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Efficacy of DNA Sampling to Monitor Population Abundance of Black Bears in the Southern Appalachians

Katie E. Settlage
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Katie E. Settlage entitled "Efficacy of DNA Sampling to Monitor Population Abundance of Black Bears in the Southern Appalachians." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

Frank T. van Manen, Major Professor

We have read this thesis and recommend its acceptance:

Joseph Clark, Lisa Muller, Michael Vaughan

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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EFFICACY OF DNA SAMPLING TO MONITOR POPULATION ABUNDANCE OF BLACK BEARS IN THE SOUTHERN APPALACHIANS

A Thesis

Presented for the

Master of Science Degree

The University of Tennessee, Knoxville

Katie E. Settlage

August 2005

DEDICATION

This thesis is dedicated to my dad, who took me to the beaches and woods and horse barns to play when most other little girls would rather stay inside.

To my mom, who instilled in me her sense of great compassion and empathy for all living things.

And to my brother, whose outdoor enthusiasm and companionship were hallmarks of my childhood in the California countryside.

ACKNOWLEDGMENTS

I would like to express my gratitude to all those who made my study not only possible, but thoroughly enjoyable. First and foremost, my major professor, Dr. Frank van Manen. I learned much under his direction, and his patience, thoughtfulness, and assistance were invaluable. Furthermore, his friendship was greatly appreciated, and some of my best memories are from days spent in the field with him. My committee also provided great counsel. Dr. Lisa Muller, Dr. Mike Vaughan and Dr. Joseph Clark were very helpful, and I especially appreciate Dr. Clark for those times I came to his office unannounced to talk about various matters. My DNA analysis was expertly conducted at the Leetown Science Center, and for that I thank Dr. Tim King, Shannon Julian, and Colleen Callahan.

My project would not have been possible without generous financial and logistical support from many different sources. Funding came from the University of Tennessee, the U.S. Geological Survey, the U.S. Forest Service, the National Park Service, Friends of the Smokies, and the Great Smoky Mountains Association. Logistical support and field personnel were provided by Georgia Department of Natural Resources, South Carolina Department of Natural Resources, North Carolina Wildlife Resources Commission, the National Park Service, and the U.S. Geological Survey. Special thanks to Larry Hartman, Kim Delozier, and Bill Stiver of the National Park Service, and Gary Peters of the U.S. Forest Service for their support of the project. I was fortunate to be involved in such a collaborative effort; with so many dedicated people caring for black bears in the southern Appalachians, their future seems optimistic.

One of the most unforgettable aspects of working on this project was all the wonderful people I got to meet. I feel like I know many graduate students before me through the stories (some true, some not!) that have been told, and I know future students will be recalling the graduate students that I have been fortunate to learn from. The art of bear trapping was taught to me by Dave and Rebecca Telesco (the bear landscape architects), Laura Thompson, and Jason Kindall. Days in the office were enlivened through their company, plus others, including Kim Asmus, Jay Clark, Jennifer and Steve Dobey, Kent Hersey, Cindy Thatcher, and Brandon Wear.

I have immense gratitude to those folks who shared in the field work to complete both the live-capture work and the DNA sampling. I spent three summers and winters in Great Smoky Mountains National Park and have received help from too many people to mention them all. However, I must specifically thank my field technicians, Bethany Bostrom, Angela Brown, Jay Clark, Justin Conley, Danny Gammons, Curtis Hill, Walt Hodges, Carrie Lowe, Jason Lupardus, Hollie Millsaps, Megan Penrod, and Roy Smith. I also thank the state agency and U.S. Forest Service personnel who collected DNA on the national forest study area. Thank you Brandon Allen, Mark Garner, Andy Gaston, Chad Grant, David Gregory, Chuck Hans, Chris Holcomb, Mark Jones, Craig Okraska, Mike Parks, Skip Still, and Doug Watson.

Last, but not least, I want to express my love and gratitude to my family. This includes all the Settlage's in Arkansas. It also includes my "family" here in Tennessee, including my fuzzy little cat that warmed my lap for many nights of journal perusing. My beautiful horse Burke, because even after 11 years of daily rides, each new day brings renewed joy, grace, and peace. But mostly my husband Mark, who is the love of

my life, my best friend, and my biggest fan. I am so glad you were here with me for this incredible journey in the Smoky Mountains…the memories of hot summer days and swims in the river, the riot of leaves on fall afternoon hikes, and the sweet smell of a cub tucked in your coat on a snowy day in February and all the more precious because you were there.

ABSTRACT

The American black bear (*Ursus americanus*) in the southern Appalachians has been the subject of intensive research. In particular, the focus has been on population monitoring using livecapture, bait stations, harvest records, and radio-active feces tagging. Genetic (DNA) sampling for mark-recapture is an emerging technique for estimating population abundance, but the efficacy of various sampling regimes for estimating populations of different densities has not been established. I conducted a pilot study to determine whether genetic sampling for population estimation is feasible to monitor black bear abundance in the southern Appalachians and to develop appropriate sampling regimes to obtain desired levels of precision. Specifically, I investigated how the density of sampling sites, number of samples analyzed, and sampling duration affect the accuracy and precision of population estimates. Research was conducted for 10 weeks from 9 June to 15 August 2003 on 2 study areas: a high-density black bear population in a portion of Great Smoky Mountains National Park (the national park study area), and a lower-density black bear population on national forest lands in North Carolina, South Carolina, and Georgia (the national forest study area). DNA was extracted from hair collected from baited barbed-wire enclosures. The average number of hair-capture sites within a typical female home range was 2.71 and 2.48 for the national park and national forest study areas, respectively. Twenty-five hair samples/week were randomly chosen for DNA analysis. Individuals were identified by their unique genetic profile obtained from 9 to 10 microsatellite loci. I identified 129 and 60 individual bears in the national park and national forest study area, respectively. Reductions in site density, subsample intensity, or sampling duration tended to produce low, heterogeneous capture probabilities,

resulting in unreliable population estimates. Sample sizes and capture probabilities were smaller and population estimates decreased as the 3 factors were reduced. Those trends were particularly apparent for the national park study area, where capture probabilities were particularly low. The combination of small sample sizes and heterogeneous capture probabilities likely were a result of an insufficient number of hair-capture sites and number of analyzed hair samples relative to the size of the sampled population. Increasing these 2 factors likely would increase sample sizes and capture probabilities and reduce heterogeneity of capture probabilities present in the data. However, increasing the number of analyzed samples also would increase costs. Because pooled sampling periods increased capture probabilities, I selected a pooled configuration for population estimation that yielded relatively high capture probabilities (3 periods comprised of 3 weeks each). I used the heterogeneity model M_h Chao, which produced an estimate of 292 bears (95% CI = 214–435) for the national park study area and 98 bears (95% CI = 76–149) for the national forest study area (density = 1.83 bears/km^2 and 0.30 bears/km^2, respectively). My results indicate that effective implementation for black bear population estimation requires careful consideration of study design. Capture probabilities of ≥20% are required to minimize bias, and this would be best achieved by analyzing more subsamples from a greater density of hair-capture sites, particularly in high-density populations. I recommend ≥4 hair-capture sites/female home range to reduce heterogeneity and a sampling duration of 6–8 weeks to reduce violation of geographic closure. In the national forest study area reasonably unbiased population estimates were achieved with 20 hair samples/week, but as many as 40 hair samples/week may be required to produce reliable estimates for the national park study area.

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CHAPTER I

INTRODUCTION

General Problem Statement

The American black bear (*Ursus americanus*) was numerous and widespread in the southern Appalachians at the time of European settlement, but subsequent unregulated exploitation led to severe population declines through the late 1800s (Clark and Pelton 1999). Bears were hunted for meat, fat, and fur, and were persecuted as pests by settlers. Furthermore, bear habitat was rapidly lost and degraded during the late 1800s and early 1900s because of farming, logging, road construction, and the introduction of the chestnut blight, which eliminated the American chestnut (*Castanea dentata*) as a key food source for bears from eastern forests (LaFollette 1974). However, during the first half of the 20th century, the U.S. government initiated programs to acquire land for the establishment of national parks and national forests, most notably Great Smoky Mountains National Park in 1934. Bear populations began to slowly recover and, by the mid 1900s, the black bear was designated a game species by most southeastern states, which resulted in further protection and regulation (Clark and Pelton 1999).

As bear populations began to recover, it became evident that there was a need for a better understanding of bear ecology to improve management. The University of Tennessee initiated research in Great Smoky Mountains National Park in the late 1960s. This research effort has provided valuable long-term information on the bear population. Research topics have been numerous and varied, including habitat use, denning ecology,

nuisance bear behavior, food habits, population monitoring, and population demographics.

During the early 1970s, there were indications of a regional decline of the bear population and managers realized that the black bear population was a shared resource (Pelton and van Manen 1996). It became clear that bear management in the southern Appalachians would benefit from closer cooperation between managers and researchers in the region. In 1976, state biologists and researchers from Georgia, North Carolina, and Tennessee formed the Tri-State Black Bear Study (Carlock et al. 1983). In subsequent years, South Carolina, the National Park Service, and the U.S. Forest Service joined the group, and they became what is now known as the Southern Appalachian Black Bear Study Group (SABBSG).

The SABBSG has consistently identified the need for an accurate population estimate for the region and the ability to track population trends over time. Currently, a bait-station survey is used by 3 of the 4 state wildlife agencies in the region to monitor bear population trends. The bait-station survey is conducted annually in the summer and consists of hanging sardine cans from trees along designated routes and counting the number of bear visits to obtain an index of population abundance. Although this technique may be useful to indicate relative changes in population size over time, it cannot be used to derive an estimate of population abundance or density (Garshelis 1990).

A regional estimate of black bear population size and density is important to guide management. Conventional mark-recapture techniques based on capture and release have been used to estimate bear populations in smaller study areas but present

logistical difficulties when applied to a large region. Live-capture techniques tend to be costly and labor intensive and often provide population estimates with relatively low precision and accuracy. Poor precision can result when sample sizes and recapture rates are low. The accuracy of estimates obtained with live-capture techniques can be affected when sampling biases, such as unequal capture probabilities, are present (Pollock et al. 1990). For example, live-capture studies on black bears frequently produce "trap smart" bears that consume bait at trap sites without being captured.

Advances in DNA technology offer alternative methods for population estimation that may help overcome problems with conventional mark-recapture techniques based on livetrapping (Taberlet et al. 1997, Woods et al. 1999, Mowat and Strobeck 2000). Whereas conventional mark-recapture studies use physical markers (e.g., ear tag, leg band, tattoo) to mark individuals, a relatively new technique is to "mark" animals based on DNA collected from hair or tissue samples. This DNA technique has advantages over live trapping, such as increased capture probability, tag permanency, reduced bias, and decreased intrusiveness (Woods et al. 1999, Mills et al. 2000). Initial research efforts were based on tissue samples to determine abundance of humpback whales (*Megaptera novaeangliae*) in the north Atlantic Ocean (Palsbøll et al. 1997) and hair samples to determine abundance of brown bears (*Ursus arctos*) in British Columbia (Mowat and Strobeck 2000, Poole et al. 2001) and Glacier National Park (Kendall et al. 2001). Since then, the DNA technique has been applied to black bears in many areas, including Tensas River National Wildlife Refuge in Louisiana (Boersen et al. 2003), Okefenokee National Wildlife Refuge and Oceola National Forest in Florida (Dobey et al. 2005), portions of the coastal plain in North Carolina (Thompson 2003; C. Tredick, Virginia Polytechnic

Institute and State University, unpublished data), and the lower peninsula of Michigan (B. Dreher, Michigan State University, unpublished data).

Justification

DNA sampling for population estimation is a promising technique to study black bears in the southern Appalachians. However, the efficacy of DNA sampling under various sampling regimes and population densities has not been established. As with any population estimation technique, DNA sampling requires an investment of time and resources and its feasibility should be assessed before a monitoring program is established. Much of the initial enthusiasm for the technique was soon tempered by the realization that DNA sampling comes with a new realm of unique challenges. For instance, the ability to identify individuals using genetic markers is hampered when quantities of DNA are low, such as when DNA is extracted from hair (Goossens et al. 1998, Taberlet et al. 1999). Small quantities of DNA are susceptible to a genotyping error known as allelic dropout, in which individuals are incorrectly identified as homozygotes when in fact they are heterozygotic (Taberlet et al. 1999). Another type of error involves the creation of false alleles due to artifacts of the polymerase chain reaction (PCR) process (Taberlet et al. 1996). Whereas genotyping errors have the potential to bias estimates of population abundance (Waits and Leberg 2000), their effect can be reduced by collecting a sufficient quantity of DNA (≥10 hairs), reducing DNA degradation by using proper collection and storage techniques, using appropriate markers with high heterozygosity, adhering to stringent laboratory protocols, and re-analyzing questionable samples (Goossens et al. 1998; Taberlet et al. 1999; Woods et al. 1999; Paetkau 2003, 2004; McKelvey and Schwartz 2004*a*,*b*).

DNA studies also require special study design consideration. Most DNA mark-recapture studies use closed models for population abundance estimation; thus, the issue of closure becomes imperative. Suggestions for minimizing geographic closure violations include scaling the size of the sampling area to be large relative to the home range of the species, using topographic features to enhance closure, defining a "core" sampling area from which estimates of density are derived, and radiocollaring animals to obtain an estimate of movement across study area boundaries (Boulanger and McLellan 2001, Boulanger et al. 2004*a*). Furthermore, it is not clearly understood what types of sampling bias can be expected with DNA sampling, and what capture probabilities can be expected under certain sampling scenarios. It is not known how sample-site density, the number of samples analyzed per sampling period (subsampling intensity), or sampling duration affect the quality of population estimates obtained from a DNA study. For instance, Boulanger et al. (2002) examined DNA mark-recapture data for grizzly bears in Canada and found that there was a trade-off between intensively sampling a small study area with many sample sites or less intensively sampling a large study area. The small study area was prone to closure violations, whereas recapture rates decreased with the large study area, thus decreasing precision.

No information is available regarding the ideal sampling design that would reduce closure violation, increase capture probabilities, and be logistically efficient for the study of black bears in the southern Appalachians. Therefore, a pilot study is needed to determine whether genetic sampling for population estimation is feasible and to develop appropriate sampling regimes to obtain desired levels of precision and identify potential sources of bias (Taberlet et al. 1999). Moreover, a pilot study would provide baseline

population estimates and would be useful to develop sampling protocols that minimize logistical issues.

Objectives

The overall goal of my study was to establish effective DNA sampling regimes and sampling protocols for estimation of black bear population abundance in the southern Appalachian region. The specific objectives of my study were to:

1) determine the influence of bear density, sample site density, length of sampling period, and the effects of subsampling on the precision and accuracy of population abundance estimates using DNA sampling;
2) evaluate the feasibility and effectiveness of DNA sampling based on comparisons with other mark-recapture techniques; and
3) provide baseline estimates of population size and density.

CHAPTER II

STUDY AREAS

Location

Two study areas were established based on relative densities of black bears in the southern Appalachian region (Fig. 1). The national park study area, in the northwest portion of Great Smoky Mountains National Park in Tennessee, had a relatively high density of black bears (Eason 2002). The sampling area covered approximately 160 km^2. The national forest study area had a relatively low density of black bears compared with the national park study area (SABBSG, unpublished data) and included portions of the Tallulah Ranger District of the Chattahoochee National Forest in Georgia, the Pickens Ranger District of the Sumter National Forest in South Carolina, and the Cheoah Ranger District of the Nantahala National Forest in North Carolina. The national forest study area was approximately 329 km^2 (see Methods for calculation of study area size).

Topography

The national park study area in Great Smoky Mountains National Park was characterized by rugged terrain consisting of long ridgelines flanked by steep coves and valleys (King and Stupka 1950). Elevations in Great Smoky Mountains National Park range from 267 to 2,025 m. Access to the national park study area was limited by only 1 improved road and a network of maintained trails.

In the national forest study area the topography was less rugged and complex compared with the national park study area. The national forest study area had a higher density of U. S. Forest Service roads than the national park study area.

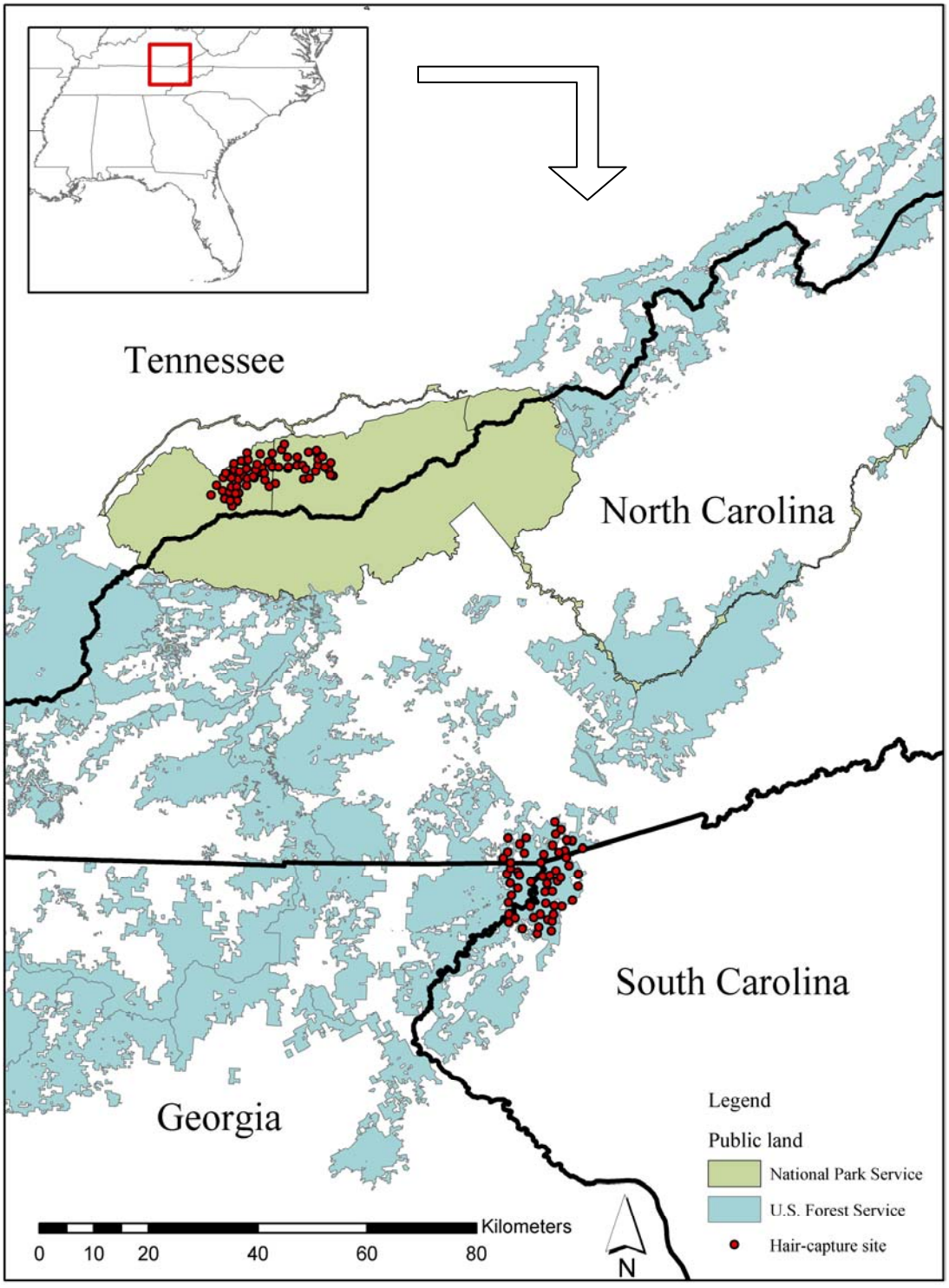


Fig. 1. Study areas to determine the feasibility of DNA sampling to estimate black bear population abundance, Great Smoky Mountains National Park, Tennessee, and portions of Chattahoochee National Forest in Georgia, Sumter National Forest in South Carolina, and Nantahala National Forest in North Carolina, 2003.

Climate

Climatic conditions in the southern Appalachians varied by season, aspect, and elevation (Shanks 1954). High elevations were generally cooler and had more precipitation than lower elevations. Some portions of Great Smoky Mountains National Park were considered temperate rainforest (Thornthwaite 1948). In low- to mid-elevation areas, the average high and low temperatures from June through August were 30.5°C and 15°C, respectively. Summer days typically were warm and humid. Rainfall in the summer occurred in the form of frequent afternoon thunderstorms, producing an average of 13.7 cm per month. During winter, average temperatures in the lower elevations ranged from 11.3°C to -2.1°C and precipitation averaged 11.9 cm of rain and 4.6 cm of snow per month (National Park Service 1997). The climate of the national forest study area was slightly moderated compared with the national park study area because of a less rugged topography and lower elevations.

Flora

The vegetation in Great Smoky Mountains National Park was diverse, with >130 native tree species and communities ranging from lowland mixed hardwoods to spruce (*Picea rubens*)-fir (*Abies fraseri*) at high elevations (King and Stupka 1950). Although much of the land was logged prior to formation of the park, substantial portions of old growth forest still remained, making this area one of the largest tracts of temperate old-growth forest in eastern North America. Sampling was restricted to the northwest quadrant of Great Smoky Mountains National Park, in areas of low to mid elevation. Vegetation in the sampling area primarily consisted of mixed hardwoods, including oaks (*Quercus* spp.), tulip poplar (*Liriodendron tulipifera*), red maple (*Acer rubrum*), sweet

gum (*Liquidambar styraciflua*), yellow buckeye (*Aesculus flava*), and dogwood (*Cornus florida*). A dense understory of rhododendron (*Rhododendron maxima*), mountain laurel (*Kalmia latifolia*), or huckleberry (*Gaylussacia* spp.) was common. Streamside areas typically were dominated by eastern hemlock (*Tsuga canadensis*), whereas ridges were dominated by pine (*Pinus* spp.). Vegetation of the national forest study area was similar to that of Great Smoky Mountains National Park, but it was actively managed by the U.S. Forest Service and tended to have a more dominant pine component.

CHAPTER III

METHODS

Approach

I constructed baited, barbed-wire enclosures (hair-capture sites) to collect black bear hair for DNA analysis (Woods et al. 1999). The DNA sampling technique uses microsatellites, which are small, non-coding, segments of DNA that consist of a series of repeated pairs of nucleotides (Sia et al. 1997). Other molecular markers, such as allozymes or mitochondrial DNA, have been used on a variety of species, but genetic variation is generally lower (McConnell et al. 1995). In contrast, microsatellites are highly variable and easier to interpret. Microsatellite analysis uses polymerase chain reactions (PCR) to amplify small or degraded DNA samples, making it suitable for use on hair (Paetkau et al. 1995, Mills et al. 2000). Because microsatellites are highly variable, researchers can use a relatively small suite of microsatellite loci to identify the unique genetic profile of an individual. The ability to identify recaptured individuals from hair samples enables the use of traditional mark-recapture models to estimate population abundance.

Sample Site Selection and Study Area Delineation

I established 65 and 60 hair-capture sites for the national park and national forest study areas, respectively. That level of sampling was based on a compromise between a recommendation of ≥4 sites/home range (Otis et al. 1978) and logistical constraints (i.e., available personnel and limited access). Elevations of sample sites ranged from approximately 360–820 m on the national park study area, and 330–930 m on the national forest study area. I calculated the effective sampling area of each site by

circumscribing arcs around each sample site, the radii of which were based on the size of an average female home range to represent the smallest expected sample range (Boersen et al. 2003, Dobey et al. 2005). For the national park study area, I set that radius to 1,635 m based on an average home range of 8.4 km^2 (minimum convex polygon method) reported for Great Smoky Mountains National Park (Beeman 1975, Garshelis 1978, Quigley 1982, Carr 1983; Fig. 2). For the national forest study area, I set the radius to 2,108 m representing an average home range of 14.0 km^2 on national forest areas in the southern Appalachians (Villarrubia 1982, Garris 1983, Beringer 1986, Seibert 1989, Reagan 1991; Fig. 3). Locations for hair-capture sites were established by generating random Universal Transverse Mercator (UTM) coordinates within the 2 study areas with ArcView® geographic information systems (GIS; ESRI, Redlands, California, USA) with the goal of creating approximately circular study areas without any gaps in sampling coverage. To facilitate field logistics, I chose sampling locations so that none were >500 m from a road or trail. The resulting dispersion of hair-capture sites yielded a sampling area of 160 km^2 and 329 km^2 for the national park and national forest study areas, respectively.

In order to examine how well the study areas met the criteria of ≥4 sites/home range suggested by Otis et al. (1978), I conducted a moving window analysis in ArcView® GIS to calculate the average number of hair-capture sites/female home range. I determined that the average number of sites within a typical home range was 2.71 and 2.48 for the national park and national forest study areas, respectively. Hair-capture site density was at the recommended level at the center of each study area, but peripheral areas typically had 1–3 hair-capture sites/female home range. However, male bears,

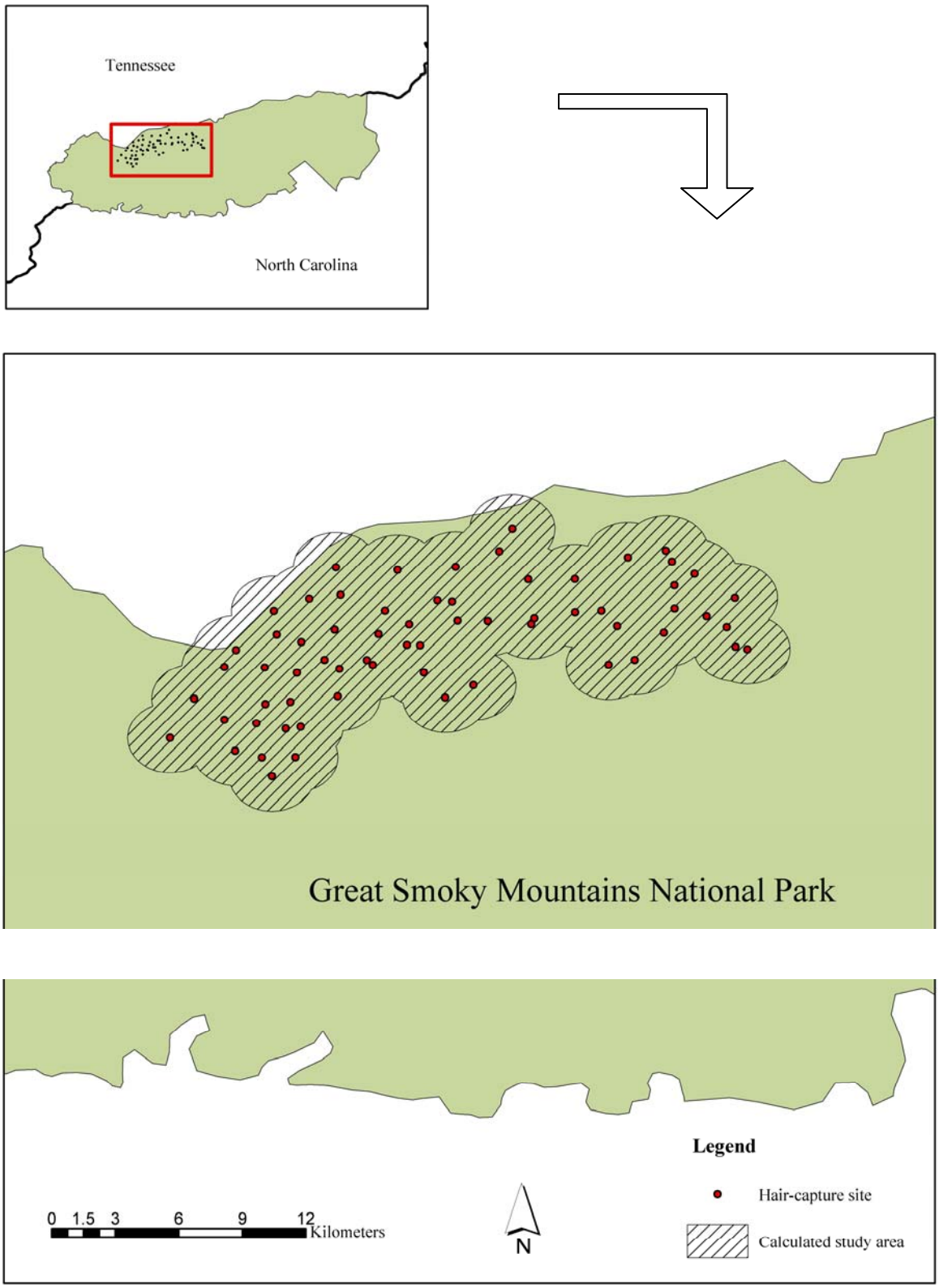


Fig. 2. Location of black bear hair-capture sites and calculated study area based on average female home-range size in the national park study area, Tennessee, 2003.

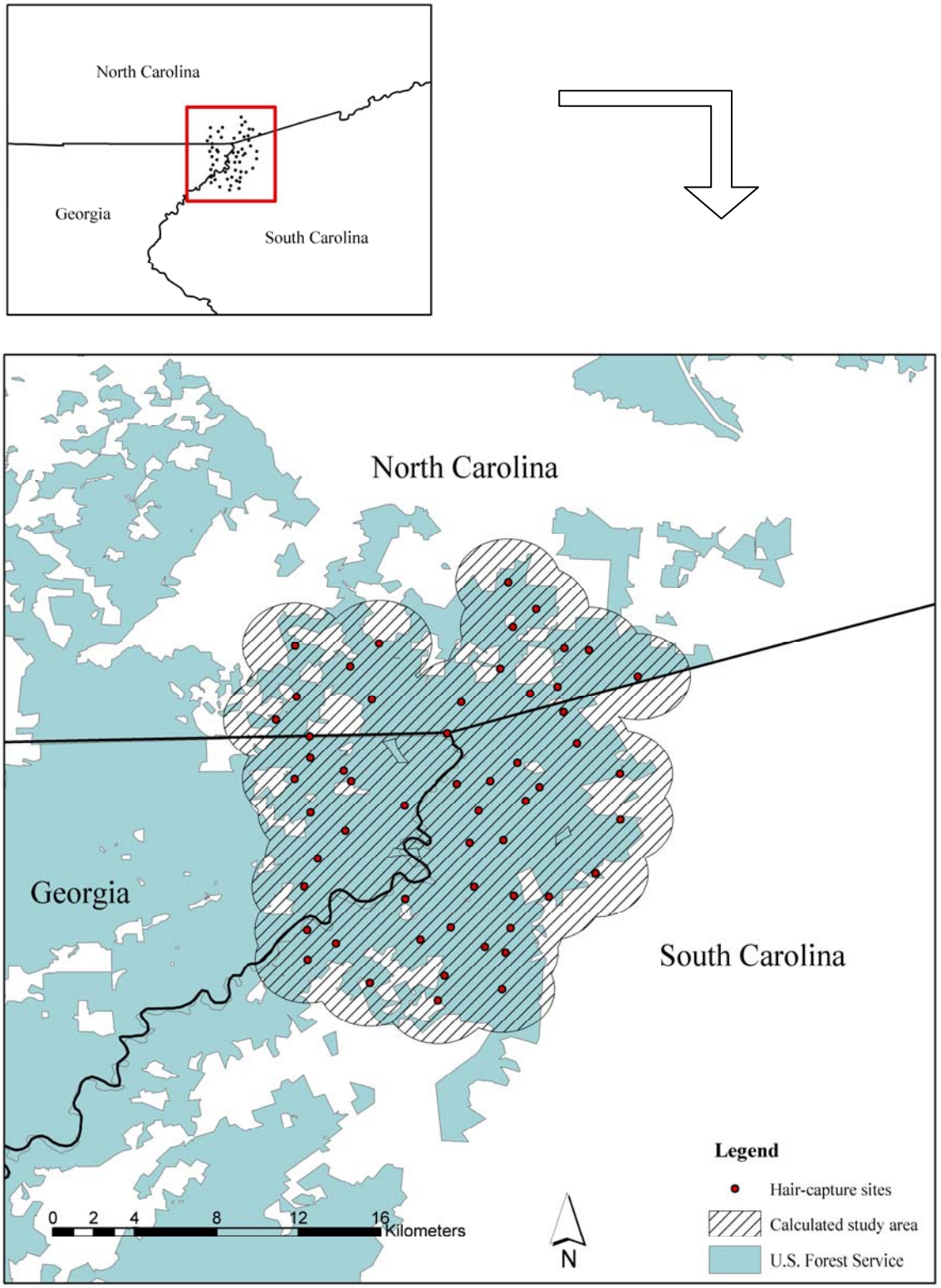


Fig. 3. Location of black bear hair-capture sites and calculated study area based on average female home-range size in the national forest study area, North Carolina, South Carolina, and Georgia, 2003.

which have larger home ranges than females, were more likely to have 4 or more hair-capture sites in their home range.

Field Sampling of DNA

Field personnel located each hair-sample site using a global positioning system (GPS) receiver (GPS 12XL; Garmin International, Olathe, Kansas, USA). Because of logistical considerations (e.g., hair-capture site coordinates occurring close to a residence or inaccessible terrain), hair-capture sites could be established anywhere within 250 m of the targeted site. In the national park study area, personnel used a network of improved and unimproved roads and >25 trails to access hair-capture sites. In the national forest study area, vehicle access was good and most hair-capture sites were accessible by short walks from maintained roads.

Each hair-capture site consisted of a barbed-wire enclosure with bait. The enclosure was constructed using 15.5-gauge barbed wire (7.5 cm spacing between each 4-pointed barb), which was stretched around 4 corner trees and nailed in place to enclose an area of approximately 5 x 5 m. The wire was placed 40–50 cm above ground and stretched tight with standard fencing tools. Bait consisting of bakery products was placed in a small waxed-paper sack and hung on a smooth wire stretched diagonally between 2 corner trees at a height and position such that a bear could not reach it without entering the enclosure. A tampon soaked in raspberry extract (Mother Murphy's Laboratories, Greensboro, North Carolina) was used as a scent lure to attract bears. As a human safety precaution, all sites were marked with orange flagging to make them more visible.

All sites were checked for hair samples and rebaited once every 7 days for 10 weeks. Each hair sample with $\geq$5 hairs was collected and individually stored in labeled

coin envelopes with desiccant (Drie-rite®, W. A. Hammond Drierite Company Ltd., Xenia, Ohio, USA). After hair collection, any remaining hairs were burned off the barbs to prevent contamination with future hair samples.

Subsampling and Microsatellite Analysis

For DNA analysis, I randomly chose 25 samples/sampling period for each study area with each sample coming from a different hair-capture site within a sampling period. Given a range of capture probabilities, this sample size consistently yielded coefficients of variation (CV) ≤20% based on simulations using the Lincoln-Peterson estimator (Chapman 1951). Thus, this number of samples was considered sufficient to examine estimates based on smaller sample sizes.

I prepared hair samples for analysis by clipping a small portion (≈ 0.5 cm) of the root end of a hair and placing it in a clear 1.5-ml boil-proof microcentrifuge tube. Five to 15 hairs were clipped from each sample (Goossens et al. 1998) and each tube containing a single sample was labeled with a unique identification number. The samples were sent to the Leetown Science Center, a U.S. Geological Survey facility in Kearneysville, West Virginia, for microsatellite DNA sequencing (Appendix A). Ten microsatellite loci were analyzed for each hair sample (Appendix A): G1A, G10B, G1D, G10C, G10L, G10X, G10M, G10P, MU23, and MU50 (Paetkau and Strobeck 1994, Paetkau et al. 1995).

Probability of Identity

An important assumption of mark-recapture models is that animals have unique marks. Although each individual has a unique genetic profile, only a certain number of loci can be analyzed because of a limited number of markers and cost restrictions. Thus, 2 individuals may share the same genetic profile at the selected markers (Mills et al.

2000). Furthermore, extracting DNA from hair or feces often results in degraded DNA, which can lead to genotyping errors due to a phenomenon known as allelic dropout. Allelic dropout occurs when only one allele of a heterozygous individual is detected, producing a false homozygote (Taberlet and Luikart 1999). Close relatives can thus be mistaken for recaptures, which will bias the population estimate low (Taberlet and Luikart 1999, Mills et al. 2000). Thus, it is important to quantify the power of the molecular markers (microsatellites) to differentiate between individuals. The probability of identity (PI) is the statistic most commonly used for this purpose and is defined as the probability of obtaining identical genotypes given certain allele frequency distributions. The frequency can be calculated for each locus by:

$$\mathrm{PI}_{\text{single locus}} = \sum p_i^4 + \sum (2 p_i p_j)^2,$$

where p_i and p_j are the frequencies of the *i*th and *j*th alleles, assuming the alleles at each locus are independent and, thus, in Hardy-Weinberg equilibrium. The overall PI across multiple loci is the product of the PIs at each locus (Taberlet and Luikart 1999). The assumption of independent loci can be easily violated, which would bias the resulting PI calculation low (Mills et al. 2000). Populations containing many siblings are a good example of a situation in which loci may not be independent. Therefore, a PI calculated for siblings gives a more conservative estimate for the number of loci needed to obtain a sufficiently low PI (Taberlet and Luikart 1999):

$$\mathrm{PI}_{sibs} = 0.25 + (0.5 \sum p_i^2) + [0.5(\sum p_i^2)^2] - (0.25 \sum p_i^4).$$

Using this formula, Taberlet and Luikart (1999) found that approximately 14 loci were needed to obtain a low probability (e.g., 0.0001) of finding 2 siblings with identical genotypes in their study population, whereas approximately 7 loci would be needed to

obtain the same PI for 2 random individuals. I used 10 loci to determine genotypes of hair samples from this study, which is considered to be sufficient for populations that do not contain many siblings (Taberlet and Luikart 1999) and has been adequate for previous studies (Thompson 2003, Paetkau 2004).

Another set of tests described by Woods et al. (1999) identifies samples that have a high probability of having the same genotype as another sample at the loci examined. The most conservative of these tests is the sibling match test (P_{sib}), which examines the probability that an individual would share a genotype with its sibling at the loci examined. The sibling match test differs from PI_{sibs} in that P_{sib} is a test on individual samples, whereas PI_{sibs} calculates a probability for the entire study population (Woods et al. 1999). For the sibling match test, it is assumed that samples are not necessarily acquired at random and may come from family groups that visit a hair-capture site together (Woods et al. 1999). The sibling match test can be calculated as:

$$P_{sib} = (1 + 2p_i + p_i^2)/4$$

for homozygotes and

$$P_{sib} = (1 + p_i + p_j + 2p_i p_j)/4$$

for heterozygotes (Woods et al. 1999). I performed the sibling match test for every sample on both study areas. Samples with a $P_{sib} > 0.05$ were not considered to be unique individuals and were excluded from analysis (Woods et al. 1999, Boersen et al. 2003, Thompson 2003).

Hardy-Weinberg and Linkage Disequilibrium

The calculation of PI is based on the assumption that the study population conforms to the Hardy-Weinberg equilibrium. A population is in Hardy-Weinberg

equilibrium when allelic and genotypic frequencies remain constant over time. This equilibrium can be violated when conditions such as mutation, migration, genetic drift, or non-random mating occur (Gillespie 1998). A population in Hardy-Weinberg equilibrium will show the following allele frequencies:

$$p^2 + 2pq + q^2 = 1,$$

where p is the frequency of allele A_i and q is the frequency of allele A_j. I used Program GENEPOP (version 3.4; Raymond and Rousset 1995) to determine if my data conformed to Hardy-Weinberg expectations. I used sequential Bonferroni adjustments, designed for use with multiple statistical tests, to determine statistical significance (Rice 1989).

Another assumption required for the analysis of genetic data is that of linkage equilibrium. When loci are in a state of linkage equilibrium, it means that alleles from different loci are independent of one another, and therefore occur in predictable frequencies as a result of recombination rates during meiosis (Gillespie 1998). Linkage disequilibrium occurs when alleles from different loci consistently occur together due to the low rate of recombination. The 10 loci used in my study were previously determined to be independent (Paetkau and Strobeck 1994, Paetkau et al. 1995). Therefore, the presence of linkage disequilibrium in my data could be indicative of sampling bias, non-random mating, sampling of siblings, the presence of immigrants, or stochastic processes (T. L. King, U.S. Geological Survey, personal communication). I used Program GENEPOP to test for linkage disequilibrium and used sequential Bonferroni adjustments to determine statistical significance.

Population Estimation

I used closed models for population estimation, principally the multiple mark-recapture models described by Otis et al. (1978). These models are based on the following assumptions (Otis et al. 1978):

(1) the population is closed to additions (births or immigrants) and deletions (deaths or emigrants),

(2) animals do not lose their marks during the experiment,

(3) all marks are correctly noted and recorded at each trapping occasion, and

(4) each animal has a constant and equal probability of capture on each trapping occasion.

Because the data were collected during a short period of time, during which no births occurred and survival was high, it is reasonable to assume that the 2 sample populations met the criteria for demographic closure. However, the assumption of geographic closure can be difficult to meet with study areas embedded within larger areas of black bear habitat (White et al. 1982). Because all bears are permanently marked with their own unique genetic code that cannot be lost, assumption 2 can reasonably be met with the DNA sampling technique. However, this assumption can be violated if errors in genotyping occur, such as allelic dropout or the presence of false alleles, but these errors are rare when enough original (i.e., template) DNA is used. Goossens et al. (1998) showed that when using 10 hairs, these errors occurred a combined total of only once out of 350 PCRs (0.29% error rate). Therefore, I did not analyze samples with ≤5 hairs. Assumption 3 was addressed based on the PI statistic. However, it is widely recognized that the assumption of equal and constant capture probability is not met in many capture-

recapture studies (White et al. 1982). For example, a capture may affect the probability of future captures of an animal. However, multiple mark-recapture models have been developed so that the assumption of equal catchability can be relaxed (Otis et al. 1978, Chao 1987). Model M_t allows capture probabilities to vary by time. This model would be useful in situations where, for example, weather conditions or different trapping methods may reduce trap activity over the course of the study. Model M_b allows capture probabilities to vary by behavioral response. The behavioral model would be useful when animals become "trap happy" or "trap shy" based on previous trapping experiences (White et al. 1982). Model M_h, the heterogeneity model, allows capture probabilities to vary by individual animal. The heterogeneity model could be used for many situations, including differences in capture probabilities because of age, sex, social dominance, or accessibility to traps (Otis et al. 1978).

Combinations of the 3 unequal capture probability models (M_{tb}, M_{th}, M_{bh}, and M_{tbh}) were not considered. I chose to focus on the single-factor models because my primary goal was to examine bias in the data, for which the single-factor models were most useful. Moreover, no estimator currently exists for model M_{tbh}. Likewise, models M_{tb} and M_{th} lack rigorous statistical estimators and generally are not as reliable as the other models for population estimation (White et al. 1982).

For sampling scenarios with low capture probabilities, I considered 2 additional multiple mark-recapture models described by Chao (1987, 1988, 1989) and Chao et al. (1992). These estimators are modifications of the M_h and M_t models described by Otis et al. (1978) and were designed to provide reliable population estimates with sparse data (i.e., animals captured only 1–2 times; Chao 1989).

Sensitivity Analysis

The sensitivity analysis was designed to examine the effects of the 3 field sampling factors on the performance of the population models. For this analysis, the following terminology applied: *factor* referred to the 3 field sampling variables that I examined (sampling duration, subsampling intensity, and trap density) and *levels* referred to the various values of these factors in the simulations. A *sampling scenario* (or *scenario*) was a single combination of levels from the factors and represented a subset of the entire dataset. To evaluate potential biases in the data and select an appropriate sampling regime for the southern Appalachians, I created 53 and 51 different sampling scenarios for the national park and national forest study area, respectively (Tables 1 and 2). I created a master dataset for each study area, which contained a bear ID number associated with each sample, the site number where the sample was collected, an assigned sample number, and the week the sample was collected. I used SAS® statistical software (SAS Institute, Cary, North Carolina, USA) to create the subsets required for the sensitivity analysis and to convert the subsets into properly formatted capture histories for population estimation in Program CAPTURE (Rexstad and Burnham 1992).

For each study area, I created 40 sampling scenarios that involved a combination of subsampling intensity and sampling duration. Twenty of those scenarios were based on each of the 10 hair-sampling periods separately (the *single–week* scenarios) and 20 involved pooling the weekly sampling periods (the *pooled sampling* scenarios). For the former, sampling duration was examined at 4 levels: 10 weeks, 8 weeks, 6 weeks, and 4 weeks. I chose those levels to examine how reductions in time investment would affect the precision and bias of the population estimates.

Table 1. Configuration of sampling duration scenarios considered for sensitivity analysis on the national park study area (NPSA) and national forest study area (NFSA), 2003. Twenty scenarios involved combinations of single weeks and subsample levels, and 20 scenarios involved pooled week configurations and subsample combinations. Each scenario was replicated 100 times in Program CAPTURE, and the number of replicates that successfully produced output for model M_h Chao are listed.

Sampling duration	**Number of subsamples**	**Number of successful replicates for M_h Chao (NPSA/ NFSA)**
10 weeks	25	100 / 100
10 weeks	20	100 / 100
10 weeks	15	100 / 100
10 weeks	10	100 / 100
10 weeks	5	93 / 99
8 weeks	25	100 / 100
8 weeks	20	100 / 100
8 weeks	15	100 / 100
8 weeks	10	86 / 100
8 weeks	5	96 / 98
6 weeks	25	100 / 100
6 weeks	20	100 / 99
6 weeks	15	100 / 100
6 weeks	10	96 / 100
6 weeks	5	97 / 95
4 weeks	25	100 / 100
4 weeks	20	86 / 100
4 weeks	15	85 / 100
4 weeks	10	91 / 93
4 weeks	5	98 / 100
3 periods of 3 weeks	75	100 / 100
3 periods of 3 weeks	60	80 / 100
3 periods of 3 weeks	45	69 / 100
3 periods of 3 weeks	30	61 / 100
3 periods of 3 weeks	15	58 / 100
3 periods of 2 weeks	50	100 / 100
3 periods of 2 weeks	40	98 / 100
3 periods of 2 weeks	30	89 / 100
3 periods of 2 weeks	20	69 / 99
3 periods of 2 weeks	10	76 / 97

Table 1. Continued.

Sampling duration	Number of subsamples	Number of successful replicates for M_h Chao (NPSA/NFSA)
4 periods of 2 weeks	50	100 / 100
4 periods of 2 weeks	40	100 / 100
4 periods of 2 weeks	30	98 / 100
4 periods of 2 weeks	20	80 / 99
4 periods of 2 weeks	10	70 / 99
5 periods of 2 weeks	50	100 / 97
5 periods of 2 weeks	40	100 / 100
5 periods of 2 weeks	30	97 / 100
5 periods of 2 weeks	20	86 / 100
5 periods of 2 weeks	10	83 / 100

Table 2. Configuration of site reduction scenarios considered for sensitivity analysis on the national park study area (NPSA) and national forest study area (NFSA), 2003. Thirteen and 11 different scenarios were considered for the NPSA and NFSA, respectively. Each scenario was replicated 100 times in Program CAPTURE, and the number of replicates that successfully produced output for model M_h Chao are listed.

Number of sites	Number of successful replicates for M_h Chao (NPSA/NFSA)
65 (NPSA only)	100
60 (NPSA only)	100
57 (NFSA only)	100
55 (NPSA only)	100
50	100 / 100
45	100 / 100
40	100 / 100
35	98 / 100
30	99 / 100
25	98 / 100
20	99 / 100
15	99 / 99
10	99 / 98
5	98 / 92

Subsampling intensity was examined at 5 levels: 25, 20, 15, 10, and 5 subsamples. I chose those levels to include a range of samples representative of typical studies based on this technique (Boersen et al. 2003, Thompson 2003, Dobey et al. 2005). Although 25 subsamples were selected each week for DNA analysis, typically only about 85% of those samples actually produced usable quantities of DNA for analysis. Therefore, my sensitivity analysis was based on the assumption that other DNA studies would have similar success rates for DNA analysis

The analysis of the single-week scenarios involved examining all possible combinations of the different levels of sampling duration and subsampling intensity, resulting in a total of 20 scenarios (Table 1). I also used various pooling configurations of the weekly sampling periods to increase sample sizes and capture probabilities within each period. The pooling allowed me to examine the effects of increased capture probabilities on model performance (Menkens and Anderson 1988). The 20 pooled sampling scenarios I examined were 5 periods of 2 weeks, 4 periods of 2 weeks, 3 periods of 2 weeks, and 3 periods of 3 weeks. Subsample levels per week for pooled sampling scenarios were pooled accordingly and thus had subsamples ranging from 10 to 50 samples for 2-week sampling periods, and 15 to 75 samples for the 3-week sampling periods (Fig. 4).

The density of hair-capture sites was examined at 13 and 11 levels for the national park and national forest study areas, respectively (Table 2). Those levels consisted of all hair-capture sites followed by stepwise reductions of 5 hair-capture sites, until only 5 hair-capture sites remained. Due to logistical difficulties encountered in the field, only 57 sites were established in the national forest study area, so the first reduction increment

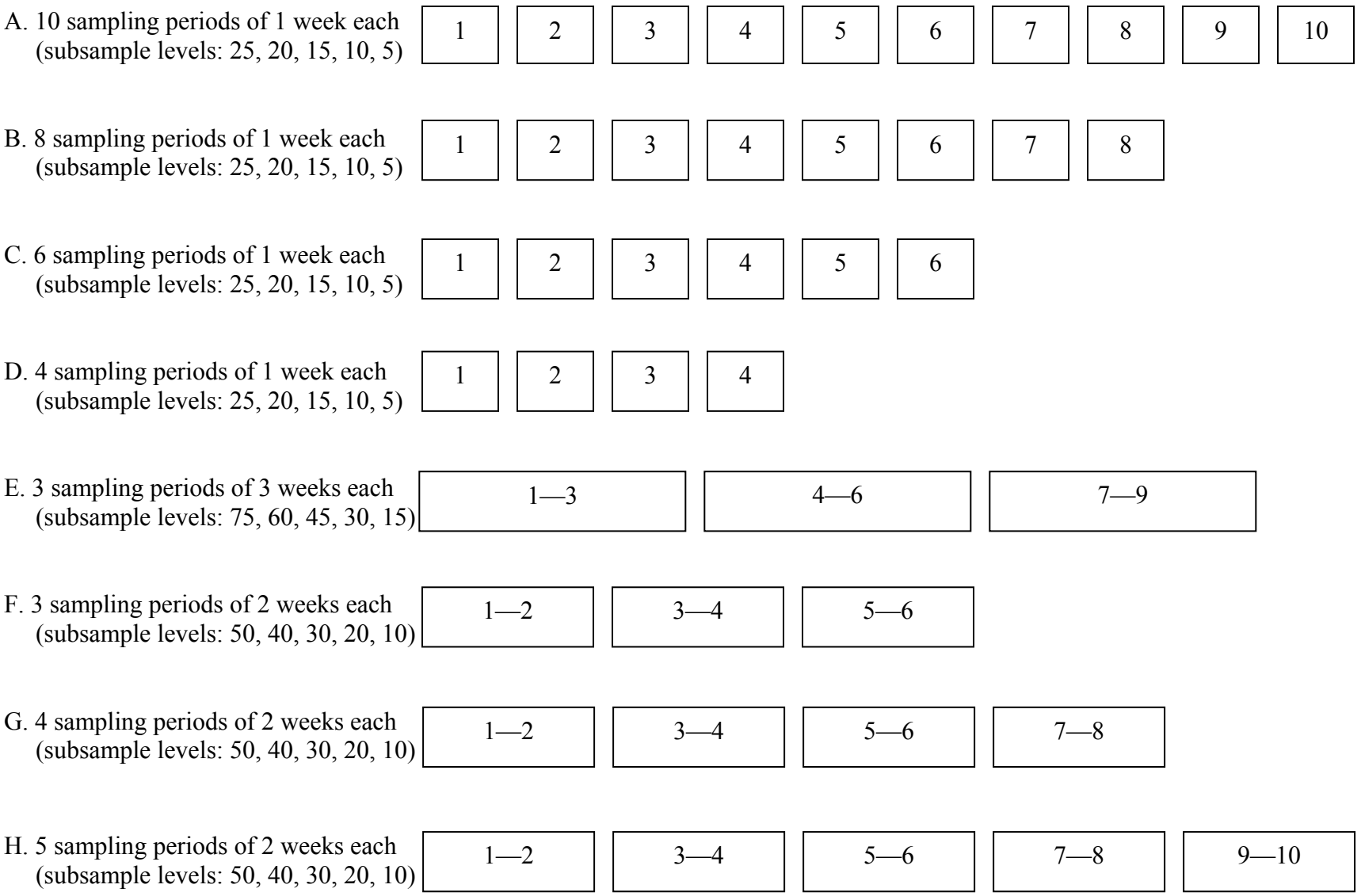


Fig. 4. Pooling configurations of 10 hair-sampling periods (weeks) for the national park and national forest study areas, 2003.

for the national forest study area was 7, and all subsequent reductions were in increments of 5. I chose this level of reduction because 5 hair-capture sites is a meaningful number in terms of field sampling logistics, but small enough to detect trends associated with reductions in the density of hair-capture sites. Those factor levels were expressed as the absolute number of hair-capture sites rather than site density. Although almost all hair-capture sites had bear activity, not all sites were originally represented in the master dataset because of random subsampling. However, for my analysis all sites had an equal probability of being selected for inclusion in a sampling scenario.

I replicated each sampling scenario 100 times to account for variability due to random sampling of data points within the master dataset. Model parameters of interest were averaged across the 100 replications and standard errors (SE) were calculated. If Program CAPTURE was unable to produce results for a scenario (e.g., if the scenario contained no recaptures), then a value of zero was assigned to the model selection criteria, and all other output parameters were averaged with the failed replications removed.

Summary statistics for each sampling scenario and population model were graphed to examine trends in parameter estimates. In particular, I focused on examining the relationship between subsample level, population estimate, and capture probability for the different combinations of sampling duration and site density.

CHAPTER IV

RESULTS

Hair Sampling

Field personnel collected 1,372 hair samples in the national park study area from 9 June to 15 August 2003. All but 1 of the 65 hair-capture sites had bear activity during ≥1 of the 10 sampling periods. The average number of samples collected/week was 137 (SE = 15.11). An average of 41 (SE = 2.06) of the 65 hair-capture sites (63%) had bear activity each week (Fig. 5). In the national forest study area, field personnel collected 584 samples from 9 June to 15 August 2003. Three hair-capture sites were omitted from the original design of 60 hair-capture sites due to sampling logistics encountered in the field. All but 10 of the 57 hair-capture sites had bear activity during ≥1 of the 10 sampling periods. The average number of samples collected per week was 58 (SE = 8.29) and an average of 24 (SE = 1.69) hair-capture sites (42%) yielded samples each week (Fig. 6). Both study areas experienced a peak in site visitation and number of samples collected during week 3 (23 June–27 June; Figs. 5 and 6).

Microsatellite Analysis

For the national park study area, 250 samples were selected for analysis, of which 204 (82%) yielded sufficient quantities of DNA for analysis. The DNA analysis identified 129 individual bears: 41 individuals (represented by 117 samples) were recaptured and 88 individuals were captured only once.

For the national forest study area, 211 samples were selected for analysis because some sampling periods did not yield 25 samples given the condition that each sample came from a different site. Of the 211 samples, 181 (86%) yielded sufficient DNA. For

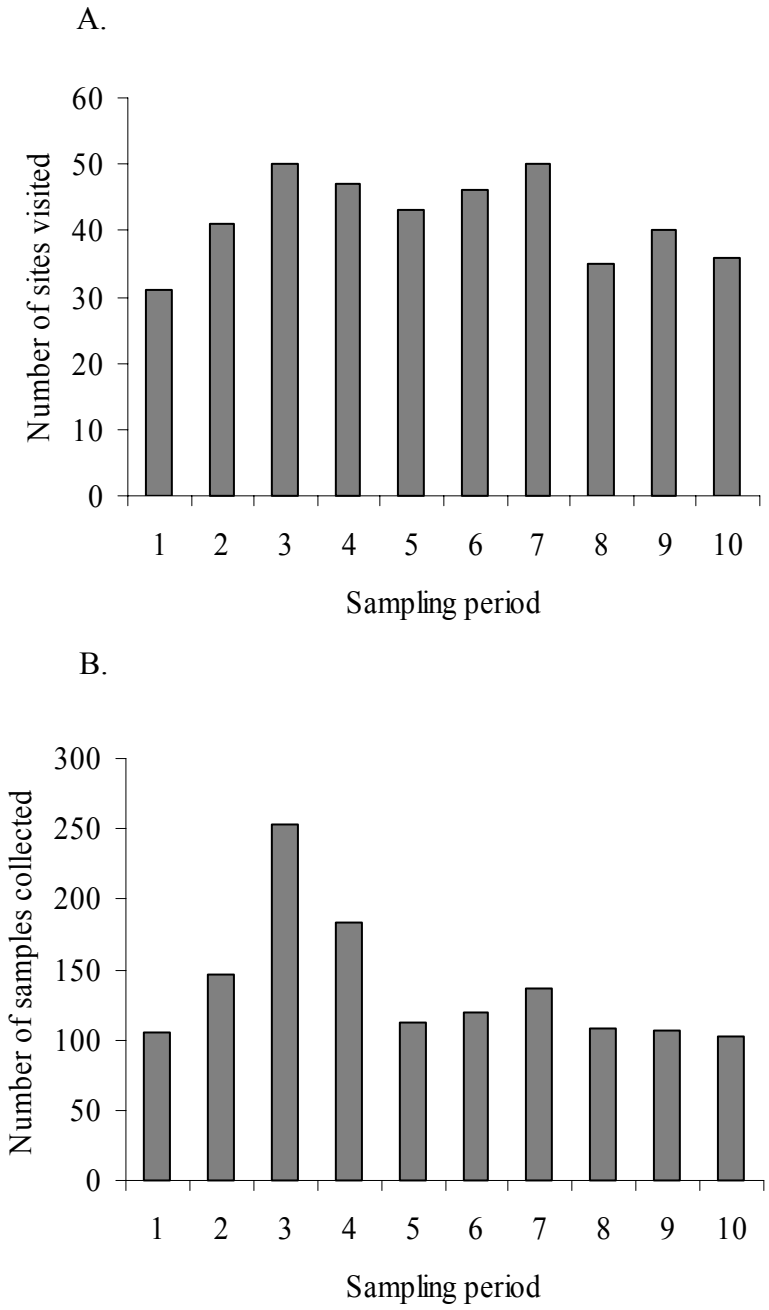


Fig. 5. Summary of hair-sampling results for the national park study area, 2003. A. Number of hair-capture sites visited by black bears/sampling period. B. Number of hair samples collected/sampling period.

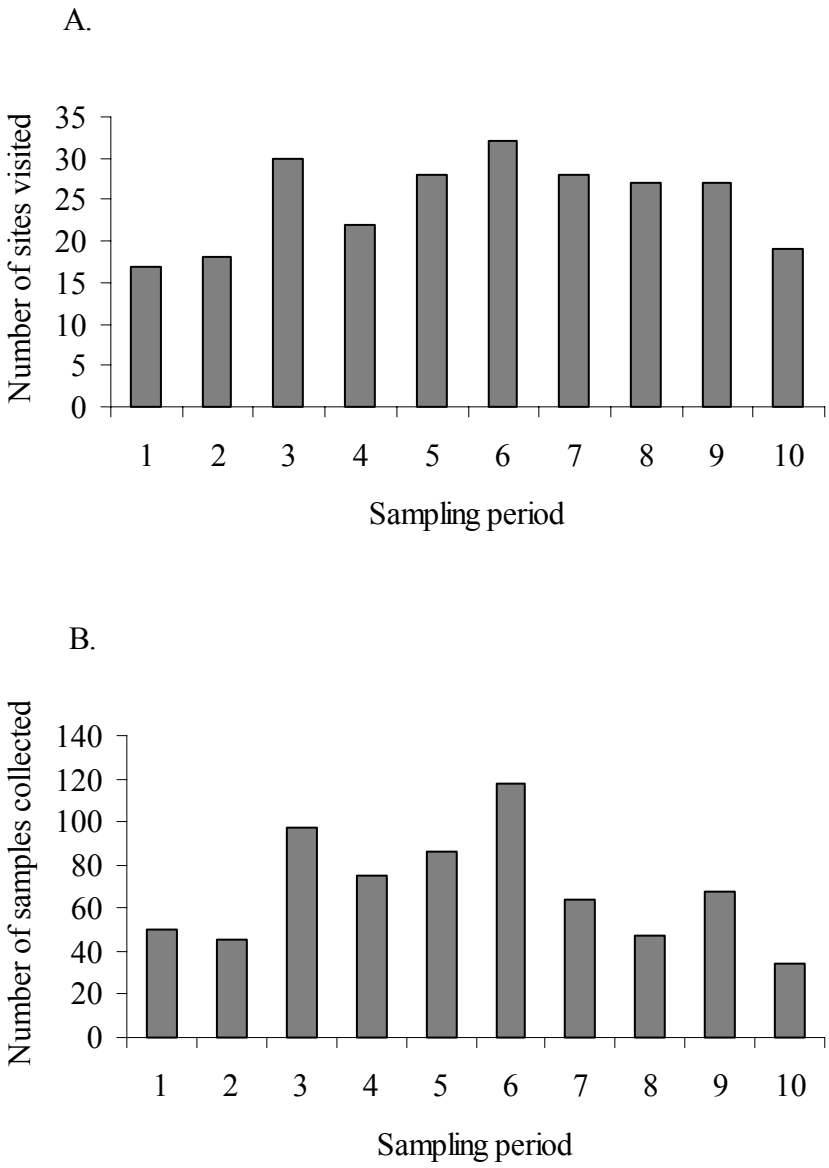


Fig. 6. Summary of hair-sampling results for the national forest study area, 2003. A. Number of hair-capture sites visited by black bears/sampling period. B. Number of hair samples collected/sampling period.

the national forest study area, a problem arose with locus MU50. This locus yielded conflicting and confusing results for some individuals. A few samples were identical at all loci except MU50. It is rare for 2 bears to be identical at 9 loci and only differ at 1 locus if they are indeed unique individuals (T. King, U.S. Geological Survey, personal communication). Therefore, it seemed unlikely that the samples in question were from different individuals. Eliminating MU50 from the analysis for all national forest study area samples and thereby allowing the questionable samples to match at the remaining 9 loci reduced the number of unique individuals from 65 to 60. The decision to remove MU50 was thus intended to reduce potential error in identification. Of the 60 individuals identified, 29 individuals (represented by 150 samples) were recaptured, and 31 individuals were captured once.

For the 10 loci analyzed for the national park study area, 4–10 alleles per locus were observed. The 9 loci analyzed for the national forest study area had 5–9 alleles per locus. I calculated allele frequencies for each locus for both study areas (Table 3).

Probability of Identity

On the national park study area, the $PI_{singlelocus}$ ranged from 0.039 to 0.264 (Table 4). The overall PI estimate was 2.17×10^{-7}, corresponding to a 1 in 4.6 million chance that an individual shared its genotype with another individual. However, the more conservative PI_{sibs} values ranged from 0.358 to 0.547. The overall PI_{sibs} was 8.63×10^{-5}, corresponding to a 1 in 11,587 chance that a bear shared its genotype with another bear. The national forest study area had $PI_{singlelocus}$ values that ranged from 0.050 to 0.209 (Table 5). The overall PI estimate was 7.57×10^{-10}, corresponding to a 1 in 1.3 billion chance that a bear shared its genotype with another bear. PI_{sibs} ranged from 0.347 to

Table 3. Observed allele frequencies for black bears on the national park study area, Tennessee, and national forest study area, North Carolina, South Carolina, Georgia, 2003.

Locus	National park study area	National forest study area
G1A		
(N)	**128**	**60**
1	0.004	0.000
2	0.043	0.167
3	0.186	0.275
4	0.271	0.092
5	0.302	0.167
6	0.186	0.300
7	0.008	0.000
G10B		
(N)	**129**	**59**
1	0.636	0.550
2	0.132	0.200
3	0.031	0.017
4	0.202	0.217
5	0.000	0.017
G10C		
(N)	**129**	**57**
1	0.008	0.000
2	0.023	0.025
3	0.008	0.000
4	0.310	0.400
5	0.368	0.150
6	0.171	0.317
7	0.035	0.000
8	0.043	0.058
9	0.035	0.000
10	0.000	0.050
G1D		
(N)	**128**	**59**
1	0.236	0.058
2	0.260	0.283
3	0.031	0.000
4	0.140	0.033
5	0.019	0.000
6	0.147	0.425
7	0.163	0.100
8	0.004	0.083
9	0.000	0.017

Table 3. (Continued).

Locus	National park study area	National forest study area
G10L		
(N)	**129**	**60**
1	0.322	0.217
2	0.012	0.000
3	0.000	0.042
4	0.143	0.092
5	0.120	0.125
6	0.050	0.083
7	0.147	0.300
8	0.140	0.075
9	0.066	0.067
G10M		
(N)	**127**	**59**
1	0.093	0.183
2	0.124	0.008
3	0.221	0.233
4	0.236	0.117
5	0.081	0.083
6	0.229	0.358
7	0.016	0.017
G10X		
(N)	**129**	**60**
1	0.124	0.108
2	0.380	0.525
3	0.058	0.017
4	0.004	0.000
5	0.132	0.050
6	0.004	0.000
7	0.004	0.117
8	0.004	0.017
9	0.291	0.167

Table 3. (Continued).

Locus	National park study area	National forest study area
MU23		
(N)	**129**	**60**
1	0.008	0.142
2	0.085	0.033
3	0.229	0.067
4	0.236	0.175
5	0.062	0.033
6	0.058	0.017
7	0.000	0.075
8	0.322	0.208
9	0.000	0.250
MU50		
(N)	**128**	
1	0.023	
2	0.062	
3	0.047	
4	0.446	
5	0.016	
6	0.039	
7	0.167	
8	0.000	
9	0.023	
10	0.128	
11	0.043	
12	0.008	
G10P		
(N)	**117**	**57**
1	0.047	0.025
2	0.050	0.008
3	0.012	0.000
4	0.012	0.250
5	0.236	0.242
6	0.186	0.125
7	0.136	0.250
8	0.136	0.042
9	0.089	0.008
10	0.004	0.000
11	0.093	0.050

Table 4. Probability of identity estimates for black bears on the national park study area, Tennessee, 2003 (n = 129).

Locus	Number of alleles	Probability of identity	Probability of identity (siblings)
G10C	9	0.113	0.411
G1A	6	0.095	0.392
G10B	4	0.264	0.547
G10M	6	0.062	0.359
G10X	9	0.112	0.410
G10L	8	0.057	0.358
G1D	8	0.065	0.362
MU23	7	0.086	0.384
MU50	10	0.088	0.398
G10P	10	0.039	0.334
Overall	7.7[a]	2.17×10^{-11} [b]	8.63×10^{-5} [b]

[a] Average number of alleles.
[b] Product of individual values.

Table 5. Probability of identity estimates for black bears on the national forest study area, North Carolina, South Carolina, and Georgia, 2003 (n = 60).

Locus	Number of alleles	Probability of identity	Probability of identity (siblings)
G10C	5	0.131	0.427
G1A	5	0.090	0.387
G10B	5	0.209	0.497
G10M	6	0.092	0.392
G10X	7	0.143	0.452
G10L	8	0.054	0.353
G1D	6	0.120	0.421
MU23	9	0.050	0.347
G10P	8	0.072	0.370
Overall	6.56[a]	7.57×10^{-10} [b]	2.78×10^{-4} [b]

[a] Average number of alleles.
[b] Product of individual values.

0.497. The overall PI_{sibs} was 2.68×10^{-4}, corresponding to a 1 in 3,731 chance of sharing the same genotype. The sibling match test (P_{sib}) indicated that all samples from both study areas had $P_{sib} < 0.05$. Thus, all samples were included in the analysis.

Hardy-Weinberg and Linkage Disequilibrium

For the national park study area, 2 loci (MU50 and MU23) tested significant (P = 0.044 and 0.044, respectively) for violations of Hardy-Weinberg expectations. However, no tests were significant ($\alpha_{crit} = 0.005$) when using the sequential Bonferroni adjustments. Similarly, for the national forest study area, MU50 and G10B loci were significant (P = 0.045 and 0.046, respectively), but not after sequential Bonferroni adjustments (α_{crit} = 0.006).

The linkage disequilibrium test for the national park study area showed associations among 8 of 45 pairs of loci ($\alpha_{crit} = 0.05$), but only 1 pair remained significant after sequential Bonferroni adjustment ($\alpha_{crit} = 0.0011$). For the national forest study area, 15 of 36 pairs of loci showed associations ($\alpha_{crit} = 0.05$) and 2 pairs remained significant after sequential Bonferroni adjustments ($\alpha_{crit} = 0.0014$).

Sensitivity Analysis

Model Selection Criteria.—For both study areas, and for all sampling scenarios, Program CAPTURE's model selection criteria did not indicate the presence of significant behavioral or temporal variations in capture probabilities. Therefore, I specifically focused on the results of the null model (M_o), the jackknife heterogeneity model (M_h jackknife), and the Chao heterogeneity model (M_h Chao). The null model and the

heterogeneity model were the 2 most frequently selected models (Table 6). The Chao models are not included in Program CAPTURE's model selection criteria.

For the single-week sampling scenarios on the national park study area, the null model was selected for all but 3 scenarios, for which the heterogeneity model was selected (10 week/25 subsample (i.e., 10w25s), 10w20s, and 8w25s). The single-week scenarios on the national forest study area were more evenly divided among the 2 models, with the heterogeneity model selected more often for scenarios with 6, 8, and 10 weeks and subsamples in the 10–25 range. For the sampling scenarios with reduced data, the null model was most often selected.

For the pooled sampling scenarios on the national park study area, the null model was selected for all but 2 scenarios (5 periods of 2 weeks each period/50 subsamples, i.e., 5p2w50s and 5p2w40s), for which the heterogeneity model was selected (Table 6). For the national forest study area, patterns were similar to the single-week data. The larger datasets (i.e., the 3 largest subsample levels; 3p3w, 4p2w, and 5p2w) were better fitted to the heterogeneity model by Program CAPTURE and the sparser datasets were better fitted to the null model. However, all 5 sampling scenarios with 3 periods of 2 weeks were best fitted to the null model.

For reductions in density of hair-capture sites on the national park study area, site levels of 40–65 and 5–35 were best fitted to the heterogeneity and null model, respectively (Table 6). Similarly, for the national forest study area the heterogeneity and null models were best fitted to site levels of 25–57 and 5–20, respectively.

Closure.—Program CAPTURE's test for population closure detected a lack of closure (i.e., P-value < 0.05) on the national park study area for only 2 scenarios: 8w25s

Table 6. Model selected by Program CAPTUREs model selection routine for each sampling scenario for the national park study area (NPSA) and national forest study area (NFSA), 2003.

Sampling scenario	NPSA model	NFSA model
10 weeks		
25 subsamples	M_h jackknife	M_h jackknife
20 subsamples	M_h jackknife	M_h jackknife
15 subsamples	M_o	M_h jackknife
10 subsamples	M_o	M_h jackknife
5 subsamples	M_o	M_o
8 weeks		
25 subsamples	M_h jackknife	M_h jackknife
20 subsamples	M_o	M_h jackknife
15 subsamples	M_o	M_h jackknife
10 subsamples	M_o	M_o
5 subsamples	M_o	M_o
6 weeks		
25 subsamples	M_o	M_h jackknife
20 subsamples	M_o	M_h jackknife
15 subsamples	M_o	M_h jackknife
10 subsamples	M_o	M_o
5 subsamples	M_o	M_o
4 weeks		
25 subsamples	M_o	M_o
20 subsamples	M_o	M_o
15 subsamples	M_o	M_o
10 subsamples	M_o	M_o
5 subsamples	M_o	M_o
3 periods of 2 weeks		
50 subsamples	M_h jackknife	M_o
40 subsamples	M_h jackknife	M_o
30 subsamples	M_o	M_o
20 subsamples	M_o	M_o
10 subsamples	M_o	M_o
3 periods of 3 weeks		
75 subsamples	M_o	M_h jackknife
60 subsamples	M_o	M_h jackknife
45 subsamples	M_o	M_o
30 subsamples	M_o	M_o
15 subsamples	M_o	M_o

Table 6. (Continued).

Sampling scenario	NPSA model	NFSA model
4 periods of 2 weeks		
50 subsamples	M_o	M_h jackknife
40 subsamples	M_o	M_h jackknife
30 subsamples	M_o	M_o
20 subsamples	M_o	M_o
10 subsamples	M_o	M_o
5 periods of 2 weeks		
50 subsamples	M_o	M_h jackknife
40 subsamples	M_o	M_h jackknife
30 subsamples	M_o	M_h jackknife
20 subsamples	M_o	M_o
10 subsamples	M_o	M_o
Sites		
65	M_h jackknife	
60	M_h jackknife	
57		M_h jackknife
55	M_h jackknife	M_h jackknife
50	M_h jackknife	M_h jackknife
45	M_h jackknife	M_h jackknife
40	M_h jackknife	M_h jackknife
35	M_o	M_h jackknife
30	M_o	M_h jackknife
25	M_o	M_h jackknife
20	M_o	M_o
15	M_o	M_o
10	M_o	M_o
5	M_o	M_o

and 8w20s. Lack of closure was detected on the national forest study area for 4 scenarios: 10w25s, 6w25s, 57 sites, and 50 sites. As site density decreased on the national forest study area, *P*-values for the closure tests increased. For the national park study area, *P*-values for the closure tests were relatively constant across varying densities of sample sites.

Comparison of Sampling Scenarios.—For both study areas, several consistent trends were apparent. The 3 field design factors of subsample intensity, site density, and sampling duration all had considerable influence on capture probabilities, population estimates, and the precision of the population estimates. However, these trends differed slightly for each of the 3 models. A reduction in subsamples, sites, or sampling duration generally resulted in a reduction of the population estimate (Figs. 7–14). This trend was most apparent with the M_h jackknife estimator (Figs. 11 and 12), although it was also present with M_o, and, to a lesser extent, M_h Chao. Population estimates generally increased within each subsample level as sampling duration increased. Similarly, scenarios with longer sampling durations generally produced larger population estimates than scenarios with shorter durations, regardless of subsample level.

The confidence intervals of the estimates produced by Program CAPTURE generally increased as the number of subsamples decreased across all sampling durations for model M_o. The opposite was true, however, for model M_h. Confidence intervals for model M_h increased as the number of subsamples increased, although they generally were not as variable across all scenarios as were the confidence intervals for M_o. Confidence intervals for the M_h Chao model were less consistent. Longer sampling durations (i.e., 8

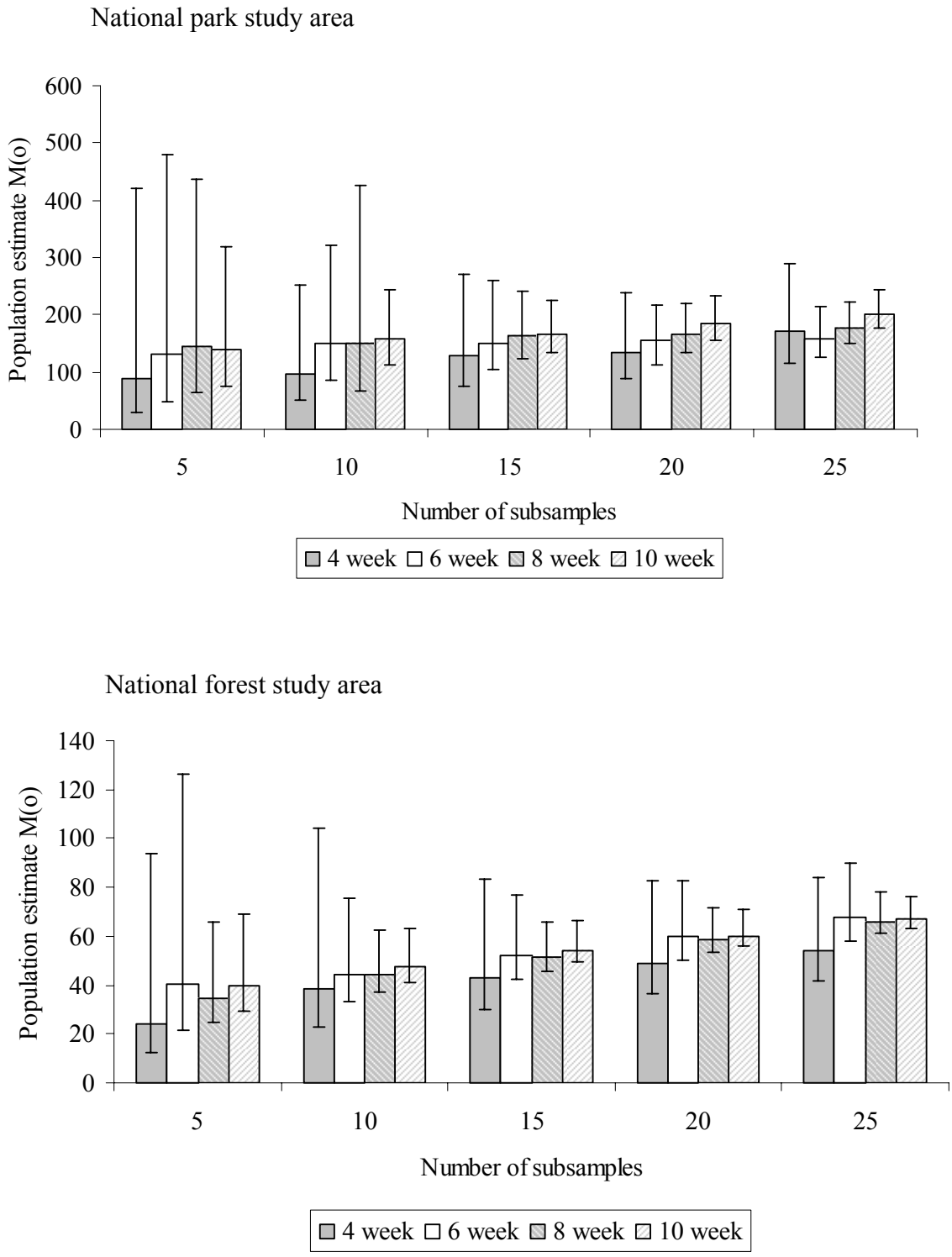
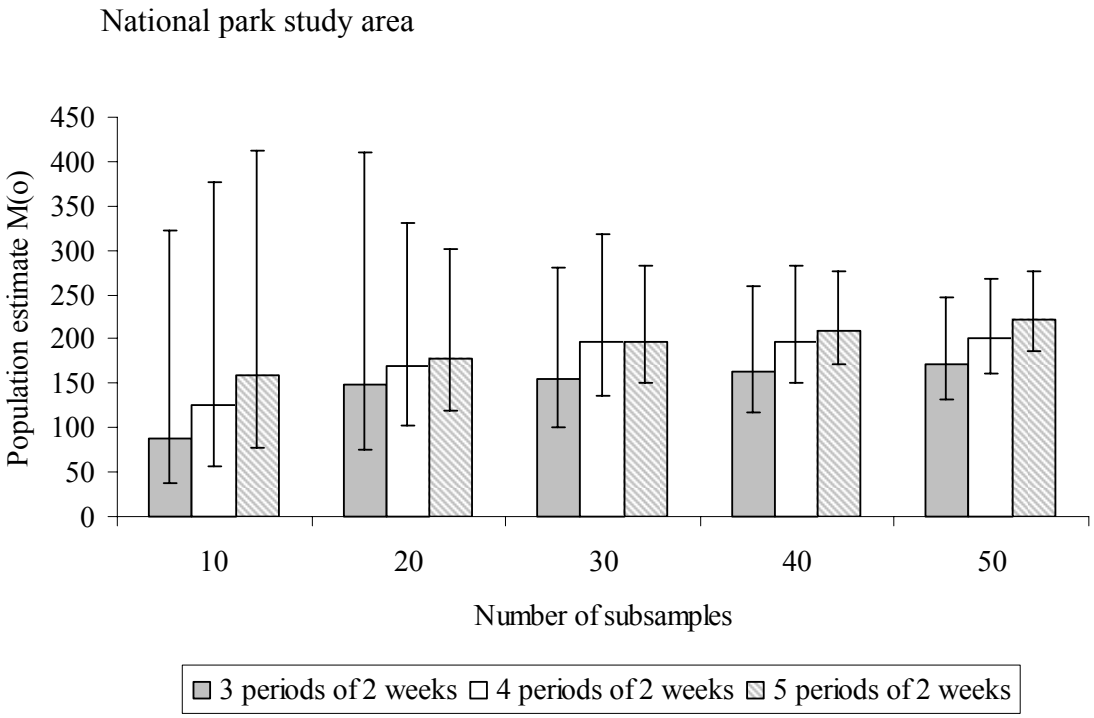


Fig. 7. Relationship between estimates of black bear population abundance (95% confidence interval, model M_o) and subsampling intensity for 4 sampling duration schemes in the national park study area and national forest study area, 2003



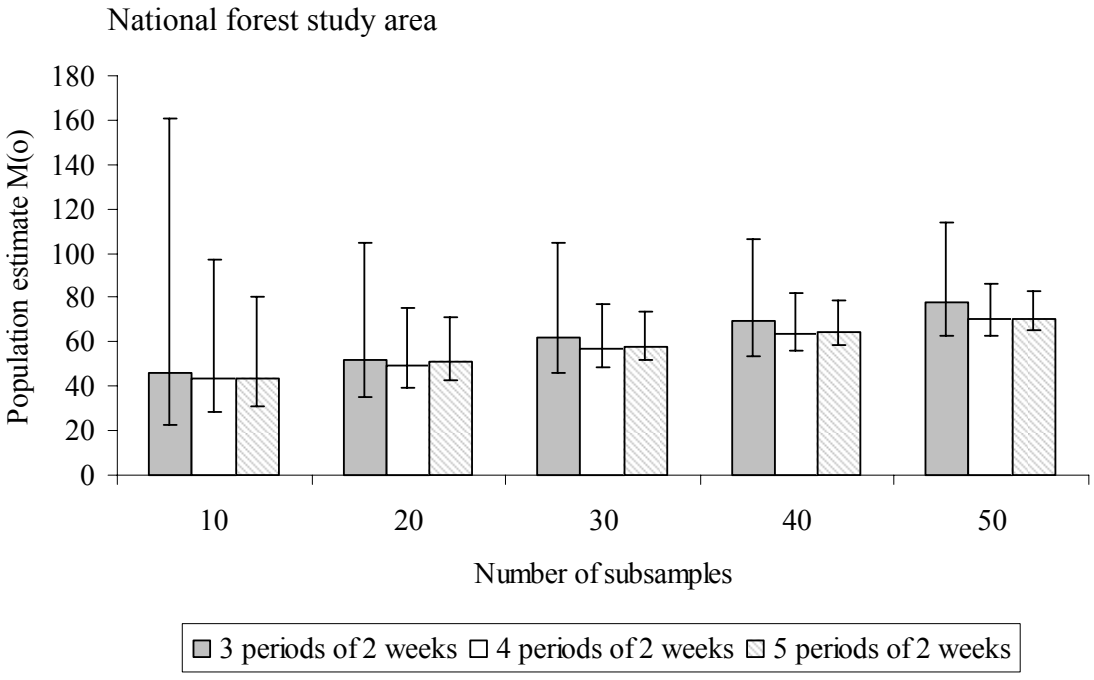


Fig. 8. Relationship between estimates of black bear population abundance (95% confidence interval, model M_o) and subsampling intensity for 3 pooled sampling duration schemes in the national park study area and national forest study area, 2003.

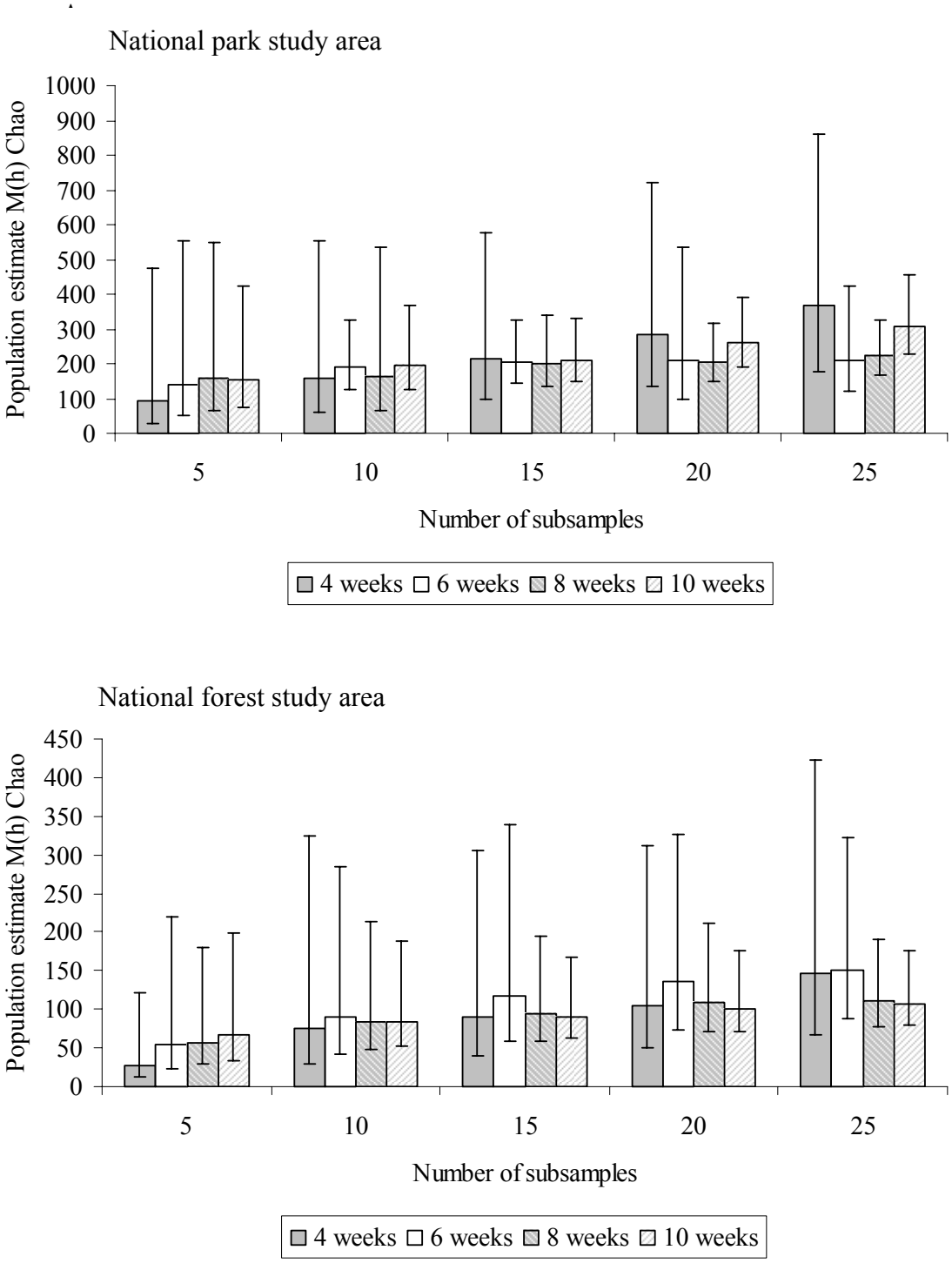
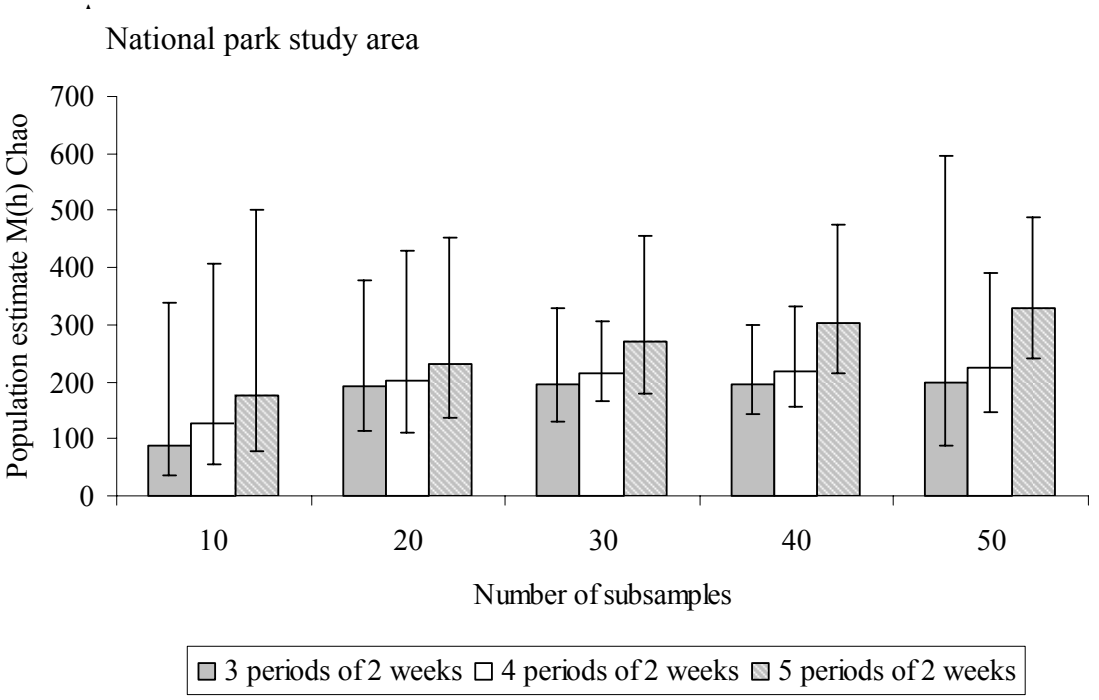


Fig. 9. Relationship between estimates of black bear population abundance (95% confidence interval, model M_h Chao) and subsampling intensity for 4 sampling duration schemes in the national park study area and national forest study area, 2003.



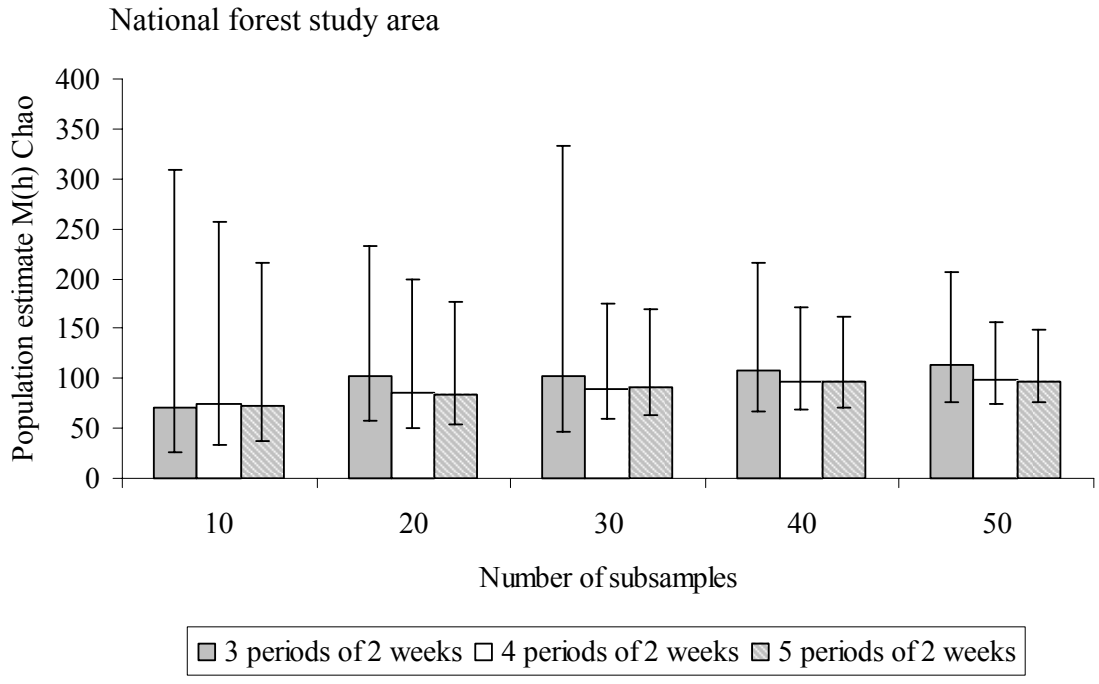


Fig. 10. Relationship between estimates of black bear population abundance (95% confidence interval, model M_h Chao) and subsampling intensity for 3 pooled sampling duration schemes in the national park study area and national forest study area, 2003.

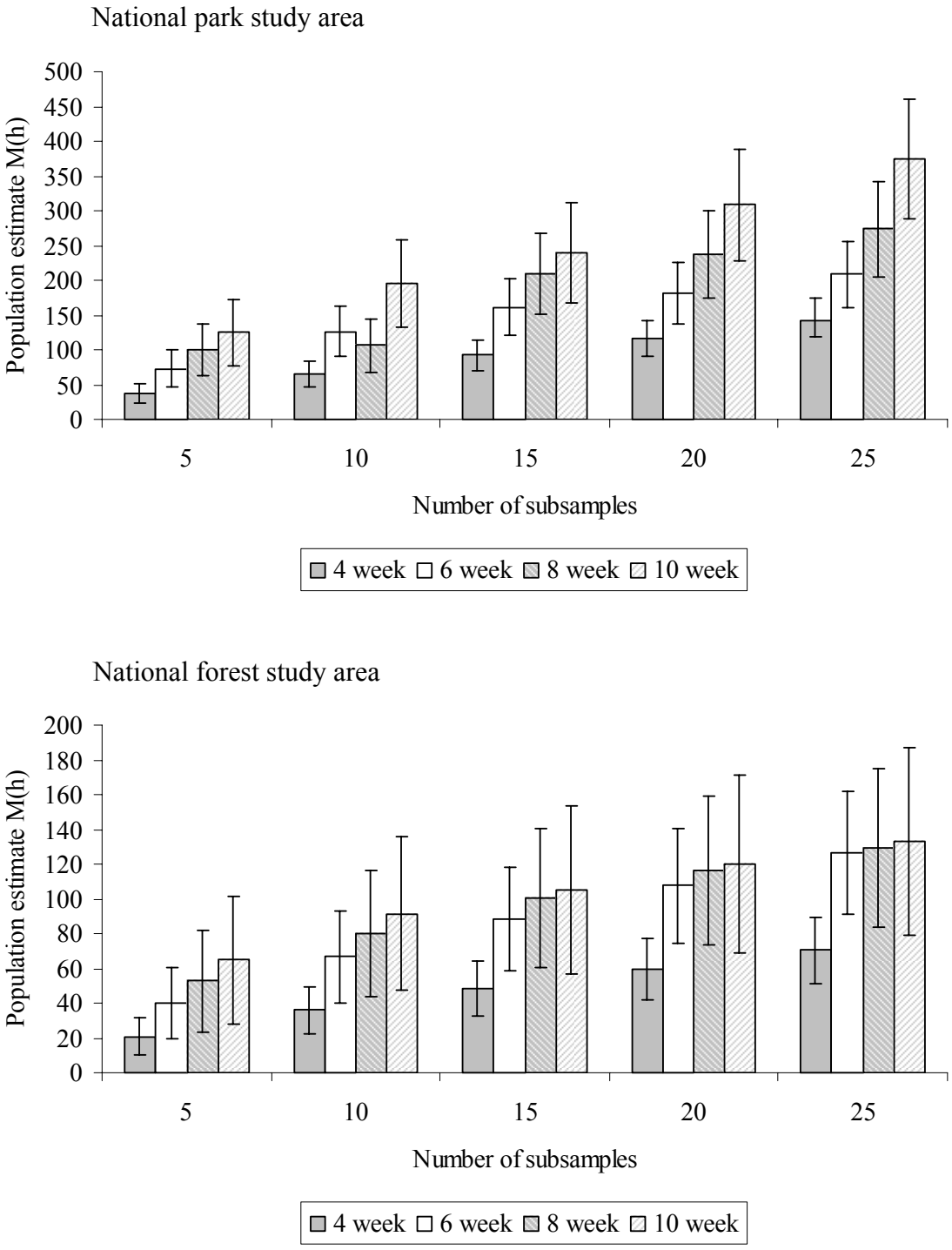


Fig. 11. Relationship between estimates of black bear population abundance (95% confidence interval, model M_h jackknife) and subsampling intensity for 4 sampling duration schemes in the national park study area and national forest study area, 2003.

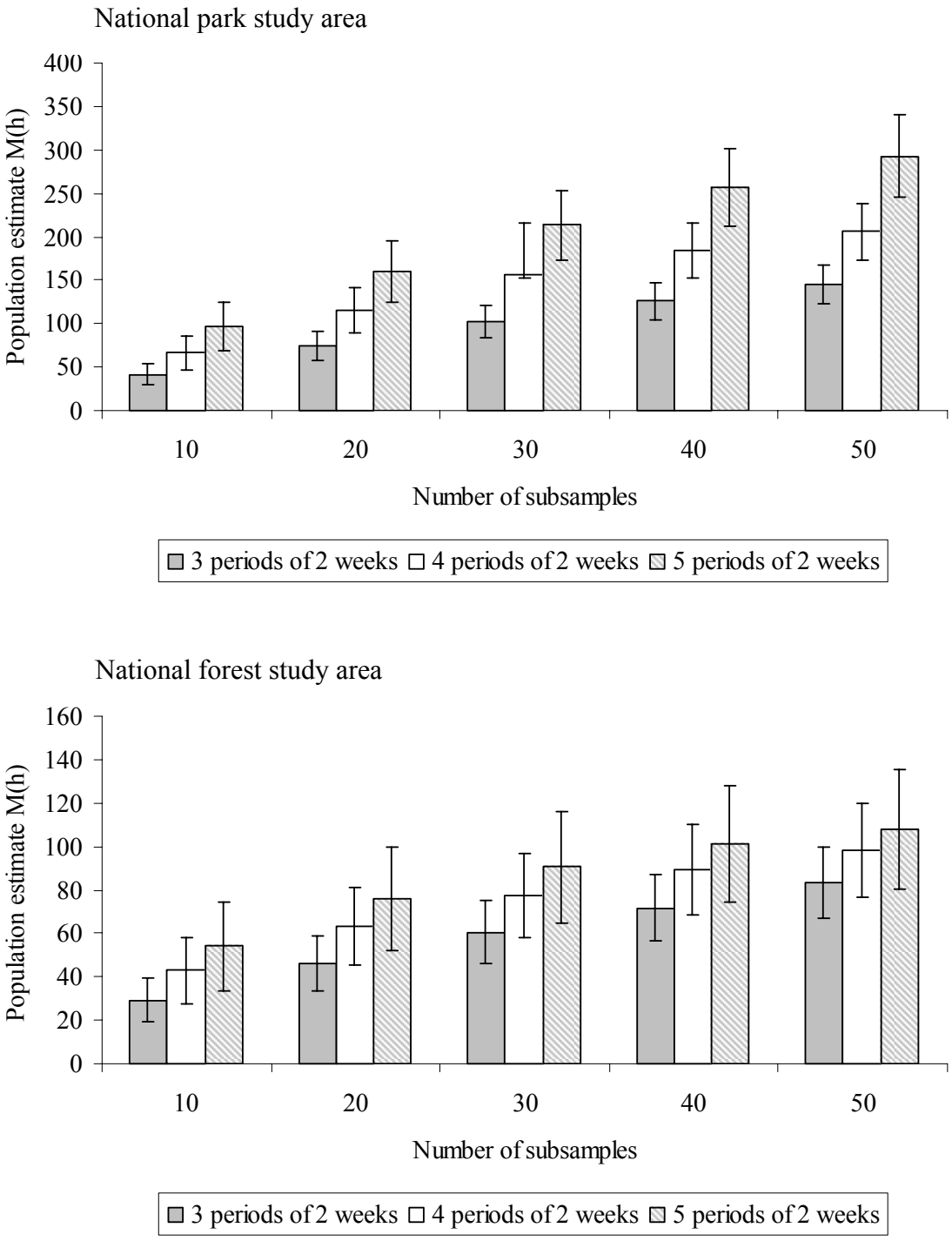


Fig. 12. Relationship between estimates of black bear population abundance (95% confidence interval, model M_h jackknife) and subsampling intensity for 3 pooled sampling duration schemes in the national park study area and national forest study area, 2003.

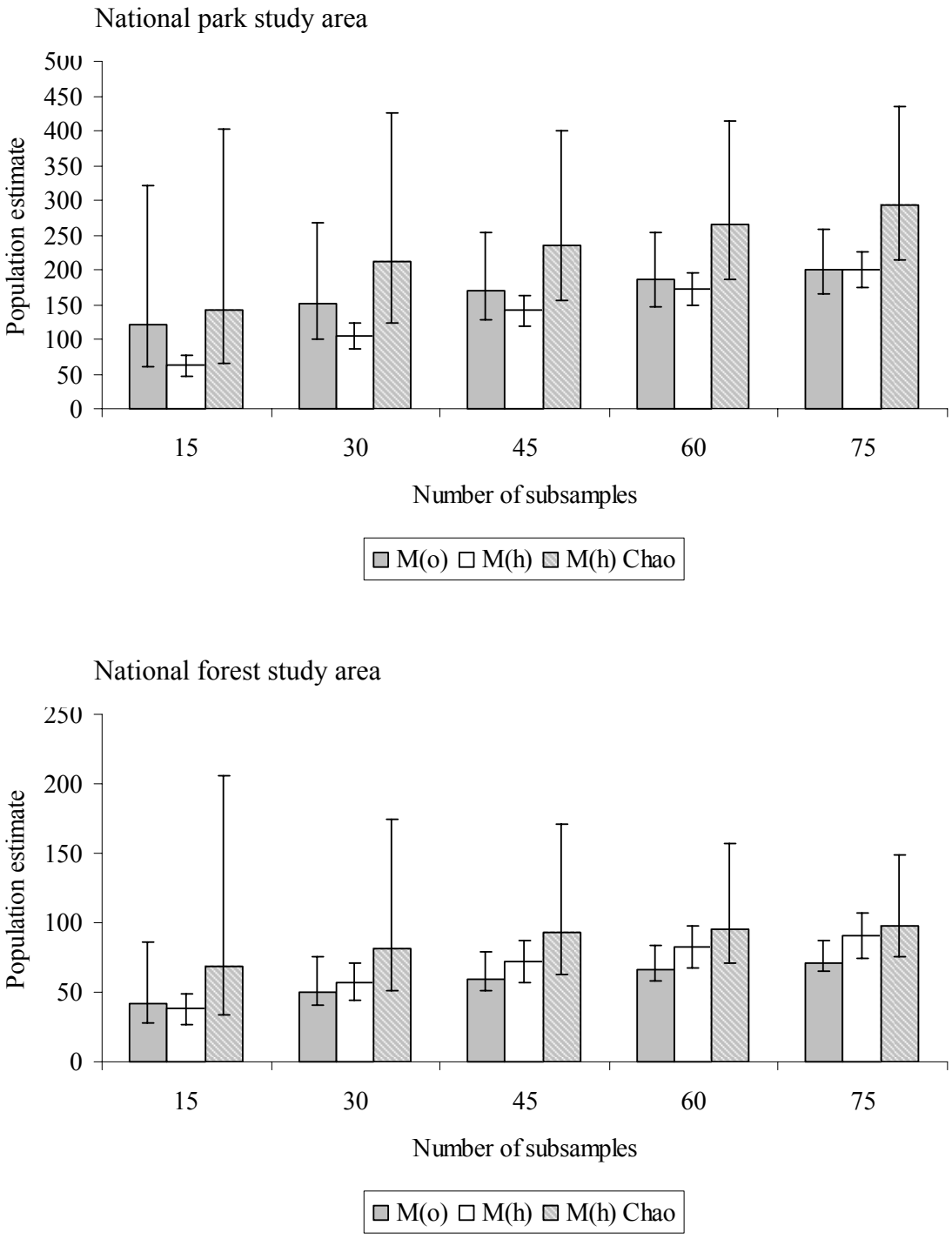


Fig. 13. Relationship between estimates of black bear population abundance (95% confidence interval, models M_o, M_h, and M_h Chao) and subsampling intensity for the pooled sampling duration scheme of 3 periods of 3 weeks in the national park study area and national forest study area, 2003.

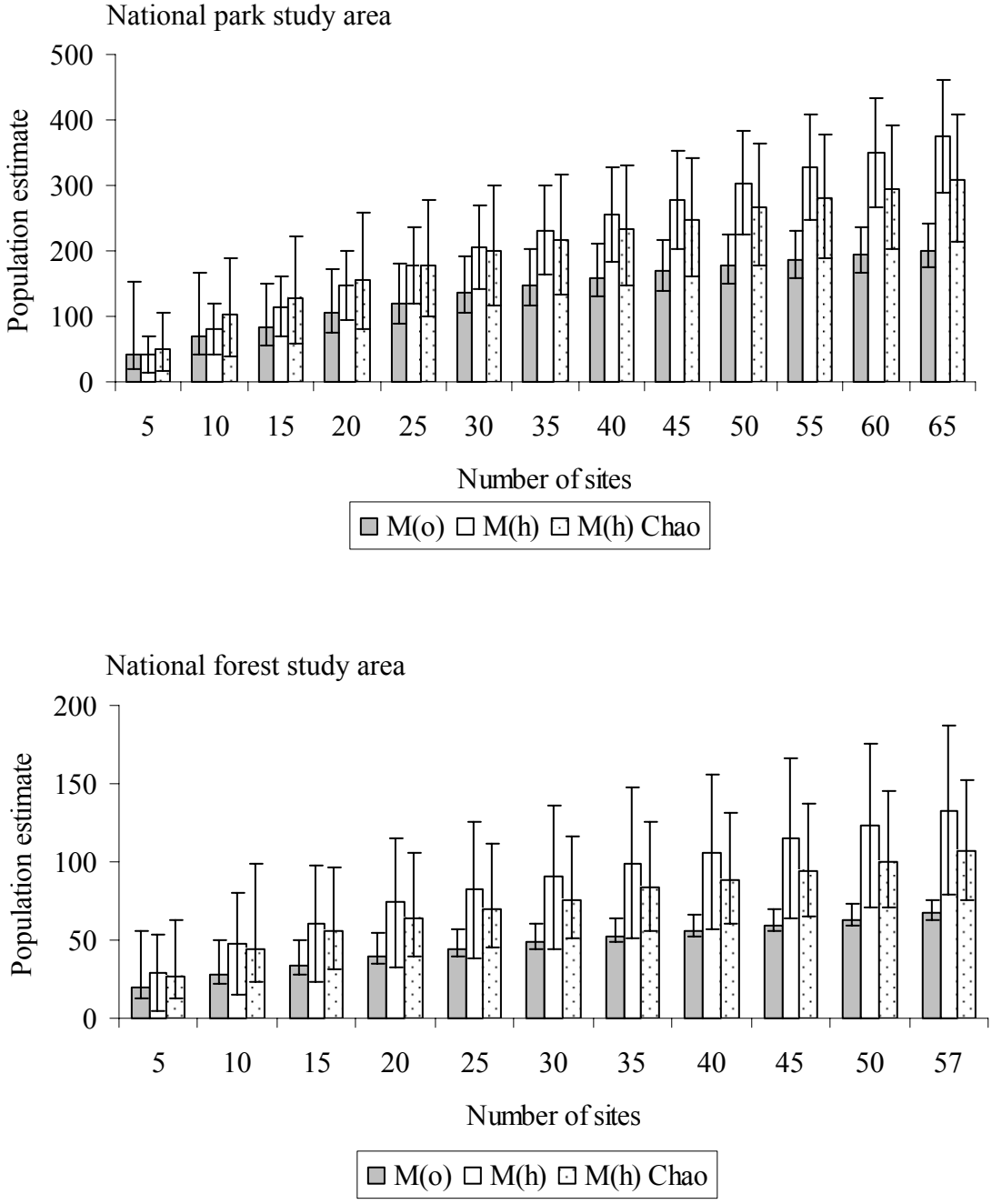


Fig. 14. Relationship between estimates of black bear population abundance (95% confidence interval, models M_o, M_h, and M_h Chao) and number of sites for the national park study area and national forest study area, 2003.

weeks, 10 weeks, and 5 periods of 2 weeks) showed increasing precision as the number of subsamples increased, but the sampling scenarios with shorter duration displayed no clear trends.

Capture probability was another parameter that displayed strong trends. For both study areas and all 3 models, subsampling intensity was closely related to capture probability. Capture probability generally increased as the number of subsamples increased (Figs. 15–17). However, contrary to that trend, capture probability tended to increase as sampling duration decreased. This trend was most evident for model M_h and was most prominent for the national park study area. Capture probabilities for the 4-week sampling scenarios appeared to be inflated compared with the 6-, 8-, and 10-week sampling scenarios, particularly for model M_h (Figs. 15B and 16B). Although capture probabilities are estimated in Program CAPTURE, no confidence intervals are provided for this statistic, so I did not examine the precision associated with these trends.

Both study areas displayed the same general trends for all 3 models, but there were some differences. Compared with the national park study area, parameter estimates in the national forest study area were slightly more uniform across scenarios (i.e., population estimates and capture probabilities produced by similar scenarios did not differ as much as those in the national park study area). Also, there generally were fewer instances of model failure for national forest study area scenarios compared with the national park study area (Tables 1 and 2). Population estimates for the national forest study area were lower than those for the national park study area and capture probabilities were greater (Figs. 7–17).

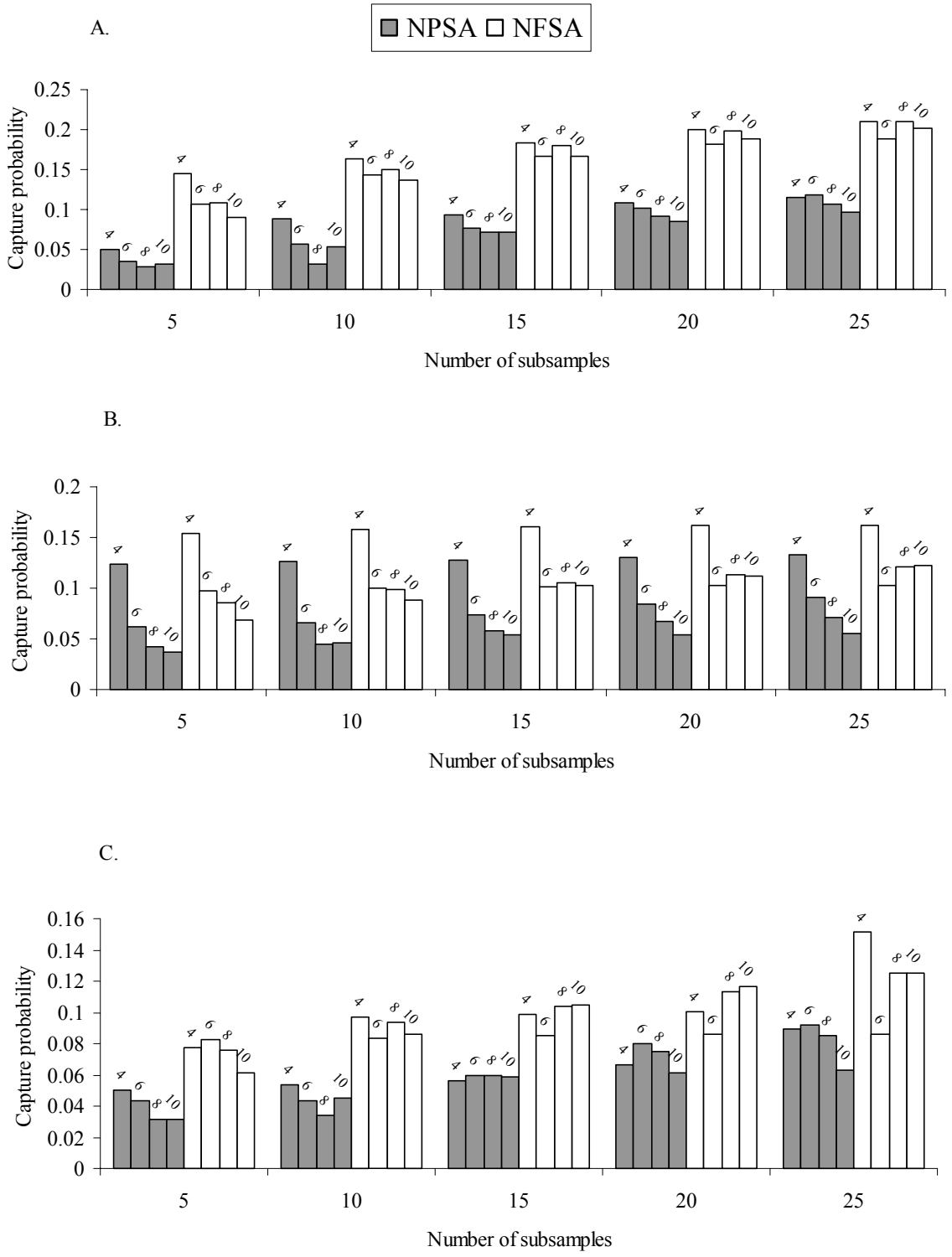


Fig. 15. Relationship between capture probability and number of subsamples for models M_o (A), M_h (B), and M_h Chao (C). The figure represents values for the single week sampling scenarios of 4 weeks, 6 weeks, 8 weeks, and 10 weeks, represented from left to right on the x-axis, for both the national park study area (NPSA) and national forest study area (NFSA), 2003.

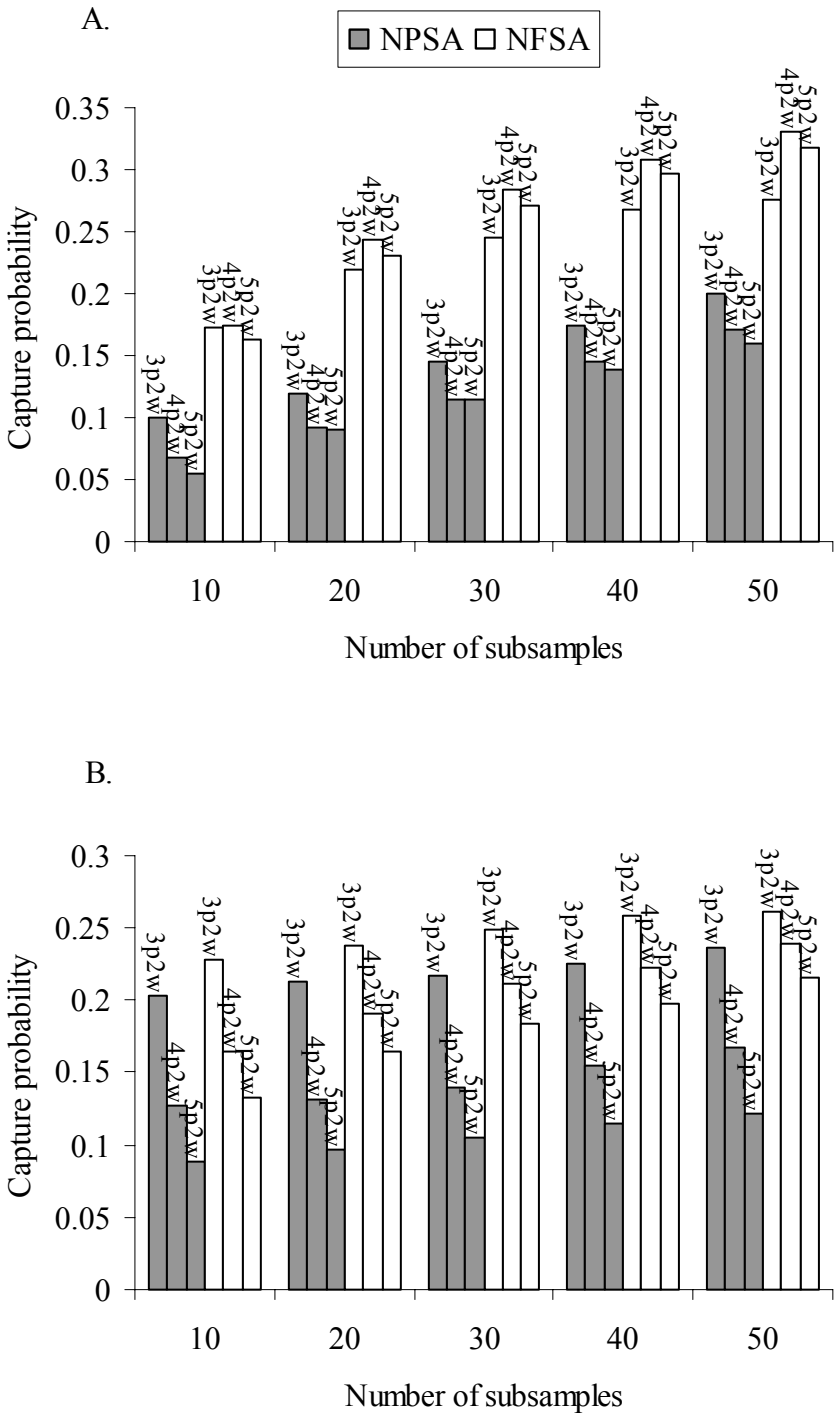


Fig. 16. Relationship between capture probability and number of subsamples for models M_o (A), M_h (B), and M_h Chao (C). The figure represents values for the 3 pooled week sampling scenarios of 3 periods of 2 weeks (i.e., 3p2w), 4p2w, and 5p2w, represented from left to right on the x-axis, for both the national park study area (NPSA) and national forest study area (NFSA), 2003.

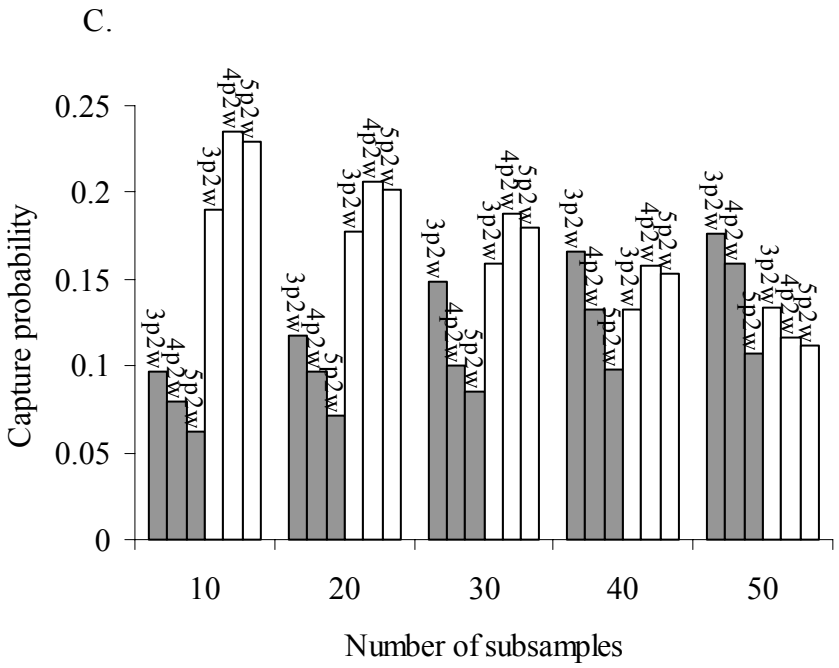
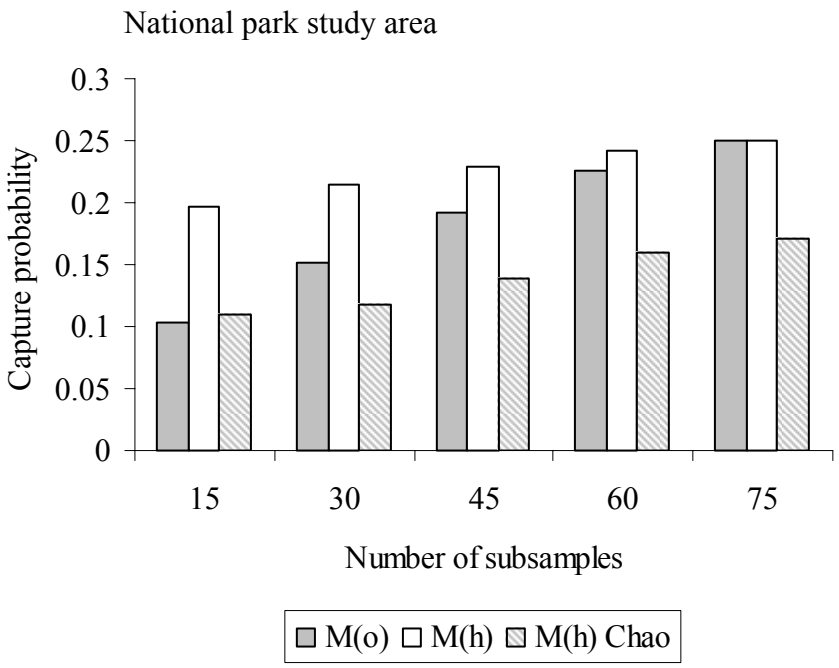


Fig. 16. Continued. (C.) M_h Chao.



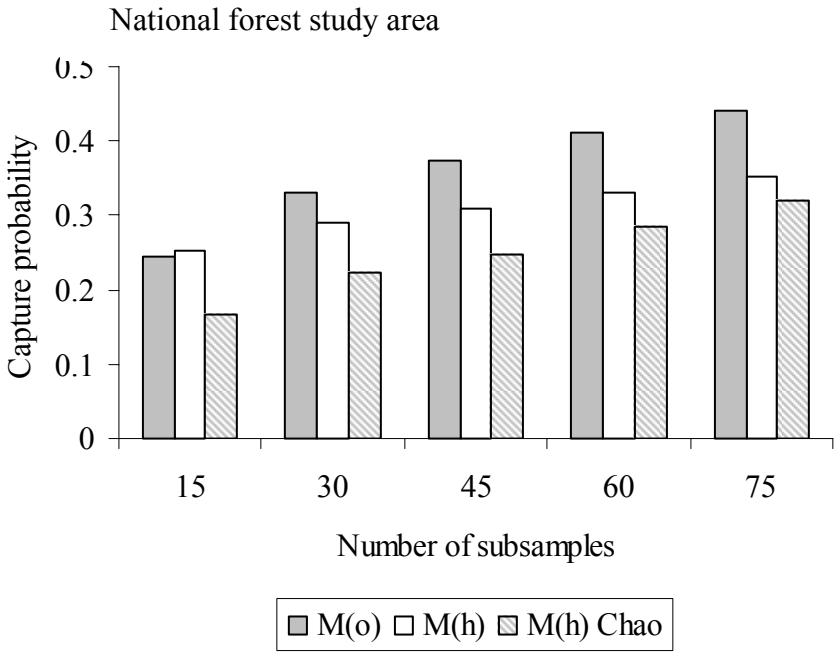


Fig. 17. Relationship between capture probability and number of subsamples for models M_o, M_h, and M_h Chao. The figure represents values for the pooled week sampling scenario 3 periods of 3 weeks for the national park study area and national forest study area, 2003.

Population Estimation

For population estimation, I chose the sampling scenario that had the largest capture probability, which was the 3 periods of 3 weeks (75 subsamples) scenario. I used model M_h Chao, which produced an estimate for the national park study area of 292 bears, with a 95% confidence interval of 214–435 and an estimated capture probability of 0.17. The estimate for the national forest study area was 98 bears, with a 95% confidence interval of 76–149 and an estimated capture probability of 0.32.

CHAPTER V

DISCUSSION

Data Quality

An estimate of animal abundance is most useful when it is both accurate and precise. Accuracy refers to how closely the estimate reflects reality, whereas precision refers to the level of uncertainty of the estimate (White et al. 1982). The precision of a population estimate is expressed through statistics such as the CV or confidence interval. Accuracy, however, is difficult to assess in studies where population size is not known. Many factors can affect the accuracy and precision of a population estimate, such as low sample sizes, biases in the data that are inherent to sampling design, or model behavior under the influence of these factors.

Sparse Data.—My data generally displayed smaller population estimates and capture probabilities as sample size decreased. Although the trends in population estimates cannot be strictly interpreted because the 95% confidence intervals of the estimates often overlapped, the consistency of these patterns suggests that small sample size contributed to bias in the population estimate. This effect also was reflected in a corresponding decrease in precision of the population estimates for models M_o and M_h Chao. Sparse data occur when sample sizes and capture probabilities are small. Closed population models require a minimum amount of input data to produce reliable population estimates that are both accurate and precise (Otis et al. 1978, White et al. 1982). For example, Otis et al. (1978) simulated a population of 400 individuals sampled over 7 sampling occasions, and partitioned their simulated data into 3 quality levels based

on capture probability: good ($\hat{p} = 0.35$), medium ($\hat{p} = 0.20$), and poor ($\hat{p} = 0.05$). They found that population estimates declined in accuracy and precision as capture probability decreased and concluded that high capture probabilities are important for reliable model performance. Similarly, Boulanger et al. (2002) stated that capture probabilities >0.20 are needed to accurately estimate a population abundance of about 100, and White et al. (1982) suggested that capture probabilities should never be <0.30 and, ideally, close to 0.50 for $N < 100$ when trapping occasions are ≤10. Those authors further stated that if these criteria can not be achieved, then it is unlikely the estimate will be precise or unbiased. White et al. (1982) concluded that, in general, as population size decreases, capture probabilities must increase in order to maintain similar model performance. These issues illustrate that prior knowledge regarding the population of interest is important before an effective mark-recapture study can be conducted.

Trends in population abundance estimates may be an indication that the modeling procedures require greater samples sizes and capture probabilities to produce unbiased population estimates. Theoretically, population estimates should approach an asymptote if a sufficient quantity of data were used for modeling. My results for the national park study area do not seem to approach a point of stabilization, whereas the larger sample size scenarios on the national forest study area seem to stabilize (Figs. 10 B and 13 B). Although capture probabilities for both study areas were relatively small, the national forest study area had higher capture probabilities than the national park study area, which would account for better model performance.

Other, more subtle, indicators of sparse data were present from my analysis. For instance, as sample sizes increased, model complexity increased (Stanley and Burnham

1998). The null model was most often selected, whereas the M_h jackknife model was selected only for a few sampling scenarios with large sample sizes and high capture probabilities. Program CAPTURE has limited power to detect variations in capture probability when capture probabilities are low (Boulanger and McLellan 2001) and it has been specifically shown that the model selection procedure has low power to detect heterogeneity when population size is <200 (Boulanger et al. 2004*a*). Thus, heterogeneity likely was present in my data, but Program CAPTURE probably could not detect it due to small sample sizes. Another indication of a sparse data effect was that model failure occurred more often with small sample scenarios (e.g., 4 week/5subsample scenario for the national park study area), which was caused by zero recaptures in the input dataset. Model failure occurred slightly more often in the national park study area, which is another indication that data from the national park study area was not as sufficient as the national forest study area for estimating abundance.

Lastly, Otis et al. (1978) suggest that adequate sampling should result in few unmarked individuals captured towards the end of the sampling period. In the national forest study area, 2 of 14 bears identified in week 9 were unmarked individuals, and 1 of 10 bears identified in week 10 was an unmarked individual. In the national park study area, however, 10 of 22 bears identified in week 9 and 13 of 21 bears identified in week 10 were initial captures. Consequently, out of 129 total bears identified in the national park study area, 18% came from the last 2 sampling periods. Thus, sampling in the national park study area may have detected an insufficient proportion of the entire study population.

Violations of Assumptions.—Aside from sparse input data, the performance of the models may be explained in part due to violations of assumptions. Primarily, the presence of heterogeneity can cause notable bias (Cormack 1968). In general, the magnitude and direction of bias introduced by heterogeneity can be difficult to predict (Otis et al. 1978). However, in my study, insufficient sampling (particularly for the smaller sampling scenarios) likely led to recapturing the same individuals repeatedly while neglecting to capture others, leading to an underestimate of abundance.

The trend of increasing population estimates as sampling duration increased likely can be attributed to violations in geographic closure. As the number of sampling periods increased, some marked bears likely moved out of the study population, whereas the probability of capturing bears previously outside the study area increased. This process resulted in decreasing estimates of capture probability and increasing population estimates as sampling duration increased, as was the case for my study. This effect was particularly amplified for model M_h, as evidenced by more distinct trends in population estimates and capture probabilities with increasing sampling duration. Those high capture probabilities may be related to the heterogeneity models adjusting capture probabilities upward to account for animals in the population that are less likely to be captured. Alternatively, the high values may be associated with high visitation on both study areas during week 3, which may have influenced capture probabilities and model performance for the 4-week sampling scenarios. However, no temporal or behavioral effects were detected by Program CAPTURE. The trends that resulted from closure violations were more apparent for the national park study area than the national forest study area, particularly for the longer durations and larger subsamples (Figs. 7 and 8).

This could be the product of a lower-density bear population combined with a study area that is surrounded by less contiguous bear habitat compared with the national park study area.

The trend of increasing population estimates as the number of subsamples increased is related to the link between subsamples and hair-capture sites. Reducing sites or samples resulted in reducing the effective study area size, thus violating the assumption of a known and defined study area. This effect was exacerbated due to heterogeneity, which may have been caused by insufficient availability of hair-capture sites. Because subsamples and sites were inextricably linked, reducing the number of subsamples in effect reduces the number of sites, which makes site availability even less sufficient. For example, Fig. 18 illustrates the impact of the linkage between hair-capture sites and subsamples on the effective study area size. Scenarios in which either subsamples or sites were reduced essentially produced population estimates for smaller study areas. As a result, the estimates using model M_h Chao for the 3 examples varied widely (16 bears, 161 bears, and 309 bears, respectively). I speculate that the lower population estimates produced from the reduced sampling scenarios may have corresponded to different and smaller study areas.

Study Design and Implementation

Several aspects of my study design potentially impacted the accuracy and precision of the population abundance estimates. I will focus on 3 factors that directly affect the ability of a DNA study to produce reliable population estimates: sampling intensity, genetic considerations, and geographic closure.

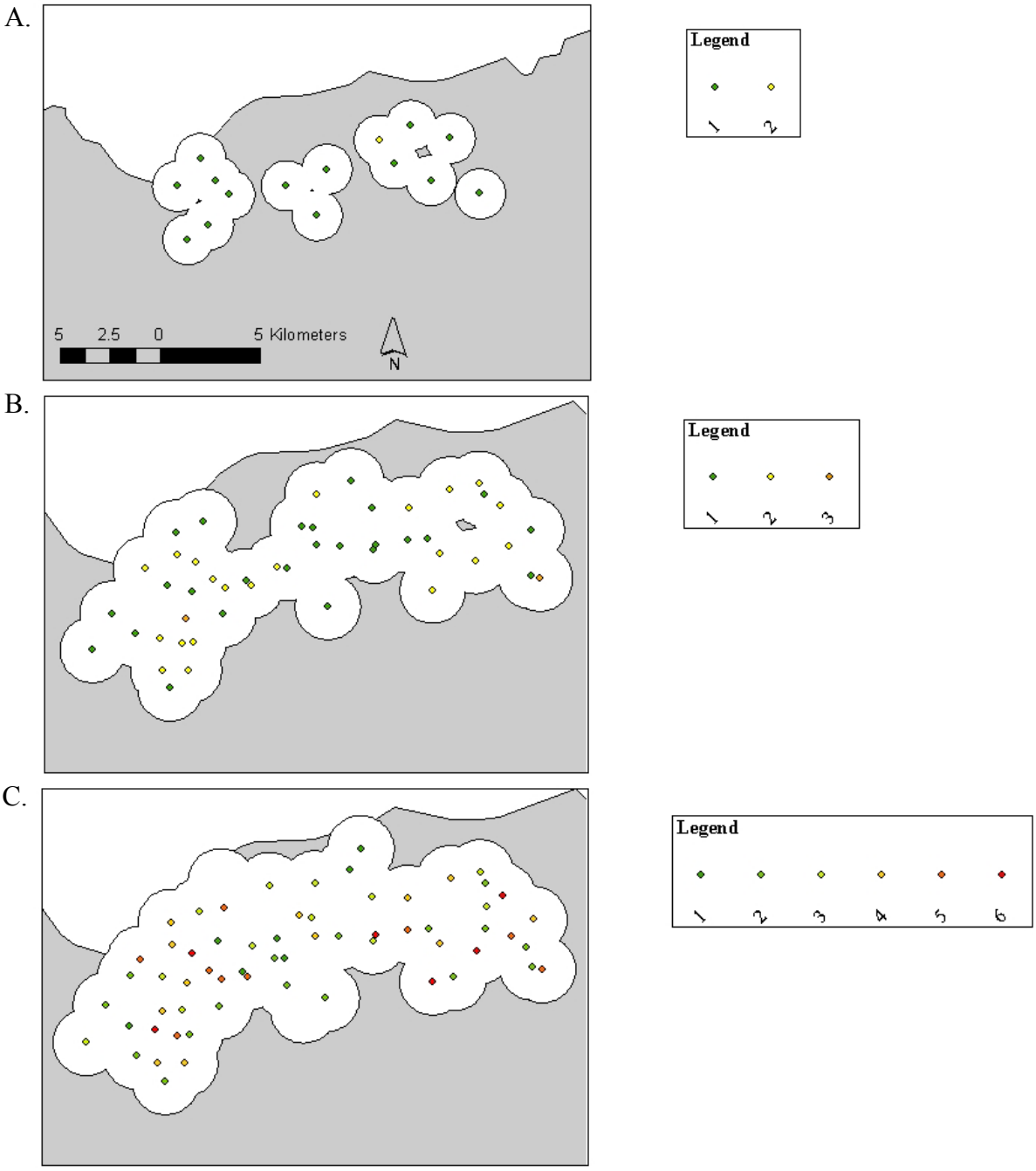


Fig.18. An example of the effect of reductions in subsample level and sampling duration on the number of hair-capture sites, national park study area, Tennessee, 2003. A. Sampling scenario: 4 weeks/5 subsamples (14 sites were sampled once and 1 site was sampled twice). B. Sampling scenario: 6 weeks/15 subsamples (25 sites were sampled once, 21 sites twice, and 3 sites 3 times). C. The complete dataset of 65 hair-capture sites, 10 weeks, and 25 subsamples per week. Seven sites were sampled once, 16 sites twice, 12 sites 3 times, 12 sites 4 times, 9 sites 5 times, and 6 sites 6 times.

Sampling Intensity.—Sampling intensity encompassed 2 factors that were closely linked in my analysis: subsampling intensity and density of hair-capture sites. A reduction in the number of hair-capture sites also resulted in a reduction in subsamples. Conversely, because I analyzed no more than 1 sample from each site per sampling period, a reduction in subsamples effectively removed a site from the landscape. Nevertheless, these were 2 distinct factors. The size of my dataset could be increased by analyzing ≥25 samples per sampling period. Selecting additional samples with the constraint that no 2 samples can come from the same site within a given week has the benefit of potentially reducing capture heterogeneity by more evenly distributing sampling effort across the study area. Analyzing more samples would likely be the most effective means of increasing capture probabilities but would also be expensive. My results may yield some insights regarding what sampling level could produce sufficient capture probabilities (Fig. 19). Both study areas showed a decreasing percent change in the population estimate as the number of samples increased. The percent change in population estimate is an indication of instability in model performance and therefore should be close to zero with sufficient sample sizes. Percent change in the estimate using model M_h Chao was approximately 11% and 2.5%, for the national park and national forest study areas, respectively, when the dataset with 3 periods of 3 weeks was reduced from 75 to 60 subsamples (i.e., 25 to 20 subsamples per week). This observation provides another indication that the bias associated with the population estimate in the national park study area was greater than in the national forest study area, likely as a result of the greater capture probabilities on the national forest study area. Therefore,

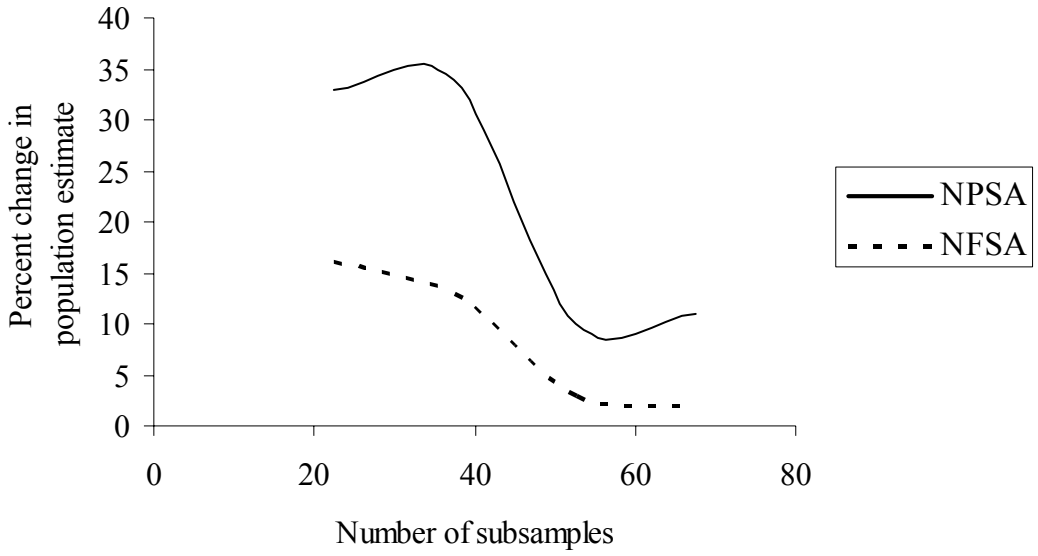


Fig. 19. Percent change in population estimate as a function of subsample intensity for model M(h) Chao (3 periods of 3 weeks sampling scheme) for the national park study area (NPSA) and national forest study area (NFSA), 2003.

depending on study objectives regarding reliability of population estimates, subsampling intensity could be reduced to 20 subsamples/week for low-density bear populations. However, subsample rates should be increased for higher-density population to produce a reliable estimate.

It is evident that subsample intensity can have a large impact on capture probabilities, and thus, model performance. Subsample intensity in my study reveals an interesting relationship: although absolute subsample levels were similar for both study areas (approximately 25/week), the proportion of subsamples analyzed from the entire number of samples collected was rather different. The total number of samples collected on the national park study area was 1,372, of which 204 (15%) were successfully analyzed. For the national forest study area, 181 of 584 samples (31%) were analyzed. Assuming that the total number of samples collected is indicative of the number of bears in the area, sampling intensity on the national forest study area was double that of the national park study area. Therefore, it is not necessarily surprising that capture probabilities for the national forest study area were approximately double that of the national park. The implication is that sampling intensity should be gauged based on the analysis of a set proportion of the total number of samples collected, and in my study it appears that the 30% level was acceptable whereas the 15% level was not fully sufficient.

My study did not achieve ≥4 sites/female home range as recommended by Otis et al. (1978) to provide sufficient trap availability to all individuals and thus reduce heterogeneity. However, this level of site density may be difficult to achieve with a wide-ranging carnivore in relatively inaccessible areas. Alternatively, I could have sampled more intensively over a smaller area. However, when sampling a small area

relative to the size of the home range, the assumption of closure is more likely violated; consequently, it may be unclear what population of animals was sampled (Boulanger et al. 2004*a*). Sampling a small area increases the influence of stochastic processes (e.g., temporary movements that occur on a small scale due to a random event, such as a small fire). DNA sampling studies on grizzly bears have been based on trapping grids to ensure intensive, uniform sampling effort and used site relocations between sampling sessions to reduce a behavioral (trap-happy) response (Mowat and Strobeck 2000; Boulanger et al. 2004*a*, 2004*b*). Those measures may help minimize capture variation (White et al. 1982), but are labor intensive and were not implemented in my study. My data did not indicate that a behavioral response was present, but capture heterogeneity likely existed. Therefore, I suggest that a trapping grid might prove useful, but moving sites may not be necessary, especially given the additional labor required to do so. Alternatively, it may be effective to detect and reduce heterogeneity by establishing a greater density of sites without restricting their placement to specific grid cells.

Sampling duration was 10 weeks for my study. My data suggest that reducing sampling duration may help reduce closure violations. Sampling duration could be reduced provided that capture probabilities and sample sizes remain sufficient. Figs. 15A. and 15C. show that capture probabilities were relatively stable across all levels of sampling duration at the 25 subsample level for both study areas. However, both study areas experienced a peak in site visitation at week 3, which resulted in a peak in capture probability for the 4-week scenarios that was apparent only at lower subsample levels. Other studies have experienced a similar peak in the number of samples collected (L. Thompson, University of Tennessee, personal communication), but it is unclear if this

pattern is related to intrinsic factors (i.e., a behavioral response) or extrinsic factors (e.g., seasonal movement patterns related to food, timing of the molt). Until this trend is better understood, I suggest a sampling duration of 4 weeks or less would likely be more susceptible to anomalies in visitation rates and would probably not be sufficient. Therefore, I suggest that sampling duration could be shortened to 6–8 weeks without a negative impact on capture probabilities. Shortening sampling duration should be combined, however, with more sites and subsamples than achieved with my study, otherwise the sparse data effect would be exacerbated. Additionally, a variety of pooled time period configurations may be considered. Pooling configurations that produce the highest capture probabilities while minimizing assumption violations should be selected for population estimation.

My study design was similar for both study areas, but study design could be tailored based on bear density. For instance, on the high-density national park study area, capture probabilities were particularly low. Therefore, increasing the number of analyzed hair samples seems to be imperative in areas with high bear densities. Capture probabilities were greater for the national forest study area, but fewer hair samples were collected there because of the low bear density. When a hair sample is collected in an area where bear density is low, the likelihood of being a recapture is greater compared with a higher-density population. However, low bear density resulted in fewer collected hair samples, with some sampling periods in the national forest study area yielding <25 samples (with the requirement that no 2 samples came from the same site). Therefore, I suggest that the low number of collected hair samples in the national forest study area may have been a limiting factor, which likely was a result of low site density. Thus,

future studies should pay particular attention to establishing more sites per female home range in low-density bear populations.

My primary goal with this analysis was to develop appropriate DNA sampling regimes to produce reliable population estimates for black bears in the southern Appalachians. However, my ability to suggest an optimal sampling scheme on the national park study area is limited because sample sizes and capture probabilities were low. However, some scenarios did provide greater capture probabilities and sample sizes (Table 7), and these scenarios again illustrate the better quantity of data obtained in the national forest study area. On the national forest study area estimates and their precision fluctuated only slightly among sampling scenarios, whereas the estimates for the national park study area were more varied. It is likely that higher overall capture probabilities in the national forest study area contributed to the relative stability of the population estimates.

However, it seems that high capture probabilities were not the only driving force behind model performance. The national park study area data illustrate this, because the estimates and CVs were very similar between the 3 periods of 3 weeks (75 samples each) scenario and the 10 weeks with 25 samples scenario, although the capture probabilities were different. Furthermore, a comparison of the 2 scenarios of 4 periods of 2 weeks (50 samples each) and 5 periods of 2 weeks (50 samples each) illustrates that estimates may increase even as capture probabilities decrease. I suggest that fluctuation of the population estimate for the national park study area may be related to many new individuals being captured, even during the final weeks of sampling. Therefore, robust estimation requires not only high capture probabilities, but also the assurance that a

Table 7. A comparison of black bear population estimates (model M_h Chao), CVs, and capture probabilities ($\hat{p}$) for DNA sampling scenarios with relatively large sample sizes in the national park study area (NPSA) and national forest study area (NFSA), 2003.

Sampling scenario	**NPSA**		**NFSA**	
	M_h population estimate (CV)	$\hat{p}$	**M_h population estimate (CV)**	$\hat{p}$
3 periods of 3 weeks/75 samples	292 (19%)	0.17	98 (18%)	0.32
3 periods of 3 weeks/60 samples	264 (21%)	0.16	96 (22%)	0.29
4 periods of 2 weeks/50 samples	216 (16%)	0.16	98 (20%)	0.23
5 periods of 2 weeks/50 samples	330 (19%)	0.11	97 (18%)	0.23
10 weeks/25 subsamples	309 (18%)	0.06	107 (21%)	0.13

sufficiently large proportion of the study population is captured.

Genetic Considerations.—One of the critical assumptions of any mark-recapture study is that all animals are uniquely marked. With the DNA technique, a low PI can lead to a biased population estimate (Waits and Leberg 2000). The PI_{sibs} for the national park study area indicated there was a 1 in 11,587 chance of an individual sharing its genotype with another individual, and a 1 in 3,731 chance for the national forest study area. These results were corroborated by the P_{sib} test. Therefore, I conclude that there was a high likelihood that each unique genotype represented a single individual.

The PI calculations are based on the assumption that alleles between different loci are independent (the linkage disequilibrium test) and that alleles are in Hardy-Weinberg proportion (Taberlet and Luikart 1999). The genetic data from both study areas met the criteria for Hardy-Weinberg equilibrium. The linkage disequilibrium test showed associations between only 1 pair of loci for the national park study area, and 2 pairs for the national forest study area. The 10 loci used in my study have been found to be independent (Paetkau and Strobeck 1994, Paetkau et al. 1995), so the significant associations found in my data may represent sampling bias, sampling of siblings, stochastic processes, or the presence of immigrants (T. King, U.S. Geological Survey, personal communication).

I used 10 loci to distinguish between individuals, but my data indicated that fewer loci could be equally sufficient. By reducing the number of loci needed to produce a genotype, both cost and error are also reduced (Taberlet and Luikart 1999, Waits and Leberg 2000, Paetkau 2004). Average observed heterozygosity (a measure of marker variability) was 0.72 for the national forest study area and 0.73 for the national park study

area. Paetkau (2003) suggested that studies on small populations ($N < 100$) are feasible with a minimum average heterozygosity of 0.69 for 6-loci systems, whereas heterozygosity should be close to 0.75 for 6-loci systems with N between 200 and 400. Therefore, I recommend that future DNA studies in the southern Appalachians use the 6 most variable loci out of the 10 used in my study. For the national park study area, those loci were G1A, G1D, G10L, G10M, G10P, and MU50, which had an average observed heterozygosity of 0.79. For the national forest study area, those loci were G1A, G1D, G10L, G10M, G10P, and MU23, which had an average heterozygosity of 0.77. Considering that MU50 was not a good marker for the national forest study area population, I recommend that future studies in the southern Appalachians avoid the use of that locus. Based on my study, an appropriate suite of 6 markers for the southern Appalachians should be chosen from G1A, G1D, G10L, G10M, G10P, MU23 and G10C.

The concerns surrounding MU50 illustrate that some markers may work well for one population and not another, although the reason why this might occur is not always clear. To examine the possibility that the questionable hair samples from the national forest study area were from a single family unit in a small geographic area, I determined the location of the hair-sample sites where the samples were taken. Those samples were distributed evenly across the entire study area, making this scenario unlikely. It is important to note that MU50 is a marker that was originally developed for use in a European brown bear (*Ursus arctos*) population (Taberlet et al. 1997). Although it may work well in most black bear populations, that marker was not effective in the national forest study area. That finding highlights the need for a more complete and well-

established genetic library for the black bear (T. King, U.S. Geological Survey, personal communication).

Much research has been conducted on the genetic aspects of non-invasive hair sampling (Goossens et al. 1997, Taberlet et al. 1997, Mills et al. 2000, Waits and Leberg 2000, Paetkau 2003). The various sources of error, such as allelic dropout and null alleles, have been clearly identified and examined. Guidelines have been established for rigorous lab protocols to detect and minimize genetic errors. Given proper lab procedures and quality hair samples, genetic error is likely to be limited (Paetkau 2003). Nevertheless, recent research continues to focus on the potential sources of bias associated with genetic error rates (McKelvey and Schwartz 2004*a*, *b*; Lukacs and Burnham 2005). Although much emphasis has been placed on genetic considerations in the past, I speculate that the future utility of the DNA technique to provide reliable population estimates mostly hinges on the ability to develop and refine field sampling protocols and models for estimation.

Geographic Closure.—Lack of geographic closure can lead to a population estimate that is biased high. The assumption of geographic closure may have been violated in my study because neither study area was separated from surrounding black bear habitat. This bias is particularly a problem with animals whose home ranges are large relative to the sampling grid (Boulanger and McLellan 2001). Program CAPTURE's test for closure for each sampling scenario rarely indicated that closure was violated on either study area, but this test is not reliable. The closure test is based on the assumption of equal catchability and violations of that assumption make the closure test

difficult to interpret. Therefore, closure should be assessed mostly from a biological basis rather than from statistical tests (Otis et al. 1978).

If indeed the assumption of geographic closure was violated, my population estimates likely are biased high (i.e., representing the superpopulation, which Kendall (1999) defined as the population of animals in the study area and surrounding region) and may not be suitable to estimate density. Because this bias could potentially be large, it is important for studies using closed models to incorporate techniques that detect and minimize closure violations. Boulanger et al. (2004*a*) used radio-collared bears to detect and model geographic closure violations. They found that the superpopulation estimates provided by Program CAPTURE were 15%–36% greater than adjusted estimates that were scaled based on the proportion of radio-marked bears on the grid at each sampling occasion. Boulanger and McLellan (2001) dealt with geographic closure violations by deriving a core population estimate, which ignored bears captured within a certain distance from the study area edge, but they cautioned that this was not a substitute for direct measurement of movements across study area boundaries. They concluded that using topographic boundaries and obtaining large sample sizes may help to detect and account for closure violations.

Closure violations may be addressed in a more indirect manner as well. For instance, DNA sampling could be implemented on an annual basis and data could be analyzed using open models (Boulanger and McLellan 2001). Alternatively, a web sampling design, originally developed for small mammals, bypasses estimates of abundance altogether and directly measures density through the use of carefully spaced traps in a web pattern (Anderson et al. 1983). However, web sampling may not be

logistically feasible to sample black bears in the mountains of the southern Appalachians, because this technique requires accurate distance measurements for trap placement from the center of the web, a very high density of traps, and all collected hair samples would have to be analyzed. Limited trail access, difficulty acquiring GPS coverage for accurate site placement, and high costs might hamper the web sampling design. Also, increasing the number of individuals captured and recaptured by analyzing more samples and establishing more hair-capture sites would reduce the need for a long sampling duration. Reduced sampling duration, combined with increased capture probabilities and grid cells that are appropriately scaled to home-range size, also may reduce closure violations (Rosenberg et al. 1995). Lastly, Kendall (1999) suggested that random movements of animals into and out of the study area reduces precision of closed models but does not introduce bias. Therefore, if density estimates are desired, site distribution should be planned to incorporate topographic boundaries to maximize closure and implement the direct accounting measures for closure violation as previously mentioned (Boulanger et al. 2004*a*). However, if DNA sampling is implemented on a region-wide basis in the southern Appalachians, then the issue of geographic closure becomes less important because sampling would occur over a large area.

Population Estimation

Estimates from DNA Sampling.—I used Program CAPTURE's model selection criteria as a guide to determine which models may be appropriate for my data. The null model was most often selected for my data. However, assumptions of the null model are restrictive and the model is typically considered unrealistic for most biological situations (Otis et al. 1978, White et al. 1982). Additionally, the model selection procedure in

Program CAPTURE often is unreliable (Otis et al. 1978, Menkins and Anderson 1988, Manning et al. 1995). The model selection procedure tends to select the null model when data are sparse but more complex models are selected with greater sample sizes (Stanley and Burnham 1998, Mowat and Strobeck 2000). For instance, Boulanger et al. (2004*a*) conducted simulations that showed CAPTURE's model selection procedure had low power to detect heterogeneity when population size was small (50–100). Thus, the frequent selection of the null model may be another indication of the sparseness of my data. Furthermore, Program CAPTURE indicated that capture probabilities likely were heterogeneous, and heterogeneity causes the estimates from M_o to be biased low (Pollock et al. 1990). My data showed that M_o indeed produced the lowest estimates compared with the 2 heterogeneity models.

Heterogeneity models are appropriate for species, such as black bears, that may show age- and sex-specific capture probabilities (White et al. 1982). Heterogeneity models also are appropriate for my study design, which may not have provided sufficient availability of hair-capture sites to all bears (Otis et al. 1978). Between these 2 models, M_h jackknife appeared to be more biased than M_h Chao, as M_h Chao showed a greater degree of stability within each subsample level (Figs. 9–13). For example, on the national forest study area the population estimates from the pooled sampling scenarios using M_h Chao (Fig. 10B.) were similar for all combinations of pooling configuration and subsample intensity, and the estimates were particularly stable for subsample levels of 20, 30, 40, and 50. In contrast, model M_h jackknife produced more variable estimates using the same data (Fig. 12B.), with estimates increasing as subsamples increased. My sensitivity analysis indicated that M_h jackknife may be more sensitive to sparse data and

may require large subsample levels and longer sampling durations to produce reliable estimates (Rosenberg et al. 1995). Furthermore, when data are sparse, the confidence interval estimates can be poor for M_h jackknife due to bias in the variance estimator (Otis et al. 1978, Burnham and Overton, 1979). My data seem to support this finding, because confidence intervals for M_h jackknife were small compared with the other 2 estimators. Therefore, I conclude that M_h Chao provided the best model for my data to estimate population abundance.

For both study areas, the largest average capture probability occurred when data were pooled for 3 periods of 3 weeks with 75 subsamples per sampling period. Although some data were lost by pooling sampling periods, I chose this scenario for population estimation because of the greater capture probabilities (0.17 and 0.32, for the national park and national forest study areas, respectively).

Comparing DNA and Live-capture Estimates.—The black bear population in the northwestern portion of Great Smoky Mountains National Park has been the subject of continuous monitoring using traditional mark-recapture techniques for 37 years. In 1968, University of Tennessee researchers initiated a live-capture study to estimate bear abundance and track population changes over time. I used the annual live-capture data to estimate population abundance using the Jolly-Seber model (open model). The 2003 Jolly-Seber population estimate (model B, constant survival rate per unit time, time-specific capture probabilities) for the 358-km^2 study area in which livetrapping took place was 215 bears (95% CI = 157–272). The study area size for the live-capture data was calculated similarly to the DNA study areas, by creating buffers around each trap-site representing the estimated radius of habitat use (Eason 2002).

The estimate of abundance from the live-capture study represents a density estimate of 0.60 bears/km^2. This density estimate is likely conservative because some portions of the study area are not adequately sampled by the linear traplines and the size of the study area was probably overestimated. The DNA study in the national park study area provided an abundance estimate of 292, which represents a density estimate of 1.83 bears/km^2, more than double that of the live-capture estimate. The national forest study area abundance estimate was 98, which represents a density estimate of 0.30 bears/km^2. The DNA density estimates may be biased high for 2 reasons. First, the closure assumption may have been violated. Second, the study area size was calculated based on female home range, and it seems probable that some male bears that were sampled had portions of their home range outside of the delineated study area. Therefore, it is likely that the actual sampled area was larger than what I estimated.

CHAPTER VI

MANAGEMENT AND RESEARCH IMPLICATIONS

Population Monitoring

Many of the difficulties associated with the DNA technique have already been discussed, but the traditional live-capture technique also poses substantial challenges. Most notably, the live-capture technique uses open population models, which tend to be less precise. The precision of the 2003 estimate from the live-capture study is acceptable (95% CI = 157–272), but this was in part due to the contribution of data from previous years. Similar precision was achieved in the DNA study with only one season of data using closed models.

The live-capture study in Great Smoky Mountains National Park is costly relative to the amount of data acquired for population estimation. Additionally, personnel must be highly trained and supervised. In contrast, a DNA study is relatively easy to conduct, is safer for personnel, and requires little personnel training. The costs associated with establishing hair-capture sites and collecting the hair samples are relatively low. The expense in DNA sampling lies in the lab costs of the DNA analysis, which typically range from $35 to $65 per sample. Hence, the cost of DNA sampling can vary widely depending to the size and scope of the study. The amount of data provided by a DNA study is therefore closely linked to budget, whereas the amount of data provided by a live-capture study is more dependent on uncontrollable factors, such as trap success. Trapping success can be hindered in a live-capture study, due in part to bears becoming trap smart. Bears are highly intelligent and in a consistently trapped population, such as in Great Smoky Mountains National Park, many bears quickly learn to take bait without

being captured. This type of bias is less likely to occur with DNA sampling, because hair-capture sites provide only a small food reward, which is not replenished daily. Likewise, bears should not have an aversion to hair-capture sites because there are no negative consequences to visiting a site. Lastly, the live-capture study area was somewhat arbitrarily defined compared with the DNA study area. This was a result of linear trapping routes along hiking trails, which were chosen to facilitate access into the relatively remote study area.

My study indicates that DNA sampling is not a panacea for the challenges surrounding the task of population estimation. Future DNA studies in the southern Appalachians will likely require greater financial resources to achieve sample sizes above that achieved in my study. DNA studies cannot provide certain information that is currently supplied by the live-capture technique, such as age, weight, or reproductive status. Nevertheless, I suggest that this technique offers a more scientifically sound alternative to the live-capture technique for the purpose of population estimation. Many of the weaknesses associated with the DNA sampling technique that I identified apply equally to live-capture data. However, the potential to address these weaknesses is greater with DNA sampling. Furthermore, it may be difficult to justify an invasive live-capture study solely for the purpose of population estimation. The DNA technique has a large advantage in this regard, with the additional benefit of potentially supplying new and useful information on gene flow and genetic structure. Therefore, with careful consideration to study design, DNA sampling may be successfully implemented for population monitoring.

Study Design and Sampling Logistics

Designing a mark-recapture study is not a simple task. My pilot study was intended to examine elements of study design so that future studies using the DNA technique can be both efficient and effective. For instance, one goal of my study was to investigate what size study area and what level of trap density can be reasonably accomplished given a certain number of personnel. My study had between 3 and 4 technicians on the national park study area, and a similar number on the national forest study area. Pairs of technicians visited between 4 and 9 hair-capture sites a day, with hiking distances ranging from 4–16 km, in a workday that typically lasted 6 hours. The amount of time spent gathering hair at a site was brief compared with the amount of time hiking between sites. Therefore, future DNA projects in the southern Appalachians likely could increase the density of hair-capture sites with that number of personnel or establish a similar number of sites with fewer personnel working independently.

If future DNA studies are designed to produce reliable density estimates, then particular attention should be paid to defining a study area. Future studies might consider generating abundance estimates for males and females separately, and defining 2 study areas sizes accordingly. This approach would improve estimation of study area size for species with differential home-range sizes between males and females. However, deriving a separate male and female estimate would be costly because additional DNA analysis would be needed to identify the sex of the animal and the number of analyzed samples would double.

In summary, I recommend that the design of future black bear DNA studies in the southern Appalachians should provide ≥4 hair-capture sites/female home range, sample

for a duration of 6–8 weeks, and analyze approximately 30% of the total samples collected. If future studies are primarily concerned with producing reliable population estimates, I suggest taking uncertainty in model selection into consideration. Program CAPTURE does not address the uncertainty in model selection. Selecting an inappropriate model can lead to substantial bias in population estimation (Stanley and Burnham 1998). One way to address this problem is to incorporate model selection uncertainty into the estimate by computing a weighted estimate that incorporates information from competing models (Buckland et al. 1997). This might be most easily accomplished using Akaike's Information Criterion found in the population modeling Program MARK (White and Burnham 1999). Program MARK also contains the Pradel model (Pradel 1996), which might prove useful for studies that are more concerned with population growth rate rather than estimates of abundance. The Pradel model is an open model, and therefore many of the issues of closure could be avoided. Similarly, the robust design (Pollock 1982) incorporates the use of both open and closed models to estimate parameters such as survival, recruitment, and abundance from data collected over a longer time period. The robust design has the advantage of using closed models to estimate capture probability variation, and open models to estimate survival, recruitment, and abundance. However, for the study of black bears this method requires at least 3 years of data. Lastly, new models have been developed that are modifications of the closed models used in this study and are designed to account for genotyping error (Lukacs and Burnham 2005).

LITERATURE CITED

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Allen, T. G. 1999. Black bear population size and habitat use on Alligator River National Wildlife Refuge, North Carolina. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Anderson, D. R., K. P. Burnham, G. C. White, and D. L. Otis. 1983. Density estimation of small-mammal populations using a trapping web and distance sampling methods. Ecology 64:674–680.

Beausoleil, R. A. 1999. Population and spatial ecology of the Louisiana black bear in a fragmented bottomland hardwood forest. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Beeman, L. E. 1975. Population characteristics, movements, and activities of the black bear (*Ursus americanus*) in the Great Smoky Mountains National Park. Dissertation, University of Tennessee, Knoxville, Tennessee, USA.

Beringer, J. J. 1986. Habitat use and response to roads by black bears in Harmon Den, Pisgah National Forest, North Carolina. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Boersen, M. R., J. D. Clark, and T. L. King. 2003. Estimating black bear population density and genetic diversity at Tensas River, Louisiana using microsatellite DNA markers. Wildlife Society Bulletin 31:197–207.

Boulanger, J., and B. N. McLellan. 2001. Closure violation in DNA-based mark-recapture estimation of grizzly bear populations. Canadian Journal of Zoology 79:642–651.

_____, B. N. McLellan, J. G. Woods, M. F. Proctor, and C. Strobeck. 2004*a*. Sampling design and bias in DNA-based capture-mark-recapture population and density estimates of grizzly bears. Journal of Wildlife Management 68:457–469.

_____, G. Stenhouse, and R. Munro. 2004*b*. Sources of heterogeneity bias when DNA mark-recapture sampling methods are applied to grizzly bear (*Ursus arctos*) populations. Journal of Mammalogy 85:618–624.

_____, G. C. White, B. N. McLellan, J. Woods, M. Proctor, and S. Himmer. 2002. A meta-analysis of grizzly bear DNA mark-recapture projects in British Columbia, Canada. Ursus 13:137–152.

Brandenburg, D. M. 1996. Effects of roads on behavior and survival of black bears in coastal North Carolina. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Buckland, S. T., K. P. Burnham, and N. H. Augustin. 1997. Model selection: an integral part of inference. Biometrics 53:603–618.

Burnham, K. P., and W. S. Overton. 1979. Robust estimation of population size when capture probabilities vary among animals. Ecology 60:927–936.

Carlock, D. M., R. H. Conley, J. M. Collins, P. E. Hale, K. G. Johnson, A. S. Johnson, and M. R. Pelton. 1983. The tri-state black bear study. Tennessee Wildlife Resources Agency Technical Report 83–9.

Carr, P. C. 1983. Habitat utilization and seasonal movements of black bears in Great Smoky Mountains National Park. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Chao, A. 1987. Estimating population size for capture-recapture data with unequal catchability. Biometrics 43:783–791.

_____. 1988. Estimating animal abundance with capture frequency data. Journal of Wildlife Management 52:295–300.

_____. 1989. Estimating population size for sparse data in capture-recapture experiments. Biometrics 45:427–438.

_____, S. M. Lee, and S. L. Jeng. 1992. Estimating population size for capture-recapture data when capture probabilities vary by time and individual animal. Biometrics 48:201–216.

Chapman, D. G. 1951. Some properties of hypergeometric distribution with applications to zoological sample censuses. University of California Publications in Statistics 1:131–160.

Clark, J. D. 1991. Ecology of two black bear (*Ursus americanus*) populations in the Interior Highlands of Arkansas. Dissertation, University of Arkansas, Fayetteville, Arkansas, USA.

_____, and M. R. Pelton. 1999. Management of a large carnivore: black bear. Pages 209–223 *in* J. D. Peine, editor. Ecosystem management for sustainability: principles and practices illustrated by a regional biosphere reserve cooperative. CRC Press, Boca Raton, Florida, USA.

Cormack, R. M. 1968. The statistics of capture-recapture methods. Oceanographer Marine Biology Annual Review 6:455–506.

Dobey, S., D. V. Masters, B. K. Scheick, J. D. Clark, M. R. Pelton, and M. E. Sunquist. 2005. Ecology of Florida black bears in the Okefenokee-Osceola ecosystem. Wildlife Monographs 158.

Eason, T. H. 2002. Population ecology of black bears in Great Smoky Mountains National Park. Dissertation, University of Tennessee, Knoxville, Tennessee, USA.

Garris, R. S. 1983. Habitat utilization and movement ecology of black bears in Cherokee National Forest. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Garshelis, D. L. 1978. Movement ecology and activity behavior of black bears in the Great Smoky Mountains National Park. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

_____. 1990. Monitoring effects of harvest on black bear populations in North America: a review and evaluation of techniques. 10th Eastern Workshop on Black Bear Research and Management 10:110–144.

Gillespie, J. H. 1998. Population genetics: a concise guide. Johns Hopkins University Press, Baltimore, Maryland, USA.

Goossens B., L. P. Waits, and P. Taberlet. 1998. Plucked hair samples as a source of DNA: reliability of dinucleotide microsatellite genotyping. Molecular Ecology 7:1237–1241.

Hellgren, E. C., and M. R. Vaughan. 1989. Demographic analysis of a black bear population in the Great Dismal Swamp. Journal of Wildlife Management 49:668–674.

Kendall, W. L. 1999. Robustness of closed capture-recapture methods to violations of the closure assumption. Ecology 80:2517–2525.

Kendall, K., J. Stetz, L. Waits, D. Roon, and M. Murphy. 2001. Non-invasive genetic sampling for forest carnivore population status at an ecosystem scale. Intermountain Journal of Sciences 7:134.

King, P. B., and A. Stupka. 1950. The Great Smoky Mountains – their geology and natural history. The Scientific Monthly 71:31–42.

Lafollette, J. D. 1974. Some aspects of the history of the black bear (*Ursus americanus*) in the Great Smoky Mountains. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Lukacs, P. M., and K. P. Burnham. 2005. Estimating population size from DNA-based closed capture-recapture data incorporating genotyping error. Journal of Wildlife Management 69:396–403.

Manning, T., W. D. Edge, and J. O. Wolf. 1995. Evaluating population-size estimators: an empirical approach. Journal of Mammalogy 76:1149–1158.

Martorello, D. A. 1998. Ecology of black bears in coastal North Carolina. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

McConnell, S. K., P. O'Reilly, L. Hamilton, J. M. Wright, and P. Bentzen. 1995. Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American and European populations. Canadian Journal of Fisheries and Aquatic Science 52:1863–1872.

McKelvey, K. S., and M. K. Schwartz. 2004*a*. Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. Journal of Wildlife Management 68:439–448.

_____, and _____. 2004*b*. Providing reliable and accurate genetic capture-mark-recapture estimates in a cost-effective way. Journal of Wildlife Management 68:453–456.

Menkens, G. E., and S. H. Anderson. 1988. Estimation of small-mammal population size. Ecology 69:1952–1959.

Mills, L. S., J. J. Citta, K. P. Lair, M. K. Schwartz, and D. A. Tallmon. 2000. Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. Ecological Applications 10:283–294.

Mowat, G., and C. Strobeck. 2000. Estimating population size of grizzly bears using hair capture, DNA profiling, and mark-recapture analysis. Journal of Wildlife Management 64:183–193.

National Park Service. 1997. Great Smoky Mountains National Park. Weather and climate information. National Park Service, Washington, D. C., USA. [online] URL: http://www.nps.gov/grsm/gsmsite/natureinfo.html#wc.

Otis, D. L., K. P. Burnham, G. C. White, and D. R. Anderson. 1978. Statistical inference from capture data on closed animal populations. Wildlife Monographs 62.

Paetkau, D. 2003. An empirical exploration of data quality in DNA-based population inventories. Molecular Ecology 12:1375–1387.

_____. 2004. The optimal number of markers in genetic capture-mark-recapture studies. Journal of Wildlife Management 68:449–452.

_____, W. Calvert, I. Sterling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian Polar Bears. Molecular Ecology 4:347–354.

_____, and C. Strobeck. 1994. Microsatellite analysis of genetic variation in black bear populations. Molecular Ecology 3:489–495.

Palsbøll, P. J., J. Allen, M. Bérubé, P. J. Clapham, T. P. Feddersen, P. S. Hammond, R. R. Hudson, H. Jørgensen, S. Katona, A. H. Larsen, F. Larsen, J. Lien, D. K. Mattila, J. Sigurjónsson, R. Sears, T. Smith, R. Sponer, P. Stevick, and N. Øien. 1997. Genetic tagging of humpback whales. Nature 388:767–769.

Pelton, M. R., and F. T. van Manen. 1996. Benefits and pitfalls of long-term research: a case study of black bears in Great Smoky Mountains. Wildlife Society Bulletin 24:443–450.

Pollock, K. H. 1982. A capture-recapture design robust to unequal probabilities of capture. Journal of Wildlife Management 46:757–760.

_____, J. D. Nichols, C. Brownie, and J. E. Hines. 1990. Statistical inference for capture-recapture experiments. Wildlife Monographs 107.

Poole, K. G., G. Mowat, and D. A. Fear. 2001. DNA-based population estimate for grizzly bears (*Ursus arctos*) in northeastern British Columbia, Canada. Wildlife Biology 7:65–76.

Pradel, R. 1996. Utilization of capture-mark-recapture for the study of recruitment and population growth rate. Biometrics 52:703–709.

Quigley, H. B. 1982. Activity patterns, movement ecology, and habitat utilization of black bears in Great Smoky Mountains National Park. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Raymond, M., and R. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

Reagan, S. R. 1991. Habitat use by female black bear in a southern Appalachian bear sanctuary. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Rexstad, E., and K. P. Burnham. 1992. User's guide for interactive Program CAPTURE. Colorado Cooperative Fish and Wildlife Research Unit, Colorado State University, Fort Collins, Colorado, USA.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Rosenberg, D. K., W. S. Overton, and R. G. Anthony. 1995. Estimation of animal abundance when capture probabilities are low and heterogeneous. Journal of Wildlife Management 59:252–261.

SAS Institute, Inc. 2001. SAS/STAT User's Guide, version 8.0, 4th edition. SAS Institute Inc., Cary, North Carolina, USA.

Seibert, S. G. 1989. Black bear habitat use and response to roads on Pisgah National Forest, North Carolina. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Shanks, R.E. 1954. Climates of the Great Smoky Mountains. Ecology 35:354–361.

Sia, E. A., S. Jinks-Robertson, and T. D. Petes. 1997. Genetic control of microsatellite stability. Mutation research 383:61–70.

Smith, T. R. 1985. Ecology of black bears in the bottomland hardwood forest in Arkansas. Dissertation, University of Tennessee, Knoxville, Tennessee, USA.

Stanley, T. R., and K. P. Burnham. 1998. Estimator selection for closed-population capture-recapture. Journal of Agricultural, Biological, and Environmental Statistics 6:131–150.

Tablerlet, P., and G. Luikart. 1999. Non-invasive genetic sampling and individual identification. Biological Journal of the Linnean Society 68:41–55.

_____, L. Waits, and G. Luikart. 1999. Non-invasive genetic sampling: look before you leap. TREE 14:323–327.

_____, S. Griffin, B. Goossens, S. Questiau, V. Manceau, N. Escaravage, L. P. Waits, and J. Bouvet. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research 24:3189–3194.

_____, J. J. Camarra, S. Griffin, E. Uhrês, O. Hanotte, L. P. Waits, C. Dubois-Paganon, T. Burke, and J. Bouvet. 1997. Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Molecular Ecology 6:869–876.

Thompson, L. M. 2003. Abundance and genetic structure of two black bear populations prior to highway construction in eastern North Carolina. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Thornthwaite, C. W. 1948. An approach toward a rational classification of climate. Geographical Review 38:55–94.

Villarrubia, C. R. 1982. Movement ecology and habitat utilization of black bears in Cherokee National Forest, Tennessee. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Waits, J. L., and P. L. Leberg. 2000. Bias associated with population estimation using molecular tagging. Animal Conservation 3:191–199.

White, G. C., D. R. Anderson, K. P. Burnham, and D. L. Otis. 1982. Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory, LA 8787-NERP, Los Alamos, New Mexico, USA.

_____, and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study Supplement 46:120–138.

Woods, J. G., D. Paetkau, D. Lewis, B. L. McLellan, M. Proctor, and C. Strobeck. 1999. Genetic tagging free-ranging black and brown bears in mark-recapture experiments. Wildlife Society Bulletin 27:616–627.

Appendix. Laboratory Protocol for Microsatellite Analysis.

MICROSATELLITE ANALYSIS

DNA Isolation

DNA was extracted from hair follicles using the InstaGene Matrix (Bio-Rad Laboratories, Hercules, California, USA). Specifically, follicles were incubated in the InstaGene Matrix in the presence of Proteinase K at 65° C overnight. This mixture was boiled (100° C) for 8–10 minutes, followed by centrifugation at 10,000–12,000 rpm. The resulting supernatant was used in PCR reactions.

Microsatellite Amplification and PCR

Microsatellite DNA amplification was performed for 10 microsatellite loci using the PCR primers described by Paetkau and Strobeck (1994) and Paetkau et al. (1995). These loci were: G1A, G1D, G10B, G10C, G10L, G10M, G10P, G10X, MU23, and MU50.

Each PCR reaction consisted of 1.5 µl of genomic DNA extract, 0.875 X PCR buffer (59 mM Tris-HCl, pH 8.3; 15 mM $(NH_4)_2SO_4$; 9mM β-mercaptoethanol; 6 mM ETDA), 2.25 mM $MgCL_2$, 0.2 mM dNTPs, 0.15-0.43 µM of each primer (forward primer fluorescently labled with TET, FAM, or HEX; Applied Biosystems (ABI), Foster City, California, USA), 1.2 units of Taq polymerase (ABI), and deionized water added to achieve the final volume of 15 µl. The amplification cycle consisted of an initial denaturing at 94° C for 2 minutes followed by 35 cycles of 94° C denaturing for 30 seconds, 56° C annealing for 30 seconds, and 72° C extension for 1 minute. Cycling culminated with a 5-minute extension at 72° C. Thermal cycling was performed in an MJ

DNA Engine PTC 200 (MJ Research, Watertown, Massachusetts, USA) configured with a heated lid.

Fragment Analysis

Generally, 1 µl of PCR product was diluted 1:1 with deionized water and thoroughly mixed. One µl of this dilution was added to 12 µl of deionized formamide and 0.5 µl of the internal size standard GENESCAN-500 (ABI). Alternatively, PCR products of separate multiplexed reactions (2–3 loci each) and multiple separate reactions (2–4) were combined and analyzed without dilution. Loci were identified in these multiplexed samples by virtue of their characteristic molecular mass and attached fluorescent label. The size standard contained DNA fragments fluorescently labeled with the dye phosphoramidite TAMRA (red). This PCR product/size standard/formamide mixture was heat denatured at 95° C for 3 minutes and placed immediately on ice for at least 5 minutes. The mixture was subjected to capillary electrophoresis on an ABI PRISM 310 Genetic Analyzer (i.e., automated sequencer). Fluorescently labeled DNA fragments were analyzed, and genotype data generated using Genescan software (ABI). GENOTYPER v. 2.0 (ABI) DNA fragment analysis software was used to score, bin, and output allelic (and genotypic) designations for each bear sample.

VITA

Katherine (Katie) Eileen Settlage was born June 8, 1979 in Greenbrae, California. She graduated from Huntsville High School, Huntsville, Arkansas in 1997. She earned a Bachelor of Science degree in Biology from the University of Arkansas, Fayetteville in May 2001. After assisting with a white-tailed deer study in South Carolina, Katie began her graduate studies at the University of Tennessee, Knoxville in August 2002. She received her Master of Science degree in Wildlife and Fisheries Science in August 2005.