Research Article

Do the Numbers and Locations of Road-Killed Anuran Carcasses Accurately Reflect Impacts of Vehicular Traffic?

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ABSTRACT Road-killed animals are easy and inexpensive to survey, and may provide information about species distributions, abundances, and mortality rates. As with any sampling method, however, we need to explore methodological biases in such data. First, how does an animal's behavior (e.g., use of the center vs. periphery of the road) influence its vulnerability to vehicular traffic? Second, how rapidly do post-mortem processes (scavenging by other animals, destruction or displacement by subsequent vehicles) change the numbers and locations of roadkills? Our surveys of anurans on a highway in tropical Australia show that different anuran species are distributed in different ways across the width of the road, and that locations of live versus dead animals sometimes differ within a species. Experimental trials show that location on the road affects the probability of being hit by a vehicle, with anurans in the middle of the road begin hit 35% more often than anurans on the edges; thus, center-using species are more likely to be hit than edge-using taxa. The magnitude of post-mortem displacement and destruction by subsequent vehicles depended on anuran species and body size. The mean parallel displacement distance was 122.7 cm, and carcasses of thin-skinned species exhibited greater post-mortem destruction. Scavenging raptors removed 73% of carcasses, most within a few hours of sunrise. Removal rates were biased with respect to size and species. Overall, our studies suggest that investigators should carefully evaluate potential biases before using roadkill counts to estimate underlying animal abundances or mortality rates. © 2014 The Wildlife Society.

KEY WORDS *Bufo marinus*, cane toad, frog, invasive species, roadkill, scavenger.

The carcasses of road-killed animals provide abundant evidence of direct human impacts on native biota (Case 1978, Erritzoe et al. 2003, Collins and Kays 2011). In addition to simply documenting the most direct impact of roads on animals (death due to collision with vehicles), carcasses of road-killed animals can provide a wealth of other information. For example, they have provided data on the spread of disease in wild animal populations (e.g., rabies in racoons [*Procyon lotor*], Anthony et al. 1990; bovine tuberculosis in badgers [*Meles meles*], Coulsen and de Leeow 2004), changes in animal abundance (e.g., red fox [*Vulpes vulpes*], Baker et al. 2004; eastern barred bandicoots [*Perameles gunnii*], Mallick et al. 1998; white-tailed deer [*Odocoileus virginianus*], McCaffery 1973), and the impacts of invasive species on native animal populations (Burmese pythons [*Python molurus*] in the Florida Everglades; Dorcas et al. 2012). Data on roadkills also have been used to identify animal activity patterns (Henke and Montemayor 1998), to quantify species diversity (Turner et al. 2003), and to document range expansion (e.g., nine-banded armadillo [*Dasypus novemcinctus*]; Hofmann 2005). Carcasses can provide a source of gene-sequence information (Doyon et al. 2003, Russell et al. 2011) and skeletons for educational purposes (Gobalet 2003). Additionally, data on road-killed animals can be incorporated into mitigation plans (Taylor and Goldingay 2010). Increasing financial and ethically driven restrictions on interfering with live wildlife may encourage more researchers and wildlife managers to make use of animals already killed on roads as a direct source of animal specimens for study or via data collection in the form of surveys of road-killed animals.

As with any sampling method, we need to ensure that we can avoid and/or account for any significant biases associated with road-killed samples if they are to provide us with reliable information. For example, the reliability of roadkill counts as indices of underlying population parameters can be reduced by 2 main biases: the probability of contact with a vehicle (probability of being hit) and the probability of the animal carcass being detected given the animal has been hit (a combination of carcass persistence time and carcass detectability by observers). Overall rates of roadkill will depend upon traffic flow, vehicle speeds, and aspects of road construction that affect the visibility of a vehicle to an animal on the road (and vice versa). Whether or not an individual or species is hit depends upon factors such as activity rates of the

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individual or species or the ability to evade oncoming vehicles (Loughry and McDonough 1996, Aresco 2005), whereas carcass persistence time and subsequent detection of the carcass by researchers depends on events such as carcass removal by scavengers or relocation or obliteration by traffic (e.g., Flint 1926, Hodson and Snow 1965, Underhill and Angold 2000, Brzezinski et al. 2012, Hubbard and Chalfoun 2012).

If either of these probabilities varies across species, age class, sex, body size, and geographical location, and this goes unnoticed, then our data will possess systematic bias and any inferences made from those data will be misleading. For example, road mortality rates were female-biased in both turtles (Aresco 2005, Gibbs and Steen 2005) and barn owls (*Tyto alba*; Moore and Mangel 1996). Juvenile Florida scrub jays (*Aphelocoma coerulescens*) had higher road mortality rates than did adults (Mumme et al. 2000), and neonate snakes were more likely to be found as roadkill than adults (DeGregrorio et al. 2010). A meta-analysis showed that mammals of about 1.06 kg were more frequently encountered in roadkill surveys than smaller or larger taxa (Ford and Fahrig 2007). Geographical location of roads can influence wildlife mortalities (Clevenger et al. 2003), as can road surface type (Smith-Patten and Patten 2008), speed limits (Gunther et al. 1998), traffic volume, and season (da Rosa and Bager 2012, Seo et al. 2013), and these factors may interact in significant ways. Different locations are also likely to have different scavenger assemblages, and so scavenger impacts are likely to differ geographically. For example, previous studies on this topic in Florida, USA (Antworth et al. 2005), France (Guinard et al. 2012), Portugal (Santos et al. 2011), and Brazil (Teixeira et al. 2013) are not directly transferable to the Australian tropics, where scavenger assemblages include animals not found on most other continents (e.g., varanid lizards). Thus, patterns of scavenging may vary because of geographic variation in the abundance and identity of scavenger species. All such bias has the potential to undermine any study inferring the effects of traffic on populations, and understanding the nature of and magnitude of such bias can aid in the interpretation of data on roadkill counts.

In the present study, we quantified spatial patterns and detection of road-killed anurans on roads in the Australian wet-dry tropics. Specially, we addressed 3 potential sources of bias that might influence conclusions from road surveys: 1) differential vulnerability of anuran species; 2) differential persistence post-mortem due to carcass removal by passing traffic or scavengers; and 3) magnitude of destruction of carcasses by subsequent traffic and the likelihood of correctly identifying carcasses to species given the level of damage inflicted by traffic. We predicted that if some species were less adept at avoiding traffic (perhaps because of their location on their road), their rates of mortality per unit time spent on the road would be higher than for a species that selects less vulnerable locations, or detects and avoids vehicles; thus, the more at-risk taxa may be disproportionately represented among roadkill victims. In addition, if carcass persistence varied because of body size, bodily structure, or location on the road, the bodies of some species may be disproportionately likely to persist. Although most researchers would acknowledge that several processes could influence roadkill counts, the scarcity of information on the nature and magnitude of such biases makes predicting the importance of such effects impossible (Taylor and Goldingay 2010, Santos et al. 2011).

STUDY AREA

We conducted fieldwork 60 km east of Darwin (131.020 48.1900 E; 12.340 14.8100S), Northern Territory, Australia, along the Arnhem Highway, which is a major arterial road, and a connecting smaller road (Anzac Parade). Both were paved roads, with sandy to muddy verges covered with grass or gravel. This tropical area contains a diverse array of native anurans, and invasive cane toads (*Rhinella marina*) that arrived at the site in 2005 (Phillips et al. 2007) and are now abundant (Brown et al. 2011). Temperatures are high year-round (max. monthly temperatures remain above 30°C), but rainfall is concentrated in a brief (Jan–Apr) wet season (Shine and Brown 2008).

METHODS

To evaluate the importance of factors such as traffic volume and road-surface temperature on rates of roadkill and of carcass destruction, we used the effects of location within a road (center vs. middle of lane vs. edge) to quantify traits of interest without the confounding effects that would be introduced by comparisons at larger spatial scales.

Road Surface Temperature

Anurans at our study site may be attracted to and use the warm road surface as a means of thermoregulation, with the consequently longer residence times increasing the thermoregulating animal's risk of being hit by passing cars (in contrast to individuals that are simply crossing the road). To quantify surface temperatures of the road and road verge, we set out 6 transects parallel to each other (running from east to west) along Anzac Parade and perpendicular to the road. We did not use the Arnhem Highway for these measurements because of its higher traffic volume and our concern for researcher safety. Transects were spaced 6 m apart (paved road surface width = 6 m, and 3 m verge to either side), and each consisted of 9 points spaced at 1.5-m intervals, with 2 verge locations occurring 1.5 m and 3 m from the edge of the pavement. The innermost point on each transect was in the road center, the next 2 were in the middle of each lane, the third set were on the road edge (but still on the paved surface), the fourth pair and the 2 outermost points were in the surrounding grassy verge (i.e., not on pavement). At each point, we measured substrate temperature by holding a Raytek Rayngger ST2L infrared thermometer (Raytek, Santa Cruz, CA) 1 m above the ground, pointed directly downward. The resultant reading quantified temperature integrated over a 12.5-cm diameter circular area, centered on the specified transect point. We took readings once during each 1-hour period from 1800 to 0200 hours on 21 and 29 March and 5 and 13 April 2009, dates that were chosen at random.

We used a general linear mixed model using Proc MIXED (SAS Institute 1998) to assess if the road surface temperature varied with time of day, location within the 6-m distances across the road, and their interaction. To account for the non-independence resulting from repeated use of the 4 transects, we specified transect number as a random effect. Specifying location on road and sampling hour as categorical variables accounted for non-linearity in temperature across the transect and with time, respectively.

Surveys of Anuran Location on Roads

Comparisons of the numbers and locations of live versus dead anurans can clarify whether or not a frog's location on the road affects its vulnerability to vehicular traffic. To compare the distributions across the road of live versus dead anurans, we divided the width of the road into 5 sections equal in size, using only the paved road surface (i.e., excluding the gravel or grassy verges, and using road center, middle of the lane, and road edge). The 5 sections correspond to 1 center location, 2 middle of lane sections, and 2 edge sections. On 4 nights between 13 March and 19 April 2009, beginning at half an hour after dusk, we slowly drove at speeds of 10 km/hr or less along the same 25-km stretch of road to record species, locations, and status (alive vs. dead) of each frog and toad we saw. We had 1 driver and 1 observer. Although some authors have reported that the carcasses of road-killed animals are best detected by surveying on foot (Havlin 1987, Erritzoe et al. 2003, Langen et al. 2007), we found that live frogs fled from a walking observer more than from a vehicle. We also assumed detection probability by the observer to be the same between live and dead anurans.

Using survey data, we asked if the proportion of observed animals differed across the 3 sections of the road, if these proportions differed for live versus dead animals, if we found an interaction between location on the road and whether the animal was alive or dead, and tested for an effect of the 4 sampling dates using a general linear model in Proc GLM (SAS Institute 1998). We arcsine square root transformed proportion data.

Removal by Scavengers Experiment

We measured the removal rates of road-killed cane toads and 1 common native frog species (*Litoria dahlii*) by all scavenger species (including raptors). These 2 species are commonly found as roadkill, and are large enough for their displaced or partially consumed carcasses to be easily discoverable. We collected virtually undamaged carcasses of road-killed large (i.e., adult) cane toads (mean snout-urostyle length [SUL] = 100.4 mm) and *L. dahlii* (mean SUL = 46.6 mm). Because we seldom found small (i.e., juvenile) toads as roadkill, we collected live specimens (mean SUL = 54.55 mm) and euthanized them via pithing. We stored carcasses in the freezer until the day of the trial (max. 3 weeks in the freezer), at which time we defrosted them prior to placement. We placed shallow cardboard trays (75 cm by 55 cm, 1-cm deep) in transects along the road verge, and filled them with fine sand to allow identification of predators from footprints left behind. Each of the 4 transects consisted of 40 trays (spaced a minimum of 100 m apart), with transects separated by at least 1 km. We placed 2 transects along the Arnhem Highway and 2 on Anzac Parade.

We sampled 1 transect per day, with 4 transects run in the wet season (Apr 2007) and 11 in the dry (Jun and Jul 2007; for details see Beckmann and Shine 2011; no transects received the same treatment within seasons). We had 5 treatments: large toads, small toads and frogs placed alone on the trays, large toads and frogs placed together on 1 tray, and small toads and frogs placed together on a tray (because the proportion of carcasses removed by scavengers were not influenced by being placed alone vs. paired; see Beckmann and Shine 2011). We were unable to locate any juvenile toads during the wet season, thus we did not conduct trials with small toads during the wet season. We placed carcasses belly-up in the center of the tray just after sunset on the night of the trial. Initial studies included regular (every 2 hr) monitoring through the night, but low nocturnal removal rates allowed us to decrease monitoring to once just before sunrise (approx. 0600 hours). At sunrise, we replaced any carcasses removed during the night (during the first approx. 12 hr of the experiment), thereby allowing for a complete data set for both night and day active scavengers. We continued to monitor the trays once every 2 hours from dawn (0700 hours) to dusk (1900 hours). We noted when carcasses were removed, and identified scavengers by examining footprints in the sand and through direct observations. For additional detail on experimental set up, see Beckmann and Shine (2011).

Because of low removal rates during the night (see Results Section), we could not conduct statistical analysis of these data. For those individuals that were removed, we assessed if time of removal of the carcasses by daytime scavengers varied with carcass type (large cane toad, small cane toad, frog) or season (wet or dry) using a general linear model in Proc MIXED (SAS Institute 1998). We specified transect identity as a random effect. We $\ln + 1$ transformed time of removal data.

Carcass Displacement Experiment

Being run over might render an anuran unidentifiable, and/or result in its carcass being thrown off the road by passing cars. Either of these events might prevent the roadkill event from being detected and recorded by researchers. We sampled 10 transects across the width of the road spaced a minimum of 200 m apart along the Arnhem Highway, where we divided the width of the road into 5 equal sections using only the paved surface. The 5 sections correspond to 1 center location, 2 middle of lane sections, and 2 edge sections. We painted a small different colored dot in the center of each section. The posted speed limit along this section of highway was 130 km/hr. We used excess carcasses collected for the removal by scavengers experiment (above) for this experiment. We tied a piece of string to the middle of each carcass. The string was color-coded to match the painted dot and we placed the carcass on the dot of matching color, thus allowing us to determine the exact starting location of any carcasses that had been moved. We placed 1 carcass belly-down (like a live frog or toad) on each painted spot on the road at dusk. We ran 35 transects with large cane toads (mean

SUL = 110.4 mm ± 44.9), 15 with small cane toads (mean SUL = 54.6 mm ± 7.1), and 40 with *L. dahlii* frogs (mean SUL = 46.6 mm ± 16.4) over 13 days between 1 and 21 April 2009. We checked transects shortly before dawn the following morning because very few carcasses are removed by scavengers overnight (see Results Section).

Each carcass was scored for how far it had been moved along the road from its starting position, and the angle moved (0° = parallel with the road in the direction the carcass was moved). From these measurements, we calculated the distance the carcass was moved from its starting point both perpendicular and parallel to the road via trigonometry. If we could not find a carcass, we searched the road surface and the verge on either side for 10 m in all directions before scoring the carcass as lost. For carcasses that were lost, we recorded the perpendicular distance moved as the maximum width of the road (854 cm) and entered the parallel distance moved as missing data. This approach estimates the minimum distances moved.

We tested whether carcass type (large toad, small toad, frog) and location on the road (road center, middle of lane, road edge) influenced whether or not the carcass was run over by a vehicle (1) or not run over (0) on the road using a logistic regression with binomial error, the logit link function, and Type 3 tests (Proc GENMOD, SAS Institute 1998). We evaluated fixed effects using a Wald test, which follows a Chi-squared distribution.

We tested whether finding the carcass on the road was influenced by carcass type (large toad, small toad, frog) and original location (road center, middle of lane, road edge) or their interaction. For this analysis, we used a logistic regression with binomial error, the logit link function, and Type 3 tests (raw binomial data: carcass found, carcass not found; Proc GENMOD; SAS Institute 1998).

We also scored each carcass for its identifiability to species on a 1–5 scale: 1 = perfect condition, probably not run-over; 2 = run over but still easily identifiable to species (flattened and/or minor damage); 3 = difficult to identify (carcass mangled but all parts are present and skin is intact enough in places to make identification possible); 4 = impossible to identify to species (carcass severely mangled so as to be unrecognizable to species level; 5 = could not be located (thrown off the road by passing vehicles, or perhaps stuck to the vehicle). We tested whether carcass identification score (1–5) was influenced by carcass type (large toad, small toad, and small frog) and location of the carcass on the road (road center, middle of lane, road edge) and their interaction using a general linear model in Proc GLM (SAS Institute 1998). Finally, using data only from carcasses that had been hit on the road, we asked if location on the road and carcass type influenced the distances of parallel and perpendicular displacement using a general linear model in Proc GLM (SAS Institute 1998). We included these 2 measures (parallel and perpendicular distances) so as to provide guidelines to researchers on how far off the road to search for carcasses (perpendicular distance), as well as how far a carcass may be moved parallel to the road, which could influence the optimal placement of mitigation features such as tunnels.

RESULTS

Road Surface Temperature

Temperatures varied across the roadway profile, with the hard-paved areas warmer than the verge ($F_{4,\,1,929} = 1{,}225.7$, $P < 0.001$). Temperatures declined into the evening ($F_{8,\,1,929} = 573.2$, $P < 0.001$), but the rate of cooling depended on roadway position (interaction $F_{32,\,1,929} = 7.61.16$, $P < 0.001$; Fig. 1). The road center and middle of lane locations did not differ in temperature (least squares means; post hoc $P > 0.23$), but all other locations differed from each other (least squares means; all post hoc $P < 0.001$). Reflecting their initially higher temperature, center-road locations cooled on average 1.4°C/hr faster than the road edges, which cooled at a rate of 0.7°C/hr (position × time interaction). The paved road surface was always warmer than the air temperature between 1800 and 0200 hours, by 10.8°C at 1800 hours and by 4.8°C at midnight.

Surveys of Location on Road

We found 1,631 identifiable frogs and toads of 11 different species on the road during the surveys, of which 41% were dead (Table 1). We compared locations between live and dead animals only for the 6 species for which we found more than 30 individuals (Table 1).

Of the 6 species, roadkills of 2 species (*L. bicolor*, *L. caerulea*) were too scarce to compare proportions found alive versus dead; thus, we only analyzed data for animals found alive in these 2 species. Locations of live *L. bicolor* were evenly distributed across the road ($F_{2,\,6} < 0.34$, $P = 0.73$; Fig. 2F),

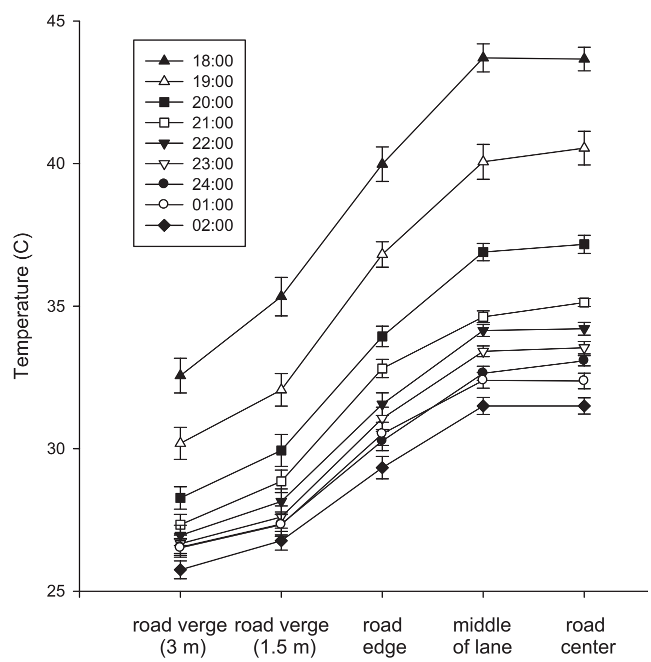

Figure 1. Hourly road surface temperature profiles on transects across a bitumen road near Middle Point Village, Northern Territory, Australia between 1800 hours and 0200 hours in 2009. Graph shows mean values (±SE) for each time period based on 6 replicates at each of 9 equally spaced points along transects perpendicular to the road. Road center, middle of lane, and road edge are on the paved road surface, whereas the 2 verge locations are 1.5 m and 3 m from the edge of the pavement.

Table 1. Number of alive, dead, and total individual number of frogs and toads found during road surveys by species, at Middle Point Village, Northern Territory, Australia during 2009.

Species	*n* alive	*n* dead	*n* total
Litoria rubella	5	0	5
Litoria caerulea	30*	19	49
Litoria dahlii	395*	244*	639
Litoria bicolor	53*	4	57
Litoria nasuta	171*	115*	286
Litoria rothii	180*	88*	268
Litoria tornieri	4	0	4
Cyclorana australis	3	3	6
Limnodynastes convexiusculus	18	9	27
Opisthodon ornatus	4	0	4
Rhinella marina	104*	181*	285

Asterisk indicates species used in statistical analyses.

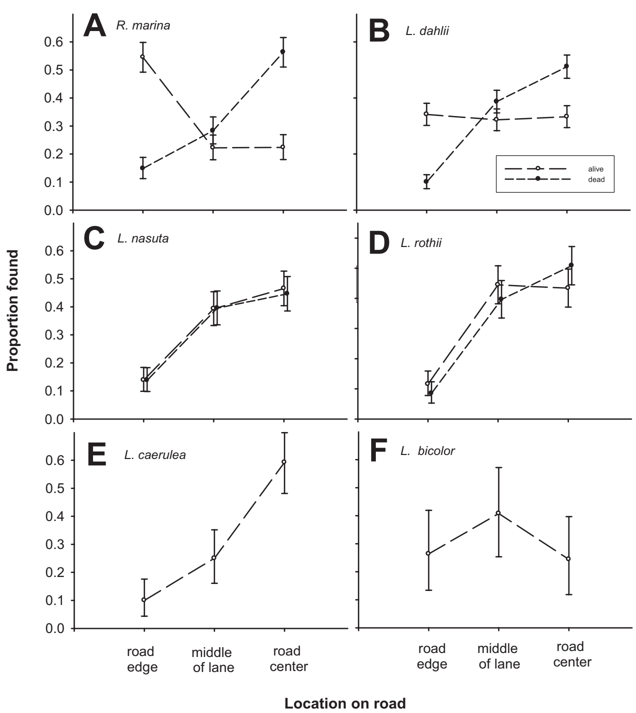

Figure 2. Back transformed mean (±SE) proportion of (B–F) frogs and (A) toads found in each section of the road (road edge, middle of lane, road center) for animals found alive (open circles), and animals found dead (closed circles) for each species analyzed, near Middle Point Village, Northern Territory, Australia in 2009.

whereas live *L. caerulea* were most often found near the center of the road ($F_{2,6} < 6.4$, $P < 0.02$; Fig. 2E). We did not find an effect of date for either species (both: $F = 0.05$, both $P > 0.9$). Post hoc tests show that proportions of live *L. caerulea* averaged 18% higher in the center of the road than in the middle or edge (both $P < 0.05$), but we did not find a difference in proportions between the road edge and middle of the lane ($P = 0.23$).

For the remaining 4 of the 6 species (*L. dahlii*, *L. nasuta*, *L. rothii*, *R. marina*), we found more than 30 individuals both alive and dead, and thus could compare locations of those found alive versus dead. Two of the native frog species (*L. nasuta*, *L. rothii*) were found both alive and dead in higher numbers toward the center of the road (both $F_{2,15} > 19$, both $P < 0.001$; Fig. 2C,D). We did not find an effect of date, status (live or dead), or the interaction between status and location on the proportion found in each section of the road (all $F > 0.01$, all $P > 0.87$). Post hoc tests for both species showed no significant difference in proportions of alive and dead animals found in the middle and center locations (both $P > 0.28$), but the proportions of both live and dead animals were on average 31% higher in the center and middle than the edge (all $P < 0.001$). For 1 native frog (*L. dahlii*) and the invasive cane toad (*R. marina*), location on the road affected the proportion of anurans that were found alive versus dead (interaction location × status, both $F_{2,15} > 18$, both $P < 0.001$; Fig. 2A,B). We did not find an effect of date for either species (both $F > 0.02$, both $P > 0.90$). Post hoc tests of the mean proportion of live or dead animals between the 3 road locations gave 15 pair-wise comparisons, of which 9 showed statistical significance in each species. For simplicity, we present only results with a greater than 10% difference in proportion of animals found alive versus dead at the same location. We also present the general trend in distributions across the road for those found alive versus dead pooled across all sampling days. Proportion of live toads were 32% more numerous on the edge of the road than in the middle or center, whereas the proportion of dead toads were 35% more numerous at the center of the road (see Fig. 2A). The road edge had 40% more live than dead toads, whereas the road center had 34% more dead than live toads. Similarly, the road edge yielded 24% more live than dead *L. dahlii*, whereas the road center had 18% more dead than live *L. dahlii* individuals. Live *L. dahlii* were equally spaced across the road, whereas the proportion of road-killed individuals was 40% higher at the road center than at the edge.

Removal by Scavengers

Only 1 of 960 carcasses placed out overnight ($n = 400$ large toads, 240 small toads, 320 frogs) was removed from its tray. The removed animal was a small toad that was taken by either a feral dog (*Canis lupus familiaris*) or dingo (*Canis lupus dingo*) based on prints found in the sand. Therefore, overall only 0.001% of the carcasses were taken by scavengers during the night.

Of 960 carcasses placed out during the day (400 large toads, 240 small toads, 320 frogs), 700 (73%) were removed by scavengers. Whistling kites (*Haliastur sphenurus*) and black kites (*Milvus migrans*) were the only scavengers that removed any of the carcasses; they often left clear foot prints on the sand in the trays, or if they seized a carcass while on the wing left a distinctive grab mark and occasional wing prints. We were unable to distinguish between the 2 raptor species by their tracks.

Length of time a carcass remained on the road did not differ among transects ($P = 0.38$). We found an interaction between carcass type and season (wet or dry) on the length of time a carcass remained on the road ($F_{1,952} = 21.23$, $P < 0.001$; Fig. 3). Post hoc comparisons revealed that the

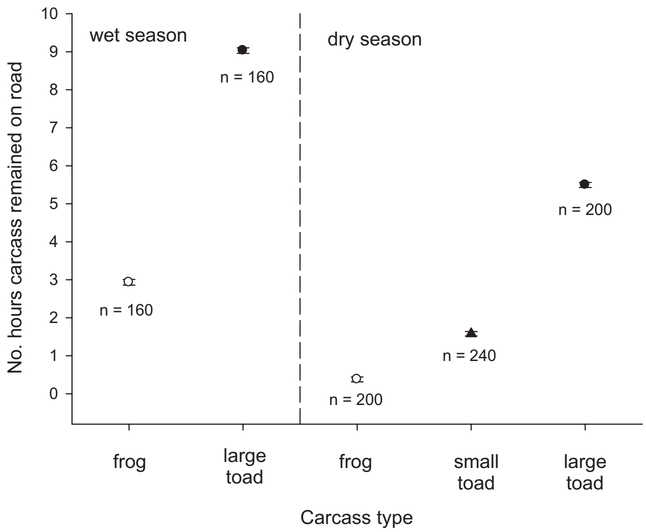

Figure 3. Back transformed mean (±SE) number of hours carcasses of frogs (open circles), large toads (closed circles), and small toads (triangle) remained on the road before being removed by scavengers during the wet and dry seasons near Middle Point Village, Northern Territory, Australia in 2009.

length of time a carcass remained near the road differed among all carcass types and seasons (all $P < 0.001$). Frog carcasses in the dry season were removed on average only 23 ± 3.6 minutes after sunrise. In contrast, frogs in the wet season remained on the road for an average of 2.9 ± 0.07 hours after sunrise. Large cane toads remained on the road longer than did frogs, especially in the wet season rather than the dry season (carcasses remained on the road for 9 ± 0.07 and 5.5 ± 0.07 hr, respectively). Small toads (dry season only) remained on the road for an average of 1.6 ± 0.06 hours.

Carcass Displacement Experiment

Most (85%) of the carcasses placed on the road were run over by passing vehicles. Carcasses on the road edge were 34% less likely to be run over than were carcasses in either the road center or in the middle of each lane ($\chi^2_2 = 106.85$, $P < 0.001$, post hoc tests both $P = 0.001$; Fig. 4A); carcasses in the road center and middle of the lane were equally likely to be hit (post hoc tests $P = 0.39$). The mean proportion of carcasses run over was 0.99, 0.98, and 0.66 for the road center, middle of the lane, and road edge locations, respectively. Neither carcass type nor the interaction between carcass position and type influenced the likelihood of a carcass being run over (carcass type: $\chi^2_2 = 0.41$, $P = 0.82$; interaction $\chi^2_4 = 2.32$, $P = 0.68$).

Of the 505 carcasses used during trials, all but 29 were found again. Carcass type influenced the likelihood of the carcass being found by the observer ($\chi^2_2 = 11.02$, $P = 0.004$). Large toads were more likely to be found than either small toads or frogs (post hoc tests both $P < 0.04$; small toads vs. frogs, post hoc $P = 0.4$). Neither position on the road nor the interaction between position and carcass type influenced the likelihood of a carcass being found (position: $\chi^2_2 = 2.24$, $P = 0.33$; interaction $\chi^2_4 = 3.89$, $P = 0.42$). The mean proportion of carcasses found was 0.99, 0.93, and 0.95 for large toads, small toads, and frogs, respectively; therefore, only 0.06% of carcasses were lost because of cars.

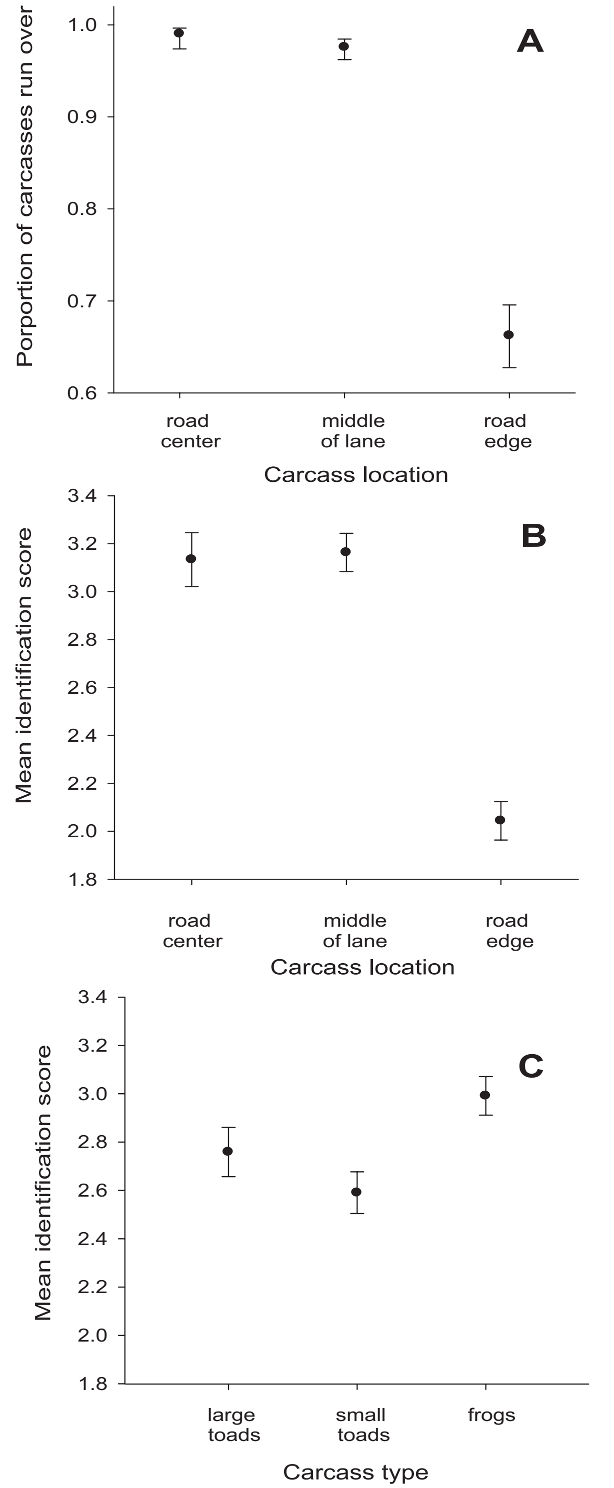

Figure 4. Back transformed mean (±SE) proportion of large toad, small toad, and frog carcasses that were run over on the road at Middle Point Village, Northern Territory, Australia in 2009, in relation to carcass location (A). Mean (±SE) carcass identification score in relation to (B) carcass location, and (C) carcass type.

The mean perpendicular displacement distance (81.1 cm) of carcasses from the starting point was not influenced by the original position on the road where the animal was placed, carcass type, or their interaction (position: $F_{2, 417} = 0.21$, $P = 0.81$; carcass type $F_{2, 417} = 0.48$, $P = 0.62$; interaction $F_{4, 417} = 2.09$, $P = 0.08$). The mean parallel displacement distance (122.7 cm) of the carcasses from the starting point was influenced by the interaction between position and carcass type; large toad carcasses initially located in the middle of the lane and in the center of the road were moved farther than either small toads or frogs ($F_{4, 390} = 4.74$, $P = 0.001$; Fig. 5).

The location of a carcass on the road influenced the identification score ($F_{2, 500} = 59.26$, $P < 0.001$; Fig. 4B), with road-edge carcasses having a lower score than those in the middle of the lane or center of the road (post hoc tests both $P < 0.001$). Carcasses in the center of the road and the middle of the lane did not differ in their identification scores (post hoc tests $P = 0.8$), with no significant interaction between carcass type and location ($F_{4, 496} = 0.46$, $P = 0.77$). Carcass type influenced mean identification score ($F_{2, 500} = 6.11$. $P = 0.002$; Fig. 4C). Frogs were more difficult to identify to species after having been run over than were either large or small toads (post hoc tests both $P < 0.001$) with the identification score for frogs being 0.3 of a category higher. Small and large toads did not differ in their mean identification scores (post hoc test $P = 0.2$).

DISCUSSION

We found large numbers of both live and dead anurans on the road at our study site. Dead individuals of all species of anurans were distributed across the width of the road in a similar pattern, with more carcasses being found in the center of the road and fewer at the edge. The pattern suggests that drivers tend to avoid the road edges, possibly to give themselves time and space to maneuver if larger animals such as kangaroos cross the road in front of them. In contrast, the distribution of live anurans across the width of the road varied among species. Native frogs showed either uniform distributions (*L. bicolor*, *L. dahlii*) or were found in higher numbers at the center than at the edge (*L. nasuta*, *L. rothii*, *L. caerulea*). Distributions of native frogs found alive closely mirrored those found dead for *L. caerulea* and *L. rothii*, but more *L. dahlii* were found dead than alive in the center of the road, and fewer were found dead than alive on the edge. This likely reflects drivers avoiding the road edge, combined with an accumulation of carcasses over time, whereas the distribution of frogs found alive represents a snapshot in time. Live cane toads showed an opposite pattern to live native frogs; cane toad numbers were higher on the road edge and lower in the center. Cane toads use the road as a travel corridor, a trait that has accelerated the rate of invasion into new areas (Brown et al. 2006). Future work could expand on Mazerolle et al. (2005) and Bouchard et al. (2009) to investigate whether invasive toads actively avoid high traffic areas of the road, and if this behavior facilitates their invasion by reducing road mortalities. Alternatively, cane toads may be avoiding the high road surface temperatures in the center of the road, whereas native frogs may be attracted to the higher surface temperatures. Future work could compare interspecific variation in frog locations to variation in thermal preferences to determine if thermal preferences influence rates of road mortality.

Although most roadkill is accidental, some drivers intentionally target certain animals (e.g., snakes and turtles; Ashley et al. 2007). Such behavior might influence the relative counts of live versus dead animals in some species compared to others. However, such bias is likely to be small. Although many Australian drivers claim to intentionally run over invasive cane toads, experimental tests show that toads are not run over more frequently than are native frogs (Beckmann and Shine 2012). Thus, the patterns revealed by our survey data likely reflect anuran behavior not driver behavior.

Although 85% of the carcasses that we set out on roads were run over, and vehicles displaced more than 85% of carcasses that they hit, this rarely resulted in a carcass being lost or becoming unidentifiable. We found almost all (94%) of the carcasses at the end of the experiment, indicating that counts of road-killed anurans at our study site are not heavily biased by removal or destruction by passing vehicles. The location of carcasses also was relatively unaffected by vehicles (mean perpendicular and parallel displacement by passing vehicles was 81 cm and 123 cm, respectively). Thus, the location of carcasses should provide reliable information on issues such as the habitat type in which the animal was killed, and the optimal locations for mitigation works such as culverts. The carcass moved the farthest by passing vehicles was a large cane toad, which was displaced 389 cm perpendicular to the road, and 2,070 cm parallel to the road. Carcasses of large cane toads were moved by vehicles farther than carcasses of smaller toads and frogs, suggesting that researchers who study roadkill locations for larger animals may need to be aware of potential bias due to displacement by vehicles (within the size range we studied; we doubt that road-killed elephants would be significantly displaced).

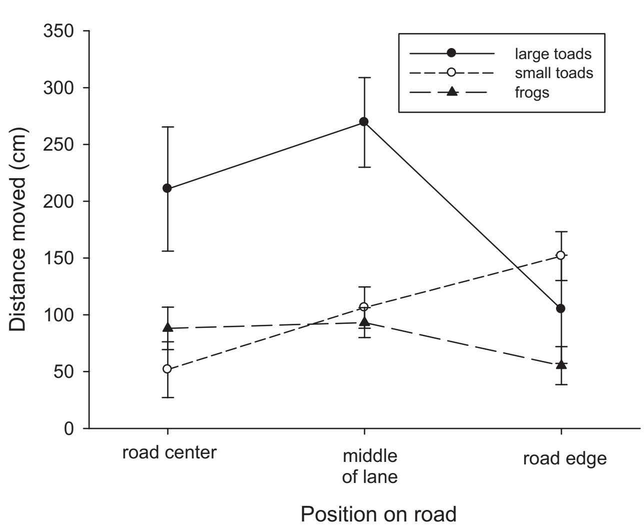

Figure 5. Mean (±SE) distance parallel to the road that large toad (closed circles), small toad (open circles), and frog (triangles) carcasses were moved by subsequent vehicles as a function of the carcass initial location on the road, at Middle Point Village, Northern Territory, Australia in 2009.

Deterioration of carcasses by passing vehicles that reduced the likelihood of positive identification was greater in the middle of the road than on the edge, consistent with the result that animals on the edge were less likely to be run over. Centrally located carcasses on average were difficult to identify, whereas edge carcasses were easily identified to species. This indicates that traffic volume may affect the ability of researchers to identify carcasses particularly for road-killed frogs that are typically smaller, less robust, and have thinner skin than cane toads. This was true even with severely mangled toad specimens, partly because the small number of anuran taxa that attain large body sizes simplifies the task of identifying toads, and native frogs in this area do not have skin similar to the distinctive toad skin.

Scavengers can have a large influence on the number of carcasses found (Antworth et al. 2005). Frogs (98%) and small cane toads (90%) were taken more often than large cane toads (43%; Beckmann and Shine 2011). Removal of carcasses by scavengers at our study site differed greatly between night and day. Virtually no carcasses were removed at night, perhaps in part because of a weather-driven population crash in dusky rats (*Rattus colletti*), a potential nocturnal scavenger, at our study site (Ujvari et al. 2011) prior to our study. Additionally, the invasion of our study site by cane toads in 2005 resulted in major population declines of native predators (Shine 2010). Some of these predators may also include roadkill in their diet. For example, scavenging has been reported in northern quolls (*Dasyurus hallucatus*; Burnett 1997, Oakwood 1997, Glen et al. 2007), varanid lizards (Shivik and Clark 1997, Cogger 2000), and snakes (Shivik and Clark 1997, DeVault and Aaron 2002). Populations of avian predators, however, do not appear to be negatively affected by cane toads (Beckmann and Shine 2009, Beckmann et al. 2011). Raptor densities were high at our field site, with up to 69 birds counted along 4 km of road at sunrise (C. Beckmann, Deakin University, personal observation). Ants also scavenged the dead anurans, but did not remove any carcasses from the trays.

Scavenging raptors were surprisingly quick to remove carcasses during the day. Removal times depended upon species and body size, with dry-season carcasses of frogs being removed on average 23 min after sunrise, toads persisting for 5.5 hr, and large toads persisting for longer than those of smaller conspecifics. Removal times also varied with season, with carcasses remaining on the road on average 2.9 hr in the wet season and 9 hr in the dry season. Higher offtake rates in the dry season probably reflect a greater abundance of raptors, raptors breeding during the dry season, and the scarcity of alternative food. Such low carcass persistence time, especially in the dry season, could introduce considerable bias to surveys of road-killed anurans undertaken during daylight hours.

Our surveys of anurans on a highway in tropical Australia show species differences in how the animals are distributed across the width of the road; and also show that location on the road can affect the probability of being hit (animals are more vulnerable in the middle of the road than on the edge). Thus, the way in which an animal's species, sex, or body size affects its response to the road surface (and thus, whether it spends time in the center vs. edge of the road) can generate substantial biases in the relationship between roadkills and underlying abundances. Even for a single age or sex class, vulnerability may shift through time as a function of physiological state. For example, reproductive male snakes may be so focused on mate-searching that they ignore oncoming vehicles, and reproductive females may be vulnerable because they select the sun-warmed road center as a warm site for nocturnal basking (Shine 1994, Brown and Shine 2004). More generally, differences in locomotor speeds, responses to approaching vehicles, and simply overall home range sizes and the extent and diurnal timing of movements can render 1 species (or age class or sex within a species) far more vulnerable to vehicular traffic than others. Ecologists who use roadkill counts as indices of abundance or distribution need to be aware of such biases.

Similar biases operate post-mortem. In our study, post-mortem displacement by passing vehicles was generally minor but sometimes precluded accurate identification. Scavengers were more important, and strong biases in removal rates (with respect to prey species, size, season, and scavenger community) mean that researchers need to evaluate rates and times of scavenging to accurately interpret data on roadkill counts. For our system, we recommend conducting anuran roadkill surveys shortly before dawn, such that the maximum number of carcasses would have accumulated, and scavengers would have removed few.

MANAGEMENT IMPLICATIONS

Data on roadkill victims should be carefully evaluated for potential biases before inferring underlying patterns of animal abundance, distribution, or rates of mortality, or using such data to make management decisions. For example, imagine that we wished to quantify the relative abundances of invasive cane toads versus native frogs at our study site. We cannot use counts of road-killed anurans as a proxy for the relative abundances of frogs and toads, even if we make the simplifying assumption that the relative densities of the different taxa are perfectly correlated with the numbers that are active on the road surface. On a per capita basis, a cane toad is less likely to be hit by a vehicle than is a frog (because the toad tends to stay near the road edge); but if hit, the toad's body will remain identifiable for longer, and is less likely to be removed by scavengers. The magnitude of post-mortem biases will shift seasonally, with more cane toads taken by scavengers during the dry season, when other prey types are scarce. The end result of these biases is to invalidate estimates of the relative numbers of toads and frogs in this system based on counting roadkills. Differences in the species' vulnerability to vehicles (caused by species-specific behavior) and rates of post-mortem disappearance (due to subsequent vehicles and scavenging) weaken any correlation between population numbers and roadkill counts. An understanding of methodological biases thus can inform the interpretation of roadkill counts, enable investigators to determine whether or not the victims of vehicular mortality provide robust evidence on underlying abundance of their study species, and assist managers to prioritize conservation problems.

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LITERATURE CITED

Anthony, J., J. Childs, G. Glass, G. Korch, L. Ross, and J. Grigor. 1990. Land use associations and changes in population indices of urban raccoons during a rabies epizootic. Journal of Wildlife Diseases 26:170–179.

Antworth, R. L., D. A. Pike, and E. E. Stevens. 2005. Hit and run: effects of scavenging on estimates of roadkilled vertebrates. Southeastern Naturalist 4:647–656.

Aresco, M. J. 2005. The effect of sex-specific terrestrial movements and roads on the sex ratio of freshwater turtles. Biological Conservation 123:37–44.

Ashley, E. P., A. Kosloski, and S. A. Petrie. 2007. Incidence of intentional vehicle–reptile collisions. Human Dimensions of Wildlife 12:137–143.

Baker, P. J., S. Harris, C. P. J. Robertson, G. Saunders, and P. C. L. White. 2004. Is it possible to monitor mammal population changes from counts of road traffic casualties? An analysis using Bristol's red foxes *Vulpes vulpes* as an example. Mammal Review 34:115–130.

Beckmann, C., M. Crossland, and R. Shine. 2011. Responses of Australian wading birds to a novel toxic prey type, the invasive cane toad (*Rhinella marina*). Biological Invasions 13:2925–2934.

Beckmann, C., and R. Shine 2009. Impact of invasive cane toads on Australian birds. Conservation Biology 23:1544–1549.

Beckmann, C., and R. Shine 2011. Toad's tongue for breakfast: exploitation of a novel prey type, the invasive cane toad, by scavenging raptors in tropical Australia. Biological Invasions 13:1447–1455.

Beckmann, C., and R. Shine 2012. Do drivers intentionally target wildlife on roads? Austral Ecology 37:629–632.

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 14:1–10.

Brown, G. P., B. L. Phillips, and R. Shine. 2011. The ecological impact of invasive cane toads on tropical snakes: field data do not support laboratory-based predictions. Ecology 92:422–431.

Brown, G. P., B. L. Phillips, J. K. Webb, and R. Shine. 2006. Toad on the road: use of roads as dispersal corridors by cane toads (*Bufo marinus*) at an invasion front in tropical Australia. Biological Conservation 133:88–94.

Brown, G. P., and R. Shine 2004. Effects of reproduction on the antipredator tactics of snakes (*Tropidonophis mairii*, Colubridae). Behavioral Ecology and Sociobiology 56:257–262.

Brzezinski, M., G. Eliava, and M. Zmihorski. 2012. Road mortality of pond-breeding amphibians during spring migrations in the Mazurian Lakeland, NE Poland. European Journal of Wildlife Research 58:685–693.

Burnett, S. 1997. Colonizing cane toads cause population declines in native predators: reliable anecdotal information and management implications. Pacific Conservation Biology 3:65–72.

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

Clevenger, A. P., B. Chruszcz, and K. E. Gunson. 2003. Spatial patterns and factors influencing small vertebrate fauna road-kill aggregations. Biological Conservation 109:15–26.

Cogger, H. G. 2000. Reptiles and amphibians of Australia. Sixth edition. Ralph Curtis Books, Sanibel Island, Florida, USA.

Collins, C., and R. Kays 2011. Causes of mortality in North American populations of large and medium-sized mammals. Animal Conservation 14:474–483.

Coulsen, N., and A. de Leeow 2004. Report on badgers and bovine Tb. Science Advisory Council, London, United Kingdom.

da Rosa, C. A., and A. Bager 2012. Seasonality and habitat types affect roadkill of neotropical birds. Journal of Environmental Management 97:1–5.

DeGregrorio, B. A., E. J. Nordberg, K. E. Stepanoff, and J. E. Hill. 2010. Patterns of snake road mortality on an isolated barrier island. Herpetological Conservation and Biology 5:441–448.

DeVault, T. L., and R. K. Aaron 2002. Scavenging by snakes: an examination of the literature. Herpetologica 58:429–436.

Dorcas, M. E., J. D. Willson, R. N. Reed, R. W. Snow, M. R. Rochford, M. A. Miller, W. E. Meshaka, P. T. Andreadis, F. J. Mazzotti, C. M. Romagosa, and K. M. Hart. 2012. Severe mammal declines coincide with proliferation of invasive Burmese pythons in Everglades National Park. Proceedings of the National Academy of Sciences 109:2418–2422.

Doyon, C., V. L. Trudeau, B. M. Hibbert, L. A. Howes, and T. W. Moon. 2003. mRNA analysis in flattened fauna: obtaining gene-sequence information from road-kill and game-hunting samples. Canadian Journal of Zoology 81:692–698.

Erritzoe, J., T. D. Mazgajski, and L. Rejt. 2003. Bird casualties on European roads—a review. Acta Ornithologica 38:77–93.

Flint, W. P. 1926. The automobile and wild life. Science 63:426–427.

Ford, A. T., and L. Fahrig 2007. Diet and body size of North American mammal road mortalities. Transportation Research Part D: Transport and Environment 12:498–505.

Gibbs, J. P., and D. A. Steen 2005. Trends in sex ratios of turtles in the United States: implications of road mortality. Conservation Biology 19:552–556.

Glen, A. S., M. N. Gentle, and C. R. Dickman. 2007. Non-target impacts of poison baiting for predator control in Australia. Mammal Review 37:191–205.

Gobalet, K. W. 2003. How to use roadkill to study the skeleton. American Biology Teacher 65:652–652.

Guinard, É., R. Julliard, and C. Barbraud. 2012. Motorways and bird traffic casualties: carcasses surveys and scavenging bias. Biological Conservation 147:40–51.

Gunther, K. A., M. J. Biel, and H. L. Robison. 1998. Factors influencing the frequency of road-killed wildlife in Yellowstone National Park. Pages 32–42 *in* International Conference on Wildlife Ecology and Transportation, 9–12 February 1998, Fort Myers, Florida, USA.

Havlin, J. 1987. Motorways and birds. Folia Zooligica 36:90–99.

Henke, S. E., and M. Montemayor 1998. Diel and monthly variations in capture success of *Phrynosoma cornutum* via road cruising in southern Texas. Herpetological Review 29:148–150.

Hodson, N. L., and D. W. Snow 1965. The road deaths enquiry, 1960–61. Bird Study 12:90–99.

Hofmann, J. E. 2005. A survey for the nine-banded armadillo (*Dasypus novemcinctus*) in Illinois. Center for Biodiversity Techincal Report 16. University of Illinois, Urbana—Champaign, Champaign, USA.

Hubbard, K. A., and A. D. Chalfoun 2012. An experimental evaluation of potential scavenger effects on snake road mortality detections. Herpetological Conservation and Biology 7:150–156.

Langen, T. A., A. Machniak, E. K. Crowe, C. Mangan, D. F. Marker, N. Liddle, and B. Roden. 2007. Methodologies for surveying herpetofauna mortality on rural highways. Journal of Wildlife Management 71:1361–1368.

Loughry, W. J., and C. M. McDonough 1996. Are road kills valid indicators of armadillo population structure? American Midland Naturalist 135:53–59.

Mallick, S. A., G. J. Hocking, and M. M. Driessen. 1998. Road-kills of the eastern barred bandicoot (*Perameles gunnii*) in Tasmania: an index of abundance. Wildlife Research 25:139–145.

Mazerolle, M. J., M. Huot, and M. Gravel. 2005. Behavior of amphibians on the road in response to car traffic. Herpetologica 61:380–388.

McCaffery, K. R. 1973. Road-kills show trends in Wisconsin deer populations. Journal of Wildlife Management 37:212–216.

Moore, T. G., and M. Mangel 1996. Traffic related mortality and the effects on local populations of barn owls *Tyto alba*. Pages 125–140 *in* G. L. Evink, P. Garrett, D. Zeigler, and J. Berry, editors. Trends in addressing transportation related wildlife mortality. Proceedings of the Transportation Related Wildlife Mortality Seminar. Florida Department of Transportation Report, FL-ER-58-96, Tallahassee, USA.

Mumme, R. L., S. J. Schoech, G. E. Woolfenden, and J. W. Fitzpatrick. 2000. Life and death in the fast lane: demographic consequences of road mortality in the Florida scrub-jay. Conservation Biology 14:501–512.

Oakwood, M. 1997. The ecology of the northern quoll *Dasyurus hallucatus*. Dissertation, Australian National University, Canberra, Australia.

Phillips, B. L., G. P. Brown, M. Greenlees, J. K. Webb, and R. Shine. 2007. Rapid expansion of the cane toad (*Bufo marinus*) invasion front in tropical Australia. Austral Ecology 32:169–176.

Russell, T. C., L. E. Neaves, and C. A. Herbert. 2011. Allocating sex in road-killed possums using PCR. Australian Mammalogy 33:1–4.

Santos, S. M., F. Carvalho, and A. Mira. 2011. How long do the dead survive on the road? Carcass persistence probability and implications for road-kill monitoring surveys. PLoS ONE 6:e25383.

SAS Institute. 1998. SAS/STAT user's guide. Version 8.0. Vol. 1. SAS Institute, Cary, North Carolina, USA.

Seo, C., J. H. Thorne, T. Choi, H. Kwon, and C.-H. Park. 2013. Disentangling roadkill: the influence of landscape and season on cumulative vertebrate mortality in South Korea. Landscape and Ecological Engineering 2013:1–13.

Shine, R. 1994. Allometric patterns in the ecology of Australian snakes. Copeia 1994:851–867.

Shine, R. 2010. The ecological impact of invasive cane toads (*Bufo marinus*) in Australia. Quarterly Review of Biology 85:253–291.

Shine, R., and G. P. Brown 2008. Adapting to the unpredictable: reproductive biology of vertebrates in the Australian wet-dry tropics. Philosophical Transactions of the Royal Society B: Biological Sciences 363:363–373.

Shivik, J. A., and L. Clark 1997. Carrion seeking in brown tree snakes: importance of olfactory and visual cues. Journal of Experimental Zoology 279:549–553.

Smith-Patten, B., and M. Patten 2008. Diversity, seasonality, and context of mammalian roadkills in the Southern Great Plains. Environmental Management 41:844–852.

Taylor, B. D., and R. L. Goldingay 2010. Roads and wildlife: impacts, mitigation and implications for wildlife management in Australia. Wildlife Research 37:320–331.

Teixeira, F. Z., A. V. P. Coelho, I. B. Esperandio, and A. Kindel. 2013. Vertebrate road mortality estimates: effects of sampling methods and carcass removal. Biological Conservation 157:317–323.

Turner, D. S., P. A. Holm, E. B. Wirt, and C. R. Schwalbe. 2003. Amphibians and reptiles of the Whetstone Mountains, Arizona. Southwestern Naturalist 48:347–355.

Ujvari, B., R. Shine, L. Luiselli, and T. Madsen. 2011. Climate-induced reaction norms for life-history traits in pythons. Ecology 92:1858–1864.

Underhill, J. E., and P. G. Angold 2000. Effects of roads on wildlife in an intensively modified landscape. Environmental Review 8:21–39.

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