



## Research article

# Sampling effects on the identification of roadkill hotspots: Implications for survey design



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## ABSTRACT

Although locating wildlife roadkill hotspots is essential to mitigate road impacts, the influence of study design on hotspot identification remains uncertain. We evaluated how sampling frequency affects the accuracy of hotspot identification, using a dataset of vertebrate roadkills ( $n = 4427$ ) recorded over a year of daily surveys along 37 km of roads. “True” hotspots were identified using this baseline dataset, as the 500-m segments where the number of road-killed vertebrates exceeded the upper 95% confidence limit of the mean, assuming a Poisson distribution of road-kills per segment. “Estimated” hotspots were identified likewise, using datasets representing progressively lower sampling frequencies, which were produced by extracting data from the baseline dataset at appropriate time intervals (1–30 days). Overall, 24.3% of segments were “true” hotspots, concentrating 40.4% of roadkills. For different groups, “true” hotspots accounted from 6.8% (bats) to 29.7% (small birds) of road segments, concentrating from <40% (frogs and toads, snakes) to >60% (lizards, lagomorphs, carnivores) of roadkills. Spatial congruence between “true” and “estimated” hotspots declined rapidly with increasing time interval between surveys, due primarily to increasing false negatives (i.e., missing “true” hotspots). There were also false positives (i.e., wrong “estimated” hotspots), particularly at low sampling frequencies. Spatial accuracy decay with increasing time interval between surveys was higher for smaller-bodied (amphibians, reptiles, small birds, small mammals) than for larger-bodied species (birds of prey, hedgehogs, lagomorphs, carnivores). Results suggest that widely used surveys at weekly or longer intervals may produce poor estimates of roadkill hotspots, particularly for small-bodied species. Surveying daily or at two-day intervals may be required to achieve high accuracy in hotspot identification for multiple species.

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## 1. Introduction

Roads are often a source of considerable wildlife mortality,

which may have significant impacts on animal populations (Coffin, 2007; Forman et al., 2003; Trombulak and Frissell, 2000). To reduce roadkill rates, many projects involve the implementation of specific mitigation measures, including the construction of wildlife crossings (e.g. over or underpasses), the use of fences to keep wildlife away from roads or to guide them to safe crossing points, and the installation of animal crossing signs (Clevenger et al., 2003; Glista et al., 2009; Mata et al., 2008). Since mitigation structures are expensive (Glista et al., 2009; Huijser et al., 2009; Iuell et al., 2003), they must be placed where they are most effective. Therefore, accurately locating roadkill hotspots, i.e. segments of roads with

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particularly high animal-vehicle collision rates (Malo et al., 2004; Ramp et al., 2005), is a key prerequisite to develop cost-effective measures to reduce animal mortality on roads (Clevenger et al., 2003; Grilo et al., 2009; Iuell et al., 2003; Ramp et al., 2005; Seiler, 2005; Van der Grift et al., 2013).

Locating roadkill hotspots generally involves the regular survey of roads for carcasses, each of which is identified and georeferenced (Clevenger et al., 2003; Ramp et al., 2005). Over repeated surveys, a large proportion of carcasses are usually recorded in a relatively small set of road segments, which are thus identified as roadkill hotspots and targeted for the implementation of mitigation measures (Glista et al., 2009; Ramp et al., 2005). Hotspots are often determined separately for different taxonomic groups, as some mitigation measures are taxon specific (Glista et al., 2009; Iuell et al., 2003). This general methodological approach has been widely used, though involving considerable variation in a number of aspects that can affect the accuracy of hotspot identification, including for instance the number of observers, and the frequency and duration of surveys, among others. However, to our knowledge, no study has yet analysed how these variations may affect estimates of the spatial mortality patterns (Teixeira et al., 2013). Although much research has been devoted to assess and correct for the effects of sampling design on overall road-related mortality (e.g. Gerow et al., 2010; Guinard et al., 2012; Teixeira et al., 2013).

Estimating roadkill hotspots may be affected by much the same errors that are known to influence the estimate of wildlife mortality on roads. Imperfect detection is one of the main problems (Guinard et al., 2012; Teixeira et al., 2013), because failure to detect carcasses can lead to underestimating road-related mortality, and it may bias estimates of spatial roadkill patterns if detection is higher in some road segments than in others. The other key problem is related to the persistence of carcasses, which eventually disappear from roads due to decomposition, or removal by scavengers, humans or vehicles (Guinard et al., 2012; Slater, 2002; Teixeira et al., 2013). As a consequence, many carcasses may be missed and road-related mortality may be underestimated when there is a long time interval between consecutive surveys, particularly in the case of small-bodied species (amphibians, reptiles, small birds, bats and small mammals) with short persistence times (Coelho et al., 2008; Santos et al., 2011; Guinard et al., 2012; Teixeira et al., 2013). This problem may affect estimates of spatial roadkill patterns, because segments with particularly high persistence times may be misidentified as sites with high mortality rates (Santos et al., 2011; Teixeira et al., 2013), thereby generating false positives (i.e., wrong identification of roadkill hotspots). In contrast, false negatives (i.e., failure to detect roadkill hotspots) may be generated when high mortality events and the subsequent removal or decay of carcasses occur in-between consecutive surveys (Guinard et al., 2012; Ratton et al., 2014; Slater, 2002), as it may be the case of amphibian mortality occurring during movements to breeding places (Hels and Buchwald, 2001). In addition to these error sources, there may be considerable random variability in the occurrence of mortality events, which may greatly affect roadkill estimates when sample sizes are small.

Considering the potential sources of errors identified, it is likely that sampling frequency should be one of the methodological decisions greatly affecting the accuracy of hotspot identification, because increasing the time interval between surveys, and thus reducing the number of survey days, may increase the likelihood of both false negative and false positive hotspots. In this paper we address this idea, evaluating how sampling frequency affects the identification of roadkill hotspots. The study was based on daily surveys of 37 km of roads, carried out during one year, which were used to set a baseline scenario for spatial mortality patterns. In a

previous paper we estimated the persistence time of different vertebrate groups, and proposed the sampling schedule required to accurately estimate the overall mortality of each group (Santos et al., 2011). We now focus on the spatial mortality patterns, aiming to: (i) estimate how the spatial accuracy of hotspot identification varies in relation to sampling frequency intervals; and (ii) how the effects of sampling frequency vary between animal groups. We expect that our study will contribute to set methodological guidelines for the accurate detection of roadkill hotspots, thereby helping to improve the spatial allocation of costly mitigation measures.

## 2. Material and methods

### 2.1. Study area

The study was conducted in southern Portugal (38°32'24" to 38°47'33"N; -08°13'33" to -07°55'45"W) within a landscape dominated by cork (*Quercus suber*) and holm oak (*Quercus rotundifolia*) open woodlands, alternating with arable fields, olive groves and vineyards. The climate is Mediterranean, with mean temperature ranging from 5.8 °C to 12.8 °C in winter (January), and from 16.3 °C to 30.2 °C in summer (July), and annual rainfall averaging 609.4 mm (Évora 1971–2000; Instituto de Meteorologia, 2010). The relief is flat to gently undulating, ranging from 150 m to 400 m above sea level. We studied 37 km of road, including sections of two national (N4 and N114) and two municipal (M528 and M370) roads. N4 and N114 have traffic volumes of 4000 to 10,000 vehicles/day, though the later includes sections with ≥10,000 vehicles/day (Estradas de Portugal, 2005). Traffic volumes are much lower in M529 (1000–4000 vehicles/day) and M3270 (<1000 vehicles/day). All roads are two-lanes wide, without central barriers, except in five road crossings. A large number of vertebrates are killed in these roads, especially small birds (44.7% of roadkills) and amphibians (28.0%) (Santos et al., 2011).

### 2.2. Datasets

The study was conducted using a baseline dataset of 4427 individual records of vertebrate carcasses (Table 1), which were detected during 368 road surveys carried out daily between January 2005 and January 2006. Surveys were made during early hours of each day by one observer driving a car at 20 km per hour while checking both sides of the road. In each daily survey, care was taken to detect all freshly road-killed vertebrates (i.e., since the previous day), as well as old carcasses remaining in the road from previous days. Each new carcass was identified to the lowest possible taxonomic (mostly species) level, and its spatial position was recorded using a GPS. Carcasses were left in the same position to determine persistence times in a concurrent study (Santos et al., 2011), but double counting was avoided using the records of location and taxonomic identity of each individual. Whenever previously recorded carcasses were not detected from the car, the observer stopped to look for remains in the pavement. This procedure was done mostly for small sized carcasses, which was expected to minimize biases potentially introduced by the lower detectability of certain animal groups from car surveys when compared with walking surveys (Langen et al., 2007; Teixeira et al., 2013). This intensive sampling schedule is expected to have detected most individuals killed due to road casualties during the study period, though we acknowledge that some small carcasses may have been missed, either because they were not detected during surveys, or because they persisted for less than the day-interval between consecutive surveys (Santos et al., 2011; Teixeira et al., 2013). Nevertheless, we assumed that this daily dataset

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