward and reverse oligonucleotide primers that are coded to attach to a specific sequence of DNA. Microsatellite markers can be created for a single locus by scanning the genome and identifying sections of tandem repeats. Once oligonucleotide primers are created and used in conjunction with PCR, they can be used to isolate and amplify known loci. The PCR product can then be run on acrylamide gels with electrophoresis and allele frequencies determined (Parker et al. 1998). The use of this technique requires that oligonucleotide primers be developed for the specific species of interest (Morin and Woodruff 1996). For the genetic differentiation and conservation genetic study of bears, at least ten forward and reverse primers have been developed previously (Paetkau and Strobeck 1994; Paetkau et al. 1995).

A pilot study conducted by Scribner and Libants (2001 unpublished data) on Michigan bears, found that five polymorphic loci (G10X, G10M, G10D, G10L, G10B;

Paetkau et al. 1995) would be sufficient in differentiating between individual black bears with an overall probability of identity of $9.96E-09$. Additionally, they analyzed 26 hair samples and were successful at obtaining genotypes at five loci in 22 of the hair samples.

When this method was first described, it was proclaimed to be an adequate alternative DNA source to blood or tissue (Taberlet et al. 2001). Subsequently, numerous studies have identified that there are many errors associated with using hair samples as a DNA source including: 1) contamination or errors made during collection and lab analysis, 2) allelic dropout, 3) null or false alleles, and 4) shadow bands causing scoring errors. Errors associated with the field collection and lab analysis can be generated by mislabeling samples, cross-contamination of multiple samples, and loading errors (Gagneux et al. 1997). Errors can be minimized by defining precise study protocols for field collection and by careful lab techniques to minimize error (Taberlet et al. 1996). Additional contamination can occur when more than one individual deposits hair at a single collection site (Gagneux et al. 1997). Contamination can be detected by the presence of more than two alleles at a single diallelic locus. Allelic dropout and null alleles are errors that occur when only one of the two alleles is amplified and detected for a single diallelic locus (Gerloff et al. 1995; Taberlet et al. 1996; Taberlet et al. 1997; Gagneux et al. 1997; Taberlet et al. 2001). Occurrence of allelic dropout increases when using small quantities of degraded DNA. This is of concern when using hair or feces as a DNA source, because the quantity of DNA can often be in the picogram range (Taberlet et al. 1996). Error arises when an individual is scored as a homozygote because only one allele is amplified. Taberlet et al. (1996) suggests regenotyping all homozygous individuals to quantify and obtain the real genotype for individuals. Additionally,

detections of allelic dropout errors can be made by examining data for a deficiency of heterozygotes (Brookfield 1996). Simulation of how allelic dropout and null alleles affect genotypes can be a useful means of estimating the magnitude of error on the outcome of population estimates (Taberlet et al. 2001). Lastly, shadow bands can cause scoring errors when genotyping individuals. This can be minimized by having more than one person score gels and by re-analysis of any disputed or inconclusive scores (Gagneux et al. 1997).

Although the analysis of hair samples for DNA fingerprinting contains potential errors, errors can be accounted for by using good lab and field practices, designed experiments to detect errors, and simulation to determine how errors affect the population estimate. By recognizing and accounting for errors in the study design phase, accurate estimates of population size are attainable using non-invasive techniques (Waits and Leburg 2000).

Population Estimate and Analysis

One assumption of using mark-recapture as a means to estimate population size is that individuals are identified correctly (White et al. 1982). Using the information of allele frequencies from a sample of individuals it is possible to calculate a probability of identity (PI) for each locus examined in the lab. This is given as the probability of two individuals sampled in a population having the exact same genotype. When analyzing several loci for a population the product of the PI for each locus gives an overall PI for the population (Paetkau et al. 1995). The PI is dependent on the amount of variation in the population. One important factor affecting PI is effective population size and in

populations of small size, allelic diversity and heterozygosity will be low (Frankham 1996). As such, more loci may be required to obtain a PI that is sufficiently small enough to differentiate individuals (Paetkau and Strobeck 1994).

Once genotypes have been determined for individual animals they can be used in a mark-recapture design to estimate population size. Woods et al. (1999), Mowat and Strobeck (2000), Poole et al. (2001), and Boerson et al. (2003) utilized genetic mark recapture techniques to obtain population estimates of brown bears in Canada and black bears in Louisiana. Palsboll et al. (1997) used molecular markers in a mark-recapture study of hump-backed whales (*Megaptera novaeangliae*) in the Atlantic Ocean using skin biopsies and sloughed skin as DNA sources. Genetic markers free from errors provide an ideal means to mark individuals because they satisfy the marking assumptions that animals do not lose their marks during the experiment, and marks are noted correctly on each sampling occasion (White et al. 1982). Genetic methods have no impact on survival, and marks can be identified and reported correctly upon recapture (Woods et al. 1999; White et al. 1982). Additionally, a non-invasive study design allows for greater sample size required for mark-recapture models and genotypes are permanent and cannot be lost over time. Because animals are not captured and handled, study designs can be established to explicitly address spatial issues necessary to meet model assumptions including population closure (Mowat and Strobeck 2000). One disadvantage of the technique is the possible under-representation of individuals that visit a site, but do not deposit hair. Additionally, bears may have an aversion to barbed wire based on previous negative experience (Woods et al. 1999). Because of the required lab

analysis, computation of a population estimate may take longer than with other conventional capture-recapture techniques.

In the general use of capture-recapture experiments it is generally recommended that the method used to mark individuals is different from the method used to recapture individuals to minimize bias (White and Schenk 2001). Utilizing the ability to capture tissue samples from harvested bears in Michigan provides a means to recapture individuals using a different method of capture.

Program MARK (White and Burnham 1999) encompasses all current methods to analyze mark-recapture data. Many different models are available in program MARK, each with different assumptions and uses, but closed capture models are most commonly used with non-invasive methodology (Woods et al. 1999; Mowat and Strobeck 2000; Boerson et al. 2003). For example, closed capture models available in program MARK are the same models that were in the older program CAPTURE (White et al 1982). Program MARK offers models that can incorporate individual heterogeneity, but assume population closure. Program MARK uses multiple competing models to determine the best fit of the data using Akaike Information Criterion (AIC). These models are ranked on their AIC score with the lowest score being the best fitting model (Burnham and Anderson 1998). These aspects make program MARK the most comprehensive software to analyze mark-recapture data (Cooch 1999).

OBJECTIVES

The main focus of this research is to develop a methodology that the MDNR can use to estimate the size of the black bear population in the NLP of Michigan. However,

non-invasive sampling techniques to estimate population abundance are relatively new and the effects of genotyping errors and sub-sampling are not well established in the literature. Therefore, quantification and simulation of errors and sub-sampling will both improve the abundance estimate of black bears in the NLP of Michigan and also contribute to the profession's understanding of this technique. The objectives of this research include:

- 1.) Perfect a methodology for collecting black bear hair and tissue samples to be genetically analyzed and used to obtain a mark-recapture based estimate of the size of the black bear population in the NLP of Michigan.
- 2.) Obtain an estimate, with confidence intervals, of the size of the black bear population in the NLP of Michigan.
- 3.) Quantify and simulate the effect of genotyping errors and sub-sampling on population abundance estimates of black bears in the NLP of Michigan
- 4.) Make recommendations to the MDNR on use of this methodology to estimate bear abundance in the NLP of Michigan

THESIS ORGANIZATION

The thesis is organized in five chapters. Chapter 1, which precedes, is a general introduction of the methodologies and literature for utilizing the non-invasive technique to estimate abundance. Chapter 2 includes the field methodologies, results and discussion for data collected in both years 2002 and 2003. Chapter 3 includes the introduction, methods, results, discussion and management implications for the population estimate. Chapter 3 only includes data collected in 2003 because data

collected in 2002 were considered preliminary and were used to perfect both the field sampling and laboratory techniques. Chapter 4 includes the introduction, methods, results, and discussion for a simulation model that we created to examine the effects of genotyping errors and sub-sampling hair samples on population estimates. The fourth chapter primarily focuses on data collected in 2003, however portions of model parameterizations were derived from data collected in 2002. Chapter 5 includes recommendations to the MDNR for the successful use of this technique and encompasses information collected throughout the duration of this research project.

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CHAPTER 2 – FIELD SAMPLING METHODOLOGIES

The main focus of this research is to develop a methodology that the MDNR can use to estimate the size of the black bear population in the NLP of Michigan. To accomplish this goal it is necessary to develop a methodology for collecting black bear hair and tissue samples in the NLP of Michigan to be genetically analyzed.

STUDY AREA

The study area is composed of 3 bear management units (BMU's) in the NLP of Michigan; Baldwin, Gladwin and Red Oak (Figure 1.1). The Baldwin BMU encompasses all of Benzie, Grand Traverse, Lake, Manistee, Osceola, and Wexford counties and that area of Newaygo County north of highway M-20. The Gladwin BMU encompasses all of Arenac, Clare, Gladwin, Iosco, and Ogemaw counties. The Red Oak BMU encompasses all of Alcona, Alpena, Antrim, Charlevoix, Cheboygan, Crawford, Emmet, Kalkaska, Missaukee, Montmorency, Oscoda, Otsego, Presque Isle, and Roscommon counties. This area is characterized by the Northern Lacustrine-Influenced ecotone as described by Albert (1995). The study area is approximately 36,848 km² with elevations ranging from 177 to 526 meters. Well drained, sandy soils in the area typically support Northern hardwoods, jack pine (*Pinus banksiana*) barrens, upland conifer forest, white pine (*Pinus strobes*) -red pine (*Pinus resinosa*) forest, hardwood-conifer swamp, and conifer swamp (Albert 1995). The landscape is highly fragmented with densities, calculated from digital coverages of county roads, streets and highways, being approximately 1 km/km² (MDNR-Land and Mineral Services Division, Resource

Mapping and Aerial Photography 1992). Approximately 11,878 km² (32.24%) of the study area is under public ownership (MDNR-Forest, Mineral and Fire Management Division, Resource Mapping and Aerial Photography 2001).

METHODS

Hair Snare Placement

There has been no similar research using hair snares in Michigan to estimate bear abundance, thus, we were limited in our knowledge of field logistics to execute this research. We developed a stratified random sampling design for each of the 2002 and 2003 field seasons to identify sampling locations while capitalizing on the information that we possessed. Additional information came from approximately 700 bait index locations established by the MDNR from 1991–2000. These locations were utilized by the MDNR to index the bear population in the NLP and could be used as point locations for hair snare sampling locations. To maximize our probability of snaring hair given the large study area, potential sites for hair snare placement were identified in a series of 5 procedures including: 1) selection of land cover/land characteristics likely to provide bear habitat, 2) determining buffers on roads to both increase the probability of bear visits and also decrease sabotage by humans, 3) selection of primarily public lands so that the number of sites was not limited by personnel access, 4) utilizing townships where hunters have harvested bear (to indicate bear presence), and 5) selection of hair snare locations. To perform these procedures we used ArcView 3.2 (Environmental Systems Research Institute, Redlands, California) using several existing digital coverages obtained from the MDNR: 1993 and 2001 Gap Analysis Program (GAP) Northern Lower Peninsula land

cover datasets (MDNR-Wildlife Bureau and MDNR-Land and Mineral Services Division, Resource Mapping and Aerial Photography 2000; MDNR-Wildlife Bureau and MDNR-Land and Mineral Services Division, Resource Mapping and Aerial Photography 2003), black bear management units (MDNR-Wildlife Bureau 2002a), Michigan (Lower Peninsula) GAP Land Stewardship Coverage (MDNR-Forest, Mineral and Fir Management Division, Resource Mapping and Aerial Photography 2001), MIRIS Base Data (MDNR-Land and Mineral Services Division, Resource Mapping and Aerial Photography 1992), and 1997–2002 black bear harvest locations (MDNR-Wildlife Bureau 2002b).

Land use/land cover characteristics are important to the distribution of bears because they represent the distribution of food resources (Rogers 1987). Therefore, hair snares should be placed in areas having land use/land cover characteristics that represent bear utilization. For the 2002 hair snare placement, we used the 1993 GAP land stewardship land cover digital coverage and for the 2003 hair snare placement we used the 2001 GAP land stewardship land cover digital coverage. The definitions of land use/land cover classifications differed between coverages (Appendix 2.1 and 2.2). We selected the following land cover/land use categories in both years: aspen (*Populus tremuloides*) / birch (*Betula papyrifera*), emergent wetland vegetation (emergent wetland/wet meadow, lowland broad-leaved deciduous shrub, lowland broad-leaved evergreen shrub, lowland broad-leaved evergreen shrub, other lowland shrub), and mixed lowland (mixed lowland conifer/hardwood, mixed lowland hardwood) (L. Visser, MDNR, person. comm. 2002). By selecting these vegetation types, we isolated areas with vegetation types likely to provide bear habitat.

Although, black bears in the NLP of Michigan show little avoidance of roads (L. Visser, MDNR, person. comm. 2002), we chose to set snares an arbitrary distance away from roads to minimize the occurrence of human tampering with snares. In 2002 we placed a buffer of 100 meters around all county roads, and a buffer of 1,000 meters was placed around highways and streets. In the summer of 2003 we placed a 500 meter buffer on all streets, county roads, and highways and removed this area from the bear land use/land cover vegetation map. Because access of field personnel was limited to public land in most cases, we also removed inaccessible private land areas from the bear land use/ land cover vegetation map.

We obtained the locations of black bear harvested from 1997–2001 and grouped these harvests by township (93 km^2). We then classified each township based on the number of bears harvested from 1997–2001. In 2002, categories were based on natural breaks in the data and included: 1–2 harvests, 3–5 harvests, 6–13 harvests, 14–25 harvests, and 26–48 harvests. These categories were used in the 2002 selection of hair snares. Before the 2003 field season, harvest locations were updated to reflect the harvests that occurred in 2002. Therefore categories for 2003 reflect the number of harvests per township from 1997–2002 and were also based on natural breaks in the data and included: 1–2 harvests, 3–10 harvests, 11–20 harvests, 21–35 harvests, and 36–59 harvests.

To create the final map that was needed to identify hair snare locations, we overlaid the townships in which bear harvests had occurred onto the map depicting accessible, potentially suitable vegetation types that were > 500 meters from county roads, streets and highways.

We then used this final map to choose hair snare locations and only considered townships that contained bear harvests from 1997–2001 for 2002 and 1997-2002 for 2003. Table 2.1, contains the frequency of harvest values per township and the resulting number of snare locations selected in 2002 and 2003 that were based on the number of snares that field personnel could operate.

Table 2.1. Categories for the number of bears harvested per township for 1997-2001 and 1997-2002 and the resulting number of hair snare locations chosen.

Number of harvests per Township 1997-2001	Number of harvests per Township 1997-2002	Number of hair snare locations chosen
0	0	0
1-2	1-2	0
3-5	3-10	1
6-13	11-20	2
14-25	21-35	3
26-48	36-59	4

After all the stratifications were completed and the number of snares for each township determined, we were ready to choose locations. We selected locations for snare placement in a series of two steps. First, we wanted to utilize MDNR bait station locations where possible, so MDNR bait index sites were then overlaid onto the townships and sites were selected meeting our criteria for the number of snares per township based on previous visitation history and proximity to other hair snare locations. Second, in the townships where MDNR bait index sites were not available, a random script was used in ArcView 3.2 whereby points meeting our criteria for the number of snares per township and a minimum spacing distance of 5 km between sites were randomly selected within our final coverage.

For the summer of 2003, we chose to keep the same snaring locations that we used in the summer of 2002. However, we added a number of snares to reflect the new harvest categories updated from harvests that occurred in 2002.

Verification of Sampling Design

Etter et al. (2002) conducted a research experiment from 1991–2000 to examine population dynamics and movements of bear in the NLP. Researchers radio-marked 126 bears (64 males, 62 females) varying from 1 year of age to 19 years of age for females and 1 year of age to 9 years of age for males. Telemetry locations were taken from a fixed wing aircraft to the nearest quarter, quarter section using a GPS. Locations were also taken from the ground by triangulation and point locations derived from a minimum of 2 radio-bearings and estimated using LOCATE II (Nams 1990). Locations with error > 16 ha were deleted from analysis. A total of 4,873 locations (2,999 locations for females and 1,874 locations for males) were taken from 5/15/1991 – 11/19/2000.

We used radio telemetry locations to verify stratifications and snare placement by examining the distribution of locations on private and public land, proximity of telemetry locations to streets, roads and highways, land use/land cover characteristics of the telemetry locations, and the overlap between the telemetry locations and the 2003 snare locations. Telemetry locations used in this analysis include all telemetry locations and a subset of locations collected during the months of June and July (i.e. months that we snared hair) for all years of the study.

To examine bear usage of both private and public lands, we overlaid all the telemetry locations and the subset of telemetry locations on the public land coverage and

determined the number of telemetry locations that were taken on public land, and calculated the percentage of telemetry locations on public land. To examine the proximity of all telemetry locations and the subset of telemetry locations to roads, streets and highways, we overlaid the map with the 500-meter road buffer on all county roads, streets and highways with the telemetry locations. We then determined the percentage of telemetry locations within 500 meters of county roads, streets and highways.

To associate and summarize the different land use/land cover characteristics with the telemetry locations we overlaid the telemetry locations with the 1993 land use/land cover digital coverage map (MDNR-Wildlife Bureau and MDNR-Land and Mineral Services Division, Resource Mapping and Aerial Photography 2000) and associated the vegetation type with the telemetry location. We then summarized the percentage of telemetry locations in each vegetation classification.

Lastly, to examine the area sampled compared to the telemetry locations, we first buffered all snare locations in 2003 to include the size of a female home range (131 km^2 ; Clark and Smith 1994). We then overlaid the telemetry locations onto the buffered area and calculated the percentage of telemetry locations within the buffered area.

Field Location of Hair Snare Sites

After snaring locations were selected, each snare was given a unique identification name and UTM location. Snares were setup and operated by field personnel from Michigan State University, numerous Department of Natural Resources field offices, United States Forest Service, Little Traverse Bay Bands of Odawa Indians, Little River Band of Ottawa Indians and the Grand Traverse Band of Ottawa and

Chippewa Indians. We held training sessions before the 2002 and 2003 field seasons to ensure consistency of field techniques.

To assist the field crew in random site location in the field, we used Topo USA 3.0 (DeLorme©, Yarmouth, ME, USA) with Garmin III© GPS (Garmin International, Olathe, KS, USA) units. Field personnel were instructed to drive as close to the random location as possible and then set out on foot with gear to find a suitable snare location. Criteria for site selection included, upland/lowland edges and features that channel bear travel such as streams or rivers, edges of lakes, old logging roads, power line or pipeline right-of-ways, well used deer runways, and upland ridges through swamps (L. Visser, MDNR, person. comm. 2002).

Hair Snare Configuration

In the summer of 2002 the hair snare configuration used was consistent with the design used by Woods et. al. (1999), Mowat and Strobeck (2000), Polle et al. (2001), and Boerson et al. (2003) where a single strand of barbed wire was used to form an enclosure around a suspended bait (Figure 2.1). In the summer of 2003 we used a snare configuration that included the use of two separate strands of barbed wire, one placed at a uniform height of 50 cm and the second placed at a uniform height of 20 cm (Figure 2.2; Eason et al. 2001). For both summers we used two-stranded barbed wire with four pronged barbs spaced every 13 cm.

After a general area was selected, field personnel were instructed to locate a suitable grouping of trees. Groupings were to contain ≥ 3 trees that were spaced approximately 4 m apart. If a site was suitable, but lacked a suitable configuration of

trees on which to attach the wire, metal fence posts were provided. Trees were large enough to attach barbed wire and preferably had smooth bark to assist with identification of bear claw marks. Additionally, over-hanging tree limbs were necessary to suspend baits and the ground needed to be relatively level to ensure the height of the barbed wire was consistent.

Starting at 1 of the selected trees, the barbed wire was fastened using a metal fencing staple. The wire was then hand stretched and tightened to the next tree at a height of 50 cm. In the summer of 2003 an additional wire was fastened and hand stretched around all trees at a height of 20 cm. This procedure was continued until an enclosure was formed (Figures 2.1 and 2.2). Ground irregularities, such as depressions, were filled in with woody debris to ensure that the distance between the wire and the ground remained consistent.

Baits were suspended from trees on opposite sides of the enclosure using twine and raised to a height of approximately 2–4 meters above the ground (Figures 2.1 and 2.2). Baits used in the summer of 2002 included: one-pound of bacon, liquid scents (anise, bacon, shellfish, hickory smoke; Bear Scents LLC, Lake Mills, WI, USA), chicken legs, and sausage links. In the summer of 2003 all baits were consistent including one-pound bacon and anise extract. Anise extract was contained in a film canisters with holes cut in the sides and stuffed with cotton balls. To warn humans of the presence of the hair snare, warning signs were placed on 2 trees on either side of the hair snare site and florescent flagging was tied to each section of wire at multiple locations. We recorded UTM coordinates, names of the setup crew, date, estimated time of setup, and a rough sketch of the hair snare from an aerial perspective.

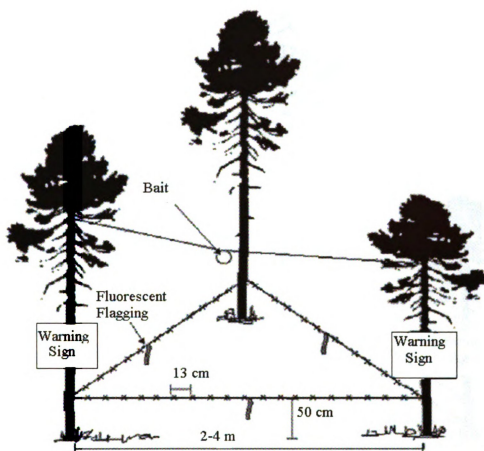


Figure 2.1. Single wired hair snare for black bear hair collection comprised of a single strand of barbed wire (13 cm barb spacing) encircling ≥ 3 trees at 50 cm from the ground. Spacing between trees is approximately 2-4 meters apart. Two warning signs and florescent flagging tied around the wire warn humans of the snare. Bait is suspended over the snare by strings attached to over-hanging limbs.

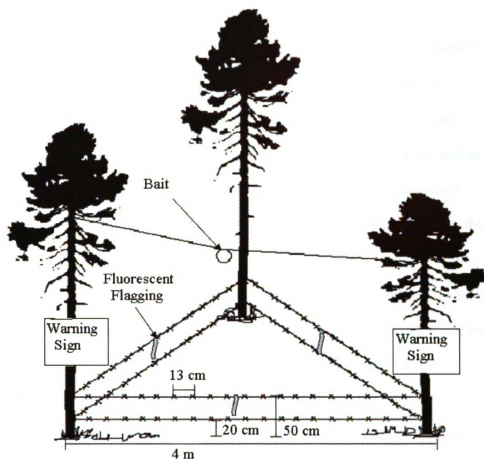


Figure 2.2. Double wired hair snare used for black bear hair collection comprised of 2 strands of barbed wire (13 cm barb spacing) encircling ≥ 3 trees at 20 cm and 50 cm from the ground. Spacing between trees is approximately 4 meters apart. Two warning signs and florescent flagging tied around the wire warn humans of the snare. Bait is suspended over the snare by strings attached to over-hanging limbs.

Checking Methods

In the summer of 2002, 2 sampling periods consisting of 4 checks in each period were conducted from May 15 – August 15, 2002. The duration between checks was 5–8 days. Upon the fourth check of the first period (checking occasions 1–4) baits were removed, but the snares remained. The snare was left without bait for one week and then revisited to replace bait and clean the wire of any hair that may have been deposited during the one week pause. Snares were then checked 4 additional times making up the second period (checking occasions 5–8).

In the summer of 2003 we conducted sampling in 1 period consisting of 5 checks from June 22 – July 26, 2003. These dates were chosen by examining the number of bear visitations per week and number of hair samples collected in the summer of 2002 (Figures 2.12 and 2.14). The duration between checks was 5–8 days.

When checking snares, field personnel evaluated whether a visitation by a bear occurred by noting claw marks on trees, absence of bait, or hair on wire. Starting at the northernmost tree or post each barb of the wire was checked by passing a white index card behind a barb and looking for the presence of hair. Once hair was found, the location was documented on the sketch, a height measurement from the ground was recorded and the hair was removed using forceps. Hair from each independent barb was considered a single sample and placed in a pre-numbered paper coin envelope. Hair samples were stored in a dry location awaiting laboratory analysis. We replenished missing baits or replaced them every two weeks if still present upon checking occasion.

In the summer of 2003, hair samples were only collected from the top strand of barbed wire and hair samples present on the lower strand of wire were noted, but not

collected. In addition, field personnel estimated the number of hairs present in each hair sample and assigned them into categories: 1–4, 5–10, 11–15 and >15 hairs. We also numerically documented the position of the hair samples on the wire by assigning a number to each barb of the snare by counting barbs from the northern most tree and proceeding clockwise.

Hunter Harvested Bear Hair and Tissue Collection

In the fall of both 2002 and 2003 tissue, hair, and tooth samples were collected from harvested bear for use as a recapture in the mark-recapture methodology. To the best of our knowledge, this recapture approach is unique and takes advantage of the mandatory bear harvest registration policy in place in Michigan. To prepare for sample collection, a training session was held for all MDNR employees who would potentially register bears. Each MDNR check station was supplied with written collection protocols and collection kits. The collection kit consisted of a 1.5 mL vile containing tissue buffer solution (Tris, ECTA, Urea, Sarcocine, NaCl, water) and two small coin envelopes for tooth and hair collection. All collection kits, including envelopes and the vile, were pre-numbered to ensure that samples collected from an individual bear were identifiable. Field personnel were instructed to collect a small muscle tissue sample (approximately 1cm by 1cm) and submerge the muscle tissue into the tissue buffer solution. Teeth were extracted and placed in a small coin envelope for subsequent aging, and hair samples were plucked so that >10 hair follicles were evident and also placed in a paper coin envelope. After collection both hair and tissue samples were frozen pending laboratory analysis.

RESULTS

Hair Snare Placement

Because of the large study area required for this project, we chose to use a stratified random design to choose the locations of hair snares. Results from the snare location process will be presented in terms of land area and how this land area was reduced for the stratification of the study area. For brevity, only the stratification results will be covered from the 2003 field season. However the results are very similar to these derived in 2002, only differing in land area values.

The overall size of the study area is 36,848 km² (Figure 1.1). The area of public land within the study area is 11,878 km² (Figure 2.3). The area of land use/land cover characteristics that we selected as potentially suitable bear habitat is approximately 5,127 km² (Figure 2.4). The area included within the 500 m road buffer around county roads, streets and highways were approximately 24,700 km² (Figure 2.5). The final area that was public land, potentially suitable bear habitat, area > 500 meters from county roads, streets and highways was 1,420 km² (Figure 2.6). This technique allowed us to narrow down the possible area to place hair snares when working with a very large study area.

Additionally, we only choose to place hair snares in townships where bear harvests had occurred from 1997–2002. There were 464 possible townships in the study area from which bears could be harvested. There were 225 different townships where harvests occurred from 1997–2002 and the frequency of harvests for each township also varied (Table 2.2 and Figure 2.8). Figure 2.7 depicts the distribution of harvest from 1997-2001 that was used to choose hair snare locations for the 2002 field season.

In some cases we were unable to meet our criteria for the number of snares per township because access was limited by private land. In addition, some townships did not have public land, and in these cases we attempted to access private lands.

Table 2.2. Number of harvests per township between 1997-2002 and the frequency of townships in each category.

Number of harvests per Township 1997-2002	Number of Townships
0	239
1-2	71
3-10	109
11-20	34
21-35	8
36-59	3

Verification of Sampling Design

The percentage of telemetry locations on public land was 63.82% (3,110 out of 4,873 telemetry locations) and 64.52% (913 out of 1,415 telemetry locations) from the subset of telemetry locations. The percentage of telemetry locations within 500 meter of county roads, street and highways was approximately 49.85% (2,429 out of 4,873 telemetry locations) and 54.28% (768 out of 1,415 telemetry locations) from the subset of telemetry locations collected in June and July.

Land use/land cover classifications of the telemetry locations cover almost all possible categories for all of the telemetry locations and the subset of telemetry locations (Tables 2.3 and 2.4). The category with the most telemetry locations included mixed lowland conifer/hardwood with 22.19% of the telemetry locations and 22.06% of the telemetry locations for all telemetry locations and the subset, respectively. For the stratified random design we selected: aspen/birch, emergent wetland vegetation

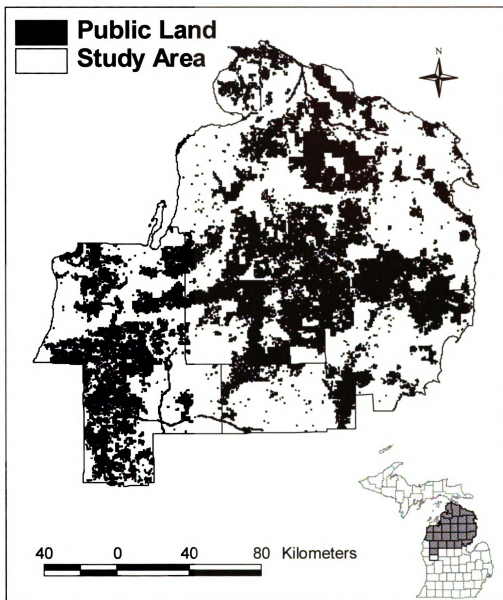


Figure 2.3. Public land ownership ($11,878 \text{ km}^2$) within the study area.

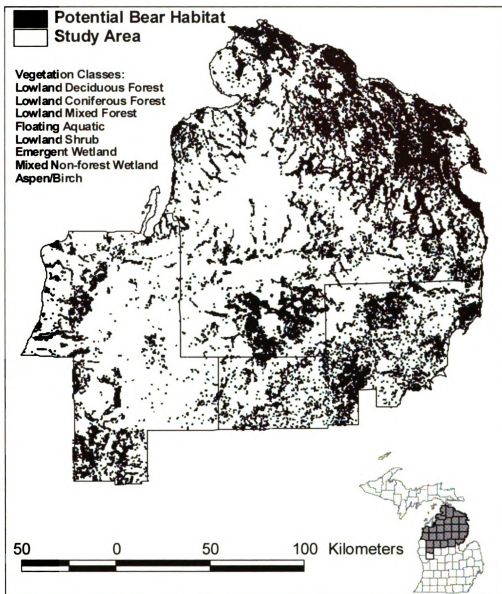


Figure 2.4. Potential bear habitat within the study area comprised of emergent wetland vegetation (emergent wetland/wet meadow, lowland broad-leaved deciduous shrub, lowland broad-leaved evergreen shrub, lowland broad-leaved evergreen shrub, other lowland shrub), mixed lowland (mixed lowland conifer/hardwood, mixed lowland hardwood), and aspen/birch vegetation categories.

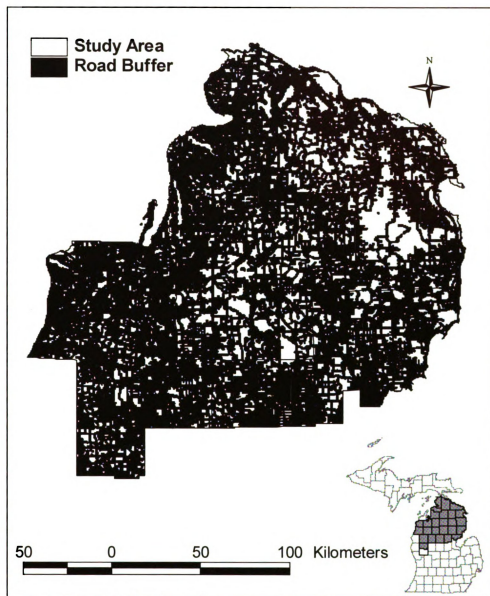


Figure 2.5. The 500 meter buffer on both sides of county roads, streets and highways within the study area.

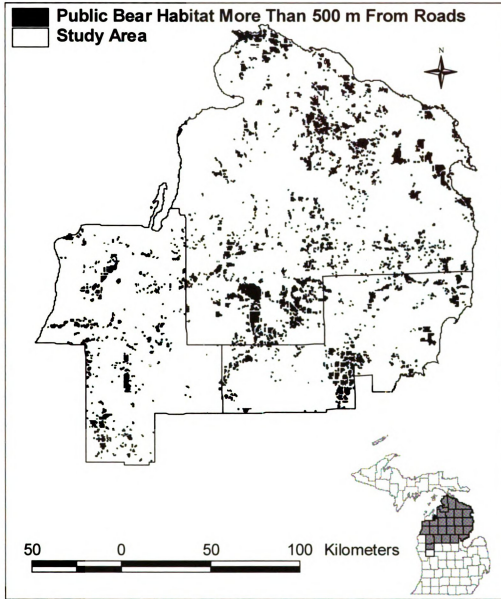


Figure 2.6. Final stratified coverage representing public land, potential bear habitat, area > 500 meters from streets, county roads, and highways. This coverage was used for the hair snare selection.

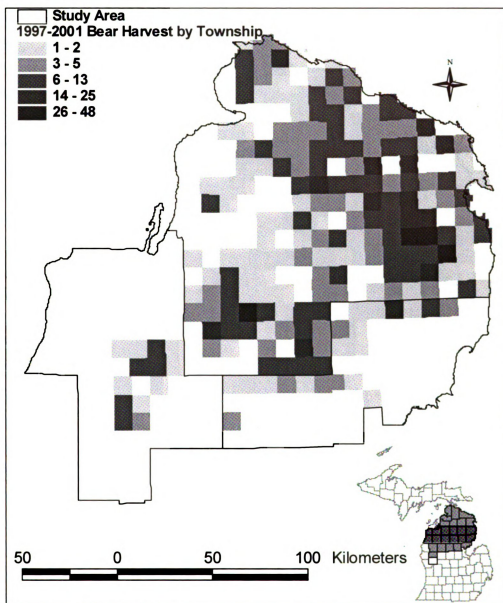


Figure 2.7. Number of bears harvested in each township within the study area from 1997-2001.

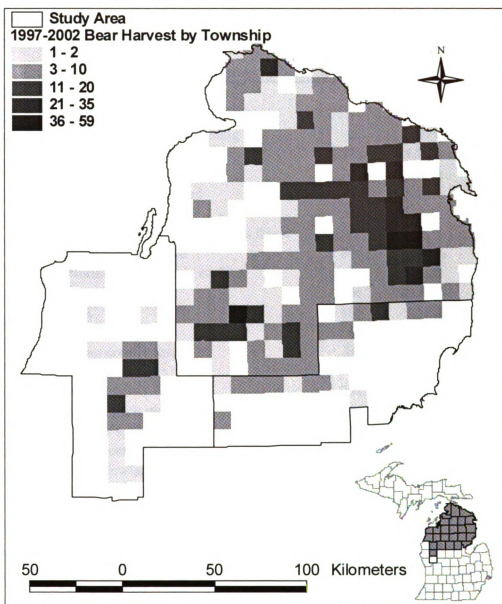


Figure 2.8. Number of bears harvested in each township within the study area from 1997-2002.

Table 2.3. Land use /land cover categories, number of telemetry locations and percent of all telemetry locations collected from 1991-2001.

Land use/cover Category	No. of Locations	Percent of Locations
High intensity urban	28	0.57%
Low intensity urban	1	0.02%
Extractive - open pit mining	0	0.00%
Agricultural crops	278	5.70%
Orchards / vineyards	0	0.00%
Herbaceous open land	281	5.77%
Shrubland	82	1.68%
Other broad-leaved deciduous forest	12	0.25%
Northern hardwood	846	17.36%
Northern hardwood / conifer	64	1.31%
Aspen / birch*	996	20.44%
Oak	166	3.41%
Oak / jack pine	6	0.12%
Other conifer forest	20	0.41%
White pine	0	0.00%
Red pine	261	5.36%
Upland jack pine	324	6.65%
Cedar / spruce / fir	2	0.04%
Emergent wetland / wet meadow*	59	1.21%
Other lowland shrub*	15	0.31%
Lowland broad-leaved deciduous shrub*	236	4.84%
Lowland broad-leaved evergreen shrub*	5	0.10%
Other forested wetland	2	0.04%
Mixed lowland hardwood*	11	0.23%
Lowland jack pine	1	0.02%
Black spruce	23	0.47%
Northern white cedar	0	0.00%
Mixed lowland conifer / hardwood*	1075	22.06%
Barren land	0	0.00%
Water	70	1.44%
Urban grasslands	4	0.08%
Lowland needle-leaved evergreen shrub	5	0.10%
Total	4873	100.00%

* - Denotes Vegetation Category selected in stratified design

Table 2.4. Land use /land cover categories, number of telemetry locations and percent of the subset of telemetry locations (June and July) collected from 1991-2001.

Land use/cover category	No. of Locations	Percent of Locations
High intensity urban	8	0.57%
Low intensity urban	1	0.07%
Extractive - open pit mining	0	0.00%
Agricultural crops	80	5.65%
Orchards / vineyards	0	0.00%
Herbaceous open land	87	6.15%
Shrubland	29	2.05%
Other broad-leaved deciduous forest	4	0.28%
Northern hardwood	243	17.17%
Northern hardwood / conifer	22	1.55%
Aspen / birch*	277	19.58%
Oak	50	3.53%
Oak / jack pine	3	0.21%
Other conifer forest	5	0.35%
White pine	0	0.00%
Red pine	83	5.87%
Upland jack pine	96	6.78%
Cedar / spruce / fir	2	0.14%
Emergent wetland / wet meadow*	14	0.99%
Other lowland shrub*	3	0.21%
Lowland broad-leaved deciduous shrub*	56	3.96%
Lowland broad-leaved evergreen shrub*	0	0.00%
Other forested wetland	1	0.07%
Mixed lowland hardwood*	1	0.07%
Lowland jack pine	0	0.00%
Black spruce	6	0.42%
Northern white cedar	0	0.00%
Mixed lowland conifer / hardwood*	314	22.19%
Barren land	0	0.00%
Water	25	1.77%
Urban grasslands	2	0.14%
Lowland needle-leaved evergreen shrub	3	0.21%
Total	1415	100.00%

* - Denotes Vegetation category selected in stratified design

(emergent wetland/wet meadow, lowland broad-leaved deciduous shrub, lowland broad-leaved evergreen shrub, lowland broad-leaved evergreen shrub, other lowland shrub), and mixed lowland (mixed lowland conifer/hardwood, mixed lowland hardwood). The percentage of telemetry locations in these categories include 47.00% and 49.19% of the telemetry locations for all telemetry locations and the subset, respectively.

By buffering all 239 hair snares set in 2003 by the radius of an adult female home range (6.46 km) we made the equivalent of the area of an adult female home range (131 km²; Figure 2.9). The total area of this buffer is approximately 18,233 km² (Figure 2.9). The percentage of telemetry locations within this buffered area was 87.83% (4,280 out of 4,873 telemetry locations) and 87.49% (1,238 out of 1,415 telemetry locations) from the subset of telemetry locations.

Hair Snaring

The total number of hair snares set, the number of checking occasions, and consequently the number of checks of hair snares differed between 2002 and 2003 (Table 2.5).

Table 2.5. Number of hair snares set, number of checking occasions, total number of checks made, number of snares that were visited by bears, total number of hair samples collected, and the percent of snares visited for the summers of 2002 and 2003.

Year	No. Snares Set	No. Checking Occasions	Total No. Checks	No. Snares Visited	Percent Visited
2002	202	8	1,616	118	58.42%
2003	239	5	1,195	122	51.05%

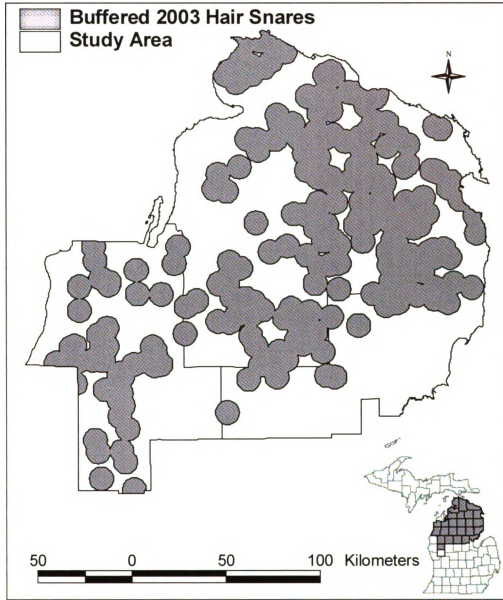


Figure 2.9. Buffer based on average size of adult female annual home range (131 km^2) around the 239 hair snares set in 2003.

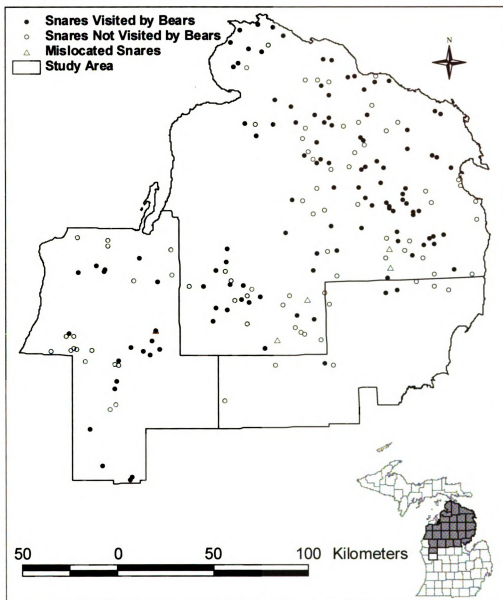


Figure 2.10. Summer of 2002 hair snare locations. Black dots refer to hair snares that were visited by bears and unfilled dots refer to snares that were not visited by bears. Documented locations of 5 snares, symbolized by a triangle and referred to as mislocated, were incorrect and the locations of these snares was approximated.

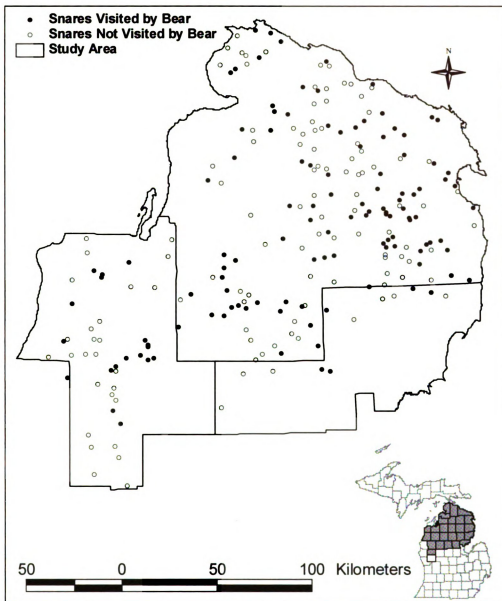


Figure 2.11. Summer of 2003 hair snare locations. Black dots represent the snares that were visited by bears and the unfilled black dots represent snares that were not visited by bears.

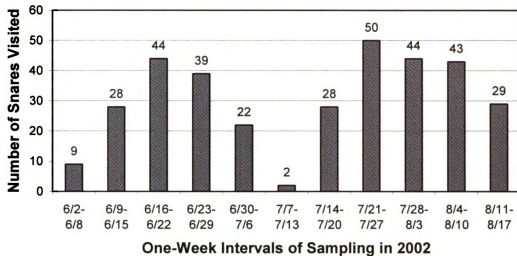


Figure 2.12. Number of hair snares visited by bears for each week of sampling in 2002. The week of 7/7 – 7/13 represents the 1-week interval between sampling periods.

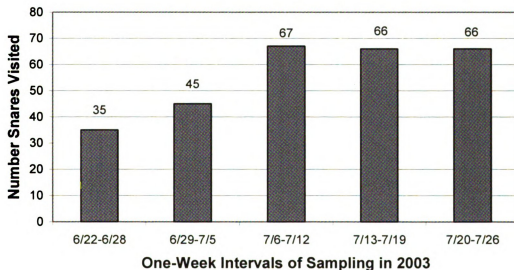


Figure 2.13. Number of hair snares visited by bears for each week of sampling in 2003.

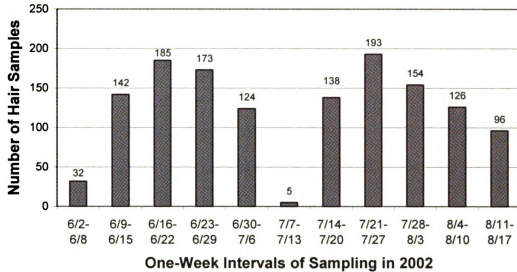


Figure 2.14. The number of hair samples collected for each one-week of sampling conducted in the summer of 2003. The week of 7/7 – 7/13 represents the 1-week interval between sampling periods.

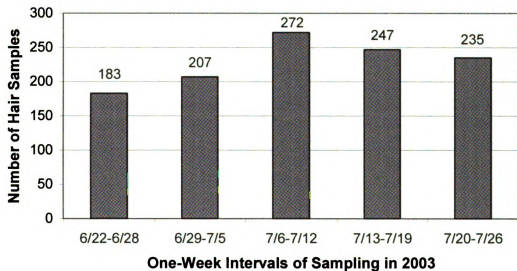


Figure 2.15. The number of hair samples collected for each one-week of sampling conducted in the summer of 2003.

A complete list of wildlife agencies and MDNR offices operating hair snares, hair snare names, year of operation, snare locations, number of bear visitations, number of hair samples collected, and county is located in Appendix 2.3 and 2.4. The distribution of snares covered much of the study area in both 2002 and 2003 (Figures 2.10 and 2.11). The number of snares visited by bears and the percent of snares visited also differed with the summer of 2003 having more visited hair snares, but the summer of 2002 having a higher percentage of snares visited (Table 2.5).

Snares were checked on approximately 7-day intervals (min = 3, max = 13 in 2002; min = 4, max = 10 in 2003). The duration of sampling in 2002 was approximately 70 days and the duration of sampling in 2003 was approximately 28 days. The number of snares visited by bears varied throughout the summer (Figures 2.12 and 2.13), as did the number of hair samples collected with a decreasing trend in the number of hair samples collected in 2002 and a slight decreasing trend in 2003 (Figures 2.14 and 2.15). We collected more hair in 2002 than we did in 2003, but the average number of hair samples collected in 2002 and 2003 was similar (Table 2.6).

Table 2.6. Total number of hair samples collected, mean, minimum and maximum number of hair samples collected given a visitation occurred for 2002 and 2003.

Year	Total No. Hair Samples Collected	Mean No. Hair sample/ Occasion	Min. No. Hair sample/ Occasion	Max. No. Hair sample/ Occasion
2002	1,368	4.01	1	24
2003	1,144	4.1	1	20

In the summer of 2003 we documented the number of hair follicles in each hair sample and placed them into categories (1–4, 5–10, 11–15, or >15 hair follicles). The

number of hair samples in each category differed with 42.83% of the hair samples having 1–4 hair follicles (Table 2.7).

Table 2.7. Number of hair samples collected in 2003 that contained 1-4, 5-10, 11-15, and >15 hair follicles.

1-4 Hair Follicles	5-10 Hair Follicles	11-15 Hair Follicles	>15 Hair Follicles	Total
490	250	130	274	1144

Hunter Harvest Tissue and Hair Collection

The duration between the summer hair snaring and the hunter harvest was 35 days in 2002 and 55 days in 2003. During the 2002 black bear harvest we collected 347 tissue samples from 351 harvested bears. Additionally, we collected hair samples from nearly all bears from which we collected tissue samples. The MDNR also submitted 5 different tissue samples from bears that were killed as nuisance bears or road kills. During the 2003 black bears harvest we collected a total of 414 tissue samples from 418 harvested bears in the NLP. We were also successful at collecting hair samples from nearly all harvested bears in 2003. In 2003 we attempted to use the tooth samples as a source for DNA and collected tooth samples from all but 2 of the 414 harvested bears from which samples were taken. In both 2002 and 2003 information on the location of the harvest was submitted by many of the hunters.

DISCUSSION

Hair Snare Placement

Design of mark-recapture experiments often follows a systematic grid design where grid size is a function of the average female bear summer home range size and suggests that 4 trapping locations be defined within each grid cell (Mowat and Strobeck 2000; White et al. 1982). This high trap density increases the probability of an animal encountering a capture location and being captured which increases the precision of the abundance estimate (White et al. 1982). Therefore, the information required to apply this technique is an estimate of an adult female summer home range and the ability to operate a large number of snares. Etter et al. (2002) estimated the annual home range size of female black bears in the NLP to be 131 km^2 . Using the systematic grid design with our study area size of $36,848 \text{ km}^2$ would require approximately 1,125 snares set across the study area, which was not feasible with available personnel. Research experiments conducted in British Columbia and Alberta, Canada (Mowat and Strobeck 2000; Poole et al. 2001), Florida (Eason et al. 2001), and Louisiana (Boerson et al. 2003) utilized the systematic grid design.

There are two primary differences between our research experiment and other research projects utilizing the systematic grid design. First, the study area that we have chosen is larger than other such research projects. Second, in most cases, study areas were made up of landownership that provided researchers with unlimited access across the entire study area (Mowat and Strobeck 2000; Poole et al. 2001; Boerson et al. 2003),

unlike our study area where only approximately one-third (11,878 km² out of 36,848 km² public land) of the study area provides unlimited access (i.e. public land).

Based on these differences, we had to adapt our study design and we chose a stratified random design to select hair snaring locations. Stratification of bear habitat within our study area is biologically relevant because not all land cover/ land use classes provide the life requisites of bears. Hirsch (1990) found that bears on Drummond Island, Michigan utilized aspen-birch, coniferous, upland hardwood, and wetland vegetation types in the spring, summer and fall seasons. In addition, telemetry data collected from 1991–2000 (Etter et al. 2002), suggested that certain land use/ land cover categories (i.e. aspen/birch and mixed lowland conifer/ hardwood) were used more than others (Tables 2.3 and 2.4).

In our study design we stratified the study area based on private land because it would be logistically difficult to sample both private and public lands on a large scale. Might we expect the sampling of primarily public lands to bias our population estimates? Verification using radio-marked animals would suggest that bears are using both public and private lands (approximately 64.52% of telemetry locations were on public land from the subset of telemetry locations). Therefore, we would not expect sampling from public land to bias population estimates.

The purpose of the road buffer was to prevent the possibility of a site being chosen at random in close proximity to major roadways in Michigan. The road buffer was mostly justified because we did not want humans vandalizing snares, and keeping the snares away from roadways would limit the finding of the snare locations. We might also expect that there is some biological relevance to a road buffer because bears might

exhibit avoidance of roads. Telemetry data collected from 1991–2000 (Etter et al. 2002) suggested that bears did not show avoidance of roads, as 54.28% of telemetry locations collected in the months of June and July were within 500 meters of county roads, streets and highways. In addition, because of the high road densities (approximately 1 km of road from every 1 km²) bears may have little choice but to utilize areas in close proximity to roads. In both years of snaring there was only one documented account of a snare being tampered by people. Therefore we might say that this road buffer was effective at keeping humans from finding and vandalizing snares. However, we did not set this up as an experiment, so inferring that the buffer was the reason for humans not tampering with snares is inconclusive.

Bears were not equally distributed across this diverse study area. Therefore we decided to stratify our study area by the townships in which harvests had occurred and the frequency of harvest from 1997–2002. This assumed that the presence of harvest in a township indicated the presence of bears within that township. By setting up our snare allocation hierarchically (i.e. more harvests in a township equates to choosing more snares), we assumed that frequencies of harvests were predictive indicators of relative bear density in that township. One therefore might expect that these areas with higher bear densities may require more snares to adequately sample the bears in the area. In addition, the population estimate generated will be used for management purposes and the primary tool that the MDNR uses to manage bears is harvest. Therefore, the population of bears from which bears were being harvested constituted the population about which the MDNR must be most informed when making management decisions (i.e. setting harvest quotas). Radio telemetry data (Etter et al. 2002) suggested that bears were

present in areas outside of the townships in which harvests have occurred since 1997–2002. Therefore, a population estimate derived for just the townships where harvests occurred from 1997–2002 might be a conservative estimate for the size of the bear population in the NLP. Conservative estimates can be important when managing a population with harvest, especially when harvest is the highest cause of mortality of bear in the NLP (Etter et al. 2002).

Ultimately we wanted to know if we were sampling in the correct locations. Once again telemetry locations collected from 1991–2000 (Etter et al. 2002) provided insight into this question. Clark and Smith (1994) and Boerson et al. (2003) determined their effective study area by placing a buffer of an adult female home range on all sampling locations. Adult female home range size was used because it is smaller than adult male home range and provides a conservative estimate of the area sampled. To examine the overlap of telemetry locations with the buffered area we overlaid the telemetry locations within the buffered area. We found that 87.49% of the telemetry locations collected in the months of June and July overlapped with this home range buffer. Therefore, we can conclude that we are sampling in areas that have been utilized by bears from 1991–2000 and this utilization provides evidence that we are sampling in the biologically relevant locations.

Hair Snaring

Utilization of non-invasive DNA sampling for abundance estimation requires the success of a field methodology for collecting hair samples. Other research experiments utilizing this methodology have been successful at both collecting hair samples and

obtaining population estimates (Mowat and Strobeck 2000; Poole et al. 2001; Eason et al. 2001; Boerson et al. 2003). The basic means to collect hair samples common to all research experiments, including this study, is a hair snare composed of 1 (Mowat and Strobeck 2000; Poole et al. 2001; Boerson et al. 2003) or 2 (Eason et al. 2001) strands of barbed wire encircling a baited location. Baits utilized by researchers varied from scents and lures (Mowat and Strobeck 2000; Poole et al. 2001) to pastries, corn and meat scraps (Boerson et al. 2003; Eason et al. 2002). Our study utilized both bacon and anise scented lure. Once a snare is baited, researchers then visit the snare at a given interval to check the wire for the presence of hair. Mowat and Strobeck (2000) and Poole et al. (2001) checked snares at 10–14 day intervals and this study and Boerson et al. (2003) checked snares at 7 day intervals. The duration of sampling also varied among studies with Eason et al. (2002) sampling for 67 days and our research experiment sampling for 55 days in 2003. The total number of hair samples collected and the number of different snares visited by bears also varied among experiments, with Mowat and Strobeck (2000) collecting the largest number of hair samples (4,245 hair samples) and Eason et al. (2002) having the largest percentage of snares visited (86.4%) in the Ocala study area (Table 2.4).

For our project, the main difference between the summers of 2002 and 2003 was the number of checks (8 checks in 2002 and 5 checks in 2003), the number of strands of barbed wire (1 strand in 2002 and 2 strands in 2003), and the total number of snares set (202 in 2002 and 239 in 2003). In 2002 a higher percentage of snares were visited by bears (58.42%), however, this may be attributed to making 5 checks in 2003 rather than 8 checks in 2002. It does not appear that 2 strands of barbed wire results in the collection

of more hair samples because the mean number of hair samples collected given a bear visitation occurred for each summer is very similar (approximately 4 hair samples). It is difficult to determine if more individual hairs were collected per sample when using the 2 strands of wire because we did not quantify the number of hairs per sample in the summer of 2002. In both summers the number of hair samples collected on each checking occasion increases each week for the first three weeks (Figures 2.14 and 2.15), which would suggest that a minimum number of sampling occasions is 3 weeks to obtain the a maximum number of visits. In addition, the number of hair samples collected per week increases to approximately the mid-point of sampling then the number of hair samples collected per week declines (Figures 2.14 and 2.15). This decline in the number of hair samples collected could possibly be attributed to bears evading the wire after encountering the wire on a previous occasion, or adverse weather conditions (i.e. rain, wind) removing the hair samples from the wire. Based on these data, it appears that the optimal time of sampling is between mid June and mid to late July (Figures 2.12 and 2.14).

Table 2.8. Summary table of other research studies, including this one, to estimate bear abundance including the author and study area, species of study, study area size, number of snares, number of snares visited, and the percent of snares visited

Research Study and Study Area	Species	Study area Size	No. of		Percent Visited
			snares	Snares Visited	
Boerson et al. (2003), Louisiana	<i>U. Americanus</i>	329 km ²	122	N/A	N/A
Mowat and Strobeck (2000), Alberta	<i>U. Arctos</i>	5,030 km ²	321	155	48.29%
Mowat and Strobeck (2000), British Columbia	<i>U. Arctos</i>	9,866 km ²	381	277	72.70%
Poole et al. (2001), British Columbia	<i>U. Arctos</i>	8,527 km ²	515	332	64.47%
Eason et al. (2002), Apalachicola, Florida	<i>U. Americanus</i>	2,196 km ²	392	123	31.38%
Eason et al. (2002), Big Cypress, Florida	<i>U. Americanus</i>	1,867 km ²	352	175	49.72%
Eason et al. (2002), Eglin, Florida	<i>U. Americanus</i>	3,764 km ²	468	117	25.00%
Eason et al. (2002), Ocala, Florida	<i>U. Americanus</i>	598 km ²	375	324	86.40%
Eason et al. (2002), Osceola, Florida	<i>U. Americanus</i>	613 km ²	408	268	65.69%
Eason et al. (2002), St. Johns, Florida	<i>U. Americanus</i>	1,581 km ²	367	71	19.35%
This study (2002), Michigan	<i>U. Americanus</i>	36,848 km ²	202	118	58.42%
This study (2003), Michigan	<i>U. Americanus</i>	36,848 km ²	239	122	51.05%

Hunter Harvested Bear Tissue Collection

Based on the high success rate (98.86% in 2002 and 99.04% in 2003) of being able to obtain muscle tissue samples from harvested bears, the MNDR has proven to be highly effective at sampling harvested bears. Before this research was conducted, we were unsure if it was necessary to collect muscle tissue samples when tooth samples could possibly be used as a DNA source, which the MDNR had traditionally collected. In 2003, there were only 2 tooth samples that were not submitted, therefore it would appear that from a field sampling standpoint (not say anything about whether DNA was actually in the tooth samples), it may not be necessary to collect muscle tissue samples and use the tooth samples as a source of DNA.

To date, no other capture-recapture studies utilizing the non-invasive methodology have used the harvest as an additional capture occasion. Results from this experiment support that collection of a DNA source from harvested bears is highly effective means to sample populations.

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CHAPTER 3 – ESTIMATION OF BLACK BEAR ABUNDANCE IN THE NORTHERN LOWER PENINSULA OF MICHIGAN USING MICROSATELLITE DNA MARKERS

INTRODUCTION

Until recently, the quantitative population management of black (*Ursus americanus*) and brown (*Ursus arctos*) bears has been a difficult task. State and federal management agencies often rely upon indirect measures of population abundance through indices and sex/age of harvested animals (D. Etter, MDNR, person. comm. 2003). Recent advancements in molecular genetics have provided a means to non-invasively estimate bear population size for conservation purposes (Boerson et al. 2003; Mowat and Strobeck 2000; Poole et al. 2001; Taberlet et al. 1997).

These methods could not be timelier for biologists attempting to manage bear populations with harvest. For many wildlife populations, management is shifting to the greater involvement of stakeholders and minimizing negative human-wildlife interactions (Riley et al. 2002). This stakeholder involvement and public interest often requires precise data in the decision making process to justify and allocate the resource. Bear management in the state of Michigan, in particular the northern Lower Peninsula (NLP), is no exception. The population of bears in the NLP is believed to be increasing and expanding into areas not occupied by bears in recent history (L. Visser, MDNR, person. comm. 2002). These population characteristics and recent changes in population management strategies have resulted in the public desire for precise scientific information.

Although non-invasive techniques have demonstrated great success and promise, they are not without their criticisms. Other researchers have identified genotyping errors

as a potential drawback to using non-invasive methods (Gagneux et al. 1997; Goossens et al. 1998). Genotyping errors result from the limited quantity and/or quality of DNA contained in hair samples that is often subjected to diverse environmental conditions associated with field collection (Taberlet and Luikart 1999). Errors result in the misidentification of individuals, which violates the capture-recapture assumptions that individuals are identified correctly and that marks are not lost (Stevick et al. 2001; White et al. 1982). This violation of assumptions results in biased population estimates that have been demonstrated to overestimate population size (Creel et al. 2003; Waits and Leburg 2000). Quantification of error is an important aspect of any research that utilizes non-invasive methods because it provides insight into the expected bias (Taberlet et al. 1999). Because of these potential drawbacks, recent statistical methods have been derived to account for genotyping error within the estimate (Lukacs and Burnham *in press*).

In the summer of 2002 we began a research project to estimate black bear abundance in the NLP of Michigan utilizing non-invasive DNA collection and capture-recapture methodologies. Although previous research had success using non-invasive methods, research using non-invasive population estimation for a bear population managed at a large spatial scale was limited. Because the bear population in the NLP is managed through harvest, we recognized a unique means to resample the population with DNA collected from hunter-harvested bears. Our objective was to estimate bear abundance in the NLP of Michigan while quantifying and accounting for genotyping error in the population estimate.

STUDY AREA

The study area is composed of 3 management units that the Michigan Department of Natural Resources (MDNR) uses to distribute hunters (Figure 1.1). This area is bounded by Lake Michigan to the west and north, Lake Huron to the east and north and increased development and agricultural lands on the south. The study area is characterized as the northern lacustrine-influenced ecotone (Albert 1995). The size is approximately $36,848 \text{ km}^2$ with elevations ranging from 177 to 526 meters. Well drained, sandy soils in the area typically support northern hardwoods, jack pine (*Pinus banksiana*) barrens, upland conifer forest, white pine (*Pinus strobes*) -red pine (*Pinus resinosa*) forest, hardwood-conifer swamp, and conifer swamp (Albert 1995). The landscape is mostly forested, but highly fragmented with densities of county roads, streets and highways approximately 1 km/km^2 (MDNR-Land and Mineral Services Division, Resource Mapping and Aerial Photography 1992). Approximately $11,878 \text{ km}^2$ (32.24%) of the study area is under public ownership (MDNR-Forest, Mineral and Fire Management Division, Resource Mapping and Aerial Photography 2001).

METHODS

Sampling Location Identification

We used a stratified random design (Ratti and Garton 1996) to derive sampling locations in our study area. Our sampling design is unique and experimental because others utilizing non-invasive marking methodologies have used a systematic grid design (Mowat and Strobeck 2000; Poole et al 2001; Boerson et al. 2003). We found the

systematic grid design impractical because private land in the study area would inhibit unlimited access and the large study area would require more sampling locations than feasibly possible to operate. We therefore used a stratified random design in a series of five procedures to identify sampling locations with ArcView 3.2 (Environmental Systems research Institute, Redlands, California) and several existing digital coverages from the MDNR spatial database. We first stratified by the frequency of bear harvest grouped by township (93 km^2) over a 5 year period (1997–2002). This stratification was used to indicate the areas of bear presence because bears are not homogeneously distributed throughout the area. We divided the frequency of harvest by township into five categories which included: 1–2, 3–10, 11–20, 21–35, and 36–59 harvests, respectively. We allocated snares to each township based on the total number of snares we could monitor and the frequency of harvest. In townships with 36–59 harvests we allocated 4 snares, 21–35 harvests 3 snares, 11–20 harvests 2 snares, 3–10 harvests 1 snare, and townships with 1–2 harvests were excluded from sampling based on logistical constraints. This hierarchical approach assumes that the frequency of harvest in each township reflects the overall density of bears within that township.

Second, we stratified for potential bear habitat within the study area. Hirsch (1990) found that bears on Drummond Island, Michigan, utilized aspen-birch, coniferous, upland hardwood, and wetland vegetation types in the spring, summer and fall seasons. We therefore, selected aspen/birch, emergent wetland, and mixed lowland vegetation classes as potential bear habitat for sampling locations.

Third, because of the high density of roads and humans in the region, we placed a road buffer of 500 meters on all county roads, streets and highways within the area.

Fourth, we selected all public land within the area so that access by field personnel was not limited. After all stratifications were made, we overlaid all of the coverages and developed a new coverage which included publicly owned potential bear habitat greater than 500 meters from county roads, streets and highways.

Lastly, we selected the sampling locations with the criteria developed based on the frequency of harvest in each township. Sampling site locations were either made at random using ArcView 3.2, or utilized existing MDNR bear bait index locations. In some instances, there was no public land in townships where historical harvest had occurred. In these townships we attempted to gain access to private lands. We used a conservative spacing distance of 5 km calculated from White et al. (1982) and based on female bear home range size for Michigan (Etter et al. 2002).

We verified our sampling locations with a subset of radio-telemetry data collected in the months June and July (1,415 locations collected from 1991–2000) for 126 radio-collared bears (64 males, 62 females; Etter et al. 2002). We found that 64.5% of locations were located on public land, so we would not expect bias by sampling public lands. We found that 49.2% of the locations were within the vegetation classifications that we included in our stratification.

Clark and Smith (1994) determined the effective sampling area for black bears by placing a buffer of an adult female home range around each sampling location. Using this methodology in our verification, we placed a buffer of an average adult female bear home range (131 km^2 ; Etter et al. 2002) around each sampling location and determined the percentage of telemetry locations that were within the buffered area. We found that 87.5% of the telemetry locations were within the buffered area. Based on the high

percentage of telemetry locations collected within the equivalent of one female home range of our sampling locations, we believe that we sampled in the proper locations.

Hair Snaring

We collected hair samples using two strands of barbed wire encircling a baited location at uniform heights of 8 cm and 20 cm (Figure 2.2). This design differs from the 1-stranded design proposed by Woods et al (1999). We attracted bears with 1-pound bacon and anise extract, which was aurally suspended over the snare. We visited snares at 5–7 day intervals for a total of 5 checking occasions (June 22 – July 26, 2003). We collected hair samples from each barb on the top strand (strand at 20 cm) independently and placed them in pre-numbered coin envelopes. Because bear cubs cannot be legally harvested and therefore included in our last capture occasion (i.e., registered harvest), we did not collect hair samples from the bottom strand of wire (strand at 8 cm) and set the top strand at 20 cm to minimize the occurrence of cubs depositing hair on the wire. Hair samples were stored in a dry location pending laboratory analysis.

Hunter Harvested Bear Tissue Collection

Current management of bear in the NLP of Michigan includes recreational harvest, which is allocated by the MDNR through a lottery with a preference point system. Because of the large number of applicants (14,330 applicants for 1,700 available licenses in 2002; Frawley 2003), the ability to successfully draw a tag may require 4–5 years. There are two bear hunting seasons offered in the study area. The first hunting season is a 7-day firearm season (19–25 September 2003) where hunters can use bait and

hounds. The second is an archery only season (3–9 October 2003) where hunters can use exclusively bait. Successful hunters must register harvested bears and hunter compliance is estimated at 98% (Visser, MDNR, person. comm.; Frawley 2002). We trained MDNR personnel for collecting muscle tissue and hand-plucked hair samples from registered bears. To determine age of harvested bears, the MDNR collects tooth samples for annuli aging. Extracted teeth contain small portions of gum tissue and saliva. We examined using collected tooth samples as the primary DNA source.

Genetic Analysis

Sample Selection

The hair snare is an effective sampling device, which can collect multiple hair samples with varying numbers of hair follicles across each checking interval. For example, Mowat and Strobeck (2000) collected 4,245 hair samples in their study of grizzly bears from British Columbia, Canada. Although this methodology is effective at collecting hair samples, it may not be possible or necessary to genetically analyze each sample. Project budgets are often limiting and the quantity of hair in each sample can affect the genotyping error rate (i.e. few follicles can increase the genotyping error rate; Goosens et al. 2000). The number of hairs collected for this project exceeded available funds for genotyping each individual hair sample. Therefore, we developed a series of selection criteria to sub-sample hair samples trying to account for the spatial and temporal components of our sampling design.

To ensure an adequate quantity of DNA, but not limit sample size, we considered only those hair samples with 5 or more hair follicles. Our first selection included a single

hair sample from each snare where a hair sample with 5 or more follicles was deposited by a bear. Because we did not move snare locations between checking occasions, it was possible for any snare to collect hair samples on a maximum of 5 occasions. To account for this temporal factor of bears visiting snares through time, our second selection criteria consisted of a single hair sample from each snare that collected hair on each checking occasion (excluding hair samples that were already selected in our first selection). The first two criteria produced a hair sample from every snare that was visited by a bear on every occasion that a bear visited, but no information about the incidence of multiple bears visiting a snare during a single checking occasion. Therefore, we randomly choose a subset of hair samples collected on the same date and location for our third selection criteria. We used these data to examine the incidence of multiple bears visiting across a single checking duration. We genotyped all muscle tissue/tooth samples obtained from harvested bears.

Microsatellite Genetic Analysis

We extracted DNA using Qiagen DNeasy® Tissue Kit (Qiagen Inc.). All hair follicles were removed and placed in a 1.5-ml centrifuge tube. DNA extractions from hair follicles occurred within approximately 7 days after collection to prevent degradation of DNA and increase amplification success (Roon et al. 2003). We used tooth samples collected from registered bears (muscle tissue samples were used when tooth samples were not available) as the DNA source from hunter-harvested bears. DNA from muscle tissue and tooth samples was quantified using a spectrophotometer and were diluted to a working concentration of 20ng/μl. The quantity of DNA obtained from hair samples was

not quantified because of the small expected quantities. After extraction, each DNA sample was placed in –70 degrees C freezer pending genetic analysis.

To differentiate individuals we genotyped extracted DNA with five polymorphic microsatellite loci including, G10X, G10L, G10D (Paetkau et al. 1995), UarMU59, and UarMU50 (Taberlet et al. 1997). Loci were amplified using polymerase chain reaction (PCR) in 10 µl reaction volumes including 1 µl 10x PCR Buffer (10mM Tris-HCl at pH 8.5, 1.5 mM MgCl₂, 50 mM KCl, 10 µg/mL nuclease-free bovine serum albumen, 0.0025% Tween-20), 200 pmol dNTPs, 10 pmol of forward and reverse fluorescently-labeled primers, 0.2 µl of commercial Taq polymerase (New England Bio Labs, Beverly, MA), additional MgCl₂, and 5µl of DNA (unknown concentration for hair and for tissue). Thermocycler conditions included a 2-minute denaturation at 94 degrees C followed by 35 (UarMU59) or 42 cycles of 30 seconds at 94 degrees C (G10L at 94 for 1 minute), 1 minute at locus-specific annealing temperature, 1 minute at 72 degrees C and one cycle of 5 additional minutes at 72 degrees C. We used denaturing acrylamide gels (6.5%) for electrophoresis visualized by LI-COR® IR² Global Edition DNA Sequencer. We used molecular weight standards and individuals of known genotypes collected from muscle tissue to standardize all genotype scores. Saga™ genotyping software (LI-COR, inc., Lincoln, NE, USA) was used to determine genotypes. All individual genotypes were manually checked by two experienced lab personnel.

Quality Control

We used quality control protocols developed by Paetkau (2003) to minimize genotyping errors and provide reliable genotypes which included, development of a

database around the field database, conservative genotype scoring, culling of samples with incomplete or contaminated genotypes, re-analysis of samples with uncertain scores, and careful scrutiny of samples with similar genotypes for the detection of genotyping errors.

We used Saga™ genotyping software to develop a database around our field database to limit notation errors made during manual entry. We initially genotyped all samples for all 5 loci and conservatively scored genotypes by not recording scores for samples that could not be confidently scored by 2 experienced lab personnel. After the initial genetic analysis we culled all samples where unambiguous and verified scores for 3 of the 5 loci were not assigned. Sample culling is important because genotyping errors are related to sample quality. Thus culling samples that do not produce genotypes from the majority of loci helps to ensure that the remaining samples are of a higher quality (Paetkau 2003). After samples were culled, we attempted to replace culled hair samples. Because we only chose a single hair sample from a visited snare on every date the snare was checked, we often had other hair samples that could replace culled samples. When possible, we choose 2 hair samples to replace a culled sample. We did this in an attempt to obtain a genotype from sites where samples were culled and minimize the possibility of a replacement sample also being culled. After all samples were genotyped a single time for all loci and all necessary culling carried out, the remaining samples with missing genotypes by locus were then reanalyzed and scored. After this reanalysis, samples that did not have confident scores for 4 loci were culled and no replacements identified and selected.

To identify and correct genotyping errors we used the mismatch methodology described by Paetkau (2003). This methodology involves the careful scrutiny of all genotypes that are nearly identical, differing by one or two loci [referred to as 1-mismatch (1-MM) and 2-mismatch (2-MM) pairs, respectively]. The basis for this data scrutiny is that the probability of genotyping errors is quite low in most cases. The most important samples in which to identify errors are those that matched, but a genotyping error caused them to miss-match, thus creating an additional individual (Paetkau 2003). We identified mismatched samples with program GENECAAP (Wilberg and Dreher 2004) and the scores of each sample in the mismatch were scrutinized by the raw genetic data for possible errors. If the mismatch could not be resolved by examining the raw genetic data, the samples were reamplified and genetically reanalyzed to determine if a genotyping error occurred. All incidences of miss-scored genotypes were noted and corrected within the database.

Genotyping Error Estimation

For purposes of population estimation, the accuracy of genotypes is critical because genotyping errors can positively bias population estimates (Waits and Leberg 2000; Creel et al. 2003). To minimize errors, Paetkau (2003) suggested careful data scrutiny, which was used in this study. We recognized an additional means to estimate genotyping errors using samples obtained from harvested bears. Upon the registration of a legally harvested bear, field personnel were instructed to collect a tissue sample and a hand-pulled hair sample. We randomly choose 110 harvested bear hair samples for our error analysis (matching muscle tissue samples were already within the genetic analysis). We handled error samples consistent with snare collected samples including extraction

and quality control protocols. Extracted hair samples were genotyped concurrent with snare-collected hair samples. To ensure that the hair and tissue samples from the same bear were independently genotyped, sample identification numbers were randomly assigned and the key was not made available to laboratory personnel.

Paetkau (2003) suggests the careful scrutiny of genotypes with the identification of mismatching samples as one means to detect and correct genotyping errors. To examine the efficacy of this methodology of error detection we matched genotypes from hair and tissue samples before the careful scrutiny of mismatches and identified and quantified inconsistencies. We then included both hair and tissue samples within the data scrutiny and corrected any inconsistencies. Finally, we matched hair and muscle tissue genotypes and quantified errors following review of data. We compared results obtained before and after review of data.

Statistical Genetic Analysis

Individual discrimination using highly polymorphic microsatellite loci necessitates that a significant number of loci be analyzed to minimize the probability of unique individuals sharing the same genotype (Waits et al. 2001). Some non-zero probability of unique individuals sharing the same genotype can result in two different bears being identified as the same individual. This can result in negative bias in population estimates because fewer individuals are uniquely identified than truly exist in the population (Mills et al. 2000). The probability of identity (PI) is a statistical measure to determine the probability of matching genotypes (Paetkau and Strobeck 1994). We calculated PI_{sibs} single locus with program GENEAP (Wilberg and Dreher 2004), which provides the probability of two full siblings in the population sharing the same genotype

for the loci examined and represents the upper range of possible *PI* values in the population (Taberlet and Luikart 1999). The following is the equation for

PIsibs single locus :

$$PIsibs_{\text{single locus}} = 0.25 + (0.5 \sum p_i^2) + [0.5(\sum p_i^2)^2] - (0.25 \sum p_i^4)$$

where p_i is the frequency of alleles and the overall *PIsibs* single locus is calculated as the product of the *PIsibs* single locus values for each locus.

Identical genotypes are assumed to be from the same individual, but because there is a probability of two individuals sharing the same genotype, statistical rigor must be used for the match declaration (Woods et al. 1999). We used P_{sib} calculations from Woods et al. (1999) in program GENEAP (Wilberg and Dreher *in press*) to calculate the probability of a matching genotype with the minimum criteria for a match of $P_{sib} < 0.05$ before a match was declared with the following equations:

$$P_{sib(Homozygotes)} = \frac{(1 + 2p_i + p_i^2)}{4}$$

$$P_{sib(Heterozygotes)} = \frac{(1 + p_i + p_j + 2p_i p_j)}{4}$$

where p_i and p_j are the frequencies of the i^{th} and j^{th} alleles. Samples that matched, but did not meet this criteria were excluded from capture histories as matching individuals (Woods et al. 1999; Mowat and Strobeck 2000; Boerson et al. 2003).

Population Estimation

Previous research using non-invasive techniques to estimate bear abundance used closed capture models in program CAPTURE (White et al. 1982; Boerson et al. 2003; Mowat and Strobeck 2000; Poole et al. 2001). For our initial population analysis we used closed capture models in program MARK (White and Burnham 1999). One of the major advantages of program MARK is the ability to create estimation models that account for a capture and behavioral response by capture occasion.

We define parameter notation as presented in Otis et al. (1978) to assist in understanding our population estimation models as follows:

- p_i Probability of an animal's initial capture in time period i
- c_i Probability of an animal's subsequent recapture in time period i
- N Population size

We created 4 *a priori* models to estimate population size by incorporating our knowledge of field methods and bear biology. All models, when applicable, were constructed to account for the harvest of bears being a different capture method than samples collected with hair snares (denoted as “hunt” in model names). We constructed our first model to account for a probability of capture that varied by occasion (model M_t , parameters $p_1, p_2, p_3, p_4, p_5, p_6, N$). Our second model accounts for a differential probability of recapture, also known as a behavioral response, which might be expected when bait is used as an attractant (model M_{b+hunt} , parameters p_{1-5}, c_{2-5}, p_6, N). Our third model accounts for a behavioral response, a time varying response, and the hunt being a separate capture method (model $M_{t+b+hunt}$, parameters $p_1, p_2, p_3, p_4, p_5, c_{2-5}, p_6,$

N). Our last model was constructed to account for a behavioral response, the harvest, and a change in the number of hair samples collected in occasions 1–3 as compared to sampling occasions 4–5 (model $M_{t1-3,4-5+b+hunt}$, parameters p_{1-3} , p_{4-5} , c_{2-5} , p_6 , N). This last model was formulated after a perceived difference in the number of hair samples collected in weeks 1–3 and 4–5 by field personnel.

Using these *a priori* models in program MARK we first derived population estimate using all 6 capture occasions. Waits and Leberg (2000) demonstrated through simulation and Creel et al. (2003) through empirical analysis that genotyping errors tend to cause a positive bias in population estimates. Upwardly biased population estimates can be particularly problematic when population estimates are used to set harvest objectives. Second, we used estimation models proposed by Lukacs and Burnham (*in press*) to account for genotyping error in the population estimate. These models are an extension of the class of closed capture models offered in program MARK (White and Burnham 1999). The major assumptions in these models are geographic and demographic closure, incorporation of enough loci to differentiate individuals, and that genotyping errors create unique genotypes that do not exist in the population (Lukacs and Burnham *in press*).

These models were written in SAS (SAS Inc. 2002), but are similar to those contained in program MARK (White and Burnham 1999) because they allow the user to account for differential probability of capture values attributed to time, and behavioral factors. Unlike the closed capture models in program MARK, the Lukacs and Burnham (*in press*) models include an additional parameter denoted as α and defined as the probability that the animal was genotyped correctly, given its first observation. The

value of α is derived using the capture histories, or incorporating a pre-measured estimate of genotyping error (Lukacs and Burnham *in press*).

Because we estimated our genotyping error rate using hair and tissue samples from harvested bears, we used a joint likelihood function to correct population estimates for our measured error rate. The following is the equation for the binomial portion of the joint likelihood function:

$$L(\alpha | n, x) = \binom{n}{x} \alpha^x (1 - \alpha)^{n - x}$$

where n is the number of samples tested and x is the number of correctly assigned genotypes. Because we incorporated a pre-measured error rate into the models, the value of α remained consistent for all models used to estimate population size.

For all models and methods of analysis we ranked competing models based on Akaike Information Criterion (AIC_c) to determine which models were best supported by the data.

RESULTS

Hair and Tissue Sample Collection

We set a total of 239 snares between 22 June 2003 and 26 July 2003. Each snare was checked 5 times for a total of 1,195 checks. We collected 1,144 hair samples from 122 (51%) snares. Laboratory examination of the hair samples determined that 687 of the 1,144 (60%) hair samples had 5 or more hair follicles. DNA was extracted from samples with 5 or more hair follicles within approximately 7 days of their collection.

We registered 423 harvested bears in the fall of 2003 and obtained a tissue sample from 421 (99.2%). We were able to obtain a quantifiable DNA product from all cases where a tooth sample was used for the DNA extraction (n = 419). In addition we obtained hand-plucked hair samples from approximately 75% of the harvested bears.

Genetic Analysis

Sample Selection and Quality Control

We genotyped 1,026 hair and tissue samples including 416 hair snare collected samples; 110 randomly selected hair samples from harvested bears for our error analysis; 421 harvested bear tissues; and 79 replacement hair samples after our quality control culling procedure (Table 3.1).

After the initial genetic analysis of all samples at 5 loci, we culled 162 samples because they lacked scores for ≥ 3 loci and 13 samples because they showed evidence of contamination (multiple genotypes indicating hair was deposited on the same wire barb by 2 or more individuals; Table 3.1). After we culled samples, we then reanalyzed inconclusive scores and genotypes that contained samples that failed to amplify. In this process we reanalyzed 238 locus genotypes from previous PCR product and re-amplified and analyzed 381 locus genotypes.

After the reanalysis, we then culled 27 samples that did not have ≥ 4 loci scored (Table 3.1). We excluded mismatches between harvested bear samples because these samples were known to be collected from different individuals. We identified 97 genotypes that differed by 1 allele and 229 samples that differed by 2 alleles. These identified mismatches include samples from harvested bear hair and tissue samples.

Table 3.1. Summary of the number of samples genotyped, number of samples culled with ≤ 3 loci score; number of samples that were contaminated; number of samples culled with ≤ 4 loci scored; number culled after mismatch examination; the total number culled and the final number used for estimation analysis for each selection category.

Selection category	Number Genotyped	No. culled w/ ≤ 3 loci scored	Number Contaminated	No. culled w/ ≤ 4 loci scored	No. culled after mismatch	Total culled	Final Number
Snared samples	416	107	12	25	6	150	266
Error hair samples	110	11	1	1	1	14	96
Bear harvest samples	421	3	0	0	0	3	417
Replacement hair samples	79	41	0	1	0	42	37
Total	1026	162	13	27	7	209	816

All mismatched samples were then examined and scrutinized for possible errors. We re-amplified 47 samples in this process to identify possible errors. We identified 55 samples where genotyping errors occurred and changed scores accordingly. In addition, we culled 7 samples based on incomplete scores and evidence of contamination (Table 3.1). We used 816 hair and tissue samples after the quality control procedures to estimate the rate of genotyping error and to estimate population size (Table 3.1).

Genotyping Error Estimation

We used 95 hair and tissue samples from harvested bears to estimate the rate of genotyping error (14 hair samples and 1 tissue sample were culled during the quality control methods). We estimated the overall misidentification rate before mismatch examination to be 20% because 19 out of the 95 pairs differed. The error rate differed across loci with the mean error rate per locus calculated at 0.044 and at 0.029 per allele (Table 3.2). The G10X locus had the highest error rate and UarMU59 locus had the lowest (Table 3.2).

The error rate decreased after we examined mismatched genotype pairs, because 15 out of 19 mismatching samples were corrected after mismatch examinations. Therefore, we did not correct 4 out of 95 sample pairs giving us an overall individual misidentification rate of 4.21% after quality control procedures. Of the 15 samples that were corrected with the mismatching procedures, 9 resulted from scoring errors differing by 2 base pairs, 1 was the result of allelic dropout, and 5 resulted from loading errors and were discovered as a result of including error samples.

Table 3.2. Summary of the genotyping error rates both before and after quality control protocols of mismatch identification. Error rates are reported per locus and per allele and reported by locus.

Locus	Error rate/locus before		Error rate/locus after		Error rate/allele before		Error rate/allele after	
	mismatch examination	mismatch examination	mismatch examination	mismatch examination	mismatch examination	mismatch examination	mismatch examination	mismatch examination
G10X	0.084		0.011		0.063		0.005	
G10D	0.032		0.011		0.021		0.011	
G10L	0.032		0.011		0.016		0.005	
UarMUS9	0.053		0.021		0.037		0.021	
UarMU50	0.021		0		0.011		0	
Mean	0.044		0.011		0.029		0.008	

The remaining 4 sample mismatches that were not corrected resulted from differences greater than 2 alleles (2 sample pairs) and pairs that differed by 2 alleles (2 sample pairs), but not corrected. The mean error rate per locus and per allele decreased as a result of examining mismatched samples (Table 3.2).

Statistical Genetic Analysis

We estimated $PIsibs$ single locus across all loci and unique genotypes at 0.00865 ($n = 544$). The number of alleles and the value of $PIsibs$ single locus differed across loci with locus G10L being the most informative based on the smallest $PIsibs$ single locus value (Table 3.3). All matches met the criteria of $P_{sib} < 0.05$ except 1 of 5 matching harvested bear genotypes. We identified 165 bears from samples collected from hair snares. We identified 412 unique genotypes from harvested bears and 33 of these genotypes were captured by hair snares. One harvested bear was deleted from the analysis because it was harvested outside of the study area in the Upper Peninsula of Michigan.

Table 3.3. Summary of the number of alleles per locus and the $PIsibs$ single locus calculations for each locus used to differentiate individuals.

Locus	No. of Alleles	$PIsibs$ single locus
G10X	10	0.4385
G10D	9	0.3876
G10L	14	0.3257
UarMU50	10	0.3635
UarMU59	7	0.4298

Population Estimate

Results of model selection based on AIC_c were very similar between the program MARK and the Lukacs and Burnham (*in press*) models (Table 3.4 and Table 3.5). The largest difference between the results was in the derived population estimates. MARK estimates were consistently larger, with the most supported model being 176 bears higher than that of the Lukacs and Burnham (*in press*) models (Table 3.4 and Table 3.5). This result was suspected because we measured a non-zero error rate within our genetic data and suspected that the MARK estimates would be inflated because of this error rate. Therefore, the remaining results and the population estimate will focus on the estimates derived from the Lukacs and Burnham (*in press*) models.

Burnham and Anderson (1998) suggest that when ΔAIC_i is less than 2 there is reasonable support for a model by the data. Two models, M_{b+hunt} and $M_{t1-3,4-5+b+hunt}$ had ΔAIC_i values less than 2 (Table 3.5). Model M_{b+hunt} appeared to have the best fit ($w_i = 0.63$) however, there was also support for model $M_{t1-3,4-5+b+hunt}$ ($w_i = 0.35$). There appears to be a behavioral response as illustrated by the strong support for both the M_{b+hunt} and $M_{t1-3,4-5+b+hunt}$ models.

Because there was support for the M_{b+hunt} , $M_{t1-3,4-5+b+hunt}$, and $M_{t+b+hunt}$ models with w_i values greater than 0, we chose to use model averaging to derive the population estimate (Buckland et al. 1997). Using this methodology we derive a population estimate of 1,880 bears (95% CI 1,454–2,306).

Table 3.4 Closed capture estimation models for adult black bears in the NLP of Michigan including model name, parameters, AIC_c , Δ_i , w_i , \hat{N} , and the 95% confidence interval. Estimates derived with closed capture estimation models in program MARK (White and Burnham 1999).

Model Name	Parameters	AIC_c^a	Δ_i^b	w_i^c	\hat{N}^d	95% CI
M_{b+hunt}	$p1-5, c2-5, p6, N$	-4124.845	0.000	0.633	2,058	1,565–2,789
$M_{l1-3,4-5+b+hunt}$	$p1-3, p4-5, c2-5, p6, N$	-4123.635	1.210	0.345	2,047	1,558–2,774
$M_{l+b+hunt}$	$p1, p2, p3, p4, p5, c2-5, p6, N$	-4118.109	6.737	0.022	2,046	1,557–2,773
M_l	$p1, p2, p3, p4, p5, p6, N$	-4043.212	81.633	0.000	1,259	1,071–1,513

^a Akaike's information criterion with an additional bias correcting term. We used AIC_c over AIC because there is no evidence of overdispersion in our data.

^b Differences in AIC_c

^c AIC_c weight, which represents the weight of evidence of one model over another. These quantities are normalized to sum to 1.

^d Population estimate

Table 3.5 Closed capture estimation models for adult black bears in the NLP of Michigan including model name, parameters, AIC_c , Δ_i , w_i , \hat{N} , and the 95% confidence interval. Estimates derived with models proposed by Lukacs and Burnham (*in press*).

Model Name	Parameters	AIC_c^a	Δ_i^b	w_i^c	\hat{N}^d	95% CI
M_{b+hunt}	P1-5, c2-5, P6, α , N	-4102.285	0.000	0.622	1,882	1,389–2,551
$M_{l1-3,4-5+b+hunt}$	P1-3, P4-5, c2-5, P6, α , N	-4101.185	1.100	0.359	1,871	1,384–2,529
$M_{l+b+hunt}$	P1, P2, P3, P4, P5, c2-5, P6, α , N	-4095.386	6.899	0.020	1,874	1,388–2,531
M_l	P1, P2, P3, P4, P5, P6, α , N	-4019.729	82.556	0.000	1,169	978–1,399

^a Akaike's information criterion with an additional bias correcting term. We used AIC_c over AIC because there is no evidence of overdispersion in our data.

^b Differences in AIC_c

^c AIC_c weight, which represents the weight of evidence of one model over another. These quantities are normalized to sum to 1.

^d Population estimate

DISCUSSION

Study Design

When estimating animal abundance it is critical to consider the spatial scale at which the species is managed. Therefore, our study design focused on the scale at which bear management decisions are made in Michigan. Other researchers conducting non-invasive DNA sampling have utilized the systematic grid design as suggested by White et al. (1982) to increase snare density and the probability of an animal encountering a trapping location (Mowat and Strobeck 2000; Poole et al. 2001; Eason et al. 2001; Boerson et al. 2003). However, all previous studies were conducted at smaller spatial scales. Mowat and Strobeck (2000) estimated grizzly bear abundance in a 9,866 km² study area in British Columbia, Canada. Boerson et al. (2003) estimated the black bear population at the 329 km² Tensas River Tract in Louisiana. Using the systematic grid design with 4 or more traps in each female bear's home range described by White et al. (1982) with our study area size of 36,848 km² would require approximately 1,125 snares. This was not feasible with available funding, personnel and private land access issues. Therefore, we used a stratified random sampling design to identify biologically relevant and accessible sampling locations. We verified our design with radio telemetry data and believe that we sampled in the proper locations.

DNA Sample Collection

Non-invasive DNA sampling with the use of hair snares is an effective means to sample bear populations. One major advantage of such a methodology is the large

sample sizes that can be obtained. We identified 165 different bears in 5 weeks of sampling. Poole et al. (2001) identified 98 different grizzly bears (*Ursus arctos*) in approximately 9 weeks of sampling. Comparable sample sizes obtained from live trapping would be hard to achieve. The MDNR live-captured and handled 126 different bears over a 10-year period from 1991–2001 in the NLP of Michigan (Etter et al. 2002). Non-invasive methods can be much more time, labor and cost effective at capturing individuals.

The new challenge with this method is to determine the best way to sub-sample hair samples if project budgets are limited. For example, we collected 1,144 hair samples and 687 had 5 or more hair follicles. Because our project budget did not allow for all hair samples to be genotyped, we developed a hair selection strategy to sub-sample our data. Boerson et al. (2003) collected 448 hair samples with greater than 10 hair follicles and choose 116 samples to derive a population estimate. Unlike other capture methods, hair snares can not, in any relative sense, be saturated. Therefore, it is possible to sample multiple individuals during a single snaring duration (i.e. that is a 7 day interval in our study). For instance, on one occasion we genotyped 7 hair samples from a single snare and detected 4 different bear genotypes. On another occasion we genotyped 14 hair samples and only detected a single genotype. Poole et al. (2001) detected as many as 7 different bears at an individual snare during a snaring session. Therefore, the researcher has the potential to impact an individual bear's probability of capture based on which hair samples are selected to be genotyped. Further work is needed to identify the optimal number of hair samples to genotype and how sub-sampling impacts population estimates.

We found the use of the harvest as an additional capture occasion to be useful for several reasons. First and most importantly, we were unable to converge on a population estimate in program MARK without harvest as a capture occasion. This can be attributed to the large sample size in which the harvest provided (approximately 22% of the estimated population). Second, we found that sufficient quantities of DNA could be extracted from bear incisors that were historically collected by the MDNR for aging bears. Excellent registration compliance by hunters (approximately 98%; Frawley 2003) and the fact that we collected DNA samples from 99.2% of the registered bears provided a large re-capture sample for our population estimate. Third, amplification success as related to the quality of DNA in teeth/tissue samples is greater than that of hair samples. We found 29 out of a possible 1,110 locus scores (2.61%) for hair samples did not amplify, while 26 out of a possible 2,080 locus scores (1.65%) for harvested bear teeth/tissue samples did not amplify. Fourth, the ability to collect both hair and tissue samples from harvested bears allowed us to quantify genotyping error, which we then used to correct the bias in our population estimate. Overall, the sampling of harvested animals, when possible, can increase sample size and data quality.

Statistical Genetic Analysis

The ability to genetically resolve unique individuals is an important aspect of non-invasive sampling. If truly unique individuals share genotypes when examined, it violates one of the assumptions of the Lukacs and Burnham (*in press*) models. Mills et al. (2000) found through simulation that truly unique, but matching genotypes biased population estimates low because fewer individuals were identified than existed in the

population. The resolution of genetic markers can be examined through the *PI* calculations. We calculated *PIsibs* single locus to be 0.00865 with a likelihood ratio of 1 in 116 of two siblings sharing the same genotype. Boerson et al. (2003) used 12 loci and estimated *PIsibs* single locus to be 0.00134, or a 1 in 745 chance of observing identical sibling genotypes.

We identified 4 out of 417 genotypes from harvested bears that matched. This suggests that we may not have used enough loci, resulting in a *PIsibs* single locus value that was not sufficiently low for our population. Our finding of matching genotypes for the 5 loci that we examined may be related to the high productivity of the NLP population of black bears. Etter et al. (2002) found that 27 of 28 radio-collared sows bred by 3 years of age and mean litter size of 76 sows was 2.6 cubs/sow. This compares to work conducted in the Upper Peninsula of Michigan where DeBruyn (1997) found the mean age of first reproduction was 4.4 years and mean litter size was 2.0–2.7 cubs/sow. Because of the high productivity of the NLP population related to younger ages at first reproduction, we might expect that the relatedness of the population is greater, resulting in greater similarity of genotypes. Therefore, future genetic identification of individuals from non-invasive sampling in the NLP of Michigan might require additional loci, or the analysis of additional loci for matching genotypes to further limit the probability of two unique bears in the population sharing identical genotypes. Overall, we believe that there is a low occurrence of matching genotypes from unique individuals. The directional bias from such matches has been demonstrated to be downward (Mills et al 2000), which provides a more conservative estimate of population size.

Quality Control and Genotyping Errors

Genotyping error has been a large focus of non-invasive methodologies because it biases population estimates. Waits and Leberg (2000) found population estimates through simulation to be biased on the magnitude of 2 times larger than true population size with a genotyping error rate of 0.05/locus. We found that the quality control protocols proposed by Paetkau (2003) substantially decreased our individual genotype misidentification rate. This can be attributed to culling of samples that did not produce confident scores, conservative scoring, and the identification of genotypes that are similar (1-MM and 2-MM sample identification). Goosens et al. (1998) documented an error rate of 4.86% per locus after extractions of 3 hair follicles and using a multi-tubes approach. This compares to our mean error rate of 4.4% per locus before the quality control protocols, but is greater than our final genotyping rate per locus of 1.1% after quality control protocols.

We found the use of hair and tissue samples from harvested bears as very beneficial for examining genotyping errors. Others have used two sources of DNA from the same individual to examine genotyping errors (Parsons 2001; Sloane et al. 2000). Interspersing error testing samples within the data increased our ability to identify potential loading errors which can result in numerous samples being misidentified. However, we still identified a non-zero probability of genotyping errors. Two of the 4 remaining errors were associated with errors that differed by more than two alleles and could potentially be corrected if we choose to examine samples that differed by 3 and 4 alleles. The time required to execute the quality control protocols was extensive, but decreased the number of genotyping errors. Because of our non-zero error rate, we

suggest using the quality control protocols in conjunction with a methodology to account for remaining errors within the estimate.

Population Estimate

Biased population estimates caused by genotyping error have raised suspicion about the efficacy of non-invasive sampling to estimate wildlife population abundance (Creel et al. 2003). This bias occurs because the capture-recapture assumptions of correct identification of individuals and tag loss are violated. Is the violation of marking assumptions with non-invasive methodologies a new phenomena, or have they been present all along with other marking methods, but too difficult to quantify? Stevick et al. (2001) examined the occurrence of natural tag misreads in a humpback whale (*Megaptera novaeangliae*) capture-recapture experiment and found an error rate of 0.125 with sub-premium photo quality. The ability to quantify and examine tag misreads is one advantage of non-invasive methodologies because it provides the opportunity to correct for assumption violation in the estimate. The effectiveness of the non-invasive technique with respect to large samples sizes and field logistics cannot be argued, but genotyping errors must be accounted for.

Lukacs and Burnham (*in press*) propose models that account for genotyping errors in the population estimate. They liken these models to other models that have been created to deal with assumption violations, such as those created by Otis et al. (1978), Huggins (1991) and Pledger (2000). One assumption that remains for all closed capture-recapture models is geographic and demographic population closure. Movement data collected by the MDNR from 1991–2001 from 126 radio-collared black bears did not

document a single bear movement outside of our study area (D. Etter, MDNR, person. comm. 2004). Therefore, it is valid to assume that Lakes Michigan and Huron to the north, east and west and areas devoid of bears to the south provide geographic population closure. In addition, survival rates during the summer months were estimated between 96% and 99% for adult and sub-adult males and females (Etter et al. 2002). Based on these findings, we feel that we met the assumption of demographic population closure.

For comparison purposes, we derived population estimates that accounted for genotyping error with the Lukacs and Burnham (*in press*) models and estimates that do not account for genotyping error in program MARK (White and Burnham 1999). We found that the AIC_c ranking of the models to be consistent between program MARK and Lukacs and Burnham (*in press*) models (Table 3.4 and Table 3.5). We found the model averaged estimate from program MARK to be 2,054 bears (95% CI 1,453–2,655). This estimate was expected to be greater than the averaged estimate obtained from the Lukacs and Burnham (*in press*) models because we violated the marking assumptions of the capture-recapture models in program MARK, and this violation has shown to overestimate population size (Waits and Leberg 2000). Thus, we feel that the estimates from the Lukacs and Burnham (*in press*) models are conservative.

Overall, the estimate of 1,880 bears (95% CI 1,454–2,306) is consistent, but slightly higher than present MDNR population models (D. Etter, MDNR, person. comm. 2004). Because we used the harvest as a recapture method, this estimate reflects the number of adult and sub-adult black bears before the 2003 hunting season. This estimate excludes the cub portion of the population because cubs can not be legally harvested and were excluded from sampling based on our hair snare design.

MANAGEMENT IMPLICATIONS

Historically, the MDNR monitored the bear population in the NLP of Michigan with a bait index. The bait index provided managers with information about the trend in the population, but did not provide estimates of population size. In addition to the bait index, management decisions were based on an empirical population model, which accounts for age- and sex-specific survival and reproduction (Garshelis and Snow 1988). The MDNR conducted a 10-year research study to collect demographic information to parameterize the population model (Etter et al. 2002). The critical parameter that was missing in the model was an initial estimate of population size. Our results can be used in the MDNR population model to aid in making decisions about future bear management.

We found great success in using harvested bears as an additional capture occasion. Utilization of this information drastically increased our sample size and allowed us to estimate genotyping errors. Collection of samples was relatively simple and proper personnel training ensured success. We found that tooth samples, that were already being taken systematically to determine age, contained adequate quantities of DNA for genotyping and can obviate collecting alternative sources of DNA (e.g. muscle tissue).

Quality control protocols proposed by Paetkau (2003) drastically reduced the amount of genotyping error in our data. The use of these protocols in conjunction with the estimation models proposed by Lukacs and Burnham (*in press*), can provide unbiased estimates of population abundance when using non-invasive techniques.

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CHAPTER 4 – PRECISION AND BIAS: CHALLENGES POSED BY NON-INVASIVE DNA SAMPLING TO ESTIMATE BLACK BEAR (*URSUS AMERICANUS*) ABUNDANCE IN THE NORTHERN LOWER PENINSULA OF MICHIGAN

INTRODUCTION

As public participation increases in the management of wildlife and the abundance of declining species becomes of greater concern, accurate abundance estimates become essential for the conservation and management planning. The use of non-invasive techniques to estimate population abundance for rare and secretive species has become a very popular and effective method to meet management needs. The non-invasive sampling of black bear (*Ursus americanus*) and brown bear (*Ursus arctos*) for purposes of abundance estimation has gained popularity in recent years. One basic methodology uses a barbed wire hair snare to collect hair samples from bears at baited locations (Woods et al. 1999). Non-invasive sampling has shown great promise in increasing sample sizes because the negative stimulus of handling is eliminated (Woods et al. 1999). In addition, more stations can be operated and study designs be spatially based because the animal is not captured in the snare (Mowat and Strobeck 2000). However, even though the non-invasive methods for sampling bears show great promise, non-invasive methods also pose new challenges. In particular, estimating the bias caused by genotyping error and changes in precision caused by sub-sampling samples must be rigorously tested.

Genotyping Error

Since recent advancements in DNA technology have provided a means to efficiently and effectively estimate the population size of large free ranging animal populations using a capture-recapture methodology, their application has been increasing for both conservation and management purposes (Boerson et al. 2003; Woods et al. 1999; Mowat and Strobeck 2000; Palsboll et al. 1997; Sloane et al. 2000). However, with the rise in popularity of non-invasive techniques utilizing hair and fecal samples as a DNA source, researchers have identified potential drawbacks, including genotyping errors, which bias population estimates (Creel et al. 2003; Waits and Leburg 2000). Research examining the cause of genotyping errors found that they result from a number of factors including the quality and quantity of the DNA (Gagneux et al. 1997). Genotyping errors result in the misidentification of individuals within the population and violate two capture-recapture assumptions: 1) the correct identification of individuals and 2) individuals do not lose their marks (White et al. 1982).

Paetkau (2003) proposed quality control lab protocols to decrease genotyping error. These protocols include development of a database around the field database, conservative genotype scoring, culling of samples with incomplete or contaminated genotypes, re-analysis of samples with questionable scores, and careful scrutiny of samples with similar genotypes for the detection of genotyping errors. These quality control methods have shown to substantially decrease genotyping error and provide reliable genotypes. However, no work has been conducted to evaluate population estimate bias attributed to using, or not using these protocols.

Sub-sampling

Non-invasive sampling methods can often result in large numbers of potential DNA samples. For example, Mowat and Strobeck (2000) collected 4,245 hair samples from black bear (*Ursus americanus*) and grizzly bears (*Ursus arctos*) in British Columbia, Canada. It is well known that multiple bears can visit a snaring location during a sampling interval. Poole et al. (2001) detected as many as 7 different bears during a 12-day snaring session. Conversely, it is possible to detect the same individual when multiple hair samples are genetically analyzed. We detected the same individual black bear when we genotyped 14 hair samples from the same site in Michigan. Precision of capture-recapture population estimates is a function of the number of individuals sampled within the population, or the probability of capture (Otis et al. 1978). The finding of multiple hair samples from the same individual does not increase the information for the population estimate. Rather it increases the cost of the project. Conversely, if multiple bears are detected when multiple hair samples are genotyped, the precision of the population estimate will increase. Overall, the researcher can influence an individual's probability of capture and the precision of an estimate if not all samples are analyzed (i.e. sub-sampling DNA samples). The affect of sub-sampling hair samples must be better explored and through this exploration better decisions can be made about the number of hair samples to sub-sample.

Non-invasive Research

In the summer of 2002 we began a research project in the northern Lower Peninsula (NLP) of Michigan to estimate black bear abundance. Because this black bear

population is managed by the MDNR through regulated harvest, it was important that we obtain an unbiased and precise population estimate. However, given the concerns about bias caused by genotyping error and a limited project budget that did not allow us to genotype all hair samples, we wanted to examine through simulation the affects of quality control methods proposed by Paetkau (2003) and sub-sampling hair samples on population estimates.

METHODS

Genotyping Error Simulation

Successful hunters are required to register all harvested bears and compliance is estimated at 98% (L. Visser, MDNR, person. comm.; Frawley 2002). We collected muscle tissue and hair samples from harvested bears to examine rates of genotyping error. We assigned different identification numbers to hair and tissue samples from the same bear and extracted DNA from hair samples with 5 or more hair follicles 7 or more days after collection. We then used the quality control protocols proposed by Paetkau (2003) for both error examination and genetic analysis for purposes of population estimation. First, we developed a database around the field database to minimize the probability of transcriptional errors associated with data entry. Second, we genotyped 110 hair and 110 tissue samples (1 each from 110 different bears) at 5 microsatellite loci including G10X, G10L, G10D (Paetkau et al. 1995), UarMU59, and UarMU50 (Taberlet et al. 1997). Two experienced lab personnel conservatively scored genotypes and unassigned scores were left blank in the database. Third, we conducted an initial cull of samples that did not have scores for 3 or more loci from our error analysis. Fourth, we

attempted to obtain a genotype using a second round of PCR for remaining samples that did not have scores assigned. Fifth, we culled samples that did not have scores for 4 or more loci. We then made our first evaluation of error by matching the remaining hair and tissue samples from the same bear. We matched 95 sample pairs (15 samples were culled) and found 19 out of 95 (20%) pairs differed and we calculated the error rate per allele for all loci. We found the mean error rate across all loci to be 0.029/allele.

We then went through the mismatching protocols described by Paetkau (2003), which involves the identification of samples that differ by 1 and 2 alleles. We identified mismatches and re-examined and/or reanalyzed samples to verify scores. We again examined error after the mismatching procedures by matching hair and tissue samples from the same bear and identifying inconsistencies. We found that 4 of the 95 (4.21%) sample pairs differed and thus the error rate per allele for all loci decreased. The mean error rate across all loci decreased to 0.008/ allele.

Genotyping Error Simulation Model

To examine the affects of different rates of genotyping error, we randomly created a virtual population of 2,000 genotypes from the allele frequencies at 5 loci (G10X, G10L, G10D, UarMU59, and UarMU50) obtained from 544 bears in the NLP of Michigan (Table 4.1). We calculated the *PIsibs* single locus (Taberlet and Luikart 1999) to be 0.00865 (n = 544) across all 5 loci. We found 10 of the randomly created genotypes matched other genotypes for all 5 loci in the virtual population of 2,000 and included these matching genotypes in the simulation to examine the affect of matching genotypes on the population estimates.

Table 4.1. Allele and allele frequencies for 5 loci including G10X, G10L, G10D, UarMU50, and UarMU59 calculated from 544 bears in the northern lower peninsula of Michigan.

Locus													
G10X			G10D			G10L			UarMU50			UarMU59	
Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency
132	0.000931099	172	0.078413284	120	0.0028463	116	0.001915709	112	0.160516605				
146	0.51396648	174	0.02398524	122	0.000948767	118	0.158045977	114	0.007380074				
148	0.088454376	176	0.402214022	136	0.045540797	120	0.216475096	116	0.000922509				
152	0.017690875	178	0.008302583	138	0.132827324	122	0.116858238	120	0.480627306				
154	0.154562384	180	0.044280443	140	0.045540797	124	0.310344828	122	0.126383764				
158	0.061452514	182	0.098708487	144	0.028462998	130	0.000957854	124	0.105166052				
160	0.000931099	184	0.1900369	150	0.042694497	132	0.026819923	126	0.11900369				
162	0.106145251	186	0.131918819	152	0.196394687	134	0.030651341	-	-				
164	0.002793296	188	0.022140221	154	0.205882353	138	0.036398467	-	-				
166	0.051210428	-	-	156	0.113851992	140	0.101532567	-	-				
168	0.001862197	-	-	158	0.083491461	-	-	-	-				
-	-	-	-	160	0.001897533	-	-	-	-				
-	-	-	-	162	0.098671727	-	-	-	-				
-	-	-	-	164	0.000948767	-	-	-	-				

We wrote the simulation in SAS (PROC IML; SAS Inc. 2002), whereby we randomly sampled our virtual population with a capture probability of 0.10 on each of 5 sampling occasions with the assignment of a uniform random number to each genotype on each occasion. Genotypes were captured on each occasion if the random number was less than or equal to the capture probability (0.10). To introduce genotyping errors into the captured genotypes, we assigned each allele in the captured genotypes a uniform random number. If the random number was less than or equal to the pre-specified error rate (0.029/ allele or 0.008/ allele) for the particular simulation, the allele was randomly replaced by a different possible allele for the locus using an additional uniform random number routine. Genotypes with and without errors were then matched across all occasions and capture histories formed for purposes of population estimation. We estimated population size using closed capture estimation models similar to models in program CAPTURE (Rexstad and Burnham 1992), but coded into the SAS environment (P. Lukacs, person. comm. 2004). We used the M_0 model because our probability of capture did not change through time (Otis et al. 1978).

We simulated each scenario of genotyping error rate (0.0/ allele, 0.029/ allele, and 0.008/ allele) 1,000 times and examined the mean, 95% confidence intervals, and estimate bias across all simulations. To examine bias, we calculated the percent relative bias (PRB; White et al. 1982):

$$PRB = \frac{\left(E(\hat{N}) - N \right)}{N} \times 100$$

where $E(\hat{N})$ is the mean estimated population size across all replications and N is the true population size, which in our simulation was 2,000.

Sub-sampling Simulation

To simulate the affects of sub-sampling, we first had to examine the incidence of multiple bears visiting a hair snare during a single checking interval. To estimate this we randomly choose 49 checking occasions where multiple hair samples were available from our hair snaring efforts in 2002 and 2003. We genotyped all hair samples (N=210) collected from the 49 occasions. The total number of hair samples genotyped for both years was 210. We then derived the probability of multiple bears visiting a site given the number of hair samples genotyped at that site (Table 4.2).

We summarized the frequency of the number of hair samples collected from our 2003 hair sample collection data by snare location (N=239) and the number of hair samples collected on each occasion (1-5 hair sampling occasions were sampled in 2003). From these data, we derived the distribution of hair samples collected across all snaring checks and locations (Table 4.2).

Sub-sampling Hair Samples Simulation Model

To examine the affects of sub-sampling hair samples we used SAS (SAS Inc. 2002) to write a bootstrapping simulation that first simulated the deposition of hair samples on a predetermined number of snaring locations and number of checking occasions, second allowed for the specification of the number of hair samples to genetically identify, and third estimated population size for each scenario of the number of hair samples genotyped.

To simulate the number of hair samples deposited on our virtual number of snares we used the frequency of the number of hair samples collected on each occasion (Table 4.2; collected from our 2003 field data) in a bootstrapping routine for 239 snares and 5

Table 4.2. Probability (%) of the number of unique animals determined for the number of hair samples observed for the given number of sites and the summary of the number of hair samples collected from 239 hair snaring locations across 5 sampling occasions (1,195 possible sampling occasions). Probabilities derived from genotyping 210 hair samples from 49 snare checking occasions.

No. of Hair Samples	Prob. of the No. of Unique Animals					No. of Sites For Prob.	Frequency (No. of Sites)	Relative Frequency	Cumulative Frequency
	1	2	3	4	5				
0	-	-	-	-	-	0	950	0.795	0.795
1	1	-	-	-	-	11	88	0.0736	0.8686
2	0.375	0.625	-	-	-	8	60	0.0502	0.9188
3	0.333	0.333	0.333	-	-	6	43	0.036	0.9548
4	0	0.5	0.5	0	-	4	17	0.0142	0.969
5	0.429	0.143	0.286	0.142	0	7	13	0.0109	0.9799
6	0	0	0.5	0.5	0	2	5	0.0042	0.9841
7	0	0.5	0	0.25	0.25	4	6	0.005	0.9891
8	1	0	0	0	0	1	4	0.0033	0.9925
9	0	0	1	0	0	2	3	0.0025	0.995
10+	0.5	0	0.5	0	0	4	6	0.005	1
Totals	---	---	---	---	---	49	1195	1	---

sampling occasions. We randomly assigned each hair sample an identification number from a virtual population of 2,000 individuals using the probability of multiple bears visiting a site given the number of hair samples genotyped (Table 4.2). We then could specify how many different hair samples were genotyped (e.g. if we specified that 1 hair sample was genotyped, then one individual per site was randomly selected, even if multiple individuals were available to be sampled). We then matched all identification numbers across the 5 occasions and 239 snaring locations to form capture histories for the purposes of population estimation. We used closed capture models written in SAS to estimate population size (P. Lukacs, person. comm. 2004). We used the M_t model because our probability of capture changed through time as a result of different numbers of individuals being randomly sampled (Otis et al. 1978). Each selection of hair samples (1, 2, 3, 4, 5, 6, and all hair samples) was simulated 1,000 times and we examined the mean and 95% confidence intervals for each scenario. In addition we plotted the standard error of the estimates to examine how precision of the estimate changes with the number of hair samples selected.

RESULTS

Genotyping Error Simulations

Mean estimated population size ($\bar{x}=1,992$) was slightly less than the true population size of 2,000 when genotyping error was 0.0/allele (Figure 4.1 and Table 4.3). Mean estimated population size was positively biased with increasing error rate. With an error rate of 0.029/allele, the simulated estimate was more than 50% larger ($N=3,214$) than the true population size ($N=2,000$; Figure 4.1 and Table 4.3). We found that the

confidence intervals around the simulated population estimates were small and attributed to the high probability of capture ($p=0.10$) used in the simulation (Figure 4.1). The standard error of the estimates increased as a result of an increase in genotyping error rate.

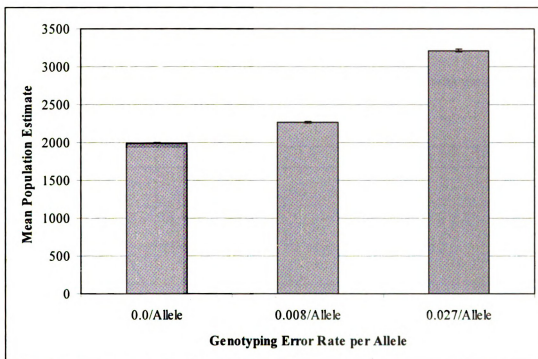


Figure 4.1. Mean estimated population size and associated 95% confidence intervals across 1,000 simulations for genotyping error rates, 0.0/allele, 0.008/allele, and 0.029/allele.

Table 4.3. Mean population size estimate, standard error of the estimates, and percent relative bias for each genotyping error rate across 1,000 simulations.

Genotyping Error Rate	Mean Population Estimate	Estimate Standard Error	Percent Relative Bias
0.0/Allele	1992	3.795	-0.39%
0.008/Allele	2269	4.820	13.46%
0.027/Allele	3214	8.615	60.70%

Sub-sampling simulations

Standard error of the estimate decreased with increasing number of hair samples genotyped (Figure 4.2 and Table 4.4). The standard error of the estimates decreased by approximately 50% by the selection of 3 hair samples ($SE=12.724$) over the selection of 1 hair sample ($SE=30.405$; Table 4.4). The selection of more than 3 hair samples did not substantially decrease the standard error of the estimates. For example, the standard error with the selection of 3 hair samples was 12.724 and the standard error with the selection of all hair samples was 12.256 (Table 4.4).

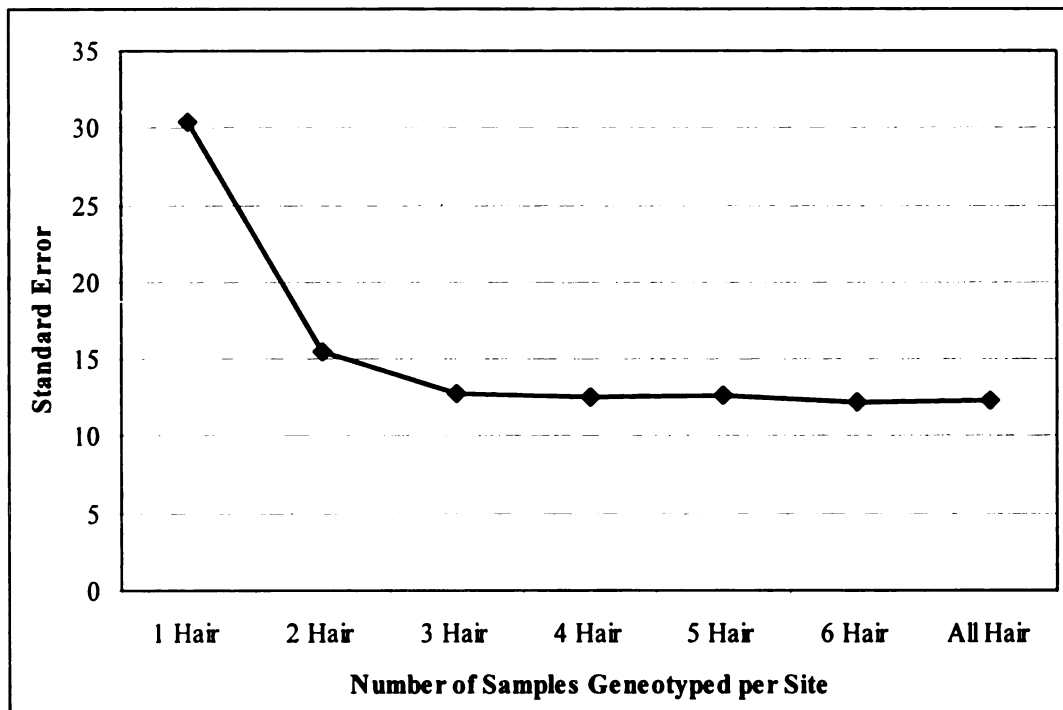


Figure 4.2. Standard error of the estimate resulting from the number of individual hair samples chosen to be genotyped across 1000 simulated data sets.

Table 4.4. Number of hair samples genotyped per site, the mean of the 1,000 population estimate replicates and the resulting standard error of the estimates.

No. of Samples Genotyped	Population Estimate	
	Mean	Standard Error
1	2209	30.405
2	2101	15.436
3	2060	12.724
4	2050	12.492
5	2060	12.636
6	2055	12.225
All	2055	12.256

DISCUSSION

Genotyping Error

Genotyping error has caused concern about the efficacy of non-invasive methods because it has the potential to bias population estimates (Creel et al. 2003). This bias is caused by genotyping errors which add new genotypes that do not exist in the population. Creel et al. (2003) found that genotyping errors overestimated wolf population size in Yellowstone by 5.5 times when using feces as a DNA source. Waits and Leberg (2000) found through simulation that population estimates were > 2 times larger when error rates were 0.05/ locus. Genetic marks differ in some aspects to that of more traditional marking techniques. One difference is in error identifiability. For example, if marks are placed and recorded on animals in the population and recaptures do not match marks that were not put into the population, the identification of an error is made. However, a genotype with an error can be extremely difficult to identify because a flawed genotype could be a possible genotype in the population.

Others have proposed methods to limit the amount of genotyping error. Taberlet et al. (1996) proposed the multi-tubes approach that involves the dilution and genetic

analysis of a DNA in multiple “tubes” to obtain reliable genotype scores. One problem with the multi-tube approach identified by Paetkau (2003) is that it can lead to increased cost to conduct such an analysis. Additionally, we found that the limited amount of DNA extracted from DNA sources such as hair, feathers or feces could also be a limiting factor when using multiple loci.

The basis behind the quality control protocols proposed by Paetkau (2003) is that not all genotypes need to be examined for potential errors. There may be genotypes that are drastically different from all other genotypes and the probability of error at multiple loci is low, therefore even if an error occurred in one of these genotypes it would have little consequence to the population estimate. Using these protocols, including sample culling and the identification of mismatching samples, we were able to drastically reduce the amount of genotyping error within our data. The overall affect of this reduction was a decrease in the bias of our population estimate as demonstrated through simulation.

However, we did not decrease the amount of genotyping error to zero and it maybe unnecessary to do so. Numerous statistical models have been developed to deal with violation of assumptions in capture-recapture estimation models (Otis et al. 1978; Huggins 1991; Pledger 2000). Currently, program CAPTURE and the closed capture models in program MARK require the assumptions that animals are identified correctly and they do not loose their marks (White et al. 1982; White and Burnham 1999). Others have been interested in tag-misreads in the application to marine mammals (Stevick et al. 2001). Lukacs and Burnham (*in press*) propose closed capture models that estimate and account for misidentification of individuals within the estimation models. The effectiveness and efficiency of non-invasive methods to collect large sample sizes of

secretive animals will ensure that such methods will increase in importance as a wildlife management tool. Therefore, we must find ways to account for potential bias caused by genotyping error. Quality control protocols proposed by Paetkau (2003) can decrease the amount of genotyping error. The estimation models by Lukacs and Burnham (*in press*) can provide unbiased estimates of population abundance accounting for genotyping error within the estimate.

An additional source of bias can exist if too few microsatellite markers are used to resolve individuals, as found with our simulations. We randomly created a population of 2,000 genotypes with allele frequencies of the 5 loci that we empirically estimated in our population estimation research in the NLP of Michigan (Table 4.1). We calculated $PIsibs_{\text{single locus}}$ across all loci with our estimation data to be 0.00865 ($n = 544$). After we created the random genotypes, we found 10 of the 2,000 genotypes matched for all 5 loci. The overall affect of these matching genotypes within the population is the downward bias in population estimates as demonstrated through our simulations. These findings are consistent with those found by Mills et al. (2000), where the downward bias increases with increasing duplication of genotypes. The overall bias caused by matching genotypes is less than the bias caused by genotyping errors and can be resolved by including additional loci in the genetic analysis.

Sub-sampling

As wildlife research budgets become increasingly limited, resource allocation becomes an important issue. The precision of a population estimate using a capture-recapture methodology is a function of the number of individuals marked in the

population reflected in the probability of capture (White et al. 1982). Given a limited research budget to genetically analyze all hair samples collected, decisions about how many hair samples to genotype should be based on the identification of unique individuals and an attempt to limit the number of duplicate genotypes. Boerson et al. (2003) collected 448 hair samples with > 10 hair follicles and choose 116 samples to derive a population estimate. Additionally, when genotyping error is present in the genetic analysis, the analysis of additional samples can increase the overall bias in the estimate. Waits and Leberg (2000) found through simulation that increasing sampling intensity resulted in increasingly biased estimates even with low rates of genotyping errors.

We propose using a simulation model to add rigor in making decisions about the number of hair samples to genotype. We found that the genetic analysis of 3 hair samples, when multiple hair samples were present, significantly decreased the standard error of the estimates. However, genotyping more than three hair samples did not significantly decrease the standard error of the estimates. These findings may be consistent with other non-invasive sampling methodologies where multiple samples are collected from the same sampling location and suggest that not all samples must be genetically analyzed.

In our simulations, we consistently overestimated the true population size. We attribute this positive bias to having very low probability of capture values. As the probability of capture values increased by sampling additional hair samples, this bias also decreased (Table 4.4). However, the general conclusions of the simulation still hold. Not

all samples must be genetically analyzed from a collection site to obtain a stabilization of estimate standard error.

Precision and bias are two very important aspects of a capture-recapture experiment (White et al. 1982). Concerns about bias attributed to genotyping error are extremely relevant and should be considered. Quality control protocols and new models that consider misidentification show great promise in obtaining reliable estimate of wildlife population through non-invasive methods (Paetkau 2003; Lukacs and Burnham *in press*). Non-invasive methods continue to be effective at sampling populations to a point where the researcher is required to make decisions about sub-sampling data. We offer insight on these difficult decisions and propose that precise estimates do not require that all DNA samples be genotyped.

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CHAPTER 5 – RECOMMENDATIONS TO THE MDNR

The overall goal of our research was to obtain a population size estimate (with confidence intervals) of black bears in the Northern Lower Peninsula (NLP) of Michigan. This information would assist the MDNR in the management of the species. We were successful in achieving an estimate, but it was not without struggle because of the large study area (36,848 km²), logistics of organizing field staff, and the recent development of non-invasive techniques. We learned a great many things while conducting this research, which can contribute to the future use of this technique. The objective of this chapter is to provide the MDNR with recommendations and estimates of resources needed to successfully execute this technique based on our observations and experiences.

Hair Snare Placement

Based on the large study area with mixed land ownership and heterogeneous bear habitat, the random stratified design was most suitable for hair snare placement (Chapter 3). We identified 239 hair snare locations using this design (Appendix 2.4). These locations were chosen at random and from the MDNR bait index locations. Because the frequency of snares was based on frequency of harvest grouped by township over a 5-year interval, it is important that the harvest frequency coverage be updated prior to the evaluation of snare locations to identify new townships where harvest was occurring.

One of the strata in our stratified random design included potential bear habitat. An evaluation of the overlap between the vegetation types and radio-telemetry data collected in the months of June and July by the MDNR (Etter et al. 2002; Chapter 2) showed that the northern hardwood vegetation class had a large number of locations

(17.17%). The northern hardwood vegetation class was not included in our stratification, but it should be considered in future stratifications.

In some situations, after all the stratifications were made, there was no sampling area left in townships where snares were to be placed. In some instances this was because of the lack of public land within the area. In particular, this occurred in the high harvest area of northern Alcona county and southern Alpena county (i.e. “club country”). In these instances, it will be important to make contact with private landowners to obtain permission for access.

Hair Snaring

Because of the large study area and the large number of snares required to obtain a population estimate, this methodology required a large number of personnel. We met these personnel needs through a multi-agency and organization effort (Appendix 2.3 and 2.4). We suggest a training session be held prior to the field collection of hair samples. This will ensure consistency of the technique in situations where personnel turnover is occurring. These training sessions should include an overview of the protocols for both snare set-up, checking of snares, and the collection and storage of hair samples (Refer to Appendix 5.1 and Appendix 5.2 for a copy of protocols and a list of supplies necessary for hair sample collection).

We found that the number of snares that a two-person crew could set in a day depended on the distance and travel time between snares. Generally, 6–10 snares could be set in 8-hours. We recommend that detailed information about the location of the snare and driving directions be documented on a standardized data collection form (refer

to Appendix 5.3 for a copy of our set-up form). Obtaining a Global Positioning System (GPS) location for each snare is also recommended.

We used a variety of baits during the 2002 field season including combinations of liquid scents, chicken, sausage, and bacon. Based on our findings we recommend that baits consist of 1-pound bacon and anise extract contained in a film canister. We recommend that baits be changed every two-weeks if the bait was not taken by a bear. Replenishing the bait helps to add fresh scent.

It was critical that collection envelopes were pre-numbered with unique numbers to ensure that samples were not confused. To expedite the sorting of hair samples in the lab, it was critical that the quantification of the number of hairs present be made at the collection. The categories that we used in 2003 included: 1-4, 5-10, 11-15 and > 15 hairs (refer to Appendix 5.4 for an example of the collection envelope). In addition, hair juxtaposition on the wire should be documented to assist in calculating the probability of hair samples collected on adjacent barbs being from the same bear. If these probabilities are of little value, this information could be deleted from the form (refer to Appendix 5.5 for a copy of the checking form).

In 2002 we set hair snares with a single strand of barbed wire and in 2003 we set snares with 2 strands of barbed wire. The double-wire hair snare was used in 2003 to try to obtain better hair samples (i.e. more hair follicles). It was difficult to determine if the single or the double wire was more successful at collecting better quality hair samples because we did not set this up as an experiment. In general, field staff showed preference towards a single wire because some believed that two wires alarmed bears resulting in a non-visitation. Future research might consist of setting two snares in close proximity,

one with a single wire, and a second with two wires. Comparisons could then be made between the effectiveness, reflected in hair quality, of one and two stands of barbed wire.

The number of sampling occasions was an important aspect of this research because it relates both to the precision of the estimate and also to the valuable time of field personnel. To gain insight into the number of sampling occasions, we examined the occurrence of new genotypes across the 5 hair snaring occasions in 2003. On average, we identified approximately 33 new individuals per week of sampling (range = 30–37). The largest number of new individuals was 37 identified in the fifth week of sampling in 2003 (Table 5.1).

Table 5.1. Number of new bears and number of previously identified bears for each sampling occasion conducted in the summer of 2003.

Sampling Occasion	No. of New Bears Identified
1	34
2	30
3	31
4	31
5	37

These data would suggest that increasing the number of sampling occasions resulted in the increased identification of new individual bears. Identifying more bears increases the precision of the estimate as demonstrated from our simulations conducted in Chapter 4. If the MDNR desires to decrease the number of sampling occasions (e.g. from 5 occasions to 4 occasions), further work should be conducted to examine the impact of such a decision with regards to population estimate precision.

Hunter Harvested Hair and Tissue Collection

In both 2002 and 2003 we had great success collecting muscle tissue samples from harvested bears. We attribute this success in part, to holding training sessions where collection protocols (Appendix 5.6) were reviewed. We recommend that these training sessions continue. The MDNR has established protocols for collecting premolars from all harvested bears for cementum ageing. Cementum ageing is conducted at Rose Lake Wildlife Disease Laboratory. In the process of collecting all bear registration materials for the MDNR, we experimented with extracting DNA from tooth samples. Extracted teeth typically contain small portions of tissue, blood and tooth pulp. We had great success (99.3%) in obtaining DNA from tooth samples and based on this success, it may not be necessary to collect muscle tissue samples from harvested bears. However, in the cases where tooth samples cannot be obtained, it will still be necessary to collect muscle tissue samples as a source of DNA. The hair sample collected from each harvested bear was used to access genotyping error and should continue to be collected. It was critical that all collection envelopes be pre-numbered individually with a consistent number for each collection set.

Sample Selection

We had greater than anticipated success at collecting hair samples from hair snares in both years (Chapter 2). This success required the development of sub-sampling protocols because of our limited budget to genotype hair samples (Chapter 3). We examined the affect of this sub-sampling on population estimates and determined that the standard error of the estimates can be drastically reduced if a maximum of three hair

samples (each individual barb equals one sample) are genotyped at a location (Chapter 4). Therefore, we recommend that future sub-sampling protocols include randomly selecting only three hair samples when 3 or more hair samples are present at a snare.

In both years, we genotyped all harvested bear muscle tissue samples. One reason that we sub-sampled hair samples was because there was a given probability that a single bear deposited hair on multiple barbs and the genetic analysis of all the hair samples would duplicate genotypes (Chapter 4). This duplication would be redundant for estimation purposes and thus waste resources that could be used to identify new genotypes. All harvested bears were assumed to be unique. Therefore, duplication was not an issue. In addition, the sample size of harvested bears was much greater than the number of different bear identified through the summer collected hair samples. We recommend that all the harvested bear samples continue to be genotyped in the future.

Genetic Analysis

We changed our genetic analysis methods between 2002 and 2003. The result of these changes was an increase in genotype amplification success from DNA samples collected in 2003 as compared to samples collected in 2002 (Table 5.2). We believe the difference in amplification success was related to three major changes. First, we extracted hair samples within approximately 7 days after their collection. Samples collected in 2002 were extracted as many as 6 months after their collection. Roon et al. (2003) demonstrated that amplification success was related to the time between collection and extraction, with longer time periods decreasing amplification success. We suggest that the short duration (7 days) between collection and extraction continue.

Table 5.2. Summary of the number of possible alleles, number of observed alleles, and the percentage of these observed alleles, for each locus used in the 2002 and 2003 hair and tissue genetic analysis.

Year 2002					Year 2003				
Locus	No. of Possible Alleles	No. of Observed Alleles	Percent Observed		Locus	No. of Possible Alleles	No. of Observed Alleles	Percent Observed	
G10X	1922	1322	68.78%		G10X	2052	1666	81.19%	
G10M	1922	1156	60.15%		G10D	2052	1734	84.50%	
G10D	1922	1420	73.88%		G10L	2052	1644	80.12%	
G10L	1922	1289	67.07%		UarMU50	2052	1684	82.07%	
G10B	1922	1272	66.18%		UarMU59	2052	1748	85.19%	
Total	9610	6459	67.21%		Total	10260	8476	82.61%	

Second, we only extracted hair samples with ≥ 5 hair follicles in 2003. In 2002 we extracted any hair sample, regardless of the number of hair follicles. Goosens et al. (1998) found that genotyping error was a function of the number of hair samples in the extraction. Error rates were greater with fewer follicles. Further work needs to be done to examine whether even better genotypes could be obtained by choosing hair samples with greater numbers of follicles (e.g. only choose samples with ≥ 10 follicles) and the impact of such protocol changes on the number of bears detected.

Third, we used quality control protocols proposed by Paetkau (2003) to increase genotyping success and reduce error (Chapter 3). We explored the affect of these protocols on estimate bias and discovered that the mismatching procedures drastically decreased estimate bias. We recommend these protocols be used in the future, but more work must be conducted to examine if a more stringent culling protocol will further reduce genotyping error.

In 2003 we found that 4 of the harvested bear genotypes matched. This finding may suggest that we used too few loci to resolve individuals because all harvested individuals are assumed to be unique. Future work may consider adding additional loci, or at least the analysis of all matching genotypes with an additional locus. The overall bias of matching genotypes of unique individuals within the population estimate was negative and we found through simulation that this bias was very slight (Chapter 4, it underestimated the population on average by 8 individuals).

Population Estimate

The effect of genotyping error on population estimates was very dramatic with positively biased population estimates (Chapter 4). Even with quality control protocols proposed by Paetkau (2003), we still had a non-zero error rate within our genetic data. Therefore, we recommend the estimation models proposed by Lukacs and Burnham (*in press*) to account for any remaining genotyping error. It is possible that these models will be incorporated in program MARK in the near future and thus easily accessed (P. Lukacs, Colorado State University, person. comm.).

Closed capture models in program MARK or the Lukacs and Burnham (*in press*) models allow the user to build models using a design matrix (White and Burnham 1999). The design matrix allows the user to account for a behavioral response, a time response, or heterogeneity in capture, for each capture occasion within the data. We found the flexibility of the design matrix to be critical when using harvest as a different capture method than that of summer collected hair samples. Any number of models can be developed, but a reasonable set of four models should be created *a priori* based on the biology and field knowledge of bears in the NLP. We found a strong behavioral response in our data, which might be expected when we use bait that offers a food reward. We did not find as strong a response related to time, but this factor should always be considered in building models in the future. Another advantage of program MARK is the ability to use individual covariates (White and Burnham 1999). We used a sexing primer for the DNA samples collected in 2003. We used sex as a covariate in the estimation models and found that these models were not substantially better than the models without sex. In

the future, we recommend that a sexing primer continue to be used because they can possibly contribute to the estimation models.

Personnel and Cost Summary

The amount of personnel hours and cost of this project is important for assessing its utility. Therefore, we summarized cost and personnel hours of the project for the MDNR's consideration. We conducted work in both the summers of 2002 and 2003. We used the summer of 2002 as a learning year to improve our methods and better understand what such research would require. We used the data collected summer/fall of 2003 to generate our population estimate using what we had learned. Therefore, our cost and personnel summary reflects the summer/fall of 2003 because it better represents what the MDNR will need to invest to reproduce this methodology.

Personnel Summary

We divided the project into a series of tasks and sub-tasks to examine the personnel hours (Appendix 5.6). The task that required the greatest number of personnel hours was the set-up and checking of the hair snares, with 2,800 estimated hours (Appendix 5.6). The task that required the least amount of time was the identification of hair snaring locations, estimated at 40 hours (Appendix 5.6). Overall, the estimated personnel hours were approximately 5,110 hours (Table 5.3). This summary includes the hours of personnel from a variety of agencies and organizations.

Cost Summary

In addition to the time of personnel, there are a variety of monetary costs required to execute this methodology. We summarized these expenses as if they were above and

Table 5.3. Summary of the personnel hours to perform the necessary tasks as reflected in the portion conducted in 2003/2004 of this project.

Task and sub-task (indented portion) to be performed	Time Required (hours)
Snare location identification	40
Summarize frequency of harvest by township	
Update or change stratifications	
Choose snaring locations	
Organize field supplies and distribute	80
Stamp envelopes	
Purchase supplies	
Make copies of field forms	
Organize supplies	
Hold training session	
Set-up and check hair snares	2,800
Set-up snares	
Check snares	
Collect hair samples from all routes and take to MSU	100
Collection of hair, tooth, and tissue from harvested bears	70
Sort and extract DNA samples for genetic analysis	320
Sort hair samples by number of follicles	
Extract DNA from hair follicles	
Extract DNA from tissue samples	
Genetic analysis of DNA samples	1,600
Organization of samples	
Preparation of database	
Initial genetic analysis	
Re-analysis to fill non-amplifications	
Verification of genotype scores	
Identification and re-analysis of mismatched samples	
Data analysis and population estimate derivation	100
Identify the genotyping error rate	
Summarize data	
Create capture histories	
Generate population estimate	
Total	5,110

beyond the cost that the MDNR would incur, if they duplicated the research themselves and did not use the services of a graduate student. Therefore, the labor associated with the MDNR doing the work was not included in this summary. We divided these costs into a number of expenses including, field supplies, harvest collection supplies, and the labor and supplies for the genetic analysis. The largest expense was the labor (estimated time of 1,600 hours) for the genetic analysis estimated at \$23,205 (Table 5.4). The estimated overall total cost of supplies and genetic analysis was \$38,945 (Table 5.4).

Table 5.4. Approximate cost for each expense for the 2003/2004 portion of the project.

Expense	Cost
Field Supplies	
Barbed wire, staples, tools, nails, flagging, etc.	\$500.00
Bait (Bacon and Anise)	\$1,372.00
Harvest Collection Supplies	\$100.00
Genetic Analysis	
Labor	\$23,205.00
Laboratory supplies	\$15,740.00
Total	\$40,917.00

To place this cost in perspective we examined the laboratory cost (supplies and labor) to identify a single bear based on our results from 2003. We identified 544 different bears from the genetic analysis of 1,026 DNA samples. Using the number of bears identified and the laboratory cost, we estimated the genetic cost to identify a single bear in 2003 to be \$71.59/bear identified.

Comparison to Current Methods and Further Work

Currently, the MDNR makes decisions about bear management in the NLP based on a population model that is verified by a bait index. The bait index does not provide an

estimate of population size, but provides a trend in the population. One of the major advantages of the bait index is that it is inexpensive to operate. However, population models can only be as accurate as the information used in their creation and no previous estimates have been derived in the NLP of Michigan. Therefore, the bait index is an inexpensive methodology, but it did not address the needs of the population model to have an initial population size estimate.

One limiting factor of the non-invasive methodology was the cost and personnel requirements. In the future the MDNR may consider using the non-invasive hair snares as a bait index. Having this methodology established would allow the MDNR to collect hair samples on a given interval to estimate abundance. This estimate would verify the index and the population model and provide an estimate of abundance. For example, the MDNR could continue to operate the 239 hair snares across the NLP and document the number of hair samples on the wire across a series of checking occasions. Instead of genotyping the hair samples, the number of hair samples could be used as an index using our data about the number of unique bears found when we genotyped all hair samples from a snare (Table 4.2). On a three-year interval, the hair samples could be collected and genetically analyzed to estimate population size. Further work would need to be conducted to establish the hair snares as a bait index, but the basic methodology has already been identified through our research.

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APPENDICES

Appendix 2.1. 1993 GAP Land Stewardship land cover/ land use categories and definitions.

Land Cover/Use Category	Land Cover/Use Definition
High Intensity Urban	Greater than 50% solid impervious cover of man-made materials, and rural improved highway surfaces with a right-of-way sufficiently clear of vegetation so that the surface is distinguishable on Thematic Mapper data.
Low Intensity Urban	Urban areas with less than 50% solid impervious cover of man-made materials. It may also contain some interspersed vegetation. Areas meeting the requirements of both urban and forested classes were included in the urban category - e.g., residential subdivision with mature tree canopy.
Orchard/Vineyard	Includes cherry and apple orchards as well as grape vineyards.
Herbaceous Openland	Non-cultivated herbaceous vegetation predominated by grasses, grass-like plants or forbs.
Shrubland	Predominated by woody vegetation, generally with several basal shoots, that generally do not exceed 20 feet in height.
Other Broad-Leaved Deciduous Forest	Upland forest with crown closure at least 40%; where deciduous species dominate the canopy; does not fall into any other more specific upland deciduous class.
Northern Hardwood	Upland forest with crown closure at least 40%; where the following species predominate or are intermixed - sugar maple, red maple, beech, and where maple or beech is a significant portion (>25%) of the canopy.

Appendix 2.1. Cont'd.

Land Cover/Use Category	Land Cover/Use Definition
Northern Hardwood/Conifer	Upland forest with crown closure at least 40%; northern hardwood forest intermixed with some conifers - e.g., northern hardwood with hemlock or northern hardwood with white or red pine.
Aspen/Birch	Upland forest with crown closure at least 40%; where aspen, white birch, and related species dominate the forest cover; includes aspen and/or white birch intermixed with pine or oak species.
Oak	Upland forest with crown closure at least 40%; where oak species dominate the forest canopy.
Oak/Jack Pine	Upland forest with crown closure at least 40%; where oak is intermixed with jack pine, and oak is predominant with jack pine component > 20% of crown closure.
Other Coniferous Forest	Upland forest with crown closure at least 40%; where coniferous species dominate the canopy; does not fall into any other more specific upland coniferous class.
White Pine	Upland forest with crown closure at least 40%; where white pine dominates the forest canopy.
Red Pine	Upland forest with crown closure at least 40%; where red pine dominates the forest canopy.
Upland Jack Pine	Upland forest with crown closure at least 40%; where jack pine dominates the forest canopy.

Appendix 2.1. Cont'd.

Land Cover/Use Category	Land Cover/Use Definition
Cedar/Spruce/Fir	Upland forest with crown closure at least 40%; where a mix of white cedar, white spruce, and balsam fir dominate the forest canopy.
Emergent Wetland/Wet Meadow	An area with water at, near, or above the land surface long enough to be capable of supporting aquatic or hydrophytic vegetation, and which has soils indicative of wet conditions; with persistent and non-persistent herbaceous plants standing above the surface of the water or soil.
Other Lowland Shrub	An area with water at, near, or above the land surface long enough to be capable of supporting aquatic or hydrophytic vegetation, and which has soils indicative of wet conditions; dominated by woody vegetation less than 20 feet tall and tree cover less than 40%; does not fall into any othermore specific lowland shrub class.
Lowland Broad-Leaved Deciduous Shrub	An area with water at, near, or above the land surface long enough to be capable of supporting aquatic or hydrophytic vegetation, and which has soils indicative of wet conditions; dominated by deciduous shrub species such as willow and alder.
Lowland Broad-Leaved Evergreen Shrub	An area with water at, near, or above the land surface long enough to be capable of supporting aquatic or hydrophytic vegetation, and which has soils indicative of wet conditions; dominated by broad-leaved evergreen shrub species such as leather-leaf and bog rosemary.

Appendix 2.1. Cont'd.

Land Cover/Use Category	Land Cover/Use Definition
Other Forested Wetland	An area with water at, near, or above the land surface long enough to be capable of supporting aquatic or hydrophytic vegetation, and which has soils indicative of wet conditions; dominated by woody perennial plants, with a canopy cover of greater than 40%, and trees reaching a mature height of at least 6 feet; does not fall into any other more specific forested wetland class.
Mixed Lowland Hardwood	An area with water at, near, or above the land surface long enough to be capable of supporting aquatic or hydrophytic vegetation, and which has soils indicative of wet conditions; where a mix of species such as elm, ash, maple and balsam poplar combine to dominate the canopy.
Lowland Jack Pine	An area with water at, near, or above the land surface long enough to be capable of supporting aquatic or hydrophytic vegetation, and which has soils indicative of wet conditions; where jack pine dominates the canopy.
Black Spruce	An area with water at, near, or above the land surface long enough to be capable of supporting aquatic or hydrophytic vegetation, and which has soils indicative of wet conditions; where black spruce dominates the canopy.
Northern White Cedar	An area with water at, near, or above the land surface long enough to be capable of supporting aquatic or hydrophytic vegetation, and which has soils indicative of wet conditions; where northern white cedar dominates the canopy.

Appendix 2.1. Cont'd.

Land Cover/Use Category	Land Cover/Use Definition
Mixed Lowland Conifer/Hardwood	An area with water at, near, or above the land surface long enough to be capable of supporting aquatic or hydrophytic vegetation, and which has soils indicative of wet conditions; where a mix of lowland conifer and hardwood dominates the canopy.
Barren Land	Land in which less than 1/3 of the area has vegetation or other cover - e.g., sand, bare soil, exposed rock. Agricultural and Wetland classes take priority if an area meets requirements for either of these and the Barren Land class.
Water	Areas of water with no vegetation present - e.g., lakes, reservoirs, rivers, ponds.
Urban Grassland	Greenspace in or near urban areas, typified by areas of closely cut lawns with shrubs or trees, such as parks, golf courses, or large lawns.
Lowland Needle-Leaved Evergreen Shrub	An area with water at, near, or above the land surface long enough to be capable of supporting aquatic or hydrophytic vegetation, and which has soils indicative of wet conditions; dominated by coniferous tree species that are permanently stunted to a height of less than 20 feet due to site conditions.

Appendix 2.2. 2000 GAP Land Stewardship land cover/ Land use categories and definitions

Land Cover/Use Category	Land Cover/Use Definition
Airports	Impervious land within airport grounds, including runways.
Road/Parking Lot	Roads or parking lots.
High Intensity Urban	Land area greater than 25% solid impervious cover made from man-made materials, other than airports, roads, or parking lots
Low Intensity Urban	Land area is greater than 10% and less than 25% man-made structures including paved and gravel roads and parking lots.
Non-vegetated Farmland	Land area tilled for crop production with less than 25% currently vegetated
Row crops	Vegetation consists of annual crops planted in rows (e.g. corn, soybeans)
Forage Crops/ Non-tilled herbaceous agriculture	Vegetation used for fodder production (e.g. alfalfa, hay). Also includes land used for pasture, or non-tilled herbaceous agriculture
Orchards/Vineyards/Nursery	Woody trees not grown for Christmas trees.
Parks/Golf Courses	Maintained for recreational purposes
Upland Shrub/Low Density Trees	The combination of woody shrubs and tree canopy (woody cover) covers more than 25% of the land area

Appendix 2.2. Cont'd.

Land Cover/Use Category	Land Cover/Use Definition
Herbaceous Openland	Less than 25% of land area consists of woody cover
Northern Hardwood Association	Combination of Maples, Beech, Basswood, White Ash, Cherry, Yellow Birch exceeds 60% of the canopy
Oak Association	Proportion of Oaks exceeds 60% of the canopy
Aspen Association	Proportion of Aspen exceeds 40% of the canopy
Other Upland Deciduous	Proportion of any other single species exceeds 60% of the canopy
Mixed Upland Deciduous	Proportion of deciduous trees exceeds 60% of the canopy
Pines	Proportion of pines exceeds 60% of the canopy
Other Upland Conifers	Proportion of non-pine upland conifers exceeds 60% of the canopy
Mixed Upland Conifers	Proportion of coniferous trees exceeds 60% of the canopy
Upland Mixed Forest	Mixed forest not falling into any other category. Proportion of conifers to deciduous ranges from 40%:60% to 60%:40%
Water	Proportion of open water exceeds 75% of land area

Appendix 2.2. Cont'd.

Land Cover/Use Category	Land Cover/Use Definition
Lowland Deciduous Forest	Proportion of deciduous trees exceeds 60% of the canopy
Lowland Coniferous Forest	Proportion of coniferous trees exceeds 60% of the canopy
Lowland Mixed Forest	Mixed forest not falling into any other category. Proportion of conifers to deciduous ranges from 40%:60% to 60%:40%
Floating Aquatic	Proportion of floating aquatic vegetation exceeds 60% of non-water cover
Lowland Shrub	Proportion of lowland shrub exceeds 60% of non-water cover
Emergent Wetland	Proportion of emergent vegetation exceeds 60% of non-water cover
Mixed Non-forest Wetland	Non-forested wetlands not falling into any other category
Sand/Soil	Land cover is formed primarily of sand or bare soil
Exposed Rock	Land cover is formed of solid rock
Mud Flats	If periodically flooded

Appendix 2.3. List of hair snare name, year, UTM location, number of visitations by bears, number of hair samples collected and the county that the hair snare was located for each management organization operating the snares.
Little Traverse Bay Bands of Odawa Indians

Snare Site Name	Year	UTM Location			No. Visits	No. Hair		County
		Zone	Easting	Northing		Samples Collected		
A1	2002	16	671871	5067066	1	2	Emmet	
A3	2002	16	662501	5064008	1	2	Emmet	
A4	2002	16	657395	5057483	2	2	Emmet	
A5	2002	16	667176	5053866	2	5	Emmet	
A6	2002	16	673906	5052667	2	6	Emmet	
A7	2002	16	661866	5046456	2	3	Emmet	
A8	2002	16	659374	5044485	5	16	Emmet	
A9	2002	16	665996	5044122	0	0	Emmet	
B1	2002	16	680029	5065597	2	2	Cheboygan	
B2	2002	16	685394	5061388	4	5	Cheboygan	
B3	2002	16	677712	5056017	0	0	Cheboygan	
Totals					21	43		

Appendix 2.3. Cont'd.
Department of Natural Resources- Mio Field Office

Snare Site Name	Year	UTM Location			No. Visits	No. Hair		County
		Zone	Eastings	Northing		Samples	Collected	
alcona_oscodaA10	2002	16	652970	4952948	0	0	0	Oscoda
alcona_oscodaB1	2002	16	752953	4952994	0	0	0	Alcona
alcona_oscodaB10	2002	16	746470	4953374	1	1	1	Oscoda
alcona_oscodaC1	2002	16	740861	4954720	0	0	0	Oscoda
alcona_oscodaC3	2002	16	746297	4958115	3	13	13	Oscoda
alcona_oscodaC5	2002	16	740859	4954731	0	0	0	Oscoda
alcona_oscodaC9	2002	16	740317	4943866	0	0	0	Oscoda
alcona1	2002	16	750063	4948398	0	0	0	Alcona
alcona11	2002	16	759688	4944247	0	0	0	Alcona
alcona30	2002	16	765638	4951822	1	1	1	Alcona
alcona32	2002	16	765172	49560361	7	59	59	Alcona
alcona34	2002	16	762991	4954828	7	35	35	Alcona
alcona36	2002	16	760193	4951388	8	49	49	Alcona
alcona5	2002	16	754170	4945758	5	15	15	Alcona
Totals					32		173	

Appendix 2.3. Cont'd.
Department of Natural Resources- Cadillac Field Office

Snare Site Name	Year	UTM Location			No. Visits	No. Hair		County
		Zone	Easting	Northing		Samples	Collected	
Q1	2002	16	637036	4927613	0	0		Missaukee
Q2	2002	16	644555	4928056	1	3		Missaukee
Q3	2002	16	658479	4929136	6	21		Missaukee
Q4	2002	16	661157	4920659	0	0		Missaukee
Q5	2002	16	651079	4916371	2	9		Missaukee
Q6	2002	16	657132	4915500	5	12		Missaukee
Q7	2002	16	649619	4909567	2	2		Missaukee
K1	2002	16	656861	4948248	4	22		Kalkaska
K2	2002	16	656455	4940944	1	2		Kalkaska
K3	2002	16	651203	4936434	2	3		Kalkaska
K4	2002	16	655880	4936018	0	0		Kalkaska
K5	2002	16	659262	4930908	0	0		Kalkaska
wexford0	2002	16	607304	4895200	8	43		Wexford
wexford11	2002	16	622080	4894146	4	6		Wexford
wexford12	2002	16	617329	4891124	2	4		Wexford
wexford2	2002	16	617925	4898599	5	22		Wexford
wexford53	2002	16	613780	4892945	3	10		Wexford
wexford70	2002	16	607304	4895200	2	6		Wexford
Totals					47		165	

Appendix 2.3. Cont'd.

Department of Natural Resources-Gladwin Field Office

Snare Site Name	Year	Zone	UTM Location		No. Visits	No. Hair		County
			Eastings	Northing		Samples Collected		
clare27	2002	16	656611	4867069	0	0		Clare
clare45	2002	16	682860	4886608	0	0		Clare
X1	2002	16	709181	4887694	2	4		Gladwin
X2	2002	16	713419	4886942	0	0		Gladwin
Totals					2	4		

Department of Natural Resources-Bay City Field Office

Snare Site Name	Year	Zone	UTM Location		No. Visits	No. Hair		County
			Eastings	Northing		Samples Collected		
R1	2002	16	703036	4910994	3	10		Roscommon
R2	2002	16	698587	4903048	0	0		Roscommon
R3	2002	16	707011	4900420	0	0		Roscommon
S4	2002	16	711257	4919336	0	0		Ogemaw
Totals					3	10		

Appendix 2.3. Cont'd.

Department of Natural Resources- Atlanta Field Office

Snare Site Name	Year	UTM Location			No. Visits	No. Hair		County
		Zone	Eastings	Northings		Samples Collected	Count	
clubs09BL06	2002	16	1	1	7	27	Alpena	Alpena
clubs19DC03	2002	16	751002	4973260	5	23	Alpena	Alpena
clubs19ST03	2002	16	760638	4979746	0	0	Alpena	Alpena
clubs26ST10	2002	16	763966	4973851	0	0	Alpena	Alpena
se_macksfF01	2002	16	717679	5013498	0	0	Presque Isle	Presque Isle
se_macksfF07	2002	16	725653	5015861	0	0	Presque Isle	Presque Isle
se_macksfG01	2002	16	728823	5003988	0	0	Montmorency	Montmorency
se_macksfG03	2002	16	728270	5006366	1	2	Montmorency	Montmorency
se_macksfG05	2002	16	726619	5007842	4	15	Montmorency	Montmorency
se_macksfH04	2002	16	723808	4999956	0	0	Montmorency	Montmorency
se_macksfI01	2002	16	720732	4992505	0	0	Montmorency	Montmorency
se_macksfI10	2002	16	727500	4991047	3	3	Montmorency	Montmorency
se_macksfJ07	2002	16	710243	4994022	2	10	Montmorency	Montmorency
se_macksfJ10	2002	16	711225	4990781	0	0	Montmorency	Montmorency
Turtle Lak04	2002	16	740530	4973437	6	53	Montmorency	Montmorency
Turtle Lak06	2002	16	741838	4371595	5	32	Montmorency	Montmorency
Turtle Lak08	2002	16	742088	4970420	7	28	Oscoda	Oscoda
Turtle Lak10	2002	16	743895	4969211	6	32	Oscoda	Oscoda
Turtle Lak11	2002	16	748872	4975069	3	8	Alpena	Alpena
Turtle Lak12	2002	16	748705	4978003	0	0	Alpena	Alpena
Totals					49	233		

Appendix 2.3. Cont'd.

Department of Natural Resources- Houghton Lake Field Office

Snare Site Name	Year	UTM Location			No. Visits	No. Hair		County
		Zone	Easting	Northing		Samples Collected		
se_rosca03	2002	16	668070	4908362	0	0		Missaukee
se_rosca10	2002	16	677706	4895377	1	4		Roscommon
se_roscaB06	2002	16	677643	4895329	0	0		Roscommon
se_roscaC05	2002	16	693107	4906596	0	0		Roscommon
se_roscaC08	2002	16	696292	4904098	0	0		Roscommon
se_roscaD03	2002	16	694490	4913174	1	1		Roscommon
se_roscaD10	2002	16	694578	4913105	0	0		Roscommon
se_roscaE10	2002	16	708580	4925204	0	0		Roscommon
Totals					2	5		

Department of Natural Resources- Traverse City Field Office

Snare Site Name	Year	UTM Location			No. Visits	No. Hair		County
		Zone	Easting	Northing		Samples Collected		
GT01	2002	16	627280	4946966	0	0		Grand Traverse
GT02	2002	16	628022	4933671	0	0		Grand Traverse
GT03	2002	16	620681	4930328	1	1		Grand Traverse
GT04	2002	16	608079	4930450	0	0		Grand Traverse
GT05	2002	16	611007	4942683	1	1		Grand Traverse
GT06	2002	16	594327	4951919	0	0		Grand Traverse
Totals					2	2		

Appendix 2.3. Cont'd.
Department of Natural Resources- Baldwin Field Office

Snare Site Name	Year	UTM Location			No. Hair		County
		Zone	Easting	Northing	No. Visits	Samples Collected	
LAKE.13	2002	16	598997	4872901	1	3	Lake
LAKE.17	2002	16	599817	4876775	2	3	Lake
LAKE.3	2002	16	596686	4861995	0	0	Lake
LAKE.30	2002	16	600529	4885534	0	0	Lake
LAKE.33	2002	16	598494	4885816	0	0	Lake
LAKE.34	2002	16	600511	4887938	3	12	Lake
LAKE.7	2002	16	599347	4864283	0	0	Lake
Totals					6	18	

Appendix 2.3. Cont'd.
Department of Natural Resources- Gaylord Field Office

Snare Site Name	Year	UTM Location			No. Visits	No. Hair		County
		Zone	Easting	Northing		Samples	Collected	
nw_macksf10E	2002	16	710182	5018239	1	2		Cheboygan
nw_macksf3E	2002	16	707351	5015691	2	2		Cheboygan
nw_macksf4B	2002	16	703694	5004892	1	1		Otsego
nw_macksf4C	2002	16	697584	5012600	0	0		Cheboygan
nw_macksf4D	2002	16	702274	5019308	1	1		Cheboygan
nw_macksf5A	2002	16	701817	4996026	0	0		Otsego
nw_macksf7A	2002	16	703748	4993949	3	7		Otsego
nw_macksf7E	2002	16	711700	5014325	2	2		Cheboygan
nw_macksf9A	2002	16	705674	4995877	3	10		Otsego
nw_macksf9B	2002	16	699215	5000116	0	0		Otsego
Totals					13		25	

Appendix 2.3. Cont'd.
Department of Natural Resources- Roscommon Field Office

Snare Site Name	Year	Zone	UTM Location			No. Visits	No. Hair		County
			Eastng	Northing			Samples Collected		
nw_rosca10	2002	16	686659	4918302		0	0		Roscommon
nw_roscaO6	2002	16	689918	4923714		0	0		Roscommon
nw_rosccO3	2002	16	674870	4922597		1	1		Roscommon
nw_roscaD10	2002	16	665694	4928717		1	5		Missaukee
nw_roscaDO1	2002	16	670022	4926910		0	0		Missaukee
nw_roscaE10	2002	16	668632	4919545		2	8		Missaukee
nw_roscaEO1	2002	16	667850	4923272		0	0		Missaukee
nw_roscaEO4	2002	16	664875	4921120		2	10		Missaukee
Totals						6	24		

Appendix 2.3. Cont'd.

United States Forest Service - Mio Ranger Station

Snare Site Name	Year	UTM Location			No. Hair		County
		Zone	Easting	Northing	No. Visits	Samples Collected	
mio_usfsStation 18	2002	16	704408	4946156	2	11	Crawford
mio_usfsStation 30	2002	16	709934	4947053	0	0	Oscoda
mio_usfsStation 40	2002	16	714788	4947620	1	1	Oscoda
mio_usfsStation 58	2002	16	715116	4934209	0	0	Oscoda
mio_usfsStation 92	2002	16	702487	4937208	6	33	Crawford
Totals					9	45	

The Grand Traverse Band of Ottawa and Chippewa Indians

Snare Site Name	Year	UTM Location			No. Hair		County
		Zone	Easting	Northing	No. Visits	Samples Collected	
BE07	2002	16	592556	4935162	6	26	Benzie
BE08	2002	16	592935	4936331	1	1	Benzie
BE09	2002	16	588568	4938361	2	3	Benzie
BE10	2002	16	579124	4934501	1	4	Benzie
BE11	2002	16	578421	4952888	0	0	Benzie
BE12	2002	16	594327	4949012	0	0	Benzie
Totals					10	34	

Appendix 2.3. Cont'd.
Little River Band of Ottawa Indians

Snare Site Name	Year	UTM Location			No. Visits	No. Hair		County
		Zone	Eastings	Northings		Samples	Collected	
manis_mason402	2002	16	575639	4893210	0	0	0	Manistee
manis_mason408	2002	16	583368	4887111	0	0	0	Lake
manis_mason410	2002	16	586871	4893176	0	0	0	Manistee
manis_mason412A	2002	16	577301	4894021	0	0	0	Manistee
manis_mason412B	2002	16	578637	4893772	0	0	0	Manistee
manis_mason503	2002	16	565307	4892750	0	0	0	Manistee
manis_mason805	2002	16	573153	4900765	0	0	0	Manistee
manis_mason810	2002	16	574631	4901948	1	2	2	Manistee
manis_mason813	2002	16	577161	4900680	0	0	0	Manistee
Totals					1		2	

Appendix 2.3. Cont'd.
Michigan State University

Snare Site Name	Year	UTM Location			No. Visits	No. Hair		County
		Zone	Easting	Northing		Samples	Collected	
B10	2002	16	709874	5030242	1	1		Cheboygan
B12	2002	16	681382	5027403	1	2		Cheboygan
B13	2002	16	690081	5023774	3	8		Cheboygan
B14	2002	16	680648	5014281	6	44		Cheboygan
B15	2002	16	692696	5015480	2	5		Cheboygan
B4	2002	16	706456	5049154	3	12		Cheboygan
B5	2002	16	711102	5048731	3	9		Cheboygan
B6	2002	16	697229	5037764	0	0		Cheboygan
B7	2002	16	703812	5037879	0	0		Cheboygan
B8	2002	16	705565	5035056	2	2		Cheboygan
B9	2002	16	709680	5036872	0	0		Cheboygan
Birch1A	2002	16	279881	4966038	7	56		Alpena
Birch2	2002	16	280368	4967650	4	8		Alpena
C1	2002	16	719413	5039957	1	2		Presque Isle
C11	2002	16	268780	5011041	0	0		Presque Isle
C12	2002	16	275375	5015056	0	0		Presque Isle
C13	2002	16	279749	5011219	1	4		Presque Isle
C14	2002	16	294907	5020129	3	11		Presque Isle
C15	2002	16	297772	5018534	1	4		Presque Isle
C2	2002	16	723270	5040164	1	2		Presque Isle

Appendix 2.3. Cont'd.
Michigan State University

Snare Site Name	Year	UTM Location			No. Visits	No. Hair		County
		Zone	Eastings	Northings		Samples	Collected	
C3	2002	16	733946	5040268	0	0		Presque Isle
C4	2002	16	733566	5037560	1	5		Presque Isle
C5A	2002	16	271936	5037531	7	76		Presque Isle
C7	2002	16	728327	5033163	1	3		Presque Isle
C8	2002	16	732526	5024768	0	0		Presque Isle
C9	2002	16	729878	5019679	2	8		Presque Isle
D1	2002	16	665470	5014350	7	21		Charlevoix
D2	2002	16	670963	5014076	0	0		Charlevoix
D3	2002	16	671474	5008280	3	8		Charlevoix
F1	2002	16	696057	5007206	0	0		Otsego
F2	2002	16	697392	4997907	3	20		Otsego
F3	2002	16	703801	4980461	0	0		Otsego
F4	2002	16	696450	4974117	0	0		Otsego
F5	2002	16	690921	4974462	1	1		Otsego
G1	2002	16	712760	4980961	4	18		Montmorency
G2A	2002	16	729407	4992606	2	5		Montmorency
G3A	2002	16	735404	4995447	2	6		Montmorency
G4A	2002	17	266067	4991612	2	19		Montmorency
G5A	2002	17	264339	4983966	5	31		Montmorency
G6	2002	16	726032	4981300	1	7		Montmorency

Appendix 2.3. Cont'd.
Michigan State University

Snare Site Name	Year	UTM Location			No. Visits	No. Hair		County
		Zone	Eastings	Northing		Samples Collected		
G7	2002	16	729358	4975737	3	17		Montmorency
G8	2002	16	725438	4972209	0	0		Montmorency
G9	2002	16	733065	4972199	1	1		Montmorency
H10	2002	17	313344	4970975	0	0		Alpena
H3	2002	17	279122	5000813	3	20		Alpena
H4	2002	17	291871	4996765	5	10		Alpena
H5A	2002	17	294617	4995746	2	5		Alpena
H7	2002	17	301645	4989879	1	4		Alpena
H8	2002	17	304916	4982946	0	0		Alpena
H9	2002	17	305968	4980057	0	0		Alpena
Hastings1	2002	17	283389	4967643	7	32		Alpena
L1	2002	16	687578	4957131	5	17		Crawford
L2	2002	16	702270	4967145	0	0		Crawford
M1	2002	16	708272	4968994	0	0		Oscoda
M2	2002	16	711510	4960966	1	1		Oscoda
M3	2002	16	727577	4960153	2	7		Oscoda
N4	2002	17	313549	4961597	0	0		Alcona
N6A	2002	17	295526	4954290	4	13		Alcona
N7	2002	17	289304	4933883	0	0		Alcona
N8A	2002	17	299678	4935608	5	16		Alcona

Appendix 2.3. Cont'd.
Michigan State University

Snare Site Name	Year	UTM Location			No. Visits	No. Hair		County
		Zone	Easting	Northing		Samples	Collected	
N9	2002	17	309206	4932450	0	0		Alcona
S1	2002	17	264493	4933193	2	4		Ogemaw
S2	2002	17	262917	4925509	3	13		Ogemaw
S3A	2002	17	268983	4926609	1	2		Ogemaw
T1	2002	17	279644	4930265	0	0		Iosco
T2	2002	17	288937	4927368	0	0		Iosco
T3	2002	17	296081	4924976	0	0		Iosco
Totals					85		385	

Appendix 2.4. List of hair snare name, year, UTM location, number of visitations by bears, number of hair samples collected and the county that the hair snare was located for each management organization operating the snares.
Department of Natural Resources - Mio Field Office

Snare Site Name	Year	UTM Location			No. Hair		County
		Zone	Easting	Northing	No. Hits	Samples Collected	
alcona_oscoda10	2003	16	742765	4939155	0	0	Oscoda
alcona_oscodaB1	2003	16	752953	4953162	2	8	Alcona
alcona_oscodaB10	2003	16	746548	4953604	1	1	Oscoda
alcona_oscodaC1	2003	16	740832	4954945	3	7	Oscoda
alcona_oscodaC3	2003	16	742179	4953082	0	0	Oscoda
alcona_oscodaC5	2003	16	741950	4949447	0	0	Oscoda
alcona_oscodaC6	2003	16	741700	4947636	0	0	Oscoda
alcona1	2003	16	750063	4948398	2	4	Alcona
alcona11	2003	16	759688	4944247	0	0	Alcona
alcona30	2003	16	765638	4951822	5	32	Alcona
alcona32	2003	16	765172	4956036	3	6	Alcona
alcona34	2003	16	762991	4954828	3	8	Alcona
alcona36	2003	16	760193	4951388	0	0	Alcona
alcona5	2003	16	754170	4945758	1	2	Alcona
Totals					14	52	

Appendix 2.4. Cont'd.

Department fo Natural Resources - Atlanta Field Office

Snare Site Name	Year	UTM Location			No. Hits	No. Hair Samples Collected	County
		Zone	Easting	Northing			
clubs09BL06	2003	16	750374	4981598	5	59	Alpena
clubs17ST01	2003	16	756559	4981053	2	2	Alpena
clubs19DC03	2003	16	751000	4973489	5	15	Alpena
clubs24ST08	2003	16	761698	4975420	0	0	Alpena
se_macksfF01	2003	16	717673	5013722	1	5	Preque Isle
se_macksfF07	2003	16	725643	5016104	1	2	Preque Isle
se_macksfG01	2003	16	728822	5004182	0	0	Montmorency
se_macksfG03	2003	16	728240	5006597	3	9	Montmorency
se_macksfG05	2003	16	726622	5008063	0	0	Montmorency
se_macksfH04	2003	16	723776	5000198	0	0	Montmorency
se_macksfI01	2003	16	720829	4992765	0	0	Montmorency
se_macksfI10	2003	16	727464	4991308	0	0	Montmorency
se_macksfJ07	2003	16	710265	4994209	0	0	Montmorency
se_macksfJ10	2003	16	711255	4990984	3	4	Montmorency
Turtle_Lak04	2003	16	740527	4973655	5	38	Montmorency
Turtle_Lak06	2003	16	741839	4971802	5	27	Montmorency
Turtle_Lak08	2003	16	742081	4970632	4	8	Oscoda
Turtle_Lak10	2003	16	743891	4969433	5	31	Oscoda
Turtle_Lak11	2003	16	748873	4975287	0	0	Alpena
Turtle_Lak12	2003	16	748704	4978206	4	41	Alpena
Totals					43	241	

Appendix 2.4. Cont'd.
Department fo Natural Resources - Houghton Lake Field Office

Snare Site Name	Year	Zone	UTM Location		No. Hits	No. Hair		County
			Eastng	Northing		Samples Collected		
se_roscA02	2003	16	671535	4903109	0	0		Roscommon
se_roscA10	2003	16	677691	4895570	0	0		Roscommon
se_roscB06	2003	16	683851	4900364	0	0		Roscommon
se_roscC05	2003	16	693085	4906789	0	0		Roscommon
se_roscC08	2003	16	696387	4904297	0	0		Roscommon
se_roscD03	2003	16	694545	4913280	0	0		Roscommon
se_roscD09	2003	16	698308	4921243	1	4		Roscommon
se_roscE10	2003	16	699886	4921769	0	0		Roscommon
se_roscHL WRS	2003	16	668670	4908592	0	0		Missaukee
Totals					1	4		

Appendix 2.4. Cont'd.

Department of Natural Resources - Baldwin Field Office

Snare Site Name	Year	UTM Location			No. Hits	No. Hair		County
		Zone	Easting	Northing		Samples Collected		
LAKE.13	2003	16	598997	4872901	0	0		Lake
LAKE.17	2003	16	599817	4876775	0	0		Lake
LAKE.30	2003	16	600529	4885534	0	0		Lake
LAKE.33	2003	16	598494	4885816	1	1		Lake
LAKE.34	2003	16	600511	4887938	3	9		Lake
LAKE.45	2003	16	600952	4870184	0	0		Lake
LAKE.7	2003	16	599347	4864283	3	8		Lake
Totals					7	18		

United States Forest Service - Baldwin Ranger Station

Snare Site Name	Year	UTM Location			No. Hits	No. Hair		County
		Zone	Easting	Northing		Samples Collected		
newaygo26	2003	16	603579	4857624	1	1		Lake
newaygo36	2003	16	600600	4845550	0	0		Newaygo
newaygo4	2003	16	586454	4851345	0	0		Newaygo
newaygo43	2003	16	602644	4839783	0	0		Newaygo
newaygo48	2003	16	607376	4825012	0	0		Newaygo
newaygoB23	2003	16	589911	4830641	0	0		Newaygo
newaygoNN	2003	16	588090	4845143	0	0		Newaygo
Totals					1	1		

Appendix 2.4. Cont'd.
Department of Natural Resources - Cadillac Field Office

Snare Site Name	Year	UTM Location				No. Hair		County
		Zone	Easting	Northing	No. Hits	Samples Collected		
K1	2003	16	656863	4948265	3	6	Kalkaska	
K2	2003	16	656992	4940966	2	8	Kalkaska	
K3	2003	16	651161	4936359	0	0	Kalkaska	
K4	2003	16	655897	4936015	2	7	Kalkaska	
K5	2003	16	662817	4944900	4	6	Kalkaska	
Q1	2003	16	634005	4923791	0	0	Missaukee	
Q2	2003	16	639568	4927130	1	1	Missaukee	
Q3	2003	16	658487	4929176	2	5	Missaukee	
Q4	2003	16	661340	4920010	1	1	Missaukee	
Q5	2003	16	651050	4916151	2	10	Missaukee	
Q6	2003	16	657131	4915506	4	27	Missaukee	
Q7	2003	16	633569	4909314	1	2	Missaukee	
wexford0	2003	16	616292	4902094	5	50	Wexford	
wexford11	2003	16	620756	4892553	4	12	Wexford	
wexford12	2003	16	617426	4891849	3	8	Wexford	
wexford2	2003	16	617753	4898543	4	7	Wexford	
wexford4	2003	16	617419	4899392	4	11	Wexford	
wexford5	2003	16	617706	4899864	5	13	Wexford	
wexford53	2003	16	613630	4893970	1	3	Wexford	
wexford70	2003	16	606318	4892359	2	8	Wexford	
Totals					50	185		

Appendix 2.4. Cont'd.
Department of Natural Resources - Gaylord Field Office

Snare Site Name	Year	Zone	UTM Location		No. Hits	No. Hair		County
			Easting	Northing		Samples Collected		
nw_macksf1E	2003	16	703802	5014806	0	0		Cheboygan
nw_macksf3E	2003	16	707351	5015691	0	0		Cheboygan
nw_macksf4B	2003	16	703694	5004892	0	0		Otsego
nw_macksf4C	2003	16	697584	5012600	0	0		Cheboygan
nw_macksf4D	2003	16	702274	5019308	0	0		Cheboygan
nw_macksf5A	2003	16	701817	4996026	1	1		Otsego
nw_macksf7A	2003	16	703748	4993949	0	0		Otsego
nw_macksf8E	2003	16	711190	5017039	1	3		Cheboygan
nw_macksf9A	2003	16	705674	4995877	0	0		Otsego
Totals					2	4		

Appendix 2.4. Cont'd.
Department of Natural Resources - Roscommon Field Office

Snare Site Name	Year	UTM Location			No. Hits	No. Hair		County
		Zone	Easting	Northing		Samples	Collected	
nw_rosca10	2003	16	686659	4918302	1	3		Roscommon
nw_rosca06	2003	16	689918	4923714	1	1		Roscommon
nw_roscco3	2003	16	674870	4922597	1	1		Roscommon
nw_roscd10	2003	16	665694	4928717	0	0		Missaukee
nw_rosdd01	2003	16	670022	4926910	0	0		Missaukee
nw_rosce10	2003	16	668632	4919545	1	3		Missaukee
nw_rosceo1	2003	16	667850	4923272	0	0		Missaukee
nw_rosceo4	2003	16	664875	4921120	1	1		Missaukee
Totals					9	0		

Appendix 2.4. Cont'd.
Department of Natural Resources - Traverse City Field Office
The Grand Traverse Bay Bands of Ottawa and Chippewa Indians

Snare Site Name	Year	Zone	UTM Location			No. Hits	No. Hair		County
			Eastings	Northing			Samples Collected		
GT01	2003	16	627280	4946966		0	0		Grand Traverse
GT03	2003	16	620681	4930328		0	0		Grand Traverse
GT04	2003	16	608079	4930450		0	0		Grand Traverse
GT07	2003	16	607221	4943255		1	1		Grand Traverse
GT08	2003	16	629348	4956128		0	0		Grand Traverse
BE07	2003	16	592556	4935162		2	3		Benzie
BE08	2003	16	592924	4936563		2	5		Benzie
BE09	2003	16	588578	4938575		0	0		Benzie
BE10	2003	16	577143	4933898		0	0		Benzie
BE11	2003	16	585240	4948110		0	0		Benzie
BE12	2003	16	584437	4955675		5	39		Benzie
Totals						10	48		

Appendix 2.4. Cont'd.
Little Traverse Bay Bands of Odawa Indians

Snare Site Name	Year	Zone	UTM Location		No. Hits	No. Hair		County
			Easting	Northing		Samples Collected		
A1	2003	16	671772	5066917	2	22		Emmet
A3	2003	16	662482	5064020	0	0		Emmet
A4	2003	16	657400	5057466	0	0		Emmet
A5	2003	16	667181	5053860	2	8		Emmet
A6	2003	16	673888	5052649	4	11		Emmet
A7	2003	16	661871	5046450	1	2		Emmet
A8	2003	16	659417	5044505	0	0		Emmet
A9	2003	16	667739	5048997	0	0		Emmet
B1	2003	16	680020	5065602	0	0		Cheboygan
B2	2003	16	685404	5061382	3	11		Cheboygan
B3	2003	16	677707	5055932	0	0		Cheboygan
Ozbourne	2003	16	653462	5048715	0	0		Emmet
TR1	2003	16	665039	5055273	0	0		Emmet
Totals					12	54		

Appendix 2.4. Cont'd.
Little River Band of Ottawa Indians

Snare Site Name	Year	UTM Location			No. Hits	No. Hair		County
		Zone	Easting	Northing		Samples	Collected	
manis_masonBBH53A	2003	16	565310	4892856	0	0	0	Manistee
manis_masonBH1112	2003	16	575542	4881622	1	4	4	Mason
manis_masonBH1217	2003	16	591477	4878662	0	0	0	Lake
manis_masonBH410A	2003	16	584630	4894533	0	0	0	Manistee
manis_masonBH41A	2003	16	577163	4893778	0	0	0	Manistee
manis_masonBH85A	2003	16	573016	4900974	1	1	1	Manistee
manis_masonBH8A	2003	16	574869	4902212	0	0	0	Manistee
manis_masonBHM3	2003	16	592121	4911813	0	0	0	Manistee
manis_masonBHT1	2003	16	592188	4901813	0	0	0	Manistee
manis_masonBHW1	2003	16	590205	4894284	0	0	0	Manistee
manis_masonBMH1	2003	16	587587	4908143	0	0	0	Manistee
manis_masonBMH7	2003	16	589245	4902631	0	0	0	Manistee
manis_masonBTH2	2003	16	577352	4921098	3	4	4	Manistee
Totals					5	9	9	

Appendix 2.4. Cont'd.
Michigan State University

Snare Site Name	Year	UTM Location			No. Hits	No. Hair		County
		Zone	Easting	Northing		Samples	Collected	
B10	2003	16	709827	5030264	4	25		Cheboygan
B12	2003	16	681363	5027421	1	2		Cheboygan
B13	2003	16	689680	5022870	4	17		Cheboygan
B14	2003	16	680648	5014281	0	0		Cheboygan
B15	2003	16	692684	5015509	2	3		Cheboygan
B20	2003	16	709648	5051150	1	4		Cheboygan
B21	2003	16	682377	5024120	0	0		Cheboygan
B22	2003	16	703520	5028750	0	0		Cheboygan
B4	2003	16	706455	5049153	0	0		Cheboygan
B5	2003	16	711014	5048705	1	3		Cheboygan
B6	2003	16	697308	5037651	0	0		Cheboygan
B7	2003	16	703862	5037900	1	2		Cheboygan
B8	2003	16	705449	5035369	0	0		Cheboygan
B9	2003	16	709621	5036856	2	8		Cheboygan
Birch1	2003	17	280196	4966279	2	5		Alcona
Birch2	2003	17	279965	4967638	0	0		Alcona
C1	2003	16	719370	5040000	0	0		Presque Isle
C11	2003	17	268781	5011018	1	3		Presque Isle
C12	2003	17	275395	5015065	4	8		Presque Isle
C13	2003	17	279968	5011248	4	13		Presque Isle

Appendix 2.4. Cont'd.
Michigan State University

Snare Site Name	Year	UTM Location			No. Hits	No. Hair		County
		Zone	Easting	Northing		Samples	Collected	
C14	2003	17	294874	5020128	1	2		Presque Isle
C15	2003	17	297819	5018533	1	4		Presque Isle
C16	2003	17	269428	5023739	2	6		Presque Isle
C2	2003	16	722750	5039499	0	0		Presque Isle
C3	2003	16	733941	5039632	1	1		Presque Isle
C4	2003	16	733680	5037738	0	0		Presque Isle
C5	2003	16	734082	5039638	3	28		Presque Isle
C7	2003	16	728324	5033155	0	0		Presque Isle
C8	2003	16	732540	5024768	0	0		Presque Isle
C9	2003	16	729898	5019648	3	14		Presque Isle
clare27	2003	16	656633	4867069	0	0		Clare
clare45	2003	16	682898	4886574	0	0		Clare
D1	2003	16	665467	5014347	2	5		Charlevoix
D2	2003	16	670933	5014123	0	0		Charlevoix
D3	2003	16	671492	5008270	0	0		Charlevoix
D4	2003	16	661549	4999315	2	5		Charlevoix
E1	2003	16	650212	4995285	0	0		Antrim
E2	2003	16	648098	4987460	1	3		Antrim
E3	2003	16	654856	4986198	0	0		Antrim
F1	2003	16	696029	5007199	0	0		Otsego

Appendix 2.4. Cont'd.
Michigan State University

Snare Site Name	Year	UTM Location			No. Hits	No. Hair		County
		Zone	Easting	Northing		Samples	Collected	
F2	2003	16	697390	4997908	1	1		Otsego
F3	2003	16	703758	4980488	0	0		Otsego
F4	2003	16	696450	4974146	0	0		Otsego
F5	2003	16	690837	4974452	1	4		Otsego
G1	2003	16	712823	4980958	3	12		Montmorency
G10	2003	16	731579	4969907	1	2		Oscoda
G11	2003	16	723442	4968748	2	14		Oscoda
G2	2003	16	729417	4992613	0	0		Montmorency
G3	2003	16	735411	4995449	0	0		Montmorency
G4	2003	17	266073	4991614	1	11		Montmorency
G5	2003	17	264342	4983983	2	10		Montmorency
G6	2003	16	726286	4981240	0	0		Montmorency
G7	2003	16	729364	4975725	3	10		Montmorency
G8	2003	16	725553	4972445	0	0		Montmorency
G9	2003	16	733169	4972097	1	1		Montmorency
H10	2003	17	313317	4971027	1	2		Alpena
H12	2003	17	277087	4999202	0	0		Alpena
H13	2003	17	299935	4986713	4	25		Alpena
H14	2003	17	286306	4982446	2	10		Alpena
H3	2003	17	279120	5000813	1	1		Alpena

Appendix 2.4. Cont'd.
Michigan State University

Snare Site Name	Year	UTM Location			No. Hair			County
		Zone	Eastings	Northing	No. Hits	Samples	Collected	
H4	2003	17	291870	4996756	0	0	0	Alpena
H5	2003	17	294625	4995750	4	9	9	Alpena
H7	2003	17	301593	4990007	2	13	13	Alpena
H8	2003	17	304913	4983076	1	2	2	Alpena
H9	2003	17	305955	4980062	0	0	0	Alpena
Hastings1	2003	17	283398	4967649	2	13	13	Alcona
L1	2003	16	687577	4957133	3	9	9	Crawford
L2	2003	16	702304	4967180	2	7	7	Crawford
M1	2003	16	708281	4968972	0	0	0	Oscoda
M10	2003	16	714875	4957738	0	0	0	Oscoda
M2	2003	16	711496	4960961	2	7	7	Oscoda
M3	2003	16	727575	4960155	0	0	0	Oscoda
M4	2003	16	706805	4960010	0	0	0	Crawford
M5	2003	16	698041	4952281	0	0	0	Crawford
M6	2003	16	703296	4974762	2	2	2	Otsego
M7	2003	16	678510	4953912	0	0	0	Crawford
M9	2003	16	670836	4969434	0	0	0	Otsego
mio_usfsStation 18	2003	16	704608	4946061	1	4	4	Crawford
mio_usfsStation 30	2003	16	709904	4947262	1	2	2	Oscoda
mio_usfsStation 40	2003	16	714945	4948070	4	10	10	Oscoda

Appendix 2.4. Cont'd.
Michigan State University

Snare Site Name	Year	UTM Location			No. Hits	Samples Collected	County
		Zone	Eastings	Northing			
mio_usfsStation 58	2003	16	715017	4934265	0	0	Oscoda
mio_usfsStation 92	2003	16	702555	4937211	0	0	Crawford
N12	2003	17	298483	4949322	3	30	Alcona
N4	2003	17	313531	4961560	0	0	Alcona
N6	2003	17	295550	4954311	3	14	Alcona
N7	2003	17	289263	4933879	0	0	Alcona
N8	2003	17	299627	4935524	1	5	Alcona
N9	2003	17	309221	4932453	1	4	Alcona
R1	2003	16	703041	4910986	1	1	Roscommon
R3	2003	16	706988	4900420	4	10	Roscommon
Reed1	2003	17	268707	4960846	0	0	Oscoda
Reed2	2003	17	270506	4957831	5	46	Oscoda
Reed3	2003	17	268090	4956763	2	7	Oscoda
S1	2003	17	264484	4933207	0	0	Oscoda
S2	2003	17	262923	4925521	0	0	Ogemaw
S3	2003	17	269009	4926648	0	0	Ogemaw
S4	2003	16	711311	4919334	2	10	Ogemaw
S5	2003	16	687726	4895776	2	10	Roscommon
S6	2003	16	674146	4892751	0	0	Roscommon
S7	2003	16	667825	4885108	0	0	Clare

Appendix 2.4. Cont'd.
Michigan State University

Snare Site Name	Year	UTM Location			No. Hair		County
		Zone	Easting	Northing	No. Hits	Samples Collected	
T1	2003	17	279678	4930206	2	6	Iosco
T2	2003	17	288921	4927404	1	2	Iosco
T3	2003	17	296000	4924979	0	0	Iosco
T4	2003	17	274196	4936265	0	0	Alcona
T5	2003	16	733356	4931944	3	9	Ogemaw
T6	2003	16	725733	4914795	0	0	Ogemaw
X1	2003	16	709163	4887655	2	6	Gladwin
X2	2003	16	713482	4886927	1	1	Gladwin
Totals					123	503	

Appendix 5.1. Hair snare set-up and checking procedures

Michigan Black Bear Hair Snaring Research Project- Procedures

- I. Setup Procedures
 - a. Trap Number and Location
 - i. All hair snare traps have an identifying number and GPS location. This number and location should be noted on the top of the data sheet along with the date in which the trap is set, and the individuals who are setting the traps.
 - b. Site Selection
 - i. Although the traps have a specific location (noted by the GPS location) the placement of the traps does not have to be in that exact location, but should be reasonably close (within a ¼ of a mile). Traps should be placed where they have the greatest probability of being visited. These would be in the following locations:
 1. Upland/lowland edges of the following habitat types:
 - a. Aspen
 - b. Northern Hardwoods (esp. cut or thinned)
 - c. Upland brush/ opening
 - d. Swamps
 2. Features to look for that would channel bear travel:
 - a. Streams or rivers
 - b. Edge of lakes
 - c. Old logging roads
 - d. Power line or pipeline right-of-ways
 - e. Well used deer runways
 - f. Upland ridges through swamps
 - c. Tree Configuration
 - i. Once a trap area has been selected a grouping of trees must be found.
 1. A suitable grouping of trees should meet the following criteria:
 - a. Look for at least three trees that could be used to form an enclosure that is at least 10 feet on each side.
 - b. Trees should optimally have smooth bark so that bear sign can be easily detected.
 - c. The ground should be as level as possible to insure that the height of the wire remains consistent.
 - d. Trees should also be chosen that contain many tree branches from which the bait can be suspended.
 - e. If a site is suitable but does not have a suitable arrangement of trees, then metal posts that have been provided can be used.
 - d. Barbed Wire Set-up

Appendix 5.1. Cont'd.

- i. Lower Wire
 - 1. Starting at one of the selected trees, the barbed wire should be fastened to the tree using a metal fencing staple. The height of the wire should be 8 inches above the ground.
 - 2. Wire should then be hand-stretched to the next tree and a measurement of 8 inches should be taken from the wire to the ground. The wire should then be attached to the second tree using a fencing staple.
 - 3. Continue to attach the wire to all of the trees, forming an enclosure.
 - 4. Inconsistencies in the ground such as low spots can be filled in with logs or other woody material to insure that the height of the wire remains 8 inches above the ground.
 - ii. Upper Wire
 - 1. Using the same method, another strand of barbed wire should be fastened to the tree at 20 inches above the ground.
- e. Warning Signs and Flagging
 - i. Once the wire has been stretched to form the enclosure:
 - 1. Florescent flagging material should be tied on the wire in at least 3 locations to warn any human individuals of its presence.
 - 2. Warning signs should be posted on 2 trees surrounding the site to warn people of the barbed wires' presence.
- f. Bait placement
 - i. Using the bacon bait provided:
 - 1. Attach string to one end of the bait.
 - 2. Throw the bait over a tree limb at least 10 feet in the air.
 - 3. Attach a second string to the bacon bait.
 - 4. Attach film canister to string beside bacon bait and put anise scent on cotton balls in canister.
 - 5. Pull the bait into the air so that it is at least 8 feet above the ground. Tie off each end.
 - 6. The bait should be at least 15-18 inches from the trunk and 24-36 inches from the nearest limb.
- g. Filling out the data sheet
 - i. Provide the trap name and date that the trap was set.
 - ii. Provide the coordinate locations of the trap using a GPS.
 - 1. These coordinates can be either in lat/long or, preferably, UTM.
 - 2. If you do not have a GPS then notify the MSU crew and they will help you out.
 - iii. Provide the names of the individuals that set up the trap.



Appendix 5.1. Cont'd.

- iv. Provide general directions to the site to assist any person that may be checking the trap, but has not previously been to the site.
- v. Give the time in which the trap setup began and the time in which the trap has been totally set and baited.
- vi. Provide any general comments that may be useful, or are pertinent (an example may be the presence of bear sign).
- vii. Give an above view sketch of how the barbed wire is configured.

Supplies list for set-up:

~~Hammer~~

Barbed wire

~~Pliers to cut wire~~

Fencing staples

~~Tape measure~~

Data sheets

Flagging material

Warning signs

Nails for signs

Metal posts

Bait string

~~Knife or scissors~~

Bacon bait

Film canisters

Anise scent

(Shading denotes supplies that are not provided by MSU)

II. Checking Procedures

a. Checking intervals

- i. Hair snaring will occur in one single period throughout the summer.
- ii. The period consists of 5 checks that are separated by 5-8 day intervals.
- iii. The MSU crew will assist in a checking schedule for all DNR bait routes once the baits have been set, if needed.

(Refer to attached sample calendar for example)

b. Checking procedures

- i. Begin by making a sketch of the barbed wire trap in the space provided on the checking sheet.
- ii. Checking the barbs
 - 1. Hair should only be collected off of the upper wire. Any hair on the lower wire should be removed and flamed only.

Appendix 5.1. Cont'd.

2. Using a white piece of paper or an index card, start at the farthest north post or tree. Check each barb for the presence of hair by placing the white piece of paper behind each barb and proceed around the trap in a clockwise direction.
 3. The barbs should be counted in the same clockwise manner.
 4. The first hair sample that is encountered will be sample one and its general location on the barbed wire should be noted on the sketch and its barb number should be recorded on the data sheet.
 5. Each barb is considered to be an independent sample and should be placed in separate envelopes.
 6. Once a hair sample is placed in an envelope, the number that is preprinted on the envelope should be recorded on the data sheet.
 7. Proceed checking each barb until you reach the starting point, making sure that each barb is independent.
- iii. Measuring the height of the hair sample
1. Using a tape measure, indicate the distance between the hair sample and ground (Although the wire was set at 20 inches, irregularities in the ground make the wire slightly different heights at different locations).
 2. This should be done for each independent hair sample.
- iv. Removing the hair
1. Hair should be removed using forceps, or hand pulled and placed into the pre-numbered envelopes and the approximate number of hairs collected should be circled on the envelope.
 2. The most important part of the hair is the white tip of the large guard hairs. It is extremely important to take good care of these hairs because they contain the most DNA material.
 3. Hair samples should be kept in a dry location and if the hair sample is wet when collected it must be dried by placing the envelopes separately in a warm dry location.
 4. The barb should be flamed with a lighter to prevent cross-contamination.
- v. Other notes:
1. Note any evidence of bears at the bait site by looking for claw marks on trees or the absence of bait in the general comments of the data sheet.

Appendix 5.1. Cont'd.

2. Note any evidence of a non-target species being present at the bait site in the comment section of the data sheet.
 3. Of particular interest is if there is evidence of black bears at the bait site, but there was no deposition of hair on any barbs.
- c. Filling out the data sheet
 - i. Begin by indicating the trap site name, the date of the visitation and the time of the visit.
 - ii. Circle the visitation number that is appropriate and indicate the name of the person collecting the sample.
 - iii. Fill in the table for hair samples, making sure to write down the envelope number, barb number, and number of hairs on the data sheet.
 - iv. Make general comments about the condition of the hair samples.
 - v. Make any general comments that might be pertinent to the study and fill them in on the comments section of the data sheet.
 - d. Bait replenishment
 - i. If the bait is absent from the site, hang a new bait and indicate the presence/absence of the bait on the data sheet.
 - ii. If bait is still present at the site, you do not have to replenish the bait.
 - e. Trap removal
 - i. At the conclusion of the sixth check, all of the barbed wire and any indication of our presence should be removed from the site.
 - ii. This includes all of the baiting materials and any flagging that marks the directions to the bait site.
 - iii. Items that should be saved include the warning signs and the barbed wire.
 - iv. All other items should be disposed of properly.

Checking Supplies List: Data sheets

Hair envelopes

~~Forceps~~

Bait

Note cards

String

~~Knife or scissors~~

~~Tape measure~~

~~Hammer~~

Nails

Staples

Film canisters

Anise scent

(Shading denotes supplies that are not provided by MSU)

Appendix 5.2. List of supplies needed for each task to be performed in the collection of hair samples and hunter harvested bear hair and muscle tissue samples.

Task to be Performed	Supply Needed
Equipment needed to find sites	Global positioning system Topographic maps
Hair Snare Equipment for one snare	60 feet of barbed wire 10 fencing nails 2-4 10 penny nails 1-2 warning signs Tape measure Hammer Fencing tool Flagging Five gallon bucket
Checking one snare for one week	One pound of bacon 20-40 feet of twine/string Anise Film canister Latex gloves Envelopes Pencil Data sheet Clipboard
Harvested Bear Hair and Tissue Collection	Envelopes Forceps Tooth extractors 10 mL vials Stamps for envelopes Buffer solution Protocols sheet

Appendix 5.3. Formed that was filled out upon the set-up of a hair snare.

Hair Snare Set-up Data Sheet - 2003

Trap Site #: _____ Date: _____ UTM: E

Date Baited: _____ N _____

Set-up Crew Names:


General Directions to Site:

Setup Information

Start Time: _____ End Time: _____

General comments:

Sketch of bait site:



Appendix 5.4. Copy of the stamp that was placed on all the hair collection envelopes.

Bear Hair

Date: _____ Envelope #: _____

Trap Site #: _____

Visitation #: 1 2 3 4 5 6 Barb #: _____

Number of Hairs: 1-4 5-10 11-15 >15

Collector: _____

Comments: _____

Appendix 5.5. Data collection form used when checking hair snares for the presence or absence of hair samples.

Hair Snare Checking Data Sheet - 2003

Trap Site #: _____ Date: _____ Time: _____

Visitation #: 1 2 3 4 5 6 Collector: _____

Total Number of Hair Samples Collected: _____

Hair Samples:

Sample #	Envelope Number	Height at Sample	Barb Number	Comments/ condition of sample
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

General comments:

Sketch of bait site, barb number, and sample number at approximate location of hair samples: (Start collecting samples at the northernmost post or tree and proceed clockwise)

↑

N

Appendix 5.6. Protocols for the collection of hair, muscle tissue, and tooth samples.

Sampling Protocols for Black Bear Tissue Samples.

Items needed:

In the materials provided by MSU you should find:

- Four envelopes. Inside of the larger envelope marked "Bear Samples", are three smaller envelopes marked "Bear Tooth", "Bear Hair" and "Bear Tissue".
- A 1.5 mL snap-top plastic tube filled with buffer. This is pre-numbered and is inside of the envelope marked "Bear Tissue".
- Scissors for cutting a small portion of clean muscle tissue.
- Tweezers/forceps to hold the muscle tissue when you cut it and put it into the tube.

To take a tissue sample:

1. Cut off a small piece of muscle tissue. Do not use soft tissues such as heart, liver, or kidneys. A piece of fresh clean muscle from the lining of the abdominal cavity, legs, or other easily accessible area from the field-dressed carcass can be used. You can also use tongue.

The cut piece of tissue should be longer than it is wide. A piece about as long as your thumb nail and no bigger around than a pencil will work nicely. Do not overfill the tube with tissue, the buffer doesn't work well if there is too much tissue in the tube. A general rule is that there should be 2 parts buffer for 1 part tissue.

After you put the tissue in the tube, please make sure the top "snaps" closed. Turn the tube upside down a few times to make sure the tissue is covered with the buffer.

2. Because we are collecting muscle tissue samples for genetic analysis, it is necessary to clean off the tweezers/forceps and scissors between bear samples. They don't have to be spotless, you can simply rinse them with water.
3. Label the envelope marked "Bear Tissue" with the Seal Number and hunter's name.
4. Place the tube inside the "Bear Tissue" envelope.
5. The tube does not need to be refrigerated or frozen. It can be stored at room temperature for several months.

Note: The buffer is made up of Tris, EDTA, UREA, Sarcocine, NaCL, and Water. It is not in any way hazardous but please don't drink it.

Appendix 5.6. Cont'd.

Sampling Protocol for Black Bear Hair Samples

1. Pull a few strands of hair from the bear - be sure to get the follicle (roots).
2. Label the envelope marked "Bear Hair" with the Seal Number and hunter's name.
3. Place the hair in the envelope marked "Bear Hair".

Sampling Protocol for Black Bear Hair Samples

1. Follow the instructions provided by the MDNR to collect a tooth.
2. Label the envelope marked "Bear Tooth" with the Seal Number and hunter's name.
3. Place the tooth in the envelope marked "Bear Tooth".
4. PLEASE BE AWARE THAT YOU ONLY NEED TO COLLECT 1 TOOTH. YOU DO

NOT NEED A TOOTH FOR THE MDNR AND ANOTHER TOOTH FOR MSU.

Final Protocol for Black Bear Samples

1. Label the envelope marked "Bear Samples" with the Seal Number and hunter's name.
2. Put the three smaller envelopes ("Bear Tissue", "Bear Hair", and "Bear Tooth") inside the envelope marked "Bear Samples".
3. Store the "Bear Samples" envelope in a dry spot.
4. Michigan State University will contact you about picking up your samples.

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