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Population and genetic impacts of a 4-lane highway on black bears in eastern North Carolina

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To the Graduate Council:

I am submitting herewith a thesis written by Jeremy Michael Nicholson entitled "Population and genetic impacts of a 4-lane highway on black bears in eastern North Carolina." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

Frank T. van Manen, Major Professor

We have read this thesis and recommend its acceptance:

Joseph D. Clark, Lisa I. Muller, Arnold M. Saxton

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

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

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Dean of the Graduate School

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POPULATION AND GENETIC IMPACTS OF A 4-LANE HIGHWAY ON BLACK BEARS IN EASTERN NORTH CAROLINA

A Thesis
Presented for the
Master of Science
Degree
University of Tennessee, Knoxville

Jeremy Michael Nicholson

August 2009

DEDICATION

This thesis is dedicated to my wife, Tonya Nicholson, whose love and support has always been appreciated and never taken for granted.

“This is the day which the lord hath made; we will rejoice and be glad in it” (Psalms 118:24)

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I am greatly indebted to my major professor, Dr. Frank van Manen, for giving me the opportunity to conduct research on such a fascinating animal and interesting project. His enthusiasm and assistance has been much needed during this challenging process. Dr. van Manen's knowledge and attention to detail distinguishes him as a researcher and his personality and sense of humor as a person. I will forever be grateful to him for this opportunity. I would also like to think my committee members, Dr. Joe Clark, Dr. Lisa Muller, and Dr. Saxton for their willingness to provide guidance in the development of this thesis.

This project would not have been possible without the funding and support from the North Carolina Department of Transportation, North Carolina Wildlife Resources Commission, and Weyerhaeuser Company. Specifically, I would like to thank Mark Jones, Colleen Olfenbuttel, Dale Turner, and Brian White of the North Carolina Wildlife Resources Commission; Bill Barber, Brian Hulka, and Darren Miller of Weyerhaeuser Company; and Ann Burroughs of the North Carolina Department of Transportation, for their logistic support during this project.

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ABSTRACT

A 19.3-km section of U.S. Highway 64 in Washington County, North Carolina was rerouted to a 4-lane, divided highway with 3 wildlife underpasses during 2001–2005. I determined the short-term population and genetic impacts of the new highway on American black bears (*Ursus americanus*). I used DNA from hair samples collected during 7 weekly sampling periods within the project area of the new highway and a nearby control area during 2000 (pre-construction phase) and 2006 (post-construction phase; $n = 70$ sites for each study area). DNA from the hair samples was used to obtain genotypes of sampled bears using 10 microsatellite markers. I created capture histories of all identified individuals and used closed mark-recapture models in Program MARK to estimate abundance. Population abundance decreased on the treatment area from 68 (CI = 53–82) before construction to 20 (CI = 14–26) after completion of the highway. On the control area, population abundance decreased from 144 to 101. Using permutation procedures, I determined that the decrease in population abundance on the treatment area was greater compared with the control area ($P = 0.0012$). Additionally, I used bear visits to the sampling sites with multi-season occupancy models in Program MARK to determine if site occupancy decreased following the construction of the highway and if any decrease was a function of distance from the highway. Following highway construction, site occupancy decreased more on the treatment area than the control area but was not a function of distance from the highway. Finally, I used the microsatellite data to compare gene flow, isolation by distance, heterozygosity, allelic diversity, population assignment, and genetic structure (F_{st}) before and after completion of the highway. I did not observe any treatment effects for these genetic measures. I speculate that displacement during the construction of the highway and

mortality due to bear-vehicle collisions contributed to the population decline and decrease in site occupancy. Although the wildlife underpasses facilitated genetic and demographic connectivity, my study indicates that the potential impact of new highways on black bear population abundance is an important consideration for transportation infrastructure planning.

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I. INTRODUCTION

The potential ecological impacts of the expanding highway infrastructure on wildlife populations have drawn the attention of natural resource managers in recent decades (Foster and Humphrey 1995, Romin and Bissonette 1996, Clevenger and Waltho 2000, Evink 2002, Cain et al. 2003). Highways can impact wildlife in 5 basic ways: (1) direct mortality, (2) direct habitat loss, (3) displacement and avoidance, (4) habitat fragmentation, and (5) associated human development (Ruediger 1998). Mortality of wildlife due to vehicle collisions can be substantial in some instances, but rarely seems to limit population size (Forman and Alexander 1998). The greater ecological impacts of highways may be more indirect, with habitat fragmentation as a primary factor (Forman and Alexander 1998). Habitat fragmentation can alter social structure within populations, disrupt population exchange, and create small and isolated populations (Frankham 1996, Lodé 2000). Those small populations may become vulnerable to extinction because of demographic processes such as excessive mortality and disruption of demographic connectivity (Lande 1988, Caughley 1994). Such populations may also experience reduced genetic exchange and thus loss of genetic diversity, which can cause inbreeding depression and ultimately lead to reduced survival and reproduction (Frankham 1996, Lodé 2000). To address ecological and human safety concerns related to wildlife use of roadways, wildlife passageways have been constructed in many areas during the last 2 decades (Reed et al. 1975, Roof and Wooding 1996, Clevenger and Waltho 2000, Ng et al. 2004). The primary purpose of wildlife passageways is to facilitate wildlife movements across highways and thus mitigate potential impacts. However, wildlife passageways are expensive to construct and their mitigation value remains undetermined for many species.

The effects of highways on wildlife have been studied for a variety of species including birds (Reijnen et al. 1995), amphibians (Mazerolle 2004), reptiles (Gibbs and Shriver 2002), small mammals (Adams and Geis 1983), and large mammals (Newmark et al. 1996). These studies reported impacts ranging from none to behavioral responses to population declines. However, due to logistical, biological, and financial constraints, few studies have been able to apply experimental designs to assess if and how wildlife populations respond to highways (van Manen et al. 2001). In fact, Fahrig and Rytwinski (2009) stated they were not aware of any before-after-control-impact (BACI) studies to determine the effects of highways on abundance of wildlife populations. Determining the effects of highways and the potential mitigation value of wildlife underpasses is of particular interest for wide-ranging carnivores because of their extensive spatial requirements (van Manen et al. 2001). The American black bear (*Ursus americanus*) is an ideal study species because populations require large areas of habitat. Also, black bears may be considered an umbrella species, whose proper habitat management also benefits many other species (Simberloff 1999).

U.S. Highway 64 in eastern North Carolina connects the city of Raleigh with the coastal area of the Outer Banks and was upgraded from a 2-lane road to a 4-lane, divided highway during the past decade. The primary reasons for this upgrade were to accommodate increasing tourism traffic and economic development of this region. A new route was selected for a 19.3-km section of U.S. Highway 64 between Roper and Cresswell, which was constructed during 2001–2005. Because this section traversed high-quality wildlife habitat with high black bear densities, 3 wildlife underpasses were incorporated into its design to mitigate potential impacts of the highway on wildlife and to increase driver safety (Scheick and Jones 1999, Jones et al.

2009). In 2000, before construction of the highway began, the University of Tennessee and the U. S. Geological Survey initiated a study in collaboration with the North Carolina Wildlife Resources Commission and the North Carolina Department of Transportation to determine the short-term impacts of U.S. Highway 64 on black bear ecology and the potential mitigation value of the wildlife underpasses (van Manen et al. 2001). The study design included data collection on a treatment area (new section of U.S. Highway 64) and a control area and during pre- and post-construction study phases. Fieldwork for the pre-construction phase started in May 2000 and was completed in June 2001 (Thompson 2003, Kindall 2004, Thompson et al. 2005, Kindall and van Manen 2007). Construction of the highway was completed in September 2005. Fieldwork for the post-construction phase was initiated in May 2006 and was completed during fall 2007. McCollister (2008) assessed the impacts of the new highway on the spatial ecology of black bears and the effectiveness of the 3 wildlife underpasses. My portion of this study focused on the population and genetic impacts of the new highway on black bears.

Objectives and Hypotheses

The objectives of my study were to:

1. determine if population abundance and site occupancy changed on the treatment area following highway construction, and
2. determine if gene flow, genetic diversity, and genetic structure of the black bear population changed on the treatment area following highway construction.

My research hypotheses were that:

(1) population abundance would decrease on the treatment area because of direct mortality caused by vehicle collisions, displacement, and habitat degradation;

(2) site occupancy and extinction on the treatment area and near the highway would decrease more compared with the control area and areas further away from the highway because of displacement and direct mortality caused by vehicle collisions;

(3a) gene flow would be reduced between the areas north and south of the highway because the highway would act as a barrier to movement;

(3b) isolation by distance would increase on the treatment area because the highway would decrease gene flow;

(3c) heterozygosity would decrease because reduced population abundance would decrease genetic variation;

(3d) allelic diversity would decrease because of reduced population abundance; and

(3e) genetic structure would increase because reduced gene flow between populations north and south of the highway would increase genetic differentiation.

II. STUDY AREA

The study area was located in Washington County in eastern North Carolina (Fig. 1). The treatment and control areas were approximately 10,750 ha and 12,270 ha, respectively (Fig. 1). The treatment area was bordered by NC Highway 32 to the north, Newland Road to the south, the town of Roper to the west, and the town of Creswell to the east. The new section of U.S. Highway 64 and the 3 wildlife underpasses were completed in September 2005. The section of U.S. Highway 64 within the treatment area was a 19.3-km divided, 4-lane roadway from Roper to Creswell. The 3 wildlife underpasses were similar in design but had different dimensions (McCollister 2008). The width ranged from 29 m for the eastern underpass to 47 m for the western underpass. Wildlife fencing (3-m high chain-link fence) extended a minimum of 800 m in both directions from the underpasses, parallel to the highway. Fencing was continuous underneath the underpasses and connected the sections that paralleled the highway. The control area was bordered by U.S. Highway 64 to the north, State Highway 99 to the south, State Highway 32 to the west, and State Road 1127 to the east (Fig. 1). The control area closely resembled the treatment area, particularly regarding the juxtaposition and area of agricultural and forested lands but did not contain a major highway (van Manen et al. 2001).

Land use on both study areas was primarily associated with forestry and agriculture. Weyerhaeuser Company was the largest landowner within the study region. Managed pine plantations provided the primary source of income and were planted as seedlings and grown in 60-yr rotations. Forest stands changed somewhat between the 2 study phases because of silvicultural practices. On the treatment area forest cover decreased from 55% in 2000 to 53% in 2006, whereas forest cover on the control area decreased from 62% in 2000 to 57% in 2006. In pine plantations, loblolly pine (*Pinus taeda*) dominated the overstory, whereas the midstory was comprised of red maple (*Acer rubrum*), yellow poplar (*Liriodendron tulipifera*), sweetgum (*Liquidambar styraciflua*), and various evergreen shrubs. Hardwood stands occurred within natural drainages and consisted of various oak species (*Quercus* spp.), yellow poplar, black gum, and sweet gum. Pine and hardwood stand had dense understory vegetation including river cane (*Arundinaria gigantea*), poison ivy (*Toxicodendron radicans*), blackberry (*Rubus* spp.), devil's walking stick (*Aralia spinosa*), grape (*Vitis rotundifolia*), red bay (*Persea borbonia*), sweetbay (*Magnolia virginiana*), and greenbriar (*Smilax* spp.). Approximately 40% of the land was used for agriculture. Common agriculture crops were corn, wheat, soybeans, peanuts, cotton, and turf grass**.**

Human development within the study region was limited to a few private homes scattered throughout the study areas and the towns of Roper and Plymouth. The human population in Washington County was relatively low (13,723; U.S. Census Bureau 2002). An extensive system of highways, unpaved roads, and canals occurred throughout the study area. Unpaved roads located on timber and agriculture lands usually were gated and locked, limiting use to landowners, hunters, and forestry and agriculture machine operators. Weyerhaeuser Company lands and numerous privately owned tracts were leased to hunting clubs.

The juxtaposition of forested and agricultural areas provided food and cover to a variety of wildlife species. Mammal species included black bear, white-tailed deer (*Odocoileus virginianus*), red wolf (*Canis rufus*), coyote (*Canis latrans*), red fox (*Vulpes vulpes*), grey fox (*Urocyon cinereoargenteus*), river otter (*Lontra canadensis*), raccoon (*Procyon lotor*), bobcat (*Lynx rufus*), beaver (*Castor canadensis*), muskrat (*Ondatra zibethicus*), opossum (*Didelphis* *virginiana*), eastern gray squirrel (*Sciurus carolinensis*), and eastern cottontail (*Sylvilagus floridanus*). Common game bird species were eastern wild turkey (*Meleagris gallopavo*), mourning dove (*Zenaida macroura*), and northern bobwhite quail (*Colinus virginianus*). Other species included waterfowl, numerous songbird species, and a variety of herpetofauna.

III. METHODS

Study Design

I used DNA collected from black bear hair samples to determine if changes in population abundance, site occupancy, gene flow, genetic variability, and genetic structure occurred following completion of the new highway. The area where the new section of U.S. Highway 64 was constructed represented the treatment area for this project. The experimental design for this study also included a control area, which was used to account for potential effects that were not due to the presence of the highway (Before-After Control-Impact design [BACI]; Green 1979). I determined the potential impacts of the highway on black bears based on comparisons among the following data groups: (1) treatment area, pre-construction phase, (2) treatment area, post-construction phase, (3) control area, pre-construction phase, (4) control area, post-construction phase (Fig. 2). I tested my research hypotheses by determining whether differences existed between data from groups 1 and 2, adjusting for any differences between the 2 data groups from the control area. Additionally, to determine changes in population structure and gene flow, I made comparisons between: (1) bears sampled on the control and treatment areas, (2) bears sampled north and south of U.S. Highway 64 on the treatment area, and (3) bears sampled north and south of a fictitious highway on the control area (Fig. 3). That fictitious highway was comparable in length and location to the new section of U.S. Highway 64 on the treatment area and was based on Thompson (2003).

Data Collection

DNA Sampling.–During the first phase of this study, 70 hair-sample sites were established on both the treatment and the control area (total n = 140; Fig. 4). Random location coordinates were generated within forested habitats only and the number of sample sites was chosen so that all bears had access to >1 site based on minimum home-range estimates (Thompson et al. 2005). Hair-sample sites were reestablished at the same locations for the second phase of the study. When low forest cover or disturbances made the original site unsuitable, the site was moved to the nearest forested area. Each hair-sample site consisted of a barbed-wire enclosure baited with bakery products (e.g., sweetrolls, donuts) and scented with raspberry extract (Mother Murphy, Greensboro, North Carolina, USA). A single strand of 15.5-gauge barbed wire was stretched around 4 corner trees 40–50 cm above the ground and marked with flagging. The size of the enclosure was approximately 5 x 5 m, or large enough to prevent bears from reaching the bait without crossing the barbed wire. Bait was suspended from a wire stretched diagonally between 2 trees and centered in the enclosure. Field personnel checked all sites for hair samples once a week for 7 weeks. Hair samples with >5 hairs were collected from the barbs, stored in labeled envelopes and kept dry using a plastic container with Drierite® (W.A. Hammond Drierite Company, Xenia, Ohio, USA) to absorb moisture. After hair collection, each station was re-baited and scented. Field personnel burned any remaining hair on barbs to prevent future mixing of DNA samples. For the first study phase, hair samples were collected during 1 October 2000–21 November 2000. During the second study phase, collection of hair samples started on 20 September 2006 with final collections occurring on 11 November 2006.

Genetic Analyses

Microsatellite Analysis.–Microsatellites represent a group of molecular markers that have been proven useful for mark-recapture experiments (Paetkau and Strobeck 1998, McKelvey and Schwartz 2004) and to assess the genetic structure of bear populations (Cegeleski et al. 2003, Jones et al. 2004, Thompson et al. 2005). The use of microsatellites has advantages over other types of markers because they are widely distributed in eukaryotic genomes, making it relatively easy to develop a suite of markers. Moreover, microsatellites are highly variable and easily interpreted and can provide a unique identification of individuals within populations (Mills et al. 2000, McKelvey and Schwartz 2004). Finally, microsatellite DNA can be amplified using polymerase chain reaction (PCR), which substantially reduces the amount and quality of DNA that is needed for analysis (Paetkau and Strobeck 1998).

During the first study phase, 25 hair samples/week were randomly chosen for microsatellite analysis, each sample from a different site. Microsatellite analysis was conducted by the U.S. Geological Survey, Leetown Science Center (Kearneysville, West Virginia, USA). Ten microsatellite loci were analyzed for each selected hair sample using the following markers: G1A, G1D, G10B, G10L, G10C, G10M, G10P, G10X, MU23, and MU50 (Paetkau and Strobeck 1994, Paetkau et al. 1995). DNA was extracted from hair roots using the InstaGene Matrix (Bio-Rad Laboratories, Hercules, California, USA) and DNA amplification was performed as described by Boersen et al. (2003). Genotype data were generated using GeneScan® Analysis Software (Applied Biosystems, Foster City, California, USA). DNA fragment analysis software (Genotyper®, version 2.0; Applied Biosystems, Foster City, California, USA) was used to score, bin, and output allelic and genotypic designations for each hair sample. The multilocus genotype of each DNA sample was used to identify newly captured individuals and recaptures during subsequent sampling occasions.

DNA analysis procedures for the second study phase were slightly different from the first study phase. I again selected 1 sample from 25 randomly chosen sample sites per week for analysis but, because of relatively low sample sizes, I selected all hair samples (*n* = 95) collected on the treatment area. I clipped and stored the roots of all hair samples. For the treatment area samples, I extracted DNA using the InstaGene Matrix. Further genetic analysis of the extracted and clipped samples was conducted by Wildlife Genetics International (WGI, Nelson, British Columbia, Canada). DNA extraction of hair samples from the treatment area was performed using QIAGEN'S DNeasy Tissue kits (Qiagen Inc., Valencia, California, USA) according to protocols established by the manufacturer (D. Paetkau, WGI, personal communication). Microsatellite analysis was initially conducted using the 7 loci with the highest variability (G1A, G1D, G10L, G10M, MU23, MU50, and G10P; Paetkau et al. 1995). Four additional loci (G10B, G10C, G10X, and the amelogenin gender marker) were analyzed for each unique individual to obtain a 10-locus genotype plus gender for all individuals. The 10-locus genotypes allowed me to compare genetic parameters based on the samples collected during the first study phase. To determine if any bears sampled during the first phase were also sampled during the second phase, WGI also obtained genotypes of 6 bears sampled during the first study phase and provided base-pair adjustments to calibrate the DNA samples from the second study phase.

Probability of Identity.–Because I used mark-recapture models to estimate population abundance, an important assumption is that individuals are uniquely marked. That assumption may be violated when 2 or more individuals share the same genotype at the examined loci. This error is referred to as a "shadow effect", which can negatively bias estimates of population abundance (Mills et al. 2000). Shadow effects occur when too few loci or loci with low heterozygosity are examined (McKelvey and Schwartz 2004). Shared genetic profiles can lead to unique individuals being classified as the same animal and mistaken as a recapture when, in fact, they are not (Mills et al. 2000). The ability of molecular markers to identify individuals can be determined using the probability of identity statistic (PI). The probability of identity is the expected frequency that 2 unrelated individuals selected at random from a population have the same genotype at multiple loci (Paetkau and Strobeck 1998). For a single locus PI can be calculated with multiple alleles as:

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

where p_i and p_j are the frequencies of the *i*th and *j*th alleles, respectively, assuming the alleles are in Hardy-Weinberg proportions (Taberlet and Luikart 1999). I calculated the overall PI for multiple loci as follows:

$$\mathrm{PI}_{\text{overall}} = \prod (\mathrm{PI}_{\text{single locus}}).$$

$\mathrm{PI}_{\text{overall}}$ values are unbiased if the multiple loci are independent (Mills et al. 2000). Within small populations, multilocus genotypes may not be independent because of shared ancestry within families or if populations are isolated (Taberlet and Luikart 1999). Therefore, as a conservative upper bound of the statistical probability of observing identical genotypes based on the sampled loci, I calculated the probability of identity between siblings ($\mathrm{PI}_{\text{sibs}}$) from the allele frequencies (Waits et al. 2001):

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

where p_i is the frequency of the *i*th allele (Taberlet and Luikart 1999). I performed all PI calculations using program GenALEx 6.1 (Peakall and Smouse 2006). For most wildlife studies, a probability of identity <0.01 should be sufficient to provide accurate results (Taberlet and Luikart 1999).

Hardy-Weinberg Equilibrium and Linkage Disequilibrium.–The computation of PI is based on the assumption that the genetic samples are in Hardy-Weinberg equilibrium and the alleles at each locus are independent (Taberlet and Luikart 1999). Deviation from these assumptions may be caused by biological factors or sampling biases and can impact the results of mark-recapture studies and genetic analyses. In a large population with random mating and no selection, mutation, or migration, the frequency of homozygotes and heterozygotes will reach Hardy-Weinberg equilibrium and remain the same across generations (Weir 1990, Connor and Hartl 2004). According to the Hardy-Weinberg law, the expected number of genotypes in the population follows:

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

where p is the frequency of the dominant allele and q is the frequency of the recessive allele (Lowe et al. 2004). I used program GENEPOP to determine if deviations from the Hardy-Weinberg equilibrium occurred by testing for heterozygote excess and heterozygote deficiency using the Markov-chain random walk algorithm (Guo and Thompson 1992). I used the Dunn-Sidak method to control the experimentwise error rate at $\alpha = 0.05$, with critical values for individual comparisons based on $\alpha = 1 - (1 - 0.05)^{1/k}$, where k is the number of individual comparisons (Sokal and Rohlf 1995).

Linkage disequilibrium occurs when there is nonrandom association between a chromosome's alleles at one locus and its alleles at another locus, resulting in certain combinations of alleles being more likely to occur together (linked) on a chromosome (Lowe et al. 2004). I used the genotypic linkage disequilibrium test in program GENEPOP to determine if alleles at each locus were independent from the other sampled loci. I again used the Dunn-Sidak method to obtain an experimentwise error rate of $\alpha = 0.05$.

Genotyping Error.–Genotyping errors occur when molecular analysis produces a genotype that does not correspond with the actual genotype of a sampled individual (Bonin et al. 2004). Genotyping errors may lead to biases in abundance estimates (McKelvey and Schwartz 2004) and unreliable inferences concerning population genetics (Bonin et al. 2004). There are several sources of genotyping error, including allelic dropout and false alleles (Taberlet and Luikart 1999, McKelvey and Schwartz 2004). Allelic dropout occurs if only 1 of 2 alleles in a heterozygote pair is amplified, thus producing a false homozygote (McKelvey and Schwartz 2004). False alleles are a result of artifacts created during amplification, which are then misinterpreted as true alleles (Taberlet and Luikart 1999). A heterozygous individual, for example, may be recorded as homozygous and thus identified as a new capture (Taberlet and Luikart 1999, McKelvey and Schwartz 2004). In a study of gray wolves (*Canis lupus*) in Yellowstone National Park, McKelvey and Schwartz (2004) found that genotyping errors could cause up to a 5.5-fold increase in population size.

To reduce genotyping errors during the first phase, only samples with ≥ 5 hairs with clearly visible roots were analyzed. Additionally, genotypes differing by only 1 or 2 alleles were genotyped a second time to minimize the likelihood of amplification errors (T. King, U.S. Geological Survey, personal communication). To estimate the error rate for microsatellite analysis performed at the U.S. Geological Survey laboratory, hair samples of 37 different black bears from Maryland were used (S. Bittner, Maryland Department of Natural Resources, unpublished data) in a double-blind test. That test was designed to match 2 sets of 7 single-locus genotypes for each bear. Although I used 3 additional loci in my study, all 7 loci analyzed for the Maryland hair samples were included in those 10 loci. Therefore, I assumed that error rates based on the 7 loci applied to the 3 additional loci as well. Of the 259 single-locus genotypes in each of the 2 sets of samples used to determine genotyping errors, only 2 were different (i.e., a per-locus error rate of 0.39%). Each of the 2 errors was limited to single individuals, likely a result of allelic dropout, and would have been reanalyzed during the error-checking protocols (Miller et al. 2002).

To reduce genotyping errors during the second study phase, WGI personnel used a 3-phase approach (D. Paetkau, WGI, personal communication). First, extracted samples were analyzed using the 7 loci with the highest variability (G1A, G1D, G10L, G10M, MU23, MU50, and G10P). Samples that initially failed to amplify at >3 loci were eliminated based on prior experience showing that such samples are prone to errors. Next, the samples that initially failed to amplify at 1–3 loci were analyzed again until a complete genotype for all 7 loci was produced, discarding any samples that still had missing data at any of the 7 loci. Individual samples that mismatched at ≤2 loci were reanalyzed to confirm their unique identity. Once the genotypes were completed, a computer search was performed for identical genotypes and individuals were defined. One sample per individual was selected to analyze 4 additional markers (G10B, G10C, G10X, and the amelogenin gender marker). Therefore, a complete 11-locus (including gender) genotype for all identified individuals was completed.

Population Responses to New Highway

Population Abundance.–I used mark-recapture models to estimate population abundance on the 2 study areas. Mark-recapture models are based on the principle that marking and recapturing only a portion of a population can be used to estimate population size (Elzinga et al. 2001). Identifying individuals based on DNA extracted from hair samples provides many advantages over conventional mark-recapture studies using livetrapping, including increased capture probability, decreased tag loss, and decreased intrusiveness (Mills et al. 2000). Mark-recapture experiments classify populations as either open or closed (Otis et al. 1978). Open population models allow for recruitment (i.e., birth or immigration) or losses (i.e., death or emigration) to occur during the course of the study, whereas closed models assume there are no population additions or losses. With samples from ≥2 sampling periods, population size can be estimated using the closed mark-recapture models of Otis et al. (1978). The general assumptions for these capture-recapture models are (Otis et al. 1978):

(1) the population is closed,

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

(3) all marks are recognized and recorded during each trapping occasion, and

(4) all members of the population have equal capture probabilities for every trapping occasion.

The closure assumption is rigid and difficult to completely satisfy in many instances (Otis et al. 1978). However, that assumption can be approximately met if data are collected within a short time period during which no births or deaths occur (Chao 2001). Hair samples were collected within a short time period (pre-construction phase: 1 October 2000–21 November 2000; post-construction phase: 20 September 2006–11 November 2006) when no births occurred. Also, sampling took place before the fall hunting season. Given high survival rates typical of black bear populations when no harvesting occurs, deaths were unlikely during the sampling periods.

Other areas occupied by black bears occurred near the study area but only a few habitat corridors connected with those areas. Therefore, given the short sampling duration, immigration and emigration likely were limited as well.

I used Program MARK (White and Burnham 1999) to estimate population abundance. Using maximum likelihood techniques, Program MARK uses capture histories to estimate capture probabilities (*p*), recapture probabilities (*c*), and population abundance (*N*). I developed a capture history for each unique genotype based on the 7 weekly sampling periods, which consisted of the numbers 1 (capture) and 0 (no capture). I pooled the capture histories for all 4 data groups and classified each capture history according to its data group (i.e., pre- or -post-construction phase and treatment or control area) using binary variables. I developed a set of a priori models to explore the effects of time (*t*), behavior (*b*), and the combination of the two on capture and recapture probabilities. To assess time effects I developed models where capture and recapture probabilities were the same but varied across sampling periods. I tested for a behavior (*b*) effect by constraining the model so capture and recapture probabilities were different. Additionally, I fitted models to determine whether capture and recapture probabilities were a function of study area (treatment or control area) or study phase (pre- or post-construction phase). To do so, I developed models where *p* and *c* were held constant or varied by study area and study phase: constant for both study area and study phase (area[.], phase[.]), different for study area but constant for study phase (area[g], phase[.]), constant for study area but different for study phase (area[.], phase [g]), and different for study area and study phase (area[g], phase[g]). Finally, I developed models combining group (study area, study phase), time, and behavior effects.

I ranked the models using Akaike's Information Criterion with a second-order correction for small sample sizes (AIC_c; Burnham and Anderson 2002). The model with the lowest AIC_c is the most parsimonious given the data, thus providing the best fit with the fewest parameters (Cooch and White 2001). Models are equally supported when the difference in the AIC_c between two models (ΔAIC_c) is ≤ 2, whereas ΔAIC_c values >2 indicate that model fit is different (Cooch and White 2001). To account for uncertainty in model selection, I used model averaging to obtain population estimates and unconditional standard errors; model averaging takes model uncertainty into account and is more likely to contain the true *N* (Burnham and Anderson 2002).

To determine if the proportional change in population abundance on the treatment area was greater than the change on the control area, I performed a permutation test using 10,000 permutations. First, I generated a random number from a normal distribution using the Box-Muller method (Press et al. 1992). For each of the 4 data groups, I multiplied the random number times the standard error of the abundance estimate and then added (or subtracted, in case of a negative random number) that value to the abundance estimate. In so doing, I obtained an abundance estimate for each data group that incorporated the variation around the point estimate based on the 95% confidence interval, which has a normal distribution (Otis et al. 1978). Next, for every permutation, I estimated the proportional change in abundance between the 2 study phases for each study area, each time also calculating the difference between those proportions for the 2 study areas. Finally, I repeated this procedure 10,000 times and determined the number of times that the proportional change in abundance on the treatment area was greater than the proportional change on the control area. A treatment effect would be evident if >95% of the permutations resulted in a greater proportional decline on the treatment area compared with the control area (i.e., $P < 0.05$).

Population Density.–Animals sampled at the edge of a study area may not spend all their time within that area. This edge effect adds to the size of the area being studied (Otis et al. 1978, Wilson and Anderson 1985). Therefore, it is important to determine the effective study area before estimating population density. First, I used ArcMap™ GIS (ESRI, Redlands, California, USA) to encircle the 70 trap sites on each study area using radii equivalent to the mean area of female home ranges during the 2 respective study phases (Kindall 2004, McCollister 2008). Home ranges were based on the 95% fixed kernel method (Powell 2000). Next, I delineated all forested tracts, including a 250-m buffer beyond the forest edge (approximately the maximum distance black bears were observed from the forest). I then defined the effective study area based on the areas surrounding the sample sites minus nonforested areas >250 m from forest edges. I determined population density for each of the 4 data groups by dividing the population estimate (N) by the effective study area. I did not statistically compare population density among the 4 data groups because home-range estimates for female bears on the control area during the second phase of the study was based on a small sample size ($n = 6$), which could have biased the effective study area calculation and thus the accuracy of the density estimate. Also, because there was no standard error associated with the density estimate, I could not test for a treatment effect in a manner similar to the method I used for population abundance.

Site Occupancy.–The presence of a road may modify an animal's behavior and may potentially alter movement patterns and cause home-range shifts away from highways (Trombulak and Frissell 2000). Studies have shown that black bears may avoid areas near paved roads with high traffic volumes (Quigley 1982, Garner 1986, Beringer et al. 1990, Fecske et al. 2002). Two processes that may be used to determine the response of a species to landscape changes are local extinction and colonization (Siramie et al. 2008). Multi-season occupancy models can be used to detect changes in occupancy (Ψ), extinction (ε), and colonization (γ) at sites between 2 or more time periods (Mackenzie et al. 2006). In multi-season occupancy models, there are primary and secondary sampling periods. There were 2 primary periods in my study (2000 and 2006) with the secondary sampling periods consisting of the 7 weeks of hair sampling during each of the 2 primary periods. I assumed that the population was closed during the secondary sampling periods and open between the primary sampling periods. Therefore, between primary periods, occupied sites may become extinct and unoccupied sites may be colonized.

I used multi-season occupancy models in Program MARK (White and Burnham 1999) to test my research hypothesis that occupancy on the treatment area decreased more than on the control area and was a function of distance from the highway. I used capture histories of the 70 sample sites on the treatment area for both study phases (14 secondary sampling periods for each site). I recorded detections as 1 and nondetections as 0. I first built models with different detection probabilities (p_i) and used Akaike's Information Criterion corrected for small sample sizes (AIC$_c$; Burnham and Anderson 2002) to determine how to best model p_i. I tested whether detection probabilities for each primary sampling period should be modeled as a function of study area (g) and time (t), or their interaction, based on secondary sampling periods (p_i[g,t,g*t] and p_i[g,t]), linear time trends (p_i[g,T$_{\text{lin}}$,g*T$_{\text{lin}}$] and p_i[g,T$_{\text{lin}}$]), asymptotic time trends (p_i[g,T$_{\text{log}}$,g*T$_{\text{log}}$] and p_i[g,T$_{\text{log}}$]; p_i[g,T$_{\text{-1/y2}}$,g*T$_{\text{-1/y2}}$] and (p_i[g,T$_{\text{-1/y2}}$]), and time only (p_i[t]).

Because local site characteristics may affect detection probability, I also tested if percent forest cover during the first (pfc1) and second study phase (pfc2) improved model fit. I mapped forested areas using the 2001 National Land Cover Data (30-m resolution; Homer et al. 2007) and combined all land-cover classes with forest cover. I excluded forest stands that had been harvested within the previous 5 years based on silvicultural records (Weyerhaeuser Company, unpublished data). Next, I calculated the proportion of forest pixels within a 270-m radius of the sample sites using ArcMap™ GIS. I used 270 m to represent the hourly movement rate of bears on our study area (Kindall 2004). Finally, I tested if the inclusion of percent forest cover (pfc1) as a covariate for occupancy increased model fit. I used pfc1 because occupancy for the first primary sampling period (Ψ_1) was estimated, whereas occupancy for the second primary sampling period (Ψ_2) was derived.

After determining how the detection probability should be modeled, I used selection procedures with AIC_c to test whether the estimates of Ψ_1, ε, and γ were different for the 2 study areas. I used model averaging to estimate Ψ_1, ε, and γ and to derive estimates for Ψ_2. I used the corresponding 95% confidence intervals of those estimates to determine whether occupancy on the treatment area decreased following highway construction and if those changes were greater than observed on the control area.

To determine if distance to the new highway was associated with any reduction in occupancy on the treatment area, I repeated the occupancy analysis based on the 70 treatment sample sites only. First, I used procedures similar to the previous occupancy analysis to determine how detection probability should be modeled and if the inclusion of covariates increased model fit. Next, I calculated the distance to the highway for each sample site using

ArcMap™ GIS. Some sample sites were at slightly different locations for the 2 study phases because habitat changes between study phases made the original site unsuitable. Therefore, I averaged the distances for both study phases. To test if occupancy decreased between the 2 sampling periods and if this decrease was a function of proximity to the highway, I used AIC_c to compare 4 a priori models that included or did not include the distance covariate for occupancy or the extinction parameter: $\Psi(.)$, $\varepsilon(.)$, $\gamma(.)$; $\Psi(\text{distance})$, $\varepsilon(.)$, $\gamma(.)$; $\Psi(.)$, $\varepsilon(\text{distance})$, $\gamma(.)$; and $\Psi(\text{distance})$, $\varepsilon(\text{distance})$, $\gamma(.)$.

No formal procedures exist to test the fit of multi-season occupancy models. Therefore, I used the approach described by MacKenzie and Bailey (2004) for single-season occupancy models. I compared the Pearson χ^2 of the global model (full parameterization) with the distribution of χ^2 values based on 10,000 parametric bootstrapping replicates. If lack of fit was evident, I used an overdispersion parameter ($\hat{c}$) to adjust the standard errors (McCullagh and Nelder 1989) and re-evaluate the model selection procedures (Burnham and Anderson 2002) using quasi-AIC ($QAIC_c$). Visits to multiple sites by 1 bear during the same secondary sampling period represent a primary source of overdispersion. Therefore, I used the genotypes of bears to estimate the proportion of bears sampled at more than 1 site during each secondary sampling period. I then calculated $\hat{c}$ as the ratio of the total number of DNA samples over the total number of samples minus those that represented bears that were sampled at >1 site during the same secondary sampling period. I calculated $\hat{c}$ for each study area and study phase and used the mean value to adjust the standard errors.

Genetic Responses to New Highway

Gene Flow.–Gene flow is the movement of individuals, or groups of individuals, from one location to another and their subsequent integration into the gene pool of their new locality (Slatkin 1987). In increasingly isolated populations, reduction in gene flow may cause genetic drift, which can lead to alleles becoming fixed (Mills and Allendorf 1996). I tested the research hypothesis that gene flow decreased between bears sampled north and south of U.S. Highway 64 after the highway was completed, adjusting for any changes in gene flow that may have occurred for bears sampled north and south of the fictitious highway on the control area.

I used program GENEPOP (version 3.1; Raymond and Rousset 1995) to calculate gene flow using the private allele method (Barton and Slatkin 1986). Private alleles are alleles only found in a single population (Lowe et al. 2004). When gene flow is low, the probability that a migrant will transport private alleles between populations is low, resulting in higher frequencies of private alleles remaining in populations (Baker 2000). Alternatively, when gene flow is high, the frequency of private alleles decreases (Baker 2000). I calculated the change in gene flow between the north and south portions of the treatment area before and after construction of the highway and did the same for the areas north and south of the fictitious highway on the control area. I then calculated the difference in the gene flow estimates between the treatment and control areas.

To test for a treatment effect, I used permutation procedures because there were no standard errors associated with the gene flow estimate and no known distribution exists. I created 100 data sets by randomly placing individual genotypes in either the north or south portion of each study area, while keeping the sample size for each portion of the study area the same as the original data. For each permutation, I estimated the change in gene flow between the 2 study phases (*Δgene flow*), each time also calculating the difference of the *Δgene flow* estimates between the 2 study areas. Finally, I used the distribution of those 100 values (difference in *Δgene flow* between the 2 study areas) to rank the observed difference in Δ*gene flow* based on the original data. That ranking represented the probability of finding a greater difference in *Δgene flow* than observed between the treatment and control areas.

Isolation by Distance.–Wright (1946) introduced the neighborhood concept, which refers to the area in which populations undergo random mating. In most instances, gene flow decreases as geographic distance increases among populations, which leads to genetic differentiation among individuals and subpopulations (Broquet et al. 2006). This process can be examined using isolation by distance models (Wright 1946). For each study area, I estimated the pair-wise genetic distances among all individual bears using program GENEALEx 6.0 (Peakall and Smouse 2005). Next, I calculated geographic distances among the sample locations of all bears using SAS software (SAS 2008). For bears sampled at multiple sites I averaged the coordinates of all sampling locations. To assess the relationship between geographic and genetic distance I used the Isolation by Distance Web Service version 3.12 (Jensen et al. 2005). This program uses major-axis regression to calculate the slope and intercept of the isolation by distance relationship and uses the Mantel test, a simple correlation method, to determine the association (r) between geographic and genetic distance (Mantel 1967). To determine if isolation by distance increased more on the treatment area than on the control area, I used Fisher's Z-test, which is an appropriate statistical method for comparing correlations. First, I used the Fisher's z-transformation to convert the r-values to z-values (Fisher 1921):

$$z = 0.5 \ln \left(\frac{1+r}{1-r} \right),$$

where r is the correlation value. Next, I tested my hypothesis by calculating:

$$\mathrm{z} = \frac{(z_1 - z_2) - (z_3 - z_4)}{\sigma_{(z_1 - z_2) - (z_3 - z_4)}},$$

where z_1 and z_2 are the z-transformed pre- and post-construction correlation values for the treatment area, respectively; z_3 and z_4 are the z-transformed pre- and post-construction correlation values for the control area, respectively; and

$$\sigma_{(z_1 - z_2) - (z_3 - z_4)} = \sqrt{\frac{1}{n_1 - 3} + \frac{1}{n_2 - 3} + \frac{1}{n_3 - 3} + \frac{1}{n_4 - 3}},$$

where n_i is the sample size for each of the 4 corresponding data groups (Dawson-Saunders and Trapp 1990). Finally, I used the z-value to determine the statistical significance of the difference in the correlations using a z-table.

Heterozygosity.–Heterozygosity is a measure of genetic variation across all loci and represents the number of heterozygous loci divided by the total number of examined loci (DeYoung and Honeycutt 2005). A reduction in heterozygosity may occur following population reduction (Franham 2002). If a treatment effect occurred, heterozygosity among bears on the treatment area would be lower for the post-construction phase compared with the pre-construction phase, adjusting for any changes in heterozygosity that occurred on the control area.

I calculated heterozygosity for each of the 10 loci for the 4 data groups using PopGen32 (version 1.31; Yeh et al. 1999). To compare changes in heterozygosity, I used a mixed model analysis of variance (Proc Mixed; SAS 2008). This approach was appropriate because I had replication based on the loci and heterozygosity is represented by a continuous number. I used area (treatment, control), period (pre- and post-construction), and area x period interaction as fixed terms and blocked on locus. A significant value for the area x period interaction would indicate a treatment effect.

Allelic Diversity.–Allelic diversity, the average number of alleles per locus, is commonly used to characterize genetic diversity (Frankham et al. 2002). With >1 locus, allelic diversity is the number of alleles averaged across all loci. Reduction in population size and habitat fragmentation negatively affects genetic diversity (Frankham et al. 2002, Stow and Briscoe 2005). I used program GENEALEx to determine allelic diversity before and after highway construction for each study area. I used a mixed model analysis of variance (Proc Mixed; SAS 2008) to compare changes in allelic diversity with area (treatment, control), period (pre- and post-construction), and area x period as fixed terms, while blocking on locus. A significant value for the interaction term would indicate a treatment effect.

Assignment Test.–An assignment test uses observed allele frequencies of 2 or more populations to calculate the expected frequency of an individual's genotype, thus identifying in which population the genotype is most likely to occur (Paetkau et al. 1995, Paetkau and Strobeck 1998). I used a priori grouping of bears based on whether they were sampled on the treatment or control area, north or south of the highway on the treatment area, or north and south of the fictitious highway on the control area. Because the probability of correct assignment increases when population are genetically more divergent (Manel et al. 2005), I hypothesized that an increase in the proportion of correctly assigned bears sampled after completion of the highway (after adjusting for any differences on the control area) would indicate a treatment effect.

Three commonly used assignment tests are based on distance, frequency, or likelihood methods. The likelihood method tends to be most accurate, particularly when a Bayesian approach is used (Cornuet et al. 1999, Eldridge et al. 2001). I used program Geneclass (version 1.02.01; Cornuet et al. 1999) to classify each individual according to its source population, using the leave-one-out option with the Bayesian method of computation. To test for a treatment effect, I used logistic regression (Proc Logistic; SAS 2008) to determine statistical differences in the proportion of individuals correctly classified from the north-south subpopulations for each study area and study phase. I used the proportion of correctly assigned individuals as the dependent variable and study area (treatment, control), study phase (pre- and post-construction phase), and the study area x study phase interaction as the independent variables.

F_{st}.–A common effect of population subdivision is an increase in heterozygosity in the metapopulation (Soulé 1987). However, increase in heterozygosity at that level occurs at the expense of decreasing genetic variation within subpopulations. Wright's (1965) F statistic, also known as the fixation index, provides a measure of the degree of genetic variation within and among populations (Yang 1998). The fixation index consists of several F statistics that can be used to compare genetic variation at 3 hierarchical levels: within subpopulations, among subpopulations, and within the total population (Connor and Hartl 2004). The F_{st} statistic measures the reduction in heterozygosity within subpopulations caused by random genetic drift and divergence among subpopulations compared with the overall population (Mills and Allendorf 1996, Hartl and Clark 1997):

$$F_{st} = \frac{H_T - \overline{H}_s}{H_T},$$

where H_T represents heterozygosity of the total population and $\overline{H}_s$ represents heterozygosity of the subpopulation. F_{st} values range between 0 and 1 (Lowe et al. 2004). Values from 0.05 to 0.15 indicate moderate genetic differentiation, values from 0.15 to 0.25 indicate great differentiation, and values >0.25 indicate extreme differentiation (Connor and Hartl 2004). For most species, values are $\leq$0.15, but values up to 0.7 have been documented (Connor and Hartl 2004). If the new highway caused an increase in the genetic differentiation among bears, I hypothesized that the F_{st} value for the post-construction phase would be greater compared with the pre-construction phase, using the areas north and south of the highway to define the subpopulations.

I calculated F_{st} values using program GENEPOP for the treatment and control areas for each study phase. I then determined the difference in F_{st} (ΔF_{st}) between the pre- and post-construction phase for the treatment area and the control area. Next, I calculated the difference in the ΔF_{st} values between the treatment and control areas. To test for a treatment effect, I performed a permutation test by creating 100 data sets and randomly placing individual genotypes in either the north or south portion of the study area in which they were captured. Sample sizes for each data group were the same as those of the original data. I calculated ΔF_{st} for each permutation and each time determined the difference in ΔF_{st} between the 2 study areas. Lastly, I used the ranking of my observed difference in ΔF_{st} relative to distribution of those values based on the permutations to determine the statistical probability of observing a greater difference in ΔF_{st} between the 2 study areas.

IV. RESULTS

DNA Sampling

During the pre-construction phase 686 and 1,240 hair samples were collected on the treatment and control areas, respectively. Fifty-five sample sites were visited during the first phase of the study for the treatment area ($\bar{x}$ = 29.3 visited sites/sampling period) and 64 sites were visited for the control area ($\bar{x}$ = 44.6 visited sites/sampling period; Table 1). The number of collected hair samples was substantially lower for the post-construction phase on both study areas. On the treatment area, only 95 hair samples were collected and only 21 of the 70 hair-sampling sites were visited by black bears ($\bar{x}$ = 5.1 visited sites/sampling period; Table 1). A total of 362 hair samples were collected on the control area from 56 sites ($\bar{x}$ = 24.0 visited sites/sampling period; Table 1).

Genetic Analyses

Microsatellite Analysis.–During the first phase of the study, microsatellite analysis for 30 (17%) hair samples from the treatment area and 22 (13%) samples from the control area failed because of insufficient DNA. From the 132 and 153 hair samples successfully analyzed, 53 and 92 individual genotypes were identified on the treatment and control areas, respectively. During the second study phase, genotypes could not be obtained for 43 (45%) samples from the treatment area and 54 (36%) samples from the control area because of insufficient DNA. An additional 4 samples from the treatment area were discarded because they lacked suitable material for analysis. The high failure rate for the treatment area likely was a result of sample quality. Twenty-three of the failed samples were classified as very poor quality ($\leq$2 hairs with follicles). The quality of many of the remaining failed samples was only fair (3 or 4 hairs with follicles, sometimes consisting of downy hair only). Generally, samples of such low quality would be discarded because of their high likelihood of failure during analysis. However, I felt it was important to analyze all samples because only 95 hair samples were collected. Forty-eight hair samples from the treatment area were successfully analyzed, which resulted in 16 unique genotypes. On the control area, 97 of 151 (64%) hair samples selected for analysis were successfully analyzed. Fifteen additional samples were analyzed to replace failed samples (if multiple samples were available from the same site and sampling period), resulting in 11 successful samples. Thus, 108 samples were successfully analyzed on the control area resulting in 65 unique genotypes. None of the genotypes obtained during the first phase of the study matched any of the genotypes from the second phase of the study.

Probability of Identity.–During the pre-construction phase of the study, overall PI and PI_{sibs} on the treatment area were 1.07×10^{-9} and 2.73×10^{-4}, respectively. On the control area, the overall PI and PI_{sibs} were 1.28×10^{-7} and 3.19×10^{-4}, respectively. During the post-construction phase of the study, overall PI and PI_{sibs} on the treatment area were 5.76×10^{-9} and 4.63×10^{-4}, respectively. On the control area, those values were 1.14×10^{-9} and 2.95×10^{-4}, respectively. Therefore, the probability of encountering identical genotypes based on the 10 loci was sufficiently low for mark-recapture analysis.

Hardy-Weinberg Equilibrium and Linkage Disequilibrium.– Following the Dunn-Sidak experimentwise error rate adjustment for each of the 4 data groups (2 study areas and 2 study phases), none of the loci showed evidence of nonrandom mating. For the first phase of the study, I observed linkage disequilibrium for 9 pairs of loci out of 45 comparisons after the Dunn-Sidak adjustment. On the control area, 4 of the pairwise comparisons showed linkage disequilibrium.

During the second phase of the study I observed linkage disequilibrium for 2 pairs of loci out of 45 comparisons on the treatment area and none on the control area.

Population Responses to New Highway

***Population Abundance*.**–Based on the AIC_c values and a combined AIC_c weight of 0.90, the 3 highest-ranked models all had different *p* (capture probability) for the pre- and post-construction phases (Models 1, 2, and 3; Table 2). The second-ranked model also included a behavioral response (Table 2), whereas the third-ranked model had different *p* and *c* for the 2 study areas. Models with time-dependent capture probabilities (Models 4 and 5; Table 2) were not well supported by the data.

Based on model averaging using the top 3 models, population abundance decreased on the treatment area from 68 (CI = 53–82; Table 3) before construction to 20 (CI = 14–26; Table 3) after construction, a proportional population reduction of 0.70. On the control area, population abundance decreased from 144 (CI = 106–183; Table 3) to 101 (CI = 73–130; Table 3), a proportional reduction of 0.30. The results of the permutation test indicated that the proportional decrease in abundance was greater for the treatment area compared with the control area in all but 12 permutations (99.88%), suggesting a treatment effect (*P* = 0.0012).

***Population Density*.**–Average female home ranges before highway construction were 3.86 km^2 and 2.70 km^2 for the treatment and control areas, respectively (McCollister 2008). Therefore, I circumscribed sample sites with radii of 1.1 km and 0.93 km to delineate effective sampling areas for the treatment and control areas, respectively. After the new highway was completed, the mean home-range areas for females were 7.97 km^2 for the treatment area and 7.37 km^2 for the control area (McCollister 2008), corresponding to radii of 1.59 km and 1.53 km, respectively. Because of the larger home ranges during the post-construction phase, the size of the effective sampling area increased on both study areas between the 2 study phases; that increase was proportionally similar on both study areas (Table 4). Population density decreased from 0.76 bears/ km² to 0.19 bears/ km² on the treatment area and from 1.47 bears/ km² to 0.81 bears/ km² on the control area (Table 4).

***Site Occupancy*.**–The Pearson χ^2 statistic of the global occupancy model suggested a lack of fit ($P = 0.036$) for the overall occupancy analysis. Therefore, I used QAIC_c and adjusted standard errors with $\hat{c}$. Based on genotypes of bears sampled during the first phase of the study, 33 of 131 ($\hat{c} = 131/[131-33] = 1.34$) DNA samples on the treatment area and 17 of 153 ($\hat{c} = 153/[153-17] = 1.13$) samples on the control area represented samples from individual bears that were also sampled at different sites during the same sampling occasion. For the second study phase, those frequencies were 4 of 48 ($\hat{c} = 48/[48-4] = 1.09$) samples on the treatment area and 5 of 107 ($\hat{c} = 107/[107-5] = 1.05$) samples on the control area. I used the mean $\hat{c}$ (1.15) to adjust for overdispersion.

For the overall occupancy analysis (testing for different changes in occupancy for the treatment and control areas), QAIC_c values indicated models with different detection probabilities for the 2 study areas represented the data better than those without (Table 5). The highest-ranked models suggest, for each study area, detection probabilities of the first few sampling occasions were lower compared with those of the subsequent sampling occasions (Table 5). Based on the QAICc values and the QAIC_c weights (Table 5), I modeled detection probabilities based on an asymptotic time trend ($\text{T}_{-1/y}{}^2$; Table 5). Addition of percent forest cover (pfc1 and pfc2) as covariates for detection resulted in substantial improvement of model fit

($\Delta QAIC_c = 6.26$; model 3 vs. model 4; Table 5). Also, using percent forest cover (pfc1) as a site covariate for occupancy increased model fit (model 1 vs. model 3; Table 5). Thus, I included these covariates in all models. Detection probabilities were moderate to high for the first primary sampling period (treatment area: $\bar{p}_1 = 0.53$; control area: $\bar{p}_1 = 0.69$) but decreased during the second sampling period (treatment area: $\bar{p}_2 = 0.19$; control area: $\bar{p}_2 = 0.41$). I fitted 8 models to test whether the parameters Ψ_1, ε, and γ were different for the treatment and the control area. The top four models (models 1–4; Table 6) exhibited similar model fit ($\Delta QAIC_c \leq 1.52$) and had a combined $QAIC_c$ weight of 0.93. Comparisons of models that included parameters estimates for study area (g) and models without separate estimates (.) did not indicate a difference between the treatment and the control area for Ψ_1 (models 1 vs. 3 and 2 vs. 4) and γ (models 1 vs. 2 and 3 vs. 4; Table 6). However, model selection results suggested that the extinction parameter for sites on the treatment area was different than sites on the control area (models 1 vs. 6, 2 vs.5, 3 vs. 8, and 4 vs. 7; Table 6). Model-averaging based on the 4 top models indicated that the confidence intervals for the extinction probability did not overlap for the treatment ($\varepsilon = 0.57$, 95% CI = 0.37–0.75) and control areas ($\varepsilon = 0.17$, 95% CI = 0.08–0.30). Confidence intervals based on model-averaged estimates of the colonization parameter (treatment area: $\gamma = 0.34$, 95% CI = 0.10–0.70; control area: $\gamma = 0.56$, 95% CI = 0.18–0.88) overlapped considerably. Model averaging of the real (Ψ_1) and derived (Ψ_2) parameter estimates indicated that occupancy of the treatment area was greater before highway construction ($\Psi_1 = 0.84$, CI = 0.71–0.92) compared with estimates following the completion of the highway ($\Psi_2 = 0.42$, CI = 0.25–0.60; Table 6). In contrast, estimates for the control area did not indicate a significant change using the same periods ($\Psi_1 = 0.91$, CI = 0.79–0.97 and $\Psi_2 = 0.81$, CI = 0.68–0.90, respectively).

Next, I tested if distance to the new highway was associated with the decrease in occupancy. The Pearson χ^2 statistic of the fully parameterized model indicated a lack of fit (P = 0.013). Therefore, I again used QAIC_c as the model selection criterion and adjusted standard errors by the mean inflation factor $\hat{c}$, which was 1.21 for the treatment area. The top models were fitted using an asymptotic time trend so I modeled detection probabilities using $p_i(T_{log})$. Using percent forest cover (pfc1) as a site covariate (model 1 vs. 2; Table 7) and as covariates for detection (pfc1 and pfc2; model 1 vs. 3; Table 7) resulted in improvement of model fit so I included them to account for habitat variability among sites. I fitted 4 models to test if the probability of site occupancy and extinction was a function of distance to the highway. The top three models (models 1–3; Table 8) exhibited similar model fit ($\Delta\,\text{QAIC}_c \leq 1.96$) and had a combined weight of 0.91. Model-averaging based on those 3 models indicated that the probability of site extinction was high ($\varepsilon = 0.62$, 95% CI = 0.45–0.77); site occupancy declined from $\Psi_1 = 0.81$ (CI = 0.68–0.90) before highway construction to $\Psi_2 = 0.35$ (CI = 0.22–0.51) after completion of the highway. Comparisons of models with or without the distance covariate indicated that the decrease in occupancy (models 1 vs. 2) and increase in site extinction (models 1 vs. 3) were not a function of distance from the highway (Table 8).

Genetic Responses to New Highway

Gene Flow.–The number of migrants per generation between the treatment and control areas decreased from 3.63 during the first phase of the study to 1.64 following highway construction. Between the 2 study phases, the number of migrants between the north and south portions of the treatment area decreased from 1.93 to 1.38 but increased from 5.37 to 10.46 migrants per generation for bears sampled in the north and south portions of the control area. Based on the permutation procedures, Δ*gene flow* did not differ between the treatment and control areas (*P* = 0.215).

***Isolation by Distance*.**–Isolation by distance increased on the treatment area from 0.168 prior to construction to 0.361 following highway construction. On the control area, isolation by distance decreased slightly between the 2 study phases from 0.058 to 0.052. However, I detected no difference in the change in isolation by distance on the treatment and control areas (*Z* = 0.60, *P* = 0.548).

***Heterozygosity*.**–Average heterozygosity on the treatment area increased from 0.651 (SD = 0.179) during the pre-construction phase to 0.70 (SD = 0.226) during the post-construction phase. On the control area, average heterozygosity decreased from 0.670 (SD = 0.179) to 0.6508 (SD = 0.179) between the 2 study phases. These changes in heterozygosity were not different for the 2 study areas (period x area interaction: *F* = 0.13, *P* = 0.720).

***Allelic Diversity*.**–Allelic diversity decreased on the treatment area from an average of 6.1 alleles per locus prior to construction to 5.0 after construction. Allelic diversity on the control area decreased from 6.9 to 6.3 alleles. Overall, allelic diversity was greater on the control area compared with the treatment area (*F* = 4.61, *P* = 0.040) and decreased marginally between the 2 study phases (*F* = 2.89, *P* = 0.100), but there was no evidence of a treatment effect (period x area interaction: *F* = 0.57, *P* = 0.460).

***Assignment Test*.**–Correct assignment of individual genotypes to the treatment and control areas decreased from 80.0% (116 of 145 individuals) for the pre-construction phase to

74.1% (60 of 81 individuals) for the post-construction phase. For individuals captured north and south of the highway on the treatment area, I observed a decrease in correct assignment from 92.4% (49 of 53 individuals) before construction to 62.5% (10 of 16 individuals) after construction. In contrast, correct assignment for the north and south portions of the control area changed little (pre-construction phase: 60.9% [37 of 65 individuals]; post-construction phase: 56.9% [56 of 92 individuals]). The results of the chi-square test indicated a difference in the proportion of individuals correctly assigned by study area ($P = 0.004$), phase ($P = 0.007$), and a treatment effect (study area x study phase interaction: $P = 0.027$). Between the 2 study phases, the percentage of individuals correctly assigned on the treatment area declined more compared with the control area. This finding was contrary to my research hypothesis but may have been influenced by the relatively small number of bears sampled during the second study phase on the treatment area ($n = 16$).

F_{st}.–Prior to the presence of the new highway, marginal population structure existed between areas north and south of the projected highway ($F_{st} = 0.0353$); the north and south portions of the control area exhibited no population structure ($F_{st} = 0.0038$). After completion of the highway, marginal population structure remained for the areas north and south of U.S. Highway 64 ($F_{st} = 0.0238$) with minimal structure for the areas north and south of the fictitious highway on the control area ($F_{st} = 0.0060$). The permutation test did not indicate a treatment effect ($P = 0.300$).

V. DISCUSSION

The decline in population abundance and site occupancy that I observed for the treatment area suggests that new highway impacted the black bear population. Overall site occupancy decreased more on the treatment area compared with the control area primarily because of site extinction. However, site occupancy near the highway was not reduced more compared with sites further away. Thus, the impact of the new highway may have occurred at a larger scale and reflect a population-level impact. This interpretation is supported by the microsatellite analysis, which indicated no recaptures of bears between the 2 sampling periods. This finding suggests that substantial population turnover occurred. I speculate that this turnover was primarily a function of mortality due to vehicle collisions and displacement.

Researchers have identified vehicle collisions as an important cause of death for a wide range of species, ranging from moose (*Alces alces*) in Kenai National Park in Alaska (Bangs et al. 1989), gray wolves (*Canis lupus*) in Minnesota (Fuller 1989), and white-tailed deer in New York (Sarbello and Jackson 1985). Based on telemetry data collected as part of this research project, McCollister (2008) documented that 4 of 8 bears with home ranges near the new highway were observed crossing the highway (n = 36 crossings; 1 crossing involved a wildlife underpass). All 4 bears were females of which 2 were confirmed to have died due to vehicle collisions on U.S. Highway 6 on 1 November 2007 and on 18 August 2008. Both mortalities occurred about 3.5 km west of the western underpass after highway monitoring ended. McCollister (2008) and C. Olfenbuttel (North Carolina Wildlife Resources Commission, personal communication) reported 6 additional mortalities due to vehicle collisions from May 2007 to November 2008. A subadult male tagged during my study was killed within 200 m from the eastern wildlife underpass on 5 May 2007. A yearling male was hit and killed by a vehicle on 26 May 2007 between the central and eastern underpass sites. Three bears (1 unmarked, young adult male on 15 August 2008, 1 adult male previously tagged during our study on 19 November 2008, and 1 unmarked, adult female on 26 November 2008) were killed <400 m east of the end of the fencing associated with the central underpass. Finally, an unmarked female bear was killed at approximately the same location as the 2 radio-collared females I mentioned previously on 10 September 2008. Thus, 7 of the 8 mortalities occurred within unfenced sections of the highway. Given the post-construction estimate of 21 bears on the treatment area, 8 mortalities in 19 months represent a substantial portion of the population. The post-construction data were collected 1 year following completion of the highway so these 8 mortalities would not have contributed to the population decline I observed on the treatment area. However, the mortality reports suggest that bear-vehicle collisions occur frequently and similar patterns likely occurred during the time period between completion of the highway and data collection, particularly considering that wildlife mortalities due to vehicle collisions may be underreported by as much as 10% to 88% (Conover et al. 1995, L-P Tardiff & Associates, Inc. 2003, Sielecki 2004). Thus, bear mortality due to vehicle collisions may have been an important contributing factor to the population decline

Mortality due to vehicle collisions alone, however, may not explain the estimated population decline from 68 to 20 individuals on the treatment area. Clearly, the potential impacts of a new highway begin during construction, which alters the local environment (Spellerberg 1998) and may include auditory and visual disturbances (Trombulak and Frissell 2000) that could lead to displacement. Whereas displacement may be reversible and immigration from adjacent populations may help augment the population over time, mortalities due to vehicle collisions likely will continue. However, the frequency of bear-vehicle collisions may decline with additional mitigation measures and as bears learn to use of the 3 wildlife underpasses over time (McCollister 2008).

I also considered if habitat loss and degradation due to the new highway could have contributed to the population decline and reduction in site occupancy. The new U.S. highway 64 section replaced approximately 49 ha of forest habitat within the treatment area. That area represented a small percentage of the forests present before the new highway was constructed and would not account for the change in population abundance that I observed. Based on analysis of telemetry data, habitat degradation neither seemed to be a major factor. Although McCollister (2008) detected that bears shifted their activity to times when traffic volume was lower, he did not observe any other impacts of the new highway on home ranges, movements, or habitat use. The power to observe treatment effects was limited for several of those analyses (McCollister 2008), but there was no clear evidence of a highway impact due to habitat degradation. Similarly, I did not observe that site occupancy decreased more for areas closer to the highway compared with areas further away from the highway, although the power for that analysis may have been low as well. Studies have shown that bear responses to highways vary greatly, depending on habitat quality, food availability, and road characteristics (Brody and Pelton 1989, Beringer et al. 1990). For example, researchers in Canada found that some grizzly bears (*Ursus arctos*), particularly subadult males, used areas near paved roads when abundant resources were available and human activity was low (Gibeau et al. 2002). These studies suggest that the benefits of obtaining certain resources may be greater than the risk of using areas near highways for certain sex and age groups. Those studies examined the responses of individual bears using telemetry data. My approach was somewhat different in that I tested for population-level effects. On the treatment area, much of the land consisted of productive agriculture crops near forest edges, which bears often used for foraging. I speculate that bears used those resources when the disturbance level from the highway was low rather than being displaced from areas near the highway altogether.

Population reduction was most evident on the treatment area but I also documented a decrease in population abundance on the control area. Therefore, it is important to consider if factors other than the highway could have caused different population declines on the treatment and the control areas and thus biased my findings. The decrease in abundance on both areas likely was influenced by changes in harvest management that occurred between the 2 study phases. In 2000, bear harvests on Weyerhaeuser Company lands were limited to 1 bear per hunt club and hunting methods consisted primarily of still hunting. Weyerhaeuser Company removed the harvest restrictions for the hunt clubs in 2001 and subsequently simply followed state regulations. Thus, more bears could be taken per hunt club and the use of dogs increased (B. Barber, Weyerhaeuser Company, personal communication). Of course, an important question is if harvest pressure on the treatment areas was greater during this period compared with the control area, as this could confound interpretation of my findings. I obtained harvest information for the period 2000–2005 from the North Carolina Wildlife Resources Commission and Weyerhaeuser Company for hunt clubs with hunting leases on the treatment and control areas. Because the leases were for areas of different sizes and 2 years of data were missing for the treatment area, I calculated the mean number of bears harvested per year per 1,000 ha. On the treatment area, 0.63 bears/year/1,000 ha were harvested, whereas the harvest rate for the control area was 1.11 bears/year/1,000 ha. The ratio of these estimates (0.63/1.11 = 0.57) was slightly higher than the baseline ratio of the 2000 population density estimates for the treatment and control areas (0.76/1.47 = 0.52). Although these harvest records are somewhat incomplete (not all hunt clubs and harvest mortalities were represented), these comparisons are based on records from the same hunt clubs on each study area and represent a large portion of the study areas. These observations suggest that harvesting occurred approximately in proportion to density and that the greater population decline on the treatment area compared with the control area was not primarily a function of differences in harvest pressure. Therefore, I conclude that the new highway likely was the primary cause for the decline in population abundance and overall site occupancy on the treatment area. Because I did not find that the decrease in occupancy was a function of distance from the highway and based on the findings of McCollister (2008), I speculate that the highway affected the population as a whole, rather than affecting individual bears that remained in the study area.

Besides demographic impacts of highways, the consequences of habitat loss and fragmentation may also manifest themselves genetically. The amount of genetic variation within local populations is primarily determined by a balance between gene flow from surrounding populations and genetic drift (Wright 1943). Accordingly, reduced gene flow due to potential barrier effects of highways may lead to decreased genetic variation within local populations (Kuehn et al. 2006). Another method to determine if the presence of the highway reduced genetic exchange was isolation by distance. Ohnishi et al. (2007) found that the genetic distance between Asiatic bears (*Ursus thibetanus*) separated by large geographic distance, but linked by sufficient travel corridors, was less than bears that were geographically close but separated by a natural barrier, which supports the inverse relationship between reduced gene flow and increased isolation by distance. Consequently, I expected the highway to reduce gene flow and increase isolation by distance on the treatment area by strengthening the association of geographic and genetic distance. Between the 2 study phases, gene flow increased substantially (from 5.37 to 10.46 migrants per generation) between the north and south portions of the control area. I speculate that the increased gene flow on the control area was attributable to the increase in the size of home ranges. As home ranges increase, gene flow also increases because individuals are more likely to come in contact with other individuals. Home-range sizes of large carnivores tend to be inversely related to population density, particularly for females (Sandell 1989), so this increase likely was a function of the lower population density during the post-construction phase (McCollister 2008). Although population decline on the treatment area was proportionally greater than the control area, gene flow decreased from 1.93 to 1.38 migrants per generation. However, I did not observe a treatment effect with regard to gene flow. Similarly, although isolation by distance more than doubled (from 0.168 to 0.361) following highway construction and decreased slightly on the control area (from 0.058 to 0.052), I did not detect a treatment effect. However, the power for that analysis was relatively low.

The F_{st} analyses indicated that minimal population structure existed for bears sampled on the north and south portions of either study area before or after highway construction, suggesting that little genetic differentiation exists within the study region. Despite low genetic differentiation, 92.4% of individuals captured north and south of the highway on the treatment area were correctly assigned to their source populations prior to highway construction. The percentage decreased substantially to 62.5% after construction. In contrast, correct assignment for the north and south portions of the control area decreased slightly from 60.9% to 56.9%. These findings do not support my research hypothesis. However, Manel et al. (2005) stated that a major shortcoming of assignment tests is that the probability of correct assignment does not depend solely on genetic differentiation but is also affected by population size. Therefore, this test may be inconclusive because the sample size for the post-construction sample on the treatment area was small (*n* = 16).

Genetic variation tends to be positively correlated with population size (Frankham et al. 2002, Dixon et al. 2007). Despite a drastic reduction in population abundance on both study areas, I did not observe a significant decrease in heterozygosity. Average heterozygosity during the second study phase was 70% and 65% for the treatment and control areas, respectively. Similar estimates of heterozygosity have been reported for other large black bear populations in the Southeast (66.3% for Okefenokee National Wildlife Refuge in Georgia; 67.9% for Osceola National Forest in Florida; Dobey [2002]). In contrast, Boersen et al. (2003) reported heterozygosity of 47.7% for the Tensas River National Wildlife Refuge in Louisiana, whereas Edwards (2002) reported 31.6% heterozygosity for bears sampled in the Mobile River area in Alabama. These latter 2 populations likely experienced substantial loss of genetic variation because of their small size and long-term isolation from other populations. If the new highway increased isolation of the populations to its north and south, the temporal scale of my study likely was too short to detect such changes. Following population reduction or isolation, allelic diversity is expected to decrease more rapidly than heterozygosity (Stow and Briscoe 2005). However, I did not observe a treatment effect for allelic diversity either. In small, isolated populations, inbreeding may occur as a random event because of a limited number of available breeding individuals (Shikano et al. 2001). One possible effect of inbreeding is an increase of homozygotes in the population. When deleterious alleles are recessive, inbreeding exposes those alleles in homozygotes, which can decrease the fitness of a population (Shikano et al. 2001). However, the results of my study suggest that the new highway did not reduce gene flow. In the short term, genetic variation has not been reduced and inbreeding is not a likely threat to the population.

The 3 wildlife underpasses may have mitigated a reduction in gene flow. Kindall and van Manen (2007) identified the western and eastern underpasses as important habitat linkages within the treatment area and with populations outside the study area. The western underpass connects Big Swamp (Bull Neck) to the control area and on to the Roanoke River, whereas the eastern underpass connects Big Swamp to the Pocosin Lakes National Wildlife Refuge and additional habitat southeast of the treatment area. Based on 1 year of monitoring with remote cameras, track surveys, and radio telemetry, black bears used the 3 underpasses on 17 occasions, likely representing at least 10 different individuals (McCollister 2008). Given that the second study phase started within a year of completion of the highway, McCollister (2008) suggested that a learning period may exist and that use of the underpasses by black bears likely will increase over time. Mills and Allendorf (1996) described the 1 migrant per generation rule for isolated populations, which states that only 1 new migrant (of either sex) into a population is needed per generation to prevent the loss of heterozygosity and polymorphism. Similarly, Miller and Waits (2003) suggested that 2 functional migrant bears per generation are needed for grizzly bear populations to maintain genetic connectivity and resist loss of genetic diversity due to inbreeding. Assuming that similar, low rates apply to black bear populations, bear use of the underpasses seemed sufficient to prevent the impacts of inbreeding in the highway project area.

VI. MANAGEMENT AND RESEARCH IMPLICATIONS

Although I did not detect any short-term genetic consequences, my findings indicate that a 4-lane, divided highway can affect black bear population abundance and occupancy. Highly mobile species that move frequently and over large distances are more likely to come in contact with highways, thus increasing the probability of mortality due to vehicle collisions (Carr and Fahrig 2001). Although not common, such mortalities have been identified as a serious long-term threat for several species such as the Florida black bear (*U. a. floridanus*), Florida panther (*Puma concolor*), and Key deer (*Odocoileus virginianus clavium*; Harris and Scheck 1991 cited in Forman et al. 2003). The results from my study indicate that vehicle collisions may have been an important cause of the reduction in population abundance on the treatment area. Because extinction is a demographic process (Allendorf and Ryman 2002), increased mortality due to new interstate or U.S. highways could impact the viability of small or declining bear populations. Although demographic exchange from adjacent populations may mitigate the impacts of the new highway in the long term, my study indicates that the construction of 4-lane highways could be detrimental to small, isolated bear populations. Clearly, for threatened or endangered populations, it would be important to include the option of no highway construction during the transportation planning process.

When populations experience habitat fragmentation and reductions in abundance, maintaining connectivity to other subpopulations may be crucial to ensure sustainability of the metapopulation by providing opportunities for exchange of demographic migrants among its subpopulations or colonization of extirpated areas (Noss et al. 1996), particularly for females. Because dispersal distances of female black bears are small (Rogers 1987), bridging larger distances between subpopulations or habitat areas will require true habitat linkages. Such habitat linkages should be of sufficient size and quality so that female bears can gradually move through the linkages over time with a low risk of mortality, particularly in highly anthropogenic landscapes.

Although demographic linkage is the more important consideration for small populations, habitat linkages can also facilitate genetic exchange among populations. Research in north-central Florida indicated that a habitat corridor connecting black bear populations centered on Osceola and Ocala National Forests was frequently used and provided a conduit for gene flow between populations that would otherwise be isolated (Dixon et al. 2007). The number of bears that used the 3 underpasses in my study (17 documented crossings of approximately 10 bears; McCollister 2008) likely was sufficient enough to facilitate gene flow within the treatment area and with other black bear populations in the Albemarle-Pamlico peninsula (Kindall and van Manen 2007). Therefore, wildlife underpasses are an important mitigation technique when genetic and demographic connectivity of black bear populations is considered a priority.

In addition to maintaining connectivity between populations, the wildlife underpasses likely reduced the number of bears that crossed the roadway and ultimately reduced mortality due to vehicle collisions. However, the number of fatal bear-vehicle collisions documented since the completion of the highway indicates that bears are not using the underpasses exclusively to cross the highway. These bear-vehicle collisions often occurred within unfenced sections of the highway. Potential improvements to the wildlife crossing structures that may increase the effectiveness of the underpasses include placing barbed-wire outriggers at the top of the fencing and burying the fencing to deter bears from climbing over or pushing under the fence (Clevenger et al. 2001, McCollister 2008). Continuous fencing between underpasses may reduce the number of bears crossing the highway and direct their movements towards the underpasses.

Several studies have found a positive correlation between driver speed and the frequency of wildlife-vehicle collisions (Allen and McCullough 1976, Case 1978, Rolley and Lehman 1992). The use of wildlife warning signs is relatively inexpensive and has been used to reduce driver speed and increase driver awareness. However, static wildlife warnings signs often are ignored by drivers and may not be effective over time (Putman 1997, Sullivan and Messmer 2003). In contrast, Hardy et al. (2006) found that wildlife advisory messages displayed on portable dynamic message signs (DMS) reduced driver speed and were more effective than static warning signs. Therefore, I suggest placing portable DMS in areas and at times when frequent bear-vehicle collisions have been documented previously.

This study was designed to determine the short-term impact of the new 4-lane highway on black bears. Although I observed demographic impacts of the new highway, the short time period between the 2 study phases may not have been sufficient to detect any genetic response. Such genetic impacts may take several generations to manifest themselves, particularly for species with long generation intervals. Using the genetic analyses I present here as a baseline, future genetic sampling could provide valuable information regarding potentially long-term impacts of the highway on this black bear population.

LITERATURE CITED

Adams, L. W., and A. D. Geis. 1983. Effects of roads on small mammals. Journal of Applied Ecology 20:403–415.

Allen, R. E., and D. R. McCullough. 1976. Deer-car accidents in southern Michigan. Journal of Wildlife Management 40:317-325.

Allendorf, F. W., and N. Ryman. 2002. The role of genetics in population viability analysis. Pages 50–85 *in* S. R Beissinger and D. R. McCullough, editors. Population viability analysis. The University of Chicago Press, Chicago, Illinois, USA.

Bangs, E. E., T. N. Bailey, and M. F. Portner. 1989. Survival rates of adult female moose on the Kenai Peninsula, Alaska. Journal of Wildlife Management 53:557–563.

Baker, A. J. 2000. Molecular methods in ecology. Blackwell Science, Oxford, United Kingdom.

Barton, N. H., and M. Slatkin. 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. Heredit*y* 56:409–15.

Beringer, J. J., S. G. Seibert, and M. R. Pelton. 1990. Incidence of road crossing by black bears on Pisgah National Forest, North Carolina. International Conference on Bear Research and Management 8:85–92.

Bonin, A., E. Bellemain, P. B. Eidesen, F. Pompanon, C. Brochmann, and P. Taberlet. 2004. How to track and assess genotyping errors in population genetics studies. Molecular Ecology 13:3261–3273.

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.

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.

Broquet, T., N. Ray, E. Petit, J. M. Fryxell, and F. Burel. 2006. Genetic isolation by distance and landscape connectivity in the American Marten (*Martes americana*). Landscape Ecology 21:877–889.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodal inference: a practical information-theoretic approach. Second edition. Springer-Verlag, New York, New York, USA.

Cain, A. T., V. R. Tuovila, D. G. Hewitt, and M. E. Tewes. 2003. Effects of a highway and mitigation projects on bobcats in Southern Texas. Biological Conservation 114:189–197.

Carr, L. W., and L. Fahrig. 2001. Effect of road traffic on two amphibian species of differing vagility. Conservation Biology 15:1071–1078.

Case, R. M. 1978. Interstate highway road-killed animals: a data source for wildlife biologists. Wildlife Society Bulletin 6:8–13.

Cegeleski, C. C., L. P. Waits, and N. J. Anderson. 2003. Assessing population structure and gene flow in Montana wolverines (*Gulo gulo*) using assignment-based approaches. Molecular Ecology 12:2907–2918.

Chao, A. 2001. An overview of closed capture-recapture models. Journal of Agricultural, Biological, and Environmental Statistics 6:158–175.

Clevenger, A. P., and N. Waltho. 2000. Factors influencing the effectiveness of wildlife underpasses in Banff National Park, Alberta, Canada. Conservation Biology 14:47–56.

Clevenger, A. P., B. Chruszcz, and K. Gunson. 2001. Highway mitigation fencing reduces wildlife-vehicle collisions. Wildlife Society Bulletin 29:646–653.

Connor, K., and D. L. Hartl. 2004. Primer of ecological genetics. Sinauer Associates, Sunderland, Massachusetts, USA.

Conover, M. R., W. C. Pitt, K. K. Kessler, T. J. DuBow and W. A. Sanborn. 1995. Review of human injuries, illnesses and economic losses caused by wildlife in the United States. Wildlife Society Bulletin 23:407–414.

Cooch, E., and G. White. 2001. Program MARK: Analysis of data from marked individuals, a gentle introduction, Second Edition. Cornell University and Colorado State University, Ithaca, New York, and Fort Collins, Colorado, USA

Cornuet, J. M., S. Piry, G. Luikart, A. Estoup, and M. Solignac M. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989–2000.

Dawson-Saunders, B, and R. G. Trapp. 1990. Basic and clinical biostatistics. Appleton and Lange, San Mateo, California, USA.

DeYoung. R. W., and R. L. Honeycutt. 2005. The molecular toolbox: genetic techniques in wildlife ecology and management. Journal of Wildlife Management 69:1362–1384.

Dixon, J. D., M. K. Oli, M. C. Wooten, T. H. Eason, J. W. McCown, and M. W. Cunningham. 2007. Genetic consequences of habitat fragmentation and loss: the case of the Florida black bear (*Ursus americanus floridanus*). Conservation Genetics 8:455–464.

Dobey, S. T. 2002. Abundance and density of Florida black bears in the Okefenokee National Wildlife Refuge Osceola National Forest. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Edwards, A. S. 2002. Ecology of the black bear (*Ursus Americans floridanus*) in southwestern Alabama. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Eldridge, M. D., J. E. Kinnear, and M. L. Onus. 2001. Source populations of dispersing rock-wallabies (*Petrogale lateralis*) identified by assignment tests on multilocus genotypic data. Molecular Ecology 10:2867–2876.

Elzinga, C. L., D. W. Salzer, J. W. Willoughby, and J. P. Gibbs. 2001. Monitoring Plant and Animal populations. Blackwell Science, Oxford, United Kingdom.

Evink, G. 2002. Interaction between roadways and wildlife ecology: a synthesis of highway practice. National Cooperative Highway Research Program, Synthesis 305. Transportation Research Board, Washington, D.C., USA.

Fahrig, L., and T. Rytwinski. 2009. Effects of roads on animal abundance: an empirical review and synthesis. Ecology and Society 14: 21 <http://www.ecologyandsociety.org/vol14/iss1/art21/>. Accessed 10 April 2009.

Fecske, D. M., R. E. Barry, F. L. Precht, H. B. Quigley, S. L. Bittner, and T. W. Webster. 2002. Habitat use by female black bears in western Maryland. Southeastern Naturalist 1:77–92.

Fisher, R. A. 1921. On the `probable error' of a coefficient of correlation deduced from a small sample. Metron 1:3–32.

Forman, R. T., and L. E. Alexander. 1998. Roads and their major ecological effects. Annual Review Ecological Systems 29:207–232

Forman, R. T. T., D. Sperling, J. A. Bissonette, A. P. Clevenger, C. D. Cutshall, V. H. Dale, L. Fahrig, R. France, C. R. Goldman, K. Heanue, J. A. Jones, F. J. Swanson, T. Turrentine, and T. C. Winter. 2003. Road Ecology: Science and Solutions. Island Press, Washington, D.C., USA.

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

Frankham, R. J. 1996. Relationship of genetic variation to population size in wildlife. Conservation Biology 10:1500–1508.

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge, United Kingdom.

Fuller, T. 1989. Population dynamics of wolves in north-central Minnesota. Wildlife Monographs 101.

Garner, N. P. 1986. Seasonal movements, habitat selection, and food habits of black bears (*Ursus americanus*) in Shenandoah National Park, Virginia. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, USA.

Gibbs, J. P., and W. G. Shriver. 2002. Estimating the effects of road mortality on turtle populations. Conservation Biology 16:1647–1652.

Gibeau, M. L., A. P. Clevenger, S. Herrero, and J. Wierzchowski. 2002. Grizzly bear response to human development and activities in the Bow River watershed, Alberta. Biological Conservation 103:227–236.

Green, R. H. 1979. Sampling design and statistical methods for environmental biologists. Wiley, New York, New York, USA.

Guo, S. W., and E. A. Thompson. 1992. A Monte Carlo method for combined segregation and linkage analysis. American Journal of Human Genetics 51:1111–1126.

Hardy, A., S. Lee, and A. F. Al-Kaisy. 2006. Effectiveness of animal advisory messages on dynamic message signs as a speed reduction tool. Transportation Research Record 1973:64–72.

Harris, L. D., and J. Scheck. 1991. From implications to applications: the dispersal corridor principle applied to the conservation of biological diversity. Pages 189–220 *in* D. A Saunders and R. J. Hobbs, editors. Nature Conservation 2: the role of corridors. Surrey Beatty, Chipping Norton, Australia. Cited in Forman et al. (2003).

Hartl, D. L., and A. G. Clark. 1997. Principles of population genetics. Third edition. Sinauer Associates, Sunderland, Massachusetts, USA.

Hedlund, J. H., P. D. Curtis, G. Curtis, and A. F. Williams. 2003. Methods to reduce traffic crashes involving deer: what works and what does not. Insurance Institute for Highway Safety, Arlington, Virginia, USA.

Homer, C., J. Dewitz, J. Fry, M. Coan, N. Hossain, C. Larson, N. Herold, A. McKerrow, J. N. VanDriel, and J. Wickman. 2007. Completion of the 2001 national land cover database for the conterminous United States. Photographic Engineering and Remote Sensing 337–341.

Jensen, J. L., A. J. Bohonak, and S. T. Kelley. 2005. Isolation by distance, Web Service, Version 3.14. BMC Genetics. < http://ibdws.sdsu.edu/>. Accessed 7 November 2008.

Jones, M. F., D. Paetkau, E. Geffen, and C. Moritz. 2004. Genetic Diversity and population structure of Tasmanian devils, the largest marsupial carnivore. Molecular Ecology 13:759–778.

Jones, M. D., F. T. van Manen, T. W. Wilson, and D. R. Cox. 2009. Establishing effective wildlife underpasses on U.S. Highway 64 in North Carolina: integrating management and science objectives. *In* J .P. Beckmann, T. Clevenger, M. Huijser, J. Hilty, and R. Ament, editors. Safe passages: highways, wildlife and habitat connectivity. Island Press, Washington, D.C., USA. (in press).

Kendall, M. G., and M. A. Stuart. 1977. The advanced theory of statistics. Volume 1. Distribution theory. 4th edition. Charles Griffin, London, United Kingdom.

Kindall, J. L. 2004. Spatial ecology of black bears prior to construction of a 4-lane highway in eastern North Carolina. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

Kindall, J. L., and F. T. van Manen. 2007. Identifying habitat linkages for black bears in eastern North Carolina. Journal of Wildlife Management 71:487–495.

Kuehn, R., K. E. Hindenlan, O. Holzgang, J. Senn, B. Stoeckle, and C. Sperisen. 2006. Genetic effect of transportation infrastructure on roe deer populations (*Capreolus capreolus*). Journal of Heredity 98:13–22.

Lande, R. 1988. Genetics and demography in biological conservation. Science 241:1455–1460.

Lodé, T. 2000. Effect of a motorway on mortality and isolation of wildlife populations. Ambio 29:163–166.

Lowe, A., S. Harris, and P. Ashton. 2004. Ecological genetics: design, analysis and application. Blackwell Publishing, Oxford, United Kingdom.

L-P Tardiff and Associates, Inc. 2003. Collisions involving motor vehicle and large animals in Canada. Final Report to Transport Canada Road Safety Directorate, Nepean, Ontario, Canada.

MacKenzie, D. I., and L. L. Bailey. 2004. Assessing the fit of site-occupancy models. Journal of Agricultural, Biological, and Environmental Statistics 9:300-318.

MacKenzie, D. I., J. D. Nichols, J. A. Royle, K. H. Pollock, L. L. Bailey, and J. E. Hines. 2006. Occupancy estimation and modeling. Academic Press, Burlington, Massachusetts, USA.

Manel, S., O. E. Gaggiotti, and R. S. Waples. 2005. Assignment methods: matching biological questions with appropriate techniques. Trends in Ecology and Evolution 20:136–142.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.

Mazerolle, M. J. 2004. Amphibian road mortality in response to nightly variations in traffic intensity. Herpetologica 60:45–53.

McCollister, M. F. 2008. Impacts of a 4-lane highway on the spatial ecology of black bears and the effectiveness of wildlife underpasses in eastern North Carolina. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

McCullagh, P., and J. A. Nelder. 1989. Generalized Linear Models. Chapman and Hall, New York, USA.

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

Miller, C. R., P. Joyce, and L. P.Waits. 2002. Assessing allelic dropout and genotype reliability using maximum likelihood. Genetics 160:357–366.

Miller, C. R., and L. P. Waits. 2003. The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): implication for conservation. Proceedings of the National Academy of Sciences 100:4334–347.

Mills, L. S., and F. W. Allendorf. 1996. The one-migrant-per-generation rule in conservation and management. Conservation Biology 10:1509–1518.

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.

Newmark, W. D., J. I. Boshe, H. I. Sariko, and G. K. Makumbule. 1996. Effects of a highway on large mammals in Mikumi National Park, Tanzania. Africa Journal of Ecology 34:15–31.

Ng, J. S., J. W. Dole, R. M. Sauvajot, S. P. Riley, and T. J. Valone. 2004. Use of highway underpasses by wildlife in southern California. Biological Conservation 115:499–507.

Noss, R. F., H. B. Quigly, M. G. Hornocker, T. Merrill, and M. K. Oli. 1996. Conservation biology and carnivore conservation in the Rocky Mountains. Conservation Biology 10:949–963.

Ohnishi, N., S. Takashi, I. Yasuyuki, and T. Oi. 2007. Low genetic diversities in isolated populations of the Asian black bear (*Ursus thibetanus*) in Japan, in comparison with large stable populations. Conservation Genetics 8:1331–1337.

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

Paetkau, D., and C. Strobeck. 1994. Microsatellite analysis of genetic variation in black bears.

Molecular ecology 4:347–354.

Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 4:347–354.

Paetkau, D., and C. Strobeck. 1998. Ecological genetic studies of bears using microsatellite analysis. Ursus 10:299–306.

Peakall, R, and P. E. Smouse. 2006. Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology 6:288–295.

Powell, R. A. 2000. Animal home ranges and territories and home range estimators. Pages 65–110 *in* L. Boitani and T. K. Fuller, editors. Research techniques in animal ecology: controversies and consequences. Columbia University, New York, New York, USA.

Press, W. , S. Teukolsky , W. Vetterling , and B. Flannery . 1992. Numerical recipes in C: the art of scientific computing. 2nd edition. Cambridge University Press, Cambridge, UK.

Putman, R. J. 1997. Deer and road traffic accidents: options for management. Journal of Environmental Management 51:43–57.

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

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

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.

Reijnen, R., R. Foppen, C. Braak, and J. Thissen. 1995. The effects of car traffic on breeding bird populations in woodland. III. Reduction of density in relation to the proximity of main roads. Journal of Applied Ecology 32:187–202.

Rogers, L. L. 1987. Effects of food supply and kinship on social behavior, movements, and population growth of black bears in northeastern Minnesota. Wildlife Monographs 97.

Rolley, R. E., and L. E. Lehman . 1992. Relationships among raccoon road-kill surveys, harvests, and traffic. Wildlife Society Bulletin 20:313–318.

Romin, L. A., and J. A. Bissonette. 1996. Temporal and spatial distribution of highway mortality of mule deer on newly constructed roads at Jordanelle Reservoir, Utah. Great Basin Naturalist 56:1–11.

Roof, J. C., and J. B. Wooding. 1996. Evaluation of S.R. 46 Wildlife Crossing. Florida Cooperative Fish and Wildlife Research Unit Technical Report #54, Gainesville, Florida, USA.

Ruediger, B. 1998. Rare carnivores and highways: moving into the 21st century. Pages 10–16 *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.

Sandell, M. 1989. The mating tactics and spacing patterns of solitary carnivores. Pages 164–182 *in* J. L. Gittleman, editor. Carnivore behaviour, ecology, and evolution. Cornell University Press, Ithaca, New York, USA.

Sarbello, W., and L. W. Jackson. 1985. Deer mortality in the town of Malone. New York Fish and Game Journal 32:141–157.

SAS Institute, Inc. 2008. SAS/Stat Users Guide, Version 9.2. Cary, North Carolina, USA.

Scheick, B. K., and M. D. Jones. 1999. Locating wildlife underpasses prior to expansion of highway 64 in North Carolina. Florida Department of Transportation, Tallahassee, Florida, USA.

Shikano, T., T. Chiyokubo, and N. Taniguchi. 2001. Temporal changes in allele frequency, genetic variation and inbreeding depression in small populations of the guppy (*Poecilia reticulate*). Heredity 86:153–160.

Sielecki, L. E. 2004. WARS 1983-2002 - Wildlife accident reporting and mitigation in British Columbia: special annual report. Ministry of Transportation, Engineering Branch. Environmental Management Section. Victoria, British Columbia, Canada.

Simberloff, D. 1999. Biodiversity and bears–a conservation paradigm shift. Ursus 11:21–28.

Siramie, C., L. Brotons, and J. L. Martin. 2008. Spatial extent of bird species response to landscape changes: colonisation/extinction dynamics at the community-level in two contrasting habitats. Ecography 31:509–518.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

Sokal, R. R,. and F. J. Rohlf. 1995. Biometry. 3rd edition W. H. Freeman and Company, San Francisco, California, USA.

Soulé, M. E. 1987. Viable Populations for Conservation, Cambridge University Press, Cambridge, United Kingdom.

Spellerberg, I. F. 1998. Ecological effects of roads and traffic: a literature review. Global Ecology and Biogeography Letters 7:317–333.

Stow, A. J., and D. A. Briscoe. 2005. Impact of habitat fragmentation on allelic diversity at microsatellite loci in Cunningham’s skink (*Egernia cunninhami*); a preliminary study. Conservation Genetics 6:455–459.

Sullivan, T. L., and T. A. Messmer. 2003. Perceptions of deer-vehicle collision management by state wildlife agency and department of transportation administrators. Wildlife Society Bulletin 31:63–73.

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

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

Thompson, L. M., F. T. van Manen, and T. L. King. 2005. Geostatistical analysis of allele presence patterns among American black bears in eastern North Carolina. Ursus 16:59–69.

Trombulak, F. C., and C. Frissell. 2000. Review of ecological effects of roads on terrestrial and aquatic communities. Conservation Biology 14:19–29.

U.S. Census Bureau. 2002. United States Census 2000. U.S. Census Bureau, Washington, D.C., USA. http://www.census.gov/main/www/cen2000.html. Accessed 2 October 2008.

van Manen, F. T., M. D. Jones, J. L. Kindall, L.M. Thompson, and B. K. Scheick. 2001.

Determining the potential mitigation effects of wildlife passageways on black bears. Proceedings of the International Conference on Wildlife Ecology, Center for Transportation and the Environment, Raleigh, North Carolina, USA.

Waits, L. P., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10:249–256.

Weir, B. S. 1990. Genetic data analysis. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.

White, G.C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46:120–138.

Wilson, K. R., and D. R. Anderson. 1985. Evaluation of two density estimators of small mammal population size. Journal of Mammalogy 66:13–21.

Wright, S. 1943. Isolation by distance. Genetics 28:114–138.

Wright, S. 1946. Isolation by distance under diverse systems of mating. Genetics 31:39–59.

Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395–420.

Yang, R. C. 1998. Estimating hierarchical F-statistics. Evolution 52:950–956.

Yeh, F. C., R. Yang, and T. Boyle. 1999. PopGene version 1.32. Microsoft Windows-based freeware for population genetic analysis. University of Alberta, Edmonton, Alberta, Canada.

APPENDICES

APPENDIX A: TABLES

Table 1. Number of hair-sample sites visited, samples collected, and samples analyzed to determine short-term impacts of a new section of U.S. Highway 64 on black bears, Washington County, North Carolina. Sampling of black bear hair occurred on a treatment area and a control area, before (pre-construction phase; 2000) and after (post-construction phase; 2006) construction of the new highway.

Study area, study phase	**Sampling occasion**	**Number of sample sites visited**	**Number of samples collected**	**Number of samples selected for analysis**
Treatment, pre-construction	1	20	61	17
	2	26	105	21
	3	30	78	25
	4	33	94	21
	5	31	108	25
	6	34	123	28
	7	31	117	26
	Subtotal	55[a]	686	163
	Average	29.3	98.0	23.3
Control, pre-construction	1	39	150	25
	2	45	171	25
	3	43	147	25
	4	47	164	25
	5	44	209	25
	6	47	198	25
	7	47	201	25
	Subtotal	64[a]	1,240	175
	Average	44.6	177.1	25.0

Table 1. (Cont.).

Study area, study phase	Sampling occasion	Number of sample sites visited	Number of samples collected	Number of samples selected for analysis
Treatment, post-construction	1	5	9	9
	2	3	4	4
	3	9	23	23
	4	4	10	10
	5	3	13	13
	6	6	20	20
	7	6	16	16
	Subtotal	21[a]	95	95
	Average	5.1	13.6	13.6
Control, post-construction	1	10	10	10
	2	33	81	25
	3	27	57	25
	4	27	57	25
	5	29	72	25
	6	24	47	23
	7	18	38	18
	Subtotal	56[a]	362	151
	Average	24.0	51.7	21.6

[a] Total number of hair-sample sites visited by black bears out of 70 sites.

Table 2. Summary of model selection procedures based on Akaike's Information Criteria (AIC_c) to determine the parameterization of closed-capture models to estimate black bear population abundance before and after completion of a new section of U.S. highway 64, Washington County, North Carolina, USA. Capture probabilities (*p*) were modeled for a treatment and a control study area (area), study phases (phase; pre-construction [2000] and post-construction [2006]), and different combinations of sampling occasion (t) and behavioral response (indicated by different recapture probability *c*) to recapture.

Model no. and model	**AIC_c**	**ΔAIC_c[a]**	**AIC_c weight**	**Model likelihood**	**Number of parameters**	**Deviance**
1. $p_{\text{phase(g),area(.)}}$	156.19	0.00	0.45	1.00	6	341.52
2. $p_{\text{phase(g),area(.)}}$, c	157.13	0.94	0.28	0.62	7	340.45
3. $p_{\text{phase(g),area(g)}}$	158.09	1.90	0.17	0.39	7	341.40
4. $p_{\text{phase(g),area(g)}}$, c	159.03	2.84	0.10	0.24	8	340.32
5. $p_{\text{phase(g,t), area(t)}}$, c	161.11	4.92	0.03	0.09	13	332.26
6. $p_{\text{phase(g,t),area(t)}}$	161.94	5.75	0.02	0.06	12	335.13
7. $p_{\text{phase(.), area(g)}}$	162.33	6.14	0.02	0.05	6	347.66
8. $p_{\text{phase(g,t,g*t), area(g,t,g*t)}}$	168.10	11.91	0.00	0.00	46	270.67

[a] Difference in AIC_c compared with lowest AIC_c model.

Table 3. Estimates of black bear population abundance for a treatment and control area and pre- (2000) and post-construction (2006) study phases to determine the short-term impacts of a new section of U.S. Highway 64 on black bears, Washington County, North Carolina.

Study area, study phase	**Population abundance**	**95% confidence interval**	**Standard error**
Treatment, pre-construction	68	53–82	7.41
Control, pre-construction	144	106–183	19.50
Treatment, post-construction	20	14–26	3.03
Control, post-construction	101	73–130	14.56

Table 4. Estimates of effective study area, population abundance, and density for a treatment and control area and pre-(2000) and post-construction (2006) study phases to determine the short-term impacts of a new section of U.S. Highway 64 on black bears, Washington County, North Carolina.

Study area, study phase	**Effective study area (km^2)**	**Population abundance (*N*)**	**Density (bears/km ^{2})**
Treatment, pre-construction	89.7	68	0.76
Control, pre-construction	97.6	144	1.47
Treatment, post-construction	108.9	21	0.19
Control, post-construction	124.1	101	0.81

Table 5. Summary of model selection procedures based on Akaike's Information Criteria (QAIC$_c$) to determine the parameterization of detection probabilities for occupancy models of black bears, treatment and control areas, Washington County, North Carolina, USA. A study area effect (g; treatment or control) was included for estimation of occupancy (Ψ), extinction (ε), and colonization (γ) parameters; percent forest cover within a 270-m radius of the sample sites during the first study phase (pfc1) was used as a site covariate in several models. Detection probabilities (p) were modeled for the first (p_1; before construction of U.S. Highway 64 [2000]) and second (p_2; after construction of U.S. Highway 64 [2006]) primary sampling periods and additive or interaction terms as a function of sampling occasion (t), linear time trend (T_{lin}), transformed time trends (T_{log} and $T_{-1/y}^2$), study area (g), and percent forest cover within a 270-m radius of the sample sites for the first (pfc1) and the second (pfc2) primary sampling period. A $\hat{c}$ adjustment of 1.15 was applied to all models.

Model no. and model[a]	**QAIC$_c$**	**ΔQAIC$_c$**[b]	**w_i**[c]	**Model likelihood**	**K**[d]
1. Ψ_1(pfc1,g) ε(g) γ(g) p_1(pfc1,g,$T_{-1/y}^2$) p_2(pfc2,g,$T_{-1/y}^2$)	1,770.00	0	0.92	1	15
2. Ψ_1(pfc1,g) ε(g) γ(g) p_1(g,$T_{-1/y}^2$) p_2(g,$T_{-1/y}^2$)	1,775.83	5.83	0.05	0.05	13
3. Ψ_1(g) ε(g) γ(g) p_1(pfc1,g,$T_{-1/y}^2$) p_2(pfc2,g,$T_{-1/y}^2$)	1,776.87	6.86	0.03	0.03	14
4. Ψ_1(g) ε(g) γ(g) p_1(g,$T_{-1/y}^2$) p_2(g,$T_{-1/y}^2$)	1,783.12	13.12	0	0	12
5. Ψ_1(g) ε(g) γ(g) p_1(g,$T_{-1/y}^2$,g*$T_{-1/y}^2$) p_2(g,$T_{-1/y}^2$,g*$T_{-1/y}^2$)	1,785.12	15.12	0	0	14
6. Ψ_1(g) ε(g) γ(g) p_1(g,T_{log}) p_2(g,T_{log})	1,790.32	20.32	0	0	12
7. Ψ_1(g) ε(g) γ(g) p_1(g,T_{log},g*T_{log}) p_2(g,T_{log},g*T_{log})	1,793.95	23.95	0	0	14
8. Ψ_1(g) ε(g) γ(g) p_1(g,T_{lin}) p_2(g,T_{lin})	1,794.29	24.29	0	0	12
9. Ψ_1(g) ε(g) γ(g) p_1(g) p_2(g)	1,797.56	27.55	0	0	10
10. Ψ_1(g) ε(g) γ(g) p_1(g,T_{lin},g*T_{lin}) p_2(g,T_{lin},g*T_{lin})	1,797.00	27.99	0	0	14
11. Ψ_1(g) ε(g) γ(g) p_1(g,t) p_2(g,t)	1,798.04	28.03	0	0	22
12. Ψ_1(g) ε(g) γ(g) p_1(g*t) p_2(g*t)	1,811.95	41.94	0	0	34
13. Ψ_1(g) ε(g) γ(g) p_1(.) p_2(.)	1,828.99	58.98	0	0	8
14. Ψ_1(g) ε(g) γ(g) p_1(t) p_2(t)	1,829.70	59.69	0	0	20

[a] Ψ_1 is the occupancy estimate for the first primary sampling period; ε is the probability of a site occupied during the first primary sampling period becoming unoccupied during the second primary sampling period; γ is the probability of a site unoccupied during the first primary sampling period becoming occupied during the second primary sampling period.

[b] Difference in QAIC$_c$ compared with lowest QAIC$_c$ model.

[c] QAIC$_c$ model weight.

[d] Number of parameters in model.

Table 6. Summary of model selection procedures using Akaike's Information Criteria ($QAIC_c$) to test hypotheses regarding the impacts of a new highway on occupancy of black bears, treatment and control areas, Washington County, North Carolina, USA. Detection probabilities were modeled for the first (p_1; before construction of U.S. highway 64 [2000]) and second (p_2; after construction of U.S. Highway 64 [2006]) primary sampling periods and as a function of study area (g; treatment area, control area). Models reflect hypotheses regarding occupancy (Ψ), extinction (ε), and colonization (γ) to test for differences between the treatment and control areas and the 2 primary sampling periods. Percent forest cover for the first study phase (pfc1) within a 270-m radius of the sample sites was used as a site covariate for occupancy; percent forest cover for the first (pfc1) and the second (pfc2) primary sampling period were used as detection covariates. A $\hat{c}$ adjustment of 1.15 was applied to all models.

Model no. and model[a]	**$QAIC_c$**	**ΔAIC_c**[b]	**w_i**[c]	**Model likelihood**	**K**[d]	**$\Psi_{1(\mathrm{treatment})}$ (SE)**[d]	**$\Psi_{2(\mathrm{treatment})}$ (SE)**[e]	**$\Psi_{1(\mathrm{control})}$ (SE)**[d]	**$\Psi_{2(\mathrm{control})}$ (SE)**[e]
1. Ψ_1(pfc1,g) ε(g) γ(g) p_1(pfc1,g,$T_{-1/y}^{2}$) p_2(pfc2,g,$T_{-1/y}^{2}$)	1,770.00	0	0.32	1	15	0.82 (0.05)	0.40 (0.08)	0.93 (0.03)	0.83 (0.05)
2. Ψ_1(pfc1,g) ε(g) γ(.) p_1(pfc1,g,$T_{-1/y}^{2}$) p_2(pfc2,g,$T_{-1/y}^{2}$)	1,770.05	0.05	0.31	0.98	14	0.82 (0.05)	0.44 (0.09)	0.93 (0.03)	0.81 (0.05)
3. Ψ_1(pfc1) ε(g) γ(g) p_1(pfc1,g,$T_{-1/y}^{2}$) p_2(pfc2,g,$T_{-1/y}^{2}$)	1,771.45	1.45	0.15	0.49	14	0.88 (0.03)	0.40 (0.08)	0.88 (0.03)	0.82 (0.05)
4. Ψ_1(pfc1) ε(g) γ(.) p_1(pfc1,g,$T_{-1/y}^{2}$) p_2(pfc2,g,$T_{-1/y}^{2}$)	1,771.53	1.52	0.15	0.47	13	0.88 (0.03)	0.44 (0.09)	0.88 (0.03)	0.79 (0.05)
5. Ψ_1(pfc1,g) ε(.) γ(.) p_1(pfc1,g,$T_{-1/y}^{2}$) p_2(g,$T_{-1/y}^{2}$)	1,774.71	4.71	0.04	0.10	13	0.82 (0.05)	0.75 (0.06)	0.93 (0.03)	0.78 (0.06)
6. Ψ_1(pfc1,g) ε(.) γ(g) p_1(pfc1,g,$T_{-1/y}^{2}$) p_2(pfc2,g,$T_{-1/y}^{2}$)	1,775.82	5.82	0.02	0.06	14	0.82 (0.05)	0.71 (0.07)	0.93 (0.03)	0.78 (0.06)
7. Ψ_1(pfc1) ε(.) γ(.) p_1(pfc1,g,$T_{-1/y}^{2}$) p_2(pfc2,g,$T_{-1/y}^{2}$)	1,776.22	6.22	0.01	0.05	12	0.88 (0.03)	0.76 (0.06)	0.88 (0.03)	0.76 (0.06)
8. Ψ_1(pfc1) ε(.) γ(g) p_1(pfc1,g,$T_{-1/y}^{2}$) p_2(pfc2,g,$T_{-1/y}^{2}$)	1,777.29	7.29	0.01	0.03	13	0.88 (0.03)	0.73 (0.06)	0.88 (0.03)	0.78 (0.06)
Model-averaged estimates (models 1–4)	(SE)					0.84 (0.05)	0.42 (0.09)	0.91 (0.03)	0.81 (0.05)

[a] Ψ_1 is the occupancy estimate for the first primary sampling period; ε is the probability of a site occupied during the first primary sampling period becoming unoccupied during the second primary sampling period; γ is the probability of a site unoccupied during the first primary sampling period becoming occupied during the second primary sampling period.

[b] Difference in $QAIC_c$ compared with lowest $QAIC_c$ model.

[c] $QAIC_c$ model weight.

[d] Number of parameters in model.

Table 7. Summary of model selection procedures based on Akaike's Information Criteria (QAIC$_c$) to determine the parameterization of detection probabilities for occupancy models of black bears, treatment area, Washington County, North Carolina, USA. Detection probabilities (*p*) were modeled for the first (p_1; before construction of U.S. Highway 64 [2000]) and second (p_2; after construction of U.S. Highway 64 [2006]) primary sampling periods and different combinations of sampling occasion (t) and time trends (T). Percent forest cover (pfc1) within a 270-m radius of the sample sites was used as a site covariate; pfc1 and pfc2 were covariates for the detection probabilities of the first and second primary sampling periods, respectively. A $\hat{c}$ adjustment of 1.21 was applied to all models.

Model no. and model [a]	**QAIC$_c$**	**Δ QAIC$_c$** [b]	**QAIC$_c$ weight**	**Model likelihood**	***K*** [c]	**QDeviance**
1. Ψ_1(pfc1), ε(.), γ(.), p_1(pfc1,T_{log}), p_2(pfc2,T_{log})	705.78	0.00	0.52	1.00	10	684.07
2. Ψ_1(.), ε(.), γ(.), p_1(pfc1,T_{log}), p_2(pfc2,T_{log})	707.99	2.21	0.17	0.33	9	688.61
3. Ψ_1(pfc1), ε(.), γ(.), p_1(T_{log}), p_2(T_{log})	708.75	2.97	0.12	0.22	8	691.65
4. Ψ_1(.), ε(.), γ(.), p_1(T_{log}), p_2(T_{log})	709.75	3.97	0.07	0.14	7	694.90
5. Ψ_1(.), ε(.), γ(.), p_1($T_{-1/y}{}^2$), p_2($T_{-1/y}{}^2$)	709.77	3.98	0.07	0.14	7	694.92
6. Ψ_1(.), ε(.), γ(.), p_1(T_{lin}), p_2(T_{lin})	711.29	5.51	0.03	0.06	7	696.44
7. Ψ_1(.), ε(.), γ(.), p_1(.), p_2(.)	712.83	7.06	0.02	0.03	5	702.39
8. Ψ_1(pfc1), ε(.), γ(.), p_1(t), p_2(t)	726.53	20.76	0.00	0.00	17	687.52

[a] Ψ_1 is the occupancy estimate for the first primary sampling period; ε is the probability of a site occupied during the first primary sampling period becoming unoccupied during the second primary sampling period; γ is the probability of a site unoccupied during the first primary sampling period becoming occupied during the second primary sampling period.

[b] Difference in QAIC$_c$ compared with lowest QAIC$_c$ model.

[c] Number of parameters in model.

Table 8. Summary of model selection procedures using Akaike's Information Criteria ($QAIC_c$) to test hypotheses regarding probability of site extinction of black bears and distance from a new highway, treatment area, Washington County, North Carolina, USA. Detection probabilities were modeled for the first (p_1; before construction of U.S. Highway 64 [2000]) and second (p_2; after construction of U.S. Highway 64 [2006]) primary sampling periods and as a function of an asymptotic time trend (T_{log}). Models reflect hypotheses regarding changes in occupancy (Ψ) and extinction (ε) between the pre- and post-treatment sampling phases as a function of distance from the highway. Distance and percent forest cover (pfc1) within a 270-m radius of the sample sites were used as a site covariate and detection probability covariates (pfc1 and pfc2). A $\hat{c}$ adjustment of 1.21 was applied to all models.

Model no. and model [a]	**$QAIC_c$**	**$\Delta QAIC_c$** [b]	**$QAIC_c$ weight**	**Model likelihood**	**K** [c]	**QDeviance**
1. Ψ_1(pfc1),ε(.), γ(.), p_1(pfc1,T_{log}) p_2(pfc2,T_{log})	702.96	0.00	0.49	1.00	10	681.26
2. Ψ_1(pfc1,distance), ε(.), γ(.), p_1(pfc1,T_{log}) p_2(pfc2,T_{log})	704.43	1.46	0.24	0.48	11	680.36
3. Ψ_1(pfc1),ε(distance), γ (.), p_1(pfc1,T_{log}) p_2(pfc2,T_{log})	704.92	1.96	0.18	0.38	11	680.86
4. Ψ_1(pfc1,distance),ε(distance), γ(.), p_1(pfc1,T_{log}) p_2(pfc2,T_{log})	706.44	3.48	0.09	0.18	12	679.99

[a] Ψ_1 is the occupancy estimate for the first primary sampling period; ε is the probability of a site occupied during the first primary sampling period becoming unoccupied during the second primary sampling period; γ is the probability of a site unoccupied during the first primary sampling period becoming occupied during the second primary sampling period.

[b] Difference in $QAIC_c$ compared with lowest $QAIC_c$ model.

[c] Number of parameters in model.

APPENDIX B: FIGURES

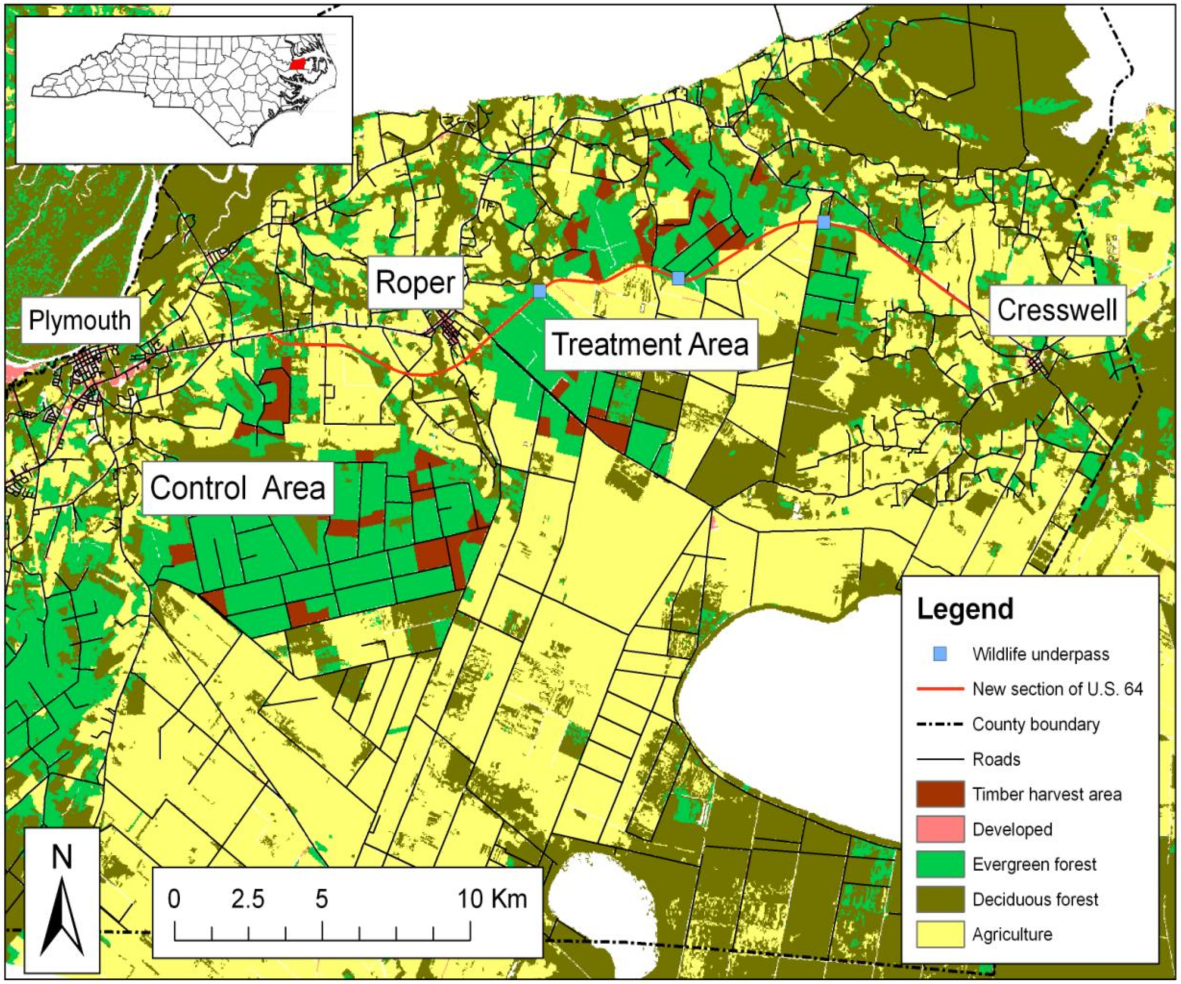

Fig. 1. Study area locations to determine the short-term impacts of a new section of U.S. Highway 64 on black bear ecology, Washington County, North Carolina, 2000–2007.

	Pre-construction phase 2000–2001	**Post-construction phase** 2006–2007
Treatment area (New highway)	Group 1	Group 2
Control area (No new highway)	Group 3	Group 4

Fig. 2. Experimental design to determine short-term impacts of a new section of U.S. Highway 64 on black bear ecology in Washington County, North Carolina, 2000–2007.

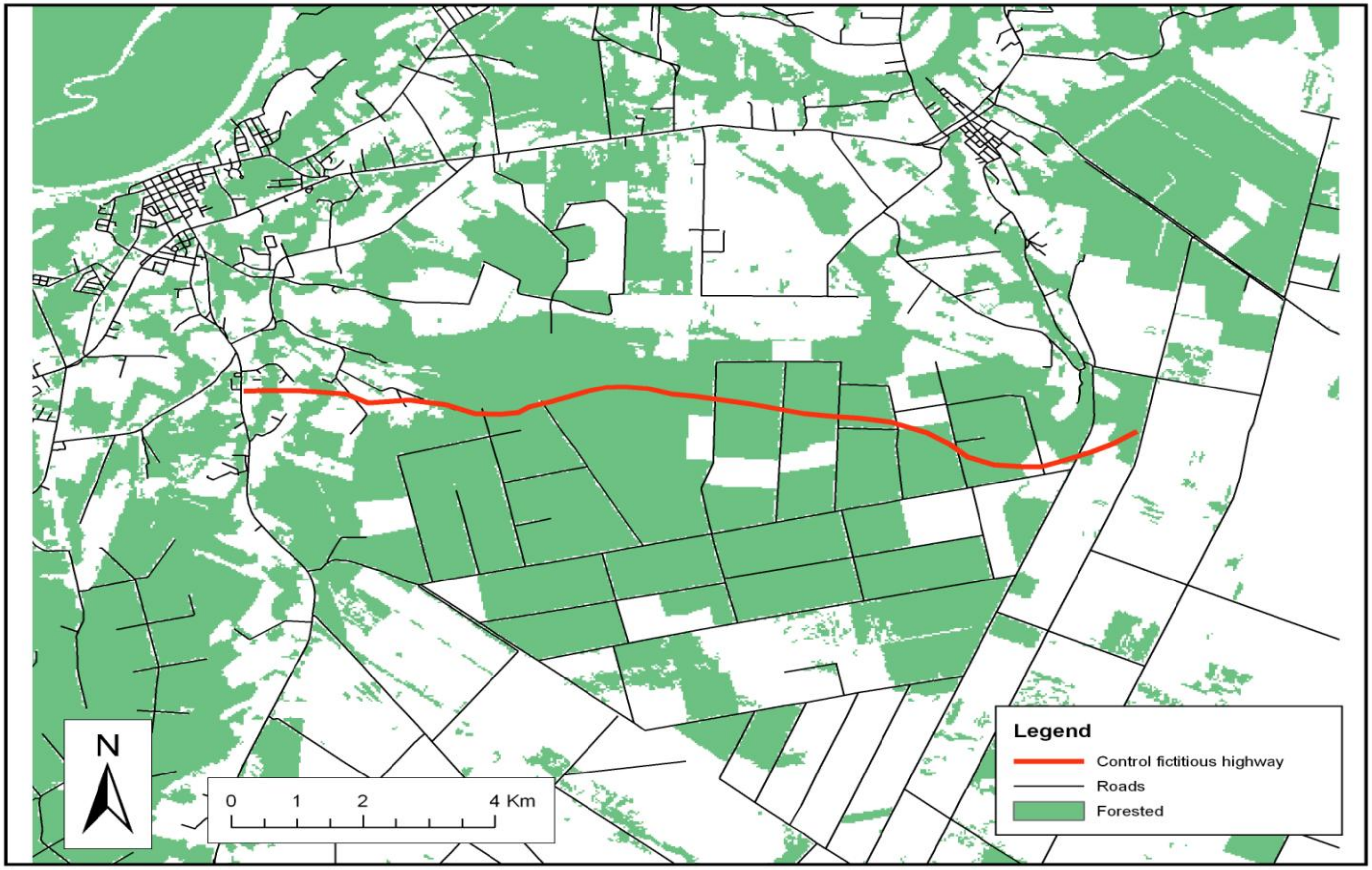

Fig. 3. Location of fictitious highway to delineate north and south sampling areas within the control area to determine the short-term impacts of a new section of U.S. Highway 64 on black bear ecology, Washington County, North Carolina, 2000–2007.

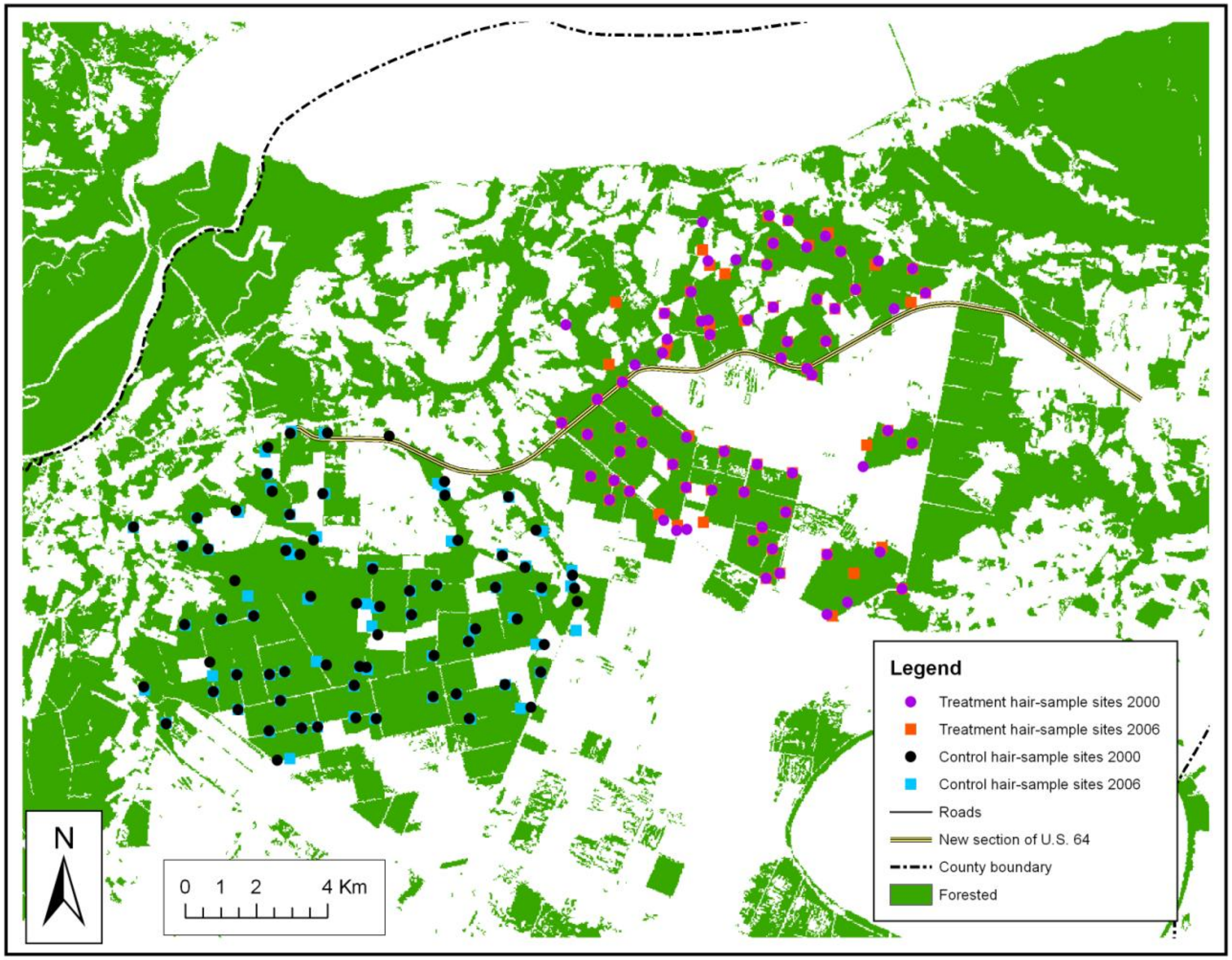

Fig. 4. Locations of black bear hair-sample sites on the treatment and control areas to determine the short-term impacts of a new section of U.S. Highway 64 on black bear ecology, Washington County, North Carolina, 2000 (pre-construction phase) and 2006 (post-construction phase).

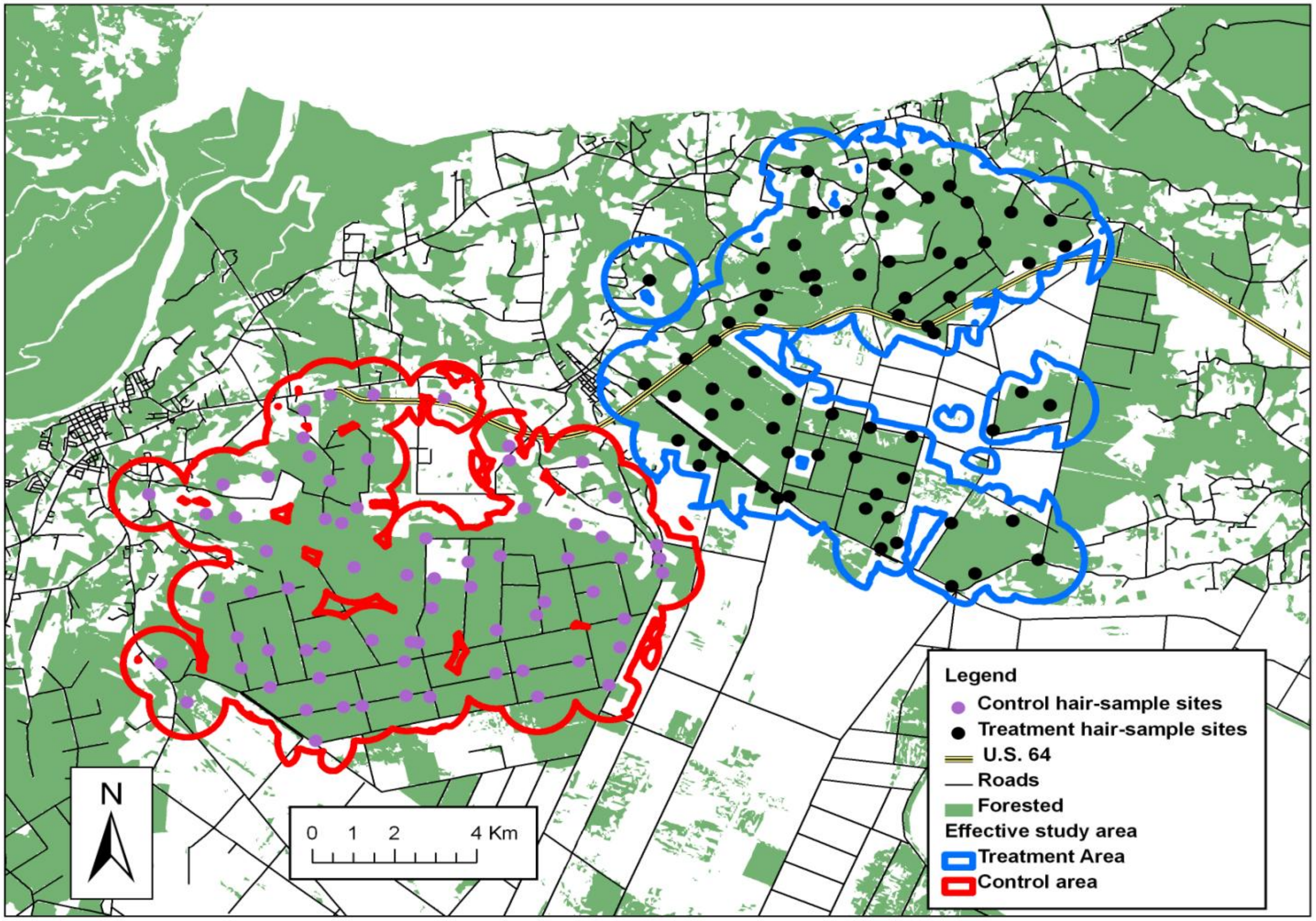

Fig. 5. Effective study areas to estimate black bear population density for a study to determine the short-term impacts of a new section of U.S. Highway 64 on black bear ecology, Washington County, North Carolina, 2000 (pre-construction phase).

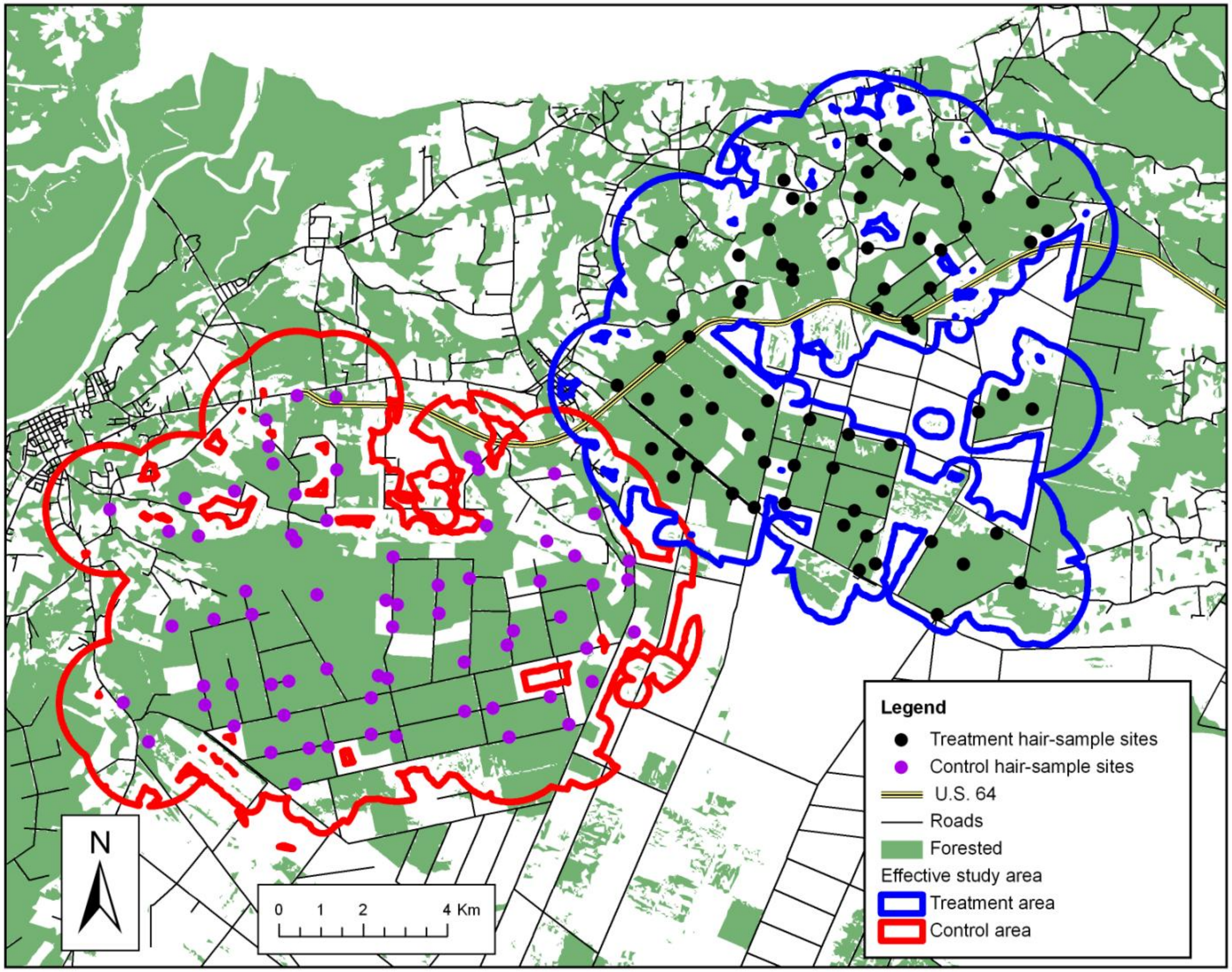

Fig. 6. Effective study area for estimation of black bear population density for a study to determine the short-term impacts of a new section of U.S. Highway 64 on black bear ecology, Washington County, North Carolina, 2006 (post-construction phase).

VITA

Jeremy Nicholson was born in Blue Ridge, Georgia on 22 September 1980. He graduated from the Fannin County High School in 1999. He moved to Tennessee in 2004 and enrolled at the University of Tennessee in the Wildlife and Fisheries Science program. While working towards his bachelor's degree, Jeremy was an active member of the University of Tennessee Wildlife and Fisheries Society and performed volunteer work for a variety of wildlife projects. He also worked for the Tennessee Wildlife Resources Agency as an intern monitoring reintroduced elk in Royal Blue and Sundquist WMAs. Jeremy graduated in May 2006 and immediately began his M.S. Thesis project as a Graduate Research Assistant in the Department of Forestry, Wildlife, and Fisheries at the University of Tennessee. Jeremy received his M.S. degree in Wildlife and Fisheries Science in August 2009.