Optimising the cost of roadkill surveys based on an analysis of carcass persistence

Dominic A.W. Henry[1,2], Wendy J. Collinson-Jonker[1,3,6*], Harriet T. Davies-Mostert[1,4], Samantha K. Nicholson[1], Lizanne Roxburgh[1], & Daniel M. Parker[3,5]

[1]Endangered Wildlife Trust, Johannesburg 1685, South Africa

[2]Statistics in Ecology, Environment and Conservation, Department of Statistical Sciences, University of Cape Town, Cape Town 7700, South Africa

[3]Wildlife and Reserve Management Research Group, Department of Zoology and Entomology, Rhodes University, Grahamstown 6140, South Africa

[4]Eugene Marais Chair of Wildlife Management, Mammal Research Institute, University of Pretoria, Pretoria 0028, South Africa

[5]School of Biology and Environmental Sciences, University of Mpumalanga, Nelspruit 1200, South Africa

[6]South African Research Chair in Biodiversity Value & Change, School of Mathematical and Natural Sciences, University of Venda, Thohoyandou, South Africa

*Correspondence should be addressed to: wendyc@ewt.org.za

- In roadkill studies there is a trade-off between survey cost and the number of carcasses detected.
- Carcass persistence can be used to inform optimal roadkill survey design.
- Survey costs can be reduced by 80% by conducting non-daily surveys based on different level of carcass persistence.
- We developed an online R Shiny web app to compare costs across different survey scenarios .

Abstract

Reliable estimates of wildlife mortality due to wildlife-vehicle collisions (WVC) are key to understanding its impact on wildlife populations and developing strategies to prevent or reduce WVC. Standardised approaches for monitoring roadkill are needed to derive robust and unbiased estimates of mortality that are comparable across different study systems and ecological contexts. When designing surveys, there is a trade-off between survey frequency (and hence logistical effort and financial cost) and carcass detection. In this regard, carcass persistence (the period a carcass remains detectable before being removed by decomposition or scavengers) is important; the longer a carcass persists, the greater the likelihood it will be detected with lower survey effort by conducting more infrequent surveys. Using multi-taxon carcass data collected over a month of repeated driven surveys, combined with five covariates (species functional group, body weight, carcass position on road, carcass condition [either flattened or not after impact], and rainfall prior to each survey), we explored the drivers of carcass persistence with the overall aim of providing information to optimise the design of carcass surveys along linear infrastructure. Our methodological approach included a survival analysis to determine carcass persistence, linear regressions to test the effect of covariates, a subsampling analysis (using field data and a simulation exercise) to assess how the proportion of carcasses detected changes according to survey frequency, and an analysis to compare the costs of surveys based on study duration, transect length and survey frequency. Mean overall carcass persistence was 2.7 days and was significantly correlated with position on road and within-functional group body weight. There was no evidence for a significant effect of rainfall, while the effect of carcass condition was weakly non-significant. The proportion of carcasses detected decreased sharply when survey intervals were longer than three days. However, we showed that survey costs can be reduced by up to 80% by conducting non-daily surveys. Expanding on the call for a standardised methodology for roadkill surveys, we propose that carcass persistence be explicitly considered during survey design. By carefully considering the objectives of the survey and characteristics of the focal taxa, researchers can substantially reduce logistical costs. In addition, we developed an R Shiny web app that can be used by practitioners to compare survey costs

across a variety of survey characteristics. This web app will allow practitioners to easily assess the trade-off between carcass detection and logistical effort.

Keywords: decomposition rates, mortality rates, multi-taxon, survey design, wildlife-vehicle collisions, vertebrates

Introduction

Wildlife vehicle collisions resulting in roadkill are one of the primary road infrastructure-associated threats to animal communities, populations, and species (Trombulak & Frissell 2000; Pinto, Clevenger, & Grilo 2020; Schwartz et al. 2020; Seiler & Bhardwaj 2020). The threat of roadkill is set to increase globally as an additional 25 million kilometres of road are expected to be added to the global road network by 2050 (Díaz et al. 2020). With this ever-expanding threat, it is increasingly important that we understand the site specific and cumulative impacts of roadkill to develop informed and effective mitigation strategies. Accurately measuring and quantifying rates of roadkill is a fundamental part of understanding which ecological and environmental factors are the most important in contributing to roadkill. There has been a call to standardise methods of roadkill surveys so that results are easily comparable across different landscapes, ecological systems, and road infrastructure configurations (W. J. Collinson et al. 2014; Jones, Borkin, & Smith 2019; Ogletree & Mead 2020).

In the past, studies quantifying rates of roadkill have varied greatly in terms of how they have investigated factors such as survey speed, number of observers, sampling frequency, number of transects, and transect distance (see Appendix S1 of Collinson et al. (2014) for details from 61 roadkill studies). In response, Collinson et al. (2014) developed a standardised protocol for counting roadkill which has been adopted in several recent studies (Collinson et al. 2015; Santos et al. 2016; Akrim et al. 2019; Mestre et al. 2019). However, an important variable not initially considered by Collinson et al. (2014) is carcass persistence, which influences the number of roadkill registered, and therefore has a bearing on the survey effort required for roadkill studies.

Carcass persistence, defined as the period a carcass remains detectable before being removed by decomposition or scavengers, is an important variable that can be used to obtain more precise mortality estimates of animal populations (Santos et al. 2011; Guinard, Julliard, & Barbraud 2012; Santos et al. 2016; Barrientos et al. 2018). For example, small-bodied species such as amphibians are likely to be heavily under-detected in roadkill surveys as their persistence on roads is assumed to be much shorter than larger, heavier-bodied animals (Teixeira et al. 2013). Weather conditions can also affect persistence in different ways (Slater, 2002; Santos et al., 2016); carcass persistence is low under wet-weather conditions, which can promote faster degradation. Rainfall runoff can also wash away carcass debris, whereas drier, hotter conditions increase persistence as carcasses desiccate (Van Der Hoeven, De Boer, & Prins 2010; Santos et al. 2016). Variation in carcass persistence across different taxa and climatic conditions can result in substantial bias in the detection and quantification of roadkill. Despite this, many studies have ignored the effects of carcass persistence, thereby implicitly assuming that carcass persistence is consistent across taxonomic groups, climate, and body size (Teixeira et al. 2013; Barrientos et al. 2018; Hastings, Barr, & Bateman 2019; Santos & Ascensão 2019).

Carcass persistence also has important consequences for the methodological design of roadkill surveys (Barrientos et al. 2018). Roadkill studies often require repeated sampling across extensive study periods to identify WVC hotspots, resulting in costly and logistically challenging surveys. Surveys that are conducted too frequently risk wasting financial resources while those that are conducted too infrequently risk underestimating the mortality of species with lower carcass persistence. Given the high risk that budgetary constraints might lead to inadequate surveys that provide insufficient guidance for development, it is essential to develop survey designs that optimise logistical capacity while minimising any bias in roadkill detection. There is currently little guidance as to how to weigh up the respective trade-offs.

Using multi-taxon carcass data collected over a month of daily surveys in the Limpopo Province of South Africa, combined with simulations and a cost analysis, we aimed to explore the factors that affect carcass persistence and demonstrate how to optimise the logistical and financial resources of carcass surveys along linear infrastructure. Our initial analysis followed a three-step approach; first, we used a survival analysis to derive carcass persistence probability curves (i.e., the duration that carcasses remain detectable during repeated driven surveys). Second, we modelled carcass persistence (measured in days) as a function of five covariates: functional group, body weight, carcass position on road, carcass condition (carcasses either flattened or not after impact), and rainfall history. We hypothesized that carcass persistence would be positively correlated with weight, and negatively related to rainfall. We also predicted that flattened carcasses, and those further away from the verge would persist for longer. Third, we modelled the number of carcass observations and species richness as a function of survey interval, ranging from 0 (i.e., daily) to every nine days. Following this, we ran a simulation to assess how the proportion of detected carcasses changes with survey interval across seven levels of mean carcass persistence ranging from 1 – 25 days. We then used the applied and simulation analysis outputs to create a roadkill study cost analysis (with an associated web application) covering a range of logistical inputs (e.g., length of road transect and study duration) to help guide the design of future roadkill studies. In addition to revealing the important drivers of carcass persistence, the analysis of field data served to ensure that our simulations, and subsequent cost analysis which made use of the simulated data, reflected conditions found in applied roadkill research.

Methods

Study area

The Greater Mapungubwe Transfrontier Conservation Area (GMTFCA), which encompasses 4,900 km^2, is situated in the northern reaches of the Limpopo Province of South Africa (Figure 1). Land-use includes nature conservation, heritage site conservation, tourism, agriculture, and mining (including associated infrastructure; Deacon et al. 2010). The study area falls within the sub-tropical region of South Africa and experiences three seasons: hot/dry (September – January), hot/wet (February – May), and cold/dry (June – August). The GMTFCA has a high species richness of reptiles (~120 species), birds (≥ 429 species) and mammals (~100 species), while amphibian species richness is relatively low (~12 species). Traffic volumes are low at approximately 220 vehicles per day (W. J. Collinson et al.

2014). The primary transect was 100 km in length and comprised three paved roads (Figure 1). A stretch of unpaved road, measuring 20 km, was also sampled.

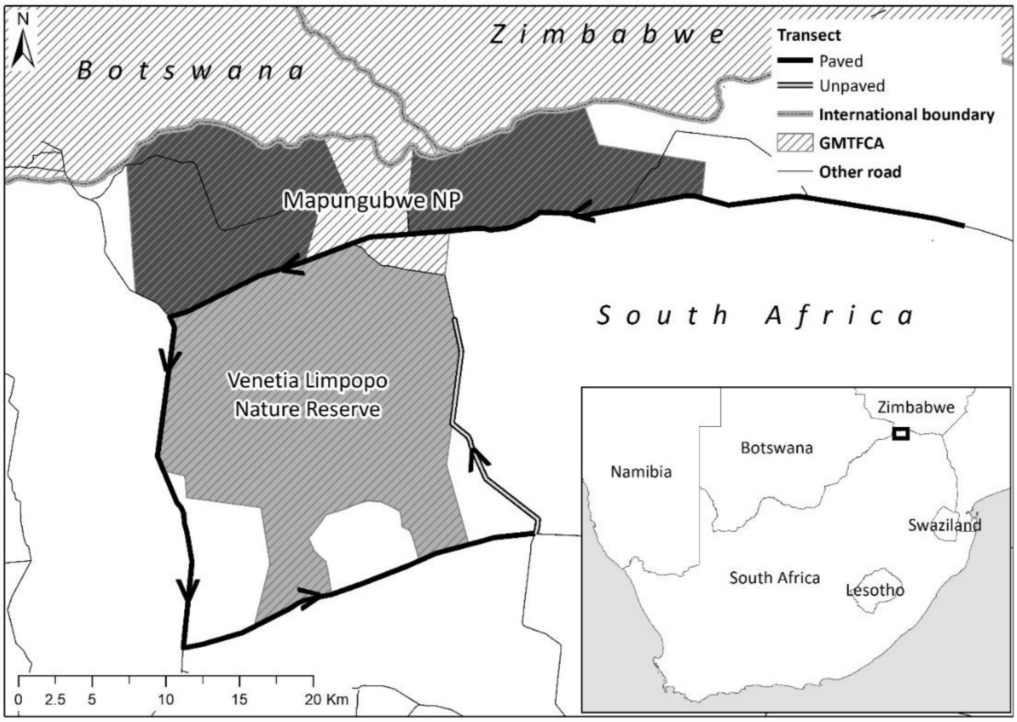

Figure 1. Map of the driven roadkill transects within the Greater Mapungubwe Transfrontier Conservation Area (GMTFCA), South Africa. Arrows depict the direction of travel for the survey route.

Data collection

Transects were driven daily to assess the persistence and decomposition of roadkill carcasses (W. J. Collinson 2013). A single trained and experienced observer completed all surveys, and drove at speeds of 40 – 50 km/h following the Collinson et al. (2014) protocol for roadkill sampling. We sampled transects in February 2011 (the hot/wet season), as this is when roadkill numbers were highest. The transect was driven daily for 30 consecutive days, commencing 1.5 hours after sunrise. The observer stopped at each carcass that was found on the road and took a photograph to verify species identity. Following that a GPS waypoint was also taken at each carcass. On subsequent days the observer used the waypoint to navigate to each recorded carcass to check if it was still present. This procedure was repeated daily until the carcass disappeared completely, which gave us a measure carcass persistence.

Analysis of field data

We assigned each carcass to one of nine groups with similar body shapes and weight ranges: amphibians, snakes, other reptiles, small mammals (10 – 70 g) , large mammals (4500 – 40 000 g), passerine birds and small (9 – 70 g), medium (100 – 170 g), and large (180 – 3100 g) non-passerine birds. We use the term 'functional group' for ease of reference from here onward to refer to these groupings but acknowledge that these are not strictly ecological functional groups. These groups were created to explore the interaction between persistence and weight within animals that had a similar body shape and structure. For a full list see Supporting Information Table S1. Prior to analysis, we removed all carcasses not identified to species level, those which were observed on the first day of surveys (which meant we only included new carcasses to avoid uncertainty in persistence from roadkill that were already on the road before we started surveys), and those species where we were not able to find published weight range information. This resulted in a final dataset of 344 unique carcass records from 73 species (Table 1).

We calculated carcass persistence probability curves using the Kaplan-Meier estimator for censored data. We created a censoring variable and right censored carcasses that continued to persist after the survey period ended. We calculated curves for all observations combined and then for each functional group using the survfit function from the *survival* R package (Therneau & Grambsch 2000). We plotted curves with 95% confidence intervals using the *ggsurvplot* function from the *survminer* R package (Kassambara & Kosinski 2019).

Generalised Linear Models (GLMs) were used to model carcass persistence (days) as a function of species weight (*weight*, grams); functional group (*FG*, nine categories); distance of carcass from road verge (*dist*, meters); whether a carcass was flattened or not after impact (*carcass condition*, two categories); whether or not there was a rainfall event in the 24 hours leading up a survey transect (*rain*, two categories); and the interaction between functional group and weight (*FG*weight*). The carcass weight variable was log-transformed before inclusion in the models to correct for the right-skew in the data. Continuous predictors were scaled before running regressions. Road type was not included as a covariate as t-test showed that there was no difference in persistence between paved and unpaved roads ($t = 0.86$, $df = 6.033$, $p = 0.42$). We used an AIC model selection procedure (Burnham & Anderson 2002) to evaluate the trade-off between complexity and goodness-of-fit of seven candidate models containing different combinations of covariates. We used the *glm* function from base R (R Core Team 2019) to run the linear models, and the *aictab* function from the *AICcmodavg* R package (Mazerolle 2019) for model selection. All plots were created using the *ggplot2* R package (Wickham 2016). Diagnostic residual plots were examined to assess model fit. Using the bubble function from the *sp* R package (Pebesma & Bivand 2005) we created a bubble plot of the residuals in geographic space to test for any spatial structure in the residuals.

Subsampling of field data

To assess how survey interval affected the proportion of carcass observations and number of species detected, we subsampled the field data to retain all observations recorded every day (complete survey dataset comprised of 30 sample days) and then with intervals of 1, 2, 3, 4, 5, 7 and 9 days. We then calculated the total number of observations and species richness for each functional group.

Simulation

In a similar manner, using parameter values that reflected the properties of the field data (i.e., similar values of observed mean carcass persistence values, total observation sample size, and study duration), we created a simulation to assess how proportion of carcasses detected varies with survey frequency across seven levels of mean carcass persistence (1, 2, 3, 5, 10, 15 and 25 days). We ran 1,000 simulations for each level of mean survival with 300 observations recorded over 30 days. For each simulation we used a Poisson distribution (λ = mean persistence, $n = 300$) to generate a random vector of carcass persistence values. We then randomly assigned each value a start day and subsequently generated an array of observations and persistence duration. Following this, we subsampled the data in the same manner as the field data to generate plots showing the proportion of carcasses detected as survey interval increased (note that in addition to the mean pattern, the simulations allowed us to estimate the associated confidence intervals).

Survey costs analysis

In the next part of the analysis we conducted a survey cost analysis that was designed to estimate the total cost of both labour and mileage of conducting a survey across a range of scenarios, each with specific inputs from a range of parameters. We calculated the total cost of a survey as the sum of mileage and labour cost, as follows:

$$mileage\ cost\ =\ transect\ distance\ (km)\ \times\ optimal\ number\ of\ surveys\ \times\ mileage\ rate\ (\$/km)$$

$$labour\ cost\ =\ \frac{total\ distance\ driven\ in\ study\ (km)}{survey\ speed\ (km/h)} \times labour\ rate\ (\$/hour)$$

$$total\ cost\ =\ mileage\ cost\ +\ labour\ cost$$

Based on the design of published roadkill studies we calculated total cost at four levels of transect distance (50, 100, 150 and 200 km). Mileage rates were set at \$0.93/km based on the USA's Internal Revenue Service 2020 mileage rates (https://www.irs.gov/pub/irs-drop/n-20-05.pdf). Labour rates were set at \$25/hour which is an average amount paid to postgraduate students for analogous ecological field surveys (based on pers. comm. with three academic staff at the following universities: University of California Davis, USA; Cardiff University, UK; and CDV Transport Research Centre. Czech republic). Survey speed was set to 40 km/h, a speed which provided a good balance between carcass detection and total survey duration (Collinson et al., 2014). The optimal number of surveys was informed by the results of the field surveys and simulation study, and was calculated as a function of: mean carcass persistence of the focal taxa (either 1, 2, 3, 5, >10 days), study duration (20, 30 or 40 days), and the proportion of acceptable total carcass detection (50 or 75% – note these are two alternatives to 100% detection that results from daily surveys under the assumption that carcasses persist for a minimum of 24 hours). Thus, if the mean carcass persistence of the focal taxa was 3 days, our simulations suggest that, to detect 75% of carcasses, surveys need to be conducted at a minimum every fifth day. At this survey interval the optimal number of surveys conducted during a 30-day study period would be 6, which is the 'optimal number of surveys' value used in the 'total cost' equation (see Supplementary Material Table S2 for a breakdown of the optimal number of surveys for each carcass persistence level at 50 and 75% detection). All R code to reproduce the outputs of the simulations and cost analysis is available at https://github.com/DomHenry/RK-survey-cost/tree/master/functions.

Results

Field data

The mean carcass persistence of the majority of functional groups was 2.7 days and ranged from 2 – 7.75 days (Table 1). The number of observations recorded for each functional group ranged from 16 (large mammals) – 66 (amphibians). Within each functional group, the distribution of persistence values exhibited noticeable right-skew (see Supplementary Material Figure S1 for frequency distribution of each functional group).

Table 1. The number of observations, proportion of total sample, and mean and standard deviation (SD) of carcass persistence times for each functional vertebrate group.

Functional group	Observations	Proportion of sample (%)	Mean (± SD)
Amphibian	66	19.19	3.03 ± 2.10
Large mammal	16	4.65	7.75 ± 9.37
Non-passerine birds (large)	29	8.43	1.93 ± 2.19
Non-passerine birds (medium)	47	13.66	2.17 ± 1.74
Non-passerine birds (small)	33	9.59	2.36 ± 2.25
Passerine birds	40	11.63	2.45 ± 2.17
Reptile	30	8.72	2.00 ± 2.10
Small mammal	28	8.14	2.43 ± 1.64
Snake	55	15.99	2.60 ± 2.97

Based on the Kaplan-Meier estimator, the median carcass persistence probability (where persistence drops below 50%) for all observations was 2 days (Figure 2) and ranged between 1 and 3 across the nine functional groups (see Supplementary Material Figure S2 for plots of each functional group).

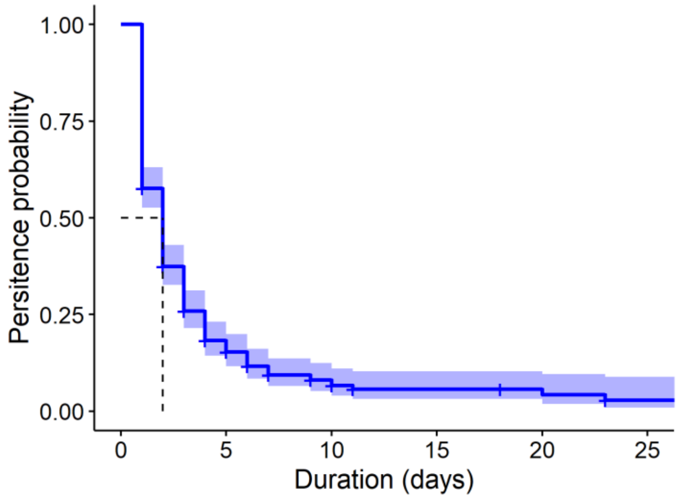

Figure 2. Carcass persistence probability curve for all observations (n = 344). Dotted line represents the median probability (i.e., where carcass persistence drops below 50%). Blue shading indicates 95% confidence intervals.

The results of the model selection identified the top model as the one that included all individual covariates as well as the interaction between weight and functional group (Table 2). The regression parameters of our chosen model indicated significant interaction effects between weight and several functional groups (Table 3). Carcass persistence increased significantly as weight increased within large mammals, small mammals, and snakes (Figure 3). There was no evidence for a significant effect of rainfall on carcass persistence while there was a positive but non-significant effect of distance to verge. The regression model explained a reasonable proportion of variation in carcass persistence ($R^2 = 0.31$). Residuals of the model were normally distributed with no extreme deviations of variance across predicted values. There was no evidence of spatial autocorrelation in the residual bubble plot (Supplementary Material Figure S3).

Table 2. Results of the AIC model selection procedure of candidate GLMs used to model carcass persistence (FG, functional group; weight, carcass weight; dist, distance to verge; rain, rainfall prior to survey; carcass condition, flattened or not; K, number of model parameters, AIC, Akaike's Information Criteria value; ΔAIC, change in AIC between sequential models; AICw, AIC weight; AICcw, AIC cumulative weight).

Candidate model	**K**	**AIC**	**ΔAIC**	**AIC_w**	**AIC_{cw}**
FG + weight + FG*weight + dist + rain + carcass condition	22	1373.32	0.00	0.78	0.78
FG + weight + dist + rain + carcass condition	14	1376.78	3.46	0.14	0.91
FG + weight + FG*weight	19	1377.70	4.38	0.09	1.00
FG + dist + rain + carcass condition	13	1387.11	13.79	0.00	1.00
weight + dist + rain + carcass condition	6	1406.35	33.04	0.00	1.00
Null	2	1428.68	55.36	0.00	1.00
dist + rain + carcass condition	5	1431.40	58.08	0.00	1.00

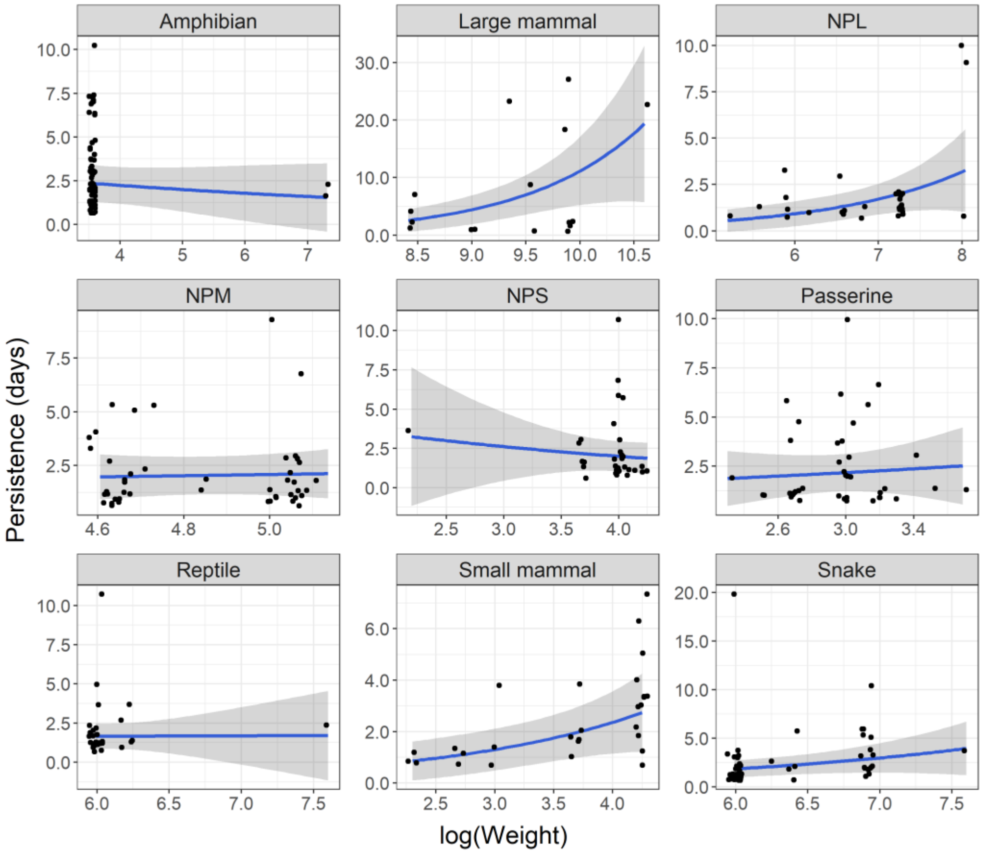

Figure. 3. Relationship between log weight (grams) and carcass persistence (days) for nine functional groups. Blue line indicates the fitted parameter slope and the shaded areas indicate 95% confidence intervals. The points indicate raw data and are jittered for visual clarity. NPL, large non-passerine birds; NPM, medium non-passerine birds; NPS, small non-passerine birds.

Table 3. Estimated regression parameters, standard errors (SE), confidence intervals and *P*-values for the GLM of the highest ranked model from the AIC model selection procedure. Note that non-interaction functional group intercept parameters are omitted from the table. NPL, large non-passerine birds; NPM, medium non-passerine birds; NPS, small non-passerine birds.

Parameter	**Estimate**	**SE**	**Lower 95% CI**	**Upper 95% CI**	***P*-value**
Intercept	1.30	0.61	0.15	2.61	< 0.05
Weight	-0.11	0.16	-0.47	0.19	0.492
Dist	0.11	0.05	0.01	0.20	< 0.05
Rain: Yes	0.13	0.18	-0.24	0.49	0.475
Carcass condition: Not flat	-0.18	0.10	-0.39	0.02	0.073
Large mammal * weight	1.04	0.30	0.46	1.64	< 0.001
NPL * weight	0.73	0.30	0.18	1.31	< 0.05
NPM * weight	0.25	0.63	-0.99	1.50	0.689
NPS * weight	-0.16	0.41	-0.97	0.67	0.701
Passerine * weight	0.33	0.49	-0.68	1.33	0.505
Reptile * weight	0.13	0.56	-1.10	1.19	0.818
Small mammal * weight	0.71	0.30	0.13	1.32	< 0.05
Snake * weight	0.59	0.28	0.03	1.16	< 0.05

Subsampling of field data

The results of varying the survey frequency showed a fairly constant rate of decrease in the proportion of carcasses detected for each functional group, although the largest decrease in proportion detected occurred between daily surveys and those with a 3-day interval (Figure 4).

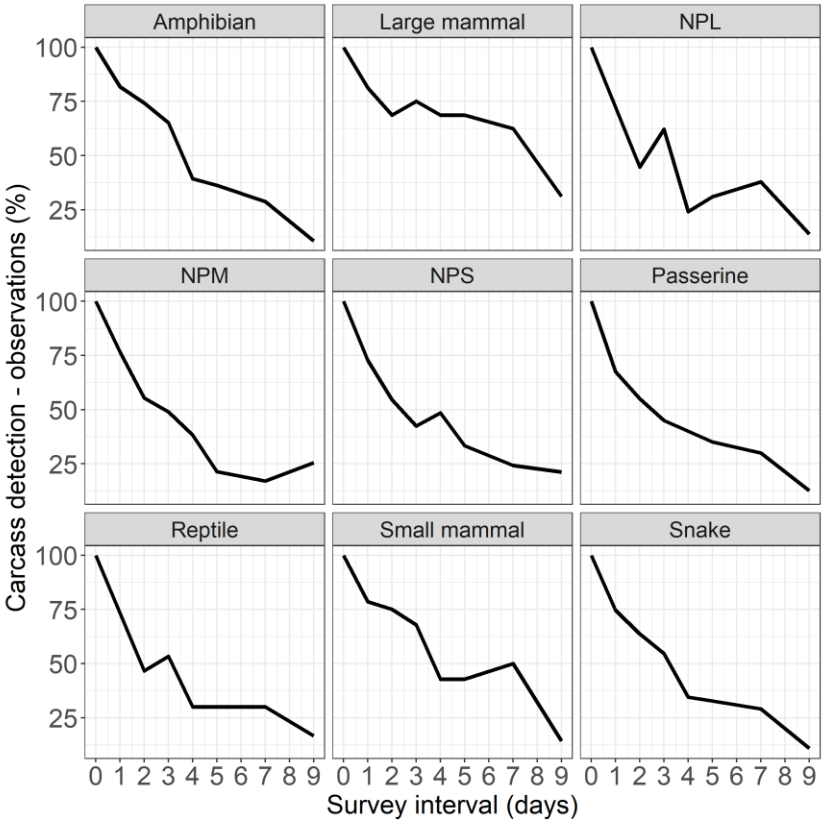

Figure. 4. Proportion of carcasses detected from the field data as a function of survey interval. NPL, large non-passerine birds; NPM, medium non-passerine birds; NPS, small non-passerine birds.

A noticeable exception was the large mammal group, which showed an initial period of decrease and then a flattening of the curve between survey intervals of two and seven days. There was also evidence that some of the curves flattened off when survey interval was greater than five days. In most cases, a loss of 50% of carcasses detected occurred when survey intervals were greater than two to four days (Figure 4). Patterns in the proportion of decrease in species richness were less consistent between functional groups. Certain groups such as passerines and snakes showed linear decreases as survey interval increased while others such as reptiles and small mammals had more fluctuating patterns (see Supplementary Material Figure S5).

Simulation

Intuitively, the simulation analysis showed a general trend of lower rates of carcass detection loss as mean carcass persistence increased (Figure 5).

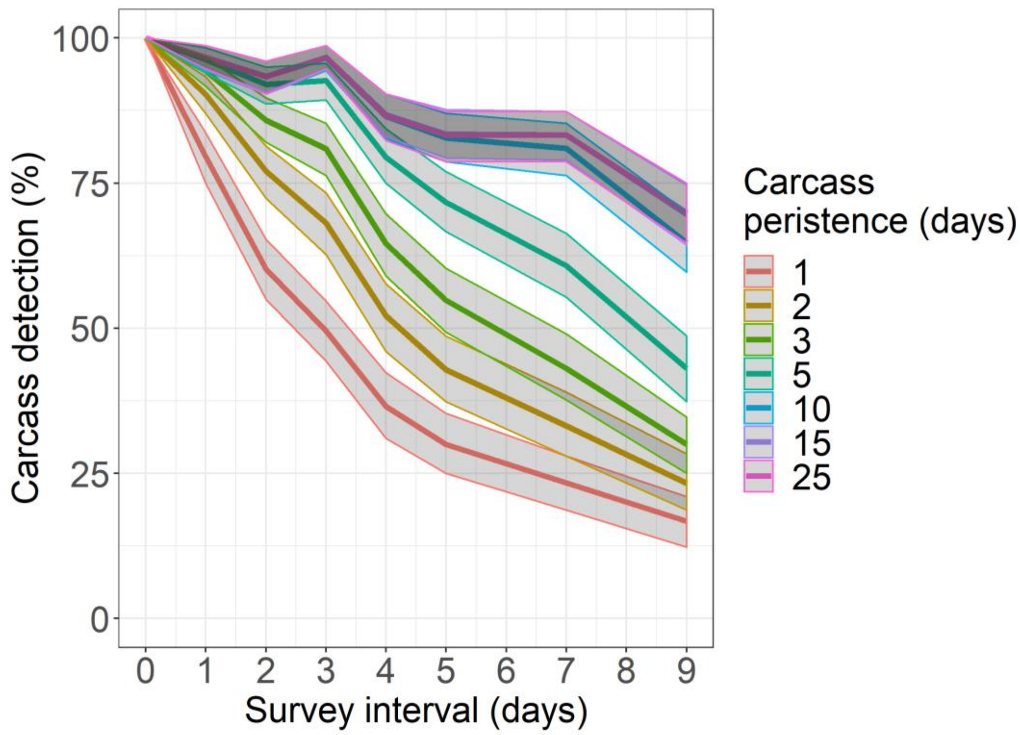

Figure. 5. Relationship between proportion of carcasses detected and survey interval across seven levels of mean carcass persistence calculated from the simulation analysis.

These results showed a good correspondence with the field data (i.e. 50% decrease for a mean carcass persistence of 2 days occurred at a 4-day survey interval). The simulations suggested that as mean carcass persistence increases above 5 days, the rates of decrease in proportion of all carcasses detected converge and become similar.

Survey costs

The range in total study cost in our analysis varied between \$156 (study duration of 20 days, transect distance of 50 km, 50% detection and mean carcass persistence >= 10) and \$6220 (study duration of 40 days, transect distance of 200 km, 75% detection and mean carcass persistence of 1 day; Figure 6).

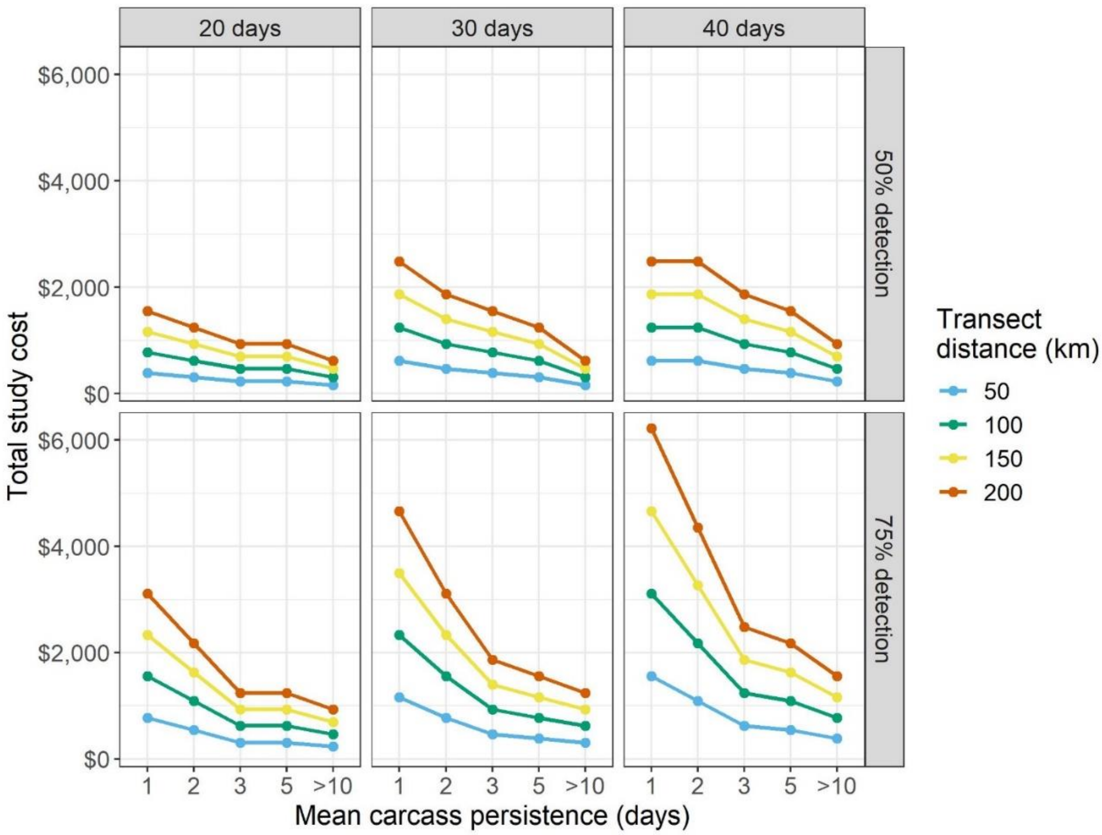

Figure. 6. The relationship between total simulated survey costs and mean carcass persistence and how these vary by transect distance and study duration.

For a given length of road requiring surveying, costs increased with study duration and level of carcasses detection required. Costs decreased as mean carcass persistence increased; this was as a result of the lower survey frequency required to detect species that persist for longer periods. The most noticeable decrease in costs occurred when going from a carcass persistence of 1 to 3 days during the 40-day study duration at 75% detection. In this instance, the costs decreased by 60% from $6220 to $2488. There were instances where costs were equivalent between two levels of mean carcass persistence (e.g., during the 20-day study period with a persistence of either 3 or 5 days). Figure 7 shows the cost of undertaking daily surveys (i.e. 100% carcass detection) using the same range of input parameters in the cost analysis (i.e., study duration and transect length).

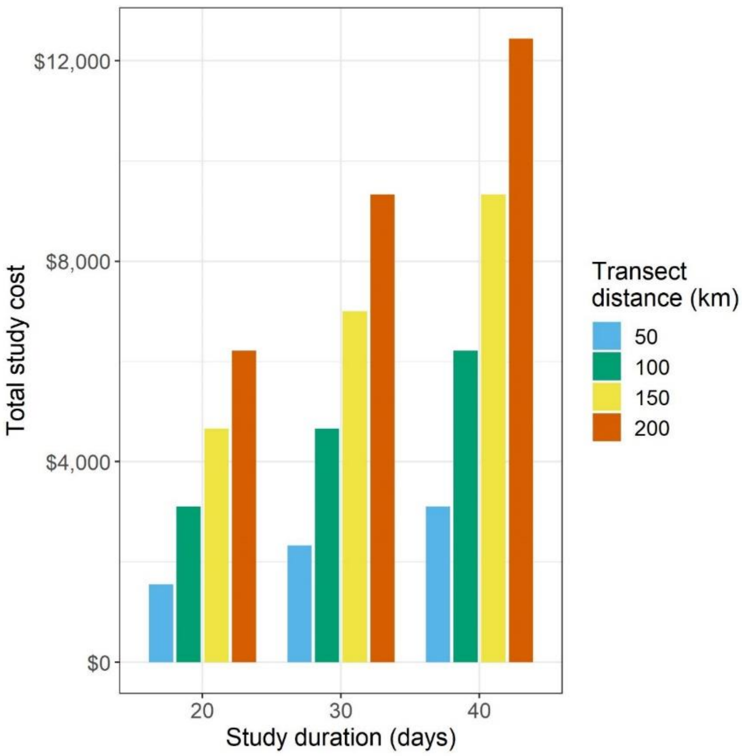

Figure. 7. The total study costs of conducting daily surveys across different combinations of study duration and transect distances.

As an example to highlight the difference in cost of adopting alternatives to daily sampling we report on the cost of surveying a 200 km transect over a 40-day period at a mean carcass persistence of three days. The daily survey cost, assuming 100% detection, was $12 440, while the costs were $2488 and $1866 at 75% and 50% detection, respectively. In this case, our results suggest that adjusting the sampling effort to decrease carcass detection by 25% results in a reduction of costs by up to 80%. We have developed an R Shiny web application that allows users to interactively explore the cost analysis presented here (users can change the values of all parameters to explore how study costs change). There are also options to download data and plots used in the analysis. The app is hosted at https://dominichenry.shinyapps.io/RK-survey-cost/ and the source code can be found at https://github.com/DomHenry/RK-survey-cost.

Discussion

Our results showed that overall carcass persistence was low, averaging only 2.7 days, although there was variation in persistence between functional groups. There was no strong evidence for an overall significant and positive effect of carcass weight on persistence but there were significant positive effects for the interaction between certain functional groups (large mammals, small mammals, large non-passerines and snakes) and weight. Carcass condition also influenced carcass persistence, in that flattened carcasses persisted for significantly longer than those that were not flattened. There was little evidence to support the effects of rainfall or distance to verge on carcass persistence. In subsampling our field data, we showed that the proportion of carcasses detected in many functional groups dropped off steeply when survey intervals were greater than three days. Survey intervals that ranged between two and four days resulted in a 50% decrease in detection for many functional groups, with large mammals being a noticeable exception. There was a general decrease in species richness as survey interval increased but the magnitude and shape of this relationship was less consistent across functional groups.

The simulation analysis matched the results from the field data; however, the simulation revealed a more gradual decrease in the proportion of carcasses detected as survey interval increased across all levels of mean carcass persistence. Interestingly, the simulations showed that when mean carcass persistence exceeded approximately five days, the rate at which observations detected decreased with increasing survey intervals converged. Using the results of the simulation to parameterise the cost analysis, we demonstrated how the survey effort affects the cost of roadkill surveys, and how this varies with study duration, transect length and mean carcass persistence of the focal taxon. Some of the most noticeable decreases in survey costs occurred when carcass persistence increased from one to three days. Under certain conditions the costs of doing non-daily surveys resulted in monetary savings of up to 80% (i.e., conducting surveys every 3 days instead of daily during a 40-day study period with transect lengths of 200 km). We encourage those interested in designing their roadkill surveys to make use of the app to compare costs across a range of values for each survey design parameter. The app allows a user to input custom values for all parameters and download the resulting plots and underlying data used to generate the comparisons.

The findings of our analyses based on our field data confirm patterns that have been illustrated in other studies. In one of the few studies to explicitly examine carcass persistence on roads, Santos et al. (2011) showed that overall persistence across multiple taxa from over 4,000 observations was approximately one day. This is shorter than our overall estimates of persistence but could be explained by the fact that 64% of their sample comprised small birds and salamanders whereas our study sample had a more even spread of observations across nine functional groups. While several studies show a positive correlation between body weight and carcass persistence (Borner et al. 2017; Santos & Ascensão 2019; Cabrera-Casas, Robayo-Palacio, & Vargas-Salinas 2020), the observed patterns from these studies are not always consistent. Interestingly, across all our samples there was not a strong effect of body weight, with only four of our functional groups showing significant relationships with carcass persistence. The lack of clear relationship between body size and persistence of a number of our functional groups (e.g., passerines, small and medium non-passerines, and reptiles) could possibly be due to functional groups that were not truly representative of typical species because presence on roads can depend on covarying factors such as season-specific behaviour (i.e. different levels of activity during reproductive and dispersal periods) (Miranda et al. 2017; Hastings et al. 2019), particularly near roads (Mkanda & Chansa 2011), and this is when road wildlife mortality rates increase (Clevenger, Chruszcz, & Gunson 2003). There was no significant effect of rainfall or distance to verge but there was support for our prediction that persistence of non-flattened carcasses is significantly lower than those that are flattened. Possible reasons for this finding include: flattened carcasses are less visible/detectable and due to desiccation, provide less nutritious food source to scavengers and are therefore not removed as frequently; and flattened carcasses may also adhere to the road surface to a greater degree. The recording of flattened versus non-flattened carcasses may also be due to survey technique; Ogletree & Mead (2020) found that more flattened carcasses were observed when walking surveys were undertaken compared to driven surveys, although this increased survey effort obviously restricts potential survey distance (Collinson et al. 2014).

Using a subsampling approach from our field data and simulation analysis we showed how, for each functional group, the proportion of carcasses detected decreased as a function of survey interval. Our results suggest that this relationship varies with carcass persistence, which itself is affected by a number of environmental variables. We demonstrate that the alternatives to conducting daily surveys, based on different levels of carcasses persistence, can result in surveys that are substantially more cost effective although carcass detection probability is lower. Researchers need to carefully consider the trade-off between carcass detection and survey cost which can be assessed using the two scenarios presented in the cost analysis (i.e., at a detection level of 50 or 75%). The overall aim, and the focal taxa of roadkill surveys also need to guide the determination of survey intervals. Our cost analysis provides a basis for how this trade-off can be addressed especially in cases where there is potential for studies to remain flexible in the configuration of parameters such as transect distance and study duration. The decision and design of roadkill-transect-lengths are usually selected on convenience of route (Collinson et al. 2019), *ad hoc* transects that have then identified specific hotspots (Silva, Crane, & Savini 2020), specification by the respective Department of Transport (for knowledge gathering; Plante et al. 2018), or knowledge of species' ranges or migration corridors (Kantola et al. 2019).

We propose that carcass persistence be taken into consideration during survey design. Efforts to design standardised roadkill data methodology will allow roadkill rates to be more comparable across time and sites. Reducing inaccuracies in reported rates of roadkill can allow for the effective evaluation of mitigation measures in different regions. Globally, there is substantial variety in roadkill data collection methods, with a variable range of durations and sampling frequencies which is a major barrier to separating the effects of sampling and those that influence the true underlying processes responsible for roadkill (Erritzoe, Mazgajski, & Rejt 2003; W. J. Collinson et al. 2014). For example, Bager & Da Rosa (2011) who sampled weekly over two years in Brazil, stated that weekly sampling was adequate for reptiles and medium-sized mammals but did not attain sampling sufficiency when all vertebrate classes were considered.

The assessment of the potential sampling biases are usually carried out in studies that aim to quantify the impacts of infrastructure such as wind-farms, power lines, fences, solar plants, or communication towers (Barrientos et al. 2017). In the context of roads, however, a more robust understanding of these biases is needed as the factors driving roadkill are far from being totally understood, and significant influences on roadkill detection further reduce our understanding of its impacts on populations. A greater understanding of carcass persistence can significantly influence the design of future projects, with major implications for the ability of researchers to manage their sampling frequencies to align with the study aims and focal animal taxa. The ability to quantify roadkill rates on impacted species will lead to an improved ability to identify mortality hotspots and implement adequate mitigation measures.

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Conflict of interest

None of the authors have any conflict of interests to declare.

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Supplementary Material

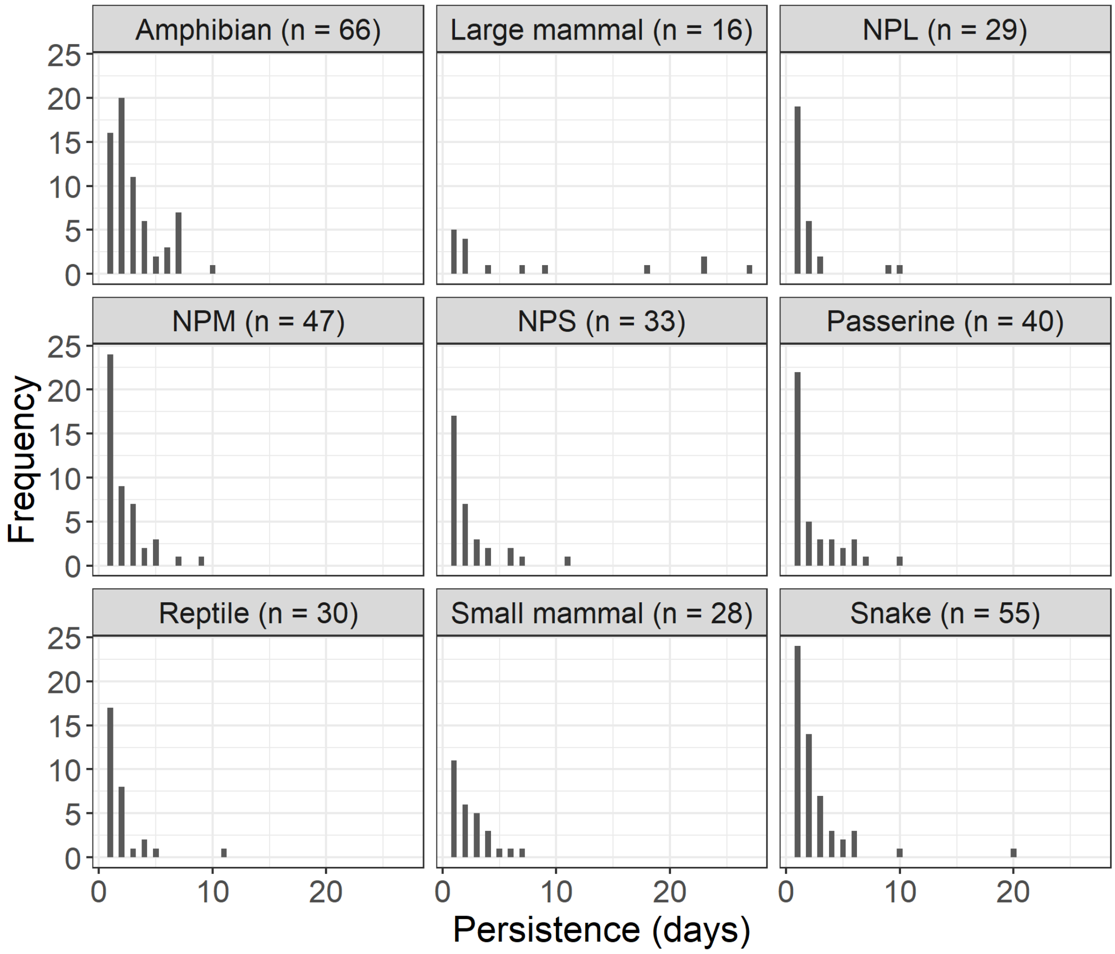
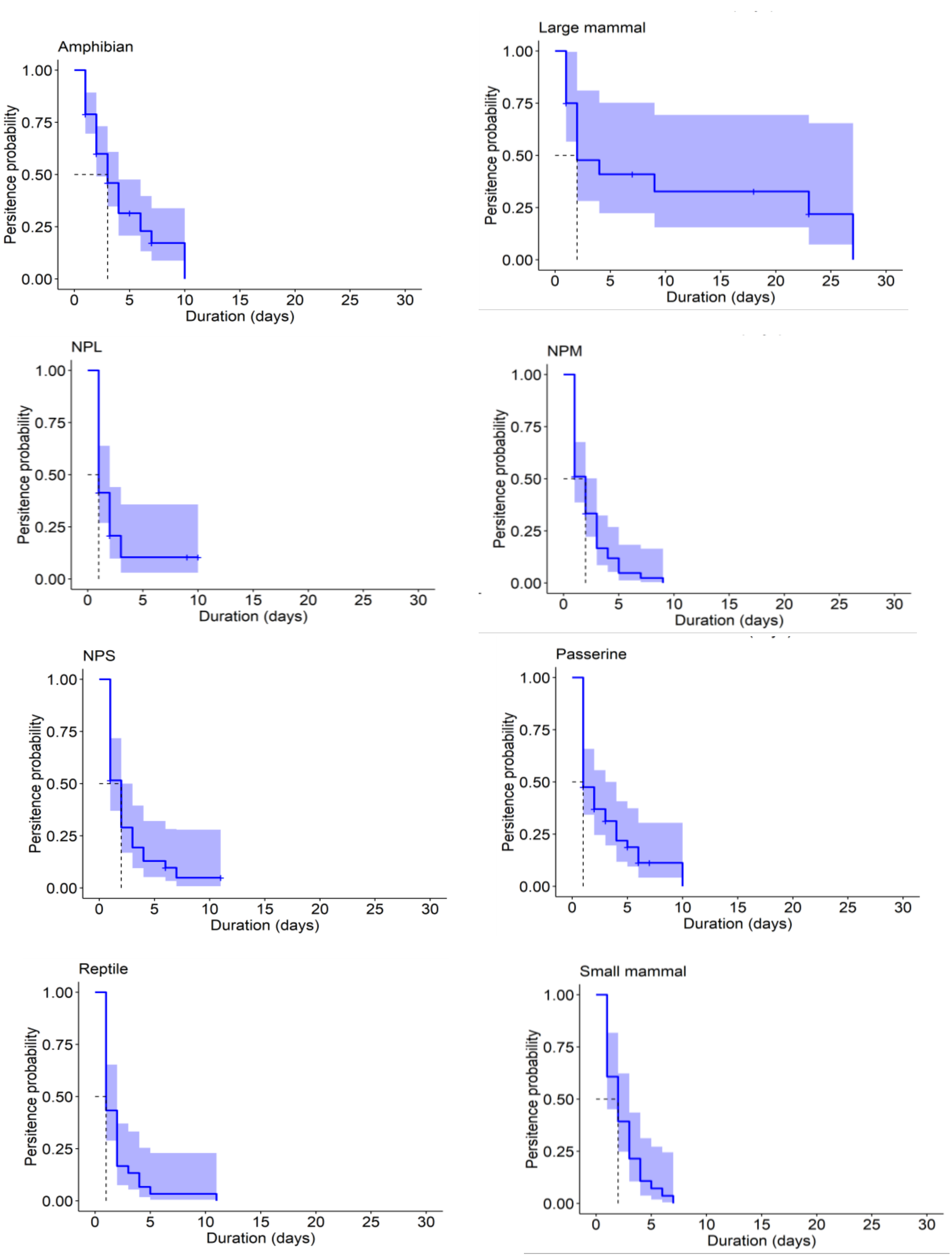


Figure. S1. Frequency distribution of carcass persistence times from data collected along repeated driven road transects (n, sample size; NPL, large non-passerine birds; NPM, medium non-passerine birds; NPS, small non-passerine birds).



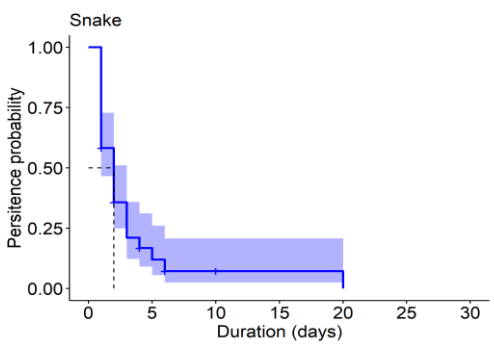


Figure. S2. Carcass persistence probability curves for each functional group. Dotted lines represent the median probability (i.e., where carcass persistence drops below 50%). Blue shading indicates 95% confidence intervals.

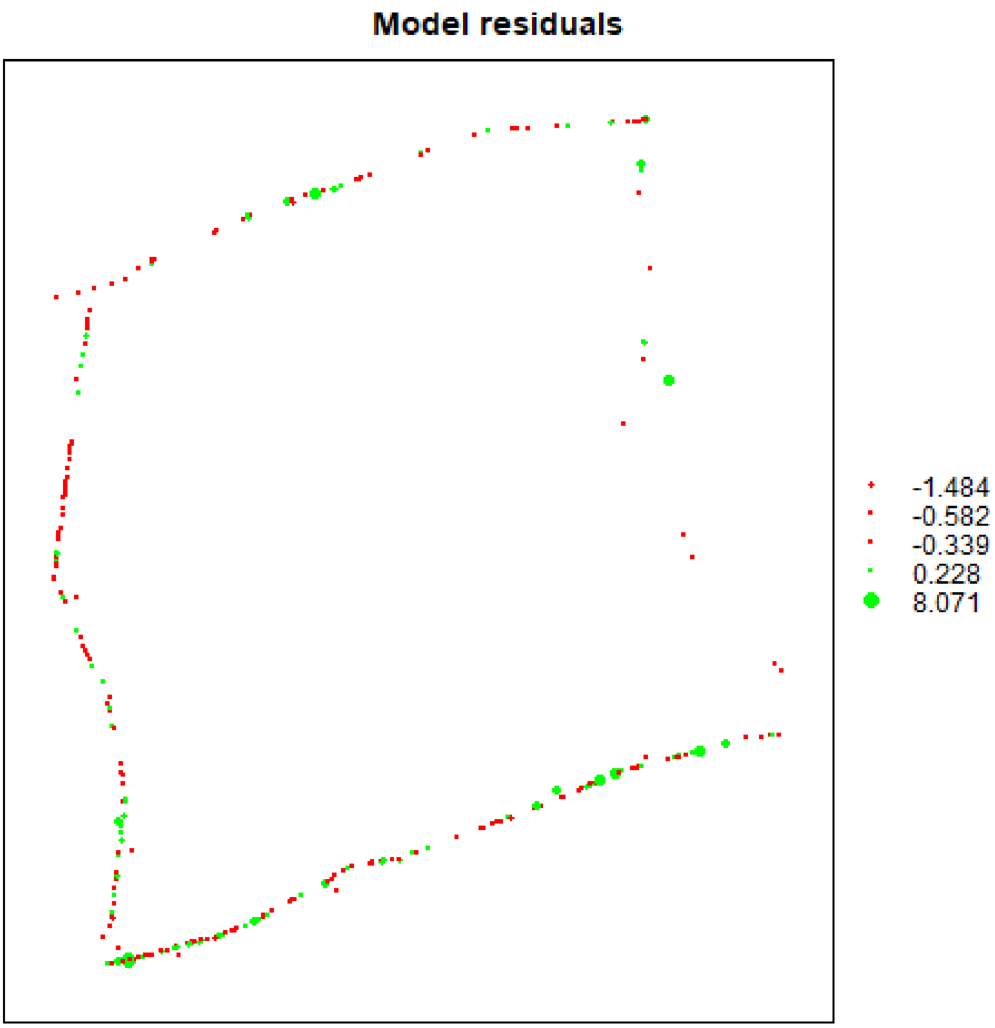


Figure. S3. Bubble plot of model residuals (used to assess spatial autocorrelation in observations) plotted in geographic space along the carcass survey transect. Each point represents a roadkill observation and the size of circles indicate magnitude of the regression residuals for that point (green, positive; red, negative).

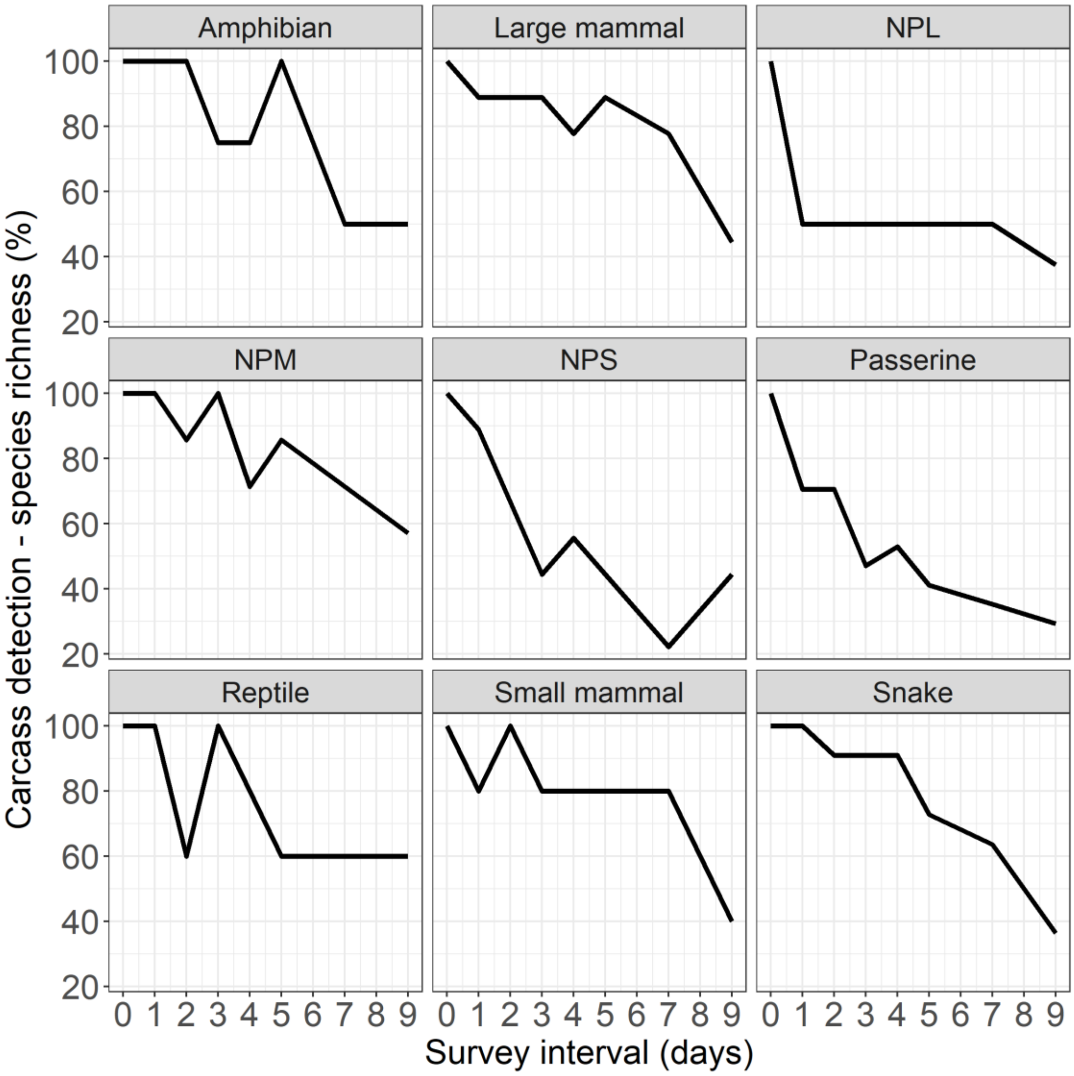


Figure. S5. Proportion of species detected as a function of survey interval for each functional group. NPL, large non-passerine birds; NPM, medium non-passerine birds; NPS, small non-passerine birds.

Table S1. Functional groups for 73 species recorded in the road driven carcass surveys. NPL, large non-passerine birds; NPM, medium non-passerine birds; NPS, small non-passerine birds.

Scientific name	Common name	Functional group
Pyxicephalus edulis	African Bull Frog	Amphibian
Bufo garmani	Eastern Olive Toad	Amphibian
Bufo rangeri	Raucous Toad	Amphibian
Proteles cristata	Aardwolf	Large mammal
Civettictis civetta	African Civet	Large mammal
Mungos mungo	Banded Mongoose	Large mammal
Otocyon megalotis	Bat-Eared Fox	Large mammal
Canis mesomelas	Black-Backed Jackal	Large mammal
Parahyaena brunnea	Brown Hyaena	Large mammal
Hystrix africaeaustralis	Cape Porcupine	Large mammal
Lepus saxatilis	Scrub Hare	Large mammal
Phacochoerus africanus	Warthog	Large mammal
Centropus burchellii	Burchell's Coucal	NPL
Numida meleagris	Helmeted Guineafowl	NPL
Pternistes natalensis	Natal Spurfowl	NPL
Ptilopsus granti	Southern White-faced Scops Owl	NPL
Bubo africanus	Spotted Eagle Owl	NPL
Burhinus capensis	Spotted Thick-Knee	NPL
Pternistes swainsonii	Swainsons Spurfowl	NPL
Bubo lacteus	Verreaux's Eagle Owl	NPL
Rhinoptilus chalcopterus	Bronze-winged Courser	NPM
Coracias garrulus	European Roller	NPM
Caprimulgus tristigma	Freckled Nightjar	NPM
Coracias caudata	Lilac-breasted Roller	NPM
Coracias naevia	Purple Roller	NPM
Turnix nana	Small Buttonquail	NPM
Tockus leucomelas	Southern Yellow-billed Hornbill	NPM
Halcyon albiventris	Brown-hooded Kingfisher	NPS
Merops nubicoides	Carmine Beeeater	NPS
Corturnix adansonii	Common Quail	NPS
Merops apiastar	European Beeeater	NPS
Caprimulgus pectoralis	Fiery-necked Nightjar	NPS
Oena capensis	Namaqua Dove	NPS
Macrodipteryx vexillarius	Pennant-winged Nightjar	NPS
Polihierax semitorquatus	Pygmy Falcon	NPS
Caprimulgus rufigena	Rufus-cheeked Nightjar	NPS

Hirundo rustica	Barn Swallow	Passerine
Prinia flavicans	Black-chested Prinia	Passerine
Uraeginthus angolensis	Blue Waxbill	Passerine
Emberiza tahapisi	Cinnamon-breasted Bunting	Passerine
Mirafra rufocinnamomea	Flappet Lark	Passerine
Emberiza flaviventris	Golden-breasted Bunting	Passerine
Sylvietta rufescens	Long-billed Crombec	Passerine
Acrocephalus palustris	Marsh Warbler	Passerine
Cisticola chinianus	Rattling Cisticola	Passerine
Lanius collurio	Red-backed Shrike	Passerine
Quelea quelea	Red-billed Quelea	Passerine
Mirafra sabota	Sabota Lark	Passerine
Vidua regia	Shaft-tailed Whydah	Passerine
Passer diffusus	Southern Grey-headed Sparrow	Passerine
Euplectus orix	Southern Red Bishop	Passerine
Chlorocichla flaviventris	Yellow-bellied Greenbul	Passerine
Serinus mozambicus	Yellow-fronted Canary	Passerine
Gerrhosaurus nigrolineatus	Black-lined Plated Lizard	Reptile
Chamaeleo dilepis	Flap-necked Chameleon	Reptile
Geochelone paradalis	Leopard Tortoise	Reptile
Lygosoma sundevallii	Sundevall's Writhing Skink	Reptile
Tatera leucogaster	Bushveld gerbil	Small mammal
Nycteris thebaica	Egyptian slit-faced bat	Small mammal
Steatomys pratensis	Fat mouse	Small mammal
Hipposideros caffer	Sundevall's roundleaf bat	Small mammal
Lamprophis capensis	Brown House Snake	Snake
Telescopus semiannulatus	Eastern Tiger Snake	Snake
Bitis caudalis	Horned Adder	Snake
Naja mossambica	Mozambique Spitting Cobra	Snake
Bitis arietans	Puff Adder	Snake
Dasypeltis scabra	Rhombic Egg-eater	Snake
Causus rhombeatus	Rhombic Nightadder	Snake
Aspidelaps scutatus	Shield Nose Snake	Snake
Naja annulifera	Snouted Cobra	Snake
Python natalensis	Southern African python	Snake
Psammophis subtaeniatus	Striped-bellied Sand Snake	Snake

Table S2. A breakdown of the optimal number of surveys (highest survey interval at each level of carcass detection) across three different study durations (i.e. the total number of surveys in that study duration across different survey intervals). Survey intervals are based on the combination of five levels of carcass persistence and two levels of carcass detection.

Persistence (days)	**Carcass detection (%)**	**Survey interval (days)**	**Optimal number of surveys**		
			40-day study duration	30-day study duration	20-day study duration
1	75	1	20	15	10
2	75	2	14	10	7
3	75	4	8	6	4
5	75	5	7	5	4
>= 10	75	8	5	4	3
1	50	3	8	8	5
2	50	4	8	6	4
3	50	6	6	5	3
5	50	8	5	4	3
>= 10	50	15	3	2	2