

ABUNDANCE AND DENSITY ESTIMATION OF THE CENTRAL GEORGIA BLACK
BEAR POPULATION

by

JOSHUA THOMAS SYLVEST

(Under the Direction of Michael Chamberlain and Robert Warren)

ABSTRACT

We used DNA-based capture-mark-recapture techniques to estimate sex-specific abundance of black bears in central Georgia. We conducted hair sampling over 2 8-week periods during the summers of 2012 and 2013 and analyzed capture histories of individual bears identified via microsatellite genotyping. We used program MARK to evaluate a set of candidate models incorporating various effects of time, behavior, and heterogeneity. There was considerable model selection uncertainty in 2012, whereas the top model in 2013 had 99% model support. The model-averaged abundance estimate for 2012 was $N = 98$ (SE = 62) for males and $N = 70$ (SE = 16) for females. In 2013, we used the top model to derive abundance estimates of $N = 70$ (SE=18) for males and $N = 69$ (SE = 18) for females. These results can be used to inform future management decisions regarding the central Georgia black bear population.

INDEX WORDS: Black bear, *Ursus americana*, capture-mark-recapture, MARK, microsatellite genotyping

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DEDICATION

I dedicate this thesis to any and all who read it with interest.

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talents and I'm proud to have worked with and learned from you both. If you'll suffer one more pun: At the end of the day, we were never "barely" getting by, we were "bearly" getting by.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Populations of large carnivores have been severely reduced across most of the United States over the past century due to indiscriminate killing and habitat degradation and fragmentation. This is particularly true of the American black bear (*Ursus americanus*), whose range across North America has become increasingly fragmented. In the eastern United States, black bears are believed to only occupy about 10% of their historic range (Maehr 1984). In the southeastern United States, it is estimated that only 30 presumably disjunct populations exist (Pelton 1990). Causes of population isolation in this region are primarily associated with anthropogenic influences on the landscape, such as forest conversion to agriculture, residential and urban development (Hellgren and Vaughan 1994), and the continued expanse of infrastructure to support these developments. Roads are considered the leading cause of habitat fragmentation and a primary barrier to bear movement in the Southeast, resulting in smaller, more isolated black bear populations (Hellgren and Maehr 1992, Brody and Pelton 1989, Beringer et al 1998, Thompson et al 2005). Similar fragmentation of other large carnivore populations in the United States has been shown to decrease fitness, as in the Florida panther (*Felis concolor coryi*; Roelke et al. 1993), and negatively affect genetic variation of mountain lions (*Puma concolor*), Scandinavian lynx (*Lynx lynx*; Spong & Hellborg 2002), and brown bears (*Ursus arctos*; Miller & Waits 2003). Habitat loss and fragmentation (Maehr 1984) coupled with

over-exploitation (Miller 1990) have resulted in several populations of black bears in the Southeast existing in isolation and contending with similar consequences.

Black Bears in the Southeast

Black bears are long-lived, have relatively long generation times, and exist at low densities. These traits are known to influence space use, colonization rates, and persistence of populations of carnivores over large areas (Brown and Nicoletto 1991). Habitat fragmentation and lack of habitat connectivity may also influence these traits of a population, ultimately resulting in reductions in gene flow (Vos et al. 2001) and genetic variability (Sherwin and Moritz 2000). Bears may be particularly susceptible to losses in genetic variability due to inherently low levels of genetic variation caused by low densities and low effective population sizes typical of large carnivores (Paetkau and Strombeck 1994).

Populations which are made smaller and more isolated by habitat fragmentation and which lack suitable movement corridors for dispersal and gene flow (Larkin et al. 2004) often become genetically distinct from the metapopulations from which they were derived. Small, isolated populations are vulnerable to local extinction due to a number of stochastic events including demographic, environmental, and genetic effects (Shaffer 1981). Among the possible genetic effects of population isolation are increases in inbreeding. Inbreeding occurs in small, isolated populations when related individuals mate with one another and may result in further reduction of genetic variability and risk of allele fixation, resulting in loss of genetic adaptability, or else fixation of unfavorable alleles whose effects are too small to be purged via selection (Keller and Waller 2002). Such fixation may lead to inbreeding depression whereby reproduction, fitness, and adaptability are all negatively affected, along with the overall viability

of populations (Lacy 1997). Dunbar et al. (1996) noted that 75% of cases of cryptorchidism and delayed testicular descent in Florida black bears came from the smaller and more fragmented populations and that inbreeding could not be ruled out as a cause. Another isolated population of Florida black bears in southern Alabama has exhibited indications of inbreeding such as lack of scrotum or testes, prolapsed rectum, and tail deformities (Kasbohm and Bentzien 1998). The levels of genetic isolation which may be responsible for these negative effects, and potentially a myriad of others, are not uncommon to other bear populations in the Southeast.

Similar to the Florida black bear, the Louisiana black bear (*U. a. luteolus*) is a subspecies of the American black bear that has been reduced to several small subpopulations within its historic range with little or no connectivity among them. Abundance estimates of the 3 extant Louisiana populations range from ~300 (Hooker 2010) in the Tensas River Basin population to ~100 in the coastal population (Triant 2001). In their genetic examination of 3 bear populations in Arkansas and 2 in Louisiana, Csiki et al. (2003) found heterozygosity values in the White River population of Arkansas and the coastal population of Louisiana, each the smallest in their respective states, to be lower than in the larger populations. Lower than average genetic diversity exhibited by these 2 populations is consistent with other southeastern black bear populations that are reproductively isolated. Dixon et al (2007) found 9 Florida black bear (*U. a. floridanus*) populations to be genetically distinct from one another despite geographic closeness. Mean expected heterozygosity, a measure of genetic variation, was found to be substantially lower in the smaller, more isolated of the Florida black bear populations. The Chassahowitzka black bear population in western Florida, which was the smallest of the 9 study populations, exhibited the lowest levels of genetic variation and greatest genetic differentiation compared to larger populations in Florida. The Highlands/Glades population in Florida exhibited similarly

low levels of genetic variability. Because these populations have been isolated for a relatively short period of time, Dixon et al. (2007) suspected inbreeding depression may have played a role in their reduction in fitness and lower than expected levels of genetic variation.

Black Bears in Georgia

Black bears in Georgia occur in 3 geographically isolated populations with presumably little or no connectivity among them, one in the northern portion of the state associated with the Appalachian Mountains, one in central Georgia associated with the Ocmulgee River drainage system, and a third population in the southern portion of the state associated with the Okefenokee Swamp. The central Georgia population (CGP) is located on core areas of contiguous forest that comprise the Oaky Woods and Ocmulgee Wildlife Management Areas (WMAs) and is believed to number around 300 individuals. However, the most recent estimates of this population place that number closer to 200 (Sanderlin 2009). Accurate estimates of population size, survival, reproduction and recruitment of the CGP is vital to effective management. This is particularly true when additional pressures such as recreational hunting and urban development are considered. Loss of genetic variation is an important factor in the long term viability of small, isolated populations such as the CGP because it causes these populations to be more vulnerable to local extinctions as a result of over-harvest and stochastic environmental pressures (Paetkau & Strobeck 1994). As a small, isolated population, the CGP has been found to exhibit high levels of genetic similarity (Miller 1995, Sanderlin 2009).

Justification

Issues such as habitat fragmentation and population isolation clearly affect many southeastern bear populations and managing bears in the face of these issues will require informed management strategies. Informed strategies are especially crucial when considering

small, reproductively isolated populations (Frankham 1995, Keller and Waller 2002). Whether setting harvest limits for healthy populations or performing population viability analyses for sensitive or threatened populations, reliable abundance estimates are a key component of nearly all wildlife management decisions. Capture-mark-recapture (CMR) is a popular and reliable technique for deriving population estimates in wildlife studies. Taberlet and Bouvet (1992) first used hair collected in the field to study bears, and advancements in genetic analysis over the past decade have allowed non-invasive, DNA-based survey methods to become widely used to survey bear populations (Boersen et al. 2003, Boulanger et al. 2008, Woods et al. 1999). DNA-based survey methods have several benefits over more traditional CMR survey methods including higher capture probabilities, decreased bias and invasiveness, and no loss of marks (Woods et al. 1999). Baited hair snares are one of the most common methods for systematic hair collection in the field and have been demonstrated to be efficient (Boulanger et al. 2004, Mowat and Strobek 2000, Mowat et al. 2005). Small amounts of DNA available in hair follicles may be amplified via polymerase chain reaction (PCR) into many copies which are then suitable for genetic analysis (Paetkau et al. 1995, Taberlet et al. 1996). Highly variable molecular markers called microsatellites are isolated and can be used to identify individual hair samples to be used in CMR analyses (McKelvey and Schwartz 2004).

The Georgia Department of Natural Resources (DNR) instituted an experimental one-day hunting season on the CGP during November 2011 and 2012. The hunts occurred on private land holdings that surround Oaky Woods and Ocmulgee WMAs. Although hunters were allowed a one bear limit, there was no quota imposed on the potential harvest as a whole. Thirty-three bears were taken during the first of the 2 experimental hunts, approximately 10% of the estimated population. An additional 14 bears were harvested in November 2012. The

continuation of these hunts will be contingent upon observed harvest rates and details of pertinent demographic factors, particularly reliable population and survival estimates. The most recent study of this population occurred 3 years ago. At that time, one set of harvest analysis models indicated that increases in harvest of 1%, 2%, and 3% each resulted in a decreasing population and that no additional harvest was sustainable (Sanderlin 2009). Observed legal harvest rates at that time averaged ~1 bear every 1 to 2 years, a considerably smaller number than the bears legally harvested in 2011 and 2012. This removal has likely had a dramatic effect on the dynamics of the population and warrants additional investigation to inform future management and conservation.

This study is being conducted in conjunction with a larger research effort to detail various aspects of the ecology and management of the CGP. Specifically, a companion study is evaluating denning ecology and describing patterns of den selection and cub survival. A doctoral research program is focusing on the effects of a highway widening project in the study area on the CGP. My objectives focus on deriving abundance and density estimates for the CGP using baited hair snares and DNA-based CMR methods.

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CHAPTER 2

ABUNDANCE AND DENSITY ESTIMATION OF THE CENTRAL GEORGIA BLACK BEAR POPULATION

Introduction

In the eastern United States, black bears are believed to only occupy about 10% of their historic range (Maehr 1984). In the southeastern United States, it is estimated that only 30 presumably disjunct populations exist (Pelton 1990). Causes of population isolation in this region are primarily associated with anthropogenic influences on the landscape, such as forest conversion to agriculture, residential and urban development (Hellgren and Vaughan 1994), and the continued expanse of infrastructure to support these developments. Roads are considered the leading cause of habitat fragmentation and a primary barrier to bear movement in the Southeast, resulting in smaller, more isolated black bear populations (Brody and Pelton 1989, Hellgren and Maehr 1992, Beringer et al 1998, Thompson et al. 2005). Similar fragmentation of other large carnivore populations in the United States has been shown to decrease fitness, as in the Florida panther (*Felis concolor coryi*; Roelke et al. 1993), and negatively affect genetic variation of mountain lions (*Puma concolor*), Scandinavian lynx (*Lynx lynx*; Spong & Hellborg 2002), and brown bears (*Ursus arctos*; Miller & Waits 2003). Habitat loss and fragmentation (Maehr 1984) coupled with over-exploitation (Miller 1990) have resulted in several populations of black bears in the Southeast existing in isolation and potentially contending with similar instances of reduced genetic variation and decreased fitness.

An absence of suitable movement corridors for dispersal and gene flow among small, isolated black bear populations may cause them to become genetically distinct from the metapopulations from which they were derived (Larkin et al. 2004). Such small populations are vulnerable to local extinction due to a number of stochastic events including demographic, environmental, and genetic effects (Shaffer 1981). Among the possible genetic effects of population isolation are losses of genetic variability and increases in inbreeding. Black bears may be particularly susceptible to losses in genetic variability due to inherently low levels of genetic variation (Paetkau and Strombeck 1994). Inbreeding occurs in small, isolated populations when related individuals mate with one another. This may result in further reduction of genetic variability and risk of allele fixation, resulting in loss of genetic adaptability, or else fixation of unfavorable alleles whose effects are too small to be purged via selection (Keller and Waller 2002). Such fixation may lead to inbreeding depression whereby reproduction, fitness, and adaptability are all negatively affected. There is evidence of inbreeding in geographically and reproductively isolated black bear populations throughout the Southeast. These include cases of cryptorchidism in small populations in Florida (Dunbar et al 1996) and lack of scrotum or testes in individuals in Alabama (Kasbohm and Bentzien 1998). Mean expected heterozygosity, a measure of genetic variation, has been found to be lowest in the smaller more isolated populations throughout the southeastern states including Florida (Dixon et al. 2007), Louisiana and Arkansas (Csiki et al. 2003). The smaller and more isolated populations of black bears in other parts of the Southeast may exhibit similar genetic effects.

Black bears in Georgia occur in 3 geographically isolated populations with little or no connectivity among them, one in the northern portion of the state associated with the Appalachian Mountains, one in central Georgia associated with the Ocmulgee River drainage

system, and a third population in the southern portion of the state associated with the Okefenokee Swamp. The central Georgia population (CGP) is located on core areas of contiguous forest that comprise the Oaky Woods and Ocmulgee Wildlife Management Areas (WMAs) and is believed to number around 300 individuals. However, the most recent estimates of this population place that number closer to 200 (Sanderlin 2009). As a small, isolated population, the CGP has been found to exhibit high levels of genetic similarity (Miller 1995, Sanderlin 2009). In addition to being a small isolated population with little genetic variability, the CGP is further threatened by a newly-implemented hunting season. The Georgia Department of Natural Resources (DNR) instituted an experimental one-day hunting season on the CGP during November 2011 and 2012; this hunt continued in 2013. Forty-eight bears were harvested over the course of these 3 hunts and this removal has likely effected the population and warrants additional investigation.

Accurate estimates of population size, survival, reproduction and recruitment of the CGP are vital to effective management. This study is being conducted in conjunction with a larger research effort to detail various aspects of the ecology and management of the CGP.

Specifically, a companion study is evaluating denning ecology and describing patterns of den selection and cub survival. A doctoral research program is focusing on the effects of a highway widening project in the study area on the CGP. My objectives focus on deriving abundance and density estimates for the CGP using DNA-based capture-recapture methods.

Study Area

This study occurred on Oaky Woods and Ocmulgee Wildlife Management Areas (WMAs) and surrounding private and state-leased lands in Bibbs, Bleckley, Houston, Pulaski, and Twiggs Counties. This area was in the Upper Coastal Plain of central Georgia southeast of the city of Macon. It was roughly bordered by GA 247 to the west, US 129 to the east, and I-16

to the north, and was surrounded almost entirely by human development (Figure 1). The WMAs comprised approximately 14,164 ha of continuous habitat separated by the Ocmulgee River. General habitat types throughout the WMAs included planted pines (*Pinus* spp) in various seral stages, bottomland hardwoods, upland pine-hardwood, cypress-gum swamps, black belt prairies, and clearcuts. Dominant tree species varied by forest type but included various oaks (*Quercus* spp.), hickories (*Carya* spp.), elms (*Ulmus* spp.), and pines mixed with sweetgum (*Liquidambar styraciflua*), American beech (*Fagus grandifolia*), dogwoods (*Cornus* spp.), persimmon (*Diospyros virginiana*), cherry (*Prunus* spp), and maples (*Acer* spp.). Common mid- and understory vegetation types included grapes (*Vitis* spp.), blueberries (*Vaccinium* spp.), holly (*Ilex* spp.), privet (*Ligustrum sinense*), hawthorne (*Crataegus* spp.), beautyberry (*Callicarpa americana*), and blackberry (*Rubus* spp.). Surrounding private lands were comprised of similar habitats but were disproportionately dominated by planted pine forests owned by Plum Creek Timber Company, the largest private landowner in the study area. Private land comprised ~27,000 ha. Common fauna throughout the study site included white-tailed deer (*Odocoileus virginianus*), raccoon (*Procyon lotor*), gray fox (*Urocyon cinereoargenteus*), coyote (*Canis latrans*), feral hog (*Sus scrofa*), bobcat (*Lynx rufus*), and black bear (*Ursus americanus*).

Methods

Hair sampling

We constructed hair snares throughout the study area such that 4 snares were placed within areas equivalent to the average size of the home range of an adult female in central Georgia (1,497 ha; Sanderlin 2009). Hair snares were placed at this density throughout the ~41,000 ha study area (Figure 1). Because female bears typically have smaller home ranges than males, placing hair snares in this fashion presumably ensured adequate access to traps for all

bears and an increased chance of being encountered (Otis et al 1978, Williams et al 2002). Hair snares were composed of 2 strands of barbed wire stretched around 3 to 4 trees to form a polygon around a bear attractant suspended in the center. We situated barbed wires at 30 and 65 cm above the ground to increase probability of hair capture for bears of varying sizes. Where topographic features prevented the bottom wire from being too far above or below the proper height, brush and debris were used to encourage bear entrance at another point (Mowat and Strobek 2000) and hair snares were made large enough so that bears would be unable to reach the bait without passing through the barbed wire. We monitored each snare for 8 consecutive weeks during the summers of 2012 and 2013 beginning in the second week of June and ending in the first week of August. We collected samples from individual barbs and stored them separately in coin envelopes in a cool, dry environment. Envelopes were labeled with a collection date and a unique week-site-sample number, differentiating between samples collected from the top or bottom wires. To ensure sample quality and prevent degradation of DNA, we monitored snares once weekly for hair samples. Dry hair samples may remain stable and suitable for DNA analysis under these conditions for decades (D. Paetkau, Wildlife Genetics International, personal communication). We re-baited hair snares with soured corn weekly and rags of raspberry scent (Mother Murphy's Laboratories, Greensboro, North Carolina) were freshened to serve as an additional lure. We used a lighter or propane torch to burn off remaining hair and prevent contamination of future samples.

Subsampling

We sent all hair samples to Wildlife Genetics International (WGI) and used a subsampling protocol to select samples for genetic analysis. During the summers of 2012 and 2013, 3,570 and 2,659 individual hair samples were collected from the hair snares, respectively.

Analysis of each sample would have been overly costly and redundant so a subsampling strategy was used to minimize cost. We assigned random numbers to each hair site/week combination which produced at least one sample. Hair samples within those site/week combinations were then assigned a second series of random numbers to avoid potential bias toward samples collected from the top or bottom wires. We selected 400 samples for subsampling which allowed 50 samples for each of the 8 weekly sampling periods. However, the number of samples collected from week to week varied and assigning random numbers in this fashion allowed that variation to be reflected in the subsampling. The subsampling intensity we used has shown to be sufficient for producing reliable population estimates (Laufenberg et al. 2013). Samples of optimal quality for this process were considered to consist of 5 or more guard hair roots, or 20 or more underfur roots. We used optimal quality samples meeting this threshold over others of lesser quality whenever possible given the subsampling strategy. Where samples of this quality were unavailable, samples meeting a less stringent threshold of 1 guard hair or 5 underfur roots were used instead.

DNA Extraction and Marker Selection

WGI used QIAGEN DNeasy Tissue kits to purify and extract DNA from hair samples using standard protocols (Paetkau 2003). Roots from individual guard hairs were clipped for extraction, whereas entire clumps of underfur were used. WGI initially attempted to use 8 microsatellites and a *ZFX/ZFY* gender marker that had successfully produced reliable individual identifications in past black bear studies from regions surrounding our study site. However, these markers were insufficient for producing accurate individual identifications in the CGP due to much lower variability compared to that of other populations in the Southeast. Therefore, 35 samples which had successfully produced a unique genotype from the original 9 markers were

used as test samples and analyzed at 15 additional markers producing a total of 23 microsatellite markers (Table 2.1; D. Paetkau, personal communication). The 23 markers were ranked according to their variability and 9 markers were selected for sufficient individual identification (*G1A*, *G10H*, *G10L*, *G10M*, *CXX20*, *MU59*, *G10X*, *CXX110*, *D1A*), as well as the *ZFX/ZFY* gender marker. In 2013, the analysis of individual identity was expanded from 10 to 11 markers, dropping *G10H* and adding *G10U* and *D123*. All 2012 samples were reanalyzed and confirmed using the new marker suite.

Microsatellite Genotyping and Error Checking

Analysis of individual identity in 2012 was performed for all DNA extracts using the suite of 9 microsatellite markers described in the marker selection process. The revised marker suite was used to identify 2013 samples and confirm 2012 samples. In 2012, WGI genotyped 438 DNA extracts at each of the markers in the first round of genotyping and 33 samples were removed for having high-confidence scores at 4 or fewer markers. In 2013, 64 of 450 DNA extracts were culled for having high-confidence scores at fewer than 5 markers. These samples were removed due to their inability to produce complete and accurate genotypes (D. Paetkau, personal communication). Re-analysis was performed at weak data points in all remaining samples from each year using 5 μ l of DNA per extraction compared to 3 μ l used in the original analysis. Standard protocols during error-checking (Paetkau 2003, Kendall et al. 2009) call for re-analysis of mismatching markers in pairs of similar genotypes. However, the dataset from this genetically similar population resulted in an overabundance of genotypes that required re-analysis and so a two-fold approach to error-checking was used instead. Genotypes that were mismatched at 1 of 10 markers in 2012 or 1 of 11 markers in 2013 were re-analyzed at the mismatching markers. Additionally, one sample from each individual in the dataset was

analyzed at an additional 3 markers (*REN145P07*, *D123*, *G10U*) in 2012 and 2 additional markers in 2013 (*REN145P07* and *G10H*). The genotypes that remained candidates for error-checking after this process were then extended to additional markers and the original mismatching markers were re-analyzed. This process is expected to have eliminated any practical risk that number of individuals identified in each year was inflated due to undetected genotyping error (D. Paetkau, personal communication).

Probability of Identity and Hardy-Weinberg Equilibrium

In populations where genetic variability is low and individuals may be closely related, misidentification may occur due to individuals sharing identical genotypes at examined loci. This phenomenon is known as the shadow effect and may result in a single individual being incorrectly identified as two individuals, causing an artificial inflation in capture probabilities and a negative bias in abundance estimates (Mills et al. 2000). The probability of identity (PI) statistic was developed by Paetkau and Strobeck (1994) to evaluate the ability of a given set of genetic markers to effectively determine individual identity. It is expressed as the likelihood that 2 individuals in a population will have the same genotype at examined loci. It can be calculated for a single locus as:

$$PI_{\text{single locus}} = \sum_i x_i^4 + \sum_i \sum_{j>i} (2x_i x_j)^2,$$

where p_i and p_j are the frequencies of the i th and j th alleles. The overall PI for multiple loci can be calculated as the product of the PI of each locus examined (Taberlet and Luikart 1999):

$$PI_{\text{overall}} = \prod (PI_{\text{single locus}}).$$

Family groups of females and cubs which move around together increase the possibility of collecting hair samples from closely related individuals and siblings. Because they are so closely related, these individuals have an increased probability of having identical genotypes at examined loci and the PI between siblings (PI_{sibs}) statistic provides the upper limit for this probability. PI_{sibs} was calculated as

$$PI_{\text{sibs}} = 0.25 + \left(0.5 \sum p_i^2\right) + \left[0.5 \left(\sum p_i^2\right)^2\right] - \left(0.25 \sum p_i^4\right),$$

where x_i was the frequency of the i th allele (Waits et al. 2001). All PI statistics are only valid if the examined loci of the genetic samples are independent and conform to Hardy-Weinberg equilibrium. Loci can be verified as independent by testing for gametic disequilibrium, the nonrandom association of alleles at different loci. We used GENEPOP 4.2 (Raymond and Rousset 1995) to test for gametic disequilibrium.

Hardy-Weinberg equilibrium is the principle that allele and genotype frequencies are expected to reach and remain at equilibrium over time in populations that exhibit random mating and no selection, mutation, immigration, or emigration. We tested the genetic samples for Hardy-Weinberg equilibrium conformation by comparing the statistical difference after sequential Bonferroni correction between observed heterozygosity (H_o) and expected heterozygosity (H_e) in GenAlEx 6.5 (Peakall and Smouse 2006). The sequential Bonferroni correction reduced pseudoreplication in multiple tests for Hardy-Weinberg proportions by dividing the α level of significance by the number of tests performed (Rice 1989). We also calculated PI statistics for the marker suite used to identify individuals in GenAlEx 6.5.

Population Abundance

Capture mark recapture (CMR) techniques estimate abundance by capturing, marking and releasing a portion of a population and comparing the proportion of marked to unmarked

individuals at a subsequent capture occasion. Advancements in genetic analysis over the past decade have allowed non-invasive, DNA-based survey methods to become widely used to survey bear populations (Boersen et al 2003, Boulanger et al 2008, Woods et al 1999) in CMR studies and offer several benefits compared to traditional physical capture and marking techniques. These benefits include higher capture probabilities, decreased bias and invasiveness, and no loss of marks (Woods et al 1999). Closed population models described by Otis et al (1978) are effective tools for modeling CMR data when the following assumptions are met:

- 1) animals do not lose their marks
- 2) animals are correctly identified
- 3) the population is demographically and geographically closed
- 4) all animals have an equal chance of being captured

Advancements in genetic analysis and stringent error-checking protocols (Paetkau 2003) ensure accurate identification of animals and because animals cannot lose their unique genotype, assumptions 1 and 2 were satisfied in our study. Because our sampling protocol was to sample the population during an 8 week period over the summer when there is no recruitment and survival is high, the demographic closure assumption was also well-satisfied. We used the time-varying capture probability test in Program CloseTest (Stanley and Richards 2004) to test for violations of the demographic closure assumption. Geographic closure can be difficult to satisfy due to potential movement on or off the sampling grid by individuals whose home ranges only partially overlap its periphery. In an effort to satisfy this assumption, we sampled all available bear habitat in the study area believed to be occupied by black bears. Animals may differ in their probability of capture for a number of reasons including differences in size, space use, positive or negative response to capture or food reward, social hierarchy, or other unidentifiable sources.

We used 2 wires per hair-snare to increase the chance of capture for bears of varying sizes and the study area was saturated with hair-snares (1 snare/1.9km²) to ensure adequate access to hair sites for bears of varying home range sizes.

We analyzed CMR data in Program MARK (White and Burnham 1999) via RMark v. 2.1.7 (Laake 2013) and R v. 3.0.3 (R Development Core Team 2014) to derive abundance estimates. We analyzed capture histories as a Huggins p and c data type and estimated abundance independently for 2012 and 2013. We estimated probability of capture (p) and recapture (c) directly, whereas abundance (N-hat) was a derived parameter. Sex was used as a grouping variable and we developed models to account for heterogeneity between genders by considering sex-specific capture and recapture probabilities. We modeled capture and recapture probabilities as independent from one another and as either time varying or constant. Time-varying models considered capture and/or recapture probabilities defined by 2 unique time periods, the first and second 4 weeks of sampling. Relatively sparse amounts of data were available for individual weeks and were insufficient for reliably estimating capture probabilities for single-week periods in fully time-varying models. By grouping the first 4 weeks and second 4 weeks, we allowed capture and recapture probabilities to differ over larger time periods, but with enough data to reliably estimate those parameters. We ranked models using Akaike's Information Criterion with a 2nd order correction for small sample sizes (AIC_c; Burnham and Anderson 2002). The model with the lowest AIC_c value is considered the best fit for the data and most parsimonious (Cooch and White 2001). When models in the candidate set exhibit differences in AIC_c (ΔAIC_c) ≤ 2 they are considered to be equally supported. We used model averaging to draw inference and derive abundance estimates from multiple models when they were equally supported. We calculated the population density using the buffer strip method (Dice 1938), whereby an effective sampling

area is determined by buffering the edge of the hair grid with an area equal to the radius of a female home range.

Results

Hair Snaring

We collected 3,570 hair samples from 126 hair snares in 2012; 100 hair snares produced at least 1 sample (79% success rate). The mean number of samples collected from sites that produced at least 1 sample during the sampling period was 36 (SD=30, range= 1-144). We added 4 additional hair snares for the 2013 sampling period and lost 1 from 2012 due to flooding. We collected 2,659 hair samples from 129 hair snares in 2013 (81% success rate). The mean number of samples collected from sites that produced at least one sample in 2013 was 23 (SD=16, range = 1-53). The mean number of samples collected per week in 2012 and 2013 was 446 (SD=151) and 332 (SD=113), respectively.

Microsatellite Genotyping

In 2012, microsatellite analysis identified 103 individuals (54 M: 49 F) from 419 capture events. The average number of captures for males and females was 3.7 (SD=3.7) and 3.3 (SD=2.3), respectively. Two females and 5 males were captured 10 or more times. The percentage of males and females that were captured only once in 2012 was 42% and 20%, respectively. In 2013 there were 99 individuals (50 M: 49 F) identified from 482 capture events, 62 of which were also detected in 2012. The average number of captures for males and females in 2013 was 8.0 (SD=8.8) and 4.1 (SD=3.6), respectively. Six females and 16 males were captured 10 or more times and the percentage of males and females captured only once was 20% and 24%, respectively.

Probability of Identity and Hardy-Weinberg Equilibrium

PI values for the 10 non-sex loci in the 11- marker suite used for identification of all samples ranged from 0.14 to 0.38 (Table 2.2). The overall probability of identity for the 10-marker suite was 1.3×10^{-6} , or a 1 in ~741,000 chance of capturing 2 individuals with the same genotype at the 10 markers used for individual identification. The PI for siblings was 1.7×10^{-3} , which represents a 1 in ~560 probability of 2 related individuals sharing the same genotype at the same 10 markers. These probabilities of observing identical genotypes using these markers were sufficiently low for reliable abundance estimates using mark-recapture techniques. Chi-square results comparing observed and expected heterozygosity at the individual loci were not significant after sequential Bonferroni correction indicating the loci used for identification did not deviate from Hardy-Weinberg Equilibrium, and that the calculated PI statistics were reliable (Table 2.3).

Population Abundance and Density Estimates

In 2012 there was considerable model selection uncertainty for the 5 candidate models (Table 2.4). The top model in 2012 modeled capture probability (p) by sex and had constant probability of recapture (c) with a model weight (w_i) of 0.31. The second highest ranked model held both p and c as constant for gender and time, whereas the third highest ranked model held p constant and allowed c to vary by 2 4-week sampling periods. These 2 models had weights of 0.27 and 0.22, respectively, making the top 3 models fairly evenly supported. ΔAIC_c values for all models in the candidate set were < 4 and were < 2 for all but the least supported model. In contrast to 2012, the highest ranked model in 2013 had a model weight of 0.99 and all remaining models had ΔAIC_c values ≥ 13 . The model-averaged abundance estimate for 2012 was $N = 98$ (SE = 62) for males and $N = 70$ (SE = 16) for females. In 2013, we used the top model to derive

abundance estimates of $N = 70$ (SE=18) for males and $N = 69$ (SE = 18) for females. We noted a potential violation of the assumption of closure in the 2012 ($\chi^2 = 62.9$, $P < 0.00001$) and 2013 ($\chi^2 = 45.6$, $P < 0.00001$) capture data, suggesting that bears may have been entering or exiting our defined study area during the sampling periods. Closure violations have the potential to inflate abundance estimates in closed capture modeling by underestimating capture probabilities.

Density was estimated for each year using the abundance estimates and an effective sampling area of 528 km². Density was estimated as 0.31 bears per km² in 2012 and 0.26 bears per km² in 2013.

Discussion

The abundance estimates for 2012 and 2013 were $N = 98$ (SE = 62) for males and 70 (SE = 16) for females and $N = 70$ (SE=18) for males and 69 (SE = 18) for females, respectively. Abundance estimates and associated standard errors were consistent for females between the 2 years. Although estimates for males between the 2 years were similar, the standard error associated with the 2012 abundance estimate was considerably greater. Reliable abundance estimates in CMR studies require adequate capture probabilities. The top model in 2012 indicated that sex-specific capture probabilities were important, suggesting heterogeneous capture probabilities between genders. Capture probabilities for females were adequate ($p = 0.17$), but were much lower for males ($p = 0.06$). Low capture probabilities for males likely inflated the number of males estimated in 2012. Higher capture probabilities in 2013 produced a lower standard error and more precise abundance estimate. The top model in 2013 modeled capture probabilities by 2 unique time periods, the first and second 4 weeks of sampling, in which the capture probabilities improved modestly from $p = 0.12$ in the first 4 week period to $p = 0.15$ in the second 4 weeks. This supports time variation in capture probabilities over the

sampling period, but our data were too sparse to develop well-supported, fully time-varying models.

We estimated a total population size of 166 in 2012 and 139 in 2013. The likely inflated estimate for males in 2012 is one possibility for the disparity between years. Likewise, the 2012 bear hunt removed 14 known individuals from the population and at least 3 bears were known to be road killed between the 2 summers in which the sampling was conducted. These 17 verified losses would represent 10% of a population of 166 and could likewise explain the lower abundance estimate in 2013.

Density estimates are often difficult to compare in a meaningful way due to differences in estimation techniques. That being said, bear density within the CGP is within the range of other populations in the Southeast (Table 2.6). Our density estimate for 2012 of 0.31 bears/km² is nearly identical to that reported by Grahl (1985) for the CGP (0.32 bears/km²), but comparing estimates from our study to his is tenuous given the differences in abundance estimation technique, scale, and amount of time elapsed since that study. The density estimate for each year was calculated using the respective abundance estimate for that year, so the 2013 density of 0.26 bears/km² is the most recent and likely more accurate since it is based on higher confidence estimates of abundance.

Program CloseTest results indicated that capture data in both years probably violated the closure assumption. This violation may have occurred as a result of bears on the periphery of the hair grid moving in and out of the sampling area. Closure violations can negatively bias capture probabilities and artificially inflate abundance estimates. A lack of closure may have contributed to the low capture probabilities and inflated abundance estimates of males in 2012. Females that were only captured once in 2012, and both males and females that were only captured once in

2013, ranged from 20-24% of individuals by gender. However, males that were captured only once in 2012 comprised 42% of the total males captured that year. While this could easily make capture probabilities difficult to estimate and probably did, it is not direct evidence of these animals moving on or off of the sampling grid as many of these cases occurred in the core area of the grid and not around the periphery.

While it is possible that bears maintaining home ranges within the study area left the sampling grid during our study period, it is also possible that individual heterogeneity in capture probabilities or some behavioral effect may have had some bears less likely to be detected. Some bears that were physically captured and radio-collared inside of the hair grid as part of the companion studies were known to be present on the grid without being sampled (M. Hooker, personal communication). While there were only a handful of these cases, it demonstrates the potential for unidentified heterogeneity effects to affect capture probabilities of individuals in our study population. Such capture heterogeneity can bias abundance estimates if it is not accounted for and, although our models were able to account for varying capture probabilities between genders, they were not sensitive to individual heterogeneity. Pledger (2000) developed mixture models to account for capture heterogeneity and improve the reliability of estimates. These models require large amounts of data but, building on our dataset, future CMR work with this population could acquire enough data to develop more complex models that would account for individual heterogeneity to a greater degree than our candidate models. Additional years of data would also allow future work to build models using Pollock's robust design (1982), which would be more robust to closure violations and able to estimate additional parameters. Hardy-Weinberg equilibrium tests revealed no evidence of nonrandom mating among the loci used for individual identification in our study. The probability of observing matching genotypes among

siblings (PI_{sibs}) in the CGP using these loci was low (0.00178), indicating that the ability to distinguish individuals was sufficient for noninvasive sampling of this population. Further, WGI extrapolated from an observed mismatch distribution to determine the probability of sampling more than 1 individual with a given genotype in the CGP and found little meaningful risk of finding a false match using our 11-marker suite (D. Paetkau, personal communication). Future noninvasive sampling of the CGP should use the new markers developed by WGI for this study.

Management Implications

This study provides an estimate of black bear abundance in central Georgia that applies to the geographic area of the sampling grid (Figure 1) and can be used to inform management decisions for the bear population within that geographic extent. At the end of the 2013 sampling period (August), we estimated 139 bears in the CGP. Observed harvest rates during the falls of 2011 and 2012 were 33 and 14 bears, respectively. Given the natural history of this species, especially its reproductive cycle, these rates are likely not sustainable. Harvest rates at the time of Sanderlin's (2009) study were 1 bear every 1 to 2 years and modeled increases in those harvest rates resulted in a decreasing population size. Miller (1990) noted a maximum sustainable harvest rate of 14% for black bear populations being managed for sustained yield under optimal conditions for reproduction, natural mortality, and assuming males to be twice as vulnerable to harvest as females. The CGP may not be a good candidate for this management technique because estimates of reproduction, survival, and recruitment are unknown. In addition, it appears based on the known harvest in the CGP during 2011-2013 that males and females are equally vulnerable to hunting mortality. Estimates of cub survival, recruitment, and overall reproductive success will help to further inform the viability of this population.

The 2013 hunt that was set for December, rather than November when the 2011 and 2012

hunts occurred, resulted in only 1 harvested bear; if this population continues to be subjected to hunting pressure, December hunt dates when bears are less active should be used to increase individual survival during hunts. However, warm periods during the winter may result in increased bear activity and otherwise offset potential benefits of late-season hunts. Adjusting the current legal size for bear harvest in the CGP from 75 pounds to a larger size may further increase individual survival in adult females. The size of breeding age females in this population varies widely and adult females have been captured that were less than 100 lbs (M. Hooker, personal communication). Perhaps increasing the minimum size of bears that can be harvested to 100 lbs or greater would prevent the harvest of females.

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Table 2.1. Number of samples analyzed at a given locus (n), number of alleles (A), expected (H_e) and observed (H_o) heterozygosity values for the 23- marker suite evaluated for efficiency to distinguish individual bears in the central Georgia black bear population and the 10 markers used for individual identification in 2012 and 2013.

| Locus | n | A | H_o | H_e |
|------------------|-----|-----|-------|-------|
| <i>D1A</i> | 189 | 4 | 0.74 | 0.70 |
| <i>G1A</i> | 189 | 5 | 0.70 | 0.68 |
| <i>D123</i> | 189 | 4 | 0.54 | 0.61 |
| <i>G10M</i> | 189 | 3 | 0.58 | 0.57 |
| <i>G10U</i> | 189 | 3 | 0.59 | 0.57 |
| <i>G10X</i> | 189 | 3 | 0.50 | 0.55 |
| <i>G10L</i> | 189 | 3 | 0.54 | 0.54 |
| <i>MU59</i> | 189 | 3 | 0.47 | 0.51 |
| <i>CXX110</i> | 189 | 2 | 0.51 | 0.50 |
| <i>CXX20</i> | 189 | 3 | 0.47 | 0.47 |
| 10-Locus Mean | | 3.3 | 0.56 | 0.57 |
| <i>REN145P07</i> | 132 | 2 | 0.50 | 0.47 |
| <i>G10H</i> | 132 | 4 | 0.42 | 0.42 |
| <i>MU23</i> | 59 | 3 | 0.34 | 0.40 |
| <i>REN144A06</i> | 51 | 3 | 0.31 | 0.39 |
| <i>CPH9</i> | 40 | 3 | 0.30 | 0.36 |
| <i>MSUT2</i> | 35 | 2 | 0.34 | 0.36 |
| <i>G10J</i> | 35 | 2 | 0.43 | 0.34 |
| <i>MU50</i> | 38 | 2 | 0.32 | 0.34 |
| <i>G10P</i> | 38 | 4 | 0.37 | 0.31 |
| <i>G10C</i> | 36 | 2 | 0.31 | 0.30 |
| <i>G10B</i> | 35 | 2 | 0.34 | 0.29 |
| <i>G1D</i> | 69 | 1 | 0.00 | 0.00 |
| <i>MU51</i> | 34 | 1 | 0.00 | 0.00 |
| 23-Locus Mean | | 2.8 | 0.42 | 0.42 |

Table 2.2. Observed (H_o) and expected (H_e) heterozygosity, number of alleles (A), probability of identity (PI), and probability of sibling identity (PI_{sibs}) statistics of microsatellite markers used to establish individual identity of black bears in the central Georgia black bear population. Overall PI and PI_{sibs} values were computed including the gender marker

| Locus | H_o | H_e | A | PI | PI_{sib} |
|--------------|-------|-------|-----|-----------------------|----------------------|
| G1A | 0.70 | 0.68 | 5 | 0.16 | 0.45 |
| G10L | 0.54 | 0.54 | 3 | 0.27 | 0.55 |
| G10M | 0.58 | 0.57 | 3 | 0.28 | 0.54 |
| CXX20 | 0.47 | 0.47 | 3 | 0.36 | 0.61 |
| MU59 | 0.47 | 0.51 | 3 | 0.37 | 0.59 |
| G10X | 0.50 | 0.55 | 3 | 0.26 | 0.54 |
| CXX110 | 0.51 | 0.50 | 2 | 0.38 | 0.59 |
| D123 | 0.54 | 0.61 | 4 | 0.22 | 0.50 |
| D1A | 0.74 | 0.70 | 4 | 0.14 | 0.44 |
| G10U | 0.59 | 0.57 | 3 | 0.27 | 0.54 |
| \bar{x} | 0.57 | 0.56 | 3.3 | 0.27 | 0.53 |
| Overall PI | - | - | - | 1.35×10^{-6} | 1.7×10^{-3} |

Table 2.3. Summary of chi-square tests with associated degrees of freedom (DF) for Hardy-Weinberg equilibrium for 10 loci used for individual identification of black bears in the central Georgia black bear population. Reported P-values are the significance values for chi-square tests comparing observed (H_O) and expected (H_E) heterozygosity for each locus.

| Locus | DF | χ^2 | P-value |
|--------|----|----------|---------|
| G1A | 6 | 4.386 | 0.625 |
| G10L | 3 | 0.284 | 0.963 |
| G10M | 3 | 2.648 | 0.449 |
| CXX20 | 3 | 2.211 | 0.530 |
| MU59 | 1 | 0.017 | 0.895 |
| G10X | 3 | 3.776 | 0.287 |
| CXX110 | 1 | 0.836 | 0.360 |
| D123 | 6 | 14.448 | 0.025 |
| D1A | 6 | 5.483 | 0.484 |
| G10U | 3 | 1.751 | 0.626 |

Table 2.4. Candidate models used to estimate abundance of the central Georgia black bear population in central Georgia, USA in 2012 and summary of model selection procedures based on corrected Akaike's Information Criteria (AIC_c). Capture probabilities (p) and recapture probabilities (c) were modeled by gender (~sex), defined as 2 unique time periods^a (~ptwoperiod or ~ctwoperiod), or constant (~1).

| Model Number | Model | AIC _c | ΔAIC _c ^b | w _i ^c | K ^d |
|--------------|------------------------------|------------------|--------------------------------|-----------------------------|----------------|
| 1 | p(~sex)c(~1) | 972.45 | 0.00000 | 0.31 | 3 |
| 4 | p(~1)c(~1) | 972.66 | 0.212008 | 0.27 | 2 |
| 3 | p(~1)c(~ctwoperiod) | 973.07 | 0.62421 | 0.22 | 3 |
| 5 | p(~ptwoperiod)c(~ctwoperiod) | 974.37 | 1.922322 | 0.11 | 4 |
| 2 | p(~ptwoperiod)c(~sex) | 975.65 | 3.203122 | 0.06 | 4 |

^aTime periods defined by first and second 4-weeks of sampling

^bRelative difference between AIC_c of model and the top model with the lowest AIC_c

^cModel weight

^dNumber of model parameters

Table 2.5. Candidate models used to estimate abundance of the central Georgia black bear population in central Georgia, USA in 2013 and summary of model selection procedures based on corrected Akaike's Information Criteria (AIC_c). Capture probabilities (p) and recapture probabilities (c) were modeled by gender (~sex), defined as 2 unique time periods^a (~ptwoperiod and ~ctwoperiod), or constant (~1).

| Model Number | Model | AIC _c | ΔAIC _c ^b | w _i ^c | K ^d |
|--------------|------------------------------|------------------|--------------------------------|-----------------------------|----------------|
| 2 | p(~ptwoperiod)c(~sex) | 916.12 | 0.00000 | 0.99 | 4 |
| 1 | p(~sex)c(~1) | 930.08 | 13.96010 | 9.2e ⁻⁴ | 3 |
| 4 | p(~1)c(~1) | 931.96 | 15.83345 | 3.6e ⁻⁴ | 2 |
| 3 | p(~1)c(~ctwoperiod) | 933.65 | 17.52722 | 1.5e ⁻⁴ | 3 |
| 5 | p(~ptwoperiod)c(~ctwoperiod) | 935.26 | 19.13434 | 6.9e ⁻⁵ | 4 |

^aTime periods defined by first and second 4-weeks of sampling

^bRelative difference between AIC_c of model and the top model with the lowest AIC_c

^cModel weight

^dNumber of model parameters

Table 2.6. Population density estimates of several black bear populations in the Southeastern United States.

| Location | Density Estimate (bears/km) | Source |
|---------------------------------------------|--------------------------------|---------------------------|
| Okefenokee Swamp, Georgia | 0.12 | Dobey et al. 2005 |
| Osceola National Forest, Florida | 0.14 | Dobey et al. 2005 |
| Ocmulgee drainage, Georgia | 0.26 | This study |
| White River NWR, Arkansas | 0.29 | Smith 1985 |
| Ocmulgee River, Georgia | 0.32 | Grahl 1985 |
| Tensas River NWR, Louisiana | 0.36 | Boersen et al. 2003 |
| Big Pocosin, North Carolina | 0.53 | Martorello 1998 |
| Tensas River Basin, Louisiana | 0.66 | Hooker 2010 |
| Great Dismal Swamp, Virginia/North Carolina | 0.47-0.68 | Hellgren and Vaughn 1989b |
| Alligator River NWR, North Carolina | 0.86 | Allen 1999 |

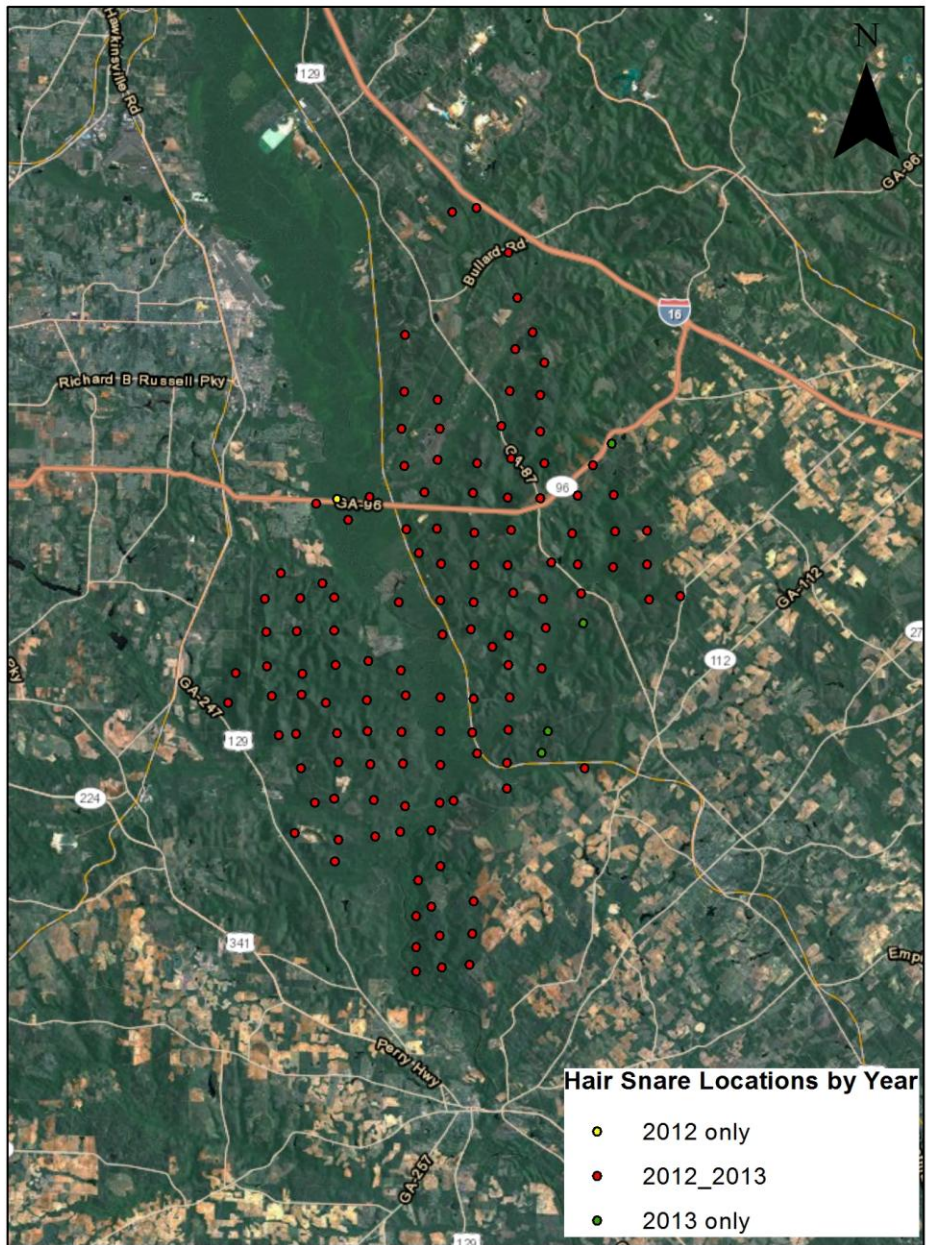


Figure 2.1. Location and distribution of hair snares used to collect bear hair in central Georgia, USA during the summers of 2012 and 2013.

CHAPTER 3

SUMMARY AND CONCLUSIONS

The abundance estimates for 2012 and 2013 were 98 (SE = 62) for males and 70 (SE = 16) for females, and 70 (SE=18) for males and 69 (SE = 18) for females, respectively.

Abundance estimates and associated standard error were consistent for females between the 2 years. While estimates for males between the 2 years were similar, the standard error associated with the 2012 abundance estimate was considerably larger. Our density estimates for 2012 and 2013 were 0.31 bears/km² and 0.26 bears/km², respectively, and were within the range of other populations in the Southeast. The density estimate for each year was calculated using the respective abundance estimate for that year, so the 2013 density of 0.26 bears/km² is the most recent and is likely more accurate since it is based on higher confidence estimates of abundance.

The total population size in 2012 and 2013 was 166 and 139, respectively. The 2012 estimate may have been inflated due to poor capture probabilities for males resulting in a high biased abundance estimate. Alternatively, the 2012 bear hunt removed 14 individuals (6M: 8F) from the population and an additional 3 (2M: 1F) bears were road killed between the 2 summers in which we conducted sampling. These 17 known losses would represent 10% of a population of 166 and could explain the lower abundance estimate in 2013.

Reliable abundance estimates in CMR studies require adequate capture probabilities. In 2012, we observed adequate capture probabilities for females ($p = 0.17$), but not for males ($p = 0.06$). The low capture probabilities for males likely inflated the estimated number of males in the 2012 population. We observed higher capture probabilities in 2013 and the resulting

abundance estimates were more precise. The top model in 2013 modeled capture probabilities by 2 unique time periods, the first and second 4 weeks of sampling, in which the capture probabilities improved modestly from $p = 0.12$ in the first 4 week period to $p = 0.15$ in the second 4 weeks. This supports time variation in capture probabilities over the sampling period, but our data were too sparse to develop well-supported, fully time-varying models.

Closed population modeling using CMR techniques assume that the population is demographically and geographically closed to gains and losses over the course of the sampling periods. Closure tests on our data in both years suggested that this assumption may have been violated, which would bias abundance estimates high. Closed population models also assume equal chance of capture for all individuals in the population. Several observations suggest that heterogeneity in capture probabilities among individuals may violate this assumption as well. For example, some bears were detected in many or all weeks whereas others were detected only once or twice; other bears which were radio-collared and known to be on the hair grid and near hair snares were not captured at all. Building on our dataset, future work would be able to use Pledger's (2000) mixture models and Pollock's (1982) robust design framework to account for capture heterogeneity and closure violations to a greater degree than our candidate models.

Our best abundance estimate at the end of the most recent sampling period (2013) was 139 black bears in a 528 km² area of central Georgia. We believe that our 2013 estimate is the most precise given the improved capture probabilities we observed that year and the resulting, more concise standard error associated with the abundance estimate. During the 1 day hunts of 2011-2013, 48 bears were harvested and half of these were females. Given the natural history of this species, especially its reproductive cycle, these rates are likely not sustainable. Estimates of cub survival, recruitment, and overall reproductive success will help to further inform the

viability of this population. The 2013 hunt that was set for December rather than November, when the 2011 and 2012 hunts occurred, resulted in only 1 harvested bear; if this population continues to be subjected to hunting pressure, later hunt dates when bears are less active should be used to increase individual survival during hunts.

Literature Cited

Pledger, S. A. 2000. Unified maximum likelihood estimates for closed capture-recapture models using mixtures. *Biometrics* 56:434–442.

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