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Using the dead to infer about the living: Amphibian roadkill spatiotemporal dynamics suggest local populations' reduction

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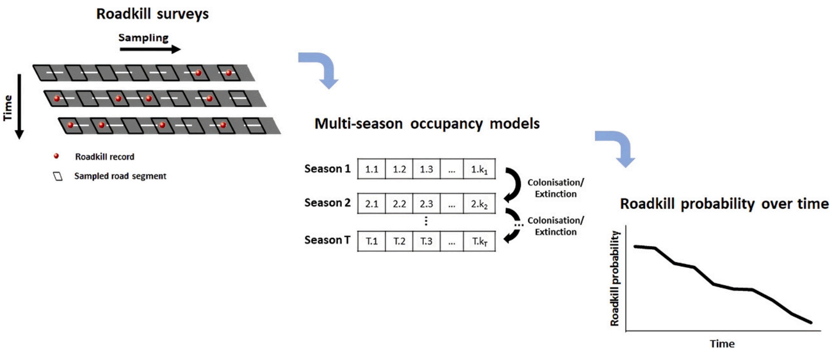
HIGHLIGHTS

- Amphibians are currently the most road-killed vertebrate group.
- We studied spatiotemporal patterns of roadkill in a Mediterranean landscape.
- We applied multi-season occupancy models to 14-year mortality data.
- Roadkill trends decreased in the study area in all analysed species.
- Assessment of amphibian populations living near roads is urgently required.

GRAPHICAL ABSTRACT



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ABSTRACT

Roads represent one of the main sources of wildlife mortality, population decline, and isolation, especially for low-vagility animal groups. It is still not clearly understood how wildlife populations respond to these negative effects over space and time. Most studies on wildlife road mortality do not consider the spatial and temporal components simultaneously, or the imperfect roadkill detection, both of which could lead to inaccurate assumptions and unreliable mitigation actions. In this study, we applied a multi-season occupancy model to a 14-year amphibian mortality dataset collected along 120 km of roads, combined with freely available landscape and remote sensing metrics, to identify the spatiotemporal patterns of amphibian roadkill in a Mediterranean landscape in Southern Portugal. Our models showed an explicit general decrease in amphibian roadkill. The Iberian painted frog (*Discoglossus galganoi*) experienced roadkill declines over time of ~70 %, while the spiny common toad (*Bufo spinosus*) and the fire salamander (*Salamandra salamandra*) had a loss of nearly 50 %, and the Southern marbled newt (*Triturus pygmaeus*) had 40 %. Despite the decreasing trend in roadkill, spatial patterns seem to be rather stable from year to year.

Multi-season occupancy models, when combined with relevant landscape and remote sensing predictors, as well as long-term monitoring data, can describe dynamic changes in roadkill over space and time. These patterns

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are valuable tools for understanding roadkill patterns and drivers in Mediterranean landscapes, enabling the differentiation of road sections with varying roadkill over time. Ultimately, this information may contribute to the development of effective conservation measures.

1. Introduction

Linear infrastructures have shaped the landscape for generations, following human development and economic growth (Forman and Alexander, 1998; Coffin, 2007; Meijer et al., 2018). Roads are one of the most widespread man-made infrastructures on Earth, responsible for fragmenting continuous habitats into smaller and less connected patches and creating barriers to animal movements (Shepard et al., 2008; Ward et al., 2015; Ibisch et al., 2016). Human pressures have intensified the expansion of road networks in natural areas, putting wildlife at a higher risk (Blondel et al., 2010; Garriga et al., 2012). Within this, roadkill (or wildlife-vehicle collisions) is the most visible direct impact of roads and contributes to the decline of species of high conservation importance (Loss et al., 2015; Bencin et al., 2019). These collisions are not randomly distributed across the landscape (Malo et al., 2004; Santos et al., 2017): generally, roadkill locations are located close to high-quality habitats, migration routes between feeding and reproduction sites, or ‘dangerous’ road sections (Carvalho and Mira, 2011; D’Amico et al., 2015). High roadkill aggregation sites (also called roadkill hotspots) are not static over space and time (Medinas et al., 2021) and tend to change their location and extent according to landscape features (Forman et al., 2003; Medinas et al., 2013), traffic density (Fahrig et al., 1995; Zimmermann Teixeira et al., 2017), road type (Clevenger et al., 2003; Medinas et al., 2013) or even weather conditions (Carvalho et al., 2017). The negative roadkill effects can endure over time, affecting the decrease of genetic structuring associated with isolation or reduced population size, and local extinctions for some species (reviewed in: Cushman, 2006; Ceia-Hasse et al., 2017). Thus, it is paramount to understand how roads impact wildlife mortality over time.

Amphibians are the most susceptible vertebrate group to die on roads due to their unique ecological, physiological, and behavioural traits (i.e., lower vagility, narrow habitat tolerances, water dependency and the necessity of seasonal migrations to complete intricate life cycles; Carr and Fahrig, 2001; Mazerolle et al., 2005; Sillero, 2008; Bouchard et al., 2009; Joly, 2019; Pinto et al., 2023). Beebee (2013) analysed amphibian roadkill at a global scale and reported that 1 % of amphibian records constituted road casualties, representing 90 % of the total vertebrate carcasses. Garriga et al. (2017) have also presented similar results for Iberian Peninsula at a local scale. Numerous studies report high mortality close to water bodies (Ascensão and Mira, 2005; Orlowski, 2007; Santos et al., 2007), near high-quality habitats (Sillero, 2008; Matos et al., 2012) and in specific times of the year related to the higher amphibian activity (Puky, 2005; Glista et al., 2008; Orlowski et al., 2008). According to the IUCN Red List, 20 of the 21 autochthonous amphibian species occurring in Portugal present declining population trends (IUCN, 2022). The persistence of amphibian populations in severely fragmented landscapes is intrinsically linked to population size, landscape types, reproduction ability, location of breeding sites, species’ dispersal capacities and road density (Carr and Fahrig, 2001). This prompts the need to understand roadkill tendencies and variations in the long term, for the implementation of more effective mitigation measures (Puky, 2005; Schmidt and Zumbach, 2008; Glista et al., 2009). In addition, studies on the roadkill effects on amphibian populations are still scarce, mainly due to the difficulties in collecting the necessary data to address these issues: a very high manpower and sampling effort are necessary to collect a small amount of populational data (Kays et al., 2011; e.g., telemetry and capture-recapture studies). Roadkill data though can be extremely valuable in allowing researchers to make inferences about the state of populations (Schwartz et al., 2020). Further, if collected over long periods of time, variations between years can provide more robust inferences on the possible impact of roads on population persistence. Temporal studies can be the key to unveiling the influence of landscape factors on roadkill spatiotemporal patterns. Moreover, by collecting data over longer periods of time, researchers increase the probability of gathering information on elusive species. The combination of long-term roadkill data with free high-resolution remote sensing data may assist in capturing environmental changes at local scales (Valerio et al., 2020). This is particularly relevant in the Mediterranean Basin, a region marked by strong seasonal variations with long and dry summers and wet winters (Goubanova and Li, 2007; Pereira et al., 2021), and for amphibians that respond not only to fine scales but are also highly vulnerable to external factors (Puky, 2005; Speybroeck et al., 2016).

In this study, we assess the spatiotemporal patterns of amphibian roadkill and their drivers in a Mediterranean landscape, during a 14-year survey period. Specifically, we implement a multi-season occupancy modelling strategy together with landscape, weather, and remote sensing covariates to explore the long-term trends and patterns of roadkill across amphibian species with different habitat requirements, life-history traits, and roadkill frequency. We expect these patterns to fluctuate over space and time, mainly due to changes in climatic factors and landscape features (Glista et al., 2008; Medinas et al., 2021).

2. Material and methods

2.1. Study area

The study was performed in the Alentejo region in Southern Portugal, along five selected road sections. The climate is Mediterranean, with mean temperatures ranging from 5.8 °C to 12.8 °C in January and from 16.5 °C to 30.2 °C in August, and annual rainfall averaging 609.4 mm (Évora 1971-2000; IPMA, 2021). The landscape is dominated by Mediterranean forests of cork (*Quercus suber*) and holm (*Quercus rotundifolia*) oaks with variable tree density, mixed with pastures for cattle grazing, composing the complex agro-silvo-pastoral system known as *montado* (Pinto-Correia et al., 2011). The landscape also includes agricultural areas (arable land, olive groves, orchards, and vineyards) in equal proportions to the forest. The topography is mainly flat with gentle slopes, ranging from 100 m to 400 m a.s.l. This study area has a well-established road network, including the main transportation corridor between Lisbon and Madrid.

2.2. Roadkill surveys

The road surveys were carried out for 14 years (from the 1st of January 2006 to the 31st of December 2011 and from the 1st of January 2013 to the 31st of December 2020), along four National Road sections (EN4, EN114, EN370 and EN18) and one Municipal Road section (EM529), comprising a total of approximately 120 km of sampled roads. All roads are two-lane wide and two (EN4 and EN114) have paved shoulders. The highway A6, although not surveyed, runs parallel to roads EN114 and EN18 (Fig. 1).

Surveys followed a standardized protocol (see Santos et al., 2011) and were performed either daily or weekly, by an experienced observer driving a car at 20–40 km/h during the first morning hours to reduce the impact of scavengers in carcass removal. Both sides of the road (including lanes and shoulders) were sampled, and all road-killed animals were collected and registered. All detected amphibian carcasses were identified to the lowest possible taxonomic level, its geographical position was recorded with a handheld GPS device and after this, carcases were removed from the road to avoid double counting during later surveys. Due to budget constraints over the study period, there were three periods where sampling did not occur (April 2013, January to March 2015, and May to September 2017, totalising nine months without surveys) and surveyed length varied over the years (Table S1 Supplementary material).

The surveys produced a roadkill dataset of 14,062 amphibian carcasses, belonging to 12 species. For the present work, we selected four anuran species: the spiny common toad (*Bufo spinosus*), the natterjack toad (*Epidalea calamita*), the Iberian spadefoot toad (*Pelobates cultripes*), and the Iberian painted frog (*Discoglossus galganoi*); and three urodele species: the fire salamander (*Salamandra salamandra*), the Iberian ribbed newt (*Pleurodeles waltl*), and the Southern marbled newt (*Triturus pygmaeus*). This selection covers species with diverse ecological specialisations (i.e., habitat generalist versus specialist, and open versus forest habitat) and with high roadkill rates (Table 1). The IUCN Red List categories for the Iberian spadefoot toad and the fire salamander are Vulnerable, the Southern marbled newt is Near Threatened, while the spiny common toad, the natterjack toad and the Iberian ribbed newt are Least Concern (IUCN, 2022).

Table 1
List of analysed species with habitat preference, habitat requirement and the total number and percentage of roadkill in the study area. Mixed habitat represents both open and forested habitat types.

Name	Habitat preference	Habitat requirement	N roadkill	% roadkill
Natterjack toad (*Epidalea calamita*)	Mixed	Generalist	6499	46.6
Fire salamander (*Salamandra salamandra*)	Forest	Specialist	2011	14.4
Spiny common toad (*Bufo spinosus*)	Forest	Generalist	1878	13.5
Iberian ribbed newt (*Plurodeles waltl*)	Pond	Specialist	1769	12.7
Iberian spadefoot toad (*Pelobates cultripes*)	Open	Generalist	1535	10.9
Iberian painted frog (*Discoglossus galganoi*)	Open	Specialist	182	1.3
Southern marbled newt (*Triturus pygmaeus*)	Mixed	Specialist	87	0.6

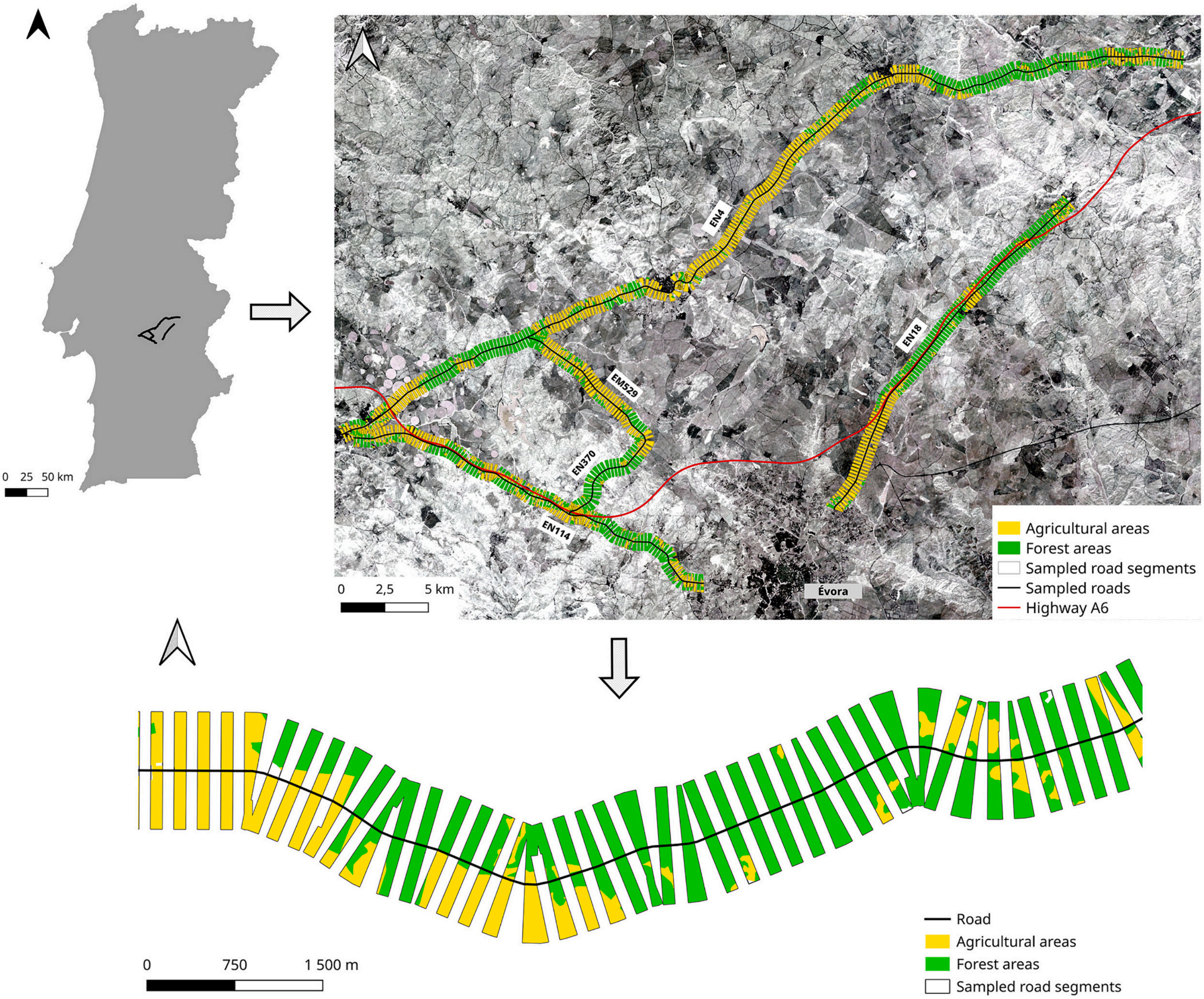

Fig. 1. Location of the study area in Southern Portugal, with sampled roads and major land cover classes. A detailed zoom highlights the 500 m buffer for each 100 m sampled road segment.

2.3. Environmental covariates and model definition

Since our selected species cover different movement distances and amphibians typically respond to micro-habitats (Langen et al., 2009), we split each road section into 100 m length contiguous segments (totalising 1182 road segments), to evaluate the spatiotemporal patterns of roadkill occurrences. To reduce spatial autocorrelation among our roadkill data, we used alternate and non-consecutive road segments (thus comprising 591 segments). We used a 500 m buffer around each road segment to extract land use and remote sensing covariates (see below) based on the species ecology (Pinto et al., 2023) and previous studies in nearby areas (Carvalho and Mira, 2011). We performed all the road segmentation and buffer creation in QGIS software (version 3.24.1; QGIS Development Team, 2022).

We modelled the spatiotemporal variation in roadkill with a multi-season occupancy model, a statistical method that estimates the likelihood of a species occurring at a specific site, while accounting for imperfect detection (MacKenzie et al., 2006). Imperfect detection (the inability to sight an individual or species when present) is tightly connected to ecological surveys and particularly to roadkill surveys, especially if a species is cryptic or of small size (Barrientos et al., 2018); when ignored, it can substantially underestimate roadkill numbers (Santos et al., 2016), ultimately leading to the imprecise implementation of mitigation measures (Santos et al., 2018). This model technique requires a repeated sampling design with a detection history during a closed period of time: thus, we considered each model season to be an entire year, where the 591 road segments (sites) were sampled 12 months each year.

This occupancy model contains four main parameters: first-year occupancy (Ψ), extinction (ε), colonisation (γ), and detection probability (ρ) (MacKenzie et al., 2003).

First-year occupancy (Ψ) represents the probability of a road segment having a roadkill in the first year, hereafter treated as the initial roadkill probability. According to Kéry and Chandler (2016), occupancy is modelled as a function of fixed site covariates (covariates that do not vary temporarily during the whole sampling period). We calculated five site covariates regarding land cover and distance to key landscape features. Concerning land cover site covariates, we used CORINE Land Cover (CLC) 2006, 2012 and 2018 to describe the land cover during 2006–2020. We merged some classes to reflect the two dominant land uses in the study area (Forest and Agriculture) and extracted the mean percentage of each land cover class from the three CORINE years for each road buffer. We have also included ‘distance to highway’ and ‘distance to ponds’ (calculated by applying Euclidean distance to a 30 m spatial resolution raster - obtained from pre-existing detailed cartography done in the framework of the LIFE LINES project – LIFE14 NAT/PT/001081) as site covariates because they represent two key features in the study area that we believe have an influence on the presence of amphibian roadkill (Ascensão and Mira, 2005; Pinto et al., 2023). Lastly, we included the percentage of tree density within each road segment, obtained by LANDSAT imagery (with 30 m pixel spatial resolution) to represent the structure of the forest (higher percentages may represent a well-established forest with a substantial canopy and a high number of individuals, meaning high-quality habitat for most amphibians; Matos et al., 2012). We performed all these operations using QGIS software (version 3.24.1; QGIS Development Team, 2022).

The next two model parameters are extinction (ε) and colonisation (γ), which translate the changes of roadkill over space and time. Extinction is the species’ probability of being road-killed in a road segment in a given year, but not the following year, considered therefore as roadkill disappearance. Colonisation specifies the species’ probability of not being road-killed in a specific road segment in a given year, but only in the subsequent year, hence considered as roadkill appearance. Since these two last parameters are related to changes within seasons, that is, between replicates of the same year (MacKenzie et al., 2006), we have used site covariates that change between years and road segments (also known as yearly site covariates). Thus, we used two remote sensing-derived indices proven to reflect determinant drivers for amphibians (Qian et al., 2007; Pinto et al., 2023): 1) mean EVI (Enhanced Vegetation Index; (Liu and Huete, 1995) representing local dynamics in primary production, and 2) mean LST (Land Surface Temperature; Hulley et al., 2019) used as an air temperature proxy. For both indices, we computed the standard deviation (SD_EVI and SD_LST) to replicate the variations throughout each year. We calculated these remote sensing indices from LANDSAT images (LANDSAT 5 and LANDSAT 8 image collections - Level 2, Tier 1) with a 30 m spatial resolution (US Geological Survey (USGS) – http://lpdaac.usgs.gov) for each year (2006–2020). We only retained high-quality images with 0 % cloud cover for the whole study area (WRS-2 scene: path 203, row 33) and we computed the mean and standard deviation for each set of images representing each year. In total, we obtained 84 images with an average of 5.6 images per year; the year 2012 had no 0 % cloud cover images available, so we decided to exclude it from the analyses (Table S2, Supplementary material). We processed all composite images and calculated the remote sensing indices in Google Earth Engine (Gorelick et al., 2017). Additionally, we have also used the aggregated total annual precipitation, obtained from monthly climatological newsletters of the Portuguese Institute for Sea and Atmosphere (IPMA, 2021) for the region (Fig. S1, Supplementary material), as a yearly site covariate since the rainiest years generally result in higher amphibian roadkill numbers (as a consequence of higher amphibian activity; Glista et al., 2008).

The last model parameter is detection probability (ρ) and refers to the probability of detecting a roadkill at a road segment during a monthly survey in a given year, given its occurrence. To account for imperfect detection, we used observation covariates to model detection probability. As our sampling frequency changed (either daily or weekly; Table S1 Supplementary material), we used sampling effort (number of sampled days per month) as an observation covariate. We considered two additional observation covariates of relevant interest to detect roadkill amphibians: 1) mean monthly precipitation (IPMA, 2021), since observer visibility may be compromised; and 2) a categorical covariate indicating whether the sampled road section had a paved shoulder or not (0 – no paved shoulder; 1 – paved shoulder). Paved shoulders expand the width of the road, which may affect observer detection performance.

The months where no sampling occurred and the road sections that were not sampled in a given month of a certain year (as the sampled road lengths varied through 2006–2020), were coded in the dataset as missing values (NA), so that estimated parameters of models would not be biased due to the heterogeneity in road surveys.

We performed all statistical analyses using the package ‘*unmarked*’ (Fiske and Chandler, 2011) within R software (version 4.1.2; R Core Team, 2021). Table 2 lists all used covariates and respective descriptions.

2.4. Model selection

We constructed our unique species’ detection history matrices with the presence or absence (or ‘NA’ whenever a road segment was not sampled in a particular month). Before modelling, we assessed collinearity using a Pearson correlation analysis between all continuous covariates and retained all covariates with a correlation higher than |0.7| (Dormann et al., 2013). We did not include highly correlated covariates in the same model (‘Forest areas’ and ‘Agriculture areas’, and ‘Tree density’ and ‘Agriculture areas’; Table S3, Supplementary material). We standardized all continuous covariates to zero mean and unit variance (Zuur et al., 2009) so that coefficients could be comparable between different types of covariates and used Akaike’s Information Criterion with correction for small sample sizes (AICc; Burnham and Anderson, 2002) to identify the most parsimonious models constructed for each of the four parameters.

We started our model selection procedure by modelling detection probability against each covariate in a univariate model. We also

Table 2
List of covariates used in the multi-season occupancy models.

Model parameter	Covariate name and code	Description	Source	References
Initial roadkill probability	Distance to ponds	Distance to nearest water body (meters)	Project LIFE LINES	N/A
	Distance to highway	Distance to highway 'A6' (meters)	Project LIFE LINES	Haklay and Weber, 2008
	Tree density	Percentage of tree density (%)	NASA	Sexton et al., 2013
	Agriculture areas	Percentage of agricultural area (%)	CORINE 2006, 2012, 2018	EEA
	Forest areas	Percentage of forest area (%)	CORINE 2006, 2012, 2018	EEA
Roadkill disappearance/ appearance	Enhanced Vegetation Index (EVI)	Mean changes in primary production content index. -1 (indicating stressed vegetation) to 1 (indicating healthier vegetation)	USGS	Liu and Huete, 1995
	Land Surface Temperature (LST)	Mean radiative temperature of land surface (°C)	Google Earth Engine (source code – Ermida et al., 2020)	Hulley et al., 2019; Ermida et al., 2020
	Total annual precipitation	Aggregated total annual precipitation for the study area region (mm)	IPMA	IPMA, 2021
	Standard deviation of Enhanced Vegetation Index (SD_EVI)	Annual variation in primary production content index	USGS	Liu and Huete, 1995
	Standard deviation of Land Surface Temperature (SD_LST)	Annual variation in radiative temperature of land surface (°C)	N/A	Hulley et al., 2019; Ermida et al., 2020
Detection probability	Sampling effort	Total number of days sampled per month	N/A	Santos et al., 2011
	Mean monthly precipitation	Mean monthly precipitation for the study area region (mm)	IPMA	IPMA, 2021
	Road shoulder	Categorical covariate indicating if a road has paved shoulder ('1') or not ('0')	N/A	N/A

constructed a model with all four model parameters constant (null model) to provide a comparison. We discarded all covariates that ranked lower than the null model and tested all the ones that ranked higher in every possible combination. The detection probability model with the highest rank was then used to model all the remaining three parameters: initial roadkill probability, roadkill disappearance and roadkill appearance, as recommended by MacKenzie et al. (2003). For each one of these, we used a similar procedure (separate selection of covariates for each parameter) while using the best model of the detection probability parameter. After finding the best singular models for each parameter, we assigned the respective covariates to a final model. This protocol was applied to each of our seven selected species. We have also performed a Variance Inflation Factor (VIF; Zuur et al., 2010) analysis on all the final models to check for possible collinearity among covariates (Table S4, Supplementary materials).

Next, we performed model validation by computing the MacKenzie and Bailey Goodness-of-fit test (MacKenzie and Bailey, 2004) using 1000 bootstrap samples for each full model. We discarded the models with a lack of fit ($p < 0.05$) or solid overdispersion (c-hat value >4). Lastly, we calculated the mean for all model parameters for each species to assess the roadkill risk over time.

3. Results

Four models presented good fit: spiny common toad ($\hat{c} = 1.52$, $p = 0.06$), fire salamander ($\hat{c} = 1.03$; $p = 0.16$), Iberian painted frog ($\hat{c} = 0.38$; $p = 0.25$) and the Southern marbled newt ($\hat{c} = 0.37$; $p = 0.22$). The three remaining models were discarded because they presented strong overdispersion and a high lack of fit: Natterjack toad ($\hat{c} = 952.5$; p = 0), Iberian spadefoot toad ($\hat{c} = 15.39$; p = 0) and the Iberian ribbed newt ($\hat{c} = 8.36$; $p = 0.01$). For this reason, the following results concern only the four species with valid models.

Mean initial roadkill probability was similar across the spiny common toad, the fire salamander, and the Iberian painted frog (0.635 ± 0.09, 0.547 ± 0.07 and 0.543 ± 0.12, respectively), while the Southern marbled newt had the lowest value (0.239 ± 0.08; Table 3). Mean roadkill disappearance probability values were low for all species, with the higher values registered for the fire salamander and the Iberian painted frog (0.102 ± 0.02 and 0.111 ± 0.03, respectively; Table 3). Mean roadkill appearance probability values were also low, with the highest values corresponding to the spiny common toad and the fire salamander (0.075 ± 0.003 and 0.043 ± 0.01, respectively; Table 3).

Table 3
Mean initial roadkill, mean roadkill disappearance, mean roadkill appearance and mean roadkill detection probabilities for the four analysed species.

	Spiny common toad	*Fire salamander*	*Iberian painted frog*	*Southern marbled newt*
Mean initial roadkill probability (ψ)	0.635 (±0.09)	0.547 (±0.07)	0.543 (±0.12)	0.239 (±0.08)
Mean roadkill disappearance probability (ε)	0.078 (±0.02)	0.102 (±0.02)	0.111 (±0.03)	0.06 (±0.06)
Mean roadkill appearance probability (γ)	0.075 (±0.03)	0.043 (±0.01)	<0.0001	<0.0001
Mean roadkill detection probability (ρ)	0.023 (±0.003)	0.028 (±0.003)	0.004 (±0.001)	0.003 (±0.001)

Mean roadkill detection probability followed the same pattern, with the spiny common toad and the fire salamander presenting higher values (0.023 ± 0.003 and 0.028 ± 0.003 respectively) than the Iberian painted frog and the Southern marbled newt (0.004 ± 0.001 and 0.003 ± 0.001, respectively; Table 3).

We detected a general decrease in the roadkill probability over time for the four species (Fig. 2). The spiny common toad revealed an approximately stable roadkill probability pattern until 2017 but experienced a major decrease in 2018 towards 2020, totalling a reduction of nearly 50 % of roadkill probability during the study period. The fire salamander had a descending trajectory in the roadkill probability pattern until 2016, where it recovered to 2009 values, but afterwards experienced a decrease towards 2020, with a total roadkill reduction of nearly 50 % as well. The Iberian painted frog and the Southern marbled newt presented a decreasing tendency in the roadkill probability patterns over the entire study period, without a single year with an ascending trajectory. The Iberian painted frog showed a roadkill probability reduction of over 70 %, with an accelerated decrease between 2014 and 2016, while the Southern marbled newt registered a more constant decrease throughout all years, totalling a roadkill reduction of 40 %. We also denoted a peak in roadkill disappearance in 2015 for all species, followed by the years 2017 and 2019 for the spiny common toad, the fire salamander, and the Southern marbled newt (although not so expressive; Fig. 3). The emergence of new roadkill in road segments over time (roadkill appearance) did not follow a general clearer pattern

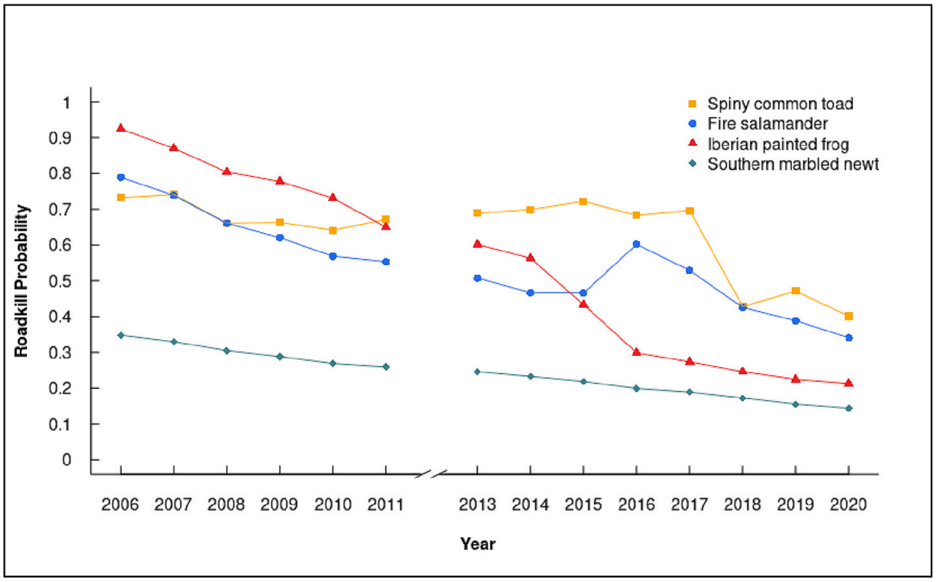

Fig. 2. Mean roadkill probabilities over the 14 studied years for the four selected species (2012 was not included in the analyses).

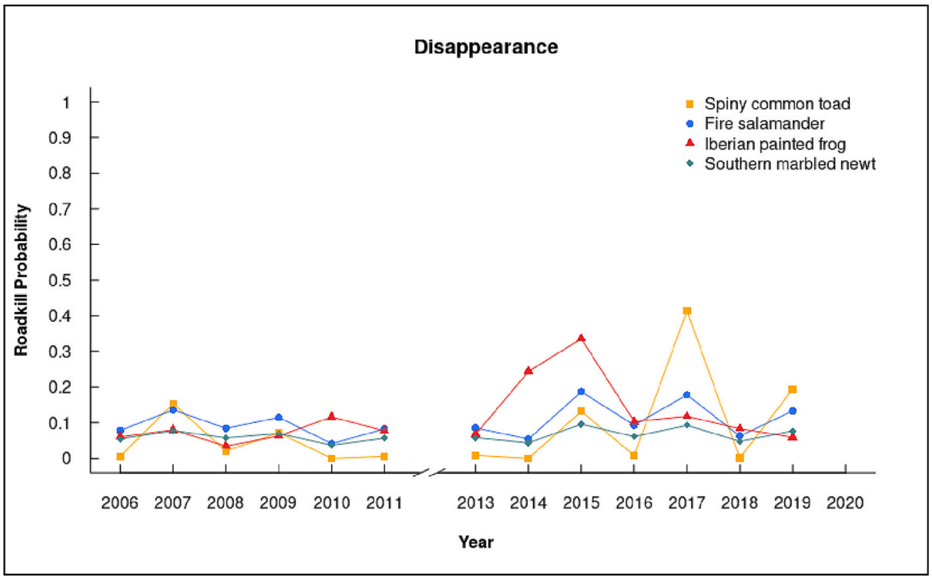

Fig. 3. Mean roadkill disappearance probabilities over the 14 studied years for the four selected species (2012 was not included in the analyses).

as roadkill disappearance, with the highest values for the spiny common toad and the fire salamander in 2015 (Fig. 4). For all species, mean roadkill disappearance probability was greater than roadkill appearance probability during the studied period (Table 3).

Concerning model covariates for the initial roadkill probability, the roadkill probability of the spiny common toad increased in road segments surrounded by forest patches (coef = 6.34; CI =1.62, 11.06) and far away from water bodies (coef = −1.85; CI = −3.34, −0.36). Road segments with higher tree density increased the roadkill probability of the Southern marbled newt (coef =0.716; CI = 0.15, 1.28). The initial roadkill probability for the fire salamander and the Iberian painted frog had no significant covariates.

Regarding the roadkill disappearance parameter, we found significant results for three of the four analysed species: the variation in the primary production decreased the probability of roadkill disappearance (that is, the roadkill in one year disappears in the following year) for the Iberian painted frog (coef = −0.514; CI = −0.91, −0.12); rain showed the same negative relation for both the spiny common toad (coef = −3.024; CI = −3.99, −2.05) and the fire salamander (coef = −0.479; CI = −0.91, −0.05); high land surface temperature decreased the probability of roadkill disappearance for the Iberian painted frog (coef = −0.758; CI = −1.42, −0.09), but increased the probability of roadkill disappearance for the spiny common toad (coef =1.07; CI = 0.57, 1.56).

For the roadkill appearance parameter, the low land surface temperature increased the probability of new road segments with roadkill for the fire salamander (coef = −2.48; CI = −3.66, −1.30).

Lastly, for the detection parameter, we observed a pattern common to all species, with high sampling effort (total number of sampled days per month) and high precipitation increasing the probability of detecting roadkill (Table 4). Also, the probability of detecting a roadkill increased in road segments with paved shoulders for the spiny common toad (coef = 0.812; CI = 0.61, 1.02) and the Iberian painted frog (coef = 0.665; CI = 0.17, 1.16) and decreased the probability for the fire salamander (coef = −0.798; CI = −0.94, −0.66) and the Southern marbled

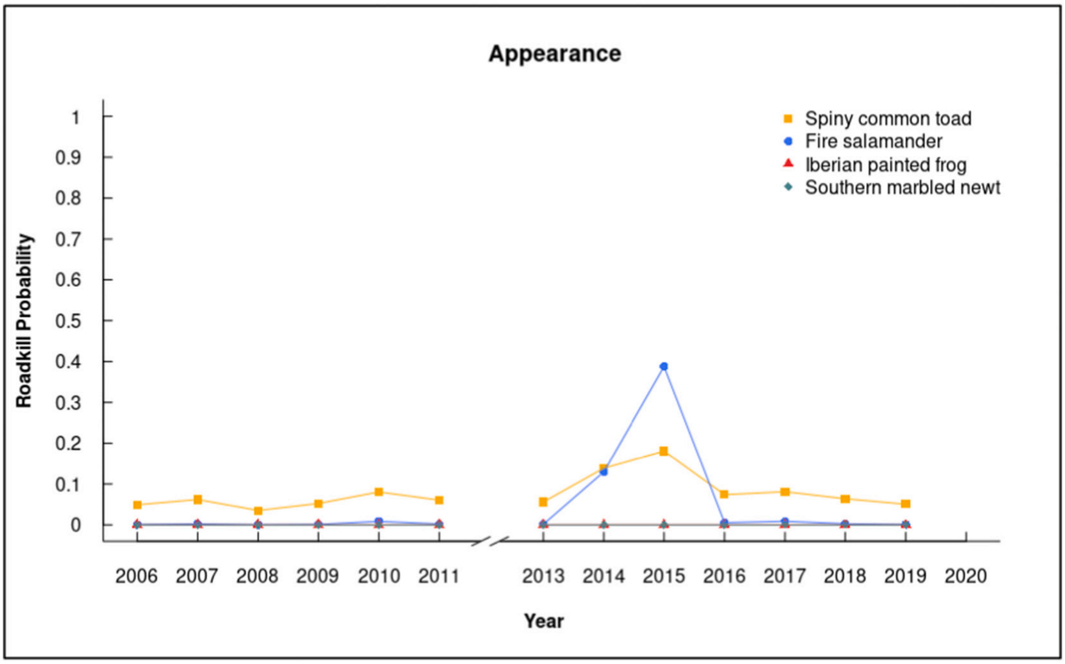

Fig. 4. Mean roadkill appearance probabilities over the 14 studied years for the four selected species (2012 was not included in the analyses).

Table 4
Coefficients (and SD) of multi-season occupancy models for the four analysed species. Statistically significant coefficients are highlighted in bold; Confidence intervals are in brackets.

	Spiny common toad	Fire salamander	Iberian painted frog	Southern marbled newt
Initial roadkill probability ψ				
Distance to ponds	**−1.85 ± 0.76** **[−3.34; −0.36]**			−0.583 ± 0.30 [−1.17; 0.01]
Distance to highway		−41.9 ± 59.4 [−158.41;74.53]	−2.24 ± 1.97 [−6.09;1.62]	
Tree density		62.7 ± 90.6 [−114.91;240.25]		**−0.785 ± 0.54** **[0.15;1.28]**
Agricultural areas				
Forest areas	**6.34 ± 2.41** **[1.62;11.06]**			
Roadkill disappearance Ɛ				
EVI		0.06 ± 0.11 [−0.15;0.27]		
LST	**1.07 ± 0.25** **[0.57;1.56]**		**−0.758 ± 0.34** **[−1.42; −0.09]**	
Total annual precipitation	**−3.02 ± 0.49** **[−3.99; −2.05]**	**−0.48 ± 0.22** **[−0.91; −0.05]**		−0.29 ± 0.74 [−2.03;1.45]
SD_EVI	−0.04 ± 0.12 [−0.28; 0.19]		**−0.514 ± 0.20** **[−0.91; −0.12]**	
SD_LST	0.17 ± 0.13 [−0.08;0.42]			
Roadkill appearance γ				
EVI				
LST	−0.511 ± 0.30 [−1.11;0.09]	**−2.48 ± 0.60** **[−3.66; −1.30]**		
Total annual precipitation				
SD_EVI				
SD_LST				
Detection probability ρ				
Sampling effort	**0.911 ± 0.05** **[0.82;1.01]**	**0.644 ± 0.04** **[0.56;0.72]**	**1.140 ± 0.14** **[0.86;1.42]**	**0.868 ± 0.19** **[−0.49;1.24]**
Mean monthly precipitation	**0.627 ± 0.03** **[0.58;0.68]**	**0.819 ± 0.03** **[0.77;0.87]**	**0.562 ± 0.06** **[0.43;0.69]**	**0.708 ± 0.09** **[0.53;0.89]**
Road shoulder	**0.812 ± 0.11** **[0.61;1.02]**	**−0.798 ± 0.07** **[−0.94; −0.66]**	**0.665 ± 0.25** **[0.17;1.16]**	**−0.756 ± 0.31** **[−1.36; −0.15]**
AIC	9518	9106	1657	894

newt (coef = −0.756; CI = −1.36, −0.15). Table 4 summarizes these results.

4. Discussion

4.1. Roadkill trends over time

We detected an overall decrease in roadkill probability over time, for the four analysed species. Four non-exclusive hypotheses can explain this: 1) application of mitigation measures; 2) historical roadkill patterns; 3) increase in traffic intensity; and 4) drought effects.

Concerning the first hypothesis, since 2018, three analysed roads (EM529, EN114 and EN4) have been subject to specific amphibian mitigation measures such as drift fences, underneath tunnels and road warning signs, as a result of a LIFE Nature and Biodiversity Program of the European Commission (LIFE LINES - LIFE14 NAT/PT/001081). Yet, these measures only totalise approximately 5 % of the 120 km of surveyed roads and were only installed in the last two years of sampling (2018). Even more, our results show a decreasing trend over the entire study period, suggesting this reason might not be a primary source for roadkill reduction.

In relation to the second hypothesis, roadkill might have decreased over time, simply because there are fewer amphibians to be roadkilled, as denoted by Fahrig and Rytwinski (2009) and Matos et al. (2012). Indeed, Cooke (2011) reported a continuous decrease in the roadkill numbers for the European common toad (*Bufo bufo*) during a twenty-year long-term study in England. This decrease was associated with population declines in nearby surveyed ponds, concluding that road mortality has played a significant role in the decline of those populations. Our raw data (roadkill) also indicates a reduction in the roadkill over the sampled years. To our knowledge, there are no studies in our study area reporting reductions in amphibian abundance caused by other factors (e.g., climate change, urbanisation, diseases, persecution, etc). Also, Orlowski (2007) and D'Amico (2009) found that higher numbers of amphibian roadkill are generally associated with higher local abundances, which may suggest that in our study area, amphibian abundances may have been declining over time.

Concerning the third possible hypothesis, and since we have no traffic data for our study area, we gathered the numbers of fuel and new vehicles sold in our region between 2006 and 2020 from the National Statistics Institute (INE, 2021). Although the pattern is cyclical (and likely correlated with past financial crises), the number of new vehicles sold between 2013 and 2020 has nearly tripled (Fig. S2, Supplementary materials). Also, fuel consumption had an ascending trajectory within the same time period (2013–2020), although not so expressive (Fig. S2, Supplementary materials). Considering these data as traffic proxies, the hypothesised potential increase in traffic may have exacerbated the barrier effect due to disturbance which may have forced existing populations to move away from roads, leading to the decline of amphibian roadkill. Fahrig et al. (1995) reported a similar response, with a decrease in amphibian road mortality directly correlated with the barrier effect caused by an increase in traffic intensity. Mazerolle (2004) also concluded that even small variations in traffic intensity can trigger distinct responses for different amphibian species. Alternatively, the potential increase in traffic may have led to a reduction in roadkill, solely due to the lack of animals to be road-killed, as referred in the aforementioned hypothesis.

As for the last hypothesis, the study area is marked by dry weather, with temperature increment and rainfall reduction recorded in the last years (Fig. S1, Supplementary materials). These weather variations may be less compatible with amphibian activity (Moss et al., 2021), which could also have led to a reduction in roadkill. The effects of droughts have also been linked to amphibian declines in other studies (Rowe et al., 2019; Zylstra et al., 2019; Moss et al., 2021).

We cannot choose a specific reason over the others: most probably the last three aforementioned reasons have contributed to shaping the roadkill patterns. The cumulative effect of road pressures (e.g., traffic intensity; species' historical roadkill patterns) in combination with other factors such as extreme climate events may pose serious threats to amphibian populations and may have helped to shape the local amphibian roadkill dynamics in our study area. This is especially alarming since our models detected that, in 14 years, the probability of the Iberian painted frog (the only amphibian species with the conservation status of Near Threatened in Portugal; Cabral et al., 2005), decreased by approximately 70 %. In the case of the spiny common toad and the fire salamander, roadkill probability decreased by nearly 50 %, and 40 % for the Southern marbled newt. More studies are needed, particularly focusing on amphibian populations inhabiting areas closer to roads, alongside with continuous roadkill monitoring, to understand the underlying factors contributing to the observed declines. Understanding the roadkill cause (e.g., increase in traffic volume, lack of suitable habitat), is pivotal for devising targeted conservation strategies.

Although we have detected peaks in roadkill disappearance and appearance probabilities, these were fairly low (< 0.12), indicating that variation in roadkill spatial patterns from year to year is unlikely, and suggests an apparent stability in roadkill spatial patterns over time.

4.2. Influence of covariates on roadkill dynamics

We have found that the drivers of amphibian roadkill spatial patterns are mainly species-specific, as reported in previous studies (e.g., Sillero, 2008; Matos et al., 2012; Pinto et al., 2023). Roadkill of the Southern marbled newt was more likely to occur in road segments crossing areas with higher tree density. Forest areas are considered high-quality habitats for many amphibian species (Sillero, 2008; Carvalho and Mira, 2011; Matos et al., 2012; Heigl et al., 2017). Although Reques (2014) did not find a relation between the occurrence of the Southern marbled newt and tree cover, our model suggests this species might occur in higher tree density areas. The spiny common toad roadkill probability also increased in road segments surrounded by forest areas, a response also reported by Matos et al. (2012) who found higher roadkill for this species in forest habitats. This is expected since the spiny common toads are known to prefer habitats with higher tree cover (Ortiz-Santaliestra, 2014). Additionally, road segments closer to water bodies decreased the roadkill probability for the spiny common toad. Although previous studies reported high mortality concentrations of this species near water bodies (Orlowski, 2007; Santos et al., 2007), the ones closest to our sampled roads are mainly used for irrigation and cattle watering, and thus might not be suitable for this species' reproduction. Spiny common toads prefer deep, permanent, and clean water bodies (Ortiz-Santaliestra, 2014) that in our study area are generally located far from the roads.

Years with less rain had road sections with a higher probability of roadkill disappearance in the next year for the spiny common toad and the fire salamander, meaning that when it rains more, the road sections with roadkill stay as roadkill for the next year. This is expected since rain mediates amphibian activity (Glista et al., 2008): increasing animal movement in turn increases roadkill risk. The low intra-annual variation in primary production (SD_EVI; i.e., humidity content in vegetation) reduced roadkill disappearance probabilities for the Iberian painted frog. Less humidity variability represents stable local conditions for amphibian movement; this is particularly relevant for the Iberian painted frog because it is more dependent on water (Martínez-Solano, 2014) than other species (e.g., spiny common toad). These road segments with low variation in humidity conditions may represent predictable movement corridors for this species, which in turn decreases the probability of roadkill disappearance at these locations from one year to the next. On the other hand, our models denoted opposite responses for high land surface temperature: a decrease in roadkill disappearance probability for the Iberian painted frog, and an increase for the spiny common toad. Amphibians are ectothermic and temperature governs their activity (Araújo et al., 2006). In fact, this trend can be linked to the patterns of drought in the study area. According to the IPMA (2023), the study area region experienced a drought event every other year between 2014 and 2020, with the years 2015 and 2019 being characterised by moderate droughts, and the year 2017 by a severe drought. The roadkill disappearance probabilities reached higher values in years characterised by drought for the aforementioned species. As stated before, Iberian painted frogs are more water dependent and thus, may be more affected by an increase in the temperature (characterised by a higher probability of roadkill disappearance), since this species typically occurs in moisture areas surrounding water bodies or ponds. However, the spiny common toad is a highly terrestrial species (seeking water bodies only for reproduction; Ortiz-Santaliestra, 2014): therefore, this species may be less exposed to temperature changes and desiccation, leading to a higher probability of roadkill disappearance in the next year.

Concerning the roadkill appearance probabilities, our models only revealed a significant covariate for one species: an increase in the probability of the emergence of new road segments with roadkill with low land surface temperature for the fire salamander. As stated before, amphibians are extremely dependent on the temperature: they typically display seasonal occurrence patterns, with a reduction or complete cessation of their activity at certain times of the year (e.g., winter and summer; Speybroeck et al., 2016). In resemblance with the spiny common toad, the fire salamander is also a highly terrestrial species. Thus, an increase in temperature, associated with increased desiccation risk, may restrain fire salamanders' movement, while lower temperatures may trigger increased movement, in turn increasing the probability of the appearance of new road segments with roadkill.

The Southern marbled newt did not respond to any of the roadkill disappearance and appearance parameters, leading to the perception that perhaps roadkill patterns are less dynamic in this species or there was not enough data to model the temporal parameter of our models. This withstands the importance of continuous sampling and long-term studies, in order to gather data on elusive species to draw better conservation management.

Overall, amphibian detection probabilities were rather low (< 0.1), thus confirming the need to correct it to avoid underestimating roadkill probability. This probability was higher with increased sampling effort. This response was expected since the increase in sampling periodicity will also increase the probability of detecting a roadkill (Santos et al., 2011). Our models also predicted a higher probability of detecting a roadkill in months with higher precipitation. Although previous studies report the opposite relation (Hels and Buchwald, 2001; Santos et al., 2011), amphibian activity is extremely dependent on rainy events in our study area. As amphibian roadkill increases with rain (as demonstrated by our models), the probability of detecting an amphibian carcass on rainy days will likely increase as well. It is true that the observer visibility may be impacted by heavier rainfall during surveys, however, carcass persistence may also increase due to reduced scavenger activity (Sergio, 2003). Concerning the influence of road shoulders on the detection probability, we detected a taxonomic pattern: anurans had higher detection probabilities on roads with paved shoulders, while urodele species had an opposite response. A possible explanation for this result could be related to the site where a given animal is road-killed and to the site's surrounding habitat. For instance, we assessed the roadkill spatial locations for all four species and detected that both the spiny common toad and the Iberian painted frog roadkill are mainly located on roads with paved shoulders (these roads cross a mix of both open forest and agriculture habitats), whereas the majority of the urodele roadkill are located on roads without paved shoulders (surrounded mainly by closed forest). This justifies the somehow order-grouped response, as the probability of detecting a species' roadkill in a given site is directly related to the probability of a carcass being present at that site and the species' preferred habitat. Therefore, caution is needed when extrapolating this result to other study areas.

5. Conclusions

In this study, we applied occupancy modelling to unveil the spatiotemporal patterns of amphibian roadkill in species with different habitat requirements. Our models explicitly show a reduction in amphibian roadkill over time for all analysed species. Although with low probabilities, roadkill disappearance dynamics seem to be more related to rainfall events and temperature and were more expressive than roadkill appearance dynamics. Our results seem to point towards an overall reduction in local amphibian populations, with little to no recruitment over time. This might be a serious challenge to the survival and maintenance of amphibian populations in landscapes such as the Mediterranean Basin.

Our study showed that occupancy models can be used to monitor spatiotemporal roadkill patterns, with fair to good results. This technique can be employed for other species and in other regions worldwide to understand the lasting effects of roads on wildlife in ever-changing landscapes. Our results may serve as a starting point to understand amphibian roadkill patterns across space and time. Future studies may investigate further the potential causes.

This work also emphasises the urgency in monitoring amphibian populations occurring near roads, in order to compare the population trends with the roadkill rates. This information would certainly be useful in providing targeted mitigation measures, depending on the cause of the decline (e.g., application of measures to reduce traffic or promote safe crossing structures between both sides of the roads). The acquisition of precise insights into the drivers of roadkill decline is imperative to formulate relevant and impactful conservation measures, particularly in critical places where roadkill is recurrent, and populations are more fragile.

CRediT authorship contribution statement

Tiago Pinto: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Neftalí Sillero:** Conceptualization, Methodology, Supervision, Writing – review & editing. **António Mira:** Funding acquisition, Methodology, Writing – review & editing. **Sara M. Santos:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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