DNA-based hair sampling to identify road crossings and estimate population size of black bears in Great Dismal Swamp National Wildlife Refuge, Virginia.

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ABSTRACT

The planned widening of U.S. Highway 17 along the east boundary of Great Dismal Swamp National Wildlife Refuge (GDSNWR) and a lack of knowledge about the refuge's bear population created the need to identify potential sites for wildlife crossings and estimate the size of the refuge's bear population. I collected black bear hair in order to collect DNA samples to estimate population size, density, and sex ratio, and determine road crossing locations for black bears (*Ursus americanus*) in GDSNWR in southeastern Virginia and northeastern North Carolina. I also investigated bear/vehicle collisions to determine patterns of road crossing.

Genetic analysis of 344 hair samples collected on 2 trapping grids identified 85 unique individuals which I used in a mark-recapture analysis. Estimated population size on the trapping grids was 105 bears (95% CI = 91-148) and average density was 0.56 bears/km^2. This density estimate projected over the entire Great Dismal Swamp ecosystem yielded a population estimate of 308 bears (550 km^2 X 0.56 bears/km^2). Similar population estimates generated by Hellgren (1988), Tredick (2005), and this study suggest a stable bear population in the Great Dismal Swamp ecosystem over a 20-year period.

I erected a 2.3-kilometer long strand of barbed wire along U. S. Highway 17 to monitor road crossing patterns near the Northwest River drainage. Genetic analysis identified 6 bears (4 males, 1 female, 1 unknown) that apparently crossed the

highway in a 10-month period. Five of 6 bears deposited hair in a 171-m section which included the Northwest River corridor. The 6 bears detected crossed the road at least 11 times.

I investigated 10 reports of bear/vehicle collisions on the periphery of the refuge from June 2000 to May 2002. Six bears (4M:1F:1 unknown) were confirmed killed during this time period. Based on reported bear/vehicle collisions from Hellgren (1988), the Virginia Department of Game and Inland Fisheries database, and this study, a minimum of 4 to 5 bears are struck by vehicles each year on the periphery of the refuge. I identified 2 areas of multiple bear/vehicle collisions: highway 58 on the north side of the refuge near Hampton Airport and Highway 17 on the eastern side of the refuge in the vicinity of the Northwest River corridor.

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I was so happy to finally be able to hire technicians the second field season. Those four people helped me get lots of work done and laugh off some of the stress of working in the hot, humid, buggy “Dismal” swamp (boy, did they get that name right!). Jamie Chronert, Pat Cuffee, Ian Allen, and Nikki Keys all worked their tails off and deserve a hearty “thank you” (now get back to work, minions!).

I would like to thank the Food Lion store in Suffolk for giving me lots of bags of cakes and doughnuts for free with no more explanation than “I’m going to use this stuff for bear bait”. Also, I appreciate the access given to us by The Nature Conservancy to their Northwest River property.

A huge thanks goes out to Dan Catlin and Jay Howell for the help they gave me with Program MARK. I’d also like to thank my committee for all their patience with me, especially since it took so long for me to wrap things up (no really, I WILL finish, I promise!).

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GENERAL INTRODUCTION

The Great Dismal Swamp National Wildlife Refuge (GDSNWR) (Fig. 1) is the last stronghold for black bears (*Ursus americanus*) in eastern Virginia. The area surrounding the GDSNWR is experiencing a phase of rapid urbanization (Hampton Roads Planning District Commission, undated). Suitable habitat for bears and other forest-dependent wildlife species in this area is shrinking rapidly (L. Culp, U.S. Fish and Wildlife Service, GDSNWR, pers. comm.). Urban and agricultural development has isolated the refuge physically, and possibly has isolated the bear population. The refuge currently is connected to other forested wetlands to the southeast by narrow linkages of similar habitat. Presumably, bears use these linkages to establish new home ranges, seek mates, and forage for seasonally or locally abundant food sources. The ability of these forested strips to maintain gene flow between bear populations is poorly understood.

Prior to this study, a recent population estimate for black bears in the Dismal Swamp was not available. Using both open and closed population mark-recapture estimators, Hellgren (1988) estimated that 262-377 bears were in the refuge in 1987. Evidence of a saturated population included a stable population growth rate (r = 0.0032). Bears dispersing from the refuge likely come into frequent conflict with people, causing an increase in real and perceived property damage as well as public safety concerns. The Virginia Department of Game and Inland Fisheries (VDGIF) has recommended a strategy of limited harvest on the refuge to reduce the number of agricultural and urban damage complaints along the periphery of the refuge (Lloyd Culp, U.S. Fish and Wildlife Service, GDSNWR, pers. comm.).

The Virginia Department of Transportation (VDOT) recently completed a highway expansion project on U.S. Highway 17 on the eastern border of the refuge (John McCambridge, VDOT, pers. comm.). This highway crosses one of the last forested strips connecting the refuge to isolated patches of habitat to the south. Previously, the road was a 2-lane, paved highway connecting the Tidewater region of Virginia with the Outer Banks of North Carolina. This route is used by commuters, tourists, and freight shipping companies and serves as a hurricane evacuation route. Originally, the existing roadbed was going to be used as the southbound route and 2 additional lanes were to be constructed beside the existing structure with a median between them. In order to avoid impacting a wetland site, the plan was modified to leave the existing roadbed in place and construct a new highway system approximately one kilometer eastward (Fig. 2). The new Highway 17 includes 2 northbound lanes, 2 southbound lanes, and an open median. Highway design includes one bridge 300 meters long and 2 large culverts intended to allow wildlife passage across the expanded roadway. The existing Highway 17 will be used for local traffic only.

Short-term black bear movement patterns related to the acquisition of food or mates may be altered by the extensive construction project. The proposed highway design will allow an increase in vehicular traffic (John McCambridge, VDOT, pers. comm.) and will be at least triple the width of the existing road. Approximately 9,500 vehicles/day traveled on Highway 17 prior to construction of the new project. It is estimated that by 2020, 18,900 vehicles/day will travel the expanded Highway 17 (John McCambridge, VDOT, pers. comm.). Not only will average daily traffic volume double, but the speed limit likely will increase from 88 km/hour to 104 km/hour. Refuge and state biologists expressed concerns that bears will be less likely to cross the new highway and that bear/vehicle collisions will become more likely when they do cross. Demographic factors such as over harvest, poaching, or disease outbreaks could negatively affect this bear population if it is isolated. Restriction of movement, combined with ongoing regional habitat loss, may cause long-term harm to the genetic viability of this bear population by increasing random genetic drift (variation in allele frequency from one generation to another due to chance fluctuations), inbreeding (breeding of 2 individuals related to each other), increased mutational load (a measure of the cost of lost alleles due to either selection or mutation)(Wilcove et al. 1986, Poethke et al. 1996), and by decreasing migration. Thus, the goal of this study was to provide up-to-date data on the size of the black bear population on GDSNWR and identify potential sites for the construction of highway wildlife underpasses.

Specific objectives were as follows:

1. Estimate the number, density, and sex ratio of black bears within the Great Dismal Swamp National Wildlife Refuge.

2. Determine road-crossing patterns of black bears along the eastern boundary of the refuge.

3. Determine distribution of bear/vehicle collisions near the refuge to identify potential travel corridors and special management areas.

STUDY AREA

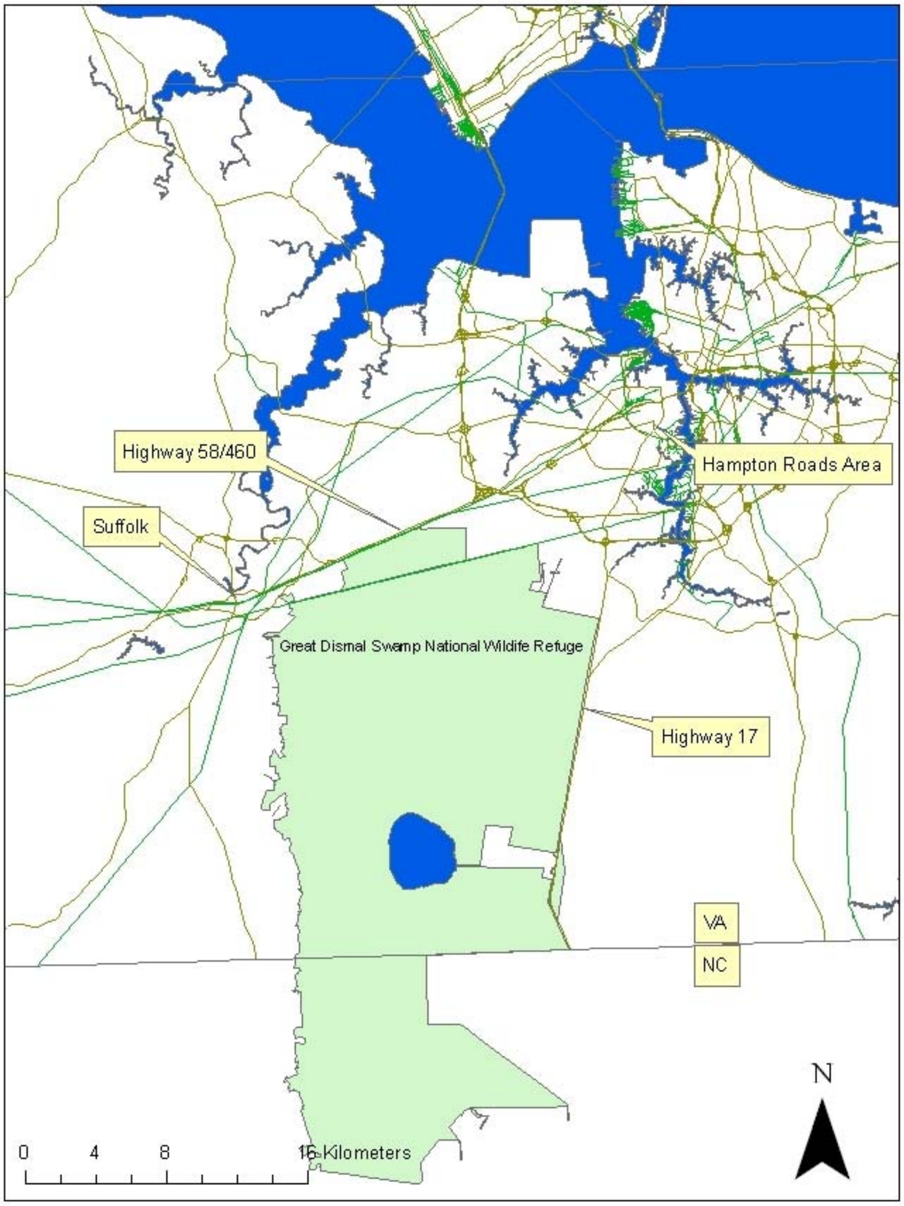
The 550-km^2 study area (hereafter referred to as the Dismal Swamp Ecosystem) includes the Great Dismal Swamp National Wildlife Refuge, Dismal Swamp State Park

(North Carolina), and various private land parcels bordering the refuge (Figure 1). The refuge is a 440-km^2 forested wetland with a highly modified hydrologic system due to numerous canals and ditches. The entire Dismal Swamp ecosystem has been logged completely several times since the 18th century (Whitehead 1972). Whitehead (1972), Musselman et al. (1977), and Gammon and Carter (1979) described the geology and major forest communities of the swamp. These communities include red maple (*Acer rubrum*), Atlantic white cedar (*Chamaecyparis thyoides*), red maple-swamp tupelo (*Nyssa aquatica*), and red maple-black gum (*N. sylvatica*). Associated tree species include sweetgum (*Liquidambar styraciflua*), redbay (*Persea borbonia*), sweetbay (*Magnolia virginiana*), and tulip poplar (*Liriodendron tulipifera*). More hydric portions of the swamp support a bald cypress (*Taxodium distichum*)-mixed gum overstory.

Pocosins, characterized by nutrient poor, peaty soils, heath-like vegetation, and a high water table occur in the Dismal Swamp. Pocosins have a pond pine (*Pinus serotina*) overstory with a high density of understory shrubs including inkberry (*Ilex glabra*), sweet gallberry (*I. coriacea*), fetterbush (*Lyonia lucida*), leucothoe (*Leucothoe* spp.), sweet pepperbush (*Clethra alnifolia*), and wax myrtle (*Myrica cerifera*). Dominant understory shrubs outside of pocosins include sweet pepperbush, blueberry (*Vaccinium* spp.), fetterbush, leucothoe, and hollies (*Ilex* spp.). Oak-beech (*Quercus-Fagus* spp.) communities exist on islands of higher ground in the refuge and on the western border of the refuge. Major vine species include poison ivy (*Rhus radicans*), greenbriar (*Smilax* spp.), wild grape (*Vitis* spp.), Japanese honeysuckle (*Lonicera japonica*), and yellow jessamine (*Gelsemium sempervirens*). Switch cane (*Arudinaria gigantea*) frequently borders roads and ditches and also forms dense stands in the understory.

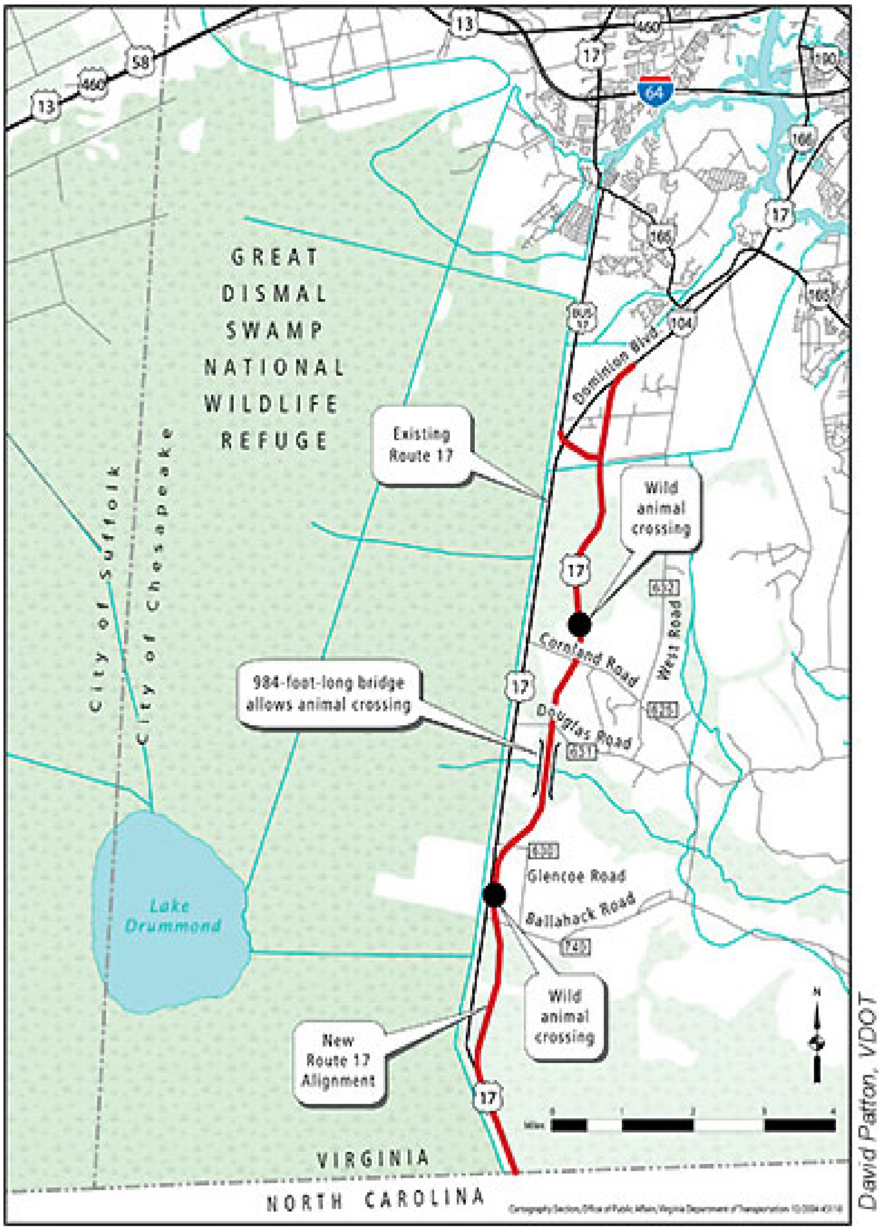
Lake Drummond, a nearly circular, shallow, natural lake roughly 4 km in diameter, lies close to the center of the refuge (Figure 1). The Suffolk escarpment, a Pleistocene beach line, forms the western geological boundary of the swamp. The refuge is bounded to the northwest and northeast by the cities of Suffolk, Portsmouth, and Chesapeake. Housing developments and large farms bound the eastern and western sides of the refuge. These farms produce a variety of agricultural crops including corn, peanuts, soybeans, wheat, and cotton (National Agricultural Statistics Service, http://www.nass.usda.gov/va/). The eastern side of the refuge is bounded by the Dismal Swamp Canal and a 4-lane highway (U. S. Highway 17) that links the heavily populated Tidewater area with the Outer Banks of North Carolina. A 2-lane highway (U. S. Highway 158) runs east to west across the southern end of the refuge. Privately owned forested areas occur south of the refuge. A 6-lane highway (U.S. Highway 58) and 2 sets of railroad tracks border the refuge to the north.

Figure 1. Great Dismal Swamp National Wildlife Refuge, VA/NC and surrounding area 2000-2002.



Map Prepared by J. Wills 4/29/2008

Figure 2. New alignment of U. S. Highway 17 with locations of wildlife crossing structures east of Great Dismal Swamp National Wildlife Refuge, VA/NC 2005.



CHAPTER 1: POPULATION SIZE, DENSITY, AND SEX RATIO OF BLACK BEARS ON GREAT DISMAL SWAMP NATIONAL WILDLIFE REFUGE

LITERATURE REVIEW

Genetic Marker Applications

The use of DNA markers to identify species, populations, sex, or individuals is a relatively new technique in the field of wildlife management, but its use is increasing as its benefits become apparent to more natural resource professionals. Researchers have extracted DNA samples from blood, skin, feces, muscle tissue, and hair strand roots. Analysis of DNA samples has been used to determine population structure, kinship, species, subspecific affinity, sex, to identify individual animals in mark/recapture population estimates and to solve wildlife forensic cases (Gerloff et al. 1995, Kohn et al. 1995, Foran et al. 1997, Reed et al. 1997, Morin et al. 1999, Woods et al. 1999, Mills et al. 2000, Mowat and Strobek 2000, Warrillow et al. 2001, and Boersen et al. 2003). Population estimates for black bears using hair traps have been obtained in various locations in North America (Woods et al. 1999, Boersen 2003, Bridges 2005, and Langer 2006) in the last several years.

Advantages of Genetic Markers

The use of genetic markers to estimate population size has several advantages over traditional mark-capture methods including decreased tag loss (i.e. genetic marks are permanent), decreased risk of injury to study animals and researchers, decreased effects of capturing and marking, and increased capture probabilities (Woods et al. 1999, Mills et al. 2000). In addition, capturing hair on barbed wire is more cost-efficient in terms of time and money than live trapping, and usually results in a larger sample size.

Disadvantages of Genetic Markers

While non-invasive genetic sampling can identify individuals for population estimation and individual sex identification, it cannot provide data on survival, sources of mortality, reproductive rates, or movement patterns that can be obtained only with radio telemetry. Analysis of DNA samples requires special care to reduce enzymatic deterioration and cross-contamination, specialized lab equipment, and specialized molecular genetics skills. The costs of analysis may vary based on the species of interest, total number of samples, and the laboratory doing the analysis. Currently, it costs $30-45 dollars per bear sample for individual identification (T. King, Conservation Genetics Laboratory, pers. comm. and D. Paetkau, Wildlife Genetics International, pers. comm.). Potential problems associated with DNA analysis are discussed in a later section.

Odor/Taste Attractant Research

Past studies have investigated responses of wildlife species to different odor or taste attractants. Bean and Mason (1995) tested white-tailed deer (*Odocoileus virginianus*) preference for natural versus artificial liquid lures designed to deliver drugs for tick control or oral contraception. They found a greater preference for liquid lures with natural flavors. Mattfield et al. (1972) found no difference in trapping success when trapping white-tailed deer in winter using traps baited with browse compared to trapping success in the summer using salt blocks. Koerth and Kroll (2000) compared white-tailed deer preference for several solid baits and found shelled corn to be the most attractive for use at remote camera stations. Mason et al. (1995) compared white-tailed deer preference for a solid bait (salt) paired with a liquid extract versus several liquid extracts presented alone or in pairs. They found that extracts paired with salt blocks enhanced attractiveness relative to salt blocks paired with propylene glycol. Also, they found that a liquid bait consisting of apple juice, glycerine, water, and sodium chloride was more attractive than a mineral block alone. Crawford and Church (1971) examined the response to sweet, sour, bitter, and salty compounds by black-tailed deer (*Odocoileus hemionus*). Rice and Church (1974) examined the response to different browse extracts by black-tailed deer. Results from both studies indicated differences in preference between males and females for different compounds.

Some studies have evaluated the relative attractiveness of several synthetic scent lures developed for coyotes (*Canis latrans*) (Roughton 1982 and Turkowski et al. 1982). Both studies indicated some lures were more effective than others, but each study tested different lures. Other studies have investigated behavioral responses by coyotes to scent lures (Martin and Fagre 1988 and Phillips et al. 1990). Both studies indicated slight differences in visitation rates and behavioral response depending on the suite of lures being tested.

Roy and Dorrance (1992) tested efficacy and selectivity of domestic chicken eggs (*Gallus domesticus*) and tallow baits used to deliver poison to rabid striped skunks (*Mephitis mephitis*). While both of these food baits were attractive, logistical constraints due to attraction of non-target species made eggs preferable over tallow baits.

McDaniel et al. (2000) tested the efficacy of five different lures used to attract lynx (*Lynx canadensis*) to hair snaring devices. Two lures were used significantly more than expected. Langer (2006) used scent lures and food rewards to attract black bears to hair traps in North Carolina. He suggested using scent lures only to minimize excessive amounts of hair samples and to avoid behavioral response from bears that received a food reward compared to bears that did not. Results from these studies indicate that many factors must be considered when choosing a lure for a given species. Factors include the attractiveness of one lure relative to another, long-term availability, price, attractiveness under various weather conditions, and target specificity.

METHODS

Hair Trap Grid Study Design

I established two hair trap grids within the refuge; one north of Lake Drummond and one south of Lake Drummond (Figure 1-1). The northern grid was 110 km^2 and the southern grid was 72 km^2. Each grid was divided into one-km^2 cells. Ideally, one hair trap corral would be placed in every other one-km^2 cell as close as possible to the center. Otis et al. (1978) recommended the placement of at least 4 traps in each animal's home range and used the following formula to calculate trap spacing

$$s = (\sqrt{2})\, W$$

where s = trap spacing and W = the radius of the average home range size. I estimated home range diameter by assuming that home ranges of adult female bears monitored by Hellgren (1988) in an earlier study in the Great Dismal Swamp were circular. I used adult female home range sizes because subadult bears do not have established home ranges, and adult males roam sufficiently large distances that such a resolution should "capture" them. Hellgren (1988) determined the median home range of adult females without early fall locations was 10.4 km^2. Early fall (16 September to 15 November) average home range size of adult females was inflated due to bears searching for locally abundant hard and soft mast (Hellgren 1988). A circular home range of 10.4 km^2 has a diameter of roughly 3.6 km. Multiplying the radius (1.8 km) by $\sqrt{2}$ results in a trap spacing no more than 2.54 km, which would ensure at least 4 traps in the average adult female's home range. Due to logistical constraints, I placed 27 and 25 hair trap in the northern and southern sections, respectively. The northern section had one hair trap/4 km^2, while the southern section had one hair trap/2.88 km^2. I used a Global Positioning System (GPS) receiver to obtain Universal Transverse Mercator (UTM) coordinates for each hair trap.

Hair Trap Design

I secured a single strand of barbed wire (15.5 gauge, double strand, 4 barbs/cluster) 15-25 meters long around a group of trees (at least 3) using fencing staples to provide a multi-sided corral (Woods et al. 1999, Mowat and Strobek 2000). Each corral had a minimum width of 4 meters. I placed the wire approximately 50 centimeters above ground level, ensuring a uniform height around the entire corral. I checked sites every 7 days beginning in late June 2001 and collected hair samples until early November 2001. I tied a short length of flagging to the center of each leg of the wire to make it visible to anyone walking through the area.

Bait Use and Placement

I used a food reward in one trapping grid and scent lure only in the other trapping grid to test for differences in the total number of bears visiting traps in each grid and the incidence of repeat visits in each area. I assumed there was no difference in bear density between the two areas. In the food reward area (the northern grid), I suspended a red plastic mesh bag containing pastries in the center of the corral roughly 2 meters above the ground. In the scent lure-only study area (the southern grid) I poured approximately 20 milliliters of commercial raspberry extract (Mother Murphy's Laboratories, Greensboro, N.C.) on a white cotton rag suspended roughly 2 meters above ground level in the center of each corral. Each site received either a new bag of pastries or fresh lure every 7 days whether or not a bear visited it.

Data Collection

It is impossible to judge in the field whether only one bear or multiple bears deposited hair on consecutive barbs; therefore, bear hair found on a barb cluster was considered an individual sample for the purpose of collection. The collectors wore latex surgical gloves during the collection process. Samples were removed from barbs with hemostats. The hemostats were flame-sterilized using a propane torch before collecting samples from a plot and between each sample to avoid cross contamination of genetic material. Barbs with bear hair also were exposed to flame for several seconds after the hair was removed. Samples were placed in a coin envelope labeled with the date, plot number, and barb number. Envelopes containing samples with fewer than 5 hair strands, with 5 to 9 strands, or with 10 or more strands were marked differently in order to eliminate unnecessary analysis of samples with insufficient genetic material. Past experience has shown that at least 5 strands with roots are necessary to obtain enough genetic material for reliable polymerase chain reaction (PCR) amplification (M. Culver, Research Associate, Virginia Polytechnic Institute and State University, pers. comm.). All samples were kept, however, in case future methods allow for smaller samples to be analyzed. All samples from a corral were placed in a labeled plastic bag. All samples were placed in a freezer within 8 hours of collection and kept frozen until processed. Eighteen sampling periods were conducted for the 2 grids combined.

Microsatellite Genotyping

DNA Isolation -.Genotyping of DNA samples was conducted by the staff of Conservation Genetics Laboratory, Leetown Science Center, Kearneysville, West Virginia. The protocol reported here is from the Leetown Science Center laboratory. DNA was extracted from black bear hair strand roots using the InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA). Hair roots were incubated in InstaGene Matrix in the presence of Proteinase K at 65°C overnight. This mixture was boiled (100°C) for 8-10 minutes, followed by centrifugation at 10,000-12,000 rpm. The resulting supernatant was used in polymerase chain reactions (PCR) to amplify microsatellite DNA loci.

Microsatellite Analysis -.Microsatellite DNA amplification was performed for 10 microsatellite loci using PCR primers described by Paetkau and Strobeck (1994), Paetkau et al. (1995), and Taberlet et al. (1997). These loci were: *G1A, G1D, G10B, G10C, G10L, G10M, G10P, G10X, MU23,* and *MU50*.

Each PCR reaction consisted of 1.5 µl of genomic DNA extract, 0.875 X PCR buffer (59 mM Tris-HCl, pH 8.3; 15 mM $(NH_4)_2SO_4$; 9 mM β-mercaptoethanol; 6 mM EDTA), 2.25 mM $MgCl_2$, 0.2 mM dNTPs, 0.15-0.43 µM of each primer (forward primer fluorescently labeled with NED, FAM, or HEX; Applied Biosystems (ABI), Foster City, CA), 1.2 units of *Taq* polymerase (ABI), and deionized water added to achieve the final volume of 15 µl. The amplification cycle consisted of an initial denaturing at 94°C for 2 minutes; followed by 35 cycles of 94°C denaturing for 30 seconds, 56°C annealing for 30 seconds, and 72°C extension for 1 minute. Cycling culminated with a 5-minute extension at 72°C. Thermal cycling was performed in an MJ DNA Engine PTC 200 (MJ Research, Watertown, MA) configured with a heated lid.

Fragment Analysis -.Generally, 1 µl of PCR product was diluted 1:1 with deionized water and thoroughly mixed. One µl of this dilution was added to 12 µl of deionized formamide and 0.5 µl of the internal size standard GENESCAN-500 (ABI). Alternatively, PCR products of separate multiplexed reactions (2-3 loci each) and multiple separate reactions (2-4) were combined and analyzed without dilution. Loci were identified in these multiplexed samples by virtue of their characteristic molecular mass and attached fluorescent label. The size standard contained DNA fragments fluorescently labeled with the dye phosphoramidite ROX (red). This PCR product/size standard/formamide mixture was heat denaturated at 95°C for 3 minutes and placed immediately on ice for at least 5 minutes. The mixture was subjected to capillary electrophoresis on an ABI PRISM 3100 Genetic Analyzer. GENOTYPER v. 2.0 (ABI) DNA fragment analysis software was used to score, bin, and output allelic (and genotypic) designations for each bear sample.

Statistical Analyses -.The multilocus genotype generated for each individual from the series of PCR amplifications was analyzed to assess the uniqueness of each hair sample. Estimates of individual pair-wise genetic distances, using the proportion of shared alleles algorithm, were calculated using a 32-bit version of Microsat 1.5d (Eric Minch, Stanford University). Pair-wise genetic distances of zero were indicative of identical multilocus genotypes.

Observed genotype frequencies were tested for consistency with Hardy-Weinberg and linkage equilibrium expectations using randomization tests implemented by

GENEPOP 3.1 (Raymond and Rousset 1995). The Hardy-Weinberg test used the Markov chain randomization test of Guo and Thompson (1992) to estimate exact two-tailed *p* values for each locus. Bonferroni adjustments (Rice 1989) were used to determine statistical significance for these tests. Linkage equilibrium tests used the randomization method of Raymond and Rousset (1995) for all pairs of loci. The amount of genetic variation in each sample was summarized by gene diversity (average expected heterozygosity).

Sex Determination

The sex-determining region on the Y chromosome (*SRY*) was used to determine sex for each DNA sample using a method developed by Taberlet et al. (1993). *SRY* primers were obtained from Sigma-Genosys (The Woodlands, TX). All samples were PCR-amplified, but only males would exhibit a PCR product for the *SRY* gene. PCR reactions were performed using a final concentration of 1 X PCR buffer, 1.5 mM $MgCl_2$, 0.05 mM dNTPs, 0.01 μM of each primer, and 0.5 units of *Taq* polymerase. Cycling conditions were an initial denaturation at 93° C for 4 minutes; 23 cycles of 93° C for 1 minutes, 50° C for 1 minute, 72° C for 1 minute; and a final extension at 72° C for 7 minutes. A second round of PCR was performed using the same conditions as above, but with nested *SRY* primers and 45 cycles.

After amplification, the PCR products were subjected to electrophoresis through a 7% native TBE polyacrylamide gel (Hoefer SE 600 gel apparatus, Amersham Pharmacia Biotech, San Francisco, CA). PCR products were visualized using a silver staining protocol as in Warrillow et al. (2001). Samples that failed to successfully identify sex were labeled as unknown. A chi-square test for equal proportions was used to determine if the male:female sex ratio differed from 1:1 (Preacher 2001).

Trapping Success and Recapture Rates

I determined trap success by dividing the number of captures of individual bears by the total number of trap-weeks and multiplying by 100 to get a percentage. I calculated the recapture rate of each grid by dividing the number of recaptures by the total number of captures and multiplying by 100.

Population and Density Estimation

I estimated population size and density for the black bear population in the GDSNWR using individuals identified from DNA analysis in a full closed heterogeneity model in Program MARK (White and Burnham 1999). Several inherent assumptions must be met when using this estimation method (White et al. 1982):

(1) The population must be closed both geographically and demographically.

(2) Animals do not lose their marks during the study.

(3) All marks are noted and recorded correctly at each sampling period.

(4) The sampling effort is sufficient to capture all individuals in the area sampled.

RESULTS

Trapping Success and Recapture Rates

I collected 344 bear hair samples from 52 hair traps. One hundred thirty-seven samples were not analyzed due to having no roots or too few roots for analysis. Another 17 samples failed to yield conclusive results during amplification and DNA analysis. Fifty-one percent of the 344 samples collected identified individual bears (176 samples/344 total samples).

In the southern grid, all of the hair traps collected bear hair at least once during the 18-week sampling period. In the northern grid, 10 of 27 hair traps (37%) did not collect hair samples during the 15-week sampling period. Five of the 10 northern grid traps with no bear hair samples were clustered together in the east central portion of the grid near Camp Ditch (Fig. 1-2). The area surrounding these 5 traps is roughly 13 km^2. The other 5 empty hair traps were scattered in various locations.

There were 66 capture events in the southern grid and 43 capture events in the northern grid. I recorded 450 trap weeks in the southern grid and 405 trap weeks in the northern grid for a total of 855 trap weeks. The capture rate (number of trap weeks/capture) was 6.8 (14.6% trap success, 66 captures/450 trap weeks) in the southern grid and 9.4 trap weeks/capture (10.6% trap success, 43 captures/405 trap weeks) in the northern grid. Eighty-five individual bears were captured 109 times in both grids combined, resulting in 7.8 trap weeks/capture and an overall trap success of 12.7% (109 captures/855 trap weeks). The capture rates in the 2 grids were not different (X^2=0.412, 1 d.f., P = 0.52). The recapture rate in the southern grid was 22% (15 recaptures of 66 total captures) and 21% in the northern grid (9 recaptures of 43 total captures). Recapture rates between grids did not appear to be different (X^2 = 1.5, 1 d.f., P = 0.22).

Most bears that were detected at >1 hair trap were detected at adjacent hair traps. One female (bear # 36) in the northern grid was detected at 2 traps 11.3 kilometers apart straight-line distance within a 3-week time period.

Hair Trap Grid Demographics

Eighty-five individual bears were identified from hair samples collected on the northern and southern grids; 51 bears were identified in the southern grid (26 males, 21 females, 4 undetermined) and 34 bears were identified in the northern grid (21 males, 13 females). No bears were detected in both grids during the sampling period. The male:female sex ratio of 1.38:1 appeared to be similar to 1:1 (X^2 = 2.08, 1 d.f., P = 0.14) (Preacher 2001).

Population Estimation

I estimated population size for each grid using Program MARK (White and Burnham 1999). Eight models were compared and ranked by MARK using AICc (Akaike Information Criterion). These models included {M(0) constant probability of capture}, M(h) heterogeneity of capture probabilities}, {M(b) behavioral response after initial capture}, {M(t) time specific variation in trapping probabilities}, {M(bh) behavioral response in presence of heterogeneity}, {M(th) time variation and individual heterogeneity}, {M(tb) time response in presence of behavior}, {M(tbh) varying time, behavior, and heterogeneity probabilities}. Model (bh) – behavior in the presence of heterogeneity - ranked highest among the eight models examined (Table 1-1). Since this model was the most heavily weighted model of the 8 models used by Program MARK, I will consider it for all population and density estimates. The population estimate for model (bh) for the southern grid was 61 bears with a standard error of 6.87 and a 95% confidence interval of 54 to 84 bears. The population estimate for the northern grid was 44 bears with a standard error of 6.22 and a 95% confidence interval of 37 to 64 bears.

Density Estimation

I used 2 different trapping grids because Lake Drummond is located in the center of the refuge and is not available as bear habitat. The northern grid was originally 110

km^2, but increased to roughly 198 km^2 by adding a 1.8-km buffer strip. The southern grid increased from 72 km^2 to roughly 150 km^2 by adding a 1.8-km buffer.

Application of Model (bh) estimated 61 bears in the southern grid, yielding an average density of 0.406 bears/km^2. The density range varied from 0.36 bears/km^2 to 0.56 bears/km^2. Application of Model (bh) estimated 44 bears for the northern grid yielding an average density of 0.22 bears/km^2. The density range for the northern grid varied from 0.18 bears/km^2 to 0.32 bears/km^2. Density in the southern grid was higher than that in the northern grid ($X^2 = 5.527$, 1 d. f., $P = 0.018$). Adding the population estimates of the 2 grids together results in 105 bears in the 348-km^2 area encompassing both grids and their 1.8-km wide buffer strips. This would yield an average density of 0.3 bears/km^2. The Dismal Swamp ecosystem area is 550 km^2. Thus, with an average bear density of 0.3 bears/km^2, the total population estimate for the ecosystem was 165 bears. Using the lowest density estimate for the northern grid (0.18 bears/km^2) and the highest density estimate for the southern grid (0.56 bears/km^2), the Dismal Swamp ecosystem population estimate ranged from 99-308 bears.

I also used the Mean Maximum Distance Moved (MMDM) method to calculate density (Wilson and Anderson 1985). Using trapping histories from 20 bears (14 males, 3 female, 3 unknown), I calculated the MMDM between hair traps by these animals during the sampling period. Fifteen bears were captured in >1 hair trap in one or multiple trap-weeks. Five of these bears were from the northern grid and 10 were from the southern grid. Five other bears visited only one hair trap over multiple sampling periods and their maximum distance moved was 0 km. Two of these bears were male, 2 were female, and the sex of 1 was unknown; 4 of 5 of these bears were from the south grid and

1 was from the north grid. The MMDM for all 20 bears was 2.79 km (Table 1-2). This resulted in a buffer strip 1.4 km wide around the grids (2.79 X 0.5). The southern grid sampling area increased to 130 km^2 and the northern grid size was increased to 177 km^2. Adding the population estimates of the 2 grids together results in 105 bears in the 307 km^2 area encompassing both grids and their 1.4 km wide buffer strips. This would yield an average density of 0.342 bears/km^2. Multiplying this density estimate by 550 km^2 resulted in a total of 188 bears in the Great Dismal Swamp Ecosystem using the MMDM buffer strip method.

DISCUSSION

Microsatellite Data Analysis

Several problems can arise when identifying individuals using genetic markers from samples with low DNA yield. Allelic dropout, the failure of 1 of 2 alleles in a pair to successfully amplify at a given locus (Navidi et al. 1992, Gerloff et al. 1995, Taberlet et al. 1996), can occur when DNA template material is minute. I largely avoided this problem by analyzing only samples of hair with at least 5 strands with roots. Another problem, false allele generation, occurs when the PCR process generates alleles at a non-target locus (Foucault et al. 1996, Taberlet et al. 1996). I overcame this problem by using an automated system (see Microsatellite genotyping) that uses a chromatogram to distinguish correct versus false alleles. A third problem, known as the shadow effect, occurs when 2 different animals are genetically indistinguishable from each other (Mills et al. 2000). This problem is most likely to occur when fewer than 7 loci are used for individual identification. I minimized this problem by using greater than 7 loci.

Hair Trap Grid Demographics

Interestingly, no bears of either sex detected in one grid were detected in the other grid or in the roadside samples (see Chapter 2) during the 18-week sampling period. Distances from hair traps in either grid to the roadside wire along Highway 17 exceeded 6 km straight-line distance. Therefore, bears with home ranges nearer the grids simply may not have traveled eastward to the highway or vice versa during the sampling period.

Lake Drummond is situated between the 2 grids. It is unlikely that a bear would swim across the lake to travel north or south. However, there is terrestrial habitat on either side of the lake linking the northern and southern portions of the refuge (Fig. 1-1). The distance between the southernmost hair traps in the northern grid and the northernmost hair traps in the southern grid was around 6 km on either side of the lake. Normal home range behavior could easily account for a lack of adult female bears showing up in both grids in an 18-week period (Hellgren 1988). Any subadult male dispersal likely had occurred prior to sampling initiated in early July (Bunnell and Tait 1985), but it is doubtful that subadult bears had established home ranges by the time sampling was initiated. It is not clear why an adult male was not detected in more than one trapping grid during this time period. Perhaps I failed to detect bears moving between grids or between a grid and the roadside wire due to DNA sample failures or related problems. Some hair samples failed to yield bear identity during the PCR process and some hair samples collected had too few roots for reliable analysis. I doubt that Lake Drummond acts as a barrier to bear movement within the refuge. Also, it is possible that a bear in one area simply failed to visit a hair trap and leave a hair sample in the second area. If more traps had been erected in both grids, individual bears would have been detected in both grids.

The sex ratio of bears detected by hair trapping appeared to be equal. Because males (especially young males) tend to travel more than females (Kemp 1976, Bunnell and Tait 1985), it would seem reasonable to expect a higher proportion of males to be sampled. The 18-week sampling period began in July, which is within the breeding period for black bears, and it is likely that subadult bears were still moving to find unoccupied home ranges.

Trapping Success and Recapture Rates

Only the northern grid had hair traps that failed to capture bear hair during the sampling period. Many factors could influence whether bears visited a particular hair trap, including established home range patterns, distribution of alternative food sources, agonistic behavior from other bears, human disturbance, or any number of unknown reasons. It is possible that bears investigated these traps, but did not enter and leave a hair sample. I made an effort to build and maintain traps to prevent bears from entering the trap without leaving a hair sample.

In the northern grid, half (5 of 10) of the empty hair traps were clustered in one area, roughly 13 km^2 (Fig. 1-2). It is difficult to explain why no bears visited 5 hair traps in such a large area. Presumably, this 13km^2 area was part of the home range of at least one bear. The same bait was used in this area as in other portions of the northern grid. Perhaps some unrecognized microhabitat feature discouraged bears from using this area (e.g. deep water, lack of preferred foods, etc.). Hellgren (1988) detected avoidance of certain habitat assemblages by bears of both sexes during different seasons. He hypothesized that this avoidance was due to food availability in other, hence preferred, habitats. The other empty hair traps were scattered in various areas of the northern grid.

Because I had 2 trap grids, I wanted to determine whether trapping success or bear visitation rates were affected by the use of a food reward (pastries) in one grid versus a scent lure only (raspberry extract) in the other. One major assumption of this test would be equal bear density and rate of movement in each grid. Bear density was estimated for the north and south grids separately. Bear density appeared to be lower in the northern grid than in the southern grid (see Results – density estimation). The number of capture events in each grid was significantly different, but the capture rates and the recapture rates between grids were not. If density was equal between the 2 grids, then perhaps bears were attracted to hair traps in the southern grid more often than in the northern grid due to the scent lure being attractive to more bears than pastries. Both the scent lure-baited trap sites and the pastry-baited sites had a visual stimulus associated with them. The pastries used in the food reward area were hung in red, plastic mesh onion bags several feet off the ground. The scent lure used in the southern grid was poured on white cotton rags and also suspended several feet off the ground. Langer (2006) tested visitation rates of hair traps baited with tangible food rewards (cups of peanut butter and honey) compared to traps baited with peanut butter-based scent lure and collected more bear hair samples from traps baited with the tangible bait than the scent lure bait. If density varies in different portions of the Great Dismal Swamp ecosystem, then testing the attractiveness of food reward versus scent lure only will be difficult to determine in different test areas. Unfortunately, I had only one field season rather than 2 to test if visitation rates were affected by choice of bait.

Population Estimation

I considered the population to be closed geographically (White et al. 1982). The refuge is surrounded by land under agricultural or urban use, effectively making it an island of suitable bear habitat in a sea of development. However, the hair trap corral grids did not cover the entire refuge and bears with home ranges on the periphery of the trap grids could come and go, potentially inflating the population density estimate and violating the geographic closure assumption. Thus, to estimate density (bears/km^2) and ensure geographic closure, I added a strip of area around the trap grid half the diameter of an average adult female home range (Dice 1938) then divided the population estimate by the area of the trap grid plus the area of the boundary strip (i.e., effective trapping area). I used home range data obtained from female bears captured by Hellgren (1988) to determine strip width. Median home range size for these bears was 10.4 km^2 and was assumed to be circular. I divided the diameter of this circle by 2 (3.6 km/2), resulting in a buffer strip 1.8 km wide. I also used the Mean Maximum Distance Moved method (Wilson and Anderson 1985) to estimate the buffer zone width from bears that visited more than 1 hair trap during the sampling period and bears that visited one hair trap multiple times.

Demographic closure may not have been complete because of immigration and emigration. However, Program MARK (White and Burnham 1999) is robust to the violation of demographic closure. I assumed that immigration and emigration were minimal and that the rates were equal. No births occurred because of the time of year, and most mortality in this population is due to fall hunting outside the refuge boundaries (Hellgren 1988, D. Martin Virginia Department of Game and Inland Fisheries, pers. comm.).

The second assumption was met because genetic markers cannot be lost. Eartags, tattoos, paint splotches, and other artificial marking techniques all have the potential to be lost, even during relatively short time periods. Other artificial markers normally require capture of the animal as well. Two potential problems may arise under this assumption when using DNA markers. First, DNA deteriorates due to cellular enzymatic action, heat, and ultraviolet radiation, leading to the potential to miss marked bears in 1 or more trapping sessions. This problem was minimized by collecting and properly storing samples every 7-10 days. Secondly, DNA markers can be misread by an inexperienced observer (perhaps leading to designating 2 unique individuals as one or designating 2 samples from 1 bear as 2 separate individuals). This problem was minimized by using an automated process. Lab personnel manually checked samples where multilocus genotypes differed by just one marker.

To minimize problems arising from violation of the assumption that all markers were correctly noted, I took several data collection precautions. Protocols for handling and labeling samples were written and all personnel involved in fieldwork were trained in the collection and recording of data. Information written on envelopes and data sheets in the field was double-checked by at least 2 people. Precautions were taken during the laboratory processing stage to ensure that samples were labeled consistently and correctly.

To ensure that all sampling effort was sufficient to capture all individuals in the area sampled, I spaced the hair traps as uniformly as possible and sampled the grids for 18 weeks. It is likely that the hair traps failed to collect samples from an unknown proportion of bears in the population, especially cubs. Also, I replaced bait/lure weekly to maintain odor and flavor attractiveness.

I estimated population size and density for each grid separately. Using the lowest density in the northern grid (0.18 bears/km^2) and the highest density estimate in the southern grid (0.56 bears/km^2) yielded an ecosystem estimate of 99-308 bears. The lower density estimate of 0.18 bears/km^2 resulted in an ecosystem estimate of 99 bears. Eighty-five individuals were identified on the 2 hair trap grids and another 6 bears were identified along U.S. Highway 17 (91 total individuals identified using genetic markers). New individual animals were being detected through the end of the sampling effort. It is unlikely that I captured every bear in the ecosystem. Therefore, the low density estimate in the northern grid is likely over-conservative. I feel that the higher density estimate produced in the southern grid is more indicative of density in the ecosystem as a whole. Therefore, I feel that the ecosystem population estimate of 308 bears is likely close to the true population. The population estimate generated by Program MARK for this study is similar to results obtained by Hellgren (1988). His population estimate ranged from 262-377 bears using an average of both open and closed population models. Hellgren (1988) used an averaged bear density (0.47 bears/km^2) derived from a combination of open and closed population models for the lower end of his population estimate. He used an average density (0.68 bears/km^2) obtained from bears in an intensively trapped section of the area south of Lake Drummond for the upper end of his population range. He considered this density to be representative of the ecosystem as a whole. The intensively trapped area corresponds roughly to the southern trap grid in my study. Tredick (2005) reported an average density of 0.59 bears/km^2. This density applied to the 550 km^2 ecosystem would yield a population estimate of 324 bears.

Similar population estimates generated by Hellgren (1988), Tredick (2005), and this study suggest a demographically stable bear population in the Great Dismal Swamp ecosystem over a 20-year period. This is not surprising given the abundance of natural and human food sources in or near the refuge and relatively little human-caused mortality within the refuge borders (D. Martin, VDGIF, pers. comm.).

Density Estimation

Due to logistical constraints, I was unable to place hair traps throughout the entire Dismal Swamp ecosystem. Instead, we chose 2 areas of contiguous bear habitat with relatively easy access to deploy hair traps in rectangular grids. Bear density may vary from one portion of the refuge to another to an unknown degree. The effective sampling area was 348 km^2 (both grids plus a 1.8-km buffer around each grid) and the ecosystem area is 550km^2. Thus, the effective sampling area included roughly 63% of the entire ecosystem area. Density for any given area within the ecosystem likely varies based on seasonal food availability (both natural and human sources). Fixed trapping grids that miss some important habitat type or traditional feeding area or that over-represent another may yield biased density estimates for such a wide-ranging species. For example, blackberries (*Rubus* spp.) provide forage during a very narrow window of time during early summer. Areas of early successional stage habitat types where blackberries thrive occur throughout the refuge, but are more abundant in some areas than others. Hair traps in close proximity to berry patches are likely to receive high bear use from late June to mid-July while hair traps in areas farther from blackberry foraging areas may see a reduction in visitation during the same time period. Anecdotally, I saw this occur while initially building hair traps during the blackberry season in the first year of the study. Bears regularly used trails I cut through dense, productive blackberry patches and left hair samples on unbaited hair traps along these trails. Hair traps built in areas away from blackberry patches were not visited by bears until baited. In the northern grid, 5 hair traps failed to collect even 1 hair sample during the entire 18-week sampling period. This most likely was due to a lack of preferred food occurring in the vicinity of this group of hair traps during my sampling period. Chi-square analysis of density between the 2 grids indicates that density varied (40.6 bears/100km^2-southern grid, 22 bears/100km^2–northern grid) and that this difference was significant ($X^2 = 5.527$, 1 d. f., $P = 0.018$). Hellgren (1988) detected a lower density of bears north of Lake Drummond compared to the area south of Lake Drummond, but he assumed this difference resulted from higher road density in the south which led to easier trapping conditions.

Bears living on the edge of the trapping grids likely used habitat outside the grids as part of their home range, which would lead to an overestimate of density. In order to mitigate this, at least to some extent, I used the Dice (1938) method to place a buffer strip around the trap grids. The buffer strip I used (1.8 km) was generated from adult female home range data collected by Hellgren (1988). This method ignored males, which usually have much larger home ranges and therefore may necessitate a wider buffer strip.

In addition to the Dice (1938) method, I investigated an alternative method of determining the trapping area buffer strip. I calculated the mean maximum distance moved (Wilson and Anderson 1985) between hair traps by 20 bears (14 males, 3 female, 3 unknown) during the sampling period. The MMDM for all 20 bears was 2.79 km

(Table 1-2) and resulting in a buffer strip 1.4 km wide around the grids (2.79 X 0.5). For the Dice method, I used only female home range data collected by Hellgren (1988). The MMDM method used a preponderance of data from males (14 males versus 3 female and 3 unknown), yet the buffer strip width was narrower. The maximum distances traveled by these males likely represents detection failure rather than a true maximum distance moved during the sampling period. Only one bear used for the MMDM method visited more than 2 hair traps during the sampling period. Tredick (2005) documented several radio-collared black bears (both male and female) not leaving hair samples at traps despite having traps in their home range. The nature of detecting animals at hair traps dictates that a particular animal visit hair traps scattered across the landscape. If the animal fails to visit a particular trap, then it obviously can't leave a sample there even if it was in the general area. In contrast, telemetry data are generally collected at least daily over the course of several months, thus providing more data points for animals with transmitters and better illustrating maximum distance moved between 2 points. To be more conservative, I used the buffer width of 1.8 km instead of 1.4 km. Regardless, it is likely that the trapping grids with boundary strips failed to fully enclose the home ranges of at least some adult males, and possibly even some females. Hellgren (1988) collected radio telemetry data from males that indicated that the home range of some bears included most of the refuge.

Since the lower density estimate of the northern grid (0.18 bears/km^2) yields an ecosystem population estimate lower than the estimate from both the northern and southern grids combined, it is likely overly conservative. Using the same trapping grids and methods in a later study, Tredick (2005) reported an average density of approximately 0.60 bears/km^2. Hellgren (1988) reported a density range between 0.47-0.68 bears/km^2 in the Dismal Swamp ecosystem. He used multiple open and closed population models to generate the lower density and used a higher population estimate south of Lake Drummond compared to the area north of Lake Drummond to generate the density estimate on the upper end of the range. Based on the data collected in this study, the density estimates for the area corresponding to the northern grid appear to be lower than those estimated by Hellgren (1988) or Tredick (2005). I believe that the higher end of the density range of the southern grid (0.56 bears/km^2) generated from this study is likely more representative of the ecosystem as a whole. Therefore, I used it to generate a population estimate of 308 bears.

MANAGEMENT IMPLICATIONS

Recreational hunting has been proposed by the Virginia Department of Game and Inland Fisheries as a way to reduce nuisance bear complaints off refuge and provide sport hunting opportunity on the refuge. In the last 2 years, limited bear hunts have taken place on the refuge, but only 1 bear was harvested in 2007 and it was under the legal weight limit. Continued bear hunting on the refuge likely can be maintained, even with higher hunter success. Some reduction in the population may be warranted, especially for adult males, to reduce agricultural crop damage outside the refuge boundaries. However, staff from both the refuge and VDGIF should work together to ensure that harvest seasons and methods of take favor males and protect females as much as is practical.

There are concerns that the Great Dismal Swamp bear population is at risk due to its isolation from other bear populations in the region (Tredick 2005). The ideal situation would be to provide habitat connectivity between the Dismal Swamp and areas to the south such as Pocosin Lakes and Alligator River National Wildlife Refuges. However, this is impractical due to the expense of obtaining connecting land parcels and the presence of many land-owning entities who would have to be convinced to sell their land along a select corridor between these bear population centers.

Proctor et al. (2004) suggested that probability of extinction for small, isolated grizzly bear (*Ursus arctos*) populations in the northern Rocky Mountains could be reduced through re-establishment or enhancement of natural population interchange. In an attempt to enhance one of these populations, Kasworm et al. (2006) translocated 4 subadult female grizzly bears into the Cabinet-Yaak grizzly bear population of northern Montana over a period of 4 years. Three of 4 transplanted bears remained in the area and at least one reproduced, indicating some measure of success. Natural population interchange is preferable, but is difficult to achieve (Proctor 2004). I recommend artificial population interchange between the Dismal Swamp and populations to the south to mitigate for the low level of natural migration if evidence of isolation, such as reduced genetic diversity, becomes evident. Bears in the Great Dismal Swamp and Pocosin Lakes/Alligator River complex could be captured and transported to their respective release sites by state or federal wildlife agencies. Exchanging bears between refuges could increase gene flow and would be more practical than establishing natural travel corridors given the logistical difficulty and expense of purchasing suitable land and overcoming obstacles such as major rivers and transportation corridors. This method would require multiple agency (both state and federal) cooperation over a long time frame.

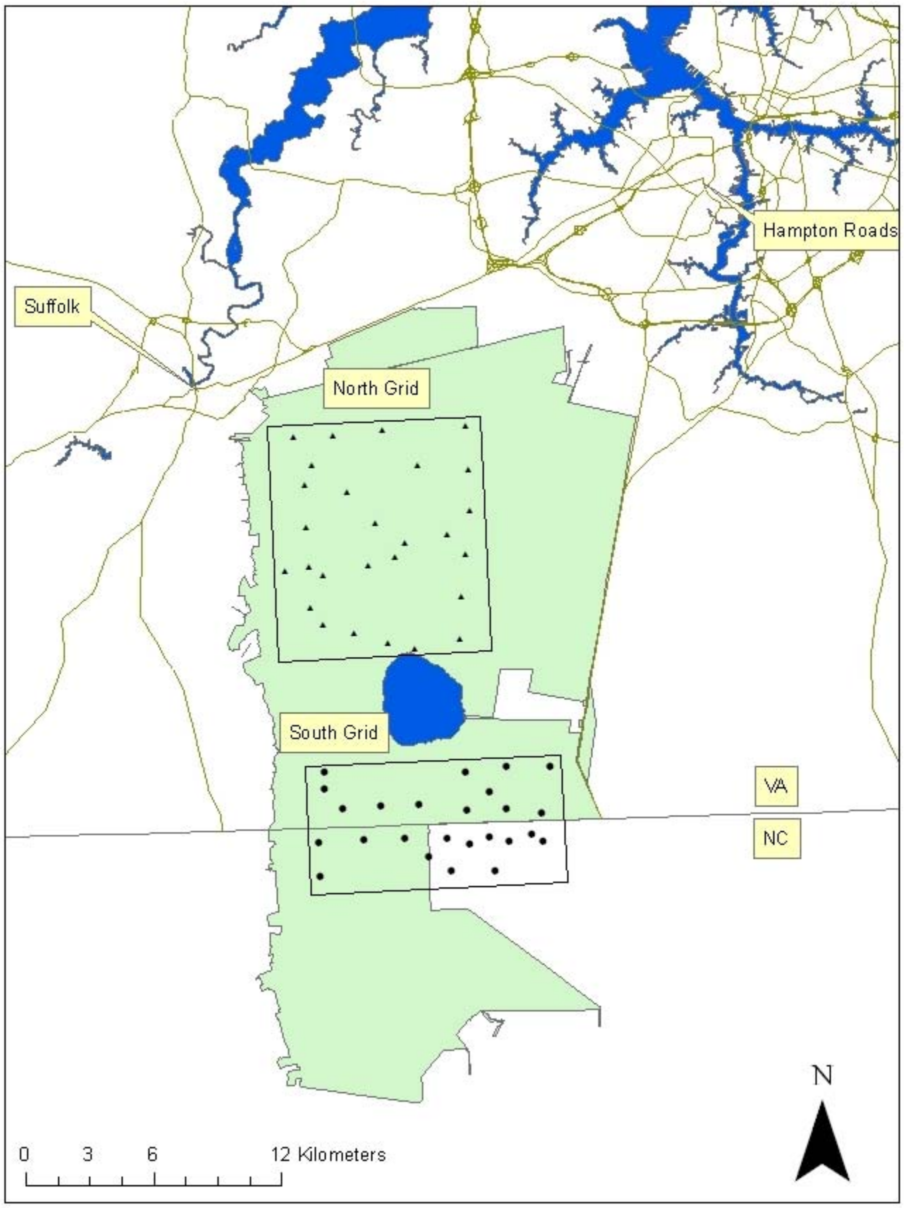
Table 1-1. Rank of population models in Program MARK for bears on Great Dismal Swamp National Wildlife Refuge, VA/NC 2000-2002.

Model	AICc	Delta AICc	AICc Weight	Model Likelihood	# Parameters	Deviance
M(bh)}	202.583	0.00	0.875	1.0000	6	182.088
{M(th)}	207.564	4.98	0.072	0.0829	22	154.453
{M(tbh)}	209.306	6.72	0.03	0.0347	23	154.133
{M(h)}	210.109	7.53	0.02	0.0232	5	191.629
{M(b)}	215.423	12.84	0.001	0.0016	4	198.957
{M(.)}	222.482	19.89	0.000	0.0000	3	208.026
{M(t)}	222.584	20.00	0.00	0.0000	20	173.587
{M(tb)}	224.001	21.42	0.00	0.0000	21	172.948

Table 1-2. Mean maximum distance moved (MMDM) between hair traps by bears (N=20) during the 18-week sampling period on Great Dismal Swamp National Wildlife Refuge, VA/NC 2000-2002.

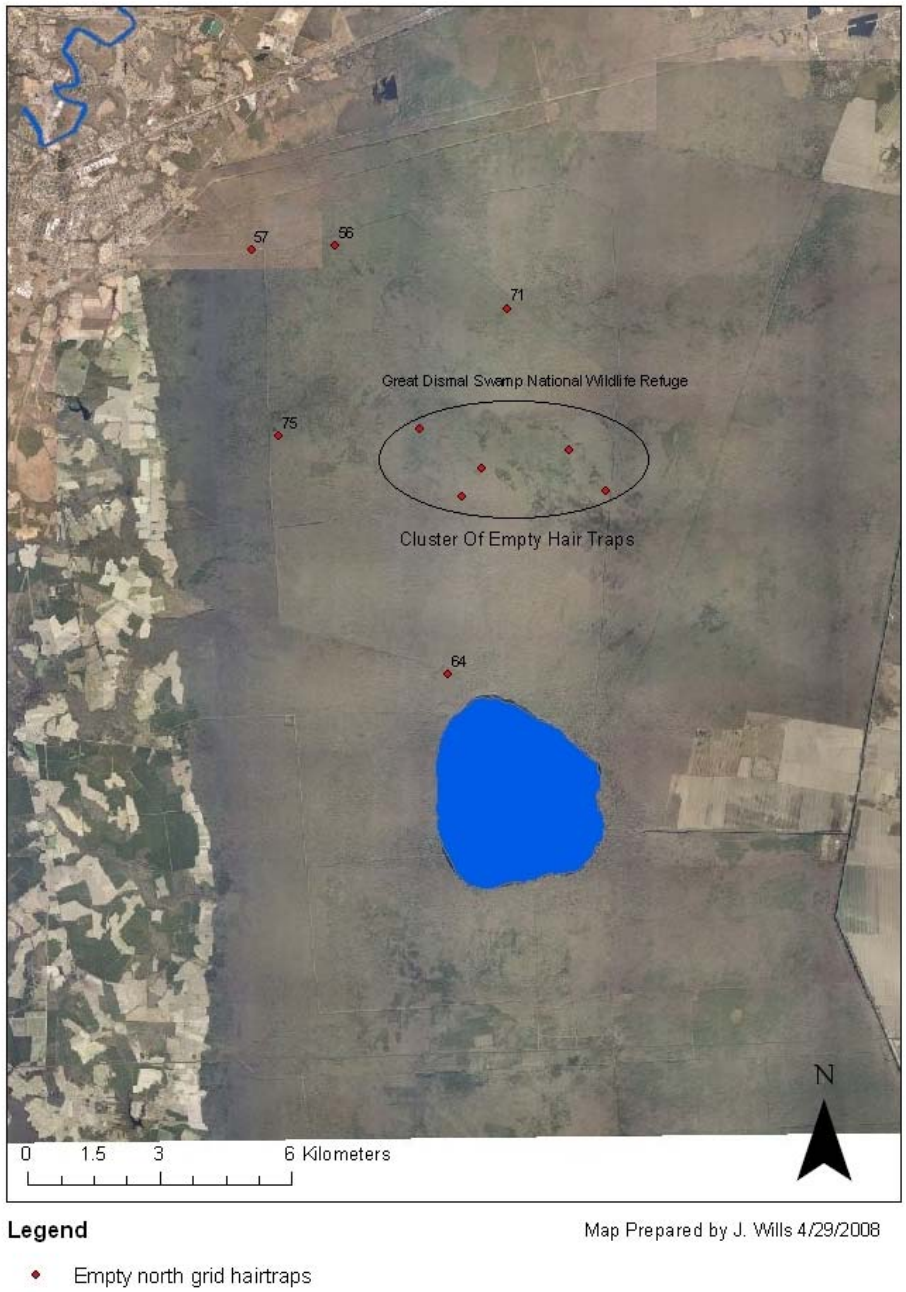
Bear #	Sex	MMDM (km)	Grid
3	M	5.21	S
4	F	0	S
5	M	7.54	S
6	U	0	S
7	U	2.08	S
10	M	2.43	S
11	U	2	S
12	M	1.24	S
16	M	4.01	S
17	M	2.77	S
19	M	0	S
20	M	0	S
21	M	0.85	S
22	M	0.85	S
25	F	0	N
30	M	2.38	N
34	M	3.97	N
36	F	11.46	N
37	M	7.17	N
38	M	1.84	N

Figure 1-1. Distribution of hair traps on Great Dismal Swamp National Wildlife Refuge, VA/NC, 2001-2002.



Map Prepared by J. Wills 4/29/2008

Figure 1-2. Hair traps on the northern grid that failed to collect hair samples in Great Dismal Swamp National Wildlife Refuge, VA/NC, 2001-2002.



CHAPTER 2: ROAD CROSSING PATTERNS OF BLACK BEARS ALONG THE EASTERN BOUNDARY OF GREAT DISMAL SWAMP NATIONAL WILDLIFE REFUGE

INTRODUCTION

There are concerns that bears in the Great Dismal Swamp may become genetically isolated as forested strips leading from the swamp are developed or bisected by high-volume roadways. Bear/vehicle collisions also may increase in frequency and result in a negative impact on the bear population, human injury, and increased damage to human property (L. Culp, U. S. Fish and Wildlife Service, pers. comm.).

Underpasses may facilitate the movement of bears both into and out of the refuge and allow for the exchange of genetic material between bears in the refuge and populations southward. Also, underpasses may reduce the likelihood of bear/vehicle collisions. Underpass placement should be based on hydrologic needs as well as seasonal and spatial use patterns of bears and other critical species (Leeson 1996). Information on travel corridor use by bears currently is unavailable for the Great Dismal Swamp ecosystem.

The Virginia Department of Transportation (VDOT) recently completed a project that modified a portion of U.S. Highway 17 on the eastern edge of the Great Dismal Swamp National Wildlife refuge (Fig. 2 – General Introduction). The road was modified from the Dominion Boulevard intersection in the city of Chesapeake south to the Virginia/North Carolina state line (approximately 19 km). The roadbed was moved approximately one kilometer east of its present position and widened from 2 lanes to 4 lanes with a median. A bridge elevated 2.4 meters above the ground and 300 meters long with barrier fencing 1.6 kilometers long on each side of the road to the north and south and 2 concrete culverts were erected in this area during 2003-2005 as part of the highway expansion project. All of these structures were designed to allow water drainage and function as wildlife crossings.

U. S. Highway 17 crosses the paleo-drainage of the Northwest River. This river corridor is one of the last forested corridors connecting the refuge to other patches of bear habitat. Also, it was an area of proposed mitigation for the highway modification project.

Monitoring patterns of road crossing by animals often is difficult. In Tennessee and North Carolina, black bears were less likely to cross roadways with higher traffic volume than roadways with lower traffic volume (Brody and Pelton 1989). In Montana, black bears avoided areas adjacent to roads (Kasworm and Manley 1989). Various methods, including radio telemetry, bear/vehicle collision data, known migration paths, attractive landscape features, analysis of landscape-scale features, and track counts, have been used to determine where animals cross roadways (Singer and Doherty 1985, Foster and Humphrey 1995, Sheick and Jones 1999). Often, more than one method is used to determine road-crossing patterns.

Corridors used by individual animals may decrease the risk of population extinction if they aid in dispersal (Beier 1993). Installing highway underpasses is a common method to minimize blockage of animal corridors by roads. An underpass is a portion of the roadway that is situated above ground level that may allow animals to cross the road, usually built over a drainage. Installation of underpasses during initial construction is far easier and less expensive than retrofitting them to existing roads (Leeson 1996). Highway underpasses designed for use by wildlife have been implemented for passage of mountain goats (*Oreamnos americanus*) (Singer and Doherty 1985), Florida panthers (*Felis concolor*) and black bears (Foster and Humphrey 1995), and various large carnivores and ungulates (Clevenger 1998). This project was designed to monitor the section of highway where the proposed bridge system was located to determine pre-construction patterns of bear travel across the roadway.

METHODS

Establishing linear barbed wire hair snare

To determine bear crossing areas along a section of Highway 17 adjacent to the GDSNWR, I placed a single strand of barbed wire (15.5 gauge, double strand, 4 barbs/cluster) on the western side of Highway 17 at least 3 meters from the edge of the road and roughly 5 meters from the eastern edge of the Dismal Swamp Canal. The wire, designated as the roadside wire, was attached to wooden and metal anchor posts beginning at the intersection of Cornland Road and Highway 17, and continued southward for 2.3 kilometers (Fig. 2-2). The wire spanned the width of the Northwest River, the site proposed for a bridge and other wildlife crossings. It was placed approximately 50 centimeters above ground level along its entire length in order to capture hair samples from bears crossing this stretch of highway. An effort was made to follow dips in the terrain along the highway caused by irregularities of the canal bank.

Wire Geo-Referencing

The roadside wire was geo-referenced by placing sequentially numbered aluminum tags and flagging on trees or anchor posts used as reference markers. I used a Global Positioning System (GPS) receiver to obtain UTM coordinates for the reference markers (Fig. 2-2). I ensured that all GPS locations were accurate within 5 meters by allowing the GPS unit enough time to average the location or by obtaining a location when satellite availability or weather conditions were more conducive to an accurate reading.

The roadside wire was regarded as having 28 sections. Section 1 was on the north end of the wire and section 28 was on the southern terminus of the monitoring wire. The distance between reference markers varied, but was approximately 80 meters. I denoted the location of all samples collected from the strands of wire in terms of the section from which they were collected.

Data Collection

I checked the 2.3-kilometer long strand of wire every 7 days from August 2001 through November 2001. From December 2001 through May 2002 it was checked every two weeks. The location of all bear hair samples was recorded and samples with 5 or more hairs were collected. Hair from a 4-barb cluster was treated as one sample, even if multiple, consecutive barbs had hair on them (see Chapter One Methods-Data Collection). Genetic analysis was performed on these samples as in Chapter One (Methods-Microsatellite Data Analysis). Each bear was assigned an identification number from the samples collected for population estimates and road crossings.

RESULTS

I collected 23 bear hair samples from the roadside barbed wire strand between August 2001 and May 2002. Twelve of 23 samples were deposited on barbs between sections 6 and 7 (Figure 2-2). Fourteen of 23 samples yielded individual identification results using genetic microsatellite analysis (Methods - Microsatellite Data Analysis Chapter 1). The other samples either had too few hair strands with roots to undergo analysis or failed to yield results. Hair samples from other species were detected (e.g., white tailed deer *Odocoileus virginianus*, gray squirrel *Sciurus carolinensis*, and raccoon *Procyon lotor*), but not recorded or collected.

Six individual bears (4 males, 1 female, 1 unknown) were identified from the 14 samples identified to individual bears. Five of 6 bears identified deposited hair on roadside wire sections 6 or 7 (approximately 171 meters in distance). One bear crossed at sections 2 and 7. One bear crossed at sections 5 and 6 and one crossed only at section 25 during this time period (Table 2-1). All crossings occurred in early spring (March-May) or in August.

The 6 identified bears crossed at least 11 times along the 2.3-kilometer roadside wire strand during the sampling period (Table 2-1). One bear crossed 3 times, 3 crossed 2 times, and 2 bears were detected crossing the wire once each.

DISCUSSION

Monitoring bear crossings along linear boundaries using barbed wire has potential to identify spatial and temporal crossing patterns, as well as providing information on the number and sex of individuals and their sex if DNA analysis is performed on hair samples (Wills and Vaughan 2002). Other techniques, such as using remote-triggered cameras, radio telemetry, historic road-kill information, known migration paths, attractive landscape features, analysis of landscape-scale features, and track counts (Singer and Doherty 1985, Foster and Humphrey 1995, Sheick and Jones 1999), may not provide the same kinds of data or may provide similar data at a much higher cost.

For example, remote-triggered cameras have a limited range of detection (normally less than 30 meters) and will not detect a bear crossing in another location outside the range of detection. Also, a photograph of a bear will not provide individual identification or sex determination unless special ear tags or other markers unique to an individual have been placed on the animal. Unique identifying ear tags have been placed on bears with subsequent detection by remote-triggered cameras (Klenzendorf 2002), but this normally requires capture and sedation of the animal and the tag can potentially be lost. Snagging hair samples from a bear with barbed wire requires no direct handling of the animal and genetic markers cannot be lost (Woods et al. 1996).

Radio telemetry may provide road crossing information for large carnivores, depending on the scale and frequency of monitoring (Paquet and Callaghan 1996, Gibeau and Herrero 1998). This technique has several disadvantages compared to the barbed wire technique. Global positioning system (GPS) collars that are monitored on a short time-scale (e.g., one location per 2 hours) may detect road crossing by bears, but not if satellite coverage is poor due to few satellites in view, inclement weather, dense tree canopy cover, or rough terrain (Gerlach and Jasumbach 1989, Rempel et al. 1995, and Gamo et al. 2000). Also, the bear must be trapped, sedated, and fitted with a transmitter. Handling individual bears is expensive, dangerous, and time consuming. Often it is difficult or too expensive to capture a sufficient number of bears to enable reasonable detection of road crossing patterns. For example, if telemetry is used to monitor road crossings, only animals with radio transmitters are included in the analysis. Often, the sample size of animals with transmitters is a small percentage of the total number of animals in such a study. As with ear tags, collars can malfunction or be slipped off by the bear, eliminating future detection of that individual. If properly installed and maintained, barbed wire is operating 24 hours/day, 7 days a week.

Barbed wire monitoring can be used in conjunction with radio telemetry or camera studies if the project requires home range, survival, habitat use, or other data collection. If a pattern is detected, effort can be concentrated in the area of interest using different monitoring techniques. For example, short stretches of barbed wire could be erected to obtain hair samples, which could be used to determine the number of individuals crossing the road (DNA fingerprinting). Additionally, infrared-triggered cameras could be placed in these areas to determine direction of travel and temporal/seasonal patterns of road crossing.

Based on the distribution of hair samples along the roadside wire, most bears during this sampling period appeared to cross the wire on the northern edge of the paleo-Northwest River corridor (Sections 1-7). In addition to the samples that yielded individual identification of bears, 4 hair samples that failed to yield individual identification were collected between sections 1 through 5. Other hair sample locations were scattered. Two hair samples came from section 11 and 1 sample came from section 27. These samples also failed to yield a positive genetic identification.

MANAGEMENT IMPLICATIONS

Ideally, the entire length of U.S. Highway 17 would allow safe crossing conditions for bears and other terrestrial wildlife. There are 2 ways of accomplishing this. Underpasses could be installed at regular intervals (e.g. every kilometer, every 2 kilometers, etc.) or the entire highway could be elevated high enough (> 2m) to allow bear passage. However this is not financially practical. The bridge system that was constructed on Highway 17 cost $4.7 million alone (S. E. Perry, Virginia Department of Transportation, pers. comm.). The entire project as constructed cost roughly $42 million. If the entire 17 km of highway had been constructed as an elevated bridge, the cost would have exceeded $294 million (S. E. Perry, Virginia Department of Transportation). Based on the limited data collected on this project, the bridge span appears to be in an area frequently used by black bears to cross the highway. Post construction monitoring over the next several years may indicate whether the crossings are truly successful at reducing bear/vehicle collisions.

This genetic technique appears to be useful for monitoring road crossings by bears in the Great Dismal Swamp and may have application for other bear populations. It may even be applicable to other mammalian species. Whether the road-crossing events observed during this study represent dispersal into or out of the Great Dismal Swamp or if they are part of individual home range movement is unknown. Movement of individuals could be monitored over time by matching DNA samples collected along roadsides to samples collected in future research projects, long-term monitoring projects, capture of nuisance animals, animals killed in vehicle collisions, depredation kills, or hunter-harvested animals. Such a system would require wildlife management agencies to maintain a long-term DNA database where an individual could be tracked over time. Since DNA samples could come from so many sources, this method would yield information useful to wildlife managers in the long run. It would allow them to identify areas of high vehicle collision risk, dispersal patterns, approximate distances traveled by nuisance bears, repatriation of habitat where bears had been extirpated, and other unforeseen applications.

Table 2-1. Road crossings by bears along US Highway 17 on the eastern border of Great Dismal Swamp NWR, VA 2001-2002.

Bear ID #	Sex	Section crossed	Period of Crossing
1	Male	6	Aug 4-8, 2001
1	Male	6	May 1-15, 2002
2	Male	2	Aug 8-15, 2001
2	Male	2	Aug 22-29, 2001
2	Male	7	April 17-May 1, 2002
42	Male	6	March 1-15, 2002
42	Male	5	April 18-May 1, 2002
43	Male	7	March 15-29, 2002
43	Male	7	April 18-May1, 2002
44	Female	25	Aug 29-Sep 12, 2001
91	Unknown	7	March 15-29, 2002

Figure 2-1. Location of roadside wire on the east side of Great Dismal Swamp National Wildlife Refuge, VA/NC, 2000-2002.

Map Prepared by J. Wills 4/29/2008

CHAPTER 3: DISTRIBUTION OF BLACK BEAR/VEHICLE COLLISIONS: AN INDICATOR OF POTENTIAL TRAVEL CORRIDORS

INTRODUCTION

Documentation of wildlife/vehicle collisions is scant, and often occurs only during the course of wildlife research projects or in response to mitigation efforts for highway expansion projects (Reed et al. 1975, Hellgren and Vaughan 1989, Beier 1995, Foster and Humphrey 1995, Gibeau and Heuer 1996, Hewitt et al. 1998, Gunther and Biel 1999, Scheick and Jones 1999, and Waller and Servheen 1999). However, identifying patterns in the distribution of bear/vehicle collisions may help identify bear travel patterns. Multiple bear/vehicle collisions along the same stretch of highway or railroad track might be indicative of a heavily used bear trail.

Documenting the occurrence of bear/vehicle collisions is difficult for a number of reasons. For instance, vehicle operators do not always report the incident to authorities or, in the case of tractor-trailers or trains, even stop after the collision. For collisions occurring at night or in poor weather conditions, the driver may not even know that the animal was a bear if it managed to run into the forest before being recovered. Some people fail to report bear mortalities because they want to keep the meat, hide, skull, or claws, and they feel that the authorities would confiscate the bear. Some people may not realize that reporting the incident is of interest to state and federal wildlife management or transportation agencies. Additionally, it is often logistically difficult for transportation, law enforcement, wildlife, or animal control officials to investigate vehicle-related bear mortalities because of poor directions to the site, a time lag between the collision and reporting date, or a lack of available personnel.

METHODS

Bear/vehicle Collision Reports

I obtained reports of bear/vehicle collisions from wildlife biologists, law enforcement officers, transportation officials, animal control officers, and private citizens. I made telephone contact with all Virginia Department of Game and Inland Fisheries game wardens, State Highway Patrol offices, Virginia Department of Transportation (VDOT) offices, and animal control offices in the cities of Suffolk and Chesapeake. I requested the above-mentioned agencies contact me when reports of bear/vehicle collisions occurred within their jurisdictions. I made myself available at all hours by giving both day and night contact phone numbers. Additionally, I supplied Virginia Department of Game and Inland Fisheries (VDGIF) wildlife biologists with mortality protocol information in cases where I was unable to investigate a bear/vehicle collision directly. I made regular inquiries to these individuals to gather data they had collected.

Data Collection

Data recorded for bear/vehicle collisions included time and date, Global Positioning System GPS location and/or reference to geographic features, and sex of the bear. I plotted kill locations on maps to help define travel routes and indicate portions of roadways where future mitigation efforts could be implemented. When possible, I searched roadsides and train tracks bordering the refuge for unreported carcasses.

RESULTS

I received reports of 6 bear/vehicle collisions between 13 June 2000 and 14 May 2002. In addition, I found 1 bear skeleton from a previous collision. Three other bears were reportedly killed or at least injured during this time period, but I was unable to confirm these collisions.

I plotted all known mortalities on a map (Fig. 3-1). One bear was struck and killed by a train on the northeastern edge of the refuge. Two bears were killed on U.S. Highway 58 on the north end of the refuge near Hampton Airport. One bear was killed on U.S. Highway 58 west of the city of Suffolk. In July 2001, project technicians discovered a bear skeleton next to U.S. Highway 17 while searching for a bear that reportedly had been killed in the vicinity the day before. The skeleton was situated near a well-used wildlife trail that crossed U.S. Highway 17. It appears to have been killed at least 1 year before its discovery. The skeleton was found along a section of road where barbed wire was used to monitor bear crossings; this section had the highest number of samples collected during the first year of monitoring (see Chapter 2-Results). The bear/vehicle collision report that led to this discovery was never confirmed. Two other bears were killed in a 2-day period in May 2002 on U.S. Highway 17 less than 1 kilometer apart.

Due to small sample size and a short 2-year period, I was unable to detect a strong seasonal pattern. I pooled data for mortalities where the date was known or at least estimated to have been within 2 weeks of being discovered. Two of the 6 mortalities occurred in May, 2 in June, 1 in July, and 1 in September.

Sex was determined by visual observation for 5 of 7 road-killed bears examined during this study; 4 were males and 1 was a female. One bear was too badly damaged by a train and decomposed to determine sex and the other was a skeleton.

DISCUSSION

Bear/vehicle collision data for the road system around the Dismal Swamp is incomplete, but available data suggest that a minimum of 4 to 5 bears are killed on the periphery of the swamp each year. The actual number of bears that die from collisions is likely higher. From June 2000 to May 2002, I documented 6 confirmed bear mortalities caused by vehicle collisions in this area. Similar results were reported by Hellgren (1988) and in the VDGIF road kill database (D. Martin, VDGIF, pers. comm.). Hellgren (1988) reported 6 road-killed males and 2 females from April 1984 to August 1986. Five males and 1 female were reported in the VDGIF road kill database from July 1996 to December 1998.

Although data on road kill locations is sparse, I identified at least 2 areas where bears were regularly hit by vehicles. The first area was on the north edge of the refuge where a multi-lane, high traffic volume roadway (U.S. Highways 58 and 460) and railroad tracks are located. This transportation system is one of the major arteries into the heavily urbanized Hampton Roads area. The average annual daily traffic volume for Highways 58/460 was 70,000 vehicles in 2006. Based on personal observations and conversations with local game wardens, wildlife biologists, and citizens, bears appear to move between the Great Dismal Swamp NWR and a 20-km^2 area of forested habitat north of U.S. Highway 58. The other area identified as having frequent bear/vehicle collisions was along Highway 17 on the eastern boundary of the refuge located just south of the Cornland Road/Highway 17 intersection. The Northwest River corridor crosses Highway 17 just south of Cornland Road. One bear skeleton was found there and one other unverified report of a bear/vehicle collision was from this area. Also, this was the area where multiple bears crossed the barbed wire used to monitor road crossings by bears (see Chapter 2-Results). I found one well-used animal trail leading from the Dismal Swamp Canal across Highway 17 in this area. All of these factors combined suggest a potential bear travel corridor and an area with a high risk of bear vehicle collisions.

Data from Hellgren (1988), the VDGIF road kill database (D. Martin, VDGIF, pers. comm.), and this study account for 21 bear mortalities from vehicle collisions. Mortalities occurred in all months except February, March, and April, which coincides with the denning period of black bears in the Dismal Swamp and other parts of the southeastern United States (Hamilton and Marchinton 1980, Smith 1986, Hellgren and Vaughan 1989, Weaver and Pelton 1994).

In addition, sex was determined for 19 of the 21 bears examined; 15 were males, 4 were females, and 2 were undetermined. Age data for these bears was lacking, making it difficult to determine whether the majority of road killed bears were subadult or adult animals.

MANAGEMENT IMPLICATIONS

Various sources indicate that the total number of bear deaths caused by vehicles near the Great Dismal Swamp NWR each year is higher than the number reported to authorities. (L. Culp, U. S. Fish and Wildlife Service, GDSNWR; T. Cherry and J. Pritchard, VDGIF; pers. comm.). Further work will be necessary to understand spatial/temporal patterns and frequency of bear/vehicle collisions on the periphery of the swamp. Other ways of expanding knowledge in this area must be developed. This could include a standardized reporting protocol, high-level, multi-agency prioritization, requests for public involvement, etc.

One area identified as a high risk collision area was on the north end of the refuge. Highway 58, a multiple lane, high traffic volume highway (average annual daily traffic volume = 70,000 vehicles) and a major freight railroad separate the refuge from a 20-km^2 patch of suitable bear habitat to the north. There are no underpasses usable by bears along this stretch of road, which forces bears determined to cross the highway to cross multiple lanes of traffic to get from one side of the road to the other, exposing them to a risk of being hit by a vehicle. Further study will be necessary to investigate effective mitigation strategies for this area. Perhaps private land in the area could be purchased by a land conservation organization such as The Nature Conservancy to preclude development and loss of bear habitat.

The other area identified as having a high risk of bear/vehicle collisions was on Highway 17 on the eastern side of the refuge in the vicinity of the Northwest River corridor. A 300-meter long bridge, 2.4 meters above the ground with barrier fencing 1.6 kilometers long on each side of the road to the north and south was erected in this area during 2003-2005 as a result of this project and as part of the highway expansion project. This may mitigate the risk of bear/vehicle collisions in this specific area. Future monitoring may indicate the wildlife underpass reduces collisions.

The effects of the current vehicle-caused mortality rate on population dynamics of the Great Dismal Swamp NWR bear population are unknown. The degree to which these mortalities negatively impact recruitment, dispersal, and gene flow must be studied more closely in order to make population management decisions. A wider highway system with increased traffic volume that causes an increase in bear mortality may have a serious impact on the ability of bears in this population to maintain genetic connectivity with other bear populations. Increased traffic volume and wider road systems also may negatively affect genetic connectivity by discouraging bears from crossing the roads at all (Brody and Pelton 1989).

In 2001, 2 bear/vehicle collisions in northern Virginia near Shenandoah National Park resulted in the death of 2 people (D. Kocka, VDGIF, pers. comm.). Three additional bear/vehicle collisions were reported on the same stretch of highway in the 6 months following the first collision involving a human fatality. An on-site examination revealed a well-worn trail used by bears to cross this highway. Any of the collisions that occurred in the Dismal Swamp area had the potential to result in human fatalities as well. From a human safety standpoint, this information highlights the need for road planners and wildlife managers to work together to reduce the risk of wildlife/vehicle collisions where possible.

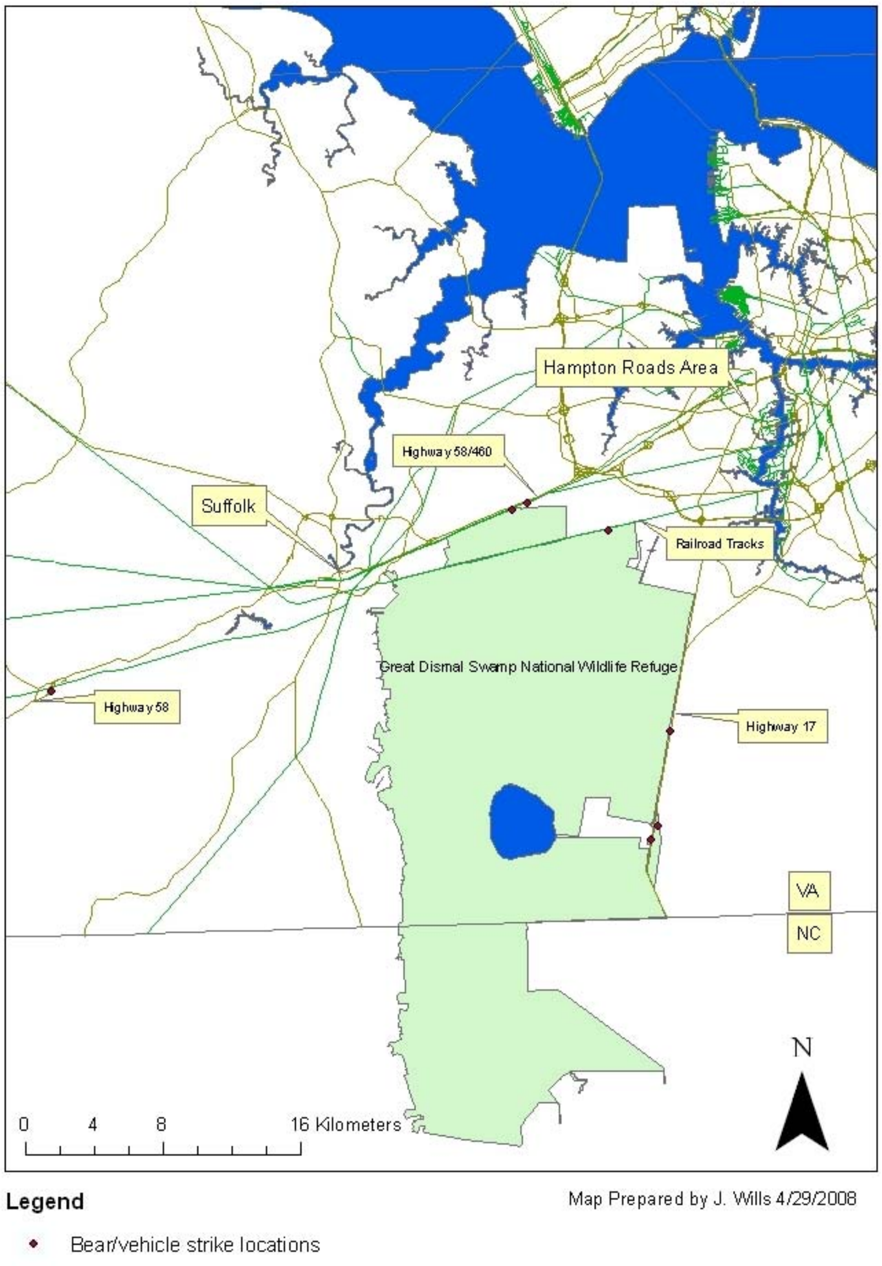
Mitigation efforts potentially could reduce the number of bear/vehicle collisions in concentrated areas with a relatively high collision rate. These efforts may include the installation of bridges, underpasses, wildlife crossing signs, or the institution of reduced speed limits (Singer and Doherty 1985, Foster and Humphrey 1995, Romin and Bissonette 1996, Clevenger 1998, and Hindelang et al. 1999). A single solution for all travel routes into and out of the Dismal Swamp may not exist. Many factors need to be studied closely, including forest stand characteristics, micro-topography features, heavily used wildlife trails, natural and man-made food sources, cover value of vegetation, etc. Future research may reveal currently unrecognized determining factors. Additionally, mitigation efforts cannot simply be installed and forgotten. A particular method or combination of methods can be monitored to allow managers to make improvements where necessary. A proactive approach to dealing with bear/vehicle collisions may protect resource agencies from future litigation stemming from injury or loss of human life in one of these situations.

Two methods of reducing or eliminating animal/vehicle collisions have been described. One approach is to place warning signs in an area where animals are likely to cross and/or institute reduced speed limits in the area. This is essentially asking motorists to voluntarily become more alert for hazardous conditions even though the hazard may not be immediately apparent. Many natural resource agencies employ this method, even though its effectiveness is either unknown or considered negligible (Romin and Bissonette 1996, Hindelang et al. 1999).

The second way to reduce collisions is to physically separate vehicular traffic from animal crossing areas. Such methods include highway underpasses and overpasses, usually with fencing along the roadway to funnel animals to desired crossing points and keep them from accidentally getting onto the roadway (Singer and Doherty 1985, Foster and Humphrey 1995, and Clevenger 1998).

Structures built for wildlife have several disadvantages. They usually cost a substantial amount of money to incorporate into road design early in a construction project (Leeson 1996). They are even more expensive and difficult to implement in an existing road system. Improper design or failure to maintain movement barriers may render the overpasses or underpasses useless or cause an even greater risk of animal/vehicle collisions (Foster and Humphrey 1995). Research of animal travel routes and landscape features must occur in order to properly place these structures where they will be most beneficial (Foster and Humphrey 1995). Post-construction monitoring must occur to verify that the structures are being used to meet the intent for which they were designed and to compare bear use in these areas with other stretches of highway (Clevenger 1998). Underpass/overpass monitoring for use by medium-sized to large animals with relatively long life spans and complex habitat requirements may take years to complete. Even then, data may indicate that animals do not use the structures because of improper design or changes in landscape-scale features (Foster and Humphrey 1995). The disadvantages of these 2 general methods of reducing animal/vehicle collisions do not automatically make them invalid mitigation options. However, managers must be willing to develop innovative methods to reduce collisions and monitor mitigation effectiveness in order to make a positive impact on the resources in question and reduce property damage and human injury.

Figure 3-1. Bear/vehicle collisions near the Great Dismal Swamp NWR VA/NC, June 2000-May 2002.



LITERATURE CITED

Bean, N. J. and J. R. Russell. 1995. Attractiveness of liquid baits containing natural and artificial sweeteners to white-tailed deer. Journal of Wildlife Management 59:610-613.

Beier, P. 1993. Determining minimum habitat areas and habitat corridors for cougars. Conservation Biology 7:94-108.

_____. 1995. Dispersal of juvenile cougars in fragmented habitat. Journal of Wildlife Management 59:228-237.

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

Bridges, A. S. 2005. Population ecology of black bears in the Alleghany Mountains of Virginia. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

Brody, A. J. and M. R. Pelton. 1989. Effects of roads on black bear movements in western North Carolina. Wildlife Society Bulletin 17:5-10.

Bunnell, F. L. and D. E. N. Tait. 1985. Mortality rates of North American bears. Arctic 38:316-323.

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

Clevenger, A. 1998. Permeability of the trans-Canada highway in Banff National Park: importance of crossing structures and factors influencing their effectiveness.

Pages 109-119 *in* G. L. Evink, P. Garrett, D. Zeigler, and J. Berry, editors. Proceedings of the International Conference on Wildlife Ecology and Transportation, FL-ER-69-98, Florida Department of Transportation, Tallahassee, Florida, USA.

Crawford, J. C. and D. C. Church. 1971. Response of black-tailed deer to various chemical taste stimuli. Journal of Wildlife Management 35:210-215.

Dice, L. R. 1938. Some census methods for mammals. Journal of Wildlife Management 2:119-130.

Felsenstein, J. 1993. PHYLIP-phylogeny inference package, version 3.5. University of Washington, Seattle, Washington. http://evolution.genetics.washington.edu/phylip.html.

Foran, D. R., S. C. Minta, and K. S. Heinmeyer. 1997. DNA-based analysis of hair to identify species and individuals for population research and monitoring. Wildlife Society Bulletin 25:840-847.

Foster, M. L. and S. R. Humphrey. 1995. Use of highway underpasses by Florida panthers and other wildlife. Wildlife Society Bulletin 23:95-100.

Foucault, F., F. Praz, C. Jaulin, and M. Amor-Gueret. 1996. Experimental limits of PCR analysis of $(CA)_n$ repeat alterations. Trends in Genetics 12:450-452.

Gamo, R. S., M. A. Rumble, F. Lindzey, M. Stefanich. 2000. GPS radio collar 3D performance as influenced by forest structure and topography. Biotelemetry 15. Proceedings of the 15th International Symposium on Biotelemetry, Juneau, Alaska, USA. May 9-14, 1999. Wageningen, The Netherlands International Society on Biotelemetry.

Gammon, P. T. and V. Carter. 1979. Vegetation mapping with seasonal color infrared photographs. Photogrammetric Engineering and Remote Sensing 45:87-97.

Gerlach, F. L. and A. E. Jasumbach. 1989. Global positioning system canopy effects study. USDA Forest Service, Technology and Development Center, Missoula, MT. 18pp.

Gerloff, U., C. Schlotterer, K. Rassmann, I. Rambold, G. Hohmann, B. Fruth, and D. Tautz. 1995. Amplification of hypervariable simple sequence repeats (microsatellites) from excremental DNA from wild living bonobos (*Pan niscus*). Molecular Ecology 4:515-518.

Gibeau, M. L. and S. Herrero. 1998. Roads, rails, and grizzly bears in the Bow River Valley, Alberta. Pages 104-108 *in* G. L. Evink, P. Garrett, D. Zeigler, and J. Berry, editors. Proceedings of the International Conference on Wildlife Ecology and Transportation, FL-ER-69-98, Florida Department of Transportation, Tallahassee, Florida, USA.

Gibeau, M. L and K. Heuer. 1996. Effects of transportation corridors on large carnivores in the Bow River Valley, Alberta. 13 pp *in* G. L Evink, P. Garrett, D. Zeigler, and J. Berry, editors. Trends in addressing transportation-related wildlife mortality. Proceedings of the Transportation-related Wildlife Mortality Seminar, FL-ER-58-96, Orlando, Florida, USA.

Gunther, K. and M. Biel. 1999. Reducing human-caused black and grizzly bear mortality along roadside corridors in Yellowstone National Park. Pages 25-28 *in* G. L Evink, P. Garrett, and D. Zeigler, editors. Proceedings of the Third

International Conference on Wildlife Ecology and Transportation, FL-ER-73-99, Florida Department of Transportation, Tallahassee, Florida, USA.

Hamilton, R. J. and R. L. Marchinton. 1980. Denning and related activities of black bears in the coastal plain of North Carolina. International Conference on Bear Research and Management 4:121-126.

Hampton Roads Planning District Commission. Undated. http://www.hrpdc.org/.

Hellgren, E. C. 1988. Ecology and physiology of a black bear population in Great Dismal Swamp and reproductive physiology in the captive female black bear. Ph. D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

_____ and M. R. Vaughan. 1989. Demographic analysis of a black bear population in the Great Dismal Swamp. Journal of Wildlife Management 53:969-977.

_____ and _____. 1989. Denning ecology of black bears in a southeastern wetland. Journal of Wildlife Management 53:347-353.

Hewitt, D. G., A. Cain, V. Tuovila, D. B. Shindle, and M. E. Tewes. 1998. Impacts of an expanded highway project on ocelots and bobcats in southern Texas and their preferences for highway crossings. Pages 126-134 *in* G. L. Evink, P. Garrett, D. Zeigler, and J. Berry, editors. Proceedings of the International Conference on Wildlife Ecology and Transportation, FL-ER-69-98, Florida Department of Transportation, Tallahassee, Florida, USA.

Hindelang, M., D. Premo, E. Rogers, and K. Premo. 1999. Addressing deer-vehicle accidents with an ecological landscape GIS approach. Pages 185-192 *in* G. L. Evink, P. Garrett, and D. Zeigler, editors. Proceedings of the Third International

Conference on Wildlife Ecology and Transportation, FL-ER-73-99, Florida Department of Transportation, Tallahassee, Florida, USA.

Kasworm, W. F. and T. L. Manley. 1989. Road and trail influences on grizzly bears and black bears in northwest Montana. Proceedings of the International Conference on Bear Research and Management 8:79-84.

Kemp, G. A. 1976. The dynamics and regulation of black bear, *Ursus americanus*, populations in northern Alberta. Proceedings of the International Conference on Bear Research and Management 3:191-197.

Klenzendorf, S. 2002. Population dynamics of Virginia's hunted black bear population. Ph. D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

Koerth, B. H. and J. C. Kroll. 2000. Bait type and timing for deer counts using cameras triggered by infrared monitors. Wildlife Society Bulletin 28:630-635.

Kohn, M. H., F. Knauer, A. Stofella, W. Schroder, and S. Paabo. 1995. Conservation genetics of the European brown bear - a study using excremental PCR of nuclear and mitochondrial sequences. Molecular Ecology 4:95-103.

Langer, T. J. 2006. Population estimates with age and genetic structure of a harvested bear population in eastern North Carolina. Ph. D. Dissertation, North Carolina State University, Raleigh, North Carolina, USA.

Leeson, B. F. 1996. Highway conflicts and resolutions in Banff National Park, Alberta, Canada. In: Evink, G. L., P. Garrett, D. Zeigler, and J. Berry, editors. Trends in addressing transportation related mortality. Proceedings of the Transportation Related Mortality Seminar, FL-ER-58-96, Florida, USA.

Martin, D. J. and D. B. Fagre. 1988. Field evaluation of a synthetic coyote attractant. Wildlife Society Bulletin 16:390-396.

Mason, J. R., N. J. Bean, L. S. Katz, and H. Hales. 1995. Development of a bait for the oral delivery of pharmaceuticals to white-tailed deer (*Odocoileus virginianus*). Pages 59-68 *in* T. J. Kreeger, editor. Contraception in Wildlife Management. U. S. General Publication Office, Washington, D.C.

Mattfield, G. F., J. E. Wiley III, and D. F. Behrend. 1972. Salt versus browse-seasonal baits for deer trapping. Journal of Wildlife Management 36:996-998.

McDaniel, G. W., K. S. McKelvey, J. R. Squires, and L. F. Ruggiero. 2000. Efficacy of lures and hair snares to detect lynx. Wildlife Society Bulletin 28:119-123.

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

Minch E., A. Ruiz-Linares, D. Goldstein, M. Feldman, and L. L. Cavalli-Sforza. Undated. MICROSAT, version 1.5. http://lotka.stanford.edu/microsat.html.

Morin, P. A., J. J. Moore, R. Chakraborty, L. Jin, J. Goodall, D. S. Woodruff. 1999. Kin selection, social structure, gene flow, and the evolution of chimpanzees. Science 265:1193-1201.

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

Musselman, L. J., D. L Nickrent, and G. F. Levy. 1977. A contribution towards a vascular flora of the Great Dismal Swamp. Rhodora 79:240-268.

National Agricultural Statistics Services. Undated. http://www.nass.usda.gov/va/

Navidi, W., N. Arnheim, M. S. Waterman. 1992. A multiple-tubes approach for accurate genotyping of very small DNA samples by using PCR: statistical considerations. American Journal of Human Genetics 50:347-359.

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

Paquet, P. C. and C. Callaghan. 1996. Effects of linear developments on winter movements of gray wolves in the Bow River Valley of Banff National Park, Alberta. 21 pp *in* G. L Evink, P. Garrett, D. Zeigler, and J. Berry, editors. Trends in addressing transportation-related wildlife mortality. Proceedings of the Transportation-related Wildlife Mortality Seminar, FL-ER-58-96, Orlando, Florida, USA.

Phillips, R. L., F. S. Blom, and R. M. Engeman. 1990. Responses of captive coyotes to chemical attractants. Pages 285-289 *in* L. R. Davis and R. E. Marsh, editors. Proceedings of the Fourteenth Vertebrate Pest Conference. University of California, Davis, California, USA.

Poethke, H. J., A. Seitz, and C. Wissel. 1996. Species survival and metapopulations: conservation implications from ecological theory. Pages 81-92 *in* J. Setelle, C. Margules, P. Poschlod, and K. Henle, editors. Species survival in fragmented landscapes. Kluwer Academic Publishers, Norwell, Massachusetts, USA.

Preacher, K.J. 2001. Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence. Available from http://www.quantpsy.org.

Proctor, M. F., C. Servheen, S. D. Miller, W. F. Kasworm, and W. L. Wakkinen. 2004. A comparative analysis of management options for grizzly bear conservation in the U.S.-Canada trans-border area. Ursus 15:145-160.

Reed, D. F., T. N Woodard, and T. M Pojar. 1975. Behavioral response of mule deer to a highway underpass. Journal of Wildlife Management 39:361-367.

Reed, J. D., D. Tollit, P. Thompson, and W. Amos. 1997. Molecular scatology: the use of molecular genetic analysis to assign species, sex, and individual identity to seal faeces. Molecular Ecology 6:225-234.

Rempel, R. S., A. R. Rodgers, and K. F. Abraham. 1995. Performance of a GPS animal location system under boreal forest canopy. Journal of Wildlife Management 59:543-551.

Rice, P. R. and D. C. Church. 1974. Taste response of deer to browse extracts, organic acids, and odors. Journal of Wildlife Management 38:830-836.

Romin, L. A. and J. A. Bissonette. 1996. Deer-vehicle collisions: status of state monitoring activities and mitigation efforts. Wildlife Society Bulletin 24:276-283.

Roughton, R. D. 1982. A synthetic alternative to fermented egg as a canid attractant. Journal of Wildlife Management 46:230-234.

Roy, L. D. and M. J. Dorrance. 1992. Efficacy and selectivity of eggs and tallow baits for skunk control. Wildlife Society Bulletin 20:326-330.

Scheick, B. and M. Jones. 1999. Locating wildlife underpasses prior to expansion on Highway 64 in North Carolina. Pages 247-252 *in* G. L Evink, P. Garrett, and D. Zeigler, editors. Proceedings of the Third International Conference on Wildlife

Ecology and Transportation, FL-ER-73-99, Florida Department of Transportation, Tallahassee, Florida, USA.

Schneider S., J. M. Kueffer, D. Roessli and L. Excoffier. 1997. Arlequin, version 1.1: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland. http://anthropologie.unige.ch/arlequin.

Simberloff, D., J. A. Farr, J. Cox, and D. W. Mehlman. 1992. Movement corridors: conservation bargains or poor investments? Conservation Biology 6:493-504.

Singer, F. J. and J. L. Doherty. 1985. Managing mountain goats at a highway crossing. Wildlife Society Bulletin 13:469-477.

Smith, T. R. 1986. Activity and behavior of denned black bears in the lower Mississippi River Valley. International Conference on Bear Research and Management 6:137-144.

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

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

Tredick, C. A. 2005. Population abundance and genetic structure of black bears in coastal North Carolina and Virginia using noninvasive genetic techniques. Master's Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA.

Turkowski, F. J., M. L Popelka, and R. W. Bullard. 1982. Efficacy of odor lures and baits for coyotes. Wildlife Society Bulletin 11:136-145.

Waller, J. and C. Servheen. 1999. Documenting grizzly bear highway crossing patterns using GPS technology. Pages 21-24 *in* G. L Evink, P. Garrett, and D. Zeigler, editors. Proceedings of the Third International Conference on Wildlife Ecology and Transportation, FL-ER-73-99, Florida Department of Transportation, Tallahassee, Florida, USA.

Warrillow, J., M. Culver, E. Hallerman, and M. R. Vaughan. 2001. Subspecific affinity of black bears in the White River National Wildlife Refuge. Journal of Heredity 92:226-233.

Weaver, K. M. and M. R. Pelton. 1994. Denning ecology of black bears in the Tensas River Basin of Louisiana. International Conference on Bear Research and Management 9:427-434.

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

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

Whitehead, D. R. 1972. Developmental and environmental history of the Dismal Swamp. Ecological Monographs 42:301-315.

Wilcove, D. S., C. H. McLellan, and A. P. Dobson. 1986. Habitat fragmentation in the temperate zone. Pages 237-256 *in* M. E. Soulé, editor. Conservation Biology:

The Science of Scarcity and Diversity. Sinauer Associates, Sunderland, Massachusetts, USA.

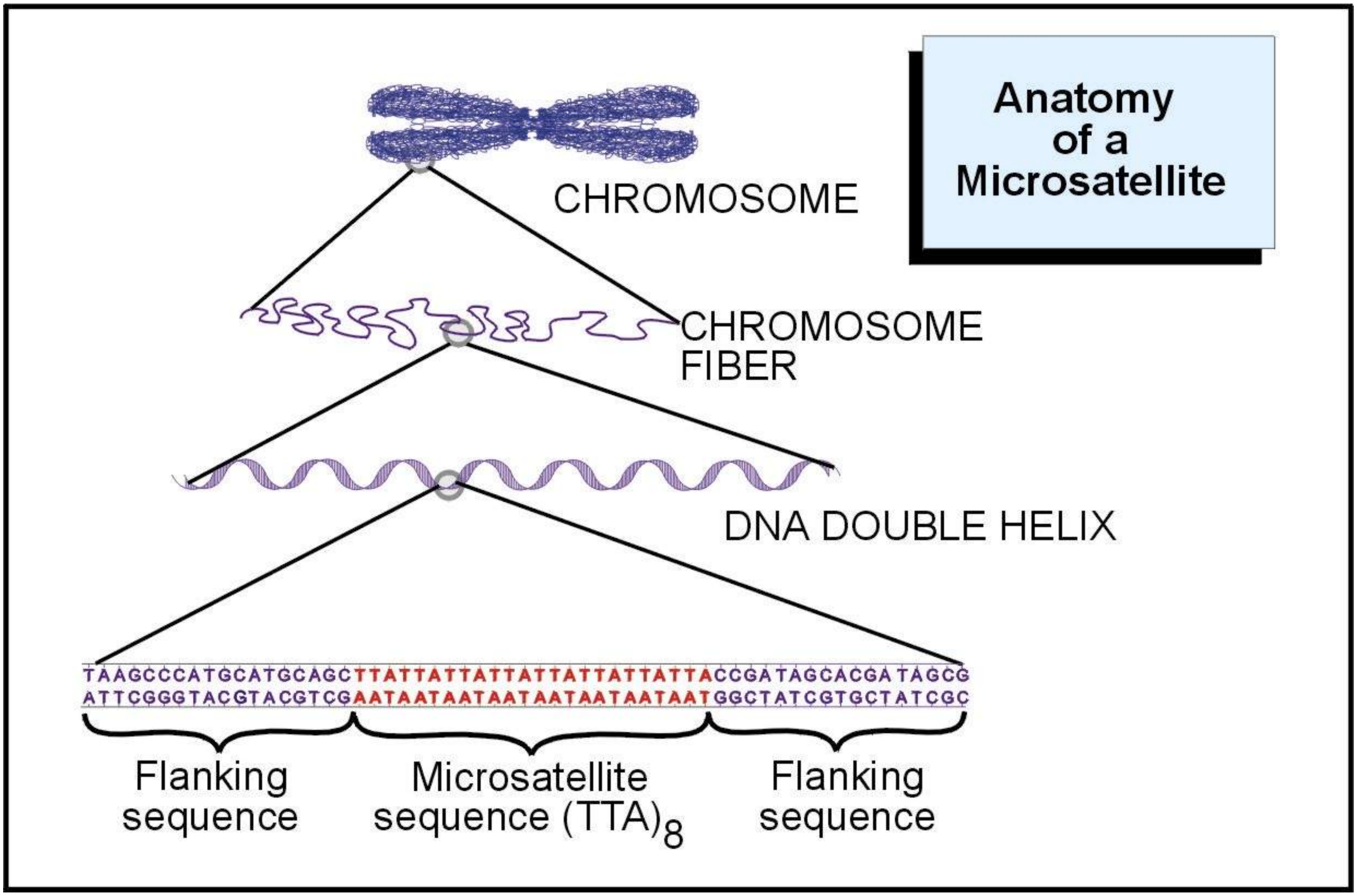
Wills, J. A. and M. R. Vaughan. 2002. Method to monitor travel corridor use by black bears along the eastern boundary of the Great Dismal Swamp National Wildlife Refuge. Proceedings of the International Conference on Ecology and Transportation, Keystone, CO, September 24-28, 2001. Raleigh, NC: Center for Transportation and the Environment, North Carolina State University (March 2002): 529-532.

Woods, J. G., B. L. McLellan, D. Paetkau, C. Strobeck, and M. Proctor. 1996. DNA fingerprinting applied to mark-recapture bear studies. International Bear News 5:9-10.

_____, D. Paetkau, D. Lewis, B. L. McLellan, M. Proctor, and C. Strobeck. 1999. Genetic tagging of free-ranging black and brown bears. Wildlife Society Bulletin 27:616-627.

APPENDIX A

Anatomy of a DNA microsatellite



APPENDIX B

Forest cover types for Great Dismal Swamp National Wildlife Refuge