

Annual patterns of small vertebrates roadkilled in a supercity of eastern China (#84573)

1

First submission

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Review the raw data.



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Structure and Criteria

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2. EXPERIMENTAL DESIGN
3. VALIDITY OF THE FINDINGS
4. General comments
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BASIC REPORTING

-  Clear, unambiguous, professional English language used throughout.
-  Intro & background to show context. Literature well referenced & relevant.
-  Structure conforms to [PeerJ standards](#), discipline norm, or improved for clarity.
-  Figures are relevant, high quality, well labelled & described.
-  Raw data supplied (see [PeerJ policy](#)).

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-  Original primary research within [Scope of the journal](#).
-  Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
-  Rigorous investigation performed to a high technical & ethical standard.
-  Methods described with sufficient detail & information to replicate.

VALIDITY OF THE FINDINGS

-  Impact and novelty not assessed. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
-  Conclusions are well stated, linked to original research question & limited to supporting results.
-  All underlying data have been provided; they are robust, statistically sound, & controlled.

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3



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Tip

Example

Support criticisms with evidence from the text or from other sources

Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.

Give specific suggestions on how to improve the manuscript

Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).

Comment on language and grammar issues

The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 - the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.

Organize by importance of the issues, and number your points

- 1. Your most important issue*
- 2. The next most important item*
- 3. ...*
- 4. The least important points*

Please provide constructive criticism, and avoid personal opinions

I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC

Comment on strengths (as well as weaknesses) of the manuscript

I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.

Annual patterns of small vertebrates roadkilled in a supercity of eastern China

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An assessment of wildlife road mortality can help develop road mitigation measures. This paper is the first to report data on wildlife-vehicle collisions (WVC) in Nanjing, a megacity in eastern China. The research was conducted on a 224.27 km stretch of nine roads in Nanjing. Between November 2020 and October 2021, we conducted 26 monitoring missions to gather roadkill carcasses so that we could analyze their temporal and spatial distribution patterns. We collected 259 carcasses of 22 different species, of which 46.42 % were mammals and 48.81% were birds. Cats and dogs are the most roadkilled mammals, and blackbirds and sparrows are the most roadkilled birds. The temporal analysis demonstrated that overall roadkills distribution was seasonal, with peak vertebrate roadkills occurring between May to July. Spatial analysis showed that the distribution patterns of vertebrate roadkills on different roads were different, generally non-random distribution and aggregation. By mapping accidents using kernel density analysis, we were able to pinpoint locations that were at high risk for roadkills. Our study suggests that the problem of car accidents due to wildlife should be a cause for concern, and the results of the analysis of temporal and spatial patterns contribute to the establishment of mitigation measures.

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Abstract

An assessment of wildlife road mortality can help develop road mitigation measures. This paper is the first to report data on wildlife-vehicle collisions (WVC) in Nanjing, a megacity in eastern China. The research was conducted on a 224.27 km stretch of nine roads in Nanjing. In the period between November 2020 and October 2021, 26 monitoring missions were conducted to gather roadkill carcasses so that we could analyze their temporal and spatial distribution patterns. We collected 259 carcasses comprising 22 different species, of which 46.42 % were mammals and 48.81% were birds. Cats and dogs are the most roadkilled mammals, and blackbirds and sparrows are the most roadkilled birds. The temporal analysis demonstrated that overall roadkills distribution was seasonal, with peak vertebrate road-kills occurring between May to July. Spatial analysis showed that the distribution patterns of vertebrate roadkills on different roads varied, with a generally non-random distribution and aggregation. By mapping accidents using kernel density analysis, we were able to pinpoint locations that were at high risk for roadkills. Our study suggests that the problem of car accidents due to wildlife should be a cause for concern, and the results of the analysis of temporal and spatial patterns contribute to the establishment of mitigation measures.

Introduction

Commented [AOA(1)]: Just a suggestion, and given how the authors have conducted a spatial analysis, having the title read "Annual patterns.." might suggest to the reader that you have only conducted temporal analysis. I recommend the title rewritten as either "Temporal and Spatial patterns of small vertebrate road kills in a supercity of eastern China" or "Assessing small vertebrate road kill patterns in a supercity of eastern China"

Commented [AOA(2)]: This term can exclude domestic animals and livestock. Consider writing "animal" in its place.

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47 Roads act as barriers, fragmenting habitats just like any other human activity that changes
 48 ecosystems, which reduces biodiversity (Ferregueti et al. 2020). The most significant ecological
 49 impact of roads, according to Coffin (2007) and Trombulak & Frissell (2000), appears to be
 50 traffic-related mortality from wildlife-vehicle collisions (WVCs, hereafter). In some cases,
 51 WVCs can have devastating effects on animal populations (Forman 2000; Forman & Alexander
 52 1998). Numerous studies conducted over the past few years has produced WVCs results for
 53 many different countries. An estimated 340 million birds are killed on U.S. roads each year (Loss
 54 et al. 2014); around 335,000 European hedgehogs are killed on the roads in the U.K. per year
 55 (Wembridge et al. 2016); and around 9 million medium-large mammals are killed in traffic
 56 accidents in Brazil every year (Pinto et al. 2022). In addition, roadkill data has been reported
 57 from Spain (Colino-Rabanal et al. 2012; Diaz-Varela et al. 2011; Lagos et al. 2012; Rodriguez-
 58 Morales et al. 2013), Poland (Tajchman et al. 2010) and Sweden (Neumann et al. 2012).

59
 60 The world's road network has been expanding steadily in recent years, and by 2050, it is
 61 anticipated that at least 25 million km will be added to the network (Laurance et al. 2014). With
 62 the continuous development of China's economy, the total mileage of China's road network and
 63 road network density is also increasing. By the end of 2020, the total mileage of China's road
 64 network reached 5.20 million km, and the density of the road network in China reached 54.15
 65 km per 100 square km. Among them, the mileage of National highways, Provincial highways
 66 and County highways was approximately 0.37 million km, 0.38 million km and 4.46 million km,
 67 respectively. Roadkills, which are one of several reasons for the loss of vertebrates, have
 68 been proven in studies to increase with the development of road networks (Forman & Alexander
 69 1998). However, there are very few studies related to wildlife roadkill in China's modern cities,
 70 and research has only been conducted in Taiwan (Lin et al. 2019), the Changbai Mountain
 71 Nature Reserve (Wang et al. 2016), the Huangnihe River Reserve (Li 2019), the Zoige Wetland
 72 National Nature Reserve (Gu et al. 2011), and the Wanglang National Nature Reserve (Zhang et
 73 al. 2018), mostly in ecologically sensitive areas and focusing on species that are endemic to
 74 China. Therefore, there is a need to investigate common wildlife and their roadkill status in a
 75 wide geographical space.

76
 77 Previous research on small vertebrates (Clevenger et al. 2003; Morelle et al. 2013) and birds
 78 (Colino-Rabanal et al. 2011; Erritzoe et al. 2003) discovered that seasonal behaviors such as
 79 dispersal and migration are linked to temporal variations in roadkill patterns. Peak periods of
 80 animal road mortality occur during periods when animals are searching for mates or increasing
 81 their foraging activity (Lagos et al. 2012; Rodriguez-Morales et al. 2013; Smith-Patten & Patten
 82 2008). Also, juvenile dispersal during periods of increased numbers of free-ranging and

Commented [AOA(5)]: Consider including a more recent study to emphasise the impact in the contemporary world.

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83 inexperienced juveniles can affect the seasonality of road mortality (Madden & Perkins 2017).
84 Roadkill patterns exhibit temporal variability, which can be understood to improve mitigation
85 methods, particularly those that can be handled in a timely manner (for example, limitation of traffic
86 intensity or speed) (D'Amico et al. 2015).

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87
88 The spatial distribution of casualties, which is arguably the most researched aspect of road
89 ecology, is another key subject in roadkill research (Jaeger & Fahrig 2004; Trombulak & Frissell
90 2000). According to certain research, roadkill of mammals and other vertebrates, such as birds,
91 does not occur randomly but rather at specific areas of the road (Coelho et al. 2008; Garriga et al.
92 2017). Mortality hotspots are stretches of road where roadkills are more common (Santos et al.
93 2015). These areas might be thought of as top priorities for the use of mitigation measures. By
94 constructing wildlife crossing structures such as tunnels, ledges in culverts, and overpasses in
95 these locations, we can reduce the amount of wildlife that is roadkilled (van der Grift et al.
96 2013).

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98 The study's aim is to present Nanjing with the initial findings regarding the spatial and
99 temporal patterns of WVCs. We monitored nine road stretches fortnightly for one year in
100 Nanjing (a supercity in eastern China). The objectives of this study were (1) to describe the
101 species composition of roadkills on the road studied; (2) to describe the seasonal pattern of
102 roadkills in mammals and birds; and (3) to describe the spatial patterns of roadkills on the
103 investigated road. For example, where are roadkill incidents gathered? Where are the located
104 mortality hotspots? How are roadkills distributed across different spatial scales? Using the
105 temporal and spatial distribution patterns in WVCs, we may also identify targeted
106 hotspots of roadkills to apply mitigating actions.

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108 Materials & Methods

109 Study area

110
111
112 The study area (Figure 1) was located in Nanjing in the southwestern Jiangsu Province (31°14' to
113 32°37' N, 118° 22' to 119°14' E). The climate is humid north subtropical with four distinct
114 seasons and abundant rainfall, with annual rainfall averaging 1106.5 mm. The annual average
115 temperature is 15.4°C, the highest annual extreme temperature is 39.7°C, and the lowest is -
116 13.1°C. Topography is flat, and elevations range from 0 to 753 m, and the average altitude is 35
117 m. Nanjing is a supercity in eastern China with a resident population of approximately 10 million

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118 people. It is also an international integrated transport hub city with a total road mileage of
 119 9,796.325 m and an extensive road network with varying road types and traffic intensities. We
 120 selected nine roads (three National highways, three Provincial highways and three Rural
 121 highways) within our study area. All nine roads are paved, with the annual average daily traffic
 122 volumes ranging from 30 000 to 60,000 vehicles per day on national highways, 10,000 to 40,000
 123 vehicles per day on provincial highways, and 5,000 to 15,000 vehicles per day on rural
 124 highways. The types of roadside habitats on these roads cover residential areas, farmland, open
 125 fields and forests. The basic conditions of the sampled roads are shown in Table 1.

126 Roadkill survey data collection

129 To gather data on road vertebrate carcasses, we ran 26 monitoring campaigns over 224.27
 130 kilometers of road, totalling 5831 km, between November 2020 and October 2021. Nine selected
 131 roads were monitored every fortnight over the course of a year. Sampling was done in good
 132 weather conditions, beginning at 9 a.m. and lasting as long as it took to complete the entire route
 133 while maintaining a speed of ≥ 40 km/h (Ferreguetti et al. 2020). Considering the safety of
 134 driving, our speed should not be too low. The survey team included drivers and observers who
 135 kept records of animal carcasses found on the survey roads. Additionally, driving recorders were
 136 installed in the automobiles to capture any roadkill events throughout the survey. All carcasses
 137 found during the survey were identified at class (to species level where possible). The Global
 138 Positioning System (GPS) was used for logging the locations of each road accident. Roadkill was
 139 georeferenced to an accuracy of 50 m. Once recorded, it was removed from the road. The
 140 accuracy of road kill hotspot detection is decreased due to the low frequency of our sample and
 141 the possibility of ignoring small-sized carcasses in car surveys (Santos et al. 2011; Santos et al.
 142 2015); nonetheless, more frequent surveys are not practical given the scope of our investigation
 143 (Garriga et al. 2017).

144 Data analysis

146 Exploratory analysis was used to calculate the absolute and relative frequencies of WVCs in
 147 order to determine the species involved and the types of roads where incidents occurred. We
 148 used the Kruskal-Wallis test to examine seasonal trends in animal roadkill at the level of species
 149 groups (mammals and birds) and small vertebrate populations.

151 We evaluated the spatial distribution of WVCs in three steps. First of all, we used the nearest
 152 neighbor distance (NND) to evaluate the distribution of roadkills on the road network (Gonser et
 153

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Commented [AOA(16)]: Name these roads.

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Commented [AOA(18)]: What constitutes "good" weather conditions?

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Commented [AOA(22)]: From which organisations?

Commented [AOA(23)]: Remember to either use "road kill" or "roadkill". Avoid using both of these.

Commented [AOA(24)]: "low frequency of sampling" or "low size of the sample"?

Commented [AOA(25)]: If possible, try clarifying this a little more. It is not an absolute necessity but I think it would help the reader.

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al. 2009). We compare the observed average distance between each incident and its nearest neighbor with the average distance that would be expected if the accidents were randomly distributed along the road (complete spatial randomness) to determine whether WVCs are aggregated along the road network (Okabe & K.Sugihara 2012). The Clark-Evans index is calculated to confirm departure from a random distribution. The index is the ratio of the observed and expected mean distances: values >1 , $=1$, <1 , indicating that the points are aggregated, randomly distributed and dispersed, respectively (Clark & Evans 1954). Secondly, to locate collision hotspots for WVCs, we employed kernel density analysis (Okabe & K.Sugihara 2012). Kernel density is the number of road-killed animals per kilometer of road. Finally, we investigate the spatial structure of WVCs at different spatial scales using the Ripley K-function, which denotes the pattern of point distribution at various scales (Mountrakis & Gunson 2009; Ripley 1976). All the spatial analyses were performed with the use of the SANET Standalone and ArcGIS 10. SANET Standalone provides tools adapted to perform spatial analysis along linear features.

Results

Species composition of roadkills

From November 2020 to October 2021, we examined a total of 5831 km through 26 surveys. A total of 293 animals (21 identified species) were collected. These included 136 mammals (6 species), 143 birds (14 species) and five reptiles (1 species) (Table 2). Birds made up 48.81% of the roadkills followed by mammals (46.42%) and reptiles (1.70%).

The most commonly mammalian roadkilled species were the cat (*Felis catus*) (28.7 % of mammalian casualties), the dog (*Canis lupus familiaris*) (26.5 %) and the weasel (*Mustela sibirica davidiana*) (9.6 %). The most common bird roadkilled species were the Blackbird (*Turdus merula*) (15.4 % of bird casualties) and the Sparrow (*Passer montanus*) (7.7 %). Together these five species accounted for 41.3 % (121) of all roadkills. The only recorded reptile species killed on the road is the Tabby-necked snake (*Rhabdophis tigrinus*).

Seasonality of roadkill numbers

Roadkill occurrences were not evenly distributed throughout the year (Fig. 2), with significant differences in the number of roadkills across months ($\chi^2 = 20.552$, $df = 11$, $p = 0.038$) and

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Commented [AOA(30)]: If this work has already been done then write in past tense.

Commented [AOA(31)]: Write this in full if it is an abbreviation or an acronym.

Commented [AOA(32)]: In the earlier comment about vertebrates (Data analysis), it would have been advisable to write either "mammals, birds and reptiles", "mammals, birds, reptiles and amphibians" or "mammals, birds and herpetofauna" as they all fall into the category of vertebrates for this study.

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Commented [AOA(33)]: If your focus is on cats and dogs for the mammals in this study, then I think it is not necessary to include this.

Commented [AOA(34)]: Use italics when writing the species scientific name.

189 seasons ($\chi^2 = 12.012$, $df = 3$, $p = 0.007$). The highest rates occurred in May (32 specimens), June
 190 (40 specimens) and July, compared to lower roadkill rates in February (13
 191 specimens), March (15 specimens) and April (15 specimens). Seasonality tests indicated that the
 192 number of road kills was significantly higher in summer and autumn than in spring and winter.
 193 Seasonality of roadkill was also shown to significantly differ between specific taxonomic groups
 (Mammals: $\chi^2 = 8.726$, $df = 3$, $p =$
 194 0.033; Birds: $\chi^2 = 11.211$, $df = 3$, $p = 0.011$). Mammals showed a higher road mortality in
 195 autumn. By contrast, birds showed peaks in summer (Fig. 3).

196
 197 Description of the spatial pattern of roadkills
 198

199 Among the different types of roads, provincial roads (5.76 ± 0.65 individuals/100km) had the
 200 highest road mortality, followed by national roads (5.38 ± 0.62 individuals/100km) and county
 201 roads (5.02 ± 0.68 individuals/100km), but no significant differences in road mortality were found
 202 among the different administrative levels of roads ($\chi^2 = 0.471$, $df = 2$, $p = 0.790$) (Fig. 4).

203
 204 We observed that carcasses were not randomly distributed along the road network. Among the
 205 nine roads investigated, the roadkills of G1, S1, and S2 showed a clustering pattern ($p < 0.05$;
 206 Table S1, Fig. S1). The heterogeneous distribution of WVCs along the road network was
 207 revealed by network kernel density analysis (Fig. 5). The shade of color in Figure 3 represented
 208 the density of roadkills. The Net-KDE results showed that the darker the road segment, the more
 209 road accidents occurred.

210
 211 For the multi-distance clustering analysis, the network K function is executed on the road network.
 212 The results of the K-function show the clustering tendency of roadkills at multiple distances (Fig.
 213 6). The distribution pattern of vertebrate roadkills on different roads varies, and it is
 214 generally non-random distribution and aggregation. On the national highway, the roadkill
 215 distribution of vertebrates on G1 and G3 is obviously clustered within 20 km; on G2, it presented
 216 a random distribution. On the provincial highway, there was significant clustering of roadkills at
 217 small spatial scales. The range of spatial scales over which clustering was significant was 0-5 km
 218 in roadkills; on a larger scale, it showed a scattered or random distribution. On the county roads,
 219 the distribution of roadkills on X2 and X3 are significantly clustered within 3 km and tend to be
 220 randomly distributed with increasing scale. On X1, it is randomly distributed in a small range,
 221 tends to be dispersed as the scale increases, and stabilizes at random after 10 km.

222

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223 Discussion

224

225 Roadkill magnitude and species affected

226

227 A total of 293 road-killed wild animals were recorded in our 12-month study, with an average
228 road mortality rate of 5.39 ± 0.37 individuals/100km. Comparison of vertebrate road mortality
229 across studies is difficult due to differences in survey methods and species conditions at survey
230 sites (Elzanowski et al. 2009; Langen et al. 2007). However, the road mortality of wild animals
231 has certainly been underestimated in these investigations. Many animals are
232 thrown far from the road after a collision due to the impact, and it is clear that these individuals
233 are often overlooked during surveys. Alternatively, animal carcasses remain on the road for
234 only a short time due to vehicle movements, rain and the activities of road scavengers. Studies
235 have shown this to be an important factor in underestimating road mortality (Pinto
236 et al. 2022). In our case, over 90 % of vertebrate carcasses remained on the road for no more than
237 5 days (well below the sampling interval of this study) (Wu & Sun, unpublished data). Research
238 approaches also play an important role in roadkill surveys. The low frequency of surveys (every
239 fortnightly) may lead to an underestimation of roadkills, as the persistence of bodies on the road
240 is much lower than the survey interval (Santos et al. 2011).

241

242 Roadkill events tend to be concentrated in one or a few species, which are usually locally
243 abundant, mobile and easily attracted by favorable resources or environmental conditions along
244 the road (Forman 2000). Cats and dogs are the most commonly killed mammals on the roads
245 sampled, probably because of their large numbers, wide distribution and high population density
(Reference?).

246 For example, the total number of owned cats in urban areas of China is estimated to be
247 149,807,371 (Li et al. 2021). The high number of dogs and cats in Nanjing is closely related to
248 the city's high population. In densely populated areas, more people treat cats and dogs as pets
249 and they can accidentally breed, be abandoned by their owners or wander off and become stray
250 animals (Ozen et al. 2016).

251

252 The blackbird and sparrow are the most commonly roadkilled birds, probably because they are
253 both resident birds with large and widely distributed populations and are the dominant species
254 among birds in Nanjing (Zhang et al. 2018). Particularly the blackbird, in contrast to other
255 avians, feeds on cereals, fruit and insects, but also on rotten and foul-smelling carrion (Zhu 2019).
256 This is why blackbirds are not only found around the mountains and forests but can also be seen
257 in residential areas. ↓

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258

259 Temporal patterns

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261 Temporal patterns of roadkill may be correlated with the phenology and activity cycles of local
262 wildlife, as well as local weather circumstances (Carvalho & Mira 2011). Some studies have
263 associated roadkill with the season (Caro et al. 2000). Generally, the peak road kills for
264 mammals appeared in summer and autumn, while the peak roadkill for birds appeared in spring
265 and summer. However, this varies depending on the species' breeding season and dispersal
266 patterns (Hell et al. 2005). For wild animals, their road mortality patterns are associated with
267 three bursts of movement in their life: the mating seasons, the weaning of young to forage with
268 their mothers, and the dispersal of juveniles (Smith-Patten & Patten 2008).

269

270 In this study, overall roadkills distribution was seasonal. May to September were months in
271 which vertebrates were found to be particularly vulnerable to road mortality. The animals'
272 heightened reproductive activity in summer and autumn may be the cause of the rise in roadkill.
273 In the spring and early summer, many taxa give birth to their young, and by the end of the
274 summer, the populations have grown significantly (Raymond et al. 2021). Not only does this
275 mean more individuals and therefore an increased likelihood of vehicle collisions, but also
276 entails a large number of inexperienced juveniles that must disperse, with an often lacking ability to
277 avoid vehicles (Legagneux & Ducatez 2013). In addition, due to the fact that parents are required
278 to forage more frequently and for longer periods of time to raise their young, some adult animals
279 may also be more susceptible to automobile accidents at this time of year (Gonser et al. 2009;
280 Hell et al. 2005).

281

282 In our study, mammalian roadkill peaks occurred in July to September and November, which is
283 probably connected to the duration of the day and the animals' seasonal activity (Tajchman et al.
284 2010). The first peak occurs in association with mammalian breeding activity. From May to
285 September, when most mammals enter the breeding season, increased breeding activity
286 (Ignatavicius & Valskys 2018), mass movements (Herr 2008) and the dispersal of young can all
287 lead to seasonal peaks in roadkill. Conversely, winter is a difficult time for many mammals and
288 corresponds to a period of dormancy or decreased activity for species (Haigh 2012). It is also a
289 time of reduced prey and food supply for many animals and as a result individuals may have to
290 travel further in search of food leading to a greater susceptibility to roadkills that may account
291 for the peak observed in mammals in November (Garriga et al. 2017).

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293 The time interval between adult breeding and juvenile dispersal is much shorter in birds
 294 compared to mammals (Dawson et al. 2001). The temporal convergence of mate search, juvenile
 295 nurturing and dispersal make it easier to observe a single-peak pattern of road mortality
 296 in birds, but the timing of the peak depends on the taxonomic unit (Raymond et al. 2021). In our
 297 study, May to July were the months when birds were particularly vulnerable to road mortality.
 298 This was also a time when birds were engaged in the breeding activity (incubation and fledging)
 299 and juveniles were dispersing (Colino-Rabanal et al. 2011; Erritzoe et al. 2003). Particularly, the
 300 high proportion of immature young people leaving the nest may be the cause of the elevated
 301 mortality (Grilo et al. 2009; Hell et al. 2005). On the other hand, during fledging, adults must
 302 frequently travel over large distances to forage for food in order to feed their young. As a result,
 303 birds may cross roads more frequently or forage among roadside vegetation, which increases the
 304 chance of roadkills (Holm & Laursen 2011; Kuitunen et al. 2003). In addition, as summer crops
 305 mature, seed-eating birds have access to more food sources close to roads, which may potentially
 306 be a factor in the increased bird mortality (da Rosa & Bager 2012). Meanwhile, we also observed
 307 a peak in road mortality in January. It may be correlated with the breeding habits of some bird
 308 species. Blackbirds and sparrows, for example, can successfully breed and raise two or more
 309 broods of young in a year, depending on weather conditions (Yuan 2017; Zhu 2019).

310 Spatial distributions

312 The distribution of the roadkills along the road network is not random, according to the spatial
 313 analyses. We indeed observed that most WVCs occurred on national and provincial roads and
 314 were highly clustered along these routes in Nanjing, southwestern Jiangsu
 315 Province. The study by Morelle et al. (Year?) in the Wallonia region of southern Belgium also found
 316 that although national roads and highways account for small parts of the total length of the road
 317 network, more than half of the WVC occur on these roads, and WVCs were highly concentrated
 318 in the highway and national roads in the south of Belgium (Morelle et al. 2013). Clevenger's (Year?)
 319 research in Western Canada found that the distribution of roadkills were uneven (Clevenger et al.
 320 2003; Morelle et al. 2013). Possible explanations for the different patterns are differences in road
 321 structure and traffic flow (Girardet et al. 2015). In our study area, the average daily traffic
 322 volume on national and provincial highways (20,000 - 50,000 vehicles/day) is two to four times
 323 higher than on rural highways (5,000 - 15,000 vehicles/day). Although we found higher roadkill
 324 mortality on national and provincial roads than on county roads, we did not find significant
 325 differences, probably because the higher road density of county roads compensates for the effects
 326 of lower traffic volumes, so animals use county roads more frequently than national and
 327 provincial roads.
 328

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329

330 The spatial distribution pattern of roadkill also shows variability between different road types. A
331 map of kernel density revealed places at higher risk of WVCs. This non-random pattern is likely
332 to be explained by road features and land cover (Gonser et al. 2009). Among our sampled roads,
333 WVCs on G1 are concentrated around Xuanwu Lake and Zijin Mountain National Forest Park.

334 The urban forest park is an important habitat for **urban animals** (Fernandez-Juricic 2004; Fontana
335 et al. 2011), in which there is a large number and variety of wildlife and high biodiversity. The
336 area is also located in the center of Nanjing, with a high population and road density, and **large**
337 traffic flow. **The increased probability** of wildlife crossing the roads in this area **makes it**,
338 prone to wildlife-vehicle collisions.

339

340 The scale of roadkills on different grades of roads **also varies**. Network nearest neighbor
341 analysis reveals that the scale of roadkill clusters was higher on national highways than on other
342 roads. On national highways, the scale of roadkill aggregation is in the range of 0-20 km, while
343 on rural highways, the scale of roadkill aggregation is only 0-5 km. Differences in vehicle speeds
344 and road characteristics are one of the reasons **behind this**. Roads with many curves and low
345 traffic speeds may result in more discontinuous roadkill patterns (i.e., roadkill aggregation on
346 smaller spatial scales) than high-speed linear highways with high speeds and little speed
347 variation (Clevenger et al. 2003).

348

349 Mitigation measures

350

351 Roadkill mitigation measures are widespread in many countries (Damarad & Bekker 2003).
352 Mitigation measures to **address** the impact of roads on wildlife need to be considered when new
353 roads are built, expanded or upgraded (Evink 2002). Common mitigation measures include
354 wildlife warning signs, road traffic volume and speed restrictions in specific seasons and
355 locations, wildlife detection systems, wildlife passages and wildlife fencing.

356

357 Our results aid in the creation of mitigating strategies to lessen roadkills in the research area.
358 Wildlife corridors at roadkilled hotspots, combined with wildlife fencing, can be effective in
359 preventing animals from entering the road. We recommend systematic and regular monitoring of
360 animal roadkill for at least a year to identify roadkilled hotspots. If roadkill can be monitored for
361 several years, the population dynamics of some species can be assessed on the basis of the results
362 (Hell et al. 2005). Temporary mitigation measures, such as speed restrictions or temporary traffic
363 control, can be implemented for hotspots where animal access is not readily available or where
364 there are seasonal peaks of WVCs. Furthermore, long-term monitoring of mitigation after

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365 implementation to determine the effectiveness of these measures and timely adjustments
366 based on actual conditions is conducive to minimizing roadkills.

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368 Conclusions

369 In our study over a 12-month period from November 2020 to October 2021, we collected 293
370 roadkilled individuals along the sampling roads in Nanjing. Temporal analysis of roadkill events
371 indicated that they were seasonal, with peaks in roadkill occurring mainly in summer and autumn
372 for birds and mammals, respectively. Spatial analysis of roadkill events showed that their spatial
373 distribution pattern on roads was generally non-random and aggregated. Our research shows that
374 wildlife-vehicle collisions should be taken seriously. We should put in place the necessary
375 mitigation measures to reduce road kill at certain times and on certain roads.

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378 Acknowledgements

379 We thank Sihan Ning, Cong Yu, Yunshu Wang, Lixin Chen and others for their help during the
380 road surveys. We are grateful to Yumeng Zhao, Yigui Zhang, Yunchao Luo and others for their
381 advice on the work. We appreciate Changjian Fu and the editors and reviewers for their valuable
382 comments and suggestions for improving the manuscript.

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535

536

Table 1 (on next page)

Table 1. Basic information of the nine sampled roads in Nanjing, China

Table 1. Basic conditions of the nine sampled roads in Nanjing, China.

Road	G1	S1	X1	G2	S2	X2	G3	S3	X3
Highway classification	National Highway	Provincial Highway	County Highway	National Highway	Provincial Highway	County Highway	National Highway	Provincial Highway	County Highway
Number of lanes	8	4/6	2	6	4	4	4/6	4	4
Presence of road medians	Yes	Yes	No	Yes	No	Yes	Yes	Partly present	Yes
Main habitat types on roadsides	residential areas, open fields	residential areas, farmland	residential areas, farmland	farmland, forests	residential areas, forests	open fields, forests	residential areas, open fields	residential areas, forests	open fields, forests
Road width	40~60m	20~50m	10~20m	30m	20m	15m	20~40m	15~35m	15~30m

Table 2(on next page)

Table 2. Frequency of small vertebrates roadkilled in the sampling roads of Nanjing, 2020-2021

1
2 Table 2. Frequency of small vertebrates roadkilled in the sampling roads of Nanjing, 2020-2021.
3

Common Name	Scientific Name	N	% of taxa	% of total kills
Mammal				
Cat	<i>Felis catus</i>	39	28.7	13.3
Dog	<i>Canis lupus familiaris</i>	36	26.5	12.3
Weasel	<i>Mustela sibirica davidiana</i>	13	9.6	3.8
Mouse	<i>Muroidea</i>	11	8.1	4.4
Hedgehog	<i>Erinaceus amurensis</i>	7	5.1	2.4
Hare	<i>Lepus sinensis</i>	1	0.7	0.3
Unidentified mammal		29	21.3	9.9
Total Mammals		136		
Bird				
Blackbird	<i>Turdus merula</i>	22	15.4	7.5
Sparrow	<i>Passer montanus</i>	11	7.7	3.8
Spotted Dove	<i>Streptopelia chinensis</i>	3	2.1	1.0
Azure-winged Magpie	<i>Cyanopica cyanus</i>	3	2.1	1.0
Light-vented Bulbul	<i>Pycnonotus sinensis</i>	3	2.1	1.0
White-cheeked Starling	<i>Sturnus cineraceus</i>	2	1.4	<1
Crested Myna	<i>Acridotheres cristatellus</i>	2	1.4	<1
Magpie	<i>Pica pica</i>	1	<1	<1
Oriental Turtle Dove	<i>Streptopelia orientalis</i>	1	<1	<1
Chicken	<i>Gallus gallus domesticus</i>	1	<1	<1
Bamboo Partridge	<i>Bambusicola thoracica</i>	1	<1	<1
Red-billed Blue Magpie	<i>Urocissa erythroryncha</i>	1	<1	<1
Red-breasted Flycatcher	<i>Ficedula albicilla</i>	1	<1	<1
White Wagtail	<i>Motacilla alba</i>	1	<1	<1
Unidentified bird		90	62.9	30.7
Total Birds		143		
Reptile				
Tabby-necked snake	<i>Rhabdophis tigrinus</i>	5	100.0	1.7
Total Reptile		5		
Unidentified body		9		
Total		293		

Figure 1

Figure1 Sample lines and roadkills for roadkill survey of small vertebrates in Nanjing

The 224.27 km road section is in bold, including the National Highway (90.60 km, blue), the Provincial Highway (84.74 km, green) and the Rural Highway (48.93 km, yellow)

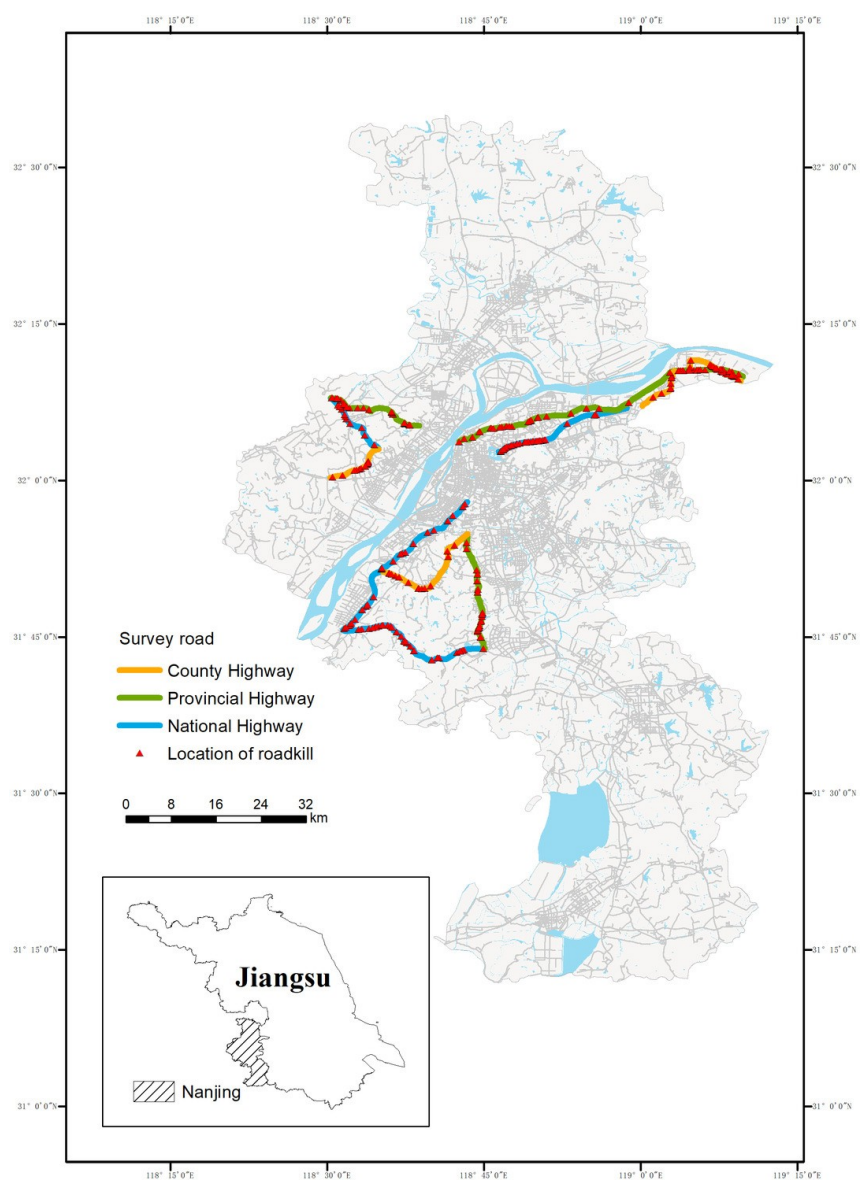


Figure 2

Figure 2. Monthly number of small vertebrate roadkills in Nanjing, November 2020 - October 2021

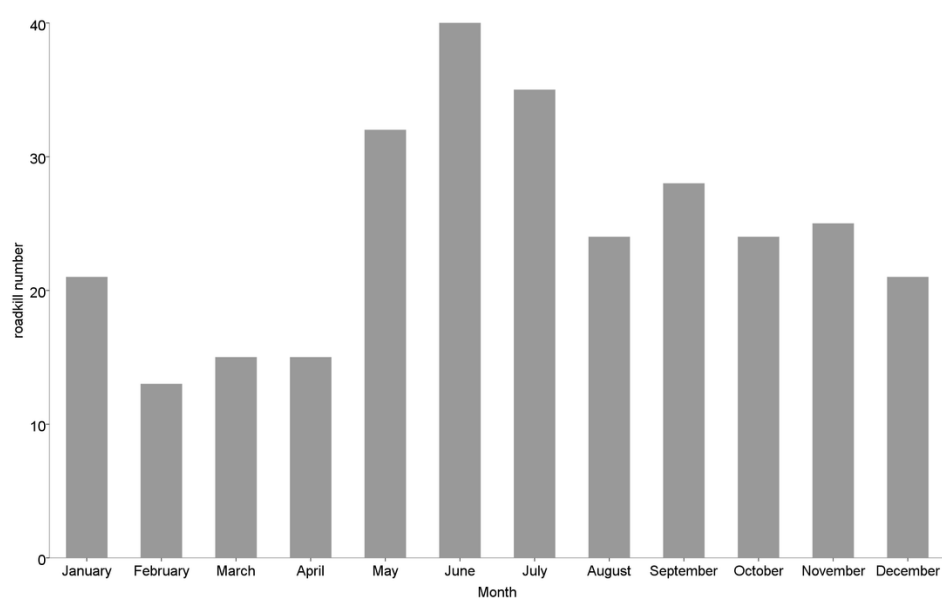


Figure 3

Figure 3. Monthly roadkill number of mammals and birds in Nanjing, November 2020 - October 2021

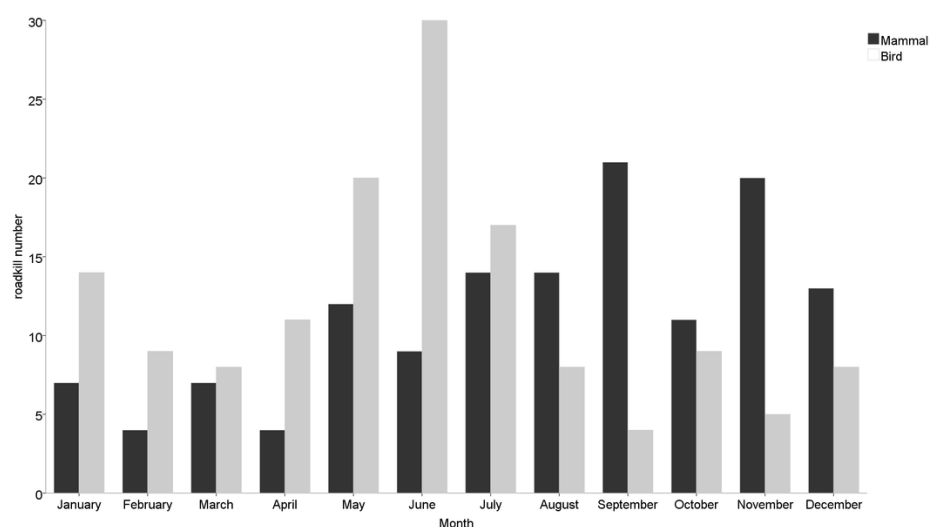


Figure 4

Figure 4. Roadkill mortality rates on different administrative levels road

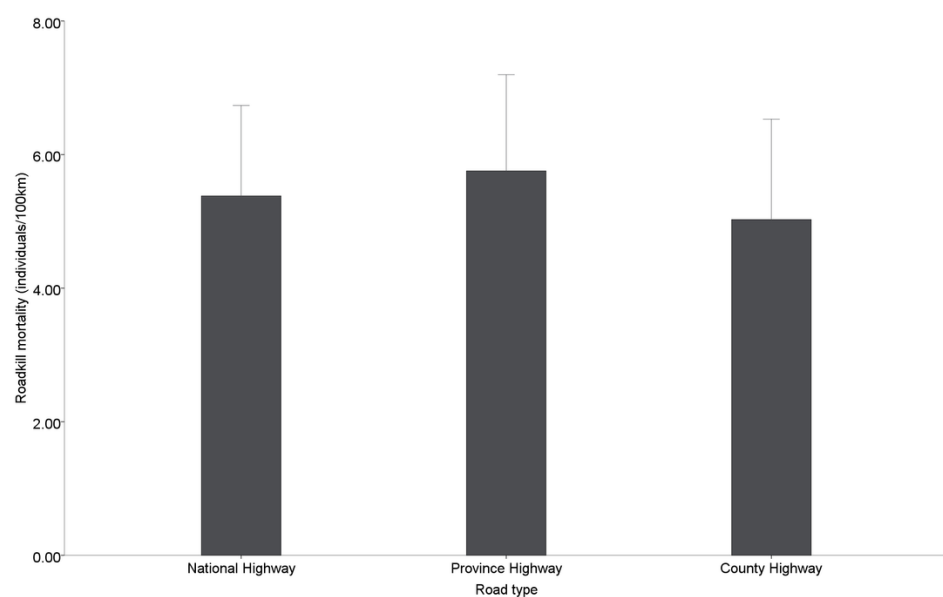


Figure 5

Figure 5. Results of network kernel density estimation

Darker colors represent higher roadkills densities.

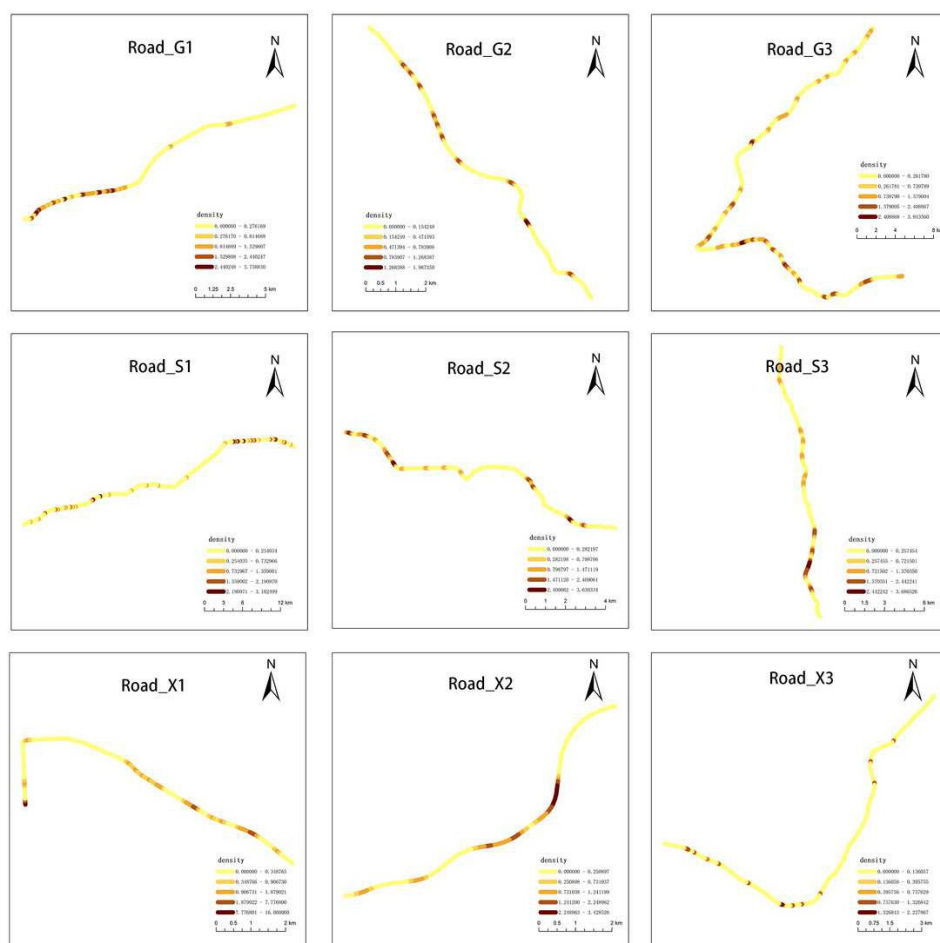


Figure 6

Figure 6. Network K-function analysis

The blue line is the observed curve, the red line is the expected curve, the green line means the upper limit of the 95 % confidence interval and the pink line means the lower limit of the 95 % confidence interval. If the observed curve is above the confidence interval, the spatial distribution pattern is clustered; below, it is dispersed; within, it is random.

