Title

Dissimilarities in behavioral responses of snakes to roads and vehicles have implications for differential impacts across species

Authors

Andrews, Kimberly M.
Gibbons, J. Whitfield

Publication Date

2005-08-29

DISSIMILARITIES IN BEHAVIORAL RESPONSES OF SNAKES TO ROADS AND VEHICLES HAVE IMPLICATIONS FOR DIFFERENTIAL IMPACTS ACROSS SPECIES

Kimberly M. Andrews (Phone: 803-725-0422, Email: Andrews@srel.edu) and **J. Whitfield Gibbons**,
Savannah River Ecology Laboratory, University of Georgia, Aiken, SC 29082

Abstract: Roads can act as a barrier to overland movement of animals by causing habitat fragmentation, disrupting landscape permeability, and having an impact on survivorship patterns and behavior. We conducted field experiments to determine how southeastern U.S. snake species with different behaviors and ecologies responded to roads. We attributed interspecific differences in how individual snakes responded to ecological and behavioral differences among the species tested. The probability that a snake would avoid entering the road rather than crossing it varied significantly among species. Smaller species showed high road avoidance behavior. We also observed significant differences in crossing speeds among species. Most nonvenomous species crossed more rapidly than venomous ones. Nonetheless, all species minimized road-crossing time by traveling at perpendicular angles. We also conducted field tests to determine how individual snakes respond to passing vehicles. We observed that most individuals of the three species tested became immobile when a vehicle passed, a non-adaptive behavior that would prolong road-crossing time of an individual and further exacerbate a species' vulnerability when crossing roads. It is essential that the differential responses of snakes and other animals to roads be identified if the direct impacts of road mortality are to be incorporated into future mitigation plans that minimize road impacts in efforts to design more effective transportation systems.

Introduction

In the United States, the road network extends approximately 6.4 million kilometers, comprising one percent of the nation's land (Forman & Alexander 1998). Roads even penetrate the nation's protected lands, with 10 percent of them occurring in national forests (Youth 1999). Although roads comprise only one percent of the land surface area in the United States, the ecological impact has been estimated to extend to 15 to 20 percent of the country's land (Forman & Alexander 1998). Development, traditionally defined in terms of structural buildings, has expanded legally to include roads (i.e., Transportation Equity Act for the 21st Century [TEA-21], 1998) due to their potential to have enormous overall impacts. Roads, despite their relative narrowness, are increasingly being recognized by biologists as having the potential to alter numerous ecosystem balances. Streams are polluted, altering water quality and faunal communities (Welsh and Ollivier 1998); animals attempt to cross the road and are hit by vehicles (e.g., Lodé 2000); and some species behaviorally avoid the road (e.g., Forman and Deblinger 2000), which potentially fragments their habitat, reducing their range, restricting gene flow, and threatening population viability. Road effects are not a static impact that only results in an immediate loss of habitat in an area; they enable human access and are a precursor to future development (Riitters and Wickham 2003)

To understand ecological impacts, we must understand not only resource-limiting aspects of habitat loss but also behavioral reactions of wildlife to such loss that determine how readily wildlife acquires necessary resources amidst landscape alteration. The degree to which the road poses a barrier to movement defines whether the bisected habitat is continuous (animals cross successfully in significant enough numbers that resources on both side of the road are still available and gene flow is sustainable) or functionally fragmented (mortality rates or behavioral avoidance is high enough that populations are isolated). If the barrier effect of the road continually prohibits immigration and emigration, this isolation will eventually affect fundamental population and community dynamics. For prudent conservation measures to be realized, a balance must be achieved between the construction of roads for domestic and commercial purposes and the persistence of intact habitats and wildlife populations. This balance can be formulated effectively only with science-based designs that are favorable to movement patterns of both humans and wildlife.

Direct effects are defined here as those that can be attributed to the road itself. The most obvious direct effects are immediate habitat loss (physical land area that the road covers) and on-road mortality. The threat of being killed on a road can be of even greater consequence if populations recover more readily from a one-time reduction in spatial resources than to the continual removal of individuals from a population. Approximately one million vertebrates die per day on U.S. roads (Lalo 1998) from the 190 million vehicles that travel the roads daily (FHWA 2001). Animals attempt to cross roads in an effort to access resources on the other side or to disperse permanently (i.e., emigrate) to escape unfavorable circumstances. The level of crossing success is dependent on the extent of human use of the road. A standard U.S. highway experiences traffic volumes of approximately 20,000 cars each day at a given location, averaging a car or truck every four seconds (Higgins 2000). In areas of lower traffic density, larger time spans between vehicles may allow greater permeability of the road to crossing animals. A developing problem is that an increasing number of roads are experiencing increasing traffic densities, decreasing these crucial windows of time (e.g., Smith and Dodd 2003). Whether an animal successfully crosses within these windows depends on their movement biology and crossing behaviors, such as the length of time required to cross the road.

Indirect effects are defined here as secondary effects that occur off road. The indirect effects of roads are more numerous and detrimental to wildlife than direct effects (Forman and Alexander 1998), although they have received less attention because most are difficult to observe and quantify. The situation is further complicated because impacts differ with species, locality, and road condition. For example, road age, substrate, and width, in addition to vehicular speeds, densities, and daily or seasonal traffic patterns (Andrews et al. 2006) add to the complexity of assessing environmental impacts of roads. The purpose of this research is to focus on factors influencing road fragmentation and investigate behavioral responses as an indicator of road permeability.

Roads can act as barriers not only when rates of mortality exceed sustainable levels such that inadequate numbers of individuals are exchanged, but also when selective (i.e., genetic or behavioral) avoidance occurs. The barrier effect of roads has tremendous implications as the pressure of isolation can reduce genetic diversity via the creation of subpopulations and also result in increased mating competition among fewer individuals. The ultimate threat of local extirpation becomes a concern if inbreeding depression results in more individuals of reduced fitness, lowering viability for the population as a whole. This indirect effect of isolation spurred by road avoidance can ultimately have bottom-up effects by altering the structure of an entire food chain. The intensity of fragmentation effects varies with each organismal group as shown by Hargis et al. (1999), wherein American marten abundance decreased in edge habitats but small mammal densities increased. Therefore, road impacts on population dynamics should be examined at the level of a particular animal group before generalizing across phylogenetic boundaries.

Snakes, the focus of this research, are an ideal group to investigate the generality of road impacts, both direct and indirect, due to (1) road mortality that has been documented for over half a century (e.g., Klauber 1939, Fitch 1949, Campbell 1953, Pough 1966, Whitecar 1973, Dodd et al. 1989, Bernardino and Dalrymple 1996, Smith and Dodd 2003) and (2) the large breadth of ecological niches represented among species. To adequately mitigate anthropogenic disturbance of wildlife patterns resulting from road development, we must understand the basics of how different groups are affected in regard to daily activities, life cycles, and migratory patterns.

Crossing speeds and angles influence the length of time required for an animal to cross the road, and, therefore, affect the animal's vulnerability to vehicular-induced mortality. For instance, snakes that cross the road at a wide angle or at a slower pace prolong the amount of time spent on the road and in the direct path of traffic. The road can become impermeable when road mortality reaches rates such that genetic interchange is reduced or halted, dividing the local population into isolated subpopulations. The threshold at which the number of snakes being killed on the road is so significant that the number crossing successfully is insufficient to maintain viable populations and would vary with species and location. While the numbers of snakes killed on roads can be appallingly high, mortality measures alone do not reveal how snake populations in surrounding habitats are truly affected.

An array of snake behaviors and physiological traits may influence a snake's use or avoidance of the road and its probability of crossing successfully, in addition to extrinsic variables (road and environmental conditions; Andrews and Gibbons 2006). Snake species demonstrate drastically different ecological strategies, ranging from fossorial and clandestine behaviors to wide-ranging habitat uses. Snakes are more vulnerable to predation when dispersing or migrating to acquire the necessary resources and have evolved adaptations to minimize the chances of being preyed upon when traveling overland (e.g., Shine and Lambeck 1985). Such strategies include crypsis (e.g., green snakes), venom (e.g., rattlesnakes), or speed (e.g., racers and coachwhips). However, species unequipped to avoid predation are less likely to cross open spaces (e.g., ringneck snakes, Fitch 1999). Some species may be more susceptible to road impacts due to ecological demands, such as home range size, that determine the degree of dispersal necessary to satisfy the critical needs of mating, foraging, and securing hibernacula (Bonnet et al. 1999). Consistencies would be expected among organisms having similar instinctive behaviors or comparable physical constraints, so that interspecific groupings would be recognizable. Consequently, snakes should exhibit varying levels of mortality and crossing rates among species that result in interspecific differences in road impacts that are reflective of natural behavioral and ecological regimes characteristic of particular species. In addition, snakes crossing the road are predicted to experience differential probabilities of mortality due to the instinctive behavior and physical ability of some species to move faster across an open space than others.

Exploring road impacts from a behavioral perspective allows determination of degrees of inhibition to and readiness of movement in the road environment, permitting a better understanding of species sensitivities. For instance, species that do cross the road are more susceptible to direct mortality. However, interspecific variation exists within that response, with species differing in the amount of time necessary to cross the road, due to speed, angle, and/or reactions to passing vehicles. Snake species that do not readily cross the road could be more directly vulnerable to barrier effects and habitat fragmentation.

We designed a two-part study to address interspecific variation in responses to the main threats presented by roads for snakes: the road itself and vehicles traveling on the road. The research objective for the road study per se was to investigate interspecific variation in how snakes behaviorally respond to the road. Based on established ecological behaviors, we hypothesized:

1. Some snake species will have a higher rate of road avoidance than others due to innate ecological inhibitions to cross open spaces.
2. Those species that cross the road readily will exhibit interspecific variation in crossing speed that reflects the variation present in movement speeds across natural substrates.
3. Snakes will cross the road at a perpendicular angle, minimizing the length of the crossing trajectory and, therefore, reducing the amount of time spent crossing the road, which would be perceived by a snake as the risk of crossing an open habitat.

The research objective for the vehicle study was to determine if snakes respond to a passing vehicle, and if this response varies across species. We hypothesized that snakes would react to the vehicle as they would an approaching predator. We predicted that species that rely on crypsis would become immobile and that species that rely on the ability to flee would exhibit flight responses.

Materials and Methods

Study site

The study was conducted on the U.S. Department of Energy's (DOE) 750-km^2 Savannah River Site (SRS) located in west-central South Carolina, USA (in parts of Aiken, Barnwell, and Allendale counties). The area is protected as a National Environmental Research Park (NERP) (Shearer and Frazer 1997) and is closed to the general public. The Wackenhut Corporation maintains security on the SRS and controls access and use of all roads. The SRS is noted for a diversity of upper coastal plain habitat types, including Carolina bay wetlands, pine and hardwood forests, cypress swamps, and sandhills, and harbors 35 native species of snakes (Gibbons and Semlitsch 1991). The field tests were conducted on a two-lane asphalt highway (1.9 km; 6 m wide) that was closed to traffic. The surrounding habitat was second-growth mixed hardwood-pine forest. The closed road allowed us to conduct testing in a situation in which behavioral responses were not disturbed by outside distractions. In addition, the vehicle tests could be carried out without regard for other traffic, and the safety of all test specimens could be assured.

Study specimens

Snakes used in the study were obtained on the SRS primarily by personnel from the Savannah River Ecology Lab (SREL). A variety of capture methods was used including aquatic minnow traps and hoop nets, drift fences with pitfall traps and terrestrial funnel traps, coverboards, standard road collecting, opportunistic captures, and time-constrained searches. After capture, snakes were held individually in snake sacks in the laboratory until testing. None of the snakes were handled or otherwise disturbed until after testing. Following testing, standard body size measurements and sex were determined. Before being released at the original capture site, all snakes were marked for future identification by cauterization (Clarke 1971) and recaptured snakes were not used in future tests. Snakes were then released at original point of capture.

Testing procedures

We tested each individual twice, from opposite sides of the road, to control for any directional component that might influence crossing behavior. An individual snake was used for a road test one time during a day in order to minimize stress on individuals. Tests in which an individual did not move after release or became defensive (vibrating tail, striking) were removed from the final analyses. Daily testing times for a particular species were based on the natural activity patterns of the species that have been reported in the general literature (Ernst and Ernst 2003) and local long-term road capture records (Gibbons and Semlitsch 1991). We tested species that are primarily nocturnal or crepuscular at dawn or at dusk. We tested typically diurnal species in the morning during summer and in the afternoon in spring and fall. We did not use specimens in the tests if they had been collected near the testing site because we assumed that the individuals would already be familiar with the area and possibly even the road itself. We also excluded specimens that appeared to be in poor health (emaciated, injured) or that were clearly gravid.

Release procedures

We constructed three release sites 12 m apart on each side of the road (figure 1; see schematic in Andrews and Gibbons 2005) at the study site. Thus, all six sites were positioned in flat, evenly vegetated shoulders with equivalent roadside habitat where similar habitat types were on adjacent and opposite sides. The use of multiple release sites minimized any potential for snakes to detect pheromone trails or other scents of previously tested snakes. We erected hardware-cloth fences (~0.5 m high and 10 m long) along the tree line at each of the six release sites to reduce the possibility that snakes would escape following the test. Observers were concealed from the test animals by a transportable blind consisting of a PVC pipe (1.6 m x 2.0 m) frame covered with camouflage fabric. The blind was placed immediately behind the hardware-cloth fence on the release side for a particular test.

Figure 1. Side shot of a release site showing fence, release pole and bucket, blind, and researcher prior to trial initiation. Two additional pairs of release sites are not shown.

We used upside-down black plastic planting pots for the release bucket in three sizes appropriate for small, medium, and large snakes. We drilled holes in the bottom of each bucket to attach string for lifting the bucket. The string was tied to a 5.1-m bamboo pole, and the bucket was placed upside-down. Thus, the observer could stand behind the blind and lift the bucket to release the snake but remain concealed during the test. To allow the snake to sample both on-road and off-road substrates before test initiation, we positioned the release bucket on the road's edge so that half was on the asphalt and half was on the vegetated roadside. A Basil 3500 cage-washing machine was used to wash each bucket between tests to eliminate the scent of previously tested snakes.

To release the snake under the bucket, we untied the snake sack and placed it under the bucket, removing it by holding a corner and sliding it out from under the bucket (tongs were used for venomous species). This procedure left the snake beneath the bucket and prevented exposure to the surrounding area prior to the test. We allowed the snake one minute to acclimate before test initiation by lifting the bucket. Defensive and search behaviors, along with their time of occurrence were recorded throughout each test in order to assess if a snake was disturbed (e.g., tail vibration, kinking, striking) and whether typical search behaviors (e.g., tongue flicking, head raising, and lateral head bobbing) were used for exploring the road environment.

Environmental variables

We recorded a suite of conditions for each test including temperatures at the release point (road, ground, and air), barometric pressure, humidity, and rainfall during the previous 24 hours, along with ranked measurements of cloud cover and wind strength. To avoid testing in temperatures outside of those of documented movement tendencies for snakes in the region (Gibbons and Semlitsch 1991; Gibbons, unpubl. data), we set a road temperature range of 15 C – 55 C (depending on and appropriate for the season). We conducted tests at times when the sun's orientation resulted in no light/shade gradient on the paired release sites opposite each other on the road, which allowed for maximum consistency of temperatures across the road-zone area.

We did not conduct tests during or immediately after rainfall. While effects of the environmental variables were analyzed, the purpose of collecting these data was to maximize standardization rather than a targeted attempt to examine environmental factors affecting road-crossing behaviors.

Road tests

Response variables of an individual were cross, avoid, or deter. Deterrence is defined here as an avoidance response in which the snake did enter the road but did not cross it and ultimately retreated, returning to the release side of the road. This testing strategy allowed us to determine if some snake species might attempt to cross the road but ultimately avoid it, in contrast to those that did not enter the road. The test was terminated when the snake reached the fence on the opposite side of the road from the release point (cross) or on the release side of the road (avoid/deter). In either case, the snake was recaptured and returned to the laboratory. For individual snakes that crossed the road, the entry and exit times and total distance were recorded for road crossing speed calculations. Additionally, the angle of the crossing trajectory relative to the road (90° = perpendicular to the lane direction) was recorded using a protractor.

We conducted a pilot study in 2002 with 27 species of snakes (*n*=225 individuals; Andrews 2004a) for the purpose of identifying target species that exhibited a range of life-history characteristics and behavioral responses to roads. After the initial testing period, we selected nine species [cottonmouth (*Agkistrodon piscivorus*), black racer (*Coluber constrictor*), canebrake rattlesnake (*Crotalus horridus*), ringneck snake (*Diadophis punctatus*), corn snake (*Elaphe guttata*), rat snake (*Elaphe obsoleta*), eastern hognose (*Heterodon platirhinos*), southern banded watersnake (*Nerodia fasciata*), southeastern crowned snake (*Tantilla coronata*)] for testing during the core season (March—November 2003) These included species that could be categorized as aquatic or terrestrial and venomous or non-venomous, and that covered a range of average adult body sizes (table 1). Data from the pilot study were not used in the core analysis, with the exception of crossing speeds and angles.

Table 1. Species of snakes selected for road tests in 2003. The black racer, canebrake rattlesnake, and rat snake were also used in the vehicle tests. Each species is categorized as (A) aquatic or (T) terrestrial, (V) venomous or (N) non-venomous, or (L) large or (S) small in average body form.

	Name	Habitat	Venom	Size
	Cottonmouth	A	V	L
	Black racer	T	N	L
	Canebrake rattlesnake	T	V	L
	Ringneck snake	T	N	S
	Corn snake	T	N	L
	Rat snake	T	N	L
	Eastern hognose	T	N	S
	Banded watersnake	A	N	L
	Southeastern crowned snake	T	N	S

We examined the influence of different variables by using a general model that incorporated all potential covariates, and category models in which variables were either classified as experimentally controlled (release site number, side of the road of release, time held in captivity, and whether the snake was initially caught on a road), physical (sex, SVL, and mass), or environmental (date, time, temperatures of road, ground and air, humidity, barometric pressure, 24-hour rainfall, wind, and cloud cover). We used stepwise regression (PROC LOGISTIC, SAS Institute, Inc., Cary, NC, 1999) to analyze for model fit and developed full models for all snakes with “species” included as a variable, and species-specific models were developed separately for each species. The use of multiple models allowed us to describe effects of covariates in greater detail for each species. Though individuals were tested twice, repeated measures designs could not be applied to the data set; therefore, models were run including all tests and only the first test of an individual, and odds ratios were calculated to investigate potential biases of carryover effects from the first test on the outcome of the second (Agresti 1996). Response probabilities were analyzed per species using Chi-square tests (PROC FREQ, SAS Institute, Inc., Cary, NC, 1999). Variable influences on crossing speeds and angles were also analyzed using stepwise regression (PROC REG, SAS Institute, Inc., Cary, NC, 1999). Interspecific differences in crossing speeds and angles were investigated using the Kruskal-Wallis test (StatSoft, Inc. Tulsa, OK, USA, 1998) after the removal of outliers (PROC UNIVARIATE, SAS Institute, Inc., Cary, NC, 1999).

Vehicle tests

We conducted the vehicle tests from early March through early November 2003. A 2002 Chevrolet Silverado 1500 pick-up truck was used for all vehicle tests to control for the event that observed behaviors were in part dependent on vehicle characteristics (e.g., size, mass). We conducted the tests on only three species (rat snakes, black racers, and canebrake rattlesnakes) that represented three distinct defensive behaviors (crypsis, speed, and venom, respectively). The same release sites and methods from the road tests were applied with the vehicle experiment. With the exception of humidity, barometric pressure, and amount of rainfall during the previous 24 hours, all other environmental variables we measured were the same as for the road tests.

The vehicle was positioned 0.3 km down the road from the release point. After the snake was contained, the observer lifted the bucket from behind the blind. The snake was not forced into the road and, therefore, had the same directional options as in the road tests (i.e., cross, avoid, deter). When the snake’s movement became consistent, the observer signaled the driver by radio to begin driving. A speed of 35 mph was maintained as the vehicle approached and passed the snake. As the vehicle approached, the observer notified the driver of the snake’s location in the road in order to minimize the distance between the passing vehicle and the snake but without jeopardizing the safety of the animal. As the snake was not always in the same physical location relative to the road in every test, distance between the snake and the vehicle could not be strictly standardized, but only minimized, and was estimated to the nearest 0.25 m. No study specimens were injured or killed during this study.

We recorded the timing of the snake’s response relative to the vehicle in terms of whether it reacted before, after, or at the moment that the vehicle passed. We also recorded if the snake exhibited no reaction, i.e., not altering speed or direction with the passing vehicle; however, we rarely observed this behavior (n=7). After the vehicle passed, we recorded any secondary response of the snake in regard to whether it continued to crawl if it had not stopped. If the snake had become immobile we recorded whether it resumed movement or continued to remain immobile. Search behaviors were recorded as in the road tests along with defense responses characteristic of the target species; rat snakes often kink as a crypsis mechanism and black racers “bow,” raising the upper half of their body. Snakes were recaptured within one minute of the vehicle passing to prevent escape. Therefore, the secondary response is a short-term observation and does not represent the maximum amount of time a snake may remain immobilized.

We used stepwise regression (PROC LOGISTIC, SAS Institute, Inc., Cary, NC, 1999) to determine if there were any covariate effects on the responses of snakes to the passing vehicle by examining both general and category models and on a grouped and individual species basis as described above in the “Road Tests” section. We again calculated odds ratios to determine the degree of consistency between the responses of an individual’s first and second test (Agresti 1996). We used Chi-square analysis (PROC FREQ, SAS Institute, Inc., Cary, NC, 1999) to examine response probabilities of each species.

Results

Road tests

Multiple analyses were run after applying exclusion criteria (n=38) to determine the consistencies in models using all tests (n= 355), and using only the first test of an individual (n=185). Due to difficulties incorporating the within-subject effects into the model itself, we used only the first test in the final analysis, although the results were similar when all tests were used. The odds ratio was marginally random (θ=1.09) but demonstrated that an individual had a greater tendency to repeat the response of the first test in the second (if θ=1, there is no correlation between the response exhibited in the first test with that in the second). In addition, when all tests were included, response was observed (p<0.02) based on which side of the road the release point was on, but no significant relationship was observed when only first tests were used.

The effect of species on road avoidance frequencies was highly significant in all models (p<0.0001); however, in the category analyses, no control or environmental variables were found to be significant. Among the measures of individual characteristics, SVL was found to be significant (p<0.05), where smaller snakes had a greater tendency to avoid the road. Single-species regressions did not yield significance for any of the variables with the exception of SVL ($p<0.05$) for canebrake rattlesnakes, in which larger specimens had a greater tendency to avoid the road. Black racers demonstrated a marginally greater avoidance tendency when tested on the west side of the road ($p=0.05$). However, if racers are removed from the sample before analysis, no effect of side of the road was observed in any of the generalized or category models. Chi-square analyses conducted on a single-species basis yielded response probabilities that deviated significantly from expected (50:50) for six of the nine species with only black racers avoiding the road less frequently than expected (figure 2). Most snakes that exhibited avoidance did not attempt to cross the road, but two species (cottonmouths and southern watersnakes) entered the road and then deterred almost 50 percent of the time; ringneck snakes deterred in 63 percent of all avoidance occurrences.

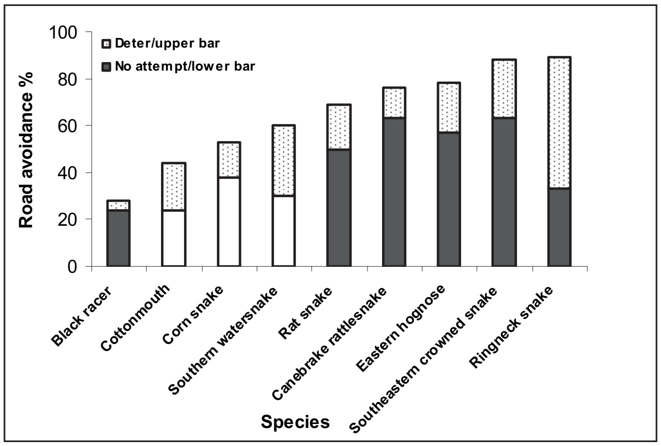

Figure 2. Road avoidance rates for nine species of southeastern snakes (adapted from Andrews and Gibbons 2005). Gray bars represent species that significantly deviated from expected ($p<0.05$). Lower bars represent individuals that retreated to the woods without entering the road (i.e., no attempt); Upper bars represent individuals that attempted but did not cross the entire road (i.e., deter). Species had a highly significant effect on crossing probability ($p<0.0001$). Sample sizes, in order by species, are black racer 54, cottonmouth 25, corn snake 13, southern watersnake 20, rat snake 26, canebrake rattlesnake 16, eastern hognose 14, southeastern crowned snake 8, ringneck snake 9.

Model results did not vary for crossing speed and angle analysis whether all tests were included or only the first tests were used. The effect of species was highly significant for crossing speed (Kruskal-Wallis test, $p<0.0001$, figure 3); five outliers were removed (black racers, $n=4$; canebrake rattlesnakes, $n=1$). SVL, mass, and road temperature significantly influenced crossing speed (SVL and mass, $p<0.01$; road temperature, $p<0.0001$). SVL and mass parameter estimates demonstrated that longer and lighter snakes move faster than did short and heavy snakes. Speed was positively correlated with road temperature across species. No species deviated significantly from a perpendicular (90°) crossing trajectory, and no species differed significantly in crossing angles ($p=0.06$) when six outliers were removed (black racer, $n=1$; corn snake, $n=1$; eastern hognose, $n=4$). Single-species regression analyses showed an effect of mass ($p<0.05$) on eastern hognose, cottonmouth, and southern watersnake. Road temperature had a significant effect specifically on cottonmouth ($p<0.01$).

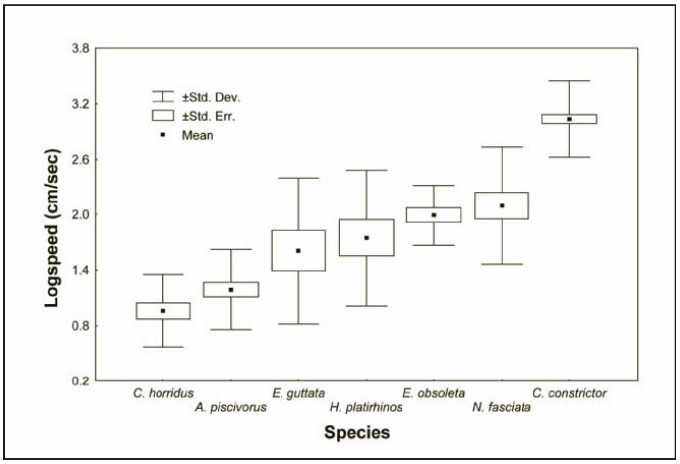

Figure 3. Crossing speeds for each of nine target species of snakes that had >10 crossing occurrences (adapted from Andrews and Gibbons 2005). Species had a highly significant ($p<0.0001$) effect on crossing speed. Sample sizes for species are canebrake rattlesnake 20, cottonmouth 29, corn snake 13, eastern hognose 14, rat snake 17, southern watersnake 19, black racer 73.

Vehicle tests

We conducted 218 trials with 113 individual snakes and found no differences between model results when all tests were used, after applying exclusion criteria (*n*=42), and only the first test of an individual (*n*=84) was used. The responses of individuals did not vary between their first and second test (ɵ=4.37). All models and analyses showed a high significance both at the species level (*p*<0.0001) and on a single-species basis (black racer, *p*<0.0001; canebrake rattlesnake, *p*=0.00; rat snake, *p*<0.0001). All canebrake rattlesnakes exhibited an immobilization response (*n*=30) and were subsequently removed from covariate analyses. Seven tests in which we observed no response to the vehicle (black racer, *n*=6; rat snake, *n*=1) are included in the presentation of the data (figure 4). However, these variations in behavior had no overall significance on the prevalence of the immobilization response for these species. None of the measured environmental, physical, or control variables had a statistically significant effect on response (*p*>0.05).

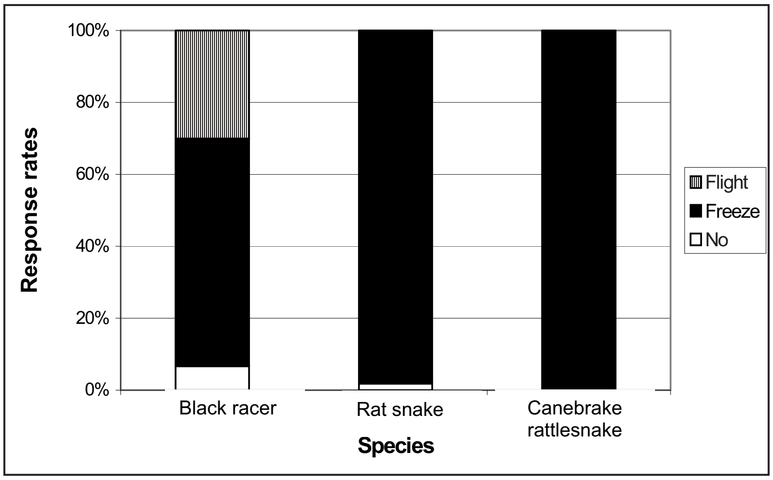

Figure 4. Vehicle response rates for three species of southeastern snakes (adapted from Andrews 2004b). All species were significant in deviating from unity. Interspecific differences were found to be highly significant (*p*<0.0001). Sample sizes in order by species, are black racer 90, rat snake 55, canebrake rattlesnake 30.

The snake's position relative to the road and the vehicle showed no effect on response to the vehicle. However, both the timing of the individual's reaction in relation to the vehicle passing and its secondary reaction after the vehicle had passed were significant for species (*p*<0.05). Black racer and rat snake were more likely to immobilize as the vehicle passed. Canebrake rattlesnakes immobilized 50 percent of the time (*n*=15 of 30; figure 5) before the vehicle reached the snake on the road. Few snakes (*n*=5 of 144, 3%) immobilized after the vehicle passed them. Sixty-two percent (*n*=89 of 144) immobilized when the vehicle passed, and 35 percent immobilized before the vehicle passed (*n*=50 of 144). Once the vehicle had passed, more than half of the snakes commenced moving again (*n*=42 of 76, 55%; figure 6), but 28 of 76 (36%) remained immobilized on the road afterwards. Both rat snakes and canebrake rattlesnakes restarted movement 65-70 percent of the time after the vehicle had passed. The highest percentage of a continued immobilization reaction occurred with black racers (*n*=11 of 28, 52%).

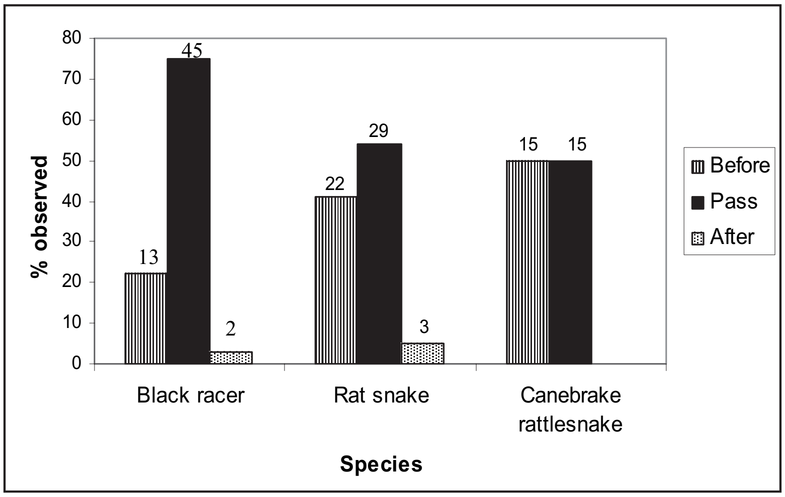

Figure 5. Timing of responses as related to a passing vehicle (adapted from Andrews 2004b). "Before" represents the proportion of responses exhibited before the vehicle passed. "Pass" represents responses exhibited at the vehicle pass. "After" represents the proportion of observed responses that occurred after the vehicle passed. Time of the reaction in relation to the vehicle passing was significant at the species level (*p*<0.05). Sample sizes are listed above the bars.

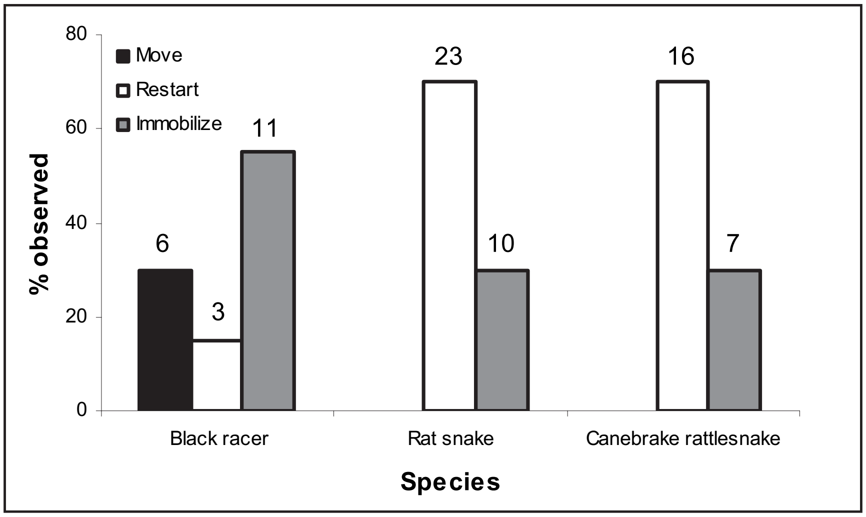

Figure 6. Secondary responses observed after the vehicle passed (adapted from Andrews 2004b). "Move" indicates that the snake fled in response to the vehicle and continued to flee after the vehicle passed. "Restart" indicates that the snake became completely immobile in response to the passing vehicle but restarted movement after the pass. "Immobilize" indicates that the snake became immobile in response to the passing vehicle and continued to freeze after the vehicle had passed. Species had a significant effect on the probability of a particular response after the vehicle passed ($p<0.05$). Sample sizes are listed above the bars.

Discussion

Road tests

Our findings supported our three hypotheses: species varied in road-crossing rates and speeds, but did not significantly vary in crossing angle. Body length comparisons conducted across species showed that smaller snake species had a greater tendency to avoid rather than cross the road. This avoidance generalization was also observed in the pilot study; data from the pilot study that were not used in analysis showed 100-percemt avoidance levels of both ringneck snakes (n=6) and southeastern crowned snakes (n=10). This finding is consistent with the observation that smaller snakes are more likely to have avian predators and are at greater risk of predation when in more exposed terrains (Fitch 1999; Gibbons and Dorcas 2005). Additionally, smaller snakes, which move shorter distances (e.g., ringneck snakes average 1-3 m/day, Fitch 1999), are less likely to encounter a road.

Ringneck snakes and southeastern crowned snakes, the smallest species in length and the ones with the highest avoidance rates, are heavily fossorial, spending predominantly more time under litter and other debris. These snakes minimize time spent in the open (e.g., ringneck snakes, Fitch 1999) and, therefore, are less likely to encounter or cross roads. We are not proposing that small snakes do not cross roads, but that road environments are not conducive to overland movement by these species. Despite the avoidance rates observed in this study, both the ringneck snake (Fitch 1999) and the southeastern crowned snake (Messenger 2004) have been observed to cross roads. In both cases, the surveyed roads bisected areas with high densities of these species. In areas with these densities, encountering the road is unavoidable for some individuals, and crossing is likely to occur in some instances.

Clear patterns did not emerge for avoidance rates across species in terms of other ecological groupings (i.e., aquatic/terrestrial, venomous/non-venomous). However, the ecological groups were not evenly represented (e.g., 2 aquatics, 6 terrestrials), so a thorough comparison could not be made. Even with comparable group sizes, it is possible that trends would not have been detectable on the group level, as ecological needs and patterns vary greatly within each group across species. Also, as road placement within a habitat is likely the key factor determining crossing probabilities, road-crossing rates cannot be generalized at this level.

Three species that showed >70-percent road avoidance (canebrake rattlesnakes, rat snakes, and eastern hognose snakes) are frequently found on the road, such that road cruising is one of the more productive techniques used to find them throughout much of their ranges. However, the observed level of avoidance in this study suggests that not all individuals that encounter roads actually cross them. Road crossings are also a consequence of home range dynamics. If snakes readily encounter the road via dispersal mechanisms, frequent road observations could be made even if only 20-30 percent of individuals cross. Thus, even the species that are more equipped to deal with the predatory threats of open spaces via body size or venom could still respond to the road as an environment to avoid.

A species-level effect of the side of the road on which the test was initiated was observed for black racers. Snakes in this study were collected from many different locations on the SRS and still exhibited species-specific tendencies; therefore, spatial displacement is not a concern in interpretation of this result. This effect suggests the potential importance of habitat cues in movement patterns in regards to directional decisions by snakes. As the study was conducted in an open outdoor environment, use of the road site by other animals could not be controlled. Therefore, trace scents from prey and predators (including other snakes) could also have influenced crossing patterns. This factor cannot be conclusively addressed from this particular study but warrants future investigation into the sensitivity of snakes to detect prior use of an area by other animals, even when the snake is placed in unfamiliar territory. Black racers also showed a significant tendency to cross the road at a higher than expected frequency. However, search behaviors were exhibited in these tests prior to crossing, demonstrating that the racers acclimated before making a directional selection. These data do not necessarily suggest that racers prefer to cross the road, or are choosing the road over the nearby forest habitat. Although it cannot be ascertained why racers showed an above-expected crossing rate, it can be concluded that the species will readily cross the road, a conclusion supported by existing road capture data of more than 1,500 racers from the SRS (Andrews and Gibbons, 2006).

Whether a snake was initially caught on the road had no significant effect on response rates although this factor was not directly tested in this study. Here again, an altered reaction to the road due to cumulative exposure could influence crossing, or avoidance, patterns at the inter- or intra-specific level. As was seen with these results, older (i.e., larger) canebrake rattlesnakes had a greater tendency to avoid the road than did younger (<1000 mm SVL) ones. Canebrake rattlesnakes, an example of a wide-ranging snake species, are inhabitants of an increasing number of areas penetrated by roads, thereby increasing the chance that an individual snake has encountered a road. Eastern diamondback rattlesnakes (*Crotalus adamanteus*) have been observed to truncate their home ranges along roads (Bruce Means, pers. comm.), and timber rattlesnakes (= canebrake rattlesnakes) have also been observed to travel parallel to country roads (e.g., Fitch 1999).

There was a strong species effect on crossing speeds, which is explainable by natural differences in body size and movement styles across species (Gibbons and Dorcas 2005). In addition to the physical implications of these species being slower due to higher length to mass ratios, venomous snakes are equipped to use venom, not flight, as their ultimate defense mechanism (Gibbons and Dorcas 2005). Therefore, these snakes are at less risk of predation than are nonvenomous species while crossing open spaces. The barrier effect can also arise with species that cross slowly (e.g., canebrake rattlesnakes), resulting in high levels of mortality, after which population stability could suffer from the pronounced loss of individuals. Fitch (1999) described the road crossing behavior of timber (= canebrake) rattlesnakes as crossing “so slowly, movement was likely to be unnoticed.” This behavior is again demonstrated in these data, not only for canebrakes, but also for our other venomous target species, the cottonmouth. The correlation between body mass and speed was negative. Long, slender snakes cross the road more quickly as observed with black racers. The three species (cottonmouths, eastern hognose snakes, and southern watersnake) for which a mass effect was shown are all stout-bodied species as adults when in physically optimal conditions (Gibbons and Dorcas 2005). Collectively, snakes moved faster at warmer road temperatures, and a specific effect was seen with cottonmouths. This general response of increased speed at warmer temperatures has been documented (e.g., Blouin-Demers et al. 2003, Heckrotte 1967). The true role of temperature in road-crossing behaviors cannot be concluded from this study as snakes were tested within constrained temperature conditions. However, as road temperature showed significance despite controlled efforts, it is likely that this factor is of considerable influence in road crossing patterns. Particular crossing frequencies have been documented to be correlated not only with season, but also during certain times of day (e.g., Klauber 1939), likely due to natural temperature fluctuations within a day (e.g., Gibbons and Semlitsch 1991).

No species deviated appreciably from a perpendicular (90°) crossing angle, and crossing angles did not vary significantly among species. This observation suggests that snakes, regardless of whether they view the road as a threat, spend no more time crossing than necessary. We observed search behaviors such as tongue flicking and head lifting in individuals at the beginning of the test, snakes were not observed to search extensively while crossing. After snakes had done initial searching and made a directional decision, they typically proceeded with consistent movement. Snakes took the shortest route possible, their inter-specific crossing speed rate notwithstanding.

In summary, highly significant levels of species-specific variation are apparent in (1) how readily a species will cross the road and (2) crossing speeds when a crossing attempt occurs. Although this study was not designed to test for importance of variables both intrinsic and extrinsic to the snake, physical features of the individual snake or species itself, certain habitat cues, and road temperatures (as a consequence of time of day or season) can potentially influence both avoidance rates and crossing speeds.

Vehicle tests

The frequency of immobilization responses was higher than initially hypothesized. Canebrake rattlesnakes, which rely on crypsis as a primary defense, did immobilize in response to the passing vehicle. Black racers had a higher immobilization response than expected, but we have also observed this behavior in close encounters in the field. Conditions in which no response was observed could not be statistically pursued due to the low sample with which this lack of exhibition was observed (*n*=7). In five of the seven tests (6 black racers and 1 rat snake), the snake was either on the road shoulder or the distance between the snake and the vehicle was 4 m or greater, suggesting that possibly if snakes can sense if they are a “safe” distance from the vehicle, they do not enact defensive behaviors. Therefore, distance between the snake and the vehicle, and the relative positions would likely have an effect on response. These data are unable to test this effect due to the lack of variance in these data for the distance between the vehicle and the snake. Studies inquiring into responses of snakes to specific distances from the vehicle are needed to determine if this factor is of significant influence.

The majority of snakes immobilized as the vehicle passed, as opposed to before or after the pass. Additionally, the majority of snakes restarted movement after the vehicle passed, suggesting that although a passing vehicle temporarily interrupts road crossing, it is a momentary reaction. However, canebrake rattlesnakes often remained immobilized for up to a minute, posing a significant extension of crossing time for species exhibiting persistent immobilization. Persistence of immobilization needs to be quantified to assess actual crossing time and mortality probabilities accurately.

Although snakes verifiably use the road for thermoregulation in some locations and under particular environmental conditions (e.g., Sullivan 1981), it is possible that immobilization behavior lends support to the belief that snakes commonly use the road for thermoregulatory purposes. Thermoregulation likely occurs at times of the day in which the road is not heavily traveled by vehicles or in regions, such as the West, where the landscape is vast and animals are more accustomed to open spaces, and in areas of reduced traffic densities.

In conclusion, vehicle responses mimic predator responses in natural habitat. The immobilization response appears to be momentary for most species. However, snakes encounter more than a single vehicle in reality; this response could significantly prolong crossing time if immobilization behaviors are repeated. It follows that the time it takes to cross the road is positively correlated with traffic density for species that immobilize in response to passing vehicles. This in-road behavior needs to be considered as a factor increasing the threat of mortality with a group that already is not adept at crossing roads due to secretive natures or applied defensive behaviors and presumed vulnerability to natural predators in open spaces.

General Conclusions

In the developing field of "road ecology" (Forman et al. 2003) an increasing number of land managers, research ecologists, environmental chemists, and hydrologists have begun to recognize the irreparable alteration to the landscape that can be caused by the nation's transportation infrastructure. To develop more environmentally sound transportation systems in the future and allow for efficient mitigation practices, we must first understand the biological impacts that result from these alterations. The research reported here was designed to identify sensitive species and the potential diversity in type and degree of road impacts across snake species.

Although a range of species behaviors is observed across snakes as a group, these data make it apparent that snakes do not deem the road area a favorable environment. It is notable to management designs that road impacts cannot be generalized even within an animal group. Perhaps some are maintaining viable populations amidst road development, but perhaps others will go locally extinct without implementation of measures minimizing road impact. The difference between the two categories needs to be apparent so that resources and future research can be prioritized for the sensitive species.

As this study was designed to investigate behavioral effects at an inter-specific level, research into intra-specific comparisons also needs to be conducted with the identified sensitive species (Andrews and Gibbons 2006). The seasonality of road mortality has been documented both across and within seasons (e.g., Case 1978, Sherbrooke 2002), but a greater understanding of the conditions of road avoidance needs to be achieved in order to document a representative section of road impacts on wildlife. Ultimately, population- and community-level assessments must occur to determine how roads are affecting ecological processes at landscape scales. The degree of permeability of the road determines whether the conduits that wildlife relies on for dispersal and survival remain open.

The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' (I found it!) but 'That's funny ...'
-Isaac Asimov (1920 - 1992)

Acknowledgments: We would first like to thank FHWA and the ICOET conference program for travel support to the 2005 meetings and the SREL Education for educational support. An infinite amount of appreciation is extended to those who have helped in the field, with a special salute to Peri Mason, Ben Lawrence, Lucas Wilkinson, and Leslie Ruyle, and to all of the SREL folks who assisted with snakes captures. Thanks to Tony Mills and Sean Poppy who have aided in site construction and Dehai Zhao for providing statistical support. We thank Wackenhut Corporation and Mack Underwood for the resources of closed roads and patience, even in moments of not understanding why one would study the age-old question of why things cross the road. We hear a few of them even swerve to miss snakes these days. Snakes were captured under scientific research permit #56-2003 from the South Carolina Department of Natural Resources, and the study was approved under University of Georgia Animal Use Proposal A2003-10135. Research was conducted with support of the Environmental Remediation Sciences Division of the Office of Biological and Environmental Research, U.S. Department of Energy, through Financial Assistance Award No. DE-FC09-96SR18546 to the University of Georgia Research Foundation. This research supports the goals of Partners in Amphibian and Reptile Conservation (PARC).

Biographical Sketches: Kimberly M. Andrews, a doctoral student in the Institute of Ecology at the University of Georgia, conducts research on road ecology and herpetology at the Savannah River Ecology Laboratory. One of her primary goals is to contribute to reptile and amphibian conservation by conducting ecological research on movement patterns and behavior in fragmented habitats and by promoting a greater understanding and appreciation of herpetofaunal ecology through public outreach and education.

J. Whitfield Gibbons, professor of ecology at the University of Georgia and head of the Environmental Outreach and Education Program at the Savannah River Ecology Laboratory, conducts research on life history and ecology of reptiles and amphibians. His primary research focus is to document and explain the distribution and abundance patterns of herpetofauna at the ecological and evolutionary levels, with an emphasis on conservation issues

References

Agresti, A. 1996. *An Introduction to Categorical Data Analysis*. John Wiley & Sons, New York.

Andrews, K. M. 2004a. Behavioral responses of snakes to road encounters: Can we generalize impacts across species? (An overview summary). In: *2003 Proceedings of the International Conference on Ecology and Transportation*, Lake Placid, NY, August 24-29 2003. Raleigh, NC: Center for Transportation and the Environment, North Carolina State University.

Andrews, K. M. 2004b. Interspecific comparisons of behavioral responses of southeastern snakes to roads. M.S. Thesis, University of Georgia. Athens, GA.

Andrews, K. M. and J. W. Gibbons. 2005 How do highways influence snake movement? Behavioral responses to roads and vehicles. *Copeia* 2005(4):771-781.

Andrews, K. M. and J. W. Gibbons. 2006. Ecological Attributes of Snakes on Roads: Sex and Body Size are Significant Within and Among Species. *Herpetological Conservation* (Urban Conservation). SSAR Special Issue.

Andrews, K. M., J. W. Gibbons, and D. M. Jochimsen. 2006. Ecological Effects of Roads on Amphibians and Reptiles: A Literature Review. *Herpetological Conservation* (Urban Conservation). SSAR Special Issue.

Bernardino, F. S., Jr. and G. H. Dalrymple. 1992. Seasonal activity and road mortality of the snakes of the Pa-hay-okee wetlands of Everglades National Park, USA. *Biological Conservation* 62:71-75.

Blouin-Demers, G., P. J. Weatherhead, and H. A. McCracken. 2003. A test of thermal coadaptation hypothesis with black rat snakes (*Elaphe obsoleta*) and northern water snakes (*Nerodia sipedon*). *Journal of Thermal Biology* 28(2003):331-340.

Bonnet, X., G. Naulleau, and R. Shine. 1999. The dangers of leaving home: dispersal and mortality in snakes. *Biological Conservation* 89: 39-50.

Campbell, H. 1953. Observations of snakes DOR in New Mexico. *Herpetologica* 9:157-160.

Case, R. M. 1978. Interstate highway road-killed animals: a data source for biologists. *Wildlife Society Bulletin* 6(1):8-13.

Clarke, D. R., Jr. 1971. Branding as a marking technique for amphibians and reptiles. *Copeia* 1971(1):148-151.

Dodd, C. K., Jr., K. M. Enge, and J. N. Stuart. 1989. Reptiles on highways in north-central Alabama, USA. *Journal of Herpetology* 23(2): 197-200.

Ernst, C. H., and E. M. Ernst. 2003. *Snakes of the United States and Canada*. Smithsonian Press, Washington, D. C.

FHWA (Federal Highway Administration). 2001. United States Department of Transportation, *Amphibian-Reptile Wall and Culverts*. http://www.fhwa.dot.gov/environment/wildlifecrossings/amphibin.htm

Fitch, H. S. 1949. Road counts of snakes in western Louisiana. *Herpetologica* 5:87-90.

Fitch, H. S. 1999. *A Kansas Snake Community: Composition and Changes Over 50 Years*. Krieger Publishing Company, Melbourne, FL.

Forman, R. T. T., and L. E. Alexander. 1998. Roads and their major ecological effects. *Annual Review of Ecological Systematics* 29:207-231.

Forman, R. T. T., and R. D. Deblinger. 2000. The ecological road-effect zone of a Massachusetts (U.S.A.) suburban highway. *Conservation Biology* 14(1):36-46.

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.

Gibbons, J. W., and M. E. Dorcas. 2005. Snakes of the Southeastern United States. University of Georgia Press. Athens, GA.

Gibbons, J. W., and R. E. Semlitsch. 1991. Guide to the Reptiles and Amphibians of the Savannah River Site. University of Georgia Press, Athens, GA.

Hargis, C. D., J. A. Bissonette, and D. L. Turner. 1999. The influence of forest fragmentation and landscape pattern on American martens. *Journal of Applied Ecology* 36:157-172.

Heckrotte, C. 1967. Relations of body temperature, size, and crawling speed of the common garter snake, *Thamnophis s. sirtalis*. *Copeia* 1967(4):759-763.

Higgins, M. 2000. ENN, *Highways Stop Wildlife Dead in their Tracks*. www.enn.com

Klauber, L. M. 1939. Studies of reptile life in the arid southwest, Part 1. Night collecting on the desert with ecological statistics. *Bulletin of the Zoological Society of San Diego* 14:2-64.

Lalo, J. 1998. The problem of roadkill. *American Forests* 50:50-52.

Lodé, T. 2000. Effect of a motorway on mortality and isolation of wildlife populations. *Ambio* 29(3):163-166.

Messenger, K. 2004. Biodiversity and movement patterns of snakes in the Carolina Sandhills Wildlife Refuge of South Carolina. North Carolina State University, unpublished report.

Pough, H. 1966. Ecological relationships of rattlesnakes on southeastern Arizona with notes on other species. *Copeia* 1966:676–683.

Ritters, K. H., and J. D. Wickham. 2003. How far to the nearest road? *Frontiers in Ecology and the Environment* 1(3):125-129.

SAS Institute, Inc. 1999. SAS/STAT software®, Release 8.1 for Windows. Cary, NC.

Shearer, C. R. H., and N. B. Frazer. 1997. The National Environmental Research Park: A new model for federal land use. *American Bar Association's Natural Resources and the Environment* 12:46-51.

Sherbrooke, W. C. 2002. Seasonally skewed sex-ratios of road collected Texas horned lizards (*Phrynosoma cornutum*). *Herpetological Review* 23(1):21-24.

Shine, R., and R. Lambeck. 1985. A radiotelemetric study of movements, thermoregulation and habitat utilization of Arafura filesnakes (Serpentes: Acrochordidae). *Herpetologica* 41(3):351-361.

Smith, L. L., and C. K. Dodd, Jr. 2003. Wildlife mortality on U.S. Highway 441 across Paynes Prairie, Alachua County, Florida. *Florida Scientist* 66(2):128-140.

Statsoft, Inc. 1998. STATISTICA for Windows. [Computer program manual]. Tulsa, OK.

Sullivan, B. K. 1981. Observed differences in body temperature and associated behavior of four snake species. *Journal of Herpetology* 15(2): 245-246.

Welsh, H. H., Jr., and L. M. Ollivier. 1998. Stream amphibians as indicators of ecosystem stress: a case study from California's redwoods. *Ecological Applications* 8(4):1118-1132.

Whitecar, T. L. 1973. Florida's 1st protected snake: the indigo. *Florida Naturalist* 46(2):23-25.

Youth, H. 1999. *Wildlife in the Fast Lane*. Zoogoer. September/October. http://nationalzoo.si.edu/Publications/ZooGoer/1999/5/wildlifelanes.cfm