Amphibian Encounter Rates on Roads with Different Amounts of Traffic and Urbanization

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Abstract: Although amphibians bave relatively high rates of road mortality in urban areas, the conditions under which traffic threatens the survival of local amphibian populations remain unclear. In the Sandhills region of North Carolina (U.S.A.), we counted living and dead amphibians along two transects (total length 165 km) established on roads in areas with varying degrees of urbanization. We found 2665 individuals of 15 species, and amphibian encounter rates declined sharply as traffic and urban development increased. Regression-tree models indicated that 35 amphibians/100 km occurred on roads with <535 vehicles/day, whereas the encounter rate decreased to only 2 amphibians/100 km on roads with >2048 vehicles/day. Although mortality rate peaked at higher traffic levels (47% dead on roads with >5200 vehicles/day), the number of dead amphibians was highest at low levels of traffic. This suggests that areas where amphibian mortality is concentrated may actually contain the largest populations remaining on a given road transect.

Keywords: amphibians, road mortality, toads, traffic, urban gradients

Tasas de Encuentro de Anfibios en Carreteras con Diferentes Cantidades de Tráfico y Urbanización

Resumen: Aunque los anfibios tienen tasas de mortalidad relativamente altas en áreas urbanas, las condiciones bajo las cuales el tráfico amenaza la supervivencia de poblaciones locales de anfibios no son claras. En la región Sandbills de Carolina del Norte (E.U.A.), contamos anfibios vivos y muertos a lo largo de dos transectos (longitud total: 165 km) establecidos en carreteras en áreas con diferentes grados de urbanización. Encontramos 2665 individuos de 15 especies, y las tasas de encuentro con anfibios declinaron abruptamente a medida que incrementaron el tráfico y el desarrollo urbano. Modelos de árbol de regresión indicaron que ocurrieron 35 anfibios/100 km con <535 vebículos/día, mientras que la tasa de encuentro disminuyó a solo 2 anfibios/100 km en carreteras con >2048 vebículos/día. Aunque la tasa de mortalidad fue máxima en niveles altos de tráfico (47% muertos en carreteras con >5200 vebículos/día), el número de anfibios muertos fue mayor en niveles bajos de tráfico. Esto sugiere que las áreas donde se concentra la mortalidad de anfibios pueden contener las mayores poblaciones remanentes en un determinado transecto de carretera.

Palabras Clave: anfibios, gradientes urbanos, mortalidad en carreteras, sapos, tráfico

Introduction

Among vertebrate groups, amphibians seem particularly vulnerable to the effects of urbanization due to their need for both terrestrial and aquatic ecosystems to complete their life cycles (Semlitsch 2000; Hamer & McDonnell 2008). This dual dependency makes amphibians sensitive to degradation of either type of habitat, and ponds, wetlands, and streams in urbanized landscapes generally contain fewer species or lower abundances of frogs, toads, and salamanders (Willson & Dorcas 2003; Pellet et al. 2004; Rubbo & Kiesecker 2005; Pillsbury & Miller 2008). Roads are one of the primary components of urban development, and when amphibians must cross roads to breed

§Address for correspondence: 5801 Mountain Island Drive, Durbam, NC 27713, U.S.A., email rws10@duke.edu Paper submitted August 24, 2009; revised manuscript accepted January 19, 2010. or disperse the animals often experience high levels of mortality (Ashley & Robinson 1996; Carr & Fahrig 2001; Puky 2006).

It is unclear, however, when road mortality will cause the decline of local amphibian populations, given the high reproductive rates of certain species and the possibility that density-dependent survivorship may compensate for individuals killed on roads (Ashley & Robinson 1996; Hels & Buchwald 2001). The absence of dead amphibians along a stretch of road cannot be interpreted by itself as a sign of secure local populations because several different mechanisms could lead to low levels of road mortality. According to conventional wisdom, few animals should be killed when vehicle traffic is infrequent. Nevertheless, the absence of road-killed amphibians on high traffic roads could mean the local population has been depleted by road mortality, or that the animals are intimidated by passing vehicles and do not attempt to cross (Seiler & Helldin 2006). Fahrig et al. (1995) counted the number of live and dead frogs and toads for six nights along roads with three different levels of traffic. They found that roads with the least amount of traffic have the highest absolute number of live and dead amphibians, even though the proportion of dead animals is at a minimum on these roads. Their findings have yet to be validated by similar investigations in other regions; thus, it remains uncertain what areas of high amphibian mortality on roads (Clevenger et al. 2003; Langen et al. 2009) reveal about the conservation status of amphibian populations in a given area.

To address this knowledge gap, we sampled live and dead amphibians in road transects along two gradients of urbanization. We expected amphibian encounter rates to decline as traffic and urbanization increased or as availability of aquatic habitats decreased. Such declines could be sharp and nonlinear if threshold levels of urbanization and habitat conditions yield rapid changes in the demography of local amphibian populations. We also hypothesized that relative mortality rates increase as vehicle traffic increases, even as total (live and dead) encounter rates fall. This follows from the expected negative relation between the frequency of passing vehicles and the probability of an amphibian crossing the road successfully. We also tested whether amphibian encounter rates on roads were significantly and positively related to detection of frog and toad choruses along the road transects (Fahrig et al. 1995).

Methods

Study Area

The original vegetation of the North Carolina Sandhills (U.S.A.) consisted of savannahs of longleaf pine (*Pinus palustris*), turkey oak (*Quercus laevis*), and wiregrass (*Aristida stricta*) in the xeric uplands and dense

"pocosin" vegetation (with a mixed pine-hardwood canopy) in the bottomlands and blackwater creeks dissecting the sand ridges (Wells & Shunk 1931; Sorrie et al. 2006). The longleaf pine ecosystem has high diversity of herbaceous plants (Peet 2006) and reptiles and amphibians (Means 2006). More than 97% of longleaf pine forests have been destroyed (Varner & Kush 2004; Van Lear et al. 2005; Frost 2006). The Sandhills contains a large extent of protected longleaf forest (approximately 100,000 ha), but this forest is now increasingly surrounded by urban development and other human activities.

Sampling Techniques

We drove at night (Shaffer & Juterbock 1994) along road transects that traversed two clear gradients of urbanization in the Sandhills (Fig. 1). The routes were designed to capture a wide range of traffic, urbanization, and habitat conditions. Average daily traffic ranged from a low of 20 to a high of 14,000 cars/day (North Carolina Department of Transportation 2005). We established the length of each transect (northern route, 75 km; southern route, 69 km) so that a round trip along one route could be made in the approximately 4 h between dusk and midnight. The majority of the two transects consisted of 2-lane paved roads. We did not survey busy highways with posted speed limits of 89 km/h (55 miles/h) or higher because these roads were unsafe to sample with our methodology.

We surveyed the northern transect only in 2006 and 2008, whereas in 2007 we surveyed the northern and southern transects in roughly equal proportions (Supporting Information). Our sampling effort in 2008 was four nights (533 km) with heavy rainfall as part of a mark-recapture study of toads. In 2006 (39 nights, 5752 km) and 2007 (46 nights, 10,371 km) we sampled without regard to precipitation. To prevent biases associated with the time that a given point along the transect was sampled, we alternated randomly among a series of standardized routes with different starting and stopping locations. An additional 21 km of roads were added to the northern transect in 2007 and 2008 to provide access to intermediate points along the main stems of that transect. Thus, the total length of the north-south transect was 165 km.

We drove the routes at a consistent speed of 48-56 km/h and tallied all living or dead amphibians encountered. Often it was not possible to identify frogs and toads to the species level because live animals tended to leave the road and dead animals were frequently unrecognizable. We were able, however, to classify most amphibians to the genus level without stopping the car, which saved considerable time. In 2008 we stopped to capture and identify every toad over 2 cm (snout vent length). These more intensive surveys (which often took >6 h) revealed that the similar morphology of the spadefoot



Figure 1. Maps of the study region showing (a) the location of the road transects with respect to local urban centers and protected areas, (b) the background gradient of average percent impervious surface calculated at a 1-km radius from each 30-m pixel, and (c) the location of the Sandbills region in North Carolina (black box).

(*Scaphiopus bolbrooki*) and *Anaxyrus* (formerly *Bufo*) spp. toads prevented accurate differentiation of these two genera while in transit. Thus, for statistical analysis, we combined all the toad data into a single value. The remaining analysis groups included true frogs (*Lithobates*), tree frogs (*Hyla* and *Acris*), and total amphibians (all species).

In 2007 and 2008 we recorded the locations of all choruses (one or more individuals) of calling frogs and toads we heard along the routes while driving with our vehicle windows down. In addition, we noted frog and toad choruses we heard along the northern route during a pair of 3-min point counts conducted in May and June 2007 for the purpose of surveying several species of nightjars (Aves: Caprimulgidae) along the northern transect (one point-count location at the midpoint of each 1-km segment of road).

Geospatial Analyses

We recorded amphibian locations as vehicle mileages from adjacent intersections. We converted these locations into digital coordinates with ArcGIS software (versions 8.2-9.3; ESRI, Redlands, California). All potential repeat observations of the same dead animal (same species or genus, same location, different trips) on a given night were removed from the data. To enable calculation of encounter rates, we broke the route into a series of 165 segments, each roughly 1 km long (range 800–1200 m). We then divided the total number of animals of a given group (e.g., tree frogs) observed on a segment by the total distance driven on that segment (number of one-way trips * length of segment). We calculated the rate of chorus detection (combined across all frog and toad species) by dividing the total number of detection episodes for each road segment by the distance driven; point counts were treated as a single traverse of the corresponding road segment. In lieu of direct estimates of the apparent size of each chorus, we treated the rate of detection of frog and toad choruses as a rough index of the number of calling males at ponds and wetlands along each section of the route, reasoning that larger populations would be detected more frequently.

We used ArcGIS to create traffic, urbanization, and aquatic habitat variables that corresponded to each of the road segments. The simplest predictor variable was average daily traffic volume, which we derived for each road segment with traffic data from the North Carolina Department of Transportation (2005). We calculated the remaining variables as average values for overlapping buffer polygons established around each road segment at 11 different radii (30, 250, and 500 m and 1, 2, 3, 4, 5, 6, 8, and 10 km). The four variables calculated at multiple extents included traffic density (a measure of road density in which traffic on roads is taken into account; Pellet et al. 2004), percent impervious surface (an index of urban development; Morse et al. 2003), percent open water (ponds and lakes), and percent wetlands.

Other researchers have documented that abiotic and biotic features characterized across relatively large

extents affect amphibian populations (Gibbs et al. 2005). Thus, rather than constraining our analyses to a single arbitrary radius (e.g., 1 km), we used a range of distances (Pellet et al. 2004). The four variables measured at different extents were highly correlated (e.g., traffic and urbanization), but we retained all the variables in their original units to maximize the clarity of our results.

Statistical Analyses

Because our response and predictor variables were spatially autocorrelated, we used an improved version of the regression-tree methodology described by De'Ath and Fabricius (2000) to analyze our amphibian encounter rate data. Regression trees recursively split the response data into increasingly homogenous groups. Each split was based on a single division among one of the predictor variables (e.g., average daily traffic >535 cars/day). The method does not assume strict independence among the data points and filters through large numbers of variables to choose the best sets of predictors in the final tree. The optimal-splitting procedure also forces the model to identify thresholds or break points in the predictor variables associated with the sharpest distinctions within the response data. We could have used newer tree-based methods (e.g., random forests and boosted regression trees) or other nonparametric techniques (e.g., Spearman's rank correlation and generalized additive models) with our data (Walsh & Kleiber 2001; Prasad et al. 2006; De'ath 2007; Elith et al. 2008), but we chose the single regression-tree approach because its outputs remain in the original units of the predictor variables and thus results are easy to interpret.

Following De'Ath and Fabricius (2000), we changed the RPART routine (Therneau & Atkinson 1997) in R (R Development Core Team 2007) so that the tree-pruning process (based on k-fold cross-validation) was repeated 200 times (each time k = 20), yielding a much more stable estimate of the most robust number of splits for the final models. For each regression tree, we calculated the apparent R^2 , which represented the amount of variation explained by the pruned tree model for the complete data set. Nevertheless, given the tendency of regression-tree models to overfit the available data (De'Ath & Fabricius 2000), we considered the overall best model for a given species group the regression tree with the highest crossvalidation R^2 (x-val R^2), which is an indication of how much variance was explained on average by regression trees formed from the k = 20 cross-validation subsets with the same set of available predictor variables. To test whether the optimally pruned models achieved higher x-val R^2 values than what would have been returned in the absence of relations between the response and predictor variables, we also implemented a Monte Carlo significance test (with 500 random permutations) for each final tree (Manly 1997).

To examine the response of amphibian encounter rates to increases in urbanization and decreases in aquatic habitat availability, we fit regression trees for each of four groupings of amphibian species with the full range of 45 variables. To enable comparison across the different spatial extents and variable categories, we also fit trees for each single predictor variable independently. To test whether amphibian mortality rates increased as a function of increasing vehicle traffic, we calculated the percentage of dead animals among the total encounters for each group of species and ran regressiontree models relating the mortality rates to the average daily traffic on the road segments. Finally, we used the same regression-tree methods to test whether the encounter rate for total amphibians was significantly and positively associated with the number of frog and toad choruses we detected along the roads during the surveys.

Results

Overview

We recorded 2665 live and dead amphibians of 15 species on the road (Supporting Information). Our observations included 2184 toads (2 spp. of Anaxyrus and Scaphiopus holbrooki), 236 tree frogs (5 spp. of Hyla and at least 1 sp. of Acris), 230 true frogs (4 spp. of Litbobates), 1 Gastrophryne carolinensis (a microhylid), 2 red salamanders (Pseudotriton ruber), and 12 dead anurans that could not be classified to the genus level. Most of the animals we found are common, widespread species, such as southern and Fowler's toads (Anaxyrus terrestris and A. fowleri), gray and green tree frogs (Hyla chrysocelis and H. cinerea), and bull and green frogs (Lithobates catesbeiana and L. clamitans). Generally, we found the greatest number of amphibians on portions of the transects that passed through low-lying swamps and riverine forests. Nevertheless, we recorded the highest encounter rate for toads (94 animals/100 km) on an upland road segment that passed through a private farm with numerous ponds. This farm was surrounded by forest within a large expanse of state-owned land.

We also detected 1002 frog and toad choruses of 14 species in 2007-2008. Cricket frogs (*Acris*; n = 400), gray tree frogs (n = 226), and green tree frogs (n = 174) constituted 80% of the total chorus detections, whereas only 59 choruses of *Anaxyrus* toads (36 *A. terrestris*, 23 *A. fowleri*) were heard along the routes. We heard three species of frogs (*Hyla andersonii*, *Pseudacris ornata*, and *Lithobates virgatipes*) that were not otherwise detected on the road during the surveys. Three other species (*Lithobates palustris*, *L. sphenocephalus*, and *Scaphiopus bolbrooki*) that were seen on the road were not detected in the chorus surveys.



Figure 2. Encounter rates on roads in 2007 relative to average daily traffic for (a) total amphibians (living and dead) along the original 75 segments of the northern transect, (b) total amphibians along the 69 segments of the southern transect (driven only in 2007) and the 21 segments of access roads (added to the northern transect in 2007), (c) dead amphibians along the northern transect, and (d) dead amphibians along the southern transect and access roads. Each point represents a single 1-km segment of road.

The number of animals we found dropped from 28 in 2006 to 7 animals/100 km in 2007. The highest encounter rates were in 2008 (64 animals/100 km), but in that year we conducted surveys only on rainy nights. We focused our regression-tree models on the combined 2006–2008 amphibian encounter rates for the northern route, which was driven in all 3 years. Even though the magnitude of encounter rates was different across years, the general patterns of amphibian responses to the traffic and urbanization variables appeared similar across the northern and southern routes in 2007 (Fig. 2).

Effects of Traffic, Urbanization, and Habitat

According to the regression-tree models, predicted toad encounter rates declined steeply when average daily traffic, traffic density, and impervious surface values were above very minimal levels. The same steep negative relations were apparent for the total amphibian group, driven by the preponderance of toads in the overall data. For example, the single-variable model that related the total number of amphibians to average daily traffic yielded an optimally pruned regression tree with two splits (*x*-val $R^2 = 0.39$, p < 0.002; Fig. 3a). When average daily traffic was <535 vehicles/day, the amphibian encounter rate was predicted to be 35 animals/100 km driven, whereas when traffic was above 2048 vehicles/day, expected amphibian encounter rate dropped to 2. Nearly identical trees (but with higher *x*-val R^2 values of 0.53 and 0.50, respectively, p < 0.002) were returned in the traffic-density (250 m radius) models for toads and total amphibians, which provided the best overall regression trees for those groups. The best impervious-surface regression tree for total amphibians (*x*-val $R^2 = 0.44$, p < 0.002), in contrast, showed that the most amphibians (45 animals/100 km) were found on roads segments with impervious surface (3-km radius) values between 0.45% and 0.59%. Above 1.8% average impervious surface at that extent, however, the predicted encounter rate decreased to 4 animals/100 km.

According to the regression-tree models, tree frog and true frog encounter rates both declined sharply in response to increased levels of impervious surface along the roads (best extent for each = 250 m). In the tree frog model, which was the best overall for that species group, encounter rates declined from 6 animals/100 km when impervious surface (250 m) was <0.49% to 0.2 animals/100 km when impervious surface was above 5.9% (*x*-val $R^2 = 0.28$, p < 0.002). In contrast, the frogs' strongest relations with traffic density occurred at 5 km for the true frogs (*x*-val $R^2 = 0.13$, p < 0.002). In both cases,



Figure 3. Regression-tree model results for (a) total amphibian (living and dead) encounter rates and (b) percentage of amphibians that were dead when encountered, both relative to average daily traffic on the transect roads (gray bars, values predicted by the pruned regression trees; points, combined [2006–2008] encounter rates [a] or mortality rates [b] for each of the 75 1-km road segments of the northern route).

and in the similar models that were returned for average daily traffic, the frog encounter rates showed a unimodal response, with peaks at low to intermediate levels of traffic.

The observed relations between encounter rates and measures of aquatic habitat (open water, wetland) varied considerably among groups and across the two variables. In the open-water models with the highest x-val R^2 , toads, tree frogs, and total amphibians responded strongly and negatively to amount of water at fairly broad extents (4-5 km). This result appeared to be related to the positive correlation between urbanization and the prevalence of ponds and lakes at these larger extents. In contrast, the best open-water model for true frogs showed a positive relation at the 500-m extent (x-val $R^2 = 0.05$, p = 0.014). All the best wetland models indicated positive relations between encounter rates and wetland coverage, and the 30-m wetland variable was the best overall model for true frogs (x-val $R^2 = 0.24$, p < 0.002), which indicated their presence was best explained by the relative presence of wetlands within the immediate vicinity of the roads.

At smaller extents (<1 km) the highest open-water and wetland concentrations occurred along road segments with low traffic density and low levels of urbanization. Nevertheless, when we considered only data from areas with few ponds and wetlands (e.g., wetland coverage <1%), there was still a clear negative effect of traffic density on amphibian encounter rate (Fig. 4).

Mixed-effect regression trees that incorporated urbanization and aquatic habitat variables were always outperformed (in terms of *x*-val R^2) by the best single-variable models. Nevertheless, the multivariable models often explained more of the variation in the full data set. For example, the optimally pruned, full-model tree for total amphibians attained an apparent R^2 of 0.79 (*x*-val $R^2 = 0.41$, p < 0.002) with three variables and four splits. According to the splitting criteria from this model (Fig. 5), amphibian encounter rates will rise to over 50 animals/100 km on road segments with low traffic density at a broad extent (6-km radius), many wetlands (500 m), and moderately low levels of impervious surface (2 km). In contrast, roads with high traffic density and high levels of impervious surface are predicted to have encounter rates of <2 animals/100 km surveyed.

For a given variable and amphibian group, the performance of the regression-tree models was fairly stable across the 11 different extents of the road-segment buffers. The differences between amphibian groups were much more pronounced than the differences between extents. The closely linked toad and total amphibian models consistently had higher values of *x*-val R^2 than models for the other groups, particularly at extents >500 m.

Traffic and Amphibian Mortality Rates

According to our regression-tree results, mortality rates (percentage of animals on each segment that were found dead) for toads, true frogs, and total amphibians all showed the expected positive relations with average daily traffic. For example, in the regression-tree model for total amphibian mortality rate, the percentage of dead animals rose from 15% when average daily traffic was <885 vehicles/day to a high of 47% when traffic was >5200 vehicles/day (x-val $R^2 = 0.07$, p = 0.008; Fig. 3b). Even though the mortality rate increased as levels of traffic increased, the encounter rate for dead amphibians was highest on roads with low levels of traffic (e.g., <1000 vehicles/day) and peaked at 16.4 dead animals/100 km on a road segment with 100 vehicles/day (Fig. 2c). In contrast to the results for total amphibians, the model of tree frog mortality showed a peak of just over 50% dead tree frogs when traffic was 758-1365 vehicles/day (x-val $R^2 = 0.09$, p = 0.014). Above and below this narrow range, mortality rates were lower. Model performance was low for all of the mortality-rate regression trees due to the high variance in the percentage of dead animals among the different sections of roads.

Frog and Toad Encounter Rates and Chorus Detection

The combined 2006–2008 amphibian encounter rates for the road segments along the northern route were significantly and positively associated with chorus detection rates measured in 2007–2008 (Supporting Information). According to the regression-tree model (two splits, *x*-val



Figure 4. Total amphibian encounter rates on roads relative to traffic density at two different levels of wetland coverage: wetlands below (low wetlands) and above (bigh wetlands) 1% within a 250-m radius of the 1-km road segments. Traffic density was calculated by multiplying (for each road in the search radius, including the transect road itself) the number of vehicles per day on the road by the area of the road (after conversion to a raster grid, units = number of 30-m pixels), and then dividing the sum of these products by the total number of 30-m pixels in the search radius.

 $R^2 = 0.29$, p < 0.002), when chorus detection rates were above 17 choruses/100 km surveyed, encounter rates for total amphibians peaked at 52 animals/100 km.

Discussion

We found that the number of amphibian encounters on roads decreased sharply as traffic and urbanization in-



creased. This relation was especially evident for toads (*Anaxyrus* and *Scaphiopus*), which were the majority of the amphibians we observed. The vulnerability of toads to vehicle traffic has been well documented in previous road-kill surveys (e.g., Van Gelder 1973; Orlowski 2007). Surveys of ponds also reveal lower abundances of breeding *Anaxyrus* (Tupper & Cook 2008) and *Scaphiopus* toads (Nystrom et al. 2002) in urban, high-traffic areas. We, however, are the first to quantify living and dead amphibians on roads across a wide gradient of traffic

Figure 5. The optimally pruned regression-tree model for total amphibians generated with all variables. Road segments for which the listed splitting criterion is true are represented at the left side of each split. Numbers at the terminal nodes are the predicted total (live and dead) amphibian encounter rate (amphibians/100 km) on road segments that fall within the conditions indicated (n, number of segments). The relative length of the branches indicates the amount of variation explained by each split. The impervious surface and wetland splitting criteria are expressed in terms of the land-cover composition of road-segment buffers at the 2-km and 500-m buffer distances, respectively. See caption of Fig. 4 for traffic density units.

levels and urbanization. We found that at traffic levels of >2000 vehicles/day, few toads (live or dead) remained on the road at night. This finding is consistent with the empirical observations of Van Gelder (1973), who calculated that 90% of adult toads in his study population would be killed each year on a road with only 3600 vehicles/day. Our observations lead us to believe that many of the toads we encountered on the road transects on summer nights were searching (or at least waiting) for insects on the road surface, in agreement with Clarke (1974). For the toads, higher levels of traffic and urbanization appeared to be associated with a 20-fold decline in encounters, whereas where there were few ponds or wetlands encounter rates decreased only by half.

The frog groups we encountered displayed negative relations with traffic and urbanization that were similar to those of toads, but the rates of decline for frogs did not appear to be as great. For the most common frogs in the Sandhills (bullfrogs, green frogs), this trend may be explained by the year-round association of these species with aquatic habitats (Rubbo & Kiesecker 2005). With little need to cross roads on a regular basis, adult bullfrogs and green frogs should be able to persist and breed in urban ponds and lakes long after more terrestrial species of frogs, toads, and salamanders are extirpated by traffic mortality. Tree frogs typically use some form of upland habitats in their life cycles, but according to our observations in the Sandhills, tree frogs tend to cross roads more quickly than true frogs and much more quickly than toads.

The best multivariable model for total amphibians counterintuitively predicted that on low-traffic, high-wetland road segments encounter rates will be highest when impervious surface values are above 0.46%. Nevertheless, the maximum value of impervious surface (2 km) within the eight segments of the tree node in question was only 0.78%. This suggests to us that the peak in amphibian encounters on roads is occurring in rural environments, where small amounts of human infrastructure are likely associated with higher densities of human-made farm ponds and associated breeding amphibians.

Our data suggest mechanisms that determine the frequency with which road-killed amphibians are encountered in different areas. As also shown by Fahrig et al. (1995) and Orlowski (2007), it appears that more dead amphibians are found on roads with light traffic than on roads with heavy traffic. Our results and those of Fahrig et al. (1995) suggest this occurs because the total number of amphibians on the road is much higher on roads with little traffic. In the Sandhills at least, total amphibian encounters will be reduced considerably long before the proportion of animals observed that are dead reaches 50%.

This finding may affect the interpretation of the results of road-kill surveys for amphibians. Roads segments where dead amphibians are frequently encountered may in fact represent the populations that are the least affected (proportionally) by road mortality along a particular survey route, with the obvious exception being roads with no traffic at all. Such populations are important to protect from additional effects of increased vehicle traffic or urbanization. Nevertheless, conservation professionals should also consider the potential benefits of highway-mitigation projects for amphibian populations that already appear to be depleted due to excessive road mortality, especially when rare species are involved and breeding and foraging habitats are otherwise available.

One limitation of our use of road transects to survey amphibians is that our results are expressed in terms of the number of amphibians encountered on the road; thus, they do not reflect directly patterns of local or regional amphibian population density along the roads. It is conceivable that amphibians living alongside busy roads have either learned individually to avoid crossing roads or have experienced selective pressure on behaviors that reduce the tendency to cross roads (Dodd et al. 1989). Amphibians and other vertebrates may also simply be deterred from crossing busy roads by a constant flow of vehicles (Seiler & Helldin 2006; Bouchard et al. 2009). Nevertheless, we found a close association between the number of amphibians seen on the road and the number of frog and toad choruses heard adjacent to the road, which suggests that road-based surveys can provide useful information regarding the relative abundance of amphibians in road-side habitats. Fahrig et al. (1995) also found that the number of amphibians on roads correlates with an index of amphibian calling activity, and Seiler and Helldin (2006) provides a list of other studies in which data from road-kill surveys appeared to correspond well with independent estimates of local or regional wildlife abundance.

Detection rates of frogs and toads either on the road or calling from roadside breeding habitats could be negatively biased in urban areas by the aural and visual interferences imposed by vehicle traffic. Heavy traffic would also tend to destroy quickly the remains of dead amphibians, suppressing road-kill counts in urban areas. Traffic volumes on most roads typically decline steeply as night progresses (e.g., Fig. 1 in Hels & Buchwald 2001), which suggests observation conditions may have been more equalized across the urban gradient during our late evening and early morning surveys. When we compared amphibian encounter rates before and after the approximate midpoint of our nightly survey efforts (22:00), the same negative relation with average daily traffic was apparent in both early-evening and late-night conditions (Fig. 6). This observation provides strong evidence that the depletion effect of roads and urbanization on amphibians that we found is more than just an expression of difficult survey conditions on roads with heavy traffic.

The strong correlation between traffic volume on roads and the amount of surrounding urban development



Figure 6. Total amphibian encounter rates (2006-2008) relative to average daily traffic within two time ranges: animals observed before and after 22:00. Each point represents the encounter rate for a single 1-km segment of road.

prevented us from distinguishing which factor affected the structure of local amphibian populations the most. Roads with heavy traffic influence amphibian mortality rates, but urbanization reduces the quantity and quality of potential breeding and foraging habitats. Regardless of the other impacts of urban development, we anticipate that areas where roads exert effects (road-effect zones) (Forman & Deblinger 2000) will at least be additive, such that mortality will be doubled in habitats within a certain distance of two busy roads. It is conceivable that overlapping road-effect zones could even have synergistic (greater than additive) effects on local populations, given the nonlinear trends in amphibian encounter rates we documented in the Sandhills.

Severe drought conditions were present across much of the southeastern United States during 2007, and the lack of rainfall is the most obvious explanation for the sharp decline in amphibian encounter rates we observed between 2006 and 2007. Including the 2008 data from surveys conducted only in the rain is unlikely to have influenced the spatial pattern of amphibian encounter rates because our survey effort in that year (as in the 2 previous years) was well balanced across the entire northern transect.

There seemed to be a substantial difference in species composition between amphibians on the road at night (primarily toads) and amphibians detected breeding in roadside aquatic habitats (primarily tree frogs and cricket frogs). This discrepancy reflects that *Anaxyrus* toads in the Sandhills region were largely finished breeding by the time we began our summer surveys. The significant positive association between toad encounter rates and chorus detection for the summer-breeding frogs indicates both groups likely share the same breeding habitats. We expected observations of salamanders would be low because most salamanders in the Sandhills breed in autumn and winter.

The bulk of the amphibians we encountered belonged to generalist species capable of breeding in permanent ponds in suburban areas. Even for these frogs and toads, it appeared that light traffic (>2000 vehicles/day) and low levels of urbanization (>1.8% impervious surface at a 3-km radius) can lead to severe reductions in local abundance along roads. In fact, we encountered no amphibians at all on 3 of the most urbanized road segments we surveyed, despite making >100 trips on warm summer nights down these roads. On the basis of our results and those of similar studies, it seems plausible that urban development and road mortality can together account for a sizeable portion of the declines in amphibian abundance that have been noted in urbanizing regions around the world.

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Supporting Information

Tables providing various parameters of the road survey methodology (Appendix S1), a detailed accounting of the amphibian species observed during the study (Appendix S2), and a figure showing the relation between total amphibian encounter rates and chorus detection rates (Appendix S3) are available as part of the online article. Authors are responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

Literature Cited

- Ashley, E. P., and J. T. Robinson. 1996. Road mortality of amphibians, reptiles and other wildlife on the long point causeway, Lake Erie, Ontario. Canadian Field-Naturalist 110:403-412.
- Bouchard, J., A. T. Ford, F. E. Eigenbrod, and L. Fahrig. 2009. Behavioral responses of northern leopard frogs (*Rana pipiens*) to roads and traffic: implications for population persistence. Ecology and Society 14:23. Available at http://www.ecologyandsociety.org/vol14/iss2/art23/
- Carr, L. W., and L. Fahrig. 2001. Effect of road traffic on two amphibian species of differing vagility. Conservation Biology **15:**1071–1078.
- Clarke, R. D. 1974. Activity and movement patterns in a population of Fowlers toad, *Bufo woodbousei fowleri*. American Midland Naturalist 92:257–274.

- Clevenger, A. P., B. Chruszczc, and K. E. Gunson. 2003. Spatial patterns and factors influencing small vertebrate fauna road-kill aggregations. Biological Conservation **109:15**–26.
- De'ath, G. 2007. Boosted trees for ecological modeling and prediction. Ecology 88:243–251.
- De'ath, G., and K. E. Fabricius. 2000. Classification and regression trees: a powerful yet simple technique for ecological data analysis. Ecology 81:3178–3192.
- Dodd, C. K., K. M. Enge, and J. N. Stuart. 1989. Reptiles on highways in north-central Alabama, USA. Journal of Herpetology 23:197-200.
- Elith, J., J. R. Leathwick, and T. Hastie. 2008. A working guide to boosted regression trees. Journal of Animal Ecology 77:802–813.
- Fahrig, L., J. H. Pedlar, S. E. Pope, P. D. Taylor, and J. F. Wegner. 1995. Effect of road traffic on amphibian density. Biological Conservation 73:177-182.
- Forman, R. T. T., and R. D. Deblinger. 2000. The ecological road-effect zone of a Massachusetts (USA) suburban highway. Conservation Biology 14:36-46.
- Frost, C. 2006. History and future of the longleaf pine ecosystem. Pages 9–42 in S. Jose, E. J. Jokela, and D. L. Miller, editors. The longleaf pine ecosystem: ecology, silviculture, and restoration. Springer-Verlag, New York.
- Gibbs, J. P., K. K. Whiteleather, and F. W. Schueler. 2005. Changes in frog and, toad populations over 30 years in New York State. Ecological Applications 15:1148-1157.
- Hamer, A. J., and M. J. McDonnell. 2008. Amphibian ecology and conservation in the urbanising world: a review. Biological Conservation 141:2432–2449.
- Hels, T., and E. Buchwald. 2001. The effect of road kills on amphibian populations. Biological Conservation 99:331–340.
- Langen, T. A., K. M. Ogden, and L. L. Schwarting. 2009. Predicting hot spots of herpetofauna road mortality along highway networks. Journal of Wildlife Management 73:104–114.
- Manly, B. F. J. 1997. Randomization, bootstrap, and Monte Carlo methods in biology. Chapman & Hall, London.
- Means, D. B. 2006. Vertebrate faunal diversity in longleaf pine ecosystems. Pages 157–213 in S. Jose, E. J. Jokela, and D. Miller, editors. The longleaf pine ecosystem: ecology, silviculture, and restoration. Springer, New York.
- Morse, C. C., A. D. Huryn, and C. Cronan. 2003. Impervious surface area as a predictor of the effects of urbanization on stream insect communities in Maine, USA. Environmental Monitoring and Assessment 89:95–127.
- Nystrom, P., L. Birkedal, C. Dahlberg, and C. Bronmark. 2002. The declining spadefoot toad *Pelobates fuscus*: calling site choice and conservation. Ecography 25:488-498.
- North Carolina (NC) Department of Transportation. 2005. Road conditions 2005. NC Department of Transportation GIS Unit, Raleigh.
- Orlowski, G. 2007. Spatial distribution and seasonal pattern in road mortality of the common toad *Bufo bufo* in an agricultural landscape of south-western Poland. Amphibia-Reptilia **28:**25–31.
- Peet, R. K. 2006. Ecological classification of longleaf pine woodlands. Pages 51-93 in S. Jose, E. J. Jokela, and D. L. Miller, editors. The longleaf pine ecosystem: ecology, silviculture, and restoration. Springer, New York.
- Pellet, J., A. Guisan, and N. Perrin. 2004. A concentric analysis of the impact of urbanization on the threatened European tree

frog in an agricultural landscape. Conservation Biology 18:1599-1606.

- Pillsbury, F. C., and J. R. Miller. 2008. Habitat and landscape characteristics underlying anuran community structure along an urban-rural gradient. Ecological Applications 18:1107–1118.
- Prasad, A. M., L. R. Iverson, and A. Liaw. 2006. Newer classification and regression tree techniques: bagging and random forests for ecological prediction. Ecosystems 9:181–199.
- Puky, M. 2006. Amphibian road kills: a global perspective. Pages 325–338 in C. L. Irwin, P. Garrett, and K. P. McDermott, editors. Proceedings of the 2005 International conference on ecology and transportation. Center for Transportation and the Environment, North Carolina State University, Raleigh, North Carolina.
- R Development Core Team. 2007. R: a language and environment for statistical computing. Version 2.6.1. R Foundation for Statistical Computing, Vienna.
- Rubbo, M. J., and J. M. Kiesecker. 2005. Amphibian breeding distribution in an urbanized landscape. Conservation Biology 19:504–511.
- Seiler, A., and J. O. Helldin. 2006. Mortality in wildlife due to transportation. Pages 165–189 in J. Davenport, and J. L. Davenport, editors. The ecology of transportation: managing mobility for the environment. Springer-Verlag, Dordrecht, The Netherlands.
- Semlitsch, R. D. 2000. Principles for management of aquatic-breeding amphibians. Journal of Wildlife Management 64:615-631.
- Shaffer, H. B., and J. E. Juterbock. 1994. Night driving. Pages 163-166 in W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster, editors. Measuring and monitoring biological diversity: standard methods for amphibians. Smithsonian Institution Press, Washington, D.C.
- Sorrie, B. A., J. B. Gray, and P. J. Crutchfield. 2006. The vascular flora of the longleaf pine ecosystem of Fort Bragg and Weymouth Woods, North Carolina. Castanea 71:127–159.
- Therneau, T. M., and E. J. Atkinson. 1997. An introduction to recursive partitioning using the RPART routines. Mayo Foundation, Rochester, Minnesota.
- Tupper, T. A., and R. P. Cook. 2008. Habitat variables influencing breeding effort in northern clade Bufo fowleri: implications for conservation. Applied Herpetology 5:101–119.
- Van Gelder, J. J. 1973. Quantitative approach to mortality resulting from traffic in a population of *Bufo bufo* L. Oecologia 13:93–95.
- Van Lear, D. H., W. D. Carroll, P. R. Kapeluck, and R. Johnson. 2005. History and restoration of the longleaf pine-grassland ecosystem: implications for species at risk. Forest Ecology and Management 211:150-165.
- Varner, J. M., and J. S. Kush. 2004. Remnant old-growth longleaf pine (*Pinus palustris* Mill.) savannas and forests of the southeastern USA: status and threats. Natural Areas Journal 24:141–149.
- Walsh, W. A., and P. Kleiber. 2001. Generalized additive model and regression tree analyses of blue shark (*Prionace glauca*) catch rates by the Hawaii-based commercial longline fishery. Fisheries Research 53:115-131.
- Wells, B. W., and I. V. Shunk. 1931. The vegetation and habitat factors of the coarser sands of the North Carolina Coastal Plain: an ecological study. Ecological Monographs 1:465–520.
- Willson, J. D., and M. E. Dorcas. 2003. Effects of habitat disturbance on stream salamanders: Implications for buffer zones and watershed management. Conservation Biology 17:763-771.

