Vol. 8: 49–60, 2009
doi: 10.3354/esr00121

ENDANGERED SPECIES RESEARCH
Endang Species Res

Printed July 2009
Published online September 9, 2008

Contribution to the Theme Section 'Bats: status, threats and conservation successes'

OPEN ACCESS

Road-killed bats, highway design, and the commuting ecology of bats

Amy L. Russell[1,3,*], Calvin M. Butchkoski[2], Leslie Saidak[1], Gary F. McCracken[1]

[1]Department of Ecology and Evolutionary Biology, 569 Dabney Hall, University of Tennessee, Knoxville, Tennessee 37996-1610, USA

[2]Pennsylvania Game Commission, Bureau of Wildlife Management, 2001 Elmerton Avenue, Harrisburg, Pennsylvania 17110-9797, USA

[3]*Present address:* Department of Biology, 1 Campus Drive, Grand Valley State University, Allendale, Michigan 49401, USA

ABSTRACT: During a *Myotis sodalis* telemetry project in Pennsylvania, USA, in 2000, road-killed *M. lucifugus* were recorded and a highway survey was initiated. The purpose of this study was to assess the level of mortality from road kills on this colony, verify which species were being killed in traffic and examine the influence of canopy height and structure on flight behavior. On 10 evenings between 15 May and 26 July 2001, bats were counted as they emerged from day roosts and crossed a heavily trafficked highway en route to foraging areas. A total of 26 442 bats were observed crossing this highway over 9.29 h of observation. Bats used canopy cover when approaching the highway from roosts. Where canopy cover was lacking adjacent to the highway, fewer bats were counted crossing; where adjacent canopy was low (≤6 m), bats crossed lower and closer to traffic. Motivated by a planned highway upgrade that would extend deforestation of the highway verge, we monitored the flight behavior of *M. lucifugus* as they crossed a 55 m mowed field. Observations of more than 1700 bats revealed that a vast majority of commuting individuals fly less than 2 m above the ground in the open field. Between 15 May and 14 September 2001, searches for road-killed bats were conducted along a section of highway crossed by large numbers of bats. We collected 27 road-killed *M. lucifugus*, 1 suspected *M. sodalis* and 1 unidentifiable *Myotis* sp. Subsequent genetic analyses of mitochondrial DNA sequences confirmed the suspect bat as a federally endangered *M. sodalis*.

KEY WORDS: Chiroptera · *Myotis lucifugus* · *Myotis sodalis* · Pennsylvania · Phylogenetics · Road kill · Commuting · Traveling

INTRODUCTION

Almost a quarter (23.8%) of the more than 1100 described species of bats are classified as threatened (Mickleburgh et al. 2002) and many threats to bat populations around the world are linked to human activities. A major threat to bats worldwide is the loss of roosting and foraging habitat, including loss or fragmentation of woodlands. Detrimental effects on wildlife from highways and traffic have been documented as resulting from habitat loss, reduced habitat quality, direct mortality (i.e. road kills) and reduced connectivity (Wilkins & Schmidly 1980, Trombulak & Frissell 2000, Bissonette 2002, Forman et al. 2003, Coffin 2007), but little research documents the effects of highways on bats (Wray et al. 2005).

The impacts of highways on bats are not immediately obvious and may differ depending on the species in question. Wray et al. (2005) analyzed impacts of a new highway on a population of endangered greater horseshoe bats *Rhinolophus ferrumequinum* in Wales. Several negative effects were documented, including the fragmentation of habitat through the addition of streetlights and loss of tree cover and the severing of commuting and foraging routes. While direct mortality from road kills was considered, there were no recorded road kills in this study. Other studies documenting road-killed bats (Kiefer et al. 1995, Lesinski 2007) show

*Email: russelam@gvsu.edu

increases in road-associated mortality in late summer, with a particularly strong impact on low-flying species. Despite these detrimental effects, some bat species may be attracted to roads and roadside habitats. Many woodland bat species forage mostly in edge habitats, such as those provided along roadsides (Grindal 1996). Zimmerman & Glanz (2000) showed that *Myotis* species, including *M. lucifugus*, *M. leibii*, and *M. septentrionalis*, are likely to use well-defined corridors, such as gravel and paved roads, probably for commuting rather than foraging (Limpens et al. 1989). Herein we document a significant impact on colonies of *M. lucifugus* and endangered *M. sodalis* from road kills.

Myotis sodalis is a small brown vespertilionid that occurs in the eastern United States from eastern Oklahoma and Kansas, northeast to Vermont, and south to northwestern Florida (Thomson 1982). It is distinguished morphologically from *M. lucifugus* by its shorter toe hairs, dull rather than glossy fur, and a small keel on the calcar that is absent in *M. lucifugus* (Thomson 1982). *M. sodalis* roosts mainly under the exfoliating bark of trees during the summer, and hibernates during the winter, primarily in well-developed limestone caves or mines. This species experienced a dramatic decline from 1960 to 2001, losing an estimated 57% of its total population (Clawson 2002). *M. sodalis* is particularly vulnerable to loss of hibernacula, as 52% of the total known population hibernates in just 7 caves and 1 abandoned mine in southern Missouri, Kentucky and southern Indiana (Clawson 2002). Summer populations also suffer from habitat degradation due to deforestation and cutting of dead and dying trees. While the major *M. sodalis* hibernacula are now protected, the continued decline of the population suggests that summer habitat protection is also vital.

We report herein on a summer nursery roost containing the morphologically similar *Myotis lucifugus* and *M. sodalis*. These colonies inhabit the attic of an abandoned one-room church at Canoe Creek and a nearby artificial roost (bat 'condo', 300 m west of the church) in Frankstown Township, Blair County, Pennsylvania, USA. The 2 roosts, located at the southern extent of Canoe Creek State Park, are separated from the colonies' primary foraging area by US Route 22 (Fig. 1). The bat condo was thought only to contain a maternity colony of *M. lucifugus*; however, a lactating female *M. sodalis* was live-trapped on 19 June 2001 using a 4.2 m^2, 2-bank, Austbat harp trap (Faunatech-Austbat) as it exited the condo, indicating potential maternal use by this species (Pennsylvania Game Commission unpubl. data). During telemetry studies in 2000 (Butchkoski & Hassinger 2002), bats were observed crossing US Route 22 as they emerged from the roosts at dusk. Road-killed bats were also noted. In May of 2002, the Pennsylvania Game Commission entered a memorandum of understanding (MOU) with the Pennsylvania Department of Transportation to assist in conducting a biological assessment (BA) for the potential impact on *M. sodalis* of highway improvements to US Route 22 at Canoe Creek. All of the data from this study period were incorporated into the BA. We present count data showing the potential impact of highway traffic on these populations and attempt to identify crucial landscape characteristics and resulting bat flight behavior that may be critical for successful conservation planning. Documented road kills are presented, illustrating the impact of this busy highway on these maternity colonies. Genetic analyses verify the identity of 1 road-killed specimen as *M. sodalis*.

MATERIALS AND METHODS

Highway crossing counts. The highway, including cleared land immediately adjacent to the pavement (i.e. verges), constituted a 20 m wide corridor. After visiting the site to ascertain where bats crossed this corridor, 4 stations covering 150 m of highway centered on the Canoe Creek/US Route 22 bridge were selected to observe flying bats silhouetted against the evening sky (Fig. 1). A surveyor was positioned at each station with a Pettersson D 100 ultrasound detector (Pettersson Elektronik AB), tally counter and survey form. Surveyors arrived 30 min before sunset for dusk counts and 60 min before sunrise for dawn counts. Sixteen crossing counts were conducted in 2001, including 10 dusk counts (15 May through 26 July) and 6 dawn counts (22 May through 24 July). Environmental variables such as temperature, wind, and sky conditions were noted prior to each count. Counts began when the first bat crossing the highway was seen or ultrasonically detected. Tallies were grouped by 10 min intervals until crossing activity ceased. Global positioning systems (Models MC-GPS, Corvallis Microtechnology) were used to document the established station locations.

Highway crossing canopy evaluation. During the course of the highway crossing counts, differences in bat numbers were observed among the 4 stations. Noticeable variance in canopy height occurred within 30 m of the north side of the highway where bats approached from the day roosts at dusk. The south side of the highway at the Canoe Creek crossing had consistently high (>20 m) canopy cover adjacent to the highway. Tree canopy heights were measured on the north and south sides of the highway with a clinometer and distance tape using standard trigonometry.

Sample collection. After each dusk and dawn count, and on additional arbitrarily selected days, the highway around the bridge was searched for road-killed

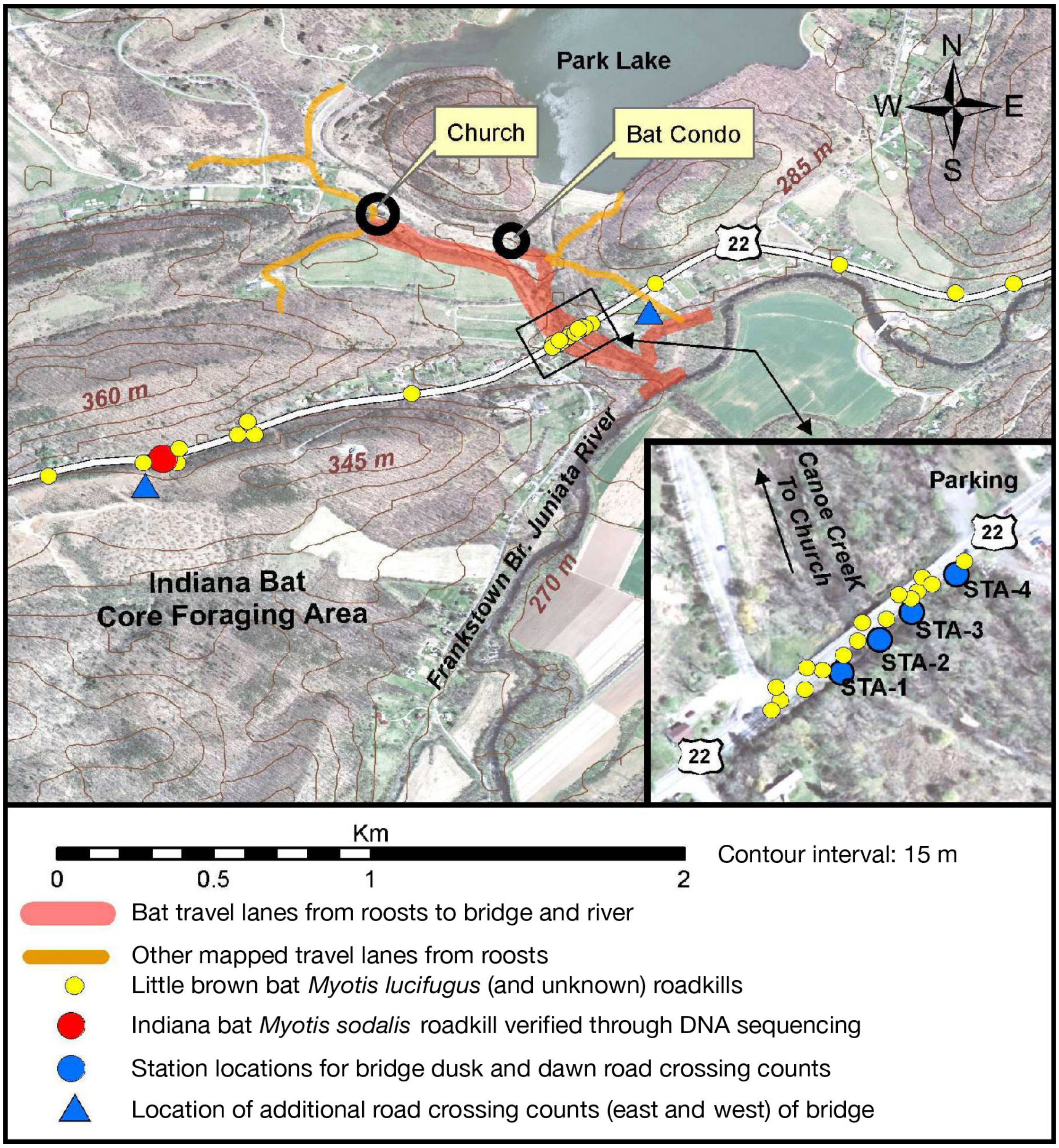

Fig. 1. Map of Canoe Creek bridge area, Pennsylvania, USA. Road-killed bat locations are given for each species, as well as the extent of the highway along which road-kills were found. Insert is close-up of bridge area and bat count stations with road-kill locations. 22: US Route 22

bats. Late in the study, this search area was extended to include 2.8 km and 1.6 km of highway to the west and east of the bridge, respectively. To conduct these searches, a team of 2 or more individuals walked the sides of the highway searching the verge and pavement for road kills. Locations of all specimens were fixed using GPS, and specimens were identified to species, sex and age, when possible. Specimens were then discarded, except 1 individual that had been run over repeatedly and was suspected of being *M. sodalis* because of a visible keeled calcar (the individual is hereafter referred to as the 'flat bat'). Upon return to the lab, approximately 20 to 25 cm^2 of wing tissue was taken from this bat and frozen at −80°C.

Tissue samples consisting of 3 mm wing biopsy punches (Worthington Wilmer & Barratt 1996) were taken from live-caught *Myotis lucifugus* (n = 7) and *M. sodalis* (n = 46) throughout the eastern USA for genetic comparison with the flat bat. Live bats were captured using hand nets or mist nets in caves or buildings and, after processing, were released on the same night at the place of capture. Tissue samples were stored on ice in the field in either NaCl-saturated 20% DMSO or silica gel dessicant.

Genetic analyses. Total genomic DNA was isolated from all samples using a DNeasy® DNA isolation kit (Qiagen). The isolation protocol for the flat bat (suspected to be *Myotis sodalis*) was modified to include an extended 72 to 96 h incubation in the ATL lysis buffer with an additional 50 µl of proteinase K. DNA for all samples was stored in 1/10 Tris-ethylenediamine-tetraacetic acid (TE) following isolation and was quantified using a Hoefer DyNA Quant® 200 fluorometer (Amersham Pharmacia). Isolates were then diluted to a standard concentration of 10 ng μl^{-1} for PCR. PCR for initial amplification and sequencing followed the protocols of Russell et al. (2005).

All sequences, including additional *Myotis* sequences from GenBank (*M. ciliolabrum*: AY460382 and AY460384; *M. evotis*: AY460367; *M. leibii*: AY460368; Rodriguez & Ammerman 2004), were aligned using the default settings in Clustal W (Thompson et al. 1994). The alignment was then edited by eye in MacClade v. 4.01 (Maddison & Maddison 2000) and cropped to a common length of 381 bp.

We used a maximum likelihood (ML) search in PAUP* v. 4.0b10 (Swofford 2002) to reconstruct the relationships among sampled *Myotis* species and to determine the species affinity of the flat bat. *M. ciliolabrum* was used as the outgroup for all other species, based on the relationships among North American *Myotis* recovered by Stadelmann et al. (2007). The initial ML heuristic search started from a neighbor-joining tree and used the HKY+Γ model, with model parameters estimated from MODELTEST v. 3.7 (Posada & Crandall 1998).

A parsimony bootstrap analysis was performed to test the hypothesis that the flat bat was *Myotis lucifugus* rather than *M. sodalis*. Because the phylogenetic analysis revealed clear genetic distinction among species, the sample size per species was pruned for the parsimony bootstrap to include only 5 individuals each of *M. lucifugus* and *M. sodalis* and 1 individual each of *M. ciliolabrum*, *M. evotis*, and *M. leibii*. The protocol for this analysis followed that detailed in Russell et al. (2008). We used Seq-Gen v. 1.3.2 (Rambaut & Grassly 1997) to simulate 100 datasets under a hypothesis defining the flat bat as a member of the *M. lucifugus* clade. ML analyses were then performed on these simulated data and on observed data, with and without the enforced constraint. The difference in likelihood scores between constrained and unconstrained searches was compared between observed and simulated datasets, with the p-value for the alternative hypothesis equal to the proportion of simulated datasets that yielded a higher difference than the observed datasets.

We also used distance-based analyses to examine patterns of species affinity for the flat bat. Genetic distances among samples of known species and from the flat bat to each sampled species were calculated in PAUP* under the evolutionary model and parameter estimates specified in MODELTEST.

Mapping of major travel corridors. To ensure that surveyors were researching the most frequently used local bat crossing area, major travel corridors were mapped as bats exited the church and condo roosts until the line of traveling bats dispersed and could no longer be followed. Over the course of several dusk emergences, a surveyor followed the traveling bats as they exited the roosts, using a GPS unit to collect GPS waypoints and track the bats. Depending on cloud cover, there was sufficient visibility to follow the bats for up to 15 to 20 min, requiring that most corridors be tracked over multiple evenings. Due to the fragmented landscape surrounding the roosts, travel corridors used by emerging bats were easily identified and followed until the bats dispersed. Waypoints were connected for each route using ArcView 3.2 (Environmental Systems Research Institute) and mapped (Fig. 1).

Population estimate of roosts. Pennsylvania Game Commission personnel counted bats exiting the condo roost on 24 May 2001; the roost was counted again by state park personnel and volunteers on 6 June 2001. On 28 June 2001, state park personnel and volunteers conducted 1 exit count at the church. These dusk counts were conducted by a number of surveyors surrounding the roost structures, each with an assigned monitoring area. The count of the larger church colony was tallied by every 10 bats and is a coarse estimate. The condo roost counts recorded each animal individually as it exited.

Behavior when crossing a field. Initiated by a planned highway upgrade that would increase deforestation at the Canoe Creek bridge area from a 20 m to a ca. 55 m wide corridor, a third colony was monitored to examine flight behavior when crossing an open field. This colony is located at the Juniata Valley High School in Huntingdon County, Pennsylvania, USA. More than 1300 *Myotis lucifugus* occupy 7 bat boxes (artificial roosts) placed 8 m high on a building and cross a 55 m mowed field upon exiting the roost (Fig. 2). Surveyors arrived at least 1 h before sunset. Two 8 m fiberglass poles were erected approximately 30 m apart and in the middle of the mowed field. Mason's string was stretched tight between the 2 poles at heights of 1, 2, 4, 6 and 8 m. Four people were positioned across the count area near the measuring strings, lying flat on the ground to minimize disturbance to the approaching bats. Each person had a separate, predetermined area to count. Bats crossing the mowed field were tallied by the height at which they passed between the poles.

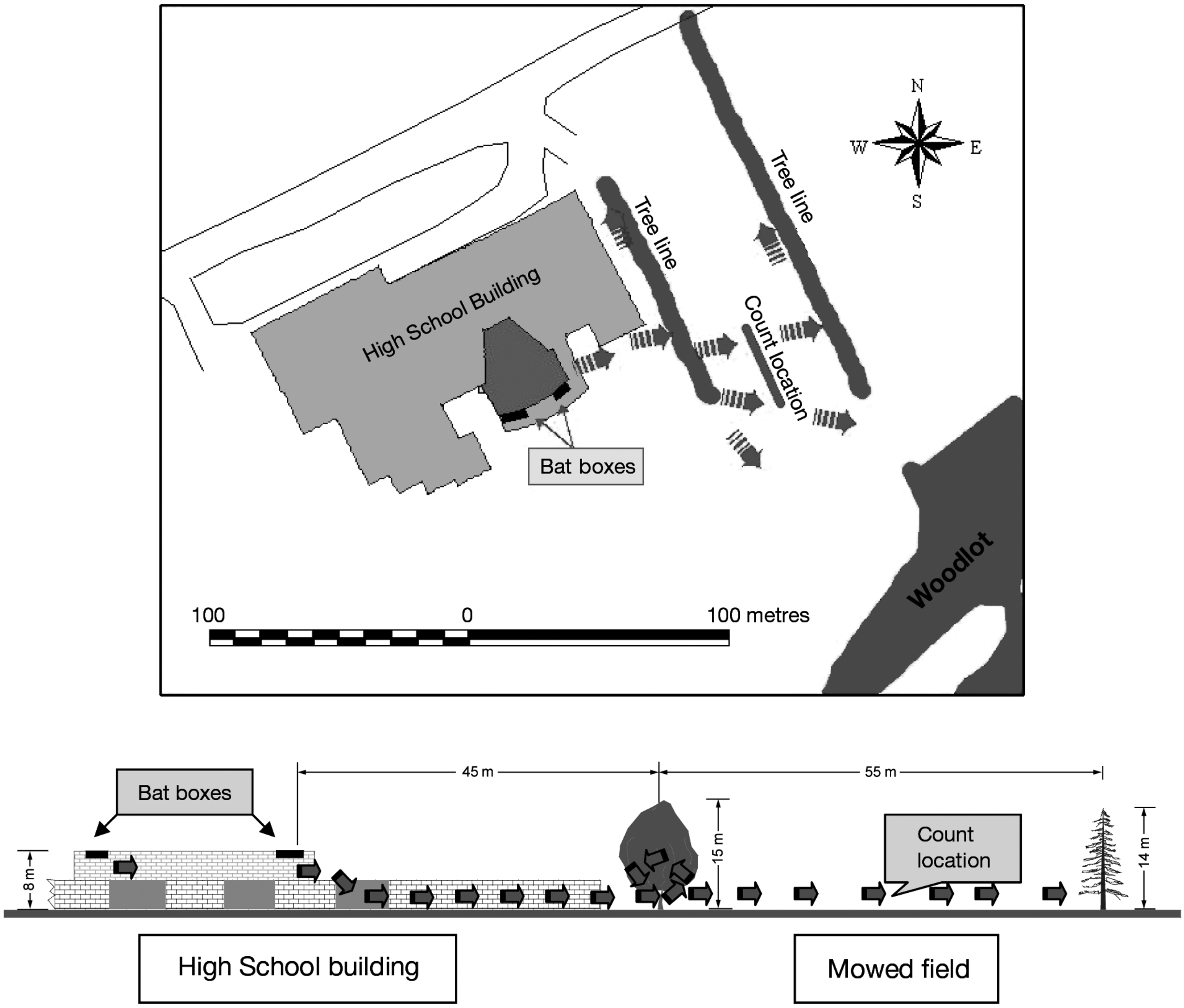

Fig. 2. Overhead and profile views of the Juniata Valley High School count location as bats (*Myotis* spp.) cross a 55 m wide mowed field. Thick arrows indicate bat travel paths

RESULTS

Population estimate of roosts

Condo exit counts were 2200 and 2115 on 24 May and 6 June 2001, respectively, giving an average of 2157. A total of 20 683 bats were counted exiting the church on 28 June 2001. The resulting total roost estimate for the church and condo roosts combined was 22 840 bats.

Mapping of major travel corridors

As bats emerged, 4 travel corridors were distinguished leading from the church roost and 2 from the bat condo (Fig. 1). From the church roost, 2 parallel travel corridors headed south along Canoe Creek and a tree-lined road to the bridge. A third corridor headed northeast and broke into sub-corridors going to the lake and to reverting fields with a small stream. The fourth church corridor headed west and then south into forest habitat where it became widely dispersed. From the condo roost, 1 corridor headed northeast to the lake, and a second headed downstream along Canoe Creek to the bridge. Downstream of the condo and along Canoe Creek, corridors from the church and condo roosts appeared to coalesce and a minor corridor headed east crossing US Route 22 east of the bridge. To evaluate these findings more thoroughly, we conducted dusk searches using bat detectors and visual observations east and west of the bridge to locate other major crossing areas. No major concentrations of bat activity were found other than at the bridge. On 17 and

18 June 2002 counts were conducted west of the bridge (Fig. 1), near where the flat bat was found in 2001, with counts of 100 and 36 bats, respectively. Forest habitat is continuous to the west, with no obvious corridors crossing the highway. Counted bats flew high, primarily traveling across the highway from hilltop to hilltop. Surveyor notes indicate that bats cross this western section of highway at random locations in the broad forested landscape; the total number of bats crossing this section may be significant but it was not possible to conduct an accurate count. The highway crossing to the east of the bridge (Fig. 1) was examined on 14 July 2003. A minor highway-crossing corridor was found at dusk, with 251 bats tallied as they crossed from forest habitat to the north to a tree line on the south side of the highway. Observations indicated that most bats stayed above the traffic at this minor crossing.

Highway crossings

Highway-crossing dusk counts ranged from 1636 to 3351 bats during each of the approximately 40 min evening observation periods (Table 1). Counts of bats crossing the highway at dawn ranged from 62 to 1114 crossings (Table 1). Counts at dawn were correlated with temperature ($r = 0.978$), with the lowest count on the coolest morning (8.9°C) and the highest count on the warmest morning (20.6°C).

Canopy heights were sampled from 30 m north of the Canoe Creek bridge where bats approached the highway from the church and condo roosts. Stns 1 and 2 had consistent canopy cover >20 m high from adjacent to the highway through 30 m to the north; Stn 3 had cover to the north only ≤3m high within 15 m of the highway and 15 to 17 m high 25 m out; Stn 4 was similar to Stn 3, except for a corridor of trees 10 to 15 m high on the east side adjacent to a small parking lot that ended with one 6 m tall tree adjacent to the highway's north verge. This tree, a common catalpa *Catalpa bignonioides*, had a narrow, 5 m diameter canopy. All stations had canopy heights of >20 m beyond 30 m to the north. Canopy located on the south side of the highway at the bridge crossing was consistently high (>20 m) and located directly adjacent to and occasionally overtop of the verge.

During dusk counts, most bats crossed the highway at Stns 1, 2, and 4 (Table 1, Fig. 1), apparently avoiding the area around Stn 3, which had the least canopy cover for approaching bats and no cover adjacent to the highway. Bats often crossed in traffic at Stn 4. Just beside and to the east of the tree line at Stn 4 a small parking lot was also avoided by bats (Fig. 1). The tree line funnels bats to the highway at the eastern-most sector of the Stn 4 count area. The canopy at that location drops to 6 m at the highway with the single catalpa tree. The bats used tree canopy along the highway crossings as cover and favored stations with canopy cover adjacent to the north verge. Where the canopy is

Table 1. *Myotis* spp. Summary of dusk and dawn crossing counts of bats over US Route 22 at Canoe Creek Bridge, Pennsylvania, USA. Duration indicates the total observation time

Date (2001)	Temp (°C)	Local sunset (h)	First/last time bat obs. (h)	Duration (h)	Stn 1	Stn 2	Stn 3	Stn 4	Total
Dusk crossings									
15 May	18.33	20:23	20:24/21:01	0.62	536	844	243	652	2275
21 May	13.33	20:29	20:18/20:57	0.65	395	534	151	556	1636
22 May	17.22	20:30	20:13/20:52	0.65	541	790	166	739	2236
23 May	15.56	20:30	20:29/21:07	0.63	517	791	199	1053	2560
18 Jun	25.56	20:47	20:47/21:27	0.67	665	784	195	701	2345
21 Jun	23.33	20:48	20:40/21:18	0.63	669	642	154	685	2150
25 Jun	21.11	20:48	20:47/21:23	0.60	638	759	139	601	2137
8 Jul	26.67	20:46	20:43/21:22	0.65	871	1060	241	1179	3351
23 Jul	27.22	20:37	20:40/21:15	0.58	847	856	142	840	2685
26 Jul	21.67	20:35	20:40/21:08	0.47	677	550	151	542	1920
				Stn totals:	6356	7610	1781	7548	
Dawn crossings									
22 May	13.33	5:51	5:16/5:56	0.67	46	62	25	145	278
23 May	8.89	5:51	5:11/5:30	0.32	9	21	15	17	62
19 Jun	14.44	5:43	5:05/5:29	0.40	82	141	23	173	419
22 Jun	20.56	5:43	5:02/5:38	0.60	263	317	123	411	1114
9 Jul	15.00	5:51	5:09/5:48	0.65	106	131	58	194	489
24 Jul	18.33	6:03	5:25/5:55	0.50	167	158	53	407	785
				Stn totals:	673	830	297	1347	26 442

high (>20 m) and within 10 m of the highway, bats cross well above the traffic; where the canopy is low (<6 m), the bats cross lower and closer to traffic and are more likely to be struck by vehicles.

The 6 m canopy height should not be confused with bats' flying height at Stn 4. Canopy structure appears to influence but does not dictate the height at which the animals travel. The canopy closest to the highway at Stn 4 is only 5 m in width and far less robust when compared to tall, mature trees. Bats at Stn 4 often approached the highway below the tree height of 6 m. During dawn counts, bats were observed flying north, high above traffic, as they exited high canopy (>20 m) adjacent to the highway while returning to the church and bat condo roosts.

Behavior when crossing a field

Myotis lucifugus at the Juniata Valley High School exited bat boxes mounted 8 m high on a building, traveled 45 m flying <4 m above the ground along the side of the building and entered a 15 m high tree line. They then exited that tree line, crossing a 55 m wide mowed field to a 14 m high tree line on the other side. The flight heights of 453 and 1346 bats were recorded on the evenings of 31 July and 8 August 2002, respectively. The low count on 31 July was due to a thunderstorm. On each evening at least 76% (79.47 and 76.59%) of the bats crossed the field at <2 m above the ground and more than 95% (96.47 and 95.61%) crossed the field flying <4 m above the ground. These heights would place the bats directly in traffic in a highway setting.

Road-killed bats

We found 29 road-killed bats: 15 *Myotis lucifugus* and 1 unidentifiable *Myotis* sp. found at the section of highway near the bridge (Stns 1 through 4), 8 *M. lucifugus* and 1 suspected *M. sodalis* (the flat bat) found on a section of highway 2.8 km to the west, and 4 *M. lucifugus* on a section 1.6 km to the east of the bridge (Table 2). The unidentified *Myotis* species was in too poor condition to be confirmed as *M. lucifugus*, but absence of a keeled calcar rejected its identification as *M. sodalis* or *M. leibii*. Therefore, it was considered to be most likely *M. lucifugu*s or *M. septentrionalis*. The suspected *M. sodalis* (the flat bat) was found 1 km to the west of the bridge, flattened and pressed into the pavement. In addition to the adult flat bat, the total of 29 road kills included 15 juvenile *M. lucifugus*, 11 adult *M. lucifugus*, 1 *M. lucifugus* of undetermined age, and 1 unidentified *Myotis* species of undetermined age. Eighteen of these bats were female, 2 were male and 9 were of undeterminable sex, including the adult flat bat.

Table 2. *Myotis* spp. Summary of road-killed bats found May to July 2001, US Route 22, Blair County, Pennsylvania, USA. Mylu: *M. lucifugus*; Myso: *M. sodalis*; Unk: unknown; Non-repro: non-reproductive; Post-lact: post-lactational

Search	Species			Sex			Age			Reproductive condition				Area checked
dates[a]	Mylu	Myso	Unk	M	F	Unk	Adult	Juv.	Unk	Non-repro	Post-lact	Unk	Total	
21 Jun	2					2	1	1		1		1	2	Bridge area
09 Jul	1		1[b]	1		1		1	1	1		1	2	Bridge area
10 Jul	1				1			1		1			1	Bridge area
11 Jul	2				1	1		1	1	1		1	2	Bridge area
14 Jul	1				1			1		1			1	Bridge area
17 Jul	1				1			1		1			1	Bridge area
18 Jul	1				1		1					1	1	Bridge area
19 Jul	2			1	1			2		1		1	2	Bridge area
23 Jul	2			2				2		2			2	Bridge area
24 Jul	1				1			1				1	1	Bridge area
29 Jul	8	1[c]			5	4	6	3		3	1	5	9	Bridge area & west
30 Jul	4				3	1	3	1		1	1	2	4	Bridge area & east
14 Sep	1				1		1			1			1	Bridge area
Total													29	

[a]No bats found at bridge area on: May 15, 21, 22, 23; June 18, 19, 22, 25; July 8, 12, 13, 15, 16, 20, 21, 22, 25, 26, 27, 28, 31; August 1. On August 22, bridge and both areas east and west of bridge were searched with no bats found

[b]Unknown *Myotis* species. Lack of keeled calcar excludes *M. sodalis* and *M. leibii*. Likely species is *M. lucifugus* or *M. septentrionalis*

[c]Bat (flat bat) verified by molecular analysis as *M. sodalis*

Genetic analysis of the flat bat

A large (14 to 47 bp) insertion in the sequenced region of the mitochondrial D-loop distinguishes *Myotis sodalis* from other *Myotis* species that have been sampled (M. Vonhof pers. comm.). The size of the insertion varies within *M. sodalis*; most (72%) have a 45 bp insertion, 15% have a 47 bp insertion, 2% have a 41 bp insertion, and 11% have a shorter 14 bp insertion. This character does not appear geographically structured within *M. sodalis* populations (data not shown). The sequence from the flat bat possessed the 47 bp insertion.

A maximum likelihood phylogenetic analysis (Fig. 3) strongly supports a species topology that is entirely consistent with that of Stadelmann et al.'s (2007) analysis of North American *Myotis*. Monophyly of *M. sodalis* inclusive of the flat bat is indicated with 100% bootstrap support. Monophyly of *M. lucifugus* exclusive of the flat bat is rather weakly supported (53% bootstrap value). Therefore, we used a parsimony bootstrap analysis to directly evaluate the hypothesis that the flat bat was *M. lucifugus* rather than *M. sodalis*. The negative log likelihood (–lnL) score of the observed edited tree (total sample size = 14) was 1269.51; this score increased to 1315.80 when the flat bat was constrained to be a member of the *M. lucifugus* clade. Comparing this observed difference in –lnL scores to a distribution from 100 simulated datasets allowed us to reject the hypothesis that the flat bat was *M. lucifugus* ($p < 0.01$).

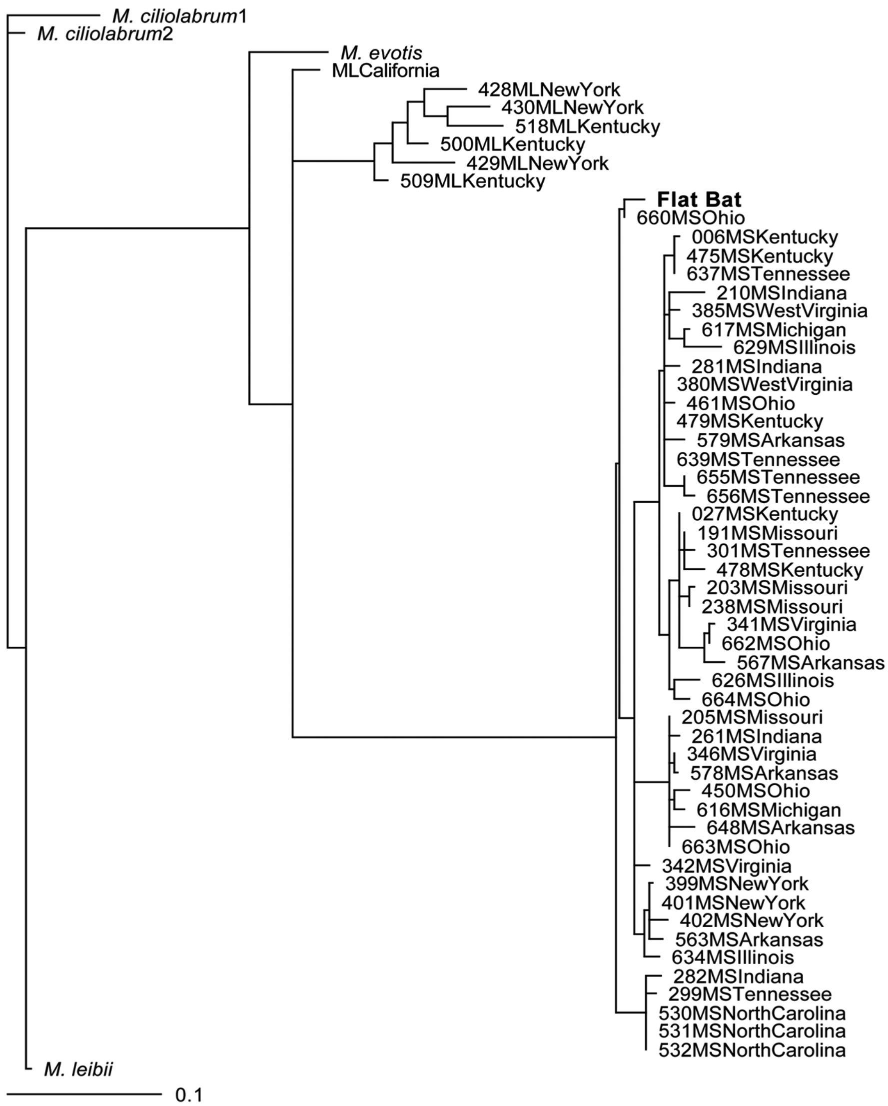

Fig. 3. *Myotis* spp. Single most likely maximum-likelihood phylogram. Haplotypes of *M. lucifugus* and *M. sodalis* are named using a 3-digit individual code, species code (ML or MS, respectively), and location code (by state). The flat bat is shown in **bold** print

Distance-based analyses (Table 3) further illustrate the species affinity of the flat bat. Average distances within species range from 3.704 % for *Myotis sodalis* to 6.384 % for *M. lucifugus*. Average interspecific distances range from 10.815 % between *M. lucifugus* and *M. evotis* to 19.076 % between *M. ciliolabrum* and *M. sodalis*. The sole exception to this pattern is the exceptionally low distance (3.786 %) between *M. ciliolabrum* and *M. leibii*, probably due to inadequate species definitions (Rodriguez & Ammerman 2004). The average distance between the flat bat and bats verified as *M. sodalis* (4.275 %) clearly falls within the range of intraspecific distances for these *Myotis* species, while that between the flat bat and bats verified as *M. lucifugus* (17.608 %) is characteristic of interspecific comparisons.

DISCUSSION

Highways have detrimental effects on terrestrial and aquatic wildlife (Trombulak & Frissell 2000, Coffin 2007). Adverse effects of highways include direct mortality and habitat loss from construction, mortality during operation from collisions with vehicles, modification of animal behavior, alteration of the physical and chemical environment, spread of exotic organisms, and increased use of areas by humans. Collision with vehicles is the primary cause of death of moose *Alces alces* in the Kenai National Wildlife Refuge in Alaska (Bangs et al. 1989) and of barn owls *Tyto alba* in the UK (Newton et al. 1991). Road kill also takes a significant toll on Iberian lynx *Felis pardina* populations in southwestern Spain (Ferreras et al. 1992), white-tailed deer *Odocoileus virginianus* in New York (Sarbello & Jackson 1985), wolves *Canis lupus* in Minnesota (Fuller 1989), and American crocodiles *Crocodylus acutus* in southern Florida (Kushlan 1988). While much documentation of road-kill mortality has focused on terrestrial mammals, birds, reptiles and amphibians, the impact of highways on bat populations has only recently been identified (Kiefer et al. 1995, Wray et al. 2006, Lesinski 2007, López et al. 2007).

Table 3. *Myotis* spp. Average maximum-likelihood distances (%) within and between species. The flat bat, listed separately, is not included in samples of named species

Species	Flat bat	*M. ciliolabrum*	*M. evotis*	*M. leibii*	*M. lucifugus*	*M. sodalis*
M. ciliolabrum	19.137	5.047				
M. evotis	16.468	12.776	–			
M. leibii	17.158	3.786	11.041	–		
M. lucifugus	17.608	15.387	10.815	14.194	6.384	
M. sodalis	4.275	19.076	16.314	17.207	16.520	3.704

We document here 26 442 crossings by bats of a 150 m stretch of US Route 22 in Blair County, Pennsylvania over a period of 9.29 h. Bats crossing this highway were mostly from colonies of *Myotis lucifugus* and *M. sodalis* located in a nearby building and artificial roost. We found 29 road-killed bats, including 1 *M. sodalis*, during 36 d of search; however, this most likely does not reflect the true impact of highway traffic on these bat colonies. Recent bird and bat impact assessments at wind farms identify problems with searcher efficiency and carcass removal by scavengers (Kunz et al. 2007, Arnett et al. 2008), which result in gross underestimates of mortality. *Myotis* species are small and carcasses may travel some distance when hit or may travel off site on the vehicle. If thrown into roadside vegetation, carcasses could be difficult to find and may be removed by scavengers such as cats, opossums, raccoons, foxes and crows.

Given the number of road-killed bats found (29 over 36 d = 0.81 road kills d^{-1}, Table 2), we estimate that, at minimum, 145 bats would be lost to traffic around this highway crossing over a 6 mo activity season (Cope & Humphrey 1977). Given the roost population estimate of 22 840 individuals, this amounts to only a 0.6 % highway mortality. However, the actual number of carcasses found is likely an underestimate of mortality and cannot be used reliably without correction. Arnett (2006) compared trained dog and human searcher efficiencies at 2 wind farm sites by placing bat carcasses of different species and stages of decay within search transects; due to varying habitat and searcher visibility, transects were mapped and 3 visibility classes (Kerns et al. 2005) assigned. Dogs consistently found more placed carcasses at the 2 sites: 71 and 81 % for dogs versus 42 and 14 % for humans for each site, respectively. If searcher efficiency for road-killed bats is at the low end (14 %) of these wind farm rates, the annual highway mortality could approach 5 % of the roost colony. It is likely that searcher efficiency in this study is even lower because (1) no searches were conducted in the adjacent cluttered roadside vegetation, where vehicle impact could throw carcasses, and (2) carcasses carried away on vehicles would never be found. Given these uncertainties, attempting to estimate actual highway mortality likely would be a costly and inconclusive task. Instead, sound management planning to guide bats above traffic or below bridges at highway crossings is clearly needed to decrease mortality.

As bats exited the church and condo roosts at dusk, they established defined travel corridors when moving to foraging areas. They consistently used canopy cover en route, making it fairly easy for surveyors to track the corridors in the surrounding fragmented landscape. This is consistent with the findings of Murray & Kurta (2004), who found that radio-tagged female *Myotis sodalis* did not fly over fields but used wooded corridors for commuting, even when a straight-line route would have been half the distance. Use of cover on these travel corridors likely provides protection from predators. This is emphasized again by our results at the bridge crossing. Bats avoided Stn 3, most likely due to lack of high canopy cover within 15 m to the north of this highway segment and lack of cover adjacent to the verge. Conversely, the tree corridor at Stn 4 provided cover for commuting bats, but did not move bats high enough to avoid traffic. At both Stns 3 and 4, the canopy gradually drops as it approaches the highway with low cover adjacent to the north verge at Stn 4 (6 m tall catalpa tree). It appears that this single tree's low cover attracts bats that use it as a jumping off point to cross the highway. This is consistent with other recent studies showing that linear elements of the landscape may funnel bats across roads and lead to a non-random distribution of road kills along roadways (Capo et al. 2006, Lesinski 2008). Our results indicate that the best landscape feature for bats commuting across a highway would be >20 m high trees immediately adjacent to the highway. The combination of cover adjacent to the highway verge, approach cover height and cover structure need to be more thoroughly researched to fully understand bat highway-crossing corridors.

One would expect that more road-killed bats would be found near Stn 4 and possibly Stn 3, due to the higher numbers of bats crossing at these areas (Fig. 1). Unfortunately, the project was not designed for this necessary precision. Future surveys should place measured survey markers along the search route for reference, especially where travel corridors are close together — such as at the bridge crossing area. The circumstances involved in the actual bat–vehicle impact were not researched here, but given the disproportionate mass involved between the 2 colliding objects, it is probable that the carcasses would not be deposited immediately at impact points; stations at the bridge crossing are likely too close in proximity to correlate with carcass locations. It is recommended that at least 100 m or more should separate research sites selected to correlate landscape with mortality.

Many questions on canopy use by commuting bats still remain. How far will bats travel across a deforested area from one high canopy to another without flying near the ground? Our data suggest 20 to 30 m in a flat landscape. How large and what structure does tree canopy need to be for flight cover? Canopy that is too dense would increase energy demands and may not be used, while canopy that is too open may reduce security and direct bats closer to the ground. Would bats readily use newly created adjacent cover for commuting if the historic corridor is destroyed? Can artificial structures and/or plantings be created to manage and direct commuting bats? It should be noted that bats at the high school were observed flying up to investigate the next highest mason line as they crossed the field, suggesting an immediate response to new structure.

The data presented here relate primarily to *Myotis* species; however, similar issues are relevant for other bat species. Searches of an 8.8 km section of US Route 322 at the Lancaster-Lebanon County border in Pennsylvania between 12 December 2006 and 22 November 2007 revealed 35 bats of at least 6 species, including *Lasiurus borealis* (n = 15), *L. cinereus* (n = 1), *M. septentrionalis* (n = 6), *M. lucifugus* (n = 6), *Eptesicus fuscus* (n = 2), *Perimyotis subflavus* (n = 4), and *Myotis* sp. (n = 1) (Pennsylvania Game Commission unpubl. data).

Many of our data were collected in an attempt to recommend bat-friendly designs for a proposed highway upgrade for US Route 22 at Canoe Creek. The Pennsylvania Department of Transportation reports that traffic demand has exceeded capacity at this location. Current average daily traffic (ADT) counts are estimated at 8569 vehicles per day (12% trucks). ADT counts are expected to increase to 12 249 vehicles per day by 2028. The proposed highway upgrade called for a ca. 55 m deforested width at the bridge with 2 traffic lanes and 1 turning lane (currently there are 2 lanes). The design would also raise the highway 3.7 m above the existing level. A new bridge over Canoe Creek would have an under-clearance of 5.7 m at its highest point. Data presented here indicate that when crossing a deforested area bats would travel closer to the ground surface, presenting a conflict between highway design and bat behavior. Removal of canopy cover used by approaching bats would likely draw them into traffic, while raising the highway surface could exacerbate the effect of canopy loss. While strict highway design standards did not allow much flexibility, various ideas were considered, including planting tall tree species near the edge of the highway, using vegetation to attempt to direct bats under the bridge, placing fencing along the sides of the bridge in an attempt to direct bat flight above traffic, and building a roost structure on the south side of the highway. Because a colony of federally endangered *Myotis sodalis* was implicated in the planning, the US Fish and Wildlife Service had jurisdiction in the formal consultation process under Section 7 of the Federal Endangered Species Act. Many of the questions posed above came into play. A solution acceptable to all parties involved could not be reached. The bridge crossing area was just one part of the project design that encompassed a 4.5 km section of US Route 22. Currently the highway upgrade has been cancelled.

We also document the utility of molecular and phylogenetic techniques for identifying the species impacted by highways. Phylogenetic methods have been applied to verify the species identity or population of origin of protected species, particularly in cetaceans, reptiles, and fish in the food and pet trades (Roman & Bowen 2000, Palsbøll et al. 2006, Baker et al. 2007, Sanders et al. 2008). To our knowledge this is the first application of these methods in studies of road kill. The physical trauma, dessication, exposure and decay experienced in most road kill cases can make DNA extraction from these tissues more difficult than from fresh tissue. We found that high-quality DNA was recoverable from wing tissue given slight modifications to the extraction protocol, specifically, extending proteinase K digestion of the tissue for up to 72 h. Although not tested here, we anticipate that intact muscle tissue should provide an additional source of high-quality DNA in road-killed specimens. Genetic identification of road-killed wildlife depends further on the quality and detail of the reference database. The database used here derived from an ongoing study of population structure in *Myotis sodalis*; the use of these data here perhaps illustrates the broader utility of such phylogeographic studies.

Acknowledgements. The field technicians deserve special thanks for enduring an irregular and demanding schedule, as well as walking the highway to find and collect road-killed bats, some of which took considerable time and effort to remove from the pavement. Field staff included Pennsylvania Game Commission biologist aides S. Wolbert, K. Chaney, M. Bazella, D. Wayland, M. Sneath and M. Ciborowski, as well as Pennsylvania Department of Transportation interns R. Adams, B. Bryan and D. McCombie. The authors thank the following individuals for donating tissue samples of *Myotis lucifugus* and *M. sodalis*: S. Amelon, J. J. Belwood, V. Brack Jr., E. Britzke, S. Carroll, T. Carter, R. Clawson, R. Currie, M. Harvey, A. Hicks, J. Kiser, A. Kurta, S. Pruitt, and C. Stihler. We also thank V. Brown for invaluable assistance in the lab. Funding for this work was provided by both the Pennsylvania Department of Transportation and the US Fish and Wildlife Service.

LITERATURE CITED

Arnett EB (2006) A preliminary evaluation on the use of dogs to recover bat fatalities at wind energy facilities. Wildl Soc Bull 34:1440–1445

Arnett EB, Brown K, Erickson WP, Fiedler J and others (2008) Patterns of bat fatalities at wind energy facilities in North America. J Wildl Manag 72:61–78

Baker CS, Cooke JG, Lavery S, Dalebout ML and others (2007) Estimating the number of whales entering trade using DNA profiling and capture-recapture analysis of market products. Mol Ecol 16:2617–2626

Bangs EE, Bailey TN, Portner MF (1989) Survival rates of adult female moose on the Kenai Peninsula, Alaska. J Wildl Manag 53:557–563

Bissonette JA (2002) Scaling roads and wildlife: the Cinderella principle. Z Jagdwiss 48S:208–214

Butchkoski CM, Hassinger JD (2002) The ecology of Indiana bats using a building as a maternity site. In: Kurta A, Kennedy J (eds) The Indiana bat: biology and management of an endangered species. Bat Conservation International, Austin, TX, p 130–142

Capo G, Chaut JJ, Arthur L (2006) Quatre ans d'étude de mortalité des Chiroptères sur deux kilomètres routiers proches d'un site d'hibernation. Symbioses 15:45–46

Clawson RL (2002) Trends in population size and current status. In: Kurta A, Kennedy J (eds) The Indiana bat: biology and management of an endangered species. Bat Conservation International, Austin, TX, p 2–8

Coffin AW (2007) From roadkill to road ecology: a review of the ecological effects of roads. J Transp Geogr 15:396–406

Cope JB, Humphrey SR (1977) Spring and autumn swarming behavior in the Indiana bat, *Myotis sodalis*. J Mammal 58:93–95

Ferreras P, Aldama JJ, Beltran JF, Delibes M (1992) Rates and causes of mortality in a fragmented population of Iberian lynx *Felis pardina* Temminck, 1824. Biol Conserv 61: 197–202

Forman RTT, Sperling D, Bissonette JA, Clevenger AP and others (2003). Road ecology: science and solutions. Island Press, Washington, DC

Fuller T (1989) Population dynamics of wolves in north-central Minnesota. Wildl Monogr 105:1–41

Grindal SD (1996) Habitat use by bats in fragmented forests. In: Barclay RMR, Brigham RM (eds) Bats and Forests Symposium, October 19–21, 1995, Victoria, British Columbia, Canada. Working Paper 23/1996, Research Branch, British Columbia Ministry of Forests, Victoria, p 260–272

Kerns JW, Erickson P, Arnett EB (2005) Bat and bird fatality at wind energy facilities in Pennsylvania and West Virginia. In: Arnett EB (ed) Relationships between bats and wind turbines in Pennsylvania and West Virginia: an assessment of bat fatality search protocols, patterns of fatality, and behavioral interactions with wind turbines. A final report submitted to the Bats and Wind Energy Cooperative. Bat Conservation International, Austin, TX, p 24–95

Kiefer A, Merz H, Rackow W, Roer H, Schlegel D (1995) Bats as traffic casualties in Germany. Myotis 32-33:215–220

Kunz TH, Arnett EB, Cooper BM, Erickson WP and others (2007) Assessing impacts of wind-energy development on nocturnally active birds and bats: a guidance document. J Wildl Manag 71:2449–2486

Kushlan JA (1988) Conservation and management of the American crocodile. Environ Manag 12:777–790

Lesinski G (2007) Bat road casualties and factors determining their number. Mammalia 71:138–142

Lesinski G (2008) Linear landscape elements and bat casualties on roads — an example. Ann Zool Fenn 45:277–280

Limpens HJGA, Helmer W, Van Winden A, Mostert K (1989) Bats (Chiroptera) and linear landscape elements: a review of our present knowledge of the importance of linear landscape elements to bats. Lutra 32:1–20

López LJ, Vargas-Contreras JA, Arroyo-Cabrales J (2007) Bat road-killing nearby 'El Volcán de los Murciélagos' cave, Calakmul, Campeche, México. Poster presentation at 14th International Bat Research Conference, Mérida, Yucatan, México

Maddison WP, Maddison DR (2000) MacClade 4: analysis of phylogeny and character evolution. Version 4.01. Sinauer Associates, Sunderland, MA

Mickleburgh SP, Hutson AM, Racey PA (2002) A review of the global conservation status of bats. Oryx 36:18–34

Murray SW, Kurta A (2004) Nocturnal activity of the endangered Indiana bat (*Myotis sodalis*). J Zool (Lond) 262:197–206

Newton I, Wylie I, Asher A (1991) Mortality causes in British barn owls *Tyto alba*, with a discussion of aldrin-dieldrin poisoning. Ibis 133:162–169

Palsbøll PJ, Berube M, Skaug HJ, Raymakers C (2006) DNA registers of legally obtained wildlife and derived products as means to identify illegal takes. Conserv Biol 20: 1284–1293

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818

Rambaut A, Grassly NC (1997) Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. Comput Appl Biosci 13:235–238

Rodriguez RM, Ammerman LK (2004) Mitochondrial DNA divergence does not reflect morphological difference between *Myotis californicus* and *Myotis ciliolabrum.* J Mammal 85:842–851

Roman J, Bowen BW (2000) The mock turtle syndrome: genetic identification of turtle meat purchased in the south-eastern United States of America. Anim Conserv 3: 61–65

Russell AL, Medellín RA, McCracken GF (2005) Genetic variation and migration in the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*). Mol Ecol 14:2207–2222

Russell AL, Goodman SM, Fiorentino I, Yoder AD (2008) Population genetic analysis of *Myzopoda* (Chiroptera: Myzopodidae) in Madagascar. J Mammal 89:209–221

Sanders JG, Cribbs JE, Fienberg HG, Hulburd GC, Katz LS, Palumbi SR (2008) The tip of the tail: molecular identification of seahorses for sale in apothecary shops and curio stores in California. Conserv Genet 9:65–71

Sarbello W, Jackson LW (1985) Deer mortality in the town of Malone. New York Fish Game J 32:141–157

Stadelmann B, Lin LK, Kunz TH, Ruedi M (2007) Molecular phylogeny of New World *Myotis* (Chiroptera: Vespertilionidae) inferred from mitochondrial and nuclear DNA genes. Mol Phylogenet Evol 43:32–48

Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony (and other methods), version 4.0b10. Sinauer Associates, Sunderland, MA

Thompson JD, Higgens DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Thomson CE (1982) *Myotis sodalis*. Mamm Species 163:1–5

Trombulak SC, Frissell CA (2000) Review of ecological effects of roads on terrestrial and aquatic communities. Conserv Biol 14:18–30

Wilkins KT, Schmidly DJ (1980) Highway mortality of vertebrates in southeastern Texas. Tex J Sci 32:343–350

Worthington Wilmer J, Barratt E (1996) A non-lethal method of tissue sampling for genetic studies of chiropterans. Bat Res News 37:1–3

Wray S, Reason P, Wells D, Cresswell W, Walker H (2005) Design, installation, and monitoring of safe crossing points for bats on a new highway scheme in Wales. In: Irwin CL, Garrett P, McDermott KP (eds) Proc 2005 Intl Conf Ecol Transport. Center for Transportation and the Environment, North Carolina State University, Raleigh, NC, p 369–379

Zimmerman GS, Glanz WE (2000) Habitat use by bats in eastern Maine. J Wildl Manag 64:1032–1040

Editorial responsibility: Stephen Rossiter, London, UK

Submitted: March 1, 2008; Accepted: July 20, 2008
Proofs received from author(s): September 4, 2008