POPULATION ABUNDANCE AND GENETIC STRUCTURE OF BLACK BEARS IN COASTAL NORTH CAROLINA AND VIRGINIA USING NONINVASIVE GENETIC TECHNIQUES

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ABSTRACT

The United States Fish and Wildlife Service (USFWS) expressed the need to develop appropriate management strategies for apparently high-density, growing black bear populations in the Roanoke-Neuse-Tar-Cape Fear ecosystem in coastal North Carolina and Virginia. In order to provide the scientific information necessary to develop these strategies, I investigated population densities and genetic structure of black bears at 3 national wildlife refuges [Great Dismal Swamp (GDSNWR), Pocosin Lakes (PLNWR), and Alligator River (ARNWR)].

Density estimates were derived from DNA samples collected noninvasively at each of the 3 refuges for 2 consecutive summers. Hair samples were analyzed for individual identification using 6-7 microsatellite markers. Estimated densities were some of the highest reported in the literature and ranged from 0.56-0.63 bears/km^2 at GDSNWR to 0.65-1.12 bears/km^2 at ARNWR to 1.23-1.66 bears/km^2 at PLNWR. Sex ratios were male-biased in all areas of all refuges.

Genetic variability and structure of bears at these refuges was assessed using 16 microsatellite markers for 40 bears from each refuge. Genetic variability of the 3 refuge populations was substantially high compared to other bear populations in North America, with observed heterozygosities ranging from 0.6729 at GDSNWR to 0.7219 at ARNWR. F_{ST} and D_S values were relatively low (0.0257-0.0895 and 0.0971-0.3640, respectively), indicating movement of bears and gene flow across the landscape is adequate to prevent high levels of genetic differentiation and structure among the refuge bears. Genetic statistics at GDSNWR indicate that this population is isolated to some degree by geography (i.e., the Albemarle Sound) and encroaching urban development (i.e., the towns of Suffolk and Chesapeake). ARNWR has

the potential to become isolated in the future if movement corridors to the south of the refuge are not maintained.

Harvest of bears is likely warranted at PLNWR and ARNWR, though extreme caution must be taken the first few seasons as hunter success will be extremely high. Further research is needed to determine population growth rates, reproductive parameters, and survival rates at all 3 refuges, particularly if a hunting season will be established and maintained in these areas. Methods for regularly monitoring bear populations at these refuges also should be incorporated into biological programs, as bears comprise a significant component of the ecosystem at these refuges and cannot be ignored when outlining management goals.

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research and thesis, but also played key roles in my formal training as a wildlife biologist and geneticist.

The grad student community in the department here at Tech has truly made Blacksburg home for me. I thank all of the students for lending an ear when I needed to bounce ideas off of someone, and for 5:00 beers to stir things up when we knew the ideas weren't bouncing anymore. The legendary Halloween parties, awesome hikes, wine tastings, Lyric movies, etc. will all be missed when I leave, but I will always have the amazing memories to take with me!

Most importantly, I thank my parents, Skip and Karen, who have supported me unconditionally in whatever I've decided to do throughout my life. I certainly would not be where I am today without their love and encouragement, and they have truly allowed me to pursue and live out my dreams.

Finally, I'd like to give a "shout out" to Mother Nature for those few days in the field when the temperature was below 100 degrees and the water was below my waist. She is a nasty little beast, but I will forever have tremendous respect for her!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

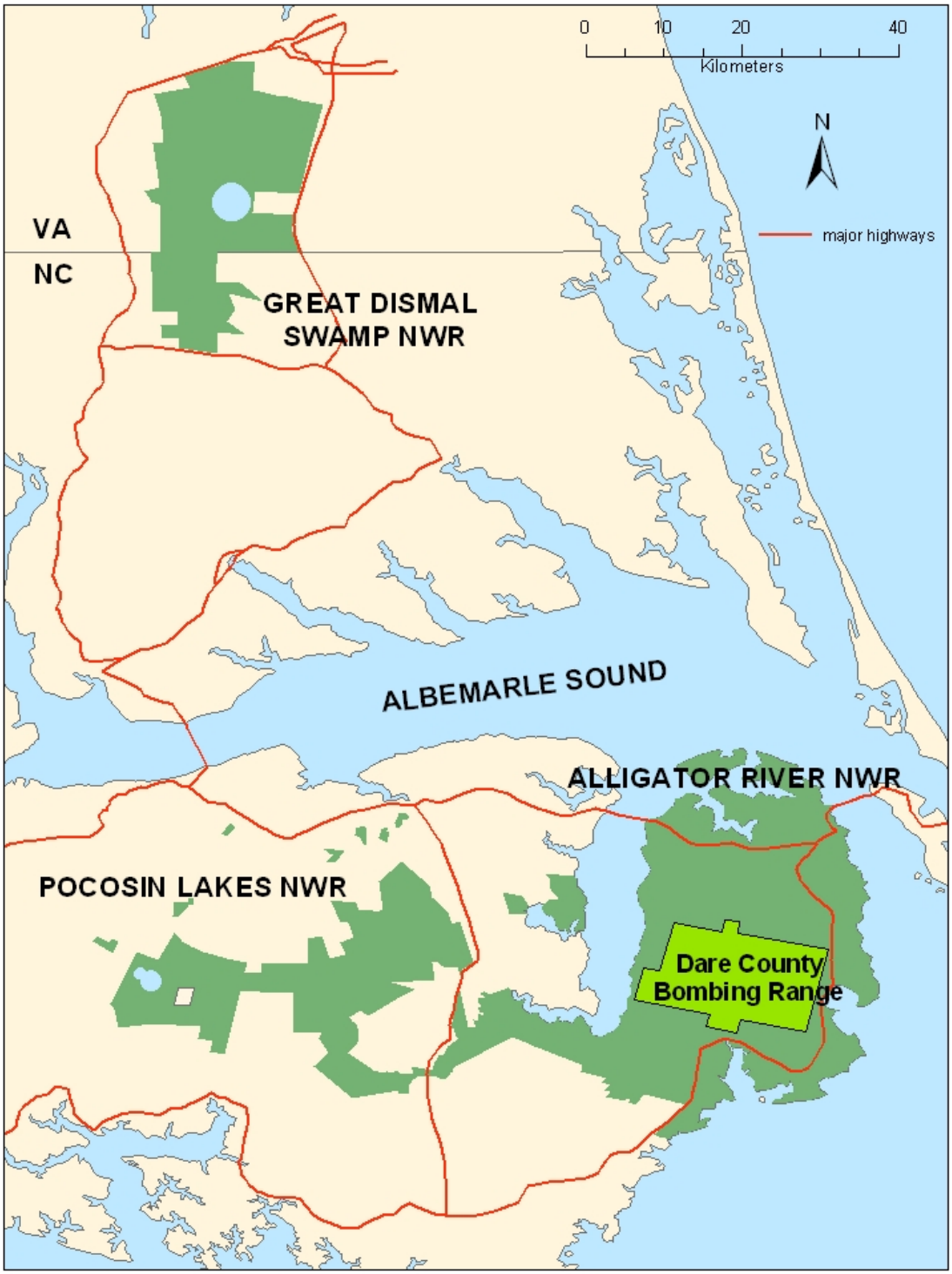
CHAPTER 1

JUSTIFICATION, OBJECTIVES, BACKGROUND, AND STUDY AREAS

JUSTIFICATION AND OBJECTIVES

Densities of black bear (*Ursus americanus)* in the Roanoke-Tar-Neuse-Cape Fear (RTNCF) ecosystem in coastal North Carolina and Virginia are some of the highest reported in North America (Martorello 1998; Allen 1999; Thompson 2003). Furthermore, United States Fish and Wildlife Service (USFWS) biologists have noted increased sightings of bears on national wildlife refuges in this region, and hunter complaints of bears taking deer carcasses and harassing them in deer stands have increased (W. Stanton, USFWS, personal communication), suggesting the bear population is approaching or surpassing cultural and biological carrying capacity for refuges and surrounding habitat in this region. Refuge managers and biologists have expressed the need to develop management strategies that will protect and perpetuate the welfare and integrity of the black bear population while minimizing potentially negative human/bear interactions that may increase with high density and growing bear populations. To develop defensible management strategies, however, reliable information regarding the population (i.e., population densities, growth rates, genetic structure, habitat requirements, etc.) is needed. The USFWS therefore commissioned this study to determine the size, density, and genetic structure of 3 unexploited black bear populations in the RTNCF ecosystem – Great Dismal Swamp National Wildlife Refuge (GDSNWR), Pocosin Lakes National Wildlife Refuge (PLNWR), and Alligator River National Wildlife Refuge (ARNWR, including the Dare County Bombing Range [DCBR]; Figure 1.1).

Figure 1.1. Study areas for a black bear project conducted on 3 national wildlife refuges (Great Dismal Swamp, Pocosin Lakes, and Alligator River) in coastal North Carolina and Virginia from 2001-2004.



Research on black bears in this region has been limited. Hellgren (1988) completed a demographic analysis of the bear population at GDSNWR during 1984-1987. Folta (1998) and Allen (1999) conducted a similar study at ARNWR from 1992-1996. Population estimates for these studies were determined using mark-recapture methods from live-captured bears, and confidence intervals were relatively large [262-377 bears (Hellgren 1988) and 372 ± 57.14 bears (Allen 1999)]. New techniques involving noninvasive collection of tissue samples (e.g., hair follicles) have been developed within the last 10 years and are proving useful for providing accurate mark-recapture estimates with relatively high precision (Woods et al. 1999, Poole et al. 2001). Genetic analysis of hair samples was used in this study to determine precise estimates of black bear numbers and densities on all 3 refuges.

A major concern in managing black bear populations on these refuges is genetic isolation and subsequent loss of genetic diversity. All 3 refuges are extensively isolated by water (i.e., ARNWR), agriculture, and human development. Preserving corridors for dispersal and migration among these refuges is essential for maintaining gene flow and preventing problems that result from loss of genetic diversity, such as inbreeding depression, reduced fecundity and survivorship, and a loss of adaptive potential (Meffe and Carroll 1997). Results of genetic analyses may also reveal the degree of isolation and gene flow among these populations, which will allow managers to assess whether or not dispersal and movement corridors between populations are effective.

My goal with this project was to provide refuge managers with the scientific data necessary to effectively manage bear populations in this region. To meet this goal, we outlined 3 primary objectives for this study:

1. Estimate the number, density, and sex ratio of black bears on these 3 refuges,

2. Assess genetic relatedness and gene flow among bears at these refuges, and
3. if warranted, develop an adaptive harvest management strategy for each refuge based on population estimates.

The methods and results for objectives 1 and 2 are discussed in chapters 2 and 3, respectively. Chapter 4 compares 2 different methods of collecting demographic data for bear populations. Chapter 5 outlines management recommendations and future research for black bear populations on these refuges based on findings from the first 2 objectives.

BACKGROUND

Black bears once inhabited contiguous forested areas throughout most of North America (Hall 1981). Due to increased human encroachment in areas where black bears exist, suitable habitat and bear numbers have declined significantly across their range (Maehr 1984; Hellgren and Maehr 1993; Pelton and van Manen 1994). Pelton (1986) suggested that black bears occupy only 5 to 10% of their historic range in the Southeast. In North Carolina and Virginia, black bears occupy the rugged, mountainous terrains in the western portions of both states and the swampy pocosins and associated wetlands along the coastal plain. In the coastal portion of Virginia, bears are limited to the Great Dismal Swamp and directly adjacent lands (Hellgren 1988); and in coastal North Carolina, bears are distributed patchily among federal lands where bears are protected (i.e., PLNWR and ARNWR), on federal forest and military lands, and on certain privately owned lands **(**Maehr 1984; Pelton and van Manen 1994).

Until the mid-1970s, black bear populations in the coastal areas of North Carolina and Virginia declined due to habitat degradation and excessive harvest (Collins 1990). In 1971, a system of 28 bear sanctuaries was established in North Carolina by the North Carolina Wildlife

Resources Commission (NCWRC) to "protect a breeding nucleus of bears" and "eventually produce a dispersing surplus [of bears] into surrounding areas" for harvest (Sanders 1978; Powell et al. 1996). Seventeen of these sanctuaries were on the coastal plain, including DCBR, situated in what is now ARNWR. Additionally, 10 coastal counties established local legislation that prohibited bear hunting during the early 1970s, including Dare County, where ARNWR was established in 1984 (Allen 1999). GDSNWR was established in 1973 and functioned as a de facto bear sanctuary since hunting was prohibited (Hellgren 1988). A portion of PLNWR was established in 1963 (as Pungo NWR), with subsequent lands being added until its establishment as it stands today in 1990. Between 1981 and 1991, occupied bear range in North Carolina increased 47% (Jones et al. 1995), indicating that this protection proved beneficial to bear populations in the Coastal Plain.

The challenge now facing managers and biologists on these refuges and sanctuaries is to maintain bear populations at a level that provides a surplus for harvest, while at the same time minimizing bear-human conflicts due to an overabundance of bears. The results of this study will assist managers by providing the scientific data necessary to meet this challenge.

STUDY AREAS

This study took place on 3 National Wildlife Refuges on the Coastal Plain of eastern North Carolina and Virginia (Figure 1.1). These areas are characterized as forested wetlands consisting primarily of short and tall pocosins, cypress-gum (*Taxodium-Nyssa spp.*) forests, freshwater pools, and farmland. Pocosins are freshwater wetlands commonly described as evergreen shrub bogs (Richardson 1983). The term pocosin comes from the Algonquin Indian word "poquoson," meaning "swamp-on-a-hill." The mosaic of coastal plain pocosin, agriculture,

and tall forests provides optimum foraging and protective habitat for black bears (Hinesley 1999).

Great Dismal Swamp National Wildlife Refuge

The 550 km^2 Great Dismal Swamp National Wildlife Refuge straddles the Virginia-North Carolina state line. It is bordered to the north by the cities of Suffolk, Chesapeake, and Portsmouth, to the east by the Dismal Swamp Canal and U.S. Highway 17, to the south by Highway 158 and a mosaic of agricultural fields and privately-held swamplands, and to the west by the Suffolk Scarp, a >200 km escarpment that runs north-south from southeastern Virginia into North Carolina (Hellgren 1988). The Albemarle Sound lies approximately 40 km south of GDSNWR and could potentially be a barrier to dispersal and movement for black bears moving to or from the other 2 refuges. Lake Drummond, a shallow, natural lake approximately 4 km in diameter, lies nearly in the center of the refuge.

The refuge is generally flat, but is characterized by an east-west gradient of approximately 19 cm/km (Gammon and Carter 1979). Mean temperatures for January and July are 5.1ºC and 26.0ºC, respectively, and annual precipitation averages 120 cm (Hellgren 1988).

Vegetation in GDSNWR consists primarily of maple-gum (*Acer-Nyssa spp.*) and cypress-gum (*Taxodium-Nyssa spp.*) forests (73%), Atlantic white cedar (*Chamacyparis thyoides*) forest (12%), and pine- and mixed-hardwood forest (14%; GDSNWR; unpublished data). Major tree species include red maple (*A. rubrum*), black gum (*N. sylvatica*), red bay (*Persea borbonia*), Atlantic white cedar, swamp tupelo (*N. aquatica*), bald cypress (*T. distichum*), and pond pine (*Pinus serotina*). Major understory shrubs include sweet pepperbush (*Clethra alnifolia*), blueberry (*Vaccinium spp.*), fetterbush (*Lyonia lucida*), hollies (*Ilex spp.*), leucothoe (*Leucothoe*

spp.), and myrtle (*Myrica cerifrea*). Major vine species include greenbriar (*Smilax spp.*), wild grape (*Vitis spp.*), Japanese honeysuckle (*Lonicera japonica*), poison ivy (*Toxicodendron radicans*), and gessamine (*Gelsemium sempervirens*). Switchcane (*Arundinaria gigantea*) forms a dense portion of forest understories (Hellgren 1988).

Pocosin Lakes National Wildlife Refuge

Pocosin Lakes National Wildlife Refuge is approximately 460 km^2 and is located in Washington, Tyrell, and Hyde counties in North Carolina. It is approximately 90 km due south of GDSNWR. The refuge is bordered by Lake Phelps and the Scuppernong River to the north, the Alligator River to the east, the Pungo River and New Lake to the south, and Pungo Lake to the west (Hinesley 1999). The refuge contains nearly 7,000 acres of freshwater lakes and pools, including Lake Phelps, Pungo Lake, and New Lake (PLNWR; unpublished data).

The swampy refuge is generally flat, but elevations range from ~6m at the highest point south of Phelps Lake to <1m in the eastern portions of the refuge (Hinesley 1999). Mean temperatures for winter and summer are approximately 3.4ºC and 28ºC, respectively, and annual precipitation ranges from 115 to 127.5 cm (Thompson 2003).

PLNWR consists primarily of high pocosins (39%), mixed- and bottomland hardwoods (27%) and grass and shrub pocosins (26%, PLNWR; unpublished data). Other major habitats include managed grassland, farmland (1%), and cypress-gum and bay forest. Major tree species are similar to GDSNWR and include red maple, black gum, swamp tupelo, red bay, loblolly bay (*Gordonia lasianthus*), Atlantic white cedar, bald cypress, loblolly pine (*P. taeda*), and pond pine. Major understory shrubs include switchcane, blueberry, fetterbush, hollies, poison ivy, titi

(*Cyrilla racemiflora*), and myrtle. Major vine species include greenbriar, wild grape, and Japanese honeysuckle. Major agricultural crops rotate between corn, soy, milo, and wheat.

Alligator River National Wildlife Refuge and Dare County Bombing Range

Alligator River National Wildlife Refuge lies on the Dare County Peninsula approximately 50 km east of PLNWR and just inland from the Outer Banks of North Carolina. A small portion of the refuge (54 km^2) is in Hyde County, with the majority (598 km^2) in Dare County. The Dare County Bombing Range (DCBR), operated by the U.S. Air Force and Navy and located in the south-central portion of ARNWR, adds 189 km^2 for a total area of approximately 841 km^2. The refuge is bounded to the north by the Albermarle Sound, to the east by the Croatan Sound, to the west by the Alligator River, and to the south by a mosaic of agricultural and privately owned forested land (Allen 1999), which potentially connects the black bear populations from PLNWR and ARNWR. U.S. Highways 64 and 264 traverse the northern and eastern sections of the refuge, respectively. A 16 km^2 farm unit is located near the northern end of the refuge.

Topography is relatively flat, with slopes ranging from 0-2% (Folta 1998). Average temperature for winter is about 7ºC, while temperatures in the summer commonly exceed 32ºC. Annual average precipitation is approximately 122.5 cm (Allen 1999).

Primary habitat types on ARNWR include pine- and mixed-hardwood forest (23%) and pine and low shrub pocosin (35%; ARNWR; unpublished data). Major tree species include pond pine, red maple, black gum, red bay, Atlantic white cedar, bald cypress. Major understory shrubs include switchcane, blueberry, blackberry, devil's walkingstick (*Aralia spinosa*),

fetterbush, hollies, titi, and myrtle. Major vine species include greenbriar, wild grape, Japanese honeysuckle, and poison ivy. Agricultural crops include corn, milo, soy, oats, and wheat.

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CHAPTER 2

POPULATION ESTIMATES, DENSITIES, AND SEX RATIOS OF BLACK BEARS ON 3 NATIONAL WILDLIFE REFUGES IN COASTAL NORTH CAROLINA AND VIRGINIA

INTRODUCTION

Screening of genetic markers for wildlife species now allows scientists to obtain precise estimates of size and density of wild animal populations based on DNA samples (e.g., hair, feces) collected noninvasively in the field (Woods et al. 1999; Mowat and Strobeck 2000; Poole et al. 2001). These methods are proving to be more effective and efficient than traditional capture-mark-recapture (CMR) techniques that require intense efforts in the field (e.g., deploying radio collars) (Mowat and Strobeck 2000). Noninvasive collection of tissue samples, such as hair and scat, does not require the costly and invasive process of physically capturing individual animals, which may limit sampling efforts to smaller areas due to cost and time constraints (Foran et al. 1997). Other advantages of noninvasive techniques include increased capture probabilities and sample sizes, decreased tag loss, and decreased effects of capture and marking (Mills et al. 2000). Once genetic material has been collected from animals, microsatellite DNA markers are used to uniquely "mark" individuals. Microsatellites are highly variable tracts of nuclear DNA consisting of short tandem repeats of 1-5 base pair motifs (e.g., CACACACA). The number of repeats at a given locus varies greatly from individual to individual, allowing easy identification of individual animals. Paetkau and Strobeck (1994) developed the first microsatellite markers that could be used to uniquely identify bears; there are now more than 20 microsatellite markers available to identify bears (Waits 1999). Capture histories of uniquely identified individuals then can be used in a CMR framework to estimate population abundance

and density. The DNA samples also can be used to identify sex of individuals and assess genetic population structure (Woods et al. 1999).

My objective with this chapter was to determine population size, density, and sex ratios of black bears on Great Dismal Swamp National Wildlife Refuge (GDSNWR), Pocosin Lakes National Wildlife Refuge (PLNWR), and Alligator River National Wildlife Refuge [ARNWR, including the Dare County Bombing Range (DCBR)] using noninvasive genetic techniques (i.e., hair snaring).

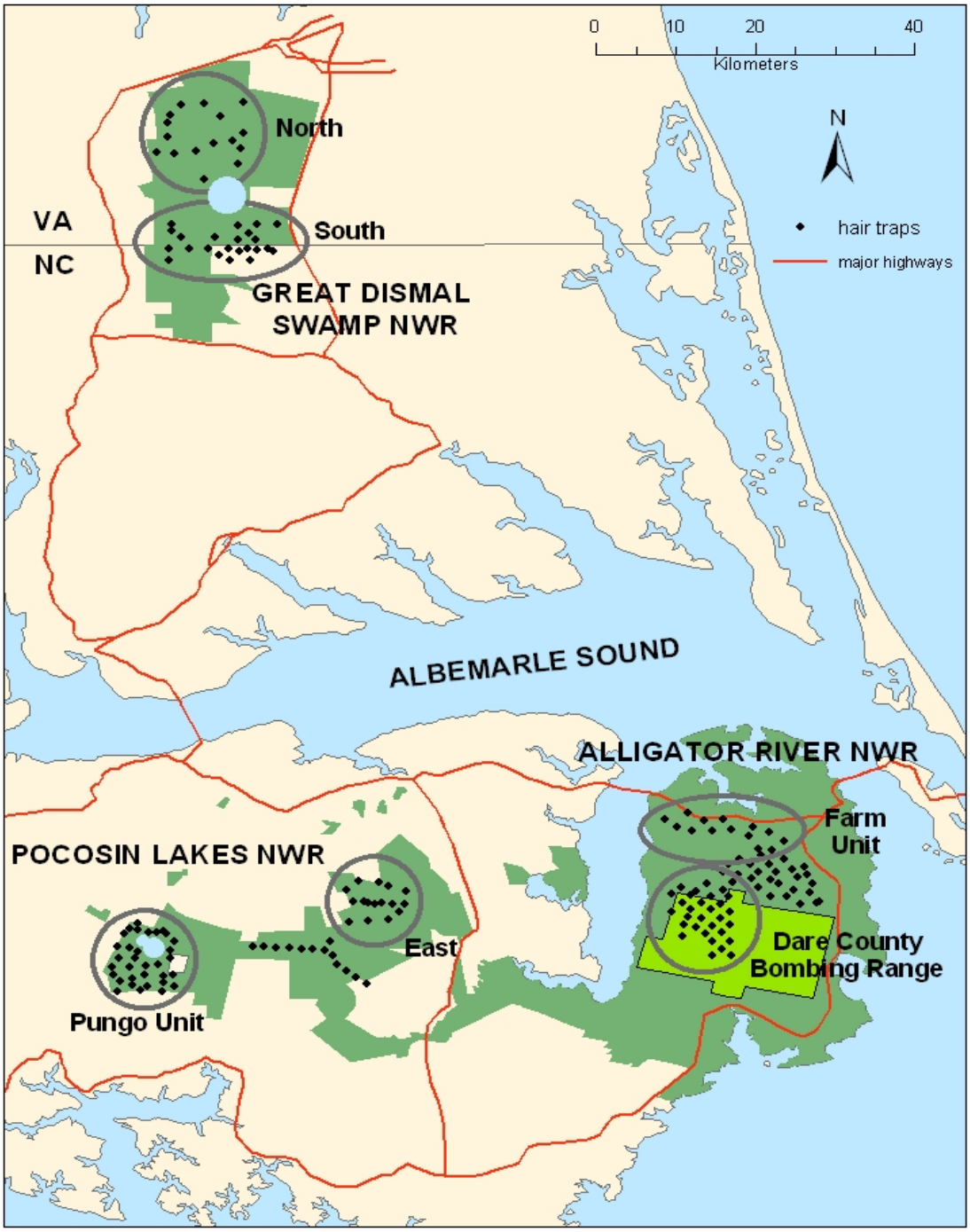
METHODS

Hair trap design

I selected specific areas on each refuge for hair trapping based on accessibility and logistic constraints. In each area, I established a grid system of 1 km^2 cells and placed hair traps near the center of every other cell. Cell size took into account home range size of bears in this area, which ranged from 1.1 km^2 to 30 km^2 (Hellgren 1988; Allen 1999). This was expected to provide at least one hair trap in each bear's home range such that each bear had a probability of being captured. Logistical constraints prevented me from following the recommendation of Otis et al. (1978) of placing 4 or more traps in each animal's home range. I used a handheld GPS unit in the field to determine exact locations of the traps.

Two grid systems were established at GDSNWR, a 75 km^2 grid south of Lake Drummond, and another 100 km^2 grid north of the lake. Traps covered approximately 175 km^2 of the 550 km^2 (31.8%) refuge (Figure 2.1). PLNWR was divided into 3 distinct grids for hair traps. These included the 60 km^2 Pungo Unit on the west end of the refuge, a 40 km^2 area on the eastern end of the refuge, and a 15 km wide transect that connected the eastern and western

Figure 2.1. Distribution of hair traps used to study population abundance and genetic relatedness of black bear populations on 3 national wildlife refuges (Great Dismal Swamp, Pocosin Lakes, and Alligator River) in coastal North Carolina and Virginia. Areas of interest for population estimation are circled in gray and labeled.



portions. Traps covered approximately 115 km^2 of the 460 km^2 (25.0%) refuge (Figure 2.1). A continuous grid (150 km^2 of the 840 km^2 refuge – 17.9%) was constructed at ARNWR and DCBR (primarily covering the western half), although the 30 km^2 farm unit on its northern end, and the western portion (60 km^2) of DCBR on the southern end, were considered distinct areas of interest for population analyses (Figure 2.1).

Hair traps were placed primarily in prime bear habitat as determined by previous studies on these refuges (Hellgren 1988; Allen 1999). Prime bear habitat consisted of areas with adequate escape cover (i.e., tall trees) and sufficient food sources, including stands of bottomland hardwood, high pocosin, bay forest, pine- and mixed-hardwoods, and cypress-gum swamps. Poor habitat (low pocosin, shrub pocosin, and marsh), in general, was not sampled. Atlantic white cedar (AWC) habitat is known to be unfavorable bear habitat in the region (Hellgren 1988; Allen 1999), but these stands were generally small and occurred within a matrix of other prime habitat. The majority of GDSNWR is composed of prime bear habitat, including maple-gum forest (62%), cypress-gum forest (11%), and pine- and mixed-hardwood forest (14%; Appendix C; GDSNWR; unpublished data). Approximately 12% of the refuge is AWC habitat. Approximately 39% of PLNWR is dominated by high pocosin habitat, followed by 27% mixed- and bottomland hardwoods (Appendix D; PLNWR; unpublished data). About 26% of PLNWR is grass and shrub pocosin, including most of the southern half of the Pungo Unit. Although samples from bears were collected on hair traps in this area, the density of bears appeared to be lower than on the remainder of the Pungo Unit and in the eastern portion of the refuge (personal observation based on visual sightings and number of hair samples collected). The western 1/3 to 1/2 of ARNWR and DCBR is comprised of a matrix of prime bear habitat, including pine- and mixed-hardwood forest (23%) and cypress-gum forest (5%). The eastern portion is comprised

primarily of lower quality bear habitat [pine and low shrub pocosin (35%) and smaller tracts of high shrub pocosin and marsh (8%; Appendix E; ARNWR; unpublished data)]. This area was not sampled with hair traps (Figure 2.1).

At each hair trap location, I made an enclosure by stringing a single strand of 15.5-gauge barbed wire around 3-4 trees and/or fenceposts at a height of ~40-50 cm from the ground. I filled in large depressions and removed excess debris from under the wire to maintain a consistent distance between the wire and the ground. I suspended bait (donuts) approximately 1.8 m above the ground in the center of each trap, and made certain that each trap was large enough to prevent bears from reaching the bait without entering the trap. I also hung a film canister with scent lure (raspberry or beef extract) at least 4 m above the ground within each trap. I used UTM coordinates of the northernmost corner of each trap as its location. This location also served as the "start" point during hair collection. I numbered barbs clockwise, starting with 1, beginning at this northernmost corner. I placed bright flagging on each trap along with signs explaining its purpose.

Hair collection

I ran hair traps for 8 weeks during the summers for 2 consecutive years at each refuge (GDSNWR: 2001-2002, PLNWR: 2002-2003, ARNWR: 2003-2004). I ran 52, 62, and 71 traps at GDSNWR, PLNWR, and ARNWR, respectively. During the second years at GDSNWR and ARNWR, only 51 and 70 traps were activated, respectively. At the beginning of each sampling season, I burned the wire to ensure no hair or genetic material was on the wire. I hung bait in the center of each trap either from a tree or from string stretched diagonally between 2 corner trees. I used only a scent lure during the second year of trapping (2002) on the northern grid at

GDSNWR, while a small food reward (cakes, donuts, etc.) was used on the southern grid. Only a small food reward was used at PLNWR in 2002. During the second year of trapping (2003) at PLNWR and both years (2003-2004) at ARNWR, I used a scent lure in combination with a small food reward to deal with the problem of baits being taken early in the sampling period, leaving nothing to attract subsequent bears to the wire. I switched lures halfway through sampling (i.e., after week 4) at each refuge each summer to prevent bears from becoming apathetic to the hair traps.

I collected hair from all traps approximately every 7 days. I removed each hair sample (a single barb with hair) with hemostats and placed it in a coin envelope. I labeled envelopes with the trap ID, date, barb number, and a number indicating the number of hairs in the sample (0 = 0-4 hairs, 1 = 5-10 hairs, 2 = 10+ hairs). After each sample was collected, I burned both the barb and hemostats to prevent cross-contamination between samples. Due to financial constraints and since more hairs (≥ 10) provide more accurate results in the lab (Goosens et al. 1998), I did not collect samples with < 5 hairs from traps that had 2 or more additional samples with ≥ 5 hairs. After all samples were collected, I maintained traps as necessary, refreshed the scent lures, and rebaited them for the next period. I stored all hair samples in a temperature-controlled, dry room until they were submitted for genetic analysis.

Genetic Analysis

Samples from 2001 at GDSNWR were prepared at Virginia Tech and analyzed at the Leetown Science Center in Kearneysville, West Virginia. Subsequent samples were sent to Wildlife Genetics International in Nelson, B.C., Canada for analysis. Genetic protocols at both labs were similar. DNA was extracted from hair follicles and polymerase chain reaction (PCR)

was used to amplify DNA. Each PCR reaction consisted of the extracted template DNA, the primers for each locus being analyzed, appropriate fluorescent markers to distinguish amplification products, *Taq* polymerase, nucleotides, and various buffers (see Paetkau et al. 1995, 1998a, 1998b, 1999 and Paetkau and Strobeck 1994 for further details). Amplification products were run through an automated DNA sequencer to generate individual genotypes. Exploratory analysis on a small number of samples from each refuge determined which of the 20 microsatellite markers for bears would be most powerful (i.e., variable) for each population. This also determined the number of loci needed to sufficiently resolve all individuals with an acceptably low probability of identity (PI; see below). For PLNWR and ARNWR, these included loci G1A, G1D, G10H, G10J, G10L, and MU50, while the loci used for GDSNWR were G1A, G10H, G10J, G10L, G10P, MU23, and MU50 (Paetkau and Strobeck 1994, Paetkau et al. 1995). Seven loci were used at GDSNWR compared to 6 at PLNWR and ARNWR since an initial subset of GDSNWR samples showed lower variability with only 6 markers. A 10-marker system (G1A, G10B, G10C, G1D, G10L, G10M, G10P, G10X, MU23, and MU50) was used at the Leetown Science Center for samples collected at GDSNWR in 2001. Sex was inferred for each individual identified using the sex determination assay developed by Ennis and Gallagher (1994). Strict laboratory protocols (see Paetkau 2003) were followed to minimize the likelihood of scoring and amplification errors.

Due to the large number of samples I collected and the expense associated with analyzing each sample (US$30-50/sample), subsampling was necessary. I set out an *a priori* subsampling scheme to prevent sampling bias. First, only high quality samples (> 5 hairs) were analyzed when > 300 samples were collected in a sampling season. If there was more than one sample at a trap in a given period, I randomly selected 2 samples for analysis. Once this was done and if

funding allowed, I randomly selected further samples for analysis. To investigate the effect of subsampling on population estimates, confidence intervals (CI), and coefficients of variation (CV), I analyzed all samples from one area of PLNWR collected during summer 2002. The results of this analysis supported the subsampling scheme outlined above (Appendix A).

Population and Density Estimation

I used full closed capture with heterogeneity models in Program MARK (White and Burnham 1999; Otis et al. 1978; Pledger 2000) to calculate population estimates for each refuge in each year. Assumptions associated with closed CMR methods include:

1) The population is closed to additions (births or immigration) and deletions (deaths or emigration) over the course of the study,
2) animals have a constant and equal probability of capture on each trapping occasion, and capture and marking do not have an effect on the animal's catchability,
3) all marks are correctly noted and recorded at each trapping occasion, and
4) animals do not lose their marks during the study.

Demographic closure was likely met in this study since sampling seasons were kept short (8 weeks during summer), no births occur during summer, and survival for bears during summer is high (Folta 1998; Bridges 2005). Geographic closure likely was violated in this study, however, since no physical boundaries delineated the study areas and bears were free to move in and out of the study areas (White et al. 1982).

A bear's genetic "mark" cannot be lost (assumption 4), but genetic errors in the lab can lead to "marking" a bear incorrectly (assumption 3). Genotyping errors usually result from amplification or scoring errors (Paetkau 2003; Mills et al. 2000; Waits and Leberg 2000;

Taberlet et al. 1996, 1999). These errors can cause bias not only in population estimates, but in calculations of genetic differentiation and gene flow as well. Amplification errors occur during the PCR process and are broken down into two types. "Allelic dropout" is the failure to amplify one allele in a heterozygote, causing it to be incorrectly scored as a homozygote. Amplification of a false allele (due to "slippage" in the PCR process) can cause a homozygote to be incorrectly scored as a heterozygote. Paetkau (2003) noted that allelic dropout is much more common, and outlined an error-checking protocol that can be used to minimize these errors in the lab. Scoring errors occur when the appearance of the raw data leads experienced workers to record an incorrect genotype (due to presence of shadow peaks, etc.). Waits and Leberg (2000) used population simulations to determine how these genotyping errors bias population estimates and found that high rates of genotyping error lead to overestimates of population size. They recommend using a few highly polymorphic loci rather than a larger number of loci with few alleles, since the chance of genotyping error increases with the number of loci used.

In some cases, the genetic markers being used may lack the power to resolve all individuals, and different animals may mistakenly exhibit the same genetic profile ("shadow effect;" Mills et al. 2000). This effect will lead to underestimates in subsequent population estimates. Researchers are able to quantify the power of various molecular markers by calculating a probability of identity (PI), which is the probability that two individuals drawn at random from a population will have the same genotype at multiple loci (Paetkau and Strobeck 1994; Woods et al. 1999; Waits and Leberg 2000; Mills et al. 2000; Waits et al. 2001). The lower the PI value for a given set of markers, the more power to distinguish between individuals. Probability of identity is calculated for a single locus with multiple alleles by:

$$\mathrm{PI}_{\text{single locus}} = \sum_i p_i^{\,4} + \sum_i \sum_{j>i} (2 p_i p_j)^2 ,$$

where p_i and p_j are the frequencies of the *i*th and *j*th alleles, assuming the allele genotypes conform to Hardy-Weinberg proportions (Paetkau and Strobeck 1994; Mills et al. 2000). The overall PI for a given population is estimated as the product of the PI values for all loci (Mills et al. 2000):

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

The assumption that loci are assorted independently must be met (linkage equilibrium), or PI values will be biased low (Mills et al. 2000).

Further bias may be introduced when populations contain many siblings (Donnelly 1995). Taberlet and Luikart (1999) outline a method for calculating PI for siblings, which represents the upper limit on the possible range of PIs in a population. The equation is given as:

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

For CMR studies, Taberlet and Luikart (1999) found that 4-8 loci should be sufficient for resolving random individuals and siblings, yielding PI as low as 0.01.

Woods et al. (1999) used a match statistic that provides PI estimates for each individual genotype rather than over all observed genotypes. Since bears often travel in family groups, and related females often occupy overlapping adjacent home ranges, P_{sib} is the most conservative PI estimate (as opposed to random or parent-offspring estimates) and is calculated for homozygotes as:

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

and for heterozygotes as:

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

Woods et al. (1999) considered genotypes that produced a $P_{sib} > 0.05$ not to be unique individuals and threw them out of consideration in their analysis. By using a sufficient number

(≥ 7; Mills et al. 2000) of highly variable markers, problems with individuals sharing the same profile can be minimized.

PI, PI*sibs*, and P_{sib} statistics were calculated for all observed genotypes. Any data with PI*sibs* > 0.01 or $P_{sib} > 0.05$ were excluded from population analyses.

It is widely recognized that equal catchability (assumption 2) commonly is not met in most CMR studies (Otis et al. 1978). Numerous models have been developed, however, that allow this assumption to be relaxed (Otis et al. 1978; White et al. 1982; White and Burnham 1999; Pledger 2000). These models allow capture probabilities to vary over time (temporally, model M_t), by trap response (behaviorally, model M_b), and by individual (heterogeneity, model M_h). Combinations of these models (M_{bh}, M_{tb}, M_{th}, and M_{tbh}) also exist. Pledger's (2000) mixture models give a framework for fitting all eight of these models (including the null model, M_o) by maximum likelihood. These were run in MARK (White and Burnham 1999) with each dataset from each refuge in each summer, as well as for each distinct area of each refuge (see above) using 2 mixtures and 8 1-week encounter occasions. Once models were run, Akaike's Information Criterion (AIC; Akaike 1973) was used to select the best model for a given dataset. AIC is an index of the balance of model fit and explanatory power related to the number of parameters in the model. In certain cases, more than 1 model may be appropriate and models may be averaged to determine parameter estimates (Burnham and Anderson 1998). For these analyses, all models with ΔAIC values < 2.0 were averaged.

Abundance estimates are generally more practical and useful when given in terms of animal densities (Otis et al. 1978). When calculating population densities, one must take into account animals whose home ranges lie partially outside the trapping grid. Abundance estimates (N) therefore actually apply to an area greater than the trapping grid itself. This is referred to as

"edge effect," and numerous methods exist to calculate this edge area. I used 2 of these methods to estimate population densities at GDSNWR, PLNWR, and ARNWR. Dice (1938) recommended placing a buffer strip around the trapping grid equal to the radius of an average home range. I used summer home ranges from Hellgren (1988) for GDSNWR and from Allen (1999) for ARNWR. No home range data were available for PLNWR, so we collected telemetry data from 9 bears to generate home range data for this refuge. The second method to calculate the edge area followed Wilson and Anderson (1985), who proposed using half the mean maximum distance moved (MMDM) by bears between hair traps to calculate a buffer strip. Bears that had a maximum distance of 0 (i.e., were captured at only 1 hair trap in multiple trapping periods) were included in the calculations. Bears that were captured only once in a summer were not included.

Sex ratios

I used the *z*-test for binomial proportions to determine if sex ratios differed from 1:1 in the genetic datasets from each refuge each year.

RESULTS

Hair collection

During 4 summers of sampling (2001-2004), I collected 5,446 hair samples from the 3 refuges. The number of samples collected at GDSNWR (204 and 223 samples in 2001 and 2002, respectively) was 4-7 times less than the number collected at PLNWR and ARNWR. I collected 823 and 1,286 samples at PLNWR in 2002 and 2003, respectively, and 1,207 and 1,703 samples at ARNWR in 2003 and 2004, respectively (Table 2.1).

The number of traps visited by bears (i.e., "hit" where bears left ≥ 1 sample) at GDSNWR also was lower than at PLNWR and ARNWR. In 2001, 39 of 52 (75%) traps were hit, and in 2002, 41 of 51 (80.4%) traps were hit at GDSNWR. Trap success [no. traps hit/no. traps available (e.g., 52 traps x 8 weeks of sampling)] at GDSNWR was 18.5% and 24.8% in 2001 and 2002, respectively. At PLNWR, 100% (62 of 62) of traps in 2002 and 98.4% (61 of 62) of traps in 2003 were hit by bears. Overall trap success at PLNWR was 55.8% in 2002 and 83.9% in 2003. All traps at ARNWR were hit in both years of hair sampling with overall trap success of 76.9% in 2003 and 88.8% in 2004 (Table 2.1).

All 427 samples collected at GDSNWR were submitted for genetic analysis, while only 1,318 of 2,109 (62.5%) of samples from PLNWR and 1,405 of 2,910 (48.3%) of samples from ARNWR were analyzed (Table 2.1).

Genetic Analysis

Since data collected at GDSNWR in 2001 were analyzed at a different lab using different genetic markers, I elected not to include these data in my analyses. From the 223 hair samples collected at GDSNWR in 2002, 67 multilocus genotypes were identified as unique individual bears. Forty bears were identified in the southern portion of the refuge and 28 bears were identified in the north (1 bear was captured in both areas). Thirty-five (15.7%) samples failed to produce complete genotypes due to insufficient amounts of DNA or mixture of DNA from 2 or more bears. Sex identification of the 67 individuals indicated a ratio of 42M:25F (South 26M:14F, North 17M:11F). PI for this dataset was 4.39×10^{-7}, P*sibs* was 0.003, and P_{sib} ranged from 0.0008-0.0105 for all genotypes, so no putative individuals were excluded from analyses (Table 2.1).

Table 2.1. Hair trap statistics for 3 summers of sampling at 3 national wildlife refuges [Great Dismal Swamp (GDSNWR), Pocosin Lakes (PLNWR), and Alligator River (ARNWR)] in coastal North Carolina and Virginia during 2002-2004.

	Year	No. of hair traps	No. of samples collected	No. of samples analyzed	Traps hit (%)	Trap success (%)	No. of bears identified	Samples failed (%)	PI[a]	PIsibs[b]
GDSNWR	2002	51	223	223	80.4	24.8	67	15.7	4.39×10^{-7}	0.003
PLNWR	2002	62	823	690	100	55.8	160	13.9	4.8×10^{-8}	0.002
PLNWR	2003	62	1,286	628	98.4	83.9	173	17.2	3.63×10^{-8}	0.002
ARNWR	2003	71	1,207	603	100	76.9	147	17.4	5.66×10^{-8}	0.002
ARNWR	2004	70	1,703	802	100	88.8	130	18.8	4.45×10^{-8}	0.002

[a]PI = Probability of identity = probability 2 individuals will share the same genotype
[b]PIsibs = Probability of identity for siblings = upper limit on possible range of PIs in a population

One-hundred sixty unique individuals (94M:66F) were identified from the 690 hair samples analyzed from PLNWR in 2002. Eighty-five bears (46M:39F) were captured on the Pungo Unit, 46 bears (31M:15F) were captured on the eastern portion of the refuge, and 29 bears (17M:12F) were captured in the middle portion. Insufficient DNA or mixed samples resulted in incomplete or failed genotype analysis for 96 (13.9%) of these samples. PI for this dataset was 4.8×10^{-8}, P*sibs* was 0.002, and P_{sib} ranged from 0.0010-0.0105 for all genotypes, so again no putative individuals were excluded from analyses (Table 2.1).

In 2003, 628 hair samples yielded 173 unique individuals (114M:59F) at PLNWR. Seventy-four of these 173 individuals (42.8%) had been identified from hair samples collected in 2002. Therefore, 99 new individuals were identified at PLNWR in 2003 (69M:30F). Eighty individuals (44M:36F) were identified on the Pungo Unit, 56 bears (41M:15F) were identified on the eastern portion of the refuge, and 37 bears (29M:8F) were captured in the middle portion. One-hundred eight (17.2%) of the total 628 hair samples from 2003 failed to produce complete genotypes in the lab. PI for this dataset was 43.63×10^{-8}, P*sibs* was 0.002, and P_{sib} ranged from 0.0010-0.0105 for all genotypes, so all putative individuals were included in population analyses (Table 2.1).

Data from ARNWR in 2003 revealed 147 unique individuals (86M:61F) from 603 hair samples. Forty-nine of these bears (35M:14F) were captured on the farm unit, while 66 (37M:29F) were captured on DCBR. One male bear was captured on both the farm unit and DCBR. The remaining 33 bears (15M:18F) were captured in the middle portion of the refuge between DCBR and the farm unit. Incomplete or failed genotype analysis occurred with 105 (17.4%) samples. PI for this dataset was 5.66×10^{-8}, P*sibs* was 0.002, and P_{sib} ranged from

0.0011-0.0058 for all genotypes, leaving all genotypes available for use in population analyses (Table 2.1).

In 2004, 130 individual bears (73M:57F) were identified from 802 hair samples at ARNWR. Sixty-nine (53.1%) of these 130 bears were captured and identified in 2003. Therefore, 61 new bears were identified at ARNWR in 2004 (34M:27F). Forty-three bears (27M:16F) were captured on the farm unit, 63 (36M:27F) were captured on DCBR, and 26 bears (12M:14F) were captured in the middle portion of the refuge between DCBR and the farm unit. Two male bears were captured on both the farm unit and DCBR. One-hundred fifty-one (18.8%) of the 802 samples from 2004 failed to produce complete genotypes in the lab. PI for this dataset was 4.45×10^{-8}, P*sibs* was 0.002, and P_{sib} ranged from 0.0011-0.0066 for all genotypes, so all samples were included in population analyses (Table 2.1).

Genotype frequencies at 1 locus (PLNWR, G10H, $p = 0.01$) did not conform to Hardy-Weinberg proportions, but the departure was not significant following sequential Bonferroni adjustment ($\alpha = 0.008$). Associations of alleles at different loci (linkage disequilibrium) were found with 12 pairs of alleles, but only 2 remained significant following sequential Bonferroni adjustment ($\alpha = 0.003$). These deviations are likely due to less than perfect random mating within the populations (D. Paetkau, Wildlife Genetics International, personal communication).

Population and Density Estimation

AIC identified model M_{tbh} as the best fitting model for data from GDSNWR in 2002. Parameters from this model failed to converge and produce appropriate estimates, however, so it was removed from consideration in the model selection routine. Estimates from models M_{th} and M_{bh} (models with $\Delta AIC \leq 2.0$) were averaged to give a population estimate of 98 bears (CV =

13%, 95% CI = 82-134; Table 2.2) at GDSNWR in 2002. Estimates from the northern and southern portions of GDSNWR were 48 bears (CV = 26%, 95% CI = 35-93) and 46 bears (CV = 9%, 95% CI = 42-62), respectively. Effective study area size from average summer home range sizes (male and female) was 167.4 km^2, yielding an overall density at GDSNWR of 0.59 bears/km^2 (Table 2.3). Using MMDM, effective study area size was 155.9 km^2, giving an overall density of 0.63 bears/km^2. Densities on the northern and southern grids using average summer home ranges were 0.56 and 0.57 bears/km^2, respectively. Using MMDM, densities were 0.61 and 0.60 bears/km^2 for the northern and southern grids, respectively.

Models $M_{th,}$ $M_{bh,}$and M_{tbh} were selected as the top models for data from PLNWR in 2002. Weighted averages of estimates from these models indicated 282 bears (CV = 25%, 95% CI = 206-518; Table 2.4). Model averages gave estimates of 103 bears (CV = 12%, 95% CI = 91-148) for the Pungo Unit and 130 bears (CV = 59%, 95% CI = 65-437) for the eastern portion of PLNWR in 2002. Estimates for the middle portion of the refuge were not calculated separately. Effective study area size generated from average summer home ranges was 132.9 km^2, giving an overall density estimate of 2.12 bears/km^2 at PLNWR in 2002 (Table 2.3). At 193.6 km^2, effective study area size using MMDM was substantially larger, yielding an overall density of 1.46 bears/km^2. Densities for the Pungo Unit and eastern portion of PLNWR using average summer home range size were 1.60 and 3.44 bears/km^2, respectively. Densities calculated using MMDM were 1.19 and 2.25 bears/km^2 for the Pungo Unit and eastern portion of PLNWR, respectively.

Models M_{bh} and M_h had the best fit to data from PLNWR in 2003, and generated an estimate of 221 bears (CV = 10%, 95% CI = 194-283; Table 2.4). Estimates for the Pungo Unit and eastern portion of PLNWR in 2003 were 101 (CV = 13%, 95% CI = 87-143) and 61 bears

Table 2.2. Population estimates from full closed captures with heterogeneity models using mixtures (Pledger 2000) for genetic capture-recapture data collected at Great Dismal Swamp National Wildlife Refuge (GDSNWR) during summer 2002. Data were analyzed in Program MARK (White and Burnham 1999) and Akaike's Information Criterion (AIC) was used for model selection. Estimates were derived for the northern and southern portions of the refuge separately, as well as using the entire dataset.

Area/year	No. of bears captured	Model	ΔAIC_c	w_i	K[a]	N[b]	CV[c]	Lower 95% CI[d]	Upper 95% CI
North 2002	28	{Mt}	0	0.629	9	60	0.30	39	116
		{Mtb}	1.0576	0.371	10	29	0.14	28	55
weighted avg.						**48**	**0.26**	**35**	**93**
South 2002	40	{Mth}	0	0.625	11	48	0.09	43	61
		{Mtbh}	1.0234	0.375	12	42	0.09	40	63
weighted avg.						**46**	**0.09**	**42**	**62**
All data 2002	67	{Mth}	0	0.642	11	112	0.15	89	158
		{Mbh}	1.1683	0.358	5	74	0.07	69	92
weighted avg.						**98**	**0.13**	**82**	**134**

[a] Number of parameters in model
[b] Estimated population size
[c] Coefficient of variation
[d] Confidence interval

Table 2.3. Density estimates calculated from genetic capture-recapture data collected on 3 national wildlife refuges (Great Dismal Swamp [GDSNWR], Pocosin Lakes [PLNWR], and Alligator River [ARNWR]) in coastal North Carolina and Virginia. Densities were determined based on effective study area (km^2) calculated using average home range size (HR) and mean maximum distance moved (MMDM) between hair traps. Due to the high CV using all the data at ARNWR in 2004, data were pooled into 2 time periods to achieve better model fit.

Refuge	Area/year	*N*[a]	Effective area (HR) (km^2)	HR density (bears/km^2)	Effective area (MMDM) (km^2)	MMDM density (bears/km^2)
GDSNWR	North 2002	48	86.1	**0.56**	79.1	**0.61**
GDSNWR	South 2002	46	81.3	**0.57**	76.8	**0.60**
GDSNWR	All data 2002	98	167.4	**0.59**	155.9	**0.63**
PLNWR	Pungo Unit 2002	103	64.5	**1.60**	86.8	**1.19**
PLNWR	East 2002	130	37.8	**3.44**	57.7	**2.25**
PLNWR	All data 2002	282	132.9	**2.12**	193.6	**1.46**
PLNWR	Pungo Unit 2003	101	64.5	**1.57**	76.6	**1.32**
PLNWR	East 2003	61	37.8	**1.61**	49.4	**1.23**
PLNWR	All data 2003	221	132.9	**1.66**	166.3	**1.33**
ARNWR	Farm Unit 2003	65	76.9	**0.84**	69.2	**0.94**
ARNWR	Bomb. Range 2003	91	88.6	**1.03**	81.5	**1.12**
ARNWR	All data 2003	217	237.3	**0.92**	220.3	**0.99**
ARNWR	Farm Unit 2004	57	76.9	**0.74**	87.2	**0.65**
ARNWR	Bomb. Range 2004	69	88.6	**0.78**	98.0	**0.70**
ARNWR	All data 2004	237	237.3	**1.00**	259.0	**0.92**
ARNWR	All data 2004 (pooled)	176	237.3	**0.74**	259.0	**0.68**

[a]Estimated population size (Tables 2.2, 2.4, 2.5)

Table 2.4. Population estimates from full closed captures with heterogeneity models using mixtures (Pledger 2000) for genetic capture-recapture data collected at Pocosin Lakes National Wildlife Refuge (PLNWR) during summer 2002 and 2003. Data were analyzed in Program MARK (White and Burnham 1999) and Akaike's Information Criterion (AIC) was used for model selection. Estimates were derived for the Pungo Unit and eastern portion of the refuge separately, as well as using the entire dataset.

Area/year	No. of bears captured	Model	ΔAIC_c	w_i	K[a]	N[b]	CV[c]	Lower 95% CI[d]	Upper 95% CI
Pungo Unit 2002	85	{Mth}	0	0.438	11	119	0.17	97	183
		{Mbh}	0.47	0.347	5	89	0.04	86	102
		{Mtbh}	1.4257	0.215	12	92	0.13	86	150
weighted avg.						**103**	**0.12**	**91**	**148**
East 2002	46	{Mh}	0	0.537	4	95	0.32	62	196
		{Mbh}	0.3001	0.463	5	170	0.76	69	717
weighted avg.						**130**	**0.59**	**65**	**437**
All data 2002	160	{Mth}	0	0.483	11	289	0.19	219	445
		{Mbh}	0.9668	0.298	5	205	0.12	177	283
		{Mtbh}	1.5757	0.220	12	373	0.45	215	996
weighted avg.						**282**	**0.25**	**206**	**518**
Pungo Unit 2003	80	{Mh}	0	0.708	4	103	0.12	88	142
		{Mbh}	1.7739	0.292	5	98	0.13	85	145
weighted avg.						**101**	**0.13**	**87**	**143**
East 2003	56	{Mbh}	0	0.557	5	62	0.08	57	82
		{Mb}	0.4603	0.443	3	59	0.05	57	72
weighted avg.						**61**	**0.07**	**57**	**77**
All data 2003	173	{Mbh}	0	0.680	5	211	0.09	188	269
		{Mh}	1.5119	0.320	4	242	0.11	207	312
weighted avg.						**221**	**0.10**	**194**	**283**

[a] Number of parameters in model
[b] Estimated population size
[c] Coefficient of variation
[d] Confidence interval

(CV = 7%, 95% CI = 57-77), respectively. Density calculated using average summer home range size (effective study area size = 132.9 km^2) was 1.66 bears/km^2 for PLNWR in 2003 (Table 2.3). A density of 1.33 bears/km^2 was generated using MMDM (effective study area size = 166.3 km^2). Densities for the Pungo Unit and eastern portion of PLNWR were 1.57 and 1.61 bears/km^2 using average summer home range size, and 1.32 and 1.23 bears/km^2 using MMDM, respectively.

MARK indicated model M_{tbh} as the best fitting model for data from ARNWR in 2003. Parameters from this model failed to converge, however, and the model was removed from consideration. Model M_{th} was selected as the next best fitting model and generated an estimate of 217 bears (CV = 13%, 95% CI = 180-293) at ARNWR in 2003 (Table 2.5). Estimates from the farm unit and DCBR were 65 bears (CV = 15%, 95% CI = 55-98) and 91 bears (CV = 13%, 95% CI = 76-127), respectively. Estimates for the middle portion of the refuge were not calculated separately. Effective study area size using average summer home range size was 237.3 km^2, resulting in an overall density estimate of 0.92 bears/km^2 for ARNWR in 2003 (Table 2.3). Density calculated using MMDM (effective study area = 220.3 km^2) was 0.99 bears/km^2. Densities for the farm unit and DCBR were 0.84 and 1.03 bears/km^2 using average summer home range size, and 0.94 and 1.12 bears/km^2 using MMDM, respectively.

For data from ARNWR in 2004, model M_{tbh} was selected as the best fitting model for the data. This model gave an estimate of 237 bears (CV = 51%, 95% CI = 148-765; Table 2.5). Model averaging yielded estimates of 57 bears (CV = 24%, 95% CI = 46-115) for the farm unit and 69 bears (CV = 7%, 95% CI = 65-87) for DCBR. Due to the high CV generated using the M_{tbh} for the entire refuge, I pooled the data into 2 time periods (first 4 weeks and second 4 weeks) and added these four models ($M_{t2bh,}$ $M_{t2h,}$ $M_{t2b,}$ and M_{t2}) to see if I could achieve a better

Table 2.5. Population estimates from full closed captures with heterogeneity models using mixtures (Pledger 2000) for genetic capture-recapture data collected at Alligator River National Wildlife Refuge (ARNWR) during summer 2003 and 2004. Data were analyzed in Program MARK (White and Burnham 1999) and Akaike's Information Criterion (AIC) was used for model selection. Estimates were derived for the Farm Unit and Dare County Bombing Range separately, as well as using the entire dataset. Due to the high CV using all the data in 2004, data were pooled into 2 time periods to achieve better model fit.

Area/year	No. of bears captured	Model	ΔAIC_c	w_i	K[a]	N[b]	CV[c]	Lower 95% CI[d]	Upper 95% CI
Farm Unit 2003	49	{Mb}	0	0.426	3	56	0.10	51	77
		{Mo}	0.0547	0.414	2	67	0.11	57	88
		{Mh}	1.9589	0.160	4	85	0.30	59	176
weighted avg.						**65**	**0.15**	**55**	**98**
Bomb. Range 2003	66	{Mth}	0	1	11	91	0.13	76	127
weighted avg.						**91**	**0.13**	**76**	**127**
All data 2003	147	{Mth}	0	1	11	217	0.13	180	293
weighted avg.						**217**	**0.13**	**180**	**293**
Farm Unit 2004	43	{Mh}	0	0.392	4	60	0.23	47	111
		{Mo}	0.6112	0.289	2	48	0.07	45	59
		{Mbh}	1.6745	0.170	5	71	0.53	47	247
		{Mb}	1.9419	0.149	3	52	0.15	45	81
weighted avg.						**57**	**0.24**	**46**	**115**
Bomb. Range 2004	63	{Mth}	0	0.718	11	68	0.04	65	77
		{Mtbh}	1.868	0.282	12	71	0.13	64	113
weighted avg.						**69**	**0.07**	**65**	**87**
All data 2004	130	{Mtbh}	0	1	12	237	0.51	148	765
weighted avg.						**237**	**0.51**	**148**	**765**
All data 2004	130	{Mt2bh}	0	0.405	6	159	0.11	140	215
(pooled)		{Mt2h}	0.462	0.322	5	145	0.04	136	164
		{Mtbh}	0.7865	0.273	12	237	0.51	148	765
weighted avg.						**176**	**0.24**	**141**	**349**

[a] Number of parameters in model
[b] Estimated population size
[c] Coefficient of variation
[d] Confidence interval

model fit. Results from these models indicated a better fit, and the weighted average of estimates from this output was 176 bears (CV = 24%, 95% CI = 141-349). Density calculated using average summer home range size (effective study area size = 237.3 km^2) was then 0.74 bears/km^2 for ARNWR in 2004 (Table 2.3). A density of 0.68 bears/km^2 was generated using MMDM (effective study area size = 259.0 km^2). Densities for the farm unit and DCBR were 0.74 and 0.78 bears/km^2 using average summer home range size, and 0.65 and 0.70 bears/km^2 using MMDM, respectively.

Sex ratios

All sex ratios of captures in all areas of all refuges were male-biased, whether significant or not. The sex ratio of captures at GDSNWR in 2002 was male-biased (1.68:1, $n = 67$, $Z = 2.08$, $p = 0.038$; Table 2.6). The sex ratio on the southern portion of the refuge showed more male-bias (1.86:1, $n = 40$, $Z = 1.9$, $p = 0.058$) than the northern portion (1.55:1, $n = 28$, $Z = 1.13$, $p = 0.258$).

Sex ratio of captures at PLNWR in 2002 were strongly male-biased in the eastern part of the refuge (2.07:1, $n = 46$, $Z = 2.36$, $p = 0.018$), but not on the Pungo Unit (1.18:1, $n = 85$, $Z = 0.759$, $p = 0.448$; Table 2.6). Similarly in 2003, sex ratios were strongly male-biased on the eastern portion of PLNWR (2.73:1, $n = 56$, $Z = 3.47$, $p = 0.001$), but were not biased on the Pungo Unit (1.22:1, $n = 80$, $Z = 0.894$, $p = 0.374$). Overall, sex ratios of captures at PLNWR were male-biased (1.42:1, $n = 160$, $Z = 2.21$, $p = 0.034$) in 2002 and strongly male-biased (1.93:1, $n = 173$, $Z = 4.18$, $p < 0.0001$) in 2003.

For ARNWR, sex ratio of captures was male-biased (1.41:1, $n = 147$, $Z = 2.06$, $p = 0.040$) overall in 2003, but less so in 2004 (1.28:1, $n = 130$, $Z = 1.40$, $p = 0.162$; Table 2.6). Sex

Table 2.6. Sex ratios of black bears sampled in noninvasive hair traps at 3 national wildlife refuges (Great Dismal Swamp [GDSNWR], Pocosin Lakes [PLNWR], and Alligator River [ARNWR]) in coastal North Carolina and Virginia. Ratios were calculated for separate areas of each refuge and each refuge overall.

Refuge	Area	Year	No. of males	No. of females	Ratio	*Z*	*p*-value
GDSNWR	North	2002	17	11	1.55:1	1.13	0.258
GDSNWR	South	2002	26	14	1.86:1	1.90	0.058
GDSNWR	Overall	2002	42	25	1.68:1	2.08	0.038
PLNWR	Pungo Unit	2002	46	39	1.18:1	0.759	0.448
PLNWR	East	2002	31	15	2.07:1	2.36	0.018
PLNWR	Overall	2002	94	66	1.42:1	2.21	0.034
PLNWR	Pungo Unit	2003	44	36	1.22:1	0.894	0.374
PLNWR	East	2003	41	15	2.73:1	3.47	0.001
PLNWR	Overall	2003	114	59	1.93:1	4.18	<0.0001
ARNWR	Farm Unit	2003	35	14	2.50:1	3.00	0.003
ARNWR	DCBR	2003	37	29	1.28:1	0.985	0.322
ARNWR	Overall	2003	86	61	1.41:1	2.06	0.040
ARNWR	Farm Unit	2004	27	16	1.69:1	1.68	0.092
ARNWR	DCBR	2004	36	27	1.33:1	1.13	0.258
ARNWR	Overall	2004	73	57	1.28:1	1.40	0.162

ratios were strongly male-biased on the farm unit in 2003 (2.50:1, $n = 49$, $Z = 3.00$, $p = 0.003$) but less so in 2004 (1.69:1, $n = 43$, $Z = 1.68$, $p = 0.092$). Sex ratios were unbiased on DCBR in both years (2003 - 1.28:1, $n = 66$, $Z = 0.985$, $p = 0.322$; 2004 – 1.33:1, $n = 63$, $Z = 1.13$, $p = 0.258$).

DISCUSSION

Violation of model assumptions

Many assumptions associated with CMR techniques can be met more easily with noninvasive techniques (Mowat and Strobeck 2000). Use of barbed wire for noninvasive collection of DNA allows better spatial coverage of a study area than traditional live traps, so the equal catchability assumption may be better met (i.e., researchers can place barbed wire in places they might not be able to place a live trap, e.g., farther from roads; Foran et al. 1997). Barbed wire traps also may create less of a sex bias in capture probabilities than do traditional live traps. Most studies employing live trapping have reported sex ratio of captures skewed towards males (Jonkel and Cowan 1971; Beecham 1980; Hellgren 1988; Allen 1999), as have studies employing baited camera stations (Mace and Waller 1997). This is also likely the case with barbed wire hair traps, though I submit this bias is less severe. Although significantly more males were captured on the eastern portion of PLNWR and the farm unit of ARNWR, captures of males and females on the Pungo Unit and DCBR were not statistically different (Table 2.6). Since these areas are close together and relatively similar, this suggests something other than trapping bias is causing the skewed sex ratios on the eastern portion of PLNWR and the farm unit of ARNWR (see further discussion below). Furthermore, the majority of traps at PLNWR and ARNWR (68-76%) caught both male and female bears, and both sexes were caught in the

same traps in the same period 11-20% of the time, indicating that males and females will use the same traps. However, most traps (46-55%) caught only 1 bear each period and more males were caught than females overall in all areas in all years (again, see further discussion below). Loss of marks is another assumption that may be better met with genetic studies since a bear's genetic profile cannot be lost (Woods et al. 1999; Mills et al. 2000).

Although many assumptions associated with CMR techniques may be better met with noninvasive techniques, problems with closure violations and correct identification of marks still exist. Demographic closure is likely to be met for this study since sampling occurred over a relatively short, 8-week period in the summer. Bears do not give birth during this time period, and survival for bears on these protected refuges during summer is known to be high ($\geq$ 85%; Hellgren 1988; Hellgren and Vaughan 1994; Folta 1998), making the number of deaths negligible for this time period. The assumption of demographic closure can be relaxed if emigration and death occur randomly among "marked" and "unmarked" bears. If immigration occurs, there will always be unmarked animals in the population, making estimates valid for subsequent sampling periods (Pollock et al. 1990). Geographic closure may be violated in this study since only portions of each study area were sampled. When this is the case, movement of animals on and off the study grid are likely to bias population estimates upward since more animals would be estimated to live in the study area than actually do (White et al. 1982). These biases can be minimized by keeping sampling duration short relative to the animal's lifespan, and by making the sampling grid large in relation to home range sizes. The 8-week sampling duration used in this study is considered relatively short for bears (Mowat and Strobeck 2000). Sampling grids ranged from 115 to 175 km^2 for the refuges, while summer home range sizes ranged from 1.1 km^2 to 30 km^2 (Hellgren 1988; Allen 1999). Kendall (1999) also looked at

violations of the closure assumption and found that completely random movement in and out of the study area does not introduce bias to estimators from closed-population methods, although precision is decreased.

All DNA samples in this study underwent a stringent error-checking protocol (Paetkau 2003) ensuring that genetic errors in the dataset were kept to a minimum. First, markers were selected to generate the most power for each dataset. Initial heterozygosity was tested for 8-10 microsatellite markers on a small set of samples from each dataset. The most variable markers were selected for use in analyses. Second, mixed samples and samples that performed poorly (i.e., worked at ≤ 3 loci) were culled. Samples that worked at 4-5 loci were re-analyzed as necessary to improve the data. If data from these samples did not improve, they were culled as well. Third, single- and double-mismatch pairs (pairs of genotypes that differ at only 1 or 2 loci) were re-analyzed for possible errors. In his review of 17 project datasets, Paetkau (2003) found 222 single-mismatch pairs prior to error-checking and 30 single-mismatch pairs following error-checking. Thus, spurious identification of 192 individuals was prevented in these studies. In our datasets, numerous scoring and amplification errors were detected and fixed, 12 single-mismatch pairs were re-analyzed and found to be due to allelic dropout at one allele, and 28 double-mismatch pairs were confirmed by multiple samples or re-analysis.

Use of food rewards and scent lures

The use of food rewards and scent lures may have introduced some bias into the sampling scheme. Most models indicated some degree of behavioral or heterogeneous response in capture probabilities, which could suggest varying responses to the bait. However, number of animals caught and number of new captures in each period for each refuge do not indicate drastic

changes in response to baiting schemes or switching lures (as was done in 2003 and 2004). Total number of captures was generally constant until period 5 or 6, at which point number of captures would taper off slightly. This happened in all areas regardless of baiting scheme (i.e., even when baits were not switched at period 5). New captures tapered off as expected through time in all areas in all years as well. The only indication of response to bait was seen in capture data from ARNWR in 2003. A slight increase in new captures occurred at period 3 for the farm unit, and total number of captures on DCBR went from an average of 21 in periods 1-4 to an average of 13 in periods 5-8. This could have been in response to the scent lure switch at period 5, the arrival of soft mast, or the tapering off of the breeding season in mid-July, but again, this response was not seen with the same baiting scheme at PLNWR.

It is noteworthy that without the use of food rewards and lures, sample sizes and capture rates would be extremely small, especially with such a highly mobile animal as the black bear. The small food reward served to keep bears interested in going into the traps rather than becoming apathetic towards a smell that provides no reward, and the scent lures provided an additional attractant to the sites once the food reward was gone. Switching lures halfway through the sampling season was intended to keep bears from becoming apathetic towards traps as well, though no substantial changes in total captures were noticeable between years when this was and was not done.

Model selection and population estimates

Program CAPTURE detected strong heterogeneity in most of the capture data from the 3 refuges. Heterogeneity models in CAPTURE tend to be biased with small capture and recapture rates, however, and models developed for sparse data tend to be imprecise (Chao 1987).

Furthermore, the model selection routine in CAPTURE has been shown to lack power (Otis et al. 1978). Mixture models incorporated in Program MARK (Pledger 2000) have been shown to be more precise than heterogeneity models in CAPTURE in most cases (Boulanger et al. 2002), and their maximum likelihood framework allows the use of more powerful model selection criteria (i.e., AIC), which is why they were chosen for use with our data.

Models generated using CMR data from each refuge generally produced precise (CV < 20%) estimates of population abundance for all areas (see Tables 2.2, 2.4, and 2.5). This was not the case for the eastern portion of PLNWR in 2002, however, and I suspect the resulting estimates from these data are biased high. Capture and recapture probabilities for this area in 2002 were low (i.e., < 10% in most cases), indicating lower quality data and leading to higher CVs for this dataset (57%; Table 2.3). This subsequently inflated estimates and confidence intervals for the entire PLNWR dataset in 2002 as well, despite small CVs for the Pungo Unit. The population estimate from the eastern portion of PLNWR in 2003 (61 bears, CV = 7%; Table 2.3) was less than half the estimate from 2002 (130 bears, CV = 59%), suggesting that 2002 data overestimated population size in this area and thus the refuge overall. It is unlikely that the population underwent a two-fold decrease in that portion of the refuge in just one year. CVs and model fits also indicate higher quality data in 2003, which likely produced more accurate estimates for the portion of the refuge sampled (221 bears, CV = 10%; Table 2.3).

Estimates generated for ARNWR in 2004 were associated with rather high CVs (i.e., > 20%). Pooling the data for the overall dataset helped to achieve better model fit, but estimates from 2003 are likely more accurate since the lower CVs indicate better data quality (Table 2.4). Results indicate data quality from DCBR was high in both years, and the best estimate likely falls between estimates from 2003 and 2004 (i.e., 69-91 bears; Table 2.4).

It is important to note that capture probabilities for cubs in hair traps is unknown. Mowat and Strobeck (2000) and Boulanger et al. (2004) demonstrated that cubs are in fact captured in hair traps, but concluded that determining the proportion captured is very difficult. If cub captures are ignored in final estimates (i.e., considered negligible or nonexistent), an overall positive bias in estimates will exist since it is probable at least a few cubs were captured and counted in the estimates (Boulanger et al. 2004). This bias is likely very low, however, since cubs represent only a small portion of bear populations (21.5%; McLellan 1989) and capture probabilities for cubs are also likely very low.

Density estimates

MMDM vs. home range density estimates. - Density estimates calculated using MMDM are highly dependent on trap spacing. If traps are spaced too far apart, bears are likely to be captured at only 1 trap or not at all, and resulting MMDM calculations will be biased. Home range data collected from telemetry likely is more accurate since locations can be collected at any time no matter where the animal is, and they are better able to pick up finer-scale movements of animals (see Chapter 4). This may not be the case for PLNWR, however, since home range data were collected only on a small sample of bears (N = 9) and the number of locations collected per animal also was small (range = 13-37; Appendix B). Calculated home ranges are likely to be underestimates with these sparse data, and are indeed smaller than other home ranges calculated in this region (Hellgren 1988; Allen 1999; Kindall 2004). This would lead to slight overestimates of density for PLNWR, so actual densities for this area likely fall between estimates given for the 2 methods (i.e., 1.23-1.66 bears/km^2).

Density estimates for the 3 refuges corresponded to estimates generated previously and for other areas in this region (Table 2.7). Density estimates for GDSNWR (0.56-0.63 bears/km^2) were nearly the same as estimates generated in the mid-1980s (0.46-0.67 bears/ km^2; Hellgren 1988) in the same area. Densities for the northern and southern portion of the refuge did not differ in this study. Hellgren (1988) calculated a difference in density for the 2 areas, but concluded the difference was not real. Density estimates from ARNWR (0.65-1.12 bears/km^2) also were similar to those found in a previous study (0.86 bears/ km^2; Allen 1999), suggesting that bear populations on these refuges have remained fairly stable for the past 10-20 years.

Densities at PLNWR (1.23-1.66 bears/km^2) were higher than those at the other 2 refuges (Table 2.3). This is likely due to large, contiguous forested tracts interspersed with small agricultural fields. Although ARNWR and GDSNWR contain large areas of contiguous forest as well, large agricultural fields lie on the periphery of these refuges and are largely inaccessible due to lack of escape cover (i.e., nearby forest). Smaller agricultural fields adjacent to contiguous forest allow safe and easy access for bears to agricultural crops (wheat, corn, soy), which they rely on as a major food source in the coastal plain (Thompson 2003). These smaller fields do not exist within the boundaries of GDSNWR, and only one contiguous farm unit exists at ARNWR. The smaller matrix of agricultural and forested land at PLNWR likely is better able to support higher bear densities (Hinesley 1999).

Major highways also bisect ARNWR and run adjacent to GDSNWR, potentially contributing to lower densities due to habitat loss and avoidance of high-traffic areas (see Figure 2.1). Increasing urban development around GDSNWR also may be decreasing habitat and limiting bear densities there. From 1990 to 2000, the cities of Suffolk and Chesapeake to the

Table 2.7. Population densities of black bear populations in the southeastern United States

Study Area	bears/km^2	Reference
GDSNWR	0.56-0.63	this study
PLNWR	1.23-1.66	this study
ARNWR	0.65-1.12	this study
GDSNWR	0.47-0.68	Hellgren 1988
Washington County, NC	1.20-1.78	Thompson 2003
ARNWR	0.86	Allen 1999
Augusta County, VA	0.63-0.96	Klenzendorf 2002
GSMNP	0.29	McLean and Pelton 1994
Gum Swamp, NC	1.35	Martorello 1998
Big Pocosin, NC	0.53	Martorello 1998

north of GDSNWR grew 22.1% and 31.1%, respectively (U.S. Census 2000). PLNWR is removed from any major urban development or highways.

Since hair traps were placed in only a portion of each refuge, it is necessary to extrapolate density estimates to obtain the number of bears for each refuge. Densities of bears are not the same in all areas of each refuge, as habitat quality varies spatially. Since hair traps were placed primarily in prime bear habitat, it is appropriate to extrapolate densities only to areas of prime bear habitat (i.e., bottomland hardwood, high pocosin, bay forest, pine- and mixed-hardwoods, and cypress-gum swamps). Although bears do exist in lower quality habitats (low pocosin, shrub pocosin, and marsh), densities in large tracts of these areas (i.e., much of the eastern portion of ARNWR and DCBR and the central portion of PLNWR) are likely much lower than densities calculated here. In order to calculate the number of bears at PLNWR, for example, densities calculated here (i.e., 1.23-1.66 bears/km^2) would be multiplied by the total area of prime bear habitat (~300 km^2 of hardwood, high pocosin, cypress-gum forests, etc.; Appendix D) for a range of 369-498 bears. Again, this range is low since bears do inhabit areas of low quality habitat, just at lower densities.

Sex ratios

Sex ratios estimated in this study ranged from 1.18M:1F on the Pungo Unit of PLNWR in 2002 to 2.73M:1F in the eastern part of PLNWR in 2003 (Table 2.6). Sex ratios were skewed at a ratio > 2M:1F for the eastern part of PLNWR in 2002 and 2003 and for the farm unit at ARNWR in 2003. Previous studies have shown that captures of bears tend to be skewed towards males (Jonkel and Cowan 1971; Lindzey and Meslow 1977; Beecham 1980), although as mentioned earlier, this may not be as severe with barbed wire hair traps. Allen (1999) captured

only 14 female bears in 5 summers of trapping on the farm unit of ARNWR. The same number of females was captured in hair traps during one summer (2003), suggesting better success at capturing females in the barbed wire traps. Live trapping in the eastern portion of PLNWR also indicated that hair traps are more successful at capturing females. Only 1 female was live-captured in the eastern portion of PLNWR (in 39 trap nights) compared to 10 males during the same time period. Hair traps, on the other hand, caught 15 females in this area each summer. In contrast to the live trapping on the eastern portion of PLNWR, live trapping on the Pungo Unit of PLNWR resulted in 10 females and 5 males with equal trapping effort, indicating there may actually be more males than females on the eastern portion of PLNWR. Visual observations in both areas of PLNWR confirmed findings from live trapping and hair trapping data (i.e., no sex ratio bias on the Pungo Unit, but significant bias in the eastern portion).

Male bears typically have larger home ranges and travel farther than females, which would potentially increase their trap encounter rates (Bunnell and Tait 1985). Habitat use segregation also may play a role in increased male captures (i.e., placement of traps near roads or high quality food sources such as agricultural fields may favor capture of males; Wielgus and Bunnell 1994; Allen 1999). Females, particularly females with cubs and yearlings, may avoid highly productive areas such as agricultural fields that are dominated by large, aggressive males for fear of injury to herself and/or her offspring. The highly skewed ratio on the farm unit of ARNWR is likely due to habitat use segregation, and may also be the case for the eastern portion of PLNWR as well, though trap spacing may have been a problem in this area. Road access was limited in this area, and the understory was impenetrable in most places, making it difficult to place traps in areas where they needed to be. The 3 roads in this area parallel each other and are approximately 2-3 km apart, so ideally some traps would have been placed halfway between

these roads (i.e., ~1 km in) to ensure full coverage. This was logistically impossible, however, and all traps in this area were set up < 400m from roads. It is therefore likely that females with small home ranges that lived > 400m from a road would not have had the opportunity to be sampled. Most roads (and traps) were adjacent to agricultural fields as well, again potentially favoring capture of males.

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CHAPTER 3

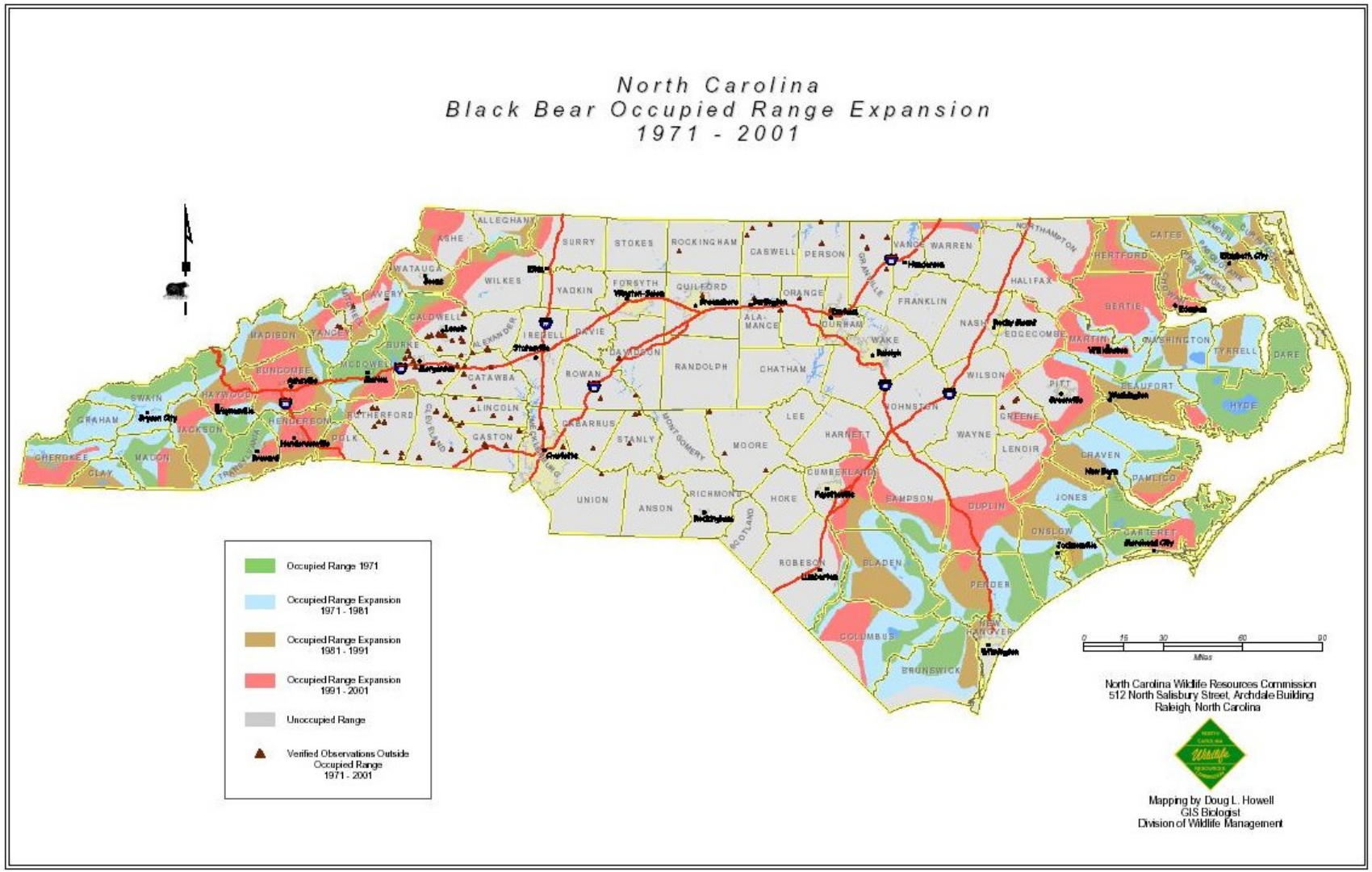
GENETIC VARIATION AND STRUCTURE OF 3 POPULATIONS OF BLACK BEARS IN COASTAL NORTH CAROLINA AND VIRGINIA

INTRODUCTION

Advances in the field of genetics have provided new tools for wildlife biologists and managers to more effectively manage wild animal populations. Understanding genetic variability and structure within and between populations of interest is important for proper management of wildlife, especially for wide-ranging species such as the black bear. As wildlife habitat increasingly becomes smaller and more fragmented, threats to the persistence of wide-ranging species increase (Harrison and Bruna 1999; Davies et al. 2001). Genetics can provide keen insight into associated impacts of these threats, including reduction of genetic variability, alteration of population structure, and changes in movement patterns of animals, which may increase isolation and loss of fitness in terms of decreased ability to adapt to changing environmental conditions (Frankham et al. 2002).

Since the early 1700s, black bear populations in the southeastern United States have undergone severe declines due to human encroachment and habitat loss (Hellgren and Maehr 1993; Maehr 1984; Pelton and van Manen 1994). Pelton (1986) suggested that black bears occupied only 5 to 10% of their historic range in the Southeast. However, the establishment of 28 bear sanctuaries throughout North Carolina increased the range of black bears there nearly 4-fold since the 1970s (from ~ 2.5 million acres to 10 million acres; Figure 3.1; North Carolina Wildlife Resources Commission; unpublished data). In the coastal portion of Virginia, however, bears are limited to the Great Dismal Swamp and directly adjacent lands (Hellgren 1988), and are susceptible to threats caused by increasing human development

Figure 3.1. Occupied black bear range expansion in North Carolina from 1971-2001.



and habitat loss and fragmentation, including reduced population size and decreased genetic diversity.

I conducted genetic analyses on black bear populations at 3 national wildlife refuges in coastal North Carolina and Virginia (Great Dismal Swamp [GDSNWR], Pocosin Lakes [PLNWR], and Alligator River [ARNWR]). Specific objectives were to determine within-population levels of genetic variation at these refuges, as well as explore genetic differentiation between populations. This information will assist in determining local and regional population structure, as well as estimating the degree of movement and gene flow across the landscape. Movement corridors and potential areas of isolation and decreased genetic variability can be identified to prevent decline and loss of black bears in this region.

METHODS

Collection of genetic material and genetic analysis

I collected black bear hair on barbed wire hair traps (Woods et al. 1999) for 8 weeks during 2 consecutive summers at each refuge. I constructed traps on a 1 km^2 grid, with traps placed approximately in the center of every other grid cell. Each hair trap consisted of a single strand of 15.5-gauge barbed wire around 3-4 trees and/or fenceposts at a height of ~40-50 cm from the ground. Bait and scent lure were hung in the center of the trap to attract bears. Hair was collected approximately every 7 days and stored in a temperature-controlled, dry room until submitted for genetic analysis. Samples were analyzed at Wildlife Genetics International in Nelson, B.C., Canada to determine individual identification. Analysis followed protocols outlined by Paetkau et al. (1995, 1998a, 1998b, 1999), Paetkau (2003), and Paetkau and Strobeck (1994). A set of 6 microsatellite DNA markers was used for samples from PLNWR and

ARNWR; these included G1A, G1D, G10H, G10J, G10L, and MU50. Seven markers were used for samples from GDSNWR (G1A, G10H, G10J, G10L, G10P, MU23, and MU50; Paetkau and Strobeck 1994, Paetkau et al. 1995). To increase the power of genetic analyses, 40 individual bears from each refuge were analyzed at an additional 9-10 loci to create 16-locus genotypes (G1A, G10C, G1D, G10H, G10J, G10L, MU50, MU59, G10P, G10B, CXX20, CXX110, G10M, MU23, G10X, and G10U; D. Paetkau, Wildlife Genetics International, personal communication). Only 39 individuals were genotyped to 16 loci at PLNWR, so 119 16-locus genotypes were used in the following analyses of genetic variability, differentiation, and population structure for the 3 refuge populations.

Statistical analyses

Hardy-Weinberg equilibrium and linkage disequilibrium. - Most statistical tests used for analyzing genetic data assume genotype frequencies conform to Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD). The Hardy-Weinberg model states that in a large population which undergoes random mating and does not experience migration, mutation, or selection, allelic and genotypic frequencies will be constantly maintained at predictable proportions (Frankham et al. 2002). Deviations of genotypes from these proportions can be the result of inbreeding, assortative mating (selection of a mate based on phenotype), the presence of null alleles, or selection within a population (Nei 1987; Hartl and Clark 1997). Linkage disequilibrium occurs when alleles at different loci are not randomly associated with each other (i.e., they occur together more often than would be expected by chance). In large, randomly breeding populations, alleles at different loci are expected to be randomly associated (Frankham et al. 2002), but sampling bias (i.e., sampling of close relatives), immigration, selection, or

stochastic processes can cause non-random associations (Frankham et al. 2002). I used Genepop 3.4 (Raymond and Rousset 2000), which uses the Markov chain method for loci with more than 4 alleles, to test for departures from HWE (Guo and Thompson 1992). I also tested for non-random associations among alleles at different loci using the linkage disequilibrium tests in Genepop 3.4. Sequential Bonferroni adjustments were made to determine the statistical significance of each test (Rice 1989).

Genetic variability. - Initial genetic variability for each refuge population was determined by calculating basic genetic parameters from the data, including allele frequencies, numbers of alleles, observed heterozygosity, and expected heterozygosity. Nei (1987) regarded allele frequencies to be the fundamental parameter in population genetic studies. For polymorphic microsatellite alleles, the frequency of an allele, A_i, in a sample of n individuals selected from a population and a locus with m codominant alleles, can be estimated by:

$$A_i = (2n_{ii} + \sum_{j \neq i} n_{ij}) / 2n \text{ (Nei 1987),}$$

where n_{ii} is the number of individuals (n) for genotype A_iA_i and n_{ij} is the number of individuals (n) for genotype A_iA_j. Observed heterozygosity (H_O) was calculated for each locus using the formula:

$$H_O = \sum_i \sum_{i \neq j} \frac{n_{lij}}{n},$$

where n_{lij} is the observed count of heterozygotes at locus l in a sample of size n. Average heterozygosity over all loci was calculated by summing individual heterozygosities at each locus and dividing by the total number of loci scored (Weir 1996). Expected heterozygosity (H_E) was calculated using the formula:

$$H_E = 1 - (n\sum_i p_i^2 - 1)/(n-1)$$

(Nei and Roychoudhury 1974), where p_i is the frequency of the *i*th allele. I also looked at the number of unique alleles (U) and the average number of alleles per locus (A) for each population.

Genetic distance. – Genetic distance statistics are useful for determining the extent of genomic differences between populations (Nei 1987). There are numerous methods for measuring genetic distance, but Nei's unbiased measure (D_S; Nei 1978) was chosen due to its low variance and its utility with fine-scale microsatellite data (Paetkau et al. 1997). Nei's unbiased measure of genetic distance was calculated for pairs of refuge populations using Popgene 1.32 (Yeh et al. 1997).

Genetic differentiation and gene flow. – Within-population departure of genotype frequencies from HW expectation can be measured using Wright's F_{IS} statistic (often referred to as the inbreeding coefficient, F). Genetic differentiation between populations is generally calculated using Wright's F_{ST} statistic (Wright 1965). F_{IS} for each population and pair-wise F_{ST} values were calculated according to Weir and Cockerham (1984) using Genepop 3.4 (Raymond and Rousset 1995). Global estimates of F_{IS} and F_{ST} also were calculated.

The degree to which genetic information is exchanged among populations determines the potential for genetic differentiation (Slatkin 1985). Wright (1931) first began attempting to quantify gene flow (i.e., exchange of genetic information) by its relation to F_{ST}. He found that the mean number of migrants, Nm, entering a subpopulation each generation is inversely proportional to the variance in gene frequencies (F_{ST}) among different subpopulations. Under the assumptions of the island model (Wright 1931), the rate of gene flow (Nm) is estimated by:

$$Nm \approx \frac{1}{4F_{ST}} - \frac{1}{4},$$

where N is the effective population size of each subpopulation, and m is the proportion of migrants entering the subpopulation. Barton and Slatkin (1986) expanded on this model and developed a method for calculating Nm from the conditional average frequency of private alleles. I estimated Nm values based on this private alleles method in Genepop 3.4 (Raymond and Rousset 1995).

Genetic structure. – Genetic structure can be defined as the distribution of alleles across a given landscape, and depends to a large degree on the amount of gene flow and genetic exchange across that landscape (Frankham et al. 2002). A high degree of genetic exchange will lead to low levels of population genetic structure, while a series of small, isolated populations will exhibit a high degree of differentiation as well as a high degree of population genetic structure. Assignment tests are a relatively new and effective method for determining the robustness of regional genetic structure. These tests use the genetic differences among populations to assign individuals (based on their genotype) to their most likely source population (Paetkau et al. 1995; Rannala and Mountain 1997; Luikart and England 1999; Davies et al. 1999; Pritchard et al. 2000). If a high degree of genetic structure exists in the populations of interest, most individuals are assigned to their correct source population. I used the software program STRUCTURE (Pritchard et al. 2000), which uses a Bayesian clustering approach to assign individual bears to a "cluster" or population of origin without regard to where individuals were sampled. I used the admixture model, which assumes that each individual derives a proportion of its membership (q) from a "mixture" of K clusters. I tested the data with independent runs for values of K between 1 and 5 and used no prior population information**.** Although alleles were assumed to be

independent for this dataset, I also tested the data using the assumption that alleles were correlated. Analyses were conducted with a burn-in length of 100,000 iterations and a Markov Chain Monte Carlo (MCMC) run length of 100,000 iterations. The best model was selected based on estimated log-likelihoods of the model probability as well as the power of the model to assign individuals to a cluster with high likelihood (i.e., ≥ 0.85).

Isolation by distance. - Genetic structure can be influenced by individual dispersal capabilities, where geographic limits to dispersal restrict gene flow and create genetic structure in an otherwise continuous population (Chambers 1995). This is referred to as isolation-by-distance (Wright 1943), which can be inferred by determining whether and to what degree geographic and genetic distances between individuals and/or populations are correlated. I used the subprogram ISOLDE in Genepop 3.4 (Raymond and Rousset 1995) to test for correlations between genetic distance and geographic distance for all refuge populations, and for all 119 individual bears. Genetic differentiation between individuals was calculated using the $\hat{a}$ parameter described by Rousset (2000). This parameter is somewhat analogous to $F_{ST}/(1- F_{ST})$. Geographic locations were calculated for each individual as the mean UTM coordinates of all hair trap sites where an individual was captured. The average sampling location of all animals at each refuge was compared with D_S and F_{ST} to test for isolation by distance among populations. A Mantel (1967) test with 1,000 permutations was used to test the statistical significance of these relationships.

RESULTS

Statistical analyses

Hardy-Weinberg equilibrium and linkage disequilibrium. – Deviance from HW proportions were observed at 2 loci (G10H, $p = 0.0002$ and CXX110, $p < 0.0001$) following sequential Bonferroni adjustments ($\alpha = 0.003$). No deviations from H-W expectations were noted in data from ARNWR following Bonferroni adjustments. Significant deviations were noted at CXX110 at GDSNWR following Bonferroni adjustments ($p < 0.0001$), and at G10H and CXX110 for PLNWR following Bonferroni adjustments ($p = 0.0004$ and $p < 0.0001$, respectively). All deviations were heterozygote deficits (PLNWR G10H - 25 observed, 34 expected; PLNWR CXX110 - 19 observed, 33 expected; GDSNWR CXX110 - 13 observed, 31 expected). Since deviations were found only at PLNWR for this locus, they are likely due to less than perfect non-random mating and will not affect subsequent analyses. Deviations at PLNWR and GDSNWR for CXX110 are indicative of a null allele at this locus, however (D. Paetkau, Wildlife Genetics International, personal communication), which may introduce bias in subsequent analyses. The severity of this bias, however, is not obvious, but power is likely increased by its inclusion, so I retained this locus for analyses.

Linkage disequilibrium was observed for 6 pairs of alleles over all populations ($\alpha = 0.05$), but only 2 of the 120 tests remained significant following sequential Bonferroni adjustments ($\alpha = 0.0004$). Non-random associations of alleles were found with 7, 10, and 10 pairs of loci at $\alpha = 0.05$ when ARNWR, PLNWR, and GDSNWR were considered separately, respectively. Two tests at ARNWR remained significant following sequential Bonferroni adjustments ($\alpha = 0.0004$). Since these disequilibria were found in only 1 population, it is unlikely these loci are physically

linked, but this is likely a result of less than perfect non-random mating (D. Paetkau, Wildlife Genetics International, personal communication).

Genetic variability. – Allele frequencies were calculated for 119 individuals from the 3 refuges (Table 3.1). At ARNWR, 4-12 alleles/locus were observed (mean = 6.56), 4-10 alleles/locus were observed at PLNWR (mean = 6.50), and 4-8 alleles/locus were observed at GDSNWR (mean = 6.13; Table 3.2). Allele frequencies ranged from 0.013-0.650 at ARNWR, 0.013-0.859 at PLNWR, and 0.013-0.650 at GDSNWR (Table 3.1). Unique alleles for each population ranged from 12 at GDSNWR to only 1 at PLNWR and 3 at ARNWR (Table 3.2). Average observed heterozygosities for ARNWR, PLNWR, and GDSNWR were 0.7219, 0.7212, and 0.6729, respectively. Expected heterozygosities were 0.7486, 0.7348, and 0.6935 for ARNWR, PLNWR, and GDSNWR, respectively (Table 3.2).

Genetic distance. – Pair-wise genetic distances between pairs of refuges were calculated based on Nei's unbiased distance (D_S). Distances were lowest between ARNWR and PLNWR (0.0971) and highest between ARNWR and GDSNWR (0.3064; Table 3.3). Distance between PLNWR and GDSNWR was 0.2087.

Genetic differentiation and gene flow. – Global estimates of F_{ST} and F_{IS} were generally low (0.0598 and 0.0362, respectively). F_{IS} values for each population were highest at ARNWR (0.0361), lowest at PLNWR (0.0191), and 0.0280 at GDSNWR (Table 3.2). Pair-wise F_{ST} were similar to D_S statistics. F_{ST} was highest between ARNWR and GDSNWR (0.0895) and lowest

Table 3.1. Allele frequencies at 16 microsatellite loci for black bears at 3 national wildlife refuges (Alligator River [ARNWR], Pocosin Lakes [PLNWR], and Great Dismal Swamp [GDSNWR]) in coastal North Carolina and Virginia.

	Population (N)		
Locus	**ARNWR**	**PLNWR**	**GDSNWR**
	40	**39**	**40**
G1A			
190	0.000	0.000	0.050
192	0.200	0.077	0.088
194	0.338	0.308	0.063
196	0.250	0.154	0.163
198	0.100	0.231	0.188
200	0.113	0.231	0.450
G10B			
152	0.063	0.064	0.000
154	0.000	0.000	0.013
156	0.450	0.346	0.300
158	0.288	0.333	0.250
160	0.113	0.141	0.263
162	0.000	0.000	0.013
164	0.088	0.115	0.138
168	0.000	0.000	0.025
G1D			
172	0.125	0.167	0.000
176	0.175	0.385	0.650
180	0.063	0.128	0.150
182	0.013	0.000	0.000
184	0.425	0.256	0.025
186	0.113	0.064	0.038
188	0.088	0.000	0.138
G10C			
209	0.000	0.000	0.225
211	0.488	0.077	0.188
213	0.038	0.026	0.025
215	0.463	0.859	0.450
217	0.000	0.000	0.050
219	0.013	0.039	0.063

Table 3.1. continued

Locus	Population (N)		
	ARNWR	PLNWR	GDSNWR
	40	39	40
G10J			
185	0.113	0.244	0.000
187	0.250	0.282	0.288
191	0.325	0.192	0.088
199	0.138	0.141	0.525
203	0.088	0.077	0.075
205	0.088	0.064	0.000
209	0.000	0.000	0.025
G10L			
135	0.025	0.026	0.100
137	0.138	0.103	0.000
139	0.088	0.026	0.050
149	0.038	0.064	0.113
151	0.125	0.180	0.250
153	0.063	0.103	0.013
155	0.188	0.167	0.450
157	0.150	0.115	0.025
159	0.188	0.218	0.000
MU50			
122	0.125	0.231	0.213
124	0.350	0.218	0.000
126	0.138	0.141	0.038
128	0.125	0.167	0.238
134	0.013	0.013	0.225
136	0.038	0.013	0.000
138	0.000	0.039	0.000
140	0.213	0.180	0.275
144	0.000	0.000	0.013
MU59			
231	0.088	0.231	0.013
233	0.050	0.013	0.000
235	0.000	0.000	0.100
237	0.063	0.039	0.075
239	0.500	0.346	0.200
241	0.050	0.026	0.013
243	0.225	0.333	0.513
245	0.025	0.013	0.088

Table 3.1. continued

Locus	Population (N)		
	ARNWR	PLNWR	GDSNWR
	40	39	40
G10P			
155	0.050	0.115	0.000
157	0.075	0.090	0.125
159	0.238	0.423	0.538
161	0.288	0.205	0.113
163	0.300	0.167	0.225
165	0.050	0.000	0.000
G10M			
206	0.013	0.090	0.038
208	0.038	0.064	0.013
210	0.288	0.103	0.113
212	0.400	0.500	0.513
214	0.163	0.090	0.038
216	0.025	0.000	0.013
218	0.075	0.154	0.275
CXX20			
123	0.338	0.192	0.088
133	0.088	0.051	0.000
137	0.050	0.051	0.075
139	0.213	0.231	0.425
141	0.138	0.192	0.088
143	0.175	0.282	0.325
CXX110			
141	0.163	0.192	0.171
143	0.225	0.154	0.026
149	0.000	0.000	0.053
151	0.000	0.013	0.276
153	0.413	0.256	0.118
155	0.163	0.180	0.250
157	0.013	0.039	0.000
159	0.000	0.051	0.013
161	0.000	0.039	0.092
163	0.013	0.064	0.000
165	0.013	0.013	0.000

Table 3.1. continued

Locus	Population (N) ARNWR	PLNWR	GDSNWR
	40	39	40
MU23			
187	0.113	0.039	0.038
189	0.000	0.000	0.125
191	0.038	0.026	0.075
195	0.250	0.295	0.538
197	0.125	0.077	0.038
201	0.175	0.180	0.050
203	0.125	0.103	0.138
205	0.175	0.244	0.000
207	0.000	0.039	0.000
G10X			
137	0.063	0.090	0.113
139	0.125	0.167	0.013
141	0.650	0.641	0.638
143	0.025	0.013	0.000
147	0.063	0.013	0.000
149	0.075	0.064	0.200
153	0.000	0.013	0.038
G10H			
235	0.013	0.000	0.000
237	0.050	0.128	0.000
239	0.088	0.077	0.013
241	0.200	0.244	0.075
243	0.213	0.154	0.400
245	0.013	0.026	0.000
247	0.013	0.000	0.163
249	0.063	0.167	0.075
251	0.050	0.000	0.013
253	0.050	0.064	0.125
255	0.100	0.064	0.000
259	0.150	0.077	0.138
G10U			
173	0.288	0.397	0.263
175	0.250	0.077	0.350
177	0.288	0.333	0.313
179	0.175	0.192	0.013
183	0.000	0.000	0.063

Table 3.2. Average observed heterozygosity (H_O), expected heterozygosity (H_E), number of unique alleles (U), average number of alleles per locus (A), and F_{IS}, a measure of nonrandom mating within populations, for black bears at 3 national wildlife refuges (Alligator River [ARNWR], Pocosin Lakes [PLNWR], and Great Dismal Swamp [GDSNWR]) in coastal North Carolina and Virginia.

	H_O	H_E	U	A	F_{IS}
ARNWR	0.7219	0.7486	3	6.56	0.0361
PLNWR	0.7212	0.7348	1	6.50	0.0191
GDSNWR	0.6729	0.6935	12	6.13	0.0280

Table 3.3. Pair-wise estimates of Nei's unbiased measure of genetic distance (D_S) for black bears on 3 national wildlife refuges (Alligator River [ARNWR], Pocosin Lakes [PLNWR], and Great Dismal Swamp [GDSNWR]) in coastal North Carolina and Virginia.

D_S	ARNWR	PLNWR	GDSNWR
ARNWR	***		
PLNWR	0.0971	***	
GDSNWR	0.3064	0.2087	***

Table 3.4. Pair-wise estimates of F_{ST} (below diagonal), a measure of genetic differentiation, and *Nm* (above diagonal), mean number of migrants per generation, for black bears on 3 national wildlife refuges (Alligator River [ARNWR], Pocosin Lakes [PLNWR], and Great Dismal Swamp [GDSNWR]) in coastal North Carolina and Virginia.

F_{ST}\Nm	ARNWR	PLNWR	GDSNWR
ARNWR	***	3.60	0.87
PLNWR	0.0257	***	0.86
GDSNWR	0.0895	0.0661	***

between ARNWR and PLNWR (0.0257; Table 3.4). F_{ST} was 0.0661 between PLNWR and GDSNWR.

Nm values also followed a similar geographic trend, ranging from 0.86 between PLNWR and GDSNWR to 3.60 between ARNWR and PLNWR (Table 3.4). *Nm* between ARNWR and GDSNWR was 0.87.

Genetic structure. - Models in program STRUCTURE assuming correlation of alleles tended to perform better than models that assumed independence of alleles. Estimated log-likelihoods of model probabilities and assignment power of the models suggested that K=3 was the appropriate number of clusters for the data (Table 3.5). Sixty-eight percent of individuals from ARNWR and PLNWR were assigned to clusters with $q \geq 0.85$, and 95% of individuals from GDSNWR were assigned to a cluster with $q \geq 0.85$ for K=3 assuming alleles were correlated (Table 3.5). Conversely, only 28% of individuals from ARNWR and PLNWR and 73% of individuals from GDSNWR were assigned to clusters with $q \geq 0.85$ at $K = 3$ when alleles were assumed to be independent. Similarly, individuals from ARNWR, PLNWR, and GDSNWR, were assigned with a greater probability (72%, 61%, and 96%, respectively) to a single cluster for K=3 assuming correlated alleles, compared to the same model assuming independent alleles (only 67%, 53%, and 88%, respectively; Table 3.6). When K was increased to 4, only 30% and 43% of individuals from ARNWR and PLNWR, respectively, were assigned to clusters with $q \geq 0.85$ (Table 3.5). Correspondingly, estimated log-likelihoods of the model probability decreased as K approached 3, and then increased again at K=4, indicating K=3 as the maximum likelihood (Table 3.5). Values of q when K=4 were also lower for all clusters (Table 3.6). For all models in all cases, individuals from GDSNWR were clustered out with higher membership proportions (q)

Table 3.5. Results of model output from program STRUCTURE for values of *K* (number of population clusters) from 1-5, and assuming allele frequencies are correlated (ac) or independent (ai). The most parsimonious model (*K*=3 ac, in bold) was chosen based on the lowest estimated log-likelihood of model probability [*ln*(Pr)] and its power to assign membership of individuals (q) with high likelihood ($q \geq 0.85$). Percentage of high likelihood membership assignments are given for each of the 3 study populations [Alligator River National Wildlife Refuge (ARNWR), Pocosin Lakes National Wildlife Refuge (PLNWR), and Great Dismal Swamp National Wildlife Refuge (GDSNWR)].

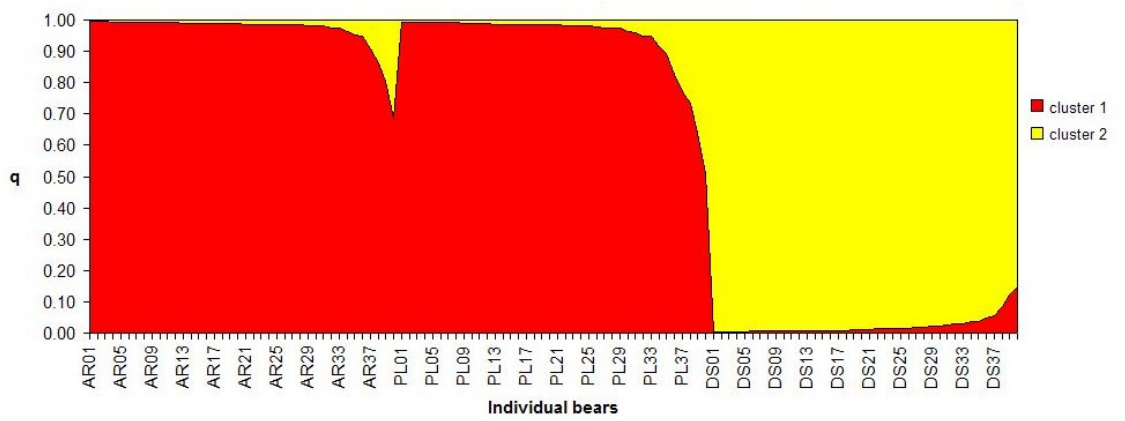
			% $q \geq 0.85$		
Model	**Estimated *ln*(Pr)**	**Variance of *ln*(Pr)**	**ARNWR**	**PLNWR**	**DSNWR**
***K*=1 ac**	-6389.4	51.3	100	100	100
***K*=1 ai**	-6390.7	54.1	100	100	100
***K*=2 ac**	-6031.4	139.9	90	88	100
***K*=2 ai**	-6030.7	140.7	90	83	95
***K*=3 ac**	**-5988.8**	**253.2**	**68**	**68**	**95**
***K*=3 ai**	-5989.6	271.5	28	28	73
***K*=4 ac**	-5996.3	406.9	30	43	93
***K*=4 ai**	-5977.5	401.5	8	10	53
***K*=5 ac**	-6112.1	680.7	NA	NA	NA
***K*=5 ai**	-5994.0	542.1	NA	NA	NA

Table 3.6. Inferred population clusters for black bears from 3 national wildlife refuges [Alligator River (ARNWR), Pocosin Lakes (PLNWR), and Great Dismal Swamp (GDSNWR)] in coastal North Carolina and Virginia. Results of model output from STRUCTURE for values of *K* (number of population clusters) from 2-4, and assuming allele frequencies are correlated (ac) or independent (ai). Note that power to resolve clusters is higher when alleles are assumed to be correlated and as the number of clusters (*K*) decreases. Results from the most parsimonious model are in bold.

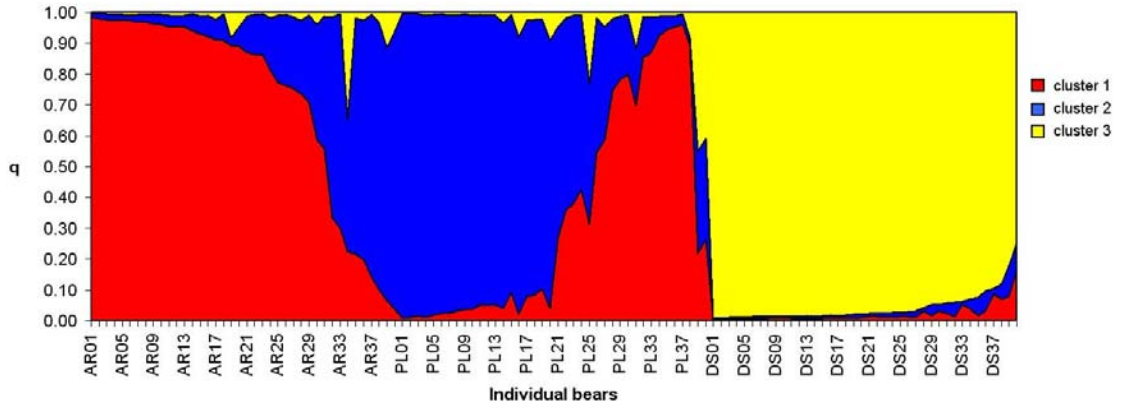
		Cluster			
Model	**Population**	**1**	**2**	**3**	**4**
***K*=2 ac**	ARNWR	0.968	0.032		
	PLNWR	0.942	0.058		
	GDSNWR	0.023	0.977		
***K*=2 ai**	ARNWR	0.952	0.048		
	PLNWR	0.923	0.077		
	GDSNWR	0.037	0.963		
***K*=3 ac**	**ARNWR**	**0.722**	**0.251**	**0.027**	
	PLNWR	**0.340**	**0.611**	**0.048**	
	GDSNWR	**0.021**	**0.021**	**0.958**	
***K*=3 ai**	ARNWR	0.673	0.237	0.090	
	PLNWR	0.349	0.528	0.122	
	GDSNWR	0.067	0.051	0.882	
***K*=4 ac**	ARNWR	0.553	0.255	0.163	0.029
	PLNWR	0.121	0.367	0.471	0.041
	GDSNWR	0.019	0.023	0.017	0.940
***K*=4 ai**	ARNWR	0.465	0.283	0.163	0.089
	PLNWR	0.153	0.340	0.397	0.110
	GDSNWR	0.064	0.065	0.050	0.821

Figure 3.2. Assignment of black bears from 3 national wildlife refuges [(Alligator River (AR), Pocosin Lakes (PL), and Great Dismal Swamp (DS)]to population clusters of origin without regard to sample origin. Output is based on results generated in STRUCTURE (Pritchard et al. 2000). Each individual bear is represented by a single vertical bar partitioned into segments based on the proportion of membership (*q)* in each cluster. a) shows assignments based on *K*=2, b) for *K*=3, and c) for *K*=4.

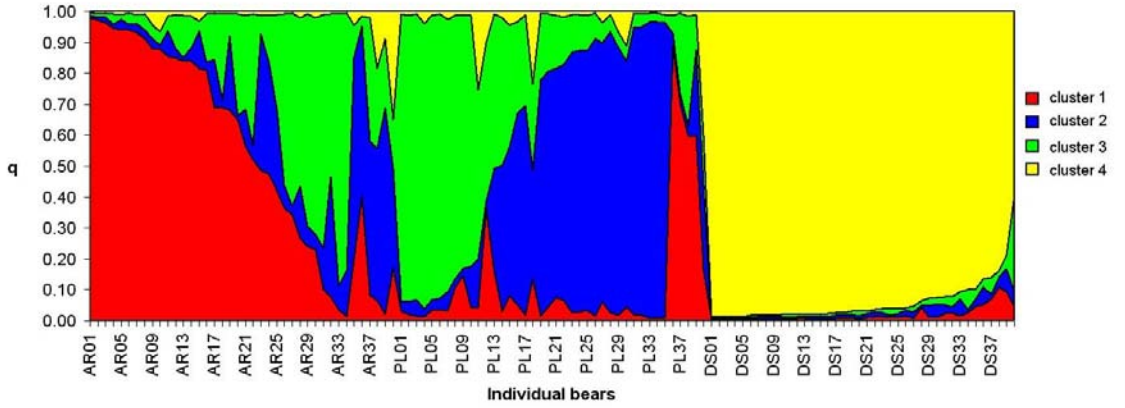
a)



b)



c)



than individuals from ARNWR and PLNWR (Figure 3.2).

Isolation by distance. – No indication of isolation-by-distance was found for the 119 individuals analyzed in this study ($p = 0.221$). In addition, no significant relationships were found between genetic and geographic distances between populations using D_S and F_{ST} ($p = 0.829$ and $p = 0.169$, respectively).

DISCUSSION

Genetic variability, differentiation, and gene flow

Genetic variability is essential for the adaptive evolution of populations in response to environmental changes (Frankham et al. 2002). The level of genetic variation within a population is determined by the opposing forces of mutation, migration, random genetic drift, and natural selection (Frankham 1996). Loss of genetic variation is generally attributed to drift due to finite population size, and genetic theory predicts that genetic variation should increase with effective population size (Soule 1976). Thus larger, contiguous populations connected to other populations by genetically effective migration are expected to have higher levels of genetic variation than smaller, isolated populations.

The 3 national wildlife refuges studied here are assumed to be part of a large, contiguous population of black bears that runs along the Coastal Plain of southeastern Virginia and eastern North Carolina (Figure 3.1). Indeed, genetic evidence presented here suggests high levels of variability and low levels of differentiation consistent with this idea. Heterozygosity levels (H_O) for black bears on these refuges are some of the highest reported in the literature (0.6729-0.7219; Table 3.7), as are the average number of alleles per locus ($A = 6.13$-6.56; Table 3.7).

Differentiation statistics (F_{ST} and D_S) are relatively low for these populations (F_{ST} range = 0.0257-0.0895; D_S range = 0.0971-0.3064).

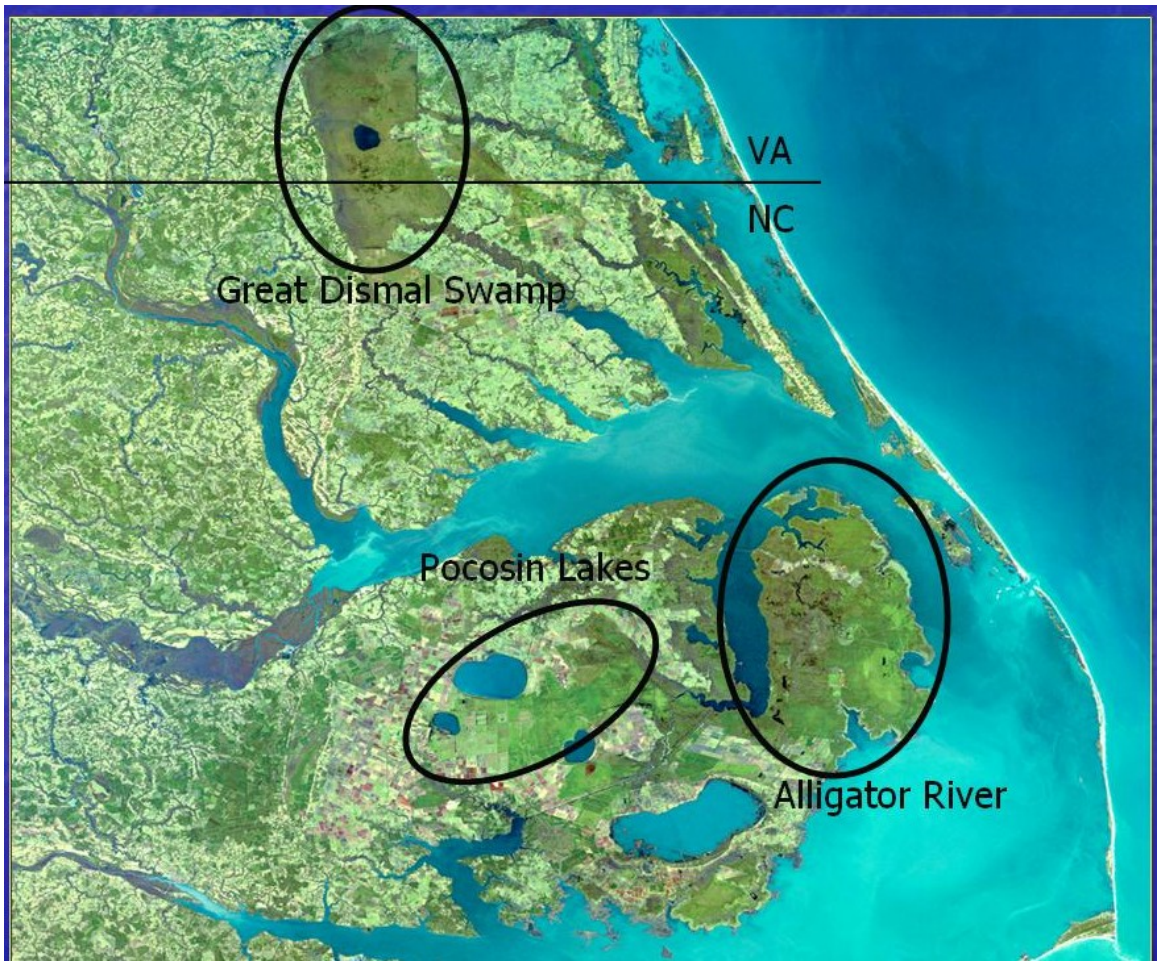
Closer examination of the data, however, reveals aspects of the genetics of these populations that may be of management concern in the not-too-distant future. Habitat fragmentation in the form of anthropogenic (e.g., roads, urban development, etc.) and geographic barriers to dispersal can contribute to declining population sizes and subsequent loss of genetic diversity. Brody and Pelton (1989) and Hellgren and Maehr (1993) found that roads and urban areas appeared to hinder the successful dispersal of black bears, which subsequently limits species distribution and gene flow (Mader 1984). This results in loss of genetic variability, which can lead to subsequent loss of fitness (reduced fecundity, decreased resistance to disease, etc.; Reed and Frankham 2003).

The beginning effects of isolation and fragmentation are evident in genetic distance and *F*-statistics from this study. Although observed and expected heterozygosities and number of alleles per locus are some of the highest reported in the literature (Table 3.7) and F_{ST} values are relatively low (0.0257-0.0895; Table 3.4), estimates of genetic variability are clearly lower for GDSNWR compared to ARNWR and PLNWR. It follows that levels of differentiation are greatest between GDSNWR and the other 2 refuges (Tables 3.3 and 3.4). Furthermore, for all models in all cases, the assignment algorithm was able to split out individuals from GDSNWR with high probability (Table 3.5 and Figure 3.2), indicating a higher level of differentiation for this population. This was not the case for ARNWR and PLNWR, however, and the assignment algorithm was not able to resolve individuals from these 2 populations effectively (Figure 3.2). Furthermore, although numbers of alleles were similar for all populations, 12 unique alleles were observed at GDSNWR, suggesting restricted gene flow for this population (Slatkin 1985).

Table 3.7. Sample size (n), observed (H_O) and expected (H_E) heterozygosities, average number of alleles per locus (A), and number of loci (l) used for microsatellite studies of black bears in the southeastern United States.

Population	***n***	H_O	H_E	***A***	**l**	**Citation**
Alligator River NWR, NC	40	0.722	0.749	6.56	16	this study
Pocosin Lakes NWR, NC	39	0.721	0.735	6.50	16	this study
Great Dismal Swamp NWR, NC/VA	40	0.673	0.694	6.13	16	this study
Washington County, NC (treatment)	66	0.667	N/A	6.00	10	Thompson 2003
Washingion County, NC (control)	115	0.664	N/A	6.90	10	Thompson 2003
Appalachicola, FL	40	0.690	0.708	5.92	12	Dixon 2004
Aucilla, FL	9	0.556	0.616	3.83	12	Dixon 2004
Big Cypress, FL	41	0.642	0.650	5.50	12	Dixon 2004
Chassahowitzka, FL	29	0.287	0.271	2.25	12	Dixon 2004
Eglin, FL	40	0.613	0.537	4.08	12	Dixon 2004
Highlands/Glades, FL	28	0.327	0.384	2.75	12	Dixon 2004
Ocala, FL	40	0.579	0.610	4.75	12	Dixon 2004
Osceola, FL	41	0.705	0.713	6.67	12	Dixon 2004
St. Johns, FL	40	0.650	0.663	5.58	12	Dixon 2004
Okefenokee NWR, GA	39	0.663	N/A	6.13	12	Dobey 2002
Tensas River, LA	36	0.576	N/A	3.80	12	Boerson et al. 2003
South Alabama	19	0.316	N/A	2.88	8	Edwards 2002
Ozark range, AR	13	0.723	0.761	5.80	5	Csiki et al. 2003
Ouachita range, AR	6	0.733	0.754	4.60	5	Csiki et al. 2003
White River NWR, LA	18	0.447	0.317	1.80	5	Csiki et al. 2003
Minnesota	10	0.576	0.772	5.60	5	Csiki et al. 2003

Figure 3.3. Satellite view of 3 national wildlife refuges (Great Dismal Swamp, Pocosin Lakes, and Alligator River) in coastal North Carolina and Virginia.



The geographic features of the study area explain many of these results (Figure 3.3). The Albemarle Sound clearly separates GDSNWR from ARNWR and PLNWR, and genetic results indicate that it acts as a barrier to dispersal for black bears. It is possible that bears cross the sound, but it is unlikely or infrequent given the data presented here. Additionally, GDSNWR is surrounded to the north by vast urban development. From 1990 to 2000, the cities of Suffolk and Chesapeake to the north of GDSNWR grew 22.1% and 31.1%, respectively (US Census 2000). This increase in human development is further fragmenting the already limited bear habitat in this area.

F_{IS} and the number of unique alleles at ARNWR may suggest the beginnings of some degree of isolation in this population as well. Slatkin (1985) determined that the number of unique or rare alleles in a population is inversely related to the rate of migration (Nm), thus the presence of 3 unique alleles at ARNWR (compared to only 1 at PLNWR) indicates that migration may be restricted. Although the F_{IS} value for ARNWR (0.0361) is not considered high, it was found to be the highest of the 3 populations examined here. This could suggest that some non-random mating or inbreeding may be occurring in the population. Again, the geographic features of ARNWR can explain the underlying nature of these numbers. The Dare County peninsula on which ARNWR sits is surrounded by water on nearly all sides. Only a thin strip of land to the south of the refuge connects ARNWR to the remainder of the mainland. This area is known to be a contiguous area of excellent bear habitat and high bear densities (Allen 1999; T. Langer, NCSU, personal communication), however, and likely contributes to the high genetic variability detected at ARNWR (H_O = 0.7219 and A = 6.56; Table 3.2). Indeed, the area to the south of ARNWR and east of PLNWR provides a corridor for migration between the 2 refuges, as evidenced by the relatively high migration rate estimated for this pair of populations

(Nm = 3.60; Table 3.4). The number of unique alleles and F_{IS} values for ARNWR, though small, illustrate the need for maintaining this corridor of contiguous bear habitat to prevent the loss of current levels of diversity.

Genetic structure

The majority of individual bears sampled were assigned with high likelihood ($q \geq 0.85$) to their correct population of origin using the assignment algorithm in STRUCTURE (Table 3.5; Figure 3.2). This was especially true for individuals from GDSNWR, of which 95% were assigned to a single population cluster at K=3, and even 93% at K=4, indicating a high degree of population structure for this population compared to ARNWR and PLNWR. The assignment algorithm was not able to resolve individuals from ARNWR and PLNWR with such a high degree of power, indicating less differentiation or structure between these 2 populations. Although most individuals (68%, Table 3.5) from these 2 populations were split into 2 separate clusters (clusters 1 and 2; Figure 3.2b) at K=3, approximately 30% of individuals from each of these populations were assigned to both populations with equal likelihood (i.e., mixed ancestry) or were assigned to the opposite cluster entirely. Results from these assignment tests are consistent with genetic distance and F-statistics given above, although it seems the assignment algorithms were able to pick out stronger signals of population structure, particularly between GDSNWR and the other 2 refuges.

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CHAPTER 4

A COMPARISON OF TELEMETRY VS. HAIR TRAPPING TO ASSESS BLACK BEAR MOVEMENTS AND HOME RANGE SIZE

INTRODUCTION

Use of noninvasive techniques to study wild animal populations is becoming more and more widespread as technology progresses. Researchers commonly use remote-sensored cameras to identify individual animals and assess movement patterns of various felid species (Karanth and Nichols 1998; Silver et al. 2004), fecal and urine samples to assess steroid hormone levels in primates (Thompson et al. 2004; Altmann et al. 2004), and hair samples to estimate abundance and relatedness of bears (Mowat and Strobeck 2000; Poole et al. 2001; Romain-Bondi 2003; Boersen et al. 2003) and other carnivore species (Mowat and Paetkau 2002; Creel et al. 2003). In many respects, these methods are preferable and more efficient than traditional capture experiments where animals are live-trapped and handled. Capture probabilities may be higher, safety of animals and researchers is increased, and costs may be lower (Foran et al. 1997; Mills et al. 2000; Mowat and Strobeck 2000). However, data collected via noninvasive methods may not be as informative as data collected by actually handling individual animals (e.g., location data collected by attaching radio transmitters, higher quality DNA or other tissue samples, etc.).

This chapter evaluates the effectiveness of hair trapping for assessing animal movement and home range size by comparison of traditional movement and home range estimation via radio telemetry. I collected hair samples on barbed wire snares (Woods et al. 1999) for 2 summers on Pocosin Lakes National Wildlife Refuge (PLNWR) to identify individual black bears based on their DNA profiles and to estimate abundance and density of bears on the refuge. I also captured and placed transmitters on 9 bears and collected location data during the summers

when hair traps were active. This allowed me the unique opportunity to compare data collected via the noninvasive hair snares and the more invasive live-capture and radio tracking of animals.

METHODS

Trapping and telemetry

I live trapped bears at PLNWR with Aldrich foot snares and culvert traps (Johnson and Pelton 1980). Trapping occurred for approximately 1 week in the fall of 2002, and 2 weeks each in late May-early June of 2003 and 2004. I sedated bears with a mixture of ketamine hydrochloride and xylazine hydrochloride (200:100 mg/ml; 1ml/45.5kg; White et al. 1996) delivered via dart pistol or jabstick. Once bears were sedated, I determined sex, reproductive status, and body condition. We attempted to weigh all bears to the nearest kg, and premolars were extracted for aging by cementum annuli analysis (Wiley 1974). Bears between 40kg and 150kg were fitted with radio collars (Advanced Telemetry Systems [ATS], Isanti, Minnesota, USA; Telonics, Mesa, Arizona, USA) equipped with breakaway cotton spacers (Hellgren et al. 1988). Bears over 150kg were fitted with eartag transmitters from ATS. I also placed tattoos on each bear's upper lip and took standard morphological measurements. Telemetry locations (1-6/week) were taken on bears from June to August 2003 and 2004.

Hair trapping occurred for 8 weeks during summers 2002 and 2003 at PLNWR. I constructed 62 hair traps on a 120 km^2 area divided into 1 km^2 grids, with traps placed approximately in the center of every other grid cell. Each hair trap consisted of a single strand of 15.5-gauge barbed wire strung around 3-4 trees and/or fenceposts at a height of ~40-50 cm from the ground. A bait and scent lure were hung in the center of the trap to attract bears. Hair was collected from the traps approximately every 7 days and stored in a temperature-controlled, dry

room until submitted for genetic analysis. Samples were analyzed at Wildlife Genetics International in Nelson, B.C., Canada to determine individual identification. Analysis followed protocols outlined by Paetkau et al. (1995, 1998a, 1998b, 1999), Paetkau (2003), and Paetkau and Strobeck (1994).

Analysis of location data

Hair trap data. – Since no bears had transmitters during summer 2002, we used only hair trapping data from 2003 for these analyses. We calculated minimum convex polygon (MCP) home ranges for all individual bears captured at > 2 hair trap locations in 2003. Distance traveled between consecutive captures (i.e., straight-line distance between hair traps where a bear was captured) and total distance traveled during 8 weeks of hair sampling (sum of straight-line distances moved between all traps where bear was captured) were calculated to determine movement patterns.

Telemetry data. - I calculated MCP home ranges for all collared bears with ≥ 10 locations per summer. For bears tracked in multiple summers, only data from 2003 were used to avoid pseudoreplication. Straight-line distance traveled between consecutive telemetry locations and the total straight-line distances traveled over the course of the summer were calculated to determine movement patterns. *T*-tests were used to determine whether home range areas and distances moved estimated using telemetry were significantly different from those estimated using hair trapping data.

Fixed-kernel home ranges were calculated for all transmittered animals using Program ABODE (Laver 2005) in ArcGIS 8.3 and the Animal Movement Extension (Hooge and

Eichenlaub 1997) and Home Range Extension (Rodgers and Carr 2001) in ArcView 3.2 (Appendix B).

Hair trap and telemetry overlap. - Telemetry locations of all transmittered animals in 2003 and 2004 were compared to locations of hair traps and hair trap captures in 2002 and 2003 to determine whether bears were captured in hair traps that fell within their home ranges.

RESULTS

Trapping and telemetry

I captured 27 bears (15M:11F:1U) 30 times during 5 weeks of trapping (87 trap nights); 19 (11F:8M) were fitted with transmitters. Dropped collars and difficulty tracking eartag transmitters resulted in sufficient telemetry data (i.e., ≥ 10 locations) for only 9 (3M:6F) bears in 2003 and 2004 (Table 4.1).

We focused trapping on 2 distinct areas of PLNWR; the 60 km^2 Pungo Unit on the western end, and a 40 km^2 area on the eastern end of the refuge. Although trapping effort was approximately equal in both areas (48 trap nights on the Pungo Unit vs. 39 on the eastern end), 19 captures of 16 bears (5M:10F:1U) were on the Pungo Unit and only 11 captures of 11 bears (10M:1F) were on the eastern portion of the refuge. Five of the latter captures were with culverts, while only 2 bears on the Pungo Unit were captured in a culvert. We were not able to collect sufficient telemetry data (i.e., ≥ 10 locations) for any bears captured on the eastern portion of the refuge.

In 2003, 628 hair samples yielded 173 unique individuals (114M:59F) at PLNWR. Forty-one (24%) of these bears were captured at > 2 traps.

Table 4.1. Age, sex, and capture histories of live-trapped black bears on Pocosin Lakes National Wildlife Refuge (PLNWR) during 2002-2004.

Bear ID	Age	Sex	Transmittered			Hair-trapped		
			2002	2003	2004	2002	2003	2004
PL2	8	F		X		X		
PL3	3	F		X	X		X	
PL19	3	M		X		X	X	
PL20	11	F		X				
PL22	U	M		X	X	X		
PL24	2	F		X	X	X	X	
PL33	2	F			X			
PL34	U	F			X	X		
PL35	2	M			X			

Analysis of location data

Hair trap data. - Average MCP home range size was 3.02 km^2 (SE = 0.617, range = 0.003-18.35; Table 4.2). Average total distance moved over 8 weeks of sampling was 8,796 m (SE = 836, range = 1,944-28,026). Distance moved between subsequent samplings (1-week intervals) ranged from 0 to 10,014 m. Maximum distance moved between subsequent samplings averaged 3,363 m (Table 4.2). Average MCP home range sizes for males (n = 34) and females (n = 7) were 3.44 km^2 and 0.86 km^2, respectively. Average total distances moved for males and females were 9,382 m and 5,912 m, respectively, while average maximum distances moved between subsequent samplings were 3,638 m and 1,953 m for males and females, respectively.

Telemetry data. - Average MCP home range size from the 9 transmittered bears was 3.7 km^2 (SE = 1.36, range = 0.461-10.88; Table 4.2). Total distance moved during summer monitoring averaged 22,855 m (SE = 5,613, range = 6,956-60,001). Distance moved between subsequent samplings (1-20 days) ranged from 50 to 4,128 m. Maximum distance moved between subsequent samplings averaged 2,510 m. Average MCP home range sizes for males (n = 3) and females (n = 6) were 8.79 km^2 and 1.16 km^2, respectively. Average total distances moved for males and females were 40,377 m and 14,094 m, respectively, while average maximum distances moved between subsequent samplings were 4,783 m and 1,373 m for males and females, respectively.

Tests of significance. – MCP home ranges calculated from hair trap captures did not differ from MCP home ranges calculated from radio telemetry data (t = -0.47, p = 0.640; Table 4.2). Total distance moved over the course of 8 weeks of hair trapping was less than total distance moved

Table 4.2. Summary of location data collected via noninvasive hair trapping and radio telemetry on Pocosin Lakes National Wildlife Refuge in 2002-2004. Home ranges were calculated using minimum convex polygons (MCP). Minimum and maximum distances moved were calculated as the distance (in meters) between subsequent trapping occasions. Standard errors are given in parentheses. Results of *t*-tests are given at the bottom.

	n	100% MCP (km^2) (SE)	Total distance (m) (SE)	Min. distance (m) (SE)	Max. distance (m) (SE)
Hair trapping	41	3.02 (0.617)	8,796 (836)	1,419 (118)	3,363 (288)
Telemetry	9	3.70 (1.36)	22,855 (5,613)	120 (31.0)	2,510 (647)
		t = -0.47, p = 0.640	t = -2.48, p = 0.037	t = 10.69, p < 0.0001	t = 1.25, p = 0.218

during summer radio tracking (t = -2.48, p = 0.037). Maximum distances moved between subsequent samplings were not different for the 2 methods (t = 1.25, p = 0.218), but minimum distances moved by hair-trapped bears were greater than for telemetered bears (t = 10.69, $p <$ 0.0001).

Hair trap and telemetry overlap. - Six of the 9 transmittered bears also were caught in hair traps (Table 4.1). Only 1 of these bears was caught at > 2 hair traps and, thus, home range size based on hair trap locations could be calculated only for this one bear (PL19; Figure 4.1). His MCP home range size from hair trapping in 2003 was 1.40 km^2, compared to 6.0 km^2 from telemetry data. Three of these 6 bears were not caught in hair traps in 2003 when they were being tracked via telemetry, though they were caught in the previous year at hair traps in or adjacent to areas where they were tracked with telemetry. This included one male whose telemetry home range clearly overlapped 7 hair traps (PL22; Figure 4.2). He was caught at only one hair trap outside his telemetry home range the year prior to being live-trapped and transmittered. The other 2 cases were females, one whose telemetry home range was off of the refuge (though she was live-trapped on the refuge; PL34; Figure 4.3), and the other whose home range likely didn't overlap any hair traps (PL2; Figure 4.4). The remaining 2 of 6 bears both telemetered and caught in hair traps were females that were caught in both 2002 and 2003 in ≤ 2 hair traps that fell within their telemetry home ranges (PL3 & PL24; Figs. 4.5 and 4.6). The final 3 transmittered bears were never caught on hair traps, despite their telemetry home ranges overlapping 1 or more hair traps (PL20, PL33, & PL35; Figs. 4.7-4.9).

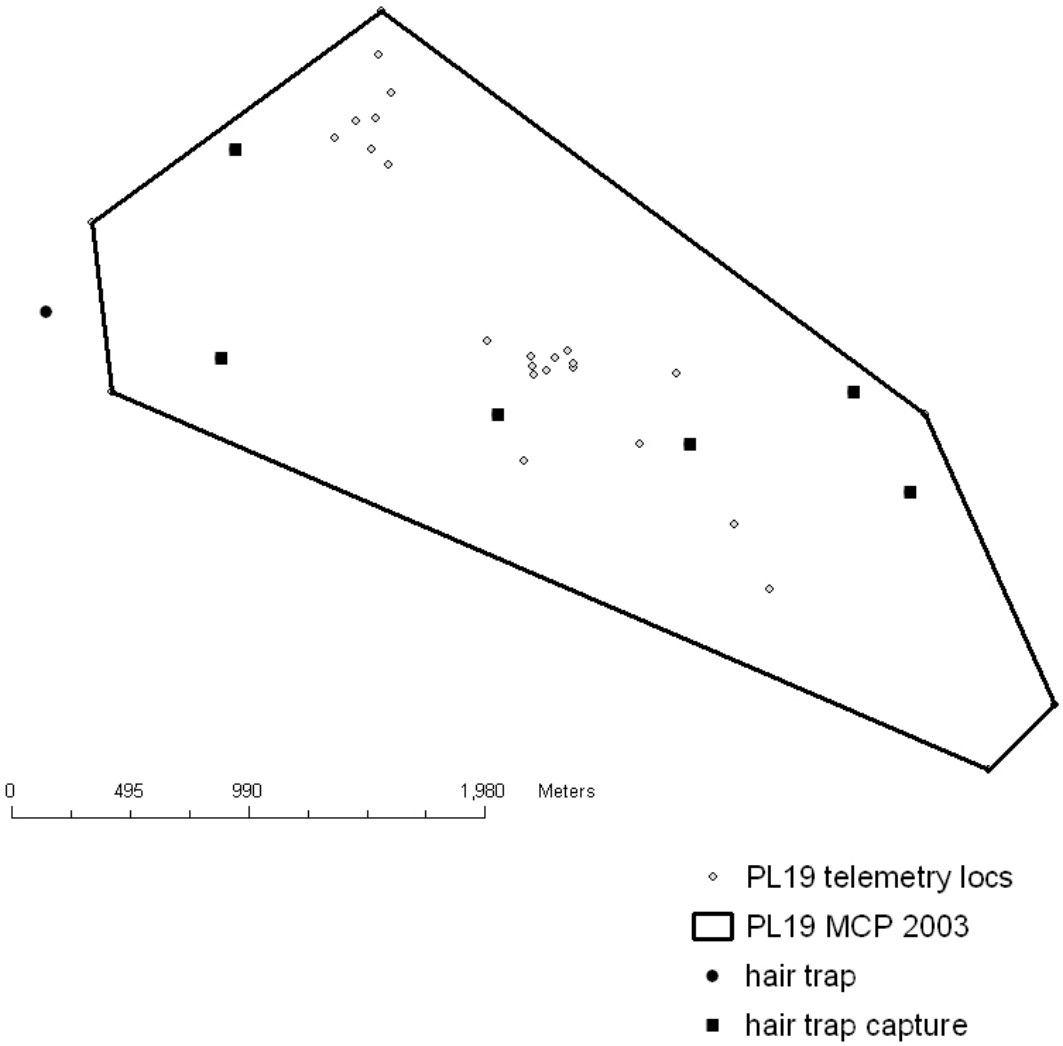

Figure 4.1. Telemetry and hair trap data collected for bear PL19, a 3-year old male, at Pocosin Lakes National Wildlife Refuge (PLNWR). MCP home range was calculated from telemetry data in 2003.

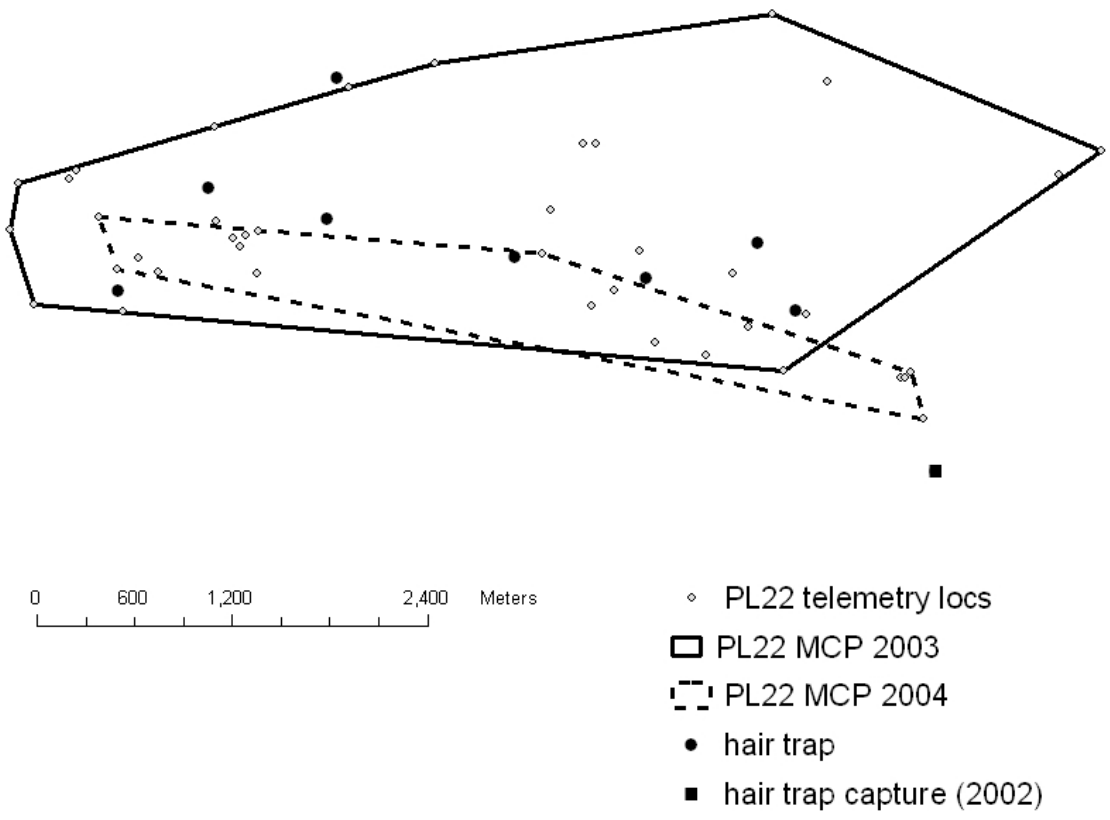

Figure 4.2. Telemetry and hair trap data collected for bear PL22, an adult male, at Pocosin Lakes National Wildlife Refuge (PLNWR). MCP home ranges were calculated from telemetry data in 2003 and 2004.

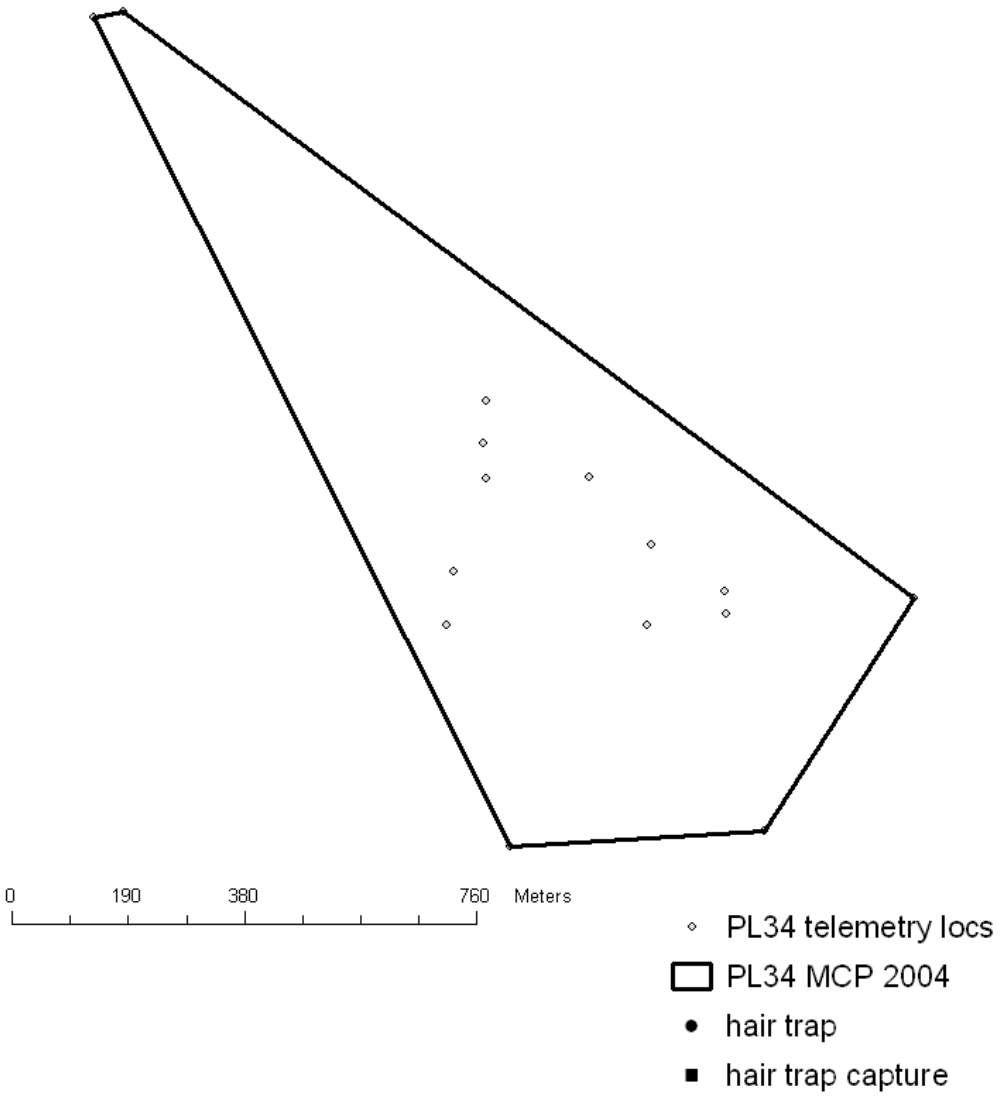

Figure 4.3. Telemetry and hair trap data collected for bear PL34, an adult female, at Pocosin Lakes National Wildlife Refuge (PLNWR). MCP home range was calculated from telemetry data in 2004.

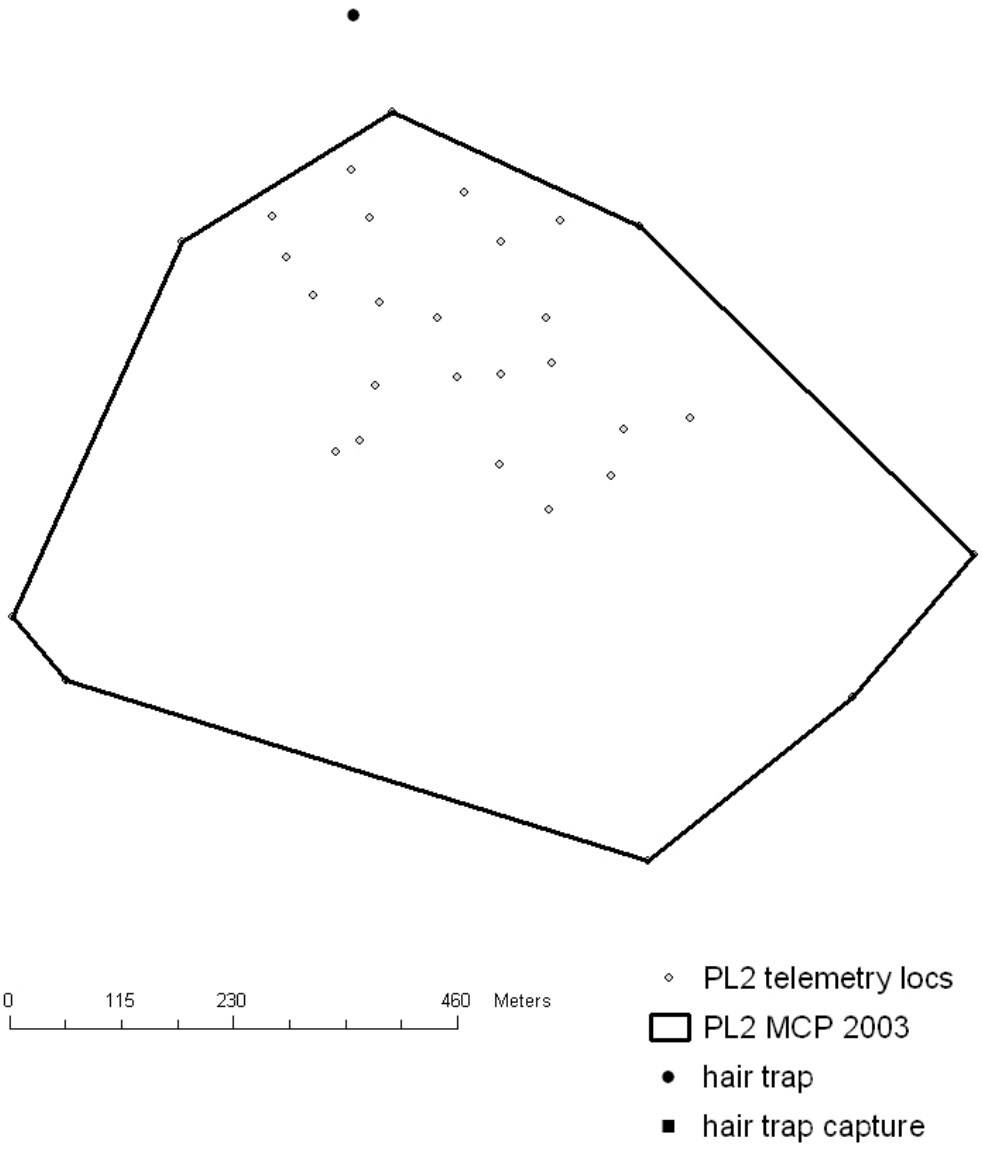

Figure 4.4. Telemetry and hair trap data collected for bear PL2, an 8-year old female, at Pocosin Lakes National Wildlife Refuge (PLNWR). MCP home range was calculated from telemetry data in 2003.

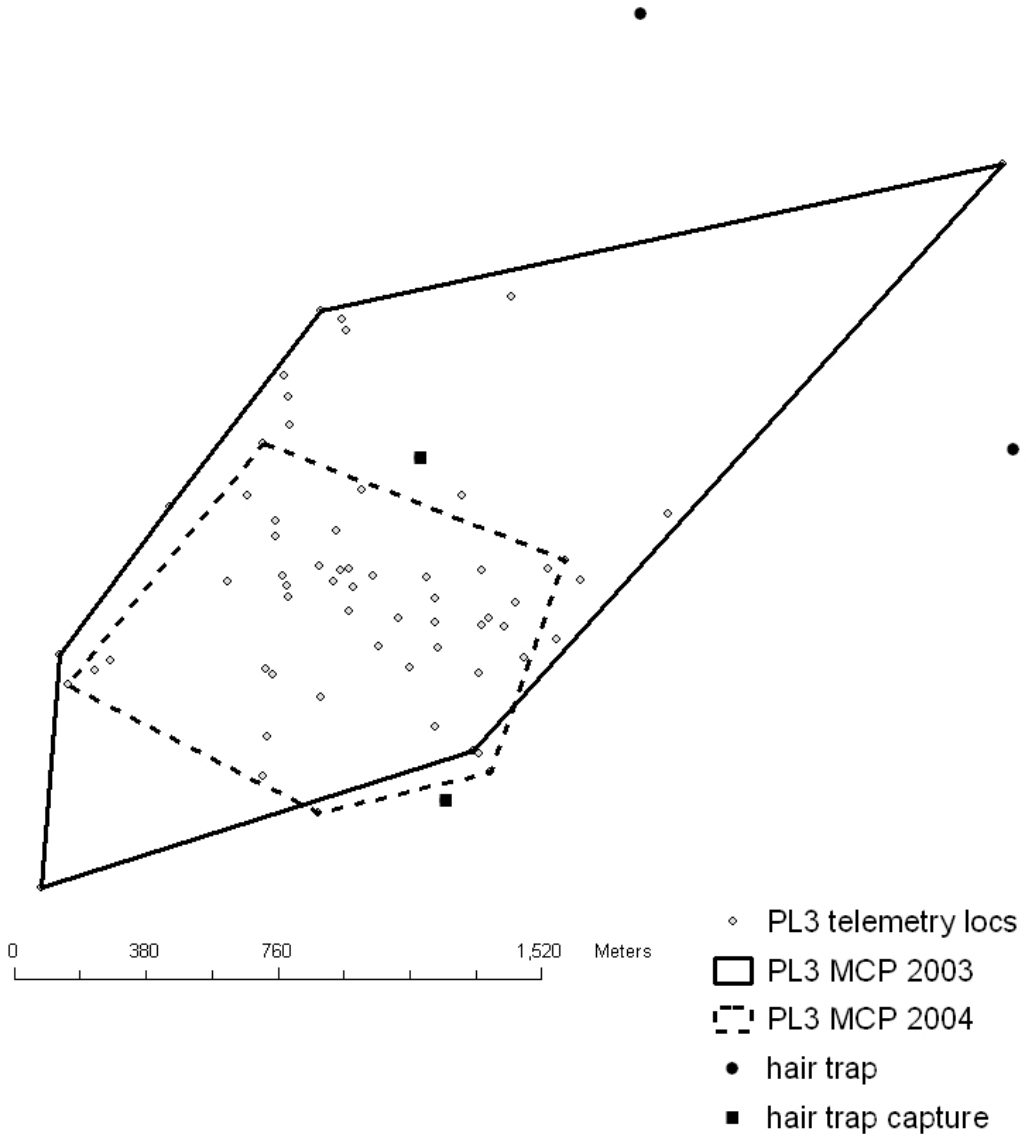

Figure 4.5. Telemetry and hair trap data collected for bear PL3, a 3-year old female, at Pocosin Lakes National Wildlife Refuge (PLNWR). MCP home ranges were calculated from telemetry data in 2003 and 2004.

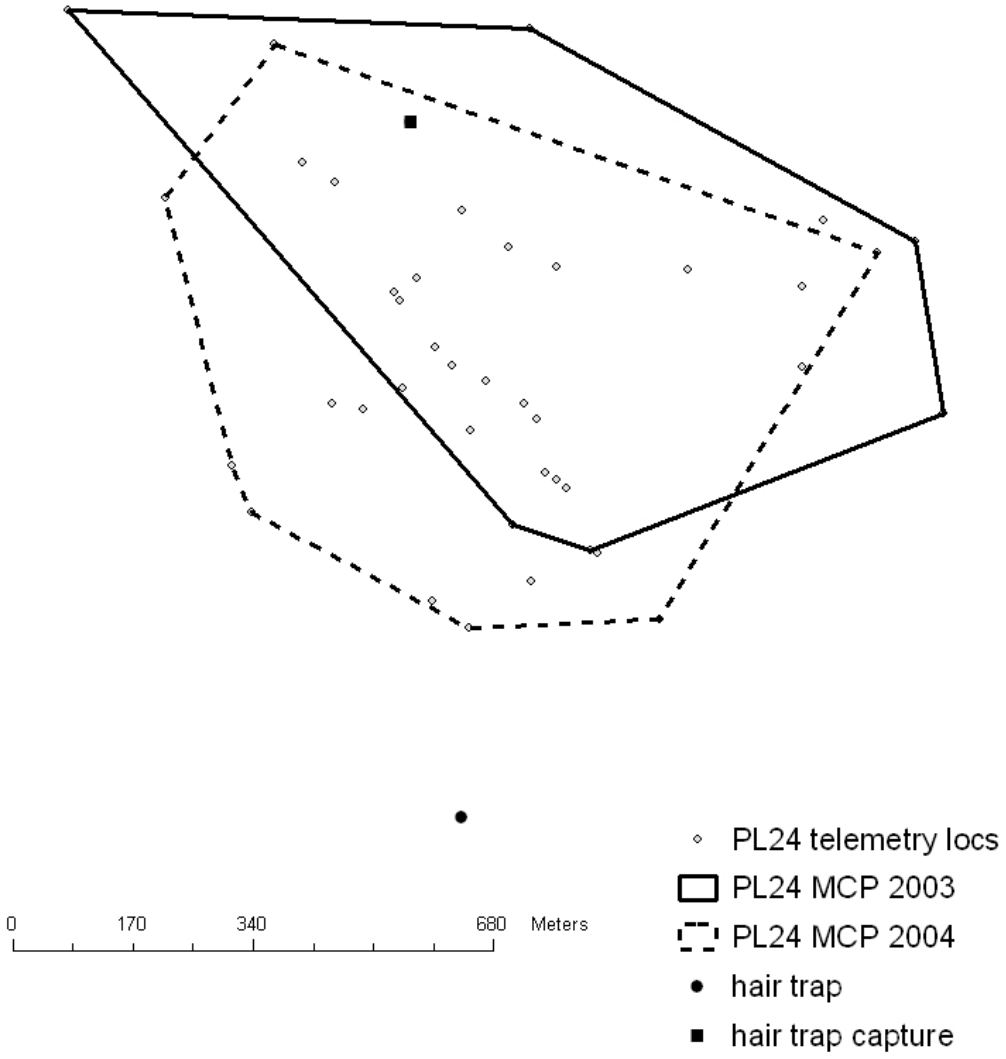

Figure 4.6. Telemetry and hair trap data collected for bear PL24, a 2-year old female, at Pocosin Lakes National Wildlife Refuge (PLNWR). MCP home ranges were calculated from telemetry data in 2003 and 2004.

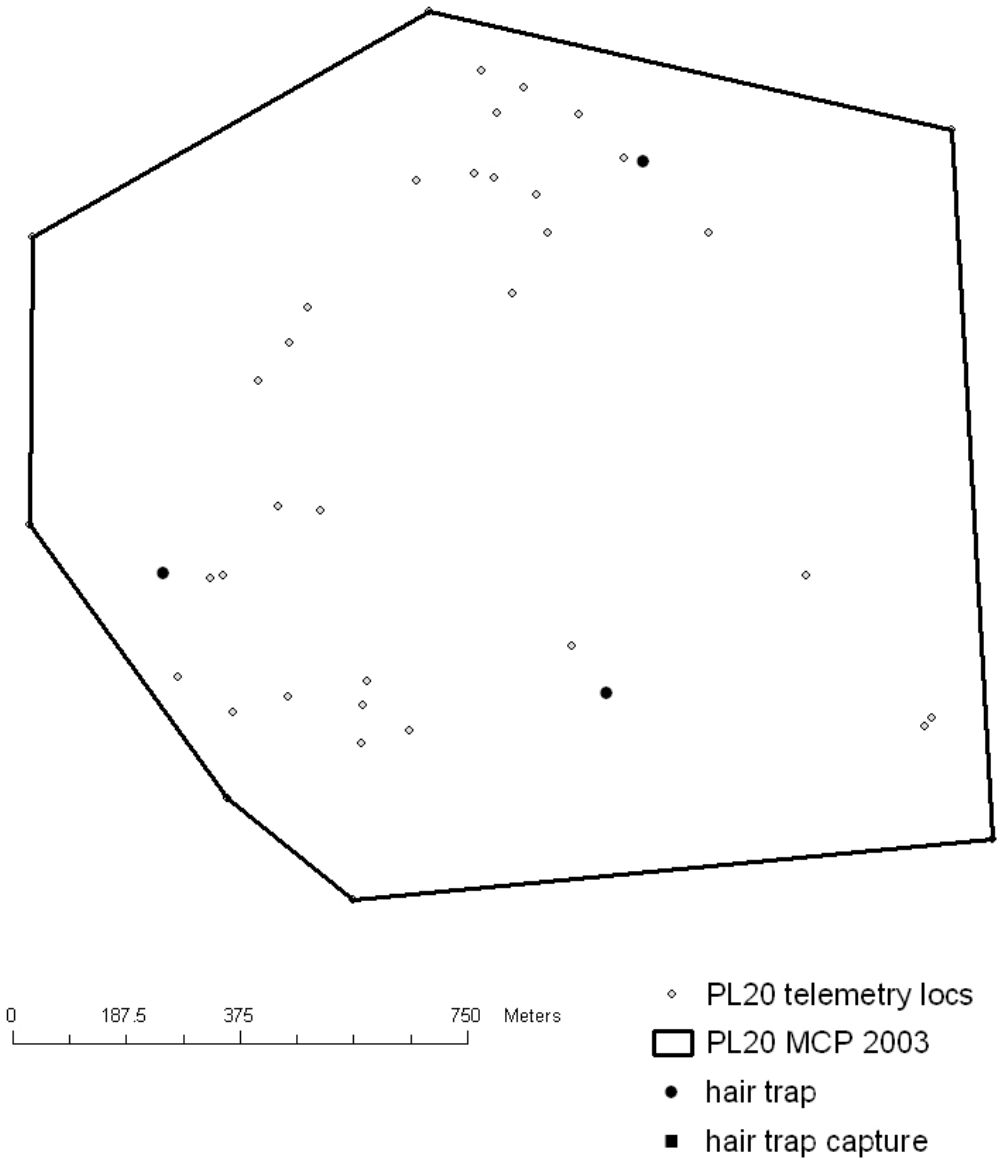

Figure 4.7. Telemetry and hair trap data collected for bear PL20, an 11-year old female, at Pocosin Lakes National Wildlife Refuge (PLNWR). MCP home range was calculated from telemetry data in 2003.

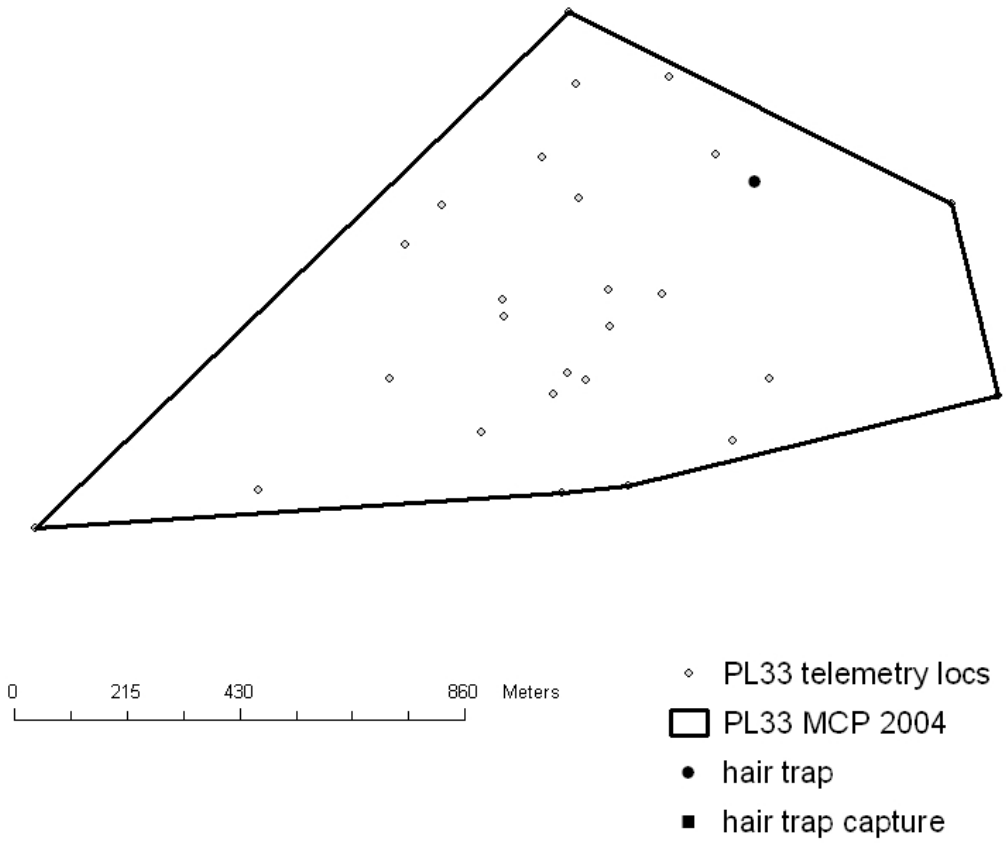

Figure 4.8. Telemetry and hair trap data collected for bear PL33, a 2-year old female, at Pocosin Lakes National Wildlife Refuge (PLNWR). MCP home range was calculated from telemetry data in 2004.

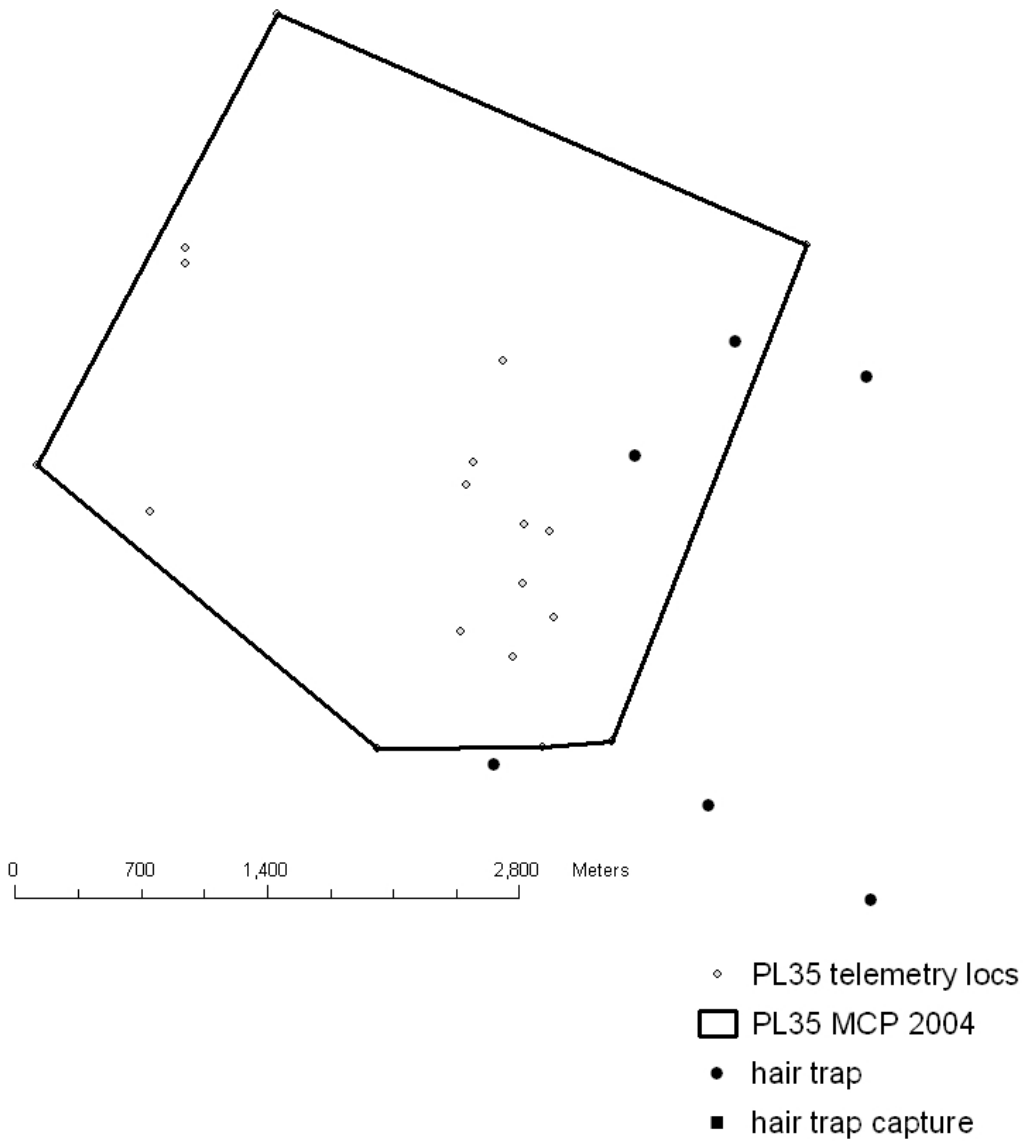

Figure 4.9. Telemetry and hair trap data collected for bear PL35, a 2-year old male, at Pocosin Lakes National Wildlife Refuge (PLNWR). MCP home range was calculated from telemetry data in 2004.

DISCUSSION

Hair-trapping vs. telemetry home ranges and movements

Home range and movement data collected from hair trapping data and radio-telemetry data clearly differed for this study (Table 4.2). Although average MCP home range sizes were similar for both methods (3.0-3.7 km^2, $p = 0.640$), average total distance moved by telemetered bears was nearly 3 times the average distance calculated from hair trapped bears, and there was a > 10-fold difference in the minimum distance moved between subsequent samplings (Table 4.2). This suggests that telemetry data is more likely to detect fine-scale movements over time. Since an animal's radio signal is continuously available for sampling, and is not restricted to a finite set of locations (as with hair trap data), it makes sense that telemetry is better able to pick up these fine-scale movements. Additionally, locating an animal in a hair trap depends on that animal actually entering the hair trap. On the other hand, once a transmitter is on an animal, location data can be taken at any time (although this does necessitate that the animal be live-trapped).

Powell (2000) stated that "a home range estimator should delimit where an animal can be found with some level of predictability." The level of predictability desired or necessary will vary based on the questions being asked. For broad-scale management of black bears, noninvasive hair trap data may be sufficient for calculating home range size. A more accurate estimate of home range size may not be necessary given management objectives. MCP home ranges calculated from hair trap data suffice to give an idea of where bears exist, home range overlap, and minimum areas traversed. More involved management objectives, such as determining habitat use and variations in seasonal movements, would require more intensive and precise sampling (i.e., denser trap spacing), but in many cases is cost-prohibitive and unnecessary.

MCP may not produce the best estimates of home range size (Harris et al. 1990; Powell 2000). This method is largely dependent on sample size, is highly sensitive to outlying data points, and often includes large areas that are never utilized by animals (Powell 2000). For purposes of this analysis, however, MCP was the most appropriate method due to its nonstatistical, simplistic qualities (Swihart and Slade 1985) and its comparibility between grid trapping and telemetry data (Jones 1983). Although MCP home ranges increase to a point with increased number of locations (Stickel 1954), they can be calculated with as few as 3 location points. More complicated statistical methods such as kernel estimators cannot perform properly with as few data points as are available with the hair trapping data from this study (i.e., 3-11 locations).

Detection issues with hair-trapping

I found 3 cases of complete non-detection of individual bears with hair traps that were known to be present via live-trapping and radio telemetry. Two of these cases involved bears PL33 and PL35 that were not live-trapped until 2004. Both of these bears were determined to be 2 years old, and thus may not have been captured in hair traps as cubs or yearlings, or moved into the area only after hair trapping was complete. The other case, however, involved an 11-year old female, PL20, whose telemetry home range overlapped 3 hair traps and who was observed frequently by project personnel and refuge staff on roads and in fields near these traps (Figure 4.7). It is possible that genotyping error could have occurred in this case, but is unlikely given the high quality sample taken from the captured bear and the strict error checking protocol followed in the lab (see Chapter 2).

One case of non-detection involved an 8-year old female (PL2) whose home range was likely so small that it did not contain any hair traps (Figure 4.4). This bear was captured on one hair trap in 2002, but not captured at all in 2003 when she was radio-collared. She was assumed to have had cubs in 2003, and thus her home range may have shifted and/or become smaller in 2003 due to her reproductive status. Her calculated home range was the smallest of any bear we captured (range 0.46-1.19 km^2), and spacing traps at this density was logistically impossible.

Two other bears were captured at a single hair trap the year prior to being live-trapped (2002), but were not captured in any hair traps the year they were transmittered (2003/2004; PL22 & PL34; Figures 4.2 and 4.3). PL34 likely shifted her home range off the refuge sometime after summer 2002 (we were unable to remove a tooth from this bear, and thus no age is available; she was lactating at the time of capture). This bear was live-trapped on the refuge in 2004, but was located and seen only off the refuge that summer. PL22 was a 400+ lb. male whose telemetry home range clearly overlapped 7 hair traps. Approximately 8% of his telemetry locations were within 100 m of a hair trap, and 55% of his telemetry locations were within 500 m of a hair trap, but hair was never left at these traps. It is possible that this bear was large enough to avoid leaving hair on the wire (by stepping over it) or that subsampling prevented any samples from this bear being analyzed in 2003 (we analyzed 2 samples/trap/period in 2003, and 72 of 164 (43.9%) samples from the 7 traps his home range overlapped), but it is most likely that some form of behavioral avoidance of hair traps was at issue.

Another case involved PL3, a 3-year old female who was not captured at a hair trap in 2002, but was captured on the 2 hair traps that fell within her telemetry home range in 2003 when she also was radio-collared (Figure 4.5). This is likely due to a home range shift as a 2-year old in 2002, but could also be due to a behavioral avoidance of hair traps.

Only 2 of 9 transmittered bears actually were captured at most or all of the hair traps that fell within their telemetry home ranges (PL19 & PL24; Figures 4.1 and 4.6).

Boulanger et al. (2004) also looked at sources of heterogeneity bias with hair sampling methods by comparing hair trapping data and radio telemetry. They found that approximately 63% of bears that encountered hair traps actually were snagged, and males encountered more traps than females. These encounter rates seem to be high compared to data collected for this study. The 9 transmittered bears from this study had approximately 168 opportunities to be captured in hair traps (# of traps in MCP home ranges x 8 sampling periods), but were captured only a combined 19 times (~ 11%). This could be due to behavioral responses to the hair traps, lack of opportunities to be trapped due to extremely small home ranges, or subsampling the data as we did (2 samples/trap/period) and missing hair trap captures of transmittered bears.

Boulanger et al. (2004) also observed a large male bear that came within 1.69 km of 17 hair traps, but was never captured. This indicates that probability of capture is not strictly limited to trap encounter rates, but is also related to bear-specific behavior. For unidentifiable forms of heterogeneity such as this (and age variation, for example) they recommend the use of heterogeneity models in Program CAPTURE or the mixture models of Pledger (2000; see Chapter 2), which will give a "less biased indication of population size" than non-heterogeneity estimators. They also recommend the use of at least 4 capture sessions and denser trap spacing to alleviate sex-specific differences in encounter rates and to ensure that all animals (including females with cubs that may have very small home ranges) have the opportunity to be captured.

Until detection rates for noninvasive hair traps can be modeled accurately, it is probably best to run hair traps while having a sample of collared animals on the trapping grid. This allows quantification of the bias that is introduced by animals that are not detected due to trap avoidance

or low trap encounter rates (i.e., animals with small home ranges). It also allows direct estimation of capture probability bias caused by closure violation (Boulanger et al. 2004).

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CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS FOR MANAGEMENT AND FUTURE RESEARCH

CONCLUSIONS

Population densities and sex ratios

Estimated densities of black bears for the 3 refuge populations studied here are some of the highest reported in the literature (Table 2.8). Estimates ranged from 0.56-0.63 bears/km^2 at GDSNWR to 0.65-1.12 bears/km^2 at ARNWR and DCBR to 1.23-1.66 bears/km^2 at PLNWR. Sex ratios at all refuges were biased towards males, particularly in the eastern portion of PLNWR and the farm unit of ARNWR. The significant excess of males in these areas is likely due to habitat use segregation, with large males excluding smaller females from highly productive agricultural fields adjacent to dense forest cover (Wielgus and Bunnell 1994). Sex ratios on the Pungo Unit of PLNWR and on DCBR did not differ significantly from 1:1 (Table 2.6).

Genetic variation and gene flow

Genetic variability of the 3 refuge populations is high compared to other bear populations in North America, with observed heterozygosities ranging from 0.6729-0.7219 (Tables 3.2 and 3.7). Movement of bears and gene flow across this landscape is adequate to prevent high levels of genetic differentiation and structure among the refuge bears. F_{ST} and D_S values were relatively low (0.0257-0.0895 and 0.0971-0.3064, respectively; Tables 3.3 and 3.4). Lower heterozygosity and *Nm* values, and higher F_{ST} and D_S values for GDSNWR indicate that this population is isolated to some degree by geography (i.e., the Albemarle Sound) and encroaching urban development (i.e., the towns of Suffolk and Chesapeake). ARNWR has the potential to

become isolated in the future if movement corridors to the south of the refuge and DCBR are not maintained.

MANAGEMENT RECOMMENDATIONS

Management of black bears on these refuges must be balanced with other refuge objectives, including optimizing habitat for species of special concern (primarily waterfowl) and providing recreational opportunities to the public (including hunting), as well as public safety concerns (i.e., too many aggressive bears on the refuge). Since each refuge has unique concerns about its bear population, I will address each one separately.

Great Dismal Swamp National Wildlife Refuge

Densities of black bears on GDSNWR appear to have remained stable over the past 20 years, but further study into demographic parameters (reproduction, survival, and population growth rate) must be completed before recommendations can be made regarding harvest of black bears at GDSNWR. This is particularly important given potential genetic concerns for this population. The 12 unique alleles and lower *Nm* values found here indicate restricted gene flow to this population, and reducing bear numbers through hunting may exacerbate this issue.

Studies should also be carried out to determine movement patterns of bears on this refuge, particularly dispersal patterns of subadult males. Movement corridors to the south and west of GDSNWR should be identified to maintain habitat connectivity with other black bear populations in the region. Although genetic concerns with this population need not be addressed at this time, regular monitoring of the population via hair snagging would be advisable to track changes in genetic variability and population size (see below).

Maintenance of prime bear habitat at GDSNWR will be most beneficial to black bears in this area (Hellgren et al. 1991). GDSNWR already is composed almost entirely of suitable bear habitat (cypress-gum swamps and mixed-hardwood forests; GDSNWR; unpublished data), but further conversion of maple and pine forests, and beginning a cooperative farm program similar to the ones that exist at PLNWR and ARNWR (i.e., establishing small tracts of farmland interspersed throughout the refuge) would benefit bears in this area even more. These recommendations are in agreement with those made by the U.S. Fish and Wildlife Service (USFWS) for management of waterfowl and other species of concern (USFWS 2002).

Pocosin Lakes National Wildlife Refuge

Complaints from deer hunters regarding aggressive bears harassing them and taking their deer carcasses have been on the rise in recent years at PLNWR (W. Stanton, PLNWR, personal communication). Human safety concerns of this magnitude suggest that bears at PLNWR have reached or even surpassed cultural carrying capacity. It may be the case, then, that harvest of bears at PLNWR is warranted. Caution must be taken during the first few harvest seasons, as bears at PLNWR are not wary of humans, and thus hunter success is likely to be extremely high the first few seasons. A limited permit hunt with a size requirement (e.g., > 300 lbs.) may be advisable for these first seasons. Regular monitoring of population numbers should be incorporated into the biological program as well (see below)

Alligator River National Wildlife Refuge and Dare County Bombing Range

Comparison of bear densities at ARNWR and DCBR from this study to those from the study conducted in the mid-1990s (Folta 1998; Allen 1999) suggests that the bear population has

remained relatively constant over the past 10 years. Reproductive and survival parameters estimated by Folta (1998) were generally high. If these parameters have remained constant, it is likely that bears at ARNWR and DCBR could sustain a limited harvest as well. Again, caution must be taken as hunter success is likely to be extremely high the first few seasons. Population monitoring via hair snares or some other means should be carried out following the first few harvest seasons.

The matrix of habitat types found at ARNWR and DCBR is highly suitable for black bears. It provides a wide variety of food and cover types for bears. Conversion of some of the pine-shrub pocosin found on the eastern portion of the Dare County Peninsula to hardwood or cypress-gum forest would benefit bears even further. Again, conversion of this nature also was recommended by the USFWS (2002) to benefit waterfowl and other species of concern.

Movement patterns and dispersal to the south of ARNWR should be analyzed to determine the degree to which bears use this "corridor" between ARNWR and PLNWR. A demographic and genetic study is currently underway at North Carolina State University in this area, and results from this study should be combined with my results to assess broader scale population trends for bears in this region.

All refuges

Black bears form a significant component of the ecosystem at these refuges and cannot be ignored when outlining management goals. Regular monitoring of these populations is necessary to prevent erosion of genetic variability and population declines, and to maintain populations at or below cultural carrying capacity. This is not only crucial for the long-term

persistence of black bears in the region, but also for the persistence of other wildlife species and for the safety and well-being of humans in the area.

With minor modifications to the methods used in this study, hair sampling can be effective for population monitoring (Settlage 2005). For example, biologists at these refuges could set up hair traps in a similar trapping grid, but over a smaller area (i.e., areas where bears are hunted) and for a shorter time period (i.e., 4-5 weeks). This could be done approximately every 2-5 years in the summer or fall as the biologists see fit. Financial constraints may be problematic, but as this technique becomes more and more widespread, costs for genetic analyses are likely to decrease substantially. Hair sampling in forested areas between refuges would be useful to determine movement and dispersal patterns and to identify potential corridors for gene flow between populations (Dixon 2004).

FUTURE RESEARCH NEEDS

Basic demographic parameters, such as reproductive rates, home range size, and survival, have not been generated for bears on PLNWR. Although I collected home range data during this study, sample sizes were small and only portions of the refuge were covered. Demographic data at GDSNWR was collected over 20 years ago, and it may be beneficial to estimate these parameters again. Population growth, survival, and reproductive rates are important parameters to monitor, particularly if managers intend to open a harvest season on these refuges.

Cooperation with private landowners and state biologists must occur to ensure the landscape on the Coastal Plain is not fragmented further to prevent movement and dispersal of bears. This is particularly true to the south of both GDSNWR and ARNWR. Identification of movement corridors and dispersal patterns of bears between refuges is critical for managing and

maintaining bears in this region. Biologists at GDSNWR also should monitor genetic parameters and population numbers closely to ensure that loss of genetic variability and population decline does not occur.

Studies incorporating human dimensions in wildlife management would assist refuge personnel with managing black bears in this region as well. For example, determining the attitudes of local landowners can help gauge if bear populations are at or above cultural carrying capacity. Managers can then work with landowners to determine management options for nuisance bears, provide optimal recreational hunting and wildlife viewing opportunities, and maintain habitat connectivity for bears on privately owned lands between the refuges.

The Outer Banks of North Carolina is a heavily visited area during the summer months, and increased traffic and development could have negative impacts on the bear populations. Expansion of Highway 64 north of PLNWR is nearly complete as of fall 2005, and plans to continue expansion of the highway through ARNWR are being generated. Furthermore, the Virginia Department of Transportation (VDOT) is planning to expand Highway 17 east of GDSNWR. A study to determine where bears cross this highway was done in 2001, and a similar study is planned in the near future at ARNWR. Follow-up studies after completion of these highway projects would be beneficial to measure their effects on the bear populations.

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APPENDIX A

Abstract of subsampling manuscript

Strategies for subsampling genetic data to estimate black bear population size

ABSTRACT

Costs for genetic analysis of hair samples collected for individual identification of bears averages approximately US$50 per sample. This can easily exceed budgetary allowances for large-scale studies or studies of high-density bear populations. We used 2 complete genetic datasets from 2 areas in the southeastern United States to explore how subsampling these data affects precision and accuracy of resulting population estimates. We used several different subsampling scenarios to create subsets of the complete datasets and compared population estimates generated from these subsets to estimates generated from the complete datasets. We found: 1) selecting only 1 sample/trap in each period biased estimates low, 2) evenly sampling over the entire sampling season is preferable to sampling more from earlier periods and complete random sampling, and 3) precision and accuracy of estimates improved as we increased the proportion of total samples used. We recommend that only high-quality samples (> 5 hair follicles) are used when budgets are constrained, and all efforts should be made to maximize capture and recapture rates in the field. Based on our data, using 2 random samples/trap/period provided the most cost effective strategy and produced estimates within 10% of those generated from complete datasets.

Key words: American black bear, budget constraints, noninvasive genetic sampling, population estimates, subsampling, *Ursus americanus*.

APPENDIX B

Home Range Data for PLNWR 2003-2004

Summer seasonal home range size (km^2) of male and female black bears at Pocosin Lakes National Wildlife Refuge, North Carolina in 2003-2004, calculated using 5 home range estimators [Minimum Convex Polygon (MCP), 95% fixed kernel (FK) in program ABODE (Laver 2005), 95% fixed kernel in the Animal Movement Extension (AME; Hooge and Eichenlaub 1997), and 95% fixed and adaptive kernel (AK) in the Home Range Extension (HRE; Rodgers and Carr 2001)].

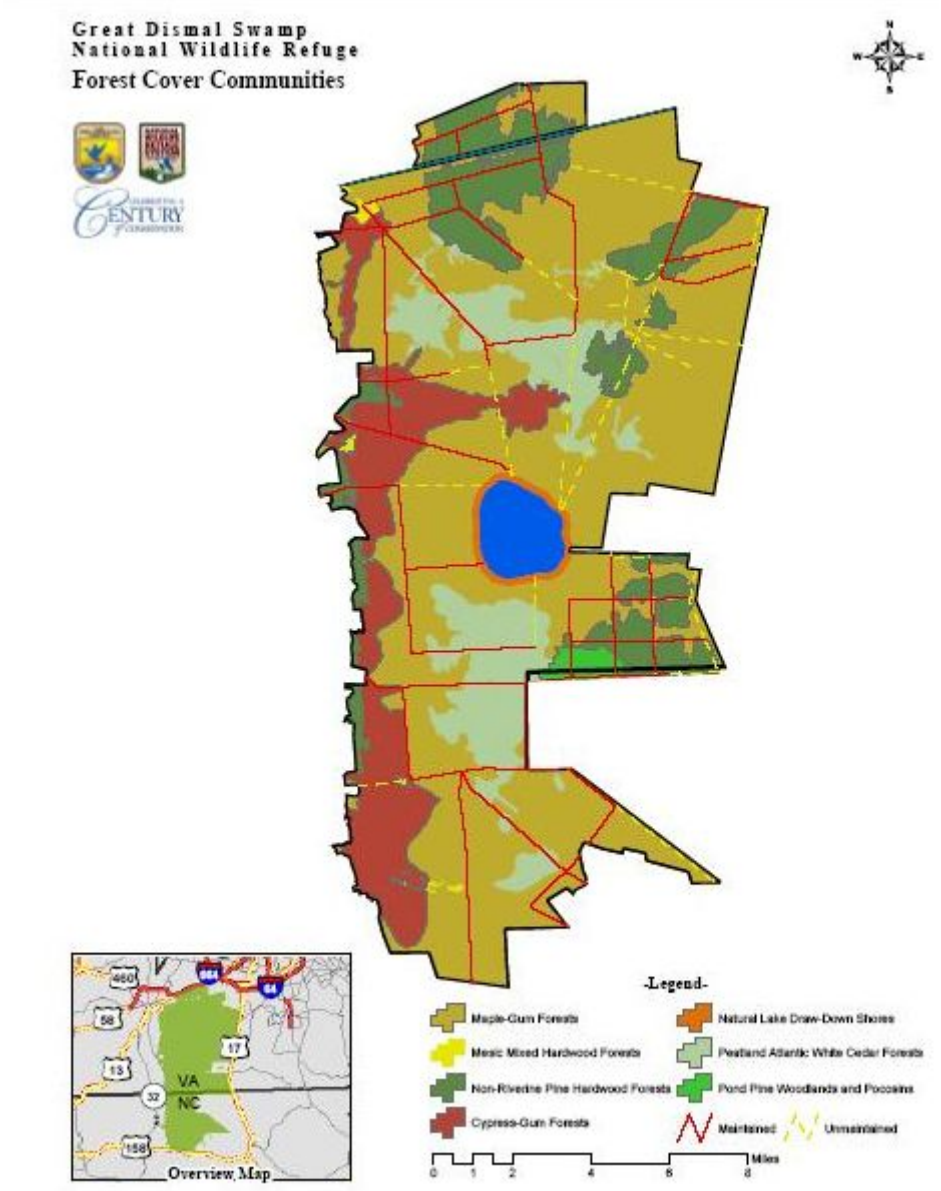
				Estimator (Area [km^2])				
Bear ID	Sex	Year	No. of locations	MCP	95% FK (ABODE)	95% FK (AME)	95% FK (HRE)	95% AK (HRE)
PL2	F	2003	30	0.461	0.499	0.476	0.861	1.192
PL20	F	2003	37	1.872	1.796	2.043	2.396	2.925
PL24	F	2003	14	0.522	0.974	1.056	1.892	2.081
PL24	F	2004	26	0.535	0.749	0.657	1.076	1.415
PL3	F	2003	33	2.448	2.006	2.483	2.905	4.236
PL3	F	2004	28	0.933	1.354	1.205	1.478	1.705
PL33	F	2004	26	0.966	1.101	1.362	2.277	3.021
PL34	F	2004	15	0.694	1.521	1.310	1.180	1.358
AVG. Female				**1.05**	**1.25**	**1.32**	**1.76**	**2.24**

APPENDIX B (cont.)
Home Range Data for PLNWR 2003-2004

Bear ID	Sex	Year	No. of locations	Estimator (Area [km^2]) MCP	95% FK (ABODE)	95% FK (AME)	95% FK (HRE)	95% AK (HRE)
PL19	M	2003	27	6.005	4.409	6.208	1.951	2.839
PL22	M	2003	27	9.479	12.852	21.347	11.546	12.460
PL22	M	2004	13	2.175	5.197	14.934	2.382	2.623
PL35	M	2004	18	10.878	11.372	13.032	18.175	23.113
AVG. Male				**7.13**	**8.46**	**13.88**	**8.51**	**10.26**
AVG. both sexes				**3.08**	**2.58**	**5.51**	**4.01**	**4.91**

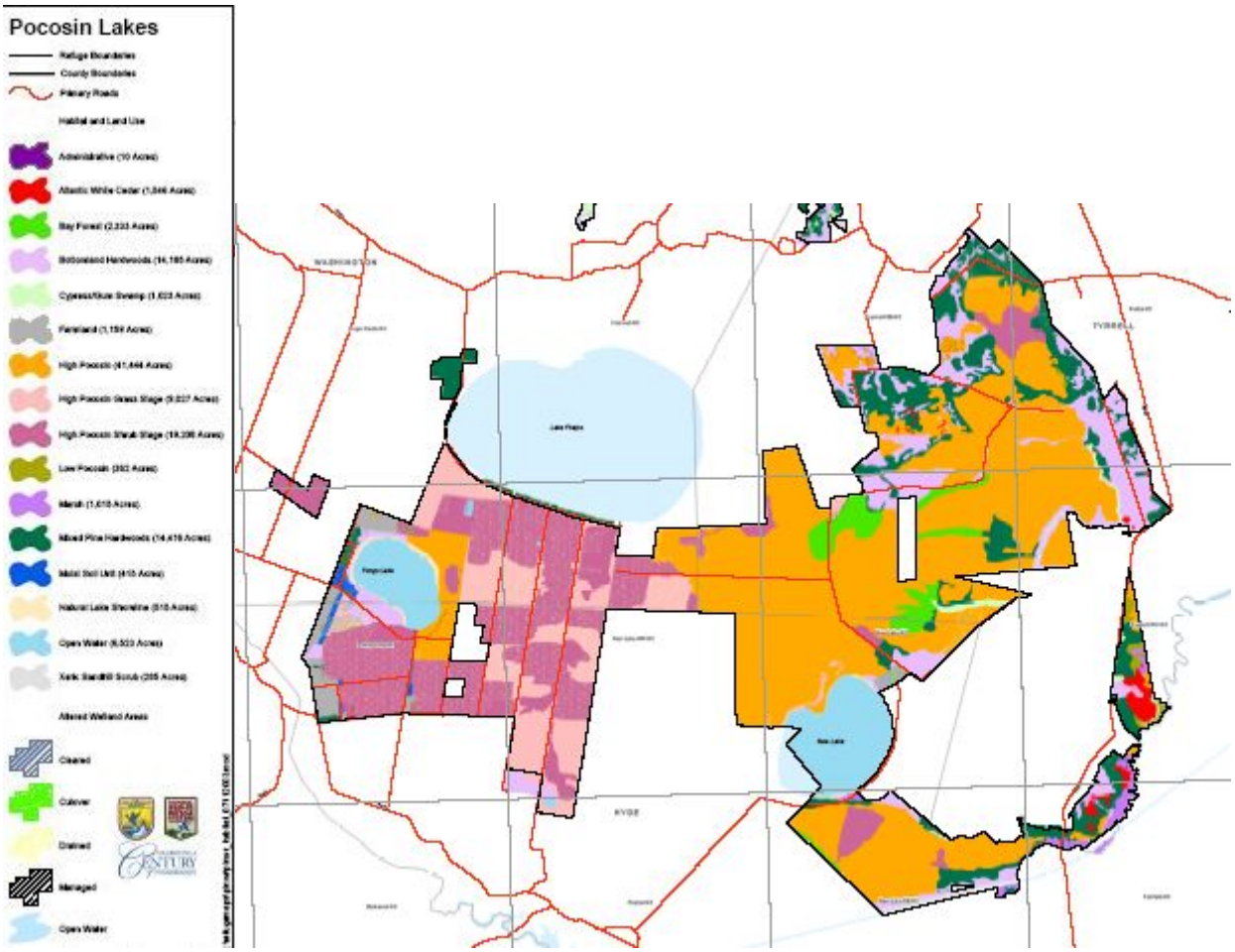
APPENDIX C

Habitat data for Great Dismal Swamp National Wildlife Refuge



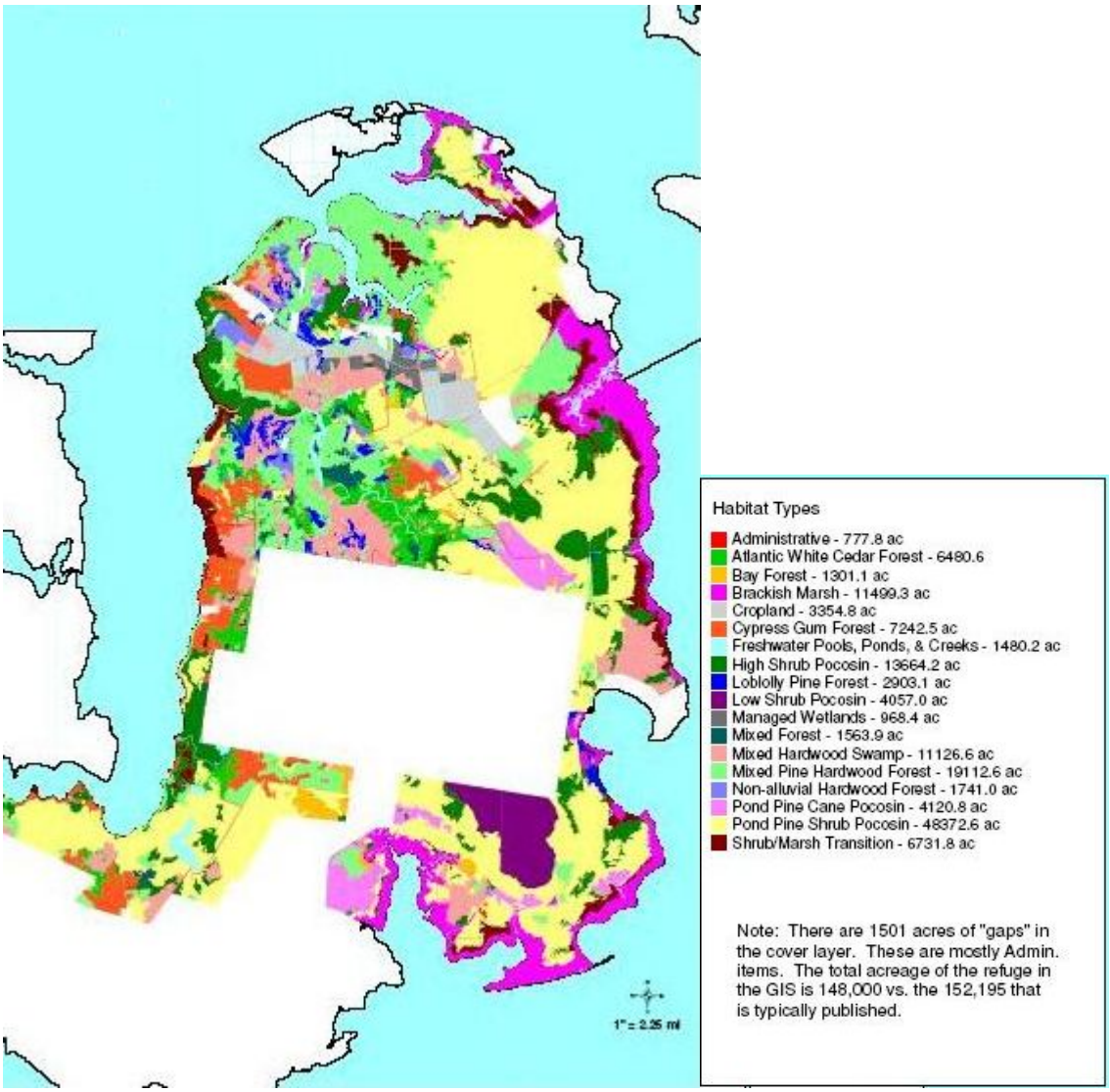
APPENDIX D

Habitat data for Pocosin Lakes National Wildlife Refuge

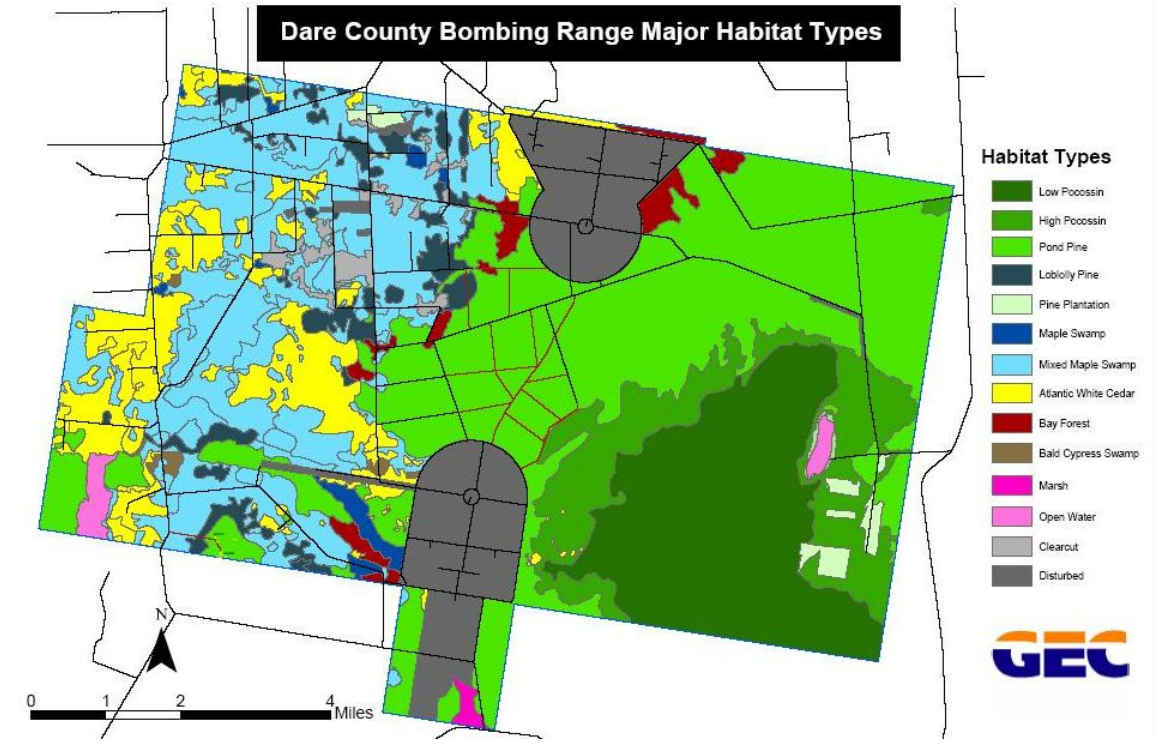


APPENDIX E

Habitat data for Alligator River National Wildlife Refuge



APPENDIX E (cont.)
Habitat data for Dare County Bombing Range



VITA

Catherine Anne Tredick, daughter of Trafton (Skip) and Karen Tredick, was born in Hampton, Virginia on November 18, 1976. She graduated from Smoky Hill High School in Aurora, Colorado in 1995. She received a Bachelor of Science degree in Biology and Environmental Science and Policy from Duke University in 1999. Following graduation, Catherine worked on a variety of wildlife field projects throughout the United States, including seabirds and rats in Hawaii, desert tortoises in Nevada, black bears in Big Bend National Park, California condors in Grand Canyon National Park, and red wolves in coastal North Carolina. She began work on her Master's degree in June 2002 and graduated from Virginia Tech with a Master of Science in Fisheries and Wildlife Sciences in Fall 2005.